Genetic Analysis and Mapping in bacteria and bacteriophage

- Bacteria and Bacteriophage
  - modern molecular genetics is founded on bacterial and bacteriophage studies
  - they have extremely short reproductive cycles
  - can be studied in pure cultures (not mixed)- all individuals are the same
  - pure cultures exhibit heritable variation (origin of this variation was once hotly debated)
  - Complex processes have evolved for the transfer of genes between individual cells in populations of bacteria
  - Mutation in bacteria
    - 98% of life on earth is microscopic
    - **Fluctuation test**: by Luria and Delbruck showed that spontaneous mutation is the primary source of genetic variation in bacteria
    - **Prototrophs**: can synthesize all essential organic compounds for growth from a 'minimal medium'
      - single carbon and nitrogen sources and inorganic ions
    - **Auxotrophs**: have lost through mutation the ability to make at least one essential organic component
    - **Culture growth**: middle of exponential growth is the ONLY reproducible physiological state for bacteria
      - typical bacterial population growth curve showing the initial lag phase, the subsequent log phase where exponential growth occurs, and the stationary phase that occurs when nutrients are exhausted
  - Dilution technique to enumerate bacterial population
    - 10-fold serial dilutions of bacteria are plated on nutrient medium
    - on the first plate 1 mL of a 10^3 fold dilution of cells was spread
    - On the second and third 1 mL of 1 in 10^4 and 10^5 was spread
    - What was the original concentration of cells?
      - plate 3 has 15 colonies so 15 x 10^5 cells per mL of undiluted cells
- Genetic recombination in bacteria
  - already known that naked DNA can be taken up by bacteria (genetic transformation)
  - Lederberg and Tatum (1946) found bacteria undergo a 'parasexual' process whereby genetic material is passed from one bacterium to another- conjugation
  - demonstrated by mixing two multiple auxotrophs and obtaining prototrophs
  - cells that donated genetic information dubbed 'fertile' or F+
  - those that received genetic information (recipients) dubbed F-
  - direct contact between donor and recipient is required (Davis U-tube experiment) via a sex-pilus
    - when a strain A and B auxotrophs are grown in a common medium but separated by a filter no genetic recombination occurs and not prototrophs are produced

- mixed auxotrophs produce prototrophs- evidence for genetic recombination
- F- factor found to be mobile and when passed to a recipient then the recipient became F+
    - now know to be a 100 kb plasmid with at least 19 genes involved in formation of the sex pilus and conjugative process
- F+ factor is replicated during transfer to recipient
- Hfr strains (high frequency of recombination)- certain genes are transferred from a donor strain at a high frequency and a non-random fashion
    - A Hfr strain has an F+ factor (plasmid) integrated into the host chromosome
        - Occasionally this will recombinant back out of the chromosome and frequently this will be imprecise and the new F+ factor will carry adjacent genes- this is called F' (F prime)
    - When F' factor is transferred to another cell by conjugation there will be two copies of the genes on the F' factor - a partial diploid or Merozygote
        - Ex) Hfr Bacteria
            - 1. Conjugation occurs between F^+ and F^- cell
            - 2. One strand of the F factor in nicked by an endonuclease and moves across the conjugation tube
            - 3. The DNA complements is synthesized on both single strands
            - 4. Movement across conjugation tube is completed DNA synthesis is completed
            - 5. Ligase closes circles; conjugants separate
                - the end result is that the F+ donor cell transmits the F plasmid and the F- recipient not becomes F+ (fertile) also
- Hfr bacteria and chromosome mapping
    - 1. F factor is integrated into the bacterial chromosome and the cell becomes an Hfr cell
        - A Hfr strain has an integrated F+ factor (plasmid)
        - Conversion of F+ to an Hfr state occurs by integration the F factor into the bacterial chromosome
        - the point of integration determines the origin (O) of transfer
    - 2. Conjugation occurs between an Hfr and F- cell. The F factor is nicked by and enzyme creating the origin of transfer of the chromosome (O)
    - 3. Chromosome transfer across the conjugation tube begins. The Hfr chromosome rotates clockwise
        - During conjugation an enzyme nicks the F factor not integrated into the host chromosome, initiating transfer of the chromosome at that point
        - Conjugation is usually interrupted prior to complete transfer
    - 4. Replication begins on both strands as chromosome transfer continues. The F factor is now on the end of the chromosome adjacent to the origin.

        - 5. Conjugation is usually interrupted before the chromosome transfer is complete.
            - Only the A and B genes are transferred to the F- cell, which may recombine with the host chromosome.
    - Figure 8-5
        - Minutes of conjugation vs relative frequency of recombination graph
            - The progressive transfer during conjugation of various genes from a specific Hfr strain of E. coli to an F- strain
            - Certain genes transfer more quickly than other and recombine more frequently
            - Others take longer to transfer and recombinants are found at a lower frequency
        - Time map of genes studied in the experiment
            - by recording the time interval between the transfer of each trait to recipients, the 'relative distance' between the genes can be estimated (in min)
    - The F' state and Merozygotes (Partial diploids)
        - 1. Excision of the F factor from the chromosome begins. During excision the F factor sometimes carries with it part of the chromosome
        - 2. Excision is complete during excision the A and E regions of the chromosome are retained in the F factor. The cell is converted to F'
            - occasionally the Hfr plasmid will recombine back out of the chromosome and frequently this will be imprecise and the new F+ factor will carry adjacent genes- this is called F' (F prime)
        - 4. The F factor replicated as one strand is transferred
            - following conjugation with an ordinary F- cell the recipient cell becomes partially diploid and is called a merozygote it also behaves as an F+ donor cell
        - 5. Replication and transfer of the F factor is complete the F- recipient has become partially diploid and is called a merozygote it is also F'
            - when an F' factor is transferred to another cell by conjugation there will be two copies of the genes on the F' factor - a partial diploid of merozygote
    - By looking for genes that reduced the frequency of integration of F+ plasmid genes called recA, recB, recC, and recD were discovered which diminish recombination about 1000 fold (similar proteins are found in Eukaryotes)
        - **RecA**- plays a role in recombination between single stranded DNA (ssDNA) and its homolog on the host chromosome
        - **RecBCD complex**- when dsDNA undergoes recombination this complex unwinds the helix to facilitate recombination via RecA
    - F factors and R plasmids
        - R plasmids contain one or more resistance factors such as antibiotic resistance genes - very important in hospitals - a BIG problem when they carry multiple drug resistances