LECTURE 18

Gene Mapping in Bacteria

A. Genetics of prokaryotes (bacteria, in this lecture) differs in context from genetics in eukaryotes

1. Salient differences between prokaryotes (bacteria, in this case) and eukaryotes

   a) No nuclear membrane
   b) No mitotic apparatus (e.g., centromere) or classical meiosis
   c) Often a singular, closed circular chromosome
   d) Genetically haploid (1N), with no truly bisexual phase

2. Types of "genes" commonly employed in bacterial genetics

   a) Antibiotic-resistant mutants
   b) Nutritional mutants (prototrophs vs. auxotrophs)
   c) Carbon source mutants
   d) A few morphological mutants (but not commonly used)

3. Parasexuality: Transformation, transduction, and conjugation

B. *Transformation*: Conversion of one genotype to another via the introduction, uptake, and recombination of exogenous DNA; the phenomenon is not limited to bacteria or to prokaryotes

1. Exogenous ("foreign") DNA is bound, then taken up across the cell membrane. Recipient cells must be "competent" and secrete a competence "factor" (a small protein) that activates 8-10 other proteins that affect cell wall recognition and membrane transfer of "foreign" DNA.

2. DNA taken into a cell is converted to single-stranded form, and then can undergo exchange with its homologous region on the recipient-cell chromosome. Exchange occurs via a classical break-and-reunion mechanism. Because the recipient chromosome is often circular, a successful exchange requires an "even" number of crossovers.

3. Note the origin of restriction enzymes: the host restriction/modification system (and the absence of such systems in eukaryotes).

4. Mapping of genes primarily is a function of co-transformation frequencies: consider that maximum size of transforming DNA is about 2% of average bacterial genome (about 3,000 genes), so on the order of 50-60 genes are transformed per transforming fragment of DNA. Because the transforming fragments are essentially random, the frequency of co-transformation is an index of spatial relationships of genes.

a) Note: this is not a linkage map! "Classical" linkage study via a standard three-factor cross can be carried out, and from such linkage data it is possible to determine linkage map "distances" (i.e., % recombination) between genes that are close together on the chromosome.

b) Note also that "linkage" groups do not really apply here as generally there is only one chromosome (i.e., only one linkage group).

C. *Transduction:* Bacterial gene transfer mediated by a bacterial virus (phage) vector

1. Introductory material

a) "Virulent" and "temperate" phages and lysogeny (the lysogenic cycle): note that some temperate (lysogenic) phages physically integrate into the bacterial chromosome (and usually at specific integration sites), whereas others can remain autonomous in the bacterial cytoplasm. Both types (integrated or not) replicate (or are replicated) at the time of bacterial cell (DNA) replication.

b) There are three types of transduction: generalized, specialized, and abortive.

2. Generalized transduction

a) "Mistaken-packaging" mechanism whereby any piece of the bacterial chromosome vectored from bacterial cell to bacterial cell during the lytic cycle of a virus. Can involve both virulent and lysogenic viruses.

b) Note that *any* bacterial gene theoretically can be transduced in this way, i.e., each gene has an equal probability of being transduced.

c) Note also that only a low number of transducing particles is produced per infected cell.

d) Mapping is in terms of co-transduction frequencies (same as with transformation), and the average size of the transduced bacterial DNA is (also) about 1-2% of the bacterial genome; can also do "clasical" three-factor mapping.

3. Specialized transduction

a) Where improper excision of an integrated prophage occurs during the lytic cycle and a small, terminal piece of bacterial DNA is incorporated into the viral chromosome.

b) Only bacterial genes on either side of the insertion site can be so transduced, and the transducing particle produced does not carry a full complement of phage genes.

c) Can co-occur in a bacterial cell with a functional phage (*helper*) so that the lytic cycle can be completed, producing a high number of transducing particles

d) The normal "insertion" site of a transducing virus of this sort can be deleted, forcing the prophage to integrate elsewhere in the bacterial chromosome. This permits fine-scale mapping of different regions of the bacterial chromosome.

e) Mapping *per se* is via co-transduction or "classical" three-point mapping.

4. Abortive transduction: where the bacterial gene(s) vectored from one bacterial cell to another fails to become incorporated in the recipient cell's chromosome via crossing over (recombination). This can occur with either generalized or specialized transduction.

D. *Conjugation*: Gene exchange between "sexually" differentiated bacterial types

1. Plasmids and episomes:

Plasmids: autonomous, extra-nuclear genetic elements
Episomes: autonomous and/or integrated, extra-nuclear elements

Conjugative plasmids/episomes:	Capable of causing transfer of genetic material from one bacterial cell to another
Non-conjugative plasmids/episomes:	Not capable of causing transfer of genetic material from one bacterial cell to another

2. The F factor: a conjugative episome, containing about 94,000 base pairs of DNA

a) F factor genes promote cell-to-cell contact (through pili) and transfer of the F episome.

b) Both the F factor and the bacterial chromosome contain IS (insertion sequences). These are relatively small, *transposable* DNA sequences that contain recognition sites (for integration of the F factor into the host chromosome) and a gene coding for a *transposase.*

3. F promotes its own transfer from F^+ cells (ones harboring the F episome) to F^- cells (ones that do not harbor the F episome.

4. Occasionally, an F factor may integrate into the host chromosome and become an *Hfr* (high frequency recombination). The integrated F factor (now called an Hfr) will then promote transfer of an entire copy of the host chromosome to a recipient cell, i.e., from an Hfr cell to an F^- cell.

5. Mapping is via "interrupted mating experiments" that generate a *time-based maps.*

a) Note that time maps are not linkage maps (although genes are co-linear). One can carry out "classical" three-factor crosses.

6. Different Hfrs have different integration sites and different orientation (clockwise or counterclockwise). It was from this genetic evidence that *circularity* of the *E. coli* chromosome was inferred. Circularity of the *E. coli* was later demonstrated visually via electron microscopy.

E. *Sexduction:* Transfer of bacterial genes by F factors (actually F' factors)

1. Occurs when an Hfr returns to the cytoplasm but accidentally includes some bacterial DNA that was adjacent to the integration site of the F factor during its stay in the host chromosome.

2. The F' behaves like an F^+ and will transfer itself (and the 'attached" bacterial gene) when in contact with an F^- cell.

3. Sexduction is an exceedingly valuable genetic tool, and can be used to make merozygotes (partial diploids) of virtually any bacterial gene.