DNA Structure and Analysis

- Required Characteristics of Genetic Material
  - replication
  - Information storage
  - Expression of information
  - Variation by mutation
- Central dogma of molecular genetics
  - Information is stored in DNA is transcribed into RNA (tRNA, mRNA, and rRNA) which are used to translate that information into proteins
  - DNA makes, RNA makes Proteins
- Friedrich Miescher 1844-1895
  - Isolated "nuclein" from nucleii of "pus cells - later na DNA but... "The many asymmetric carbon atoms (of protein) allow such a colossal number of stereoisomers that the richness and variety of hereditary transmission may find their expression in it"
- Evidence favoring DNA as Genetic Material
  - 1927- Frederick Griffith found that heat killed virulent strains of Diplococcus pneumoniae could "transform" living avirulent strains and restore pathogenicity
  - Strains of Diplococcus pneumoniae used by Griffith in his Original Transformation Experiments:
    - Serotype: IIR, IIIS
    - Colony Morphology: Rough, Smooth
    - Capsule: Absent, Present
    - Virulence: Avirulent, Virulent
- Figure 9-2
  - Smooth colony (IIIS) living IIIS (virulent) were injected into the mouse, the mouse dies
  - Rough colony (IIR) living IIR (avirulent) were injected into the mouse, the mouse lives
  - heat killed IIIS is injected into the mouse, mouse lives
  - living IIR and heat-killed IIIS are injected into the mouse, mouse dies and the analization reveals that living IIIS is recovered
- Evidence favoring DNA as genetic material
  - 1944- Avery, McCarthy and McLeod performed "genetic transformation" studies in Diplococcus pneumoniae. An extension of Griffiths experiments
  - Virulent strains from bacterial colonies that are smooth (S) in appearance whereas avirulent strains appear rough ®
  - Found that DNA alone from S strains could change R strains to smooth (figure 9-2)
  - Concluded that "A DNA is the fundamental unit of the transforming principle of Pneumococcus type III:

    - 1952- Hershey and Chase found that DNA of bacteriophage is responsible for directing reproduction of the virus during replication in E coli
- Figure 9-3a: Avery, MacLeod, McCarty- DNA = transforming principle
    - IIIS cells in liquid culture medium → put in centrifuge → IIIS cells spun to bottom of test tube → heat kill → homogenize cells recover IIIS filtrate → extract carbohydrates, lipids, and proteins → treat with protease → treat with ribonuclease → threat with deoxyribonuclease
    - results: IIR + IIIS filtrate: transformation occurs, IIR cells + protease-treated IIIS filtrate: transformation occurs, IIR + RNase-treated IIIS filtrate: transformation occurs, IIR cells + DNase-treated IIIS filtrate: NO transformation occurs
- Figure 9-3b
    - IIR cells + DNase-treated IIIS filtrate (no transformation) → Only IIR cells, Conclusion: Active factor is DNA
    - IIR + RNase-treated IIIS filtrate (transformation occurs) → IIR cells + IIIS cells, Conclusion: Active factor is NOT RNA
    - IIR cells + protease-treated IIIS filtrate (transformation occurs) → IIR cells + IIIS cells, Conclusion: Active factor is NOT protein
    - IIR + IIIS filtrate (transformation occurs) → IIR cells + IIIS cells, Control: IIIS contains active factor
- Bacteriophage attach to surface of a bacterial cell but do not enter...what does?
    - attachment of phage tail fibers to bacteria wall → phage genetic materil is injected into the bacterium → phage reproductive cycle begins → components accumulate; assembly of mature phages occurs → cell lysis occurs and new phages released
- Figure 9-5a; Set up of Hershey-Chase experiment
    - Phated are about 50% DNA and 50% protein
    - The differential labeling of DNA with P^{32} and protein with S^{35} was the key to the design of this experiment
    - Only ^{32}P labeled material was injected into the host so DNA not protein was the genetic material
- 9.4 Indirect evidence that DNA is the genetic material
    - 1: DNA is found in the nucleus where genetic material needs to function - also is protein. But protein is also in the cytoplasm whereas DNA is NOT.
    - 2: The DNA content of haploid cells is half that of diploid cells
    - 3: The action spectrum of UV light in damaging genetic material correlates with the absorption spectrum of DNA
    - 4: Recombinant DNA technology proves traits can be transferred from organism to organism by DNA alone
- Some viruses use RNA as the genetic material
    - Although the use of DNA is near universal - there are some viruses that use RNA as the genetic material
        - ex) the QB virus that infects bacteria, it uses an RNA replicase to make new copies of RNA inside the host cell

    - **Retroviruses**- have RNA as the genetic storage material but go through a DNA stage when replicating
    - this is carried out by an enzyme known as **reverse transcriptase**
- Structural analysis of DNA
    - 1953- proposal of double helix structure of DNA by Jim Watson and Francis Crick revealed a structure with required properties of genetic material
    - Know basic chemical structures of RNA and DNA. Do not need to draw but be able to recognize bases and what 5' and 3' ends are and phosphodiester bonds
    - Erwin Chargaff base composition data (1949-1953)
        - amount of A residues is proportional to about of T residues and amount of G residues is proportional to C
        - Sum of purines (A + G) = sum of pyrimidines (C + T)
        - % of G + C does not necessarily equal % of A + T
- 9.7 difference between ribose and deoxyribose
    - ribose- has OH
    - deoxyribose- has H
- Watson Crick Model of DNA
    - two long polynucleotide chains coiled around a central axis, forming a right-handed double helix
    - two chains are antiparallel (C-5' to C-3') orientations run in opposite directions
    - bases of both chains are flat perpendicular to axis stacked 0.34 nm
    - Nitrogenous bases paired via hydrogen bonds, with only A + T and G + C pairs permitted (complementarity)
    - One complete turn of the helix every 3.4 nm (10 bases/turn)
    - Major and minor grooves apparent along DNA axis
    - Helix is 2 nm in diameter
- RNA is similar to DNA but single stranded
    - RNA has ribose instead of deoxyribose and uracil instead of thymine
    - Although most RNA is single stranded, some RNA folds back on itself to make small regions of double stranded RNA (antiparallel)
    - There are three major classes of RNA
        - 1: ribosomal RNA (rRNA)
        - 2: messenger RNA (mRNA)
        - 3: transfer RNA (tRNA)
    - Several other classes of RNA - small nuclear RNA (snRNA), microRNA (miRNA) and short interfering RNA (siRNA)
- Separation of DNA molecules by Electrophoresis
    - DNA is negatively charged so when it is placed in an electrical field is will move
    - Small DNA molecules will move faster through a gel than large one
    - This is an important technique known as DNA gel electrophoresis
    - DNA samples are loaded into depressions (wells) in the gel and immersed in a buffer