Chapter 14 Lecture Notes: Microbial Genetics – Recombination and Plasmids

I. Genetic elements

A. Chromosome

B. Plasmids

1. Small, circular DNA molecules that can exist independently of the chromosome
2. Characteristics
   a) Much smaller than the chromosome (<1/20th the size), ranging in size from 200 kb to 2 kb.
   b) contain origin of replication which directs DNA replication
   c) many contain partioning systems which help partition plasmids into daughter cells during cell division
   d) Copy number is variable (1 –200 copies per cell depending on the plasmid)
   e) contain "non-essential" genes
   f) some can integrate into the chromosome
3. Types (Table 14-1)
   a) Fertility factor plasmids
      (1) carries genes for transfer to another bacterium via conjugation (see later)
      (2) can integrate into the chromosome
   b) R plasmids
      (1) carry drug resistance genes
      (2) many are conjugative
   c) Col plasmids
      carry genes for bacteriocins – bacterial proteins that destroy other proteins
   d) Virulence plasmids
      carry virulence genes in pathogenic bacteria which allow for dissemination and survival of the organisms in the body
   e) Physiological function plasmids
      (1) carry genes for degradation of compounds
      (2) nodulation and symbiotic nitrogen fixation (*Rhizobium*)
   f) Cryptic plasmids – no known function
4. Evolutionary implications/Importance
   a) To organisms as indicated above
   b) To research as vector for transferring genes into the cells (Fig. 15-11)

C. Transposable elements

1. DNA segments that are capable of transporting themselves to other locations on the chromosome (or onto plasmids) by a process called transposition
2. General characteristics
    a) carry gene(s) required for transposition (transposase)
    b) transposons may carry other genes
    c) some carry conjugation genes
    d) "ends" are composed of inverted repeats (similar sequences that are inverted) that are recognized by transposase during transposition.

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__________>          <__________
GGGGTCTAGAtransposonTCTAGACCCC
CCCCAGCTCTtransposonAGATCTGGGG
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    e) insertion site on chromosome is duplicated during insertion so that the element is flanked by direct repeats of approx. 9 basepairs (Fig. 14-9)
    f) different levels of randomness of insertion depending on element
    g) frequency of transposition = $1/10^3$ to $1/10^4$ per element per generation
    h) frequency of transposition into a certain gene = $1/10^5$ to $1/10^7$
    i) frequency of reversion = $1/10^6$ to $1/10^{10}$
3. Types
    a) Insertion sequences – very simple; carry just gene(s) required for transposition (Fig. 14-8a)
    b) Transposons (Fig. 14-8b)
        (1) carry genes in addition to transposase
        (2) can be formed by 2 insertion sequences that flank additional genes (composite transposons)
    c) Replicative vs. nonreplicative (see attached)
4. Mechanisms of replicative transposition within circular DNA molecules
    a) generation of staggered breaks in target DNA and in the transposon
    b) joining of transposons to ends of target DNA
    c) replication of transposon and staggered breaks
    d) ligation to generate a cointergrate (both molecules are joined)
    e) recombination (see later) to resolve the molecules apart
5. Evolutionary implications/importance of transposition
    a) Disruption of genes
    b) Activation of cryptic genes by promoter element in transposable element
    c) Carry genes between bacteria
    d) Mediate rearrangement of genetic material because of recombinational events between two similar sequences
    e) Tool for genetics as a mutagenesis, reporter

D. Phage

II. Genetic processes

A. Recombination

1. Process by which a new chromosome that differs from either parent DNA is formed by combining genetic material from two sources; crossing over during meiosis in eukaryotes

2. Types

a) General reciprocal (Fig. 14-2)

(1) reciprocal exchange between two homologous DNA sequences

(2) termed "general" because it can occur anywhere within the large region of homology

(3) very complex molecular process characterized by strand nicking → strand exchange and ligation → branch migration to generate a heteroduplex → resolution via endonuclease to generate recombinant molecules → ligation

(4) recombination between similar sequences on two circular molecules generates a cointegrate (2 molecules joined together)

(5) recombination between similar sequences on the same circular molecule can generate inversions or deletions of intervening sequence depending on the orientation of the homologous sequences

(6) recombination in linear molecules as per Fig. 14-2

b) Non-reciprocal

(1) nonreciprocal exchange between two homologous DNA sequences (Fig. 14-3)

(2) may be important in bacterial transformation

c) Site-specific

(1) recombination of "non-homologous" genetic material at a particular site in the chromosome (i.e. integration by phage lambda)

(2) short seq. of homology mediate recombination at an exact site

(3) important for phage

d) Replicative

(1) recombination of non-homologous genetic material accompanied by replication of genetic material

(2) important for transposons

B. Transformation
    1. Uptake of naked DNA from media/environment by a cell and stable incorporation of the material
    2. From where does the DNA come?
        a) Lysed cells release DNA fragments
        b) We provide DNA in the lab
    3. Only some cells can be transformed – they are termed competent
        a) naturally competent (some organisms take DNA without manipulation) via several different mechanisms (Fig. 14-17 ex of *Streptococcus pneumoniae*)
        b) in the lab
            (1) $CaCl_2$ treatment
            (2) electroporation
    4. Fate of DNA (Fig. 14-16 and Fig. 14-4)
        a) Linear must recombine into a stable replicon or it will be degraded
        b) Plasmids DNA can be maintained if the origin of replication functions in the host cell

C. Conjugation
    1. Transfer of genetic information via cell to cell contact
    2. Types
        a) F^+ X F^- (Fig. 14-14a)
            (1) F plasmid contains genes for sex pilus formation and plasmid transfer. During mating, F plasmid replicates by rolling circle replication and the copied strand is transferred to the F^- bacterium
            (2) chromosomal genes are rarely transferred
            (3) conversion of F^- to F^+
        b) Hfr (Fig. 14-14b)
            (1) increased frequency in the transfer of <u>chromosomal</u> genes (<u>h</u>igh <u>f</u>requency of <u>r</u>ecombination)
            (2) no conversion of F^- to F^+
            (3) F integrates into the chromosome via homologous recombination at insertion sequences → conjugation can still occur and when it does part of the chromosome is transferred with the part of the plasmid → pilus usually breaks before entire molecule is transferred
            (4) fate of the transferred DNA: degraded unless it is recombined into the chromosome
            (5) 100 minutes to transfer the entire chromosome
        c) F' X F^- (Fig. 14-15)
            (1) F' = F plasmid derivative that picked up part of the chromosome when it excised
            (2) mechanism of transfer is same as F plasmid
            (3) fate of transferred DNA = can be maintained as a plasmid
        d) other plasmids: contain a variety of mobilization sequences and genes

D. Transduction
    1. Transfer of bacterial genes by viruses
    2. Types
        a) Generalized (Fig. 14-19)
            (1) transfer of a random part of the bacterial genome when a virus mistakenly packages a bacterial DNA fragment into the capsid by mistake and then infects another cell
            (2) fate of the DNA
                (a) degraded OR
                (b) recombined into the chromosome at a homologous sequence OR
                (c) maintained as nonintegrated DNA; however, since it can not replicate, the DNA is only in one cell (a clone can not be obtained)
        b) Specialized (Fig. 14-20 and -21)
            (1) transfer of a specific part of the bacterial genome when the bacterial genes are mistakenly excised with the prophage during induction of the prophase and packaged into the capsid by mistake
            (2) fate of the DNA
                (a) can become integrated at phage integration site
                (b) can recombine into the chromosome at a homologous sequence

III. Importance of these elements and processes
A. Evolution of bacteria is in part driven by horizontal (cell to non-daughter cell) genetic exchanges described in this chapter
B. Genetic mapping of genes on the bacterial chromosome
    1. Hfr (Fig. 14-22)
    2. Transduction (closely linked genes will cotransduce at a higher frequency)
C. Recombinant DNA technology
    1. cloning of specific DNA fragments on plasmids and the transfer into bacteria via transformation and/or conjugation (Fig. 15-11)
    2. moving genes via transduction