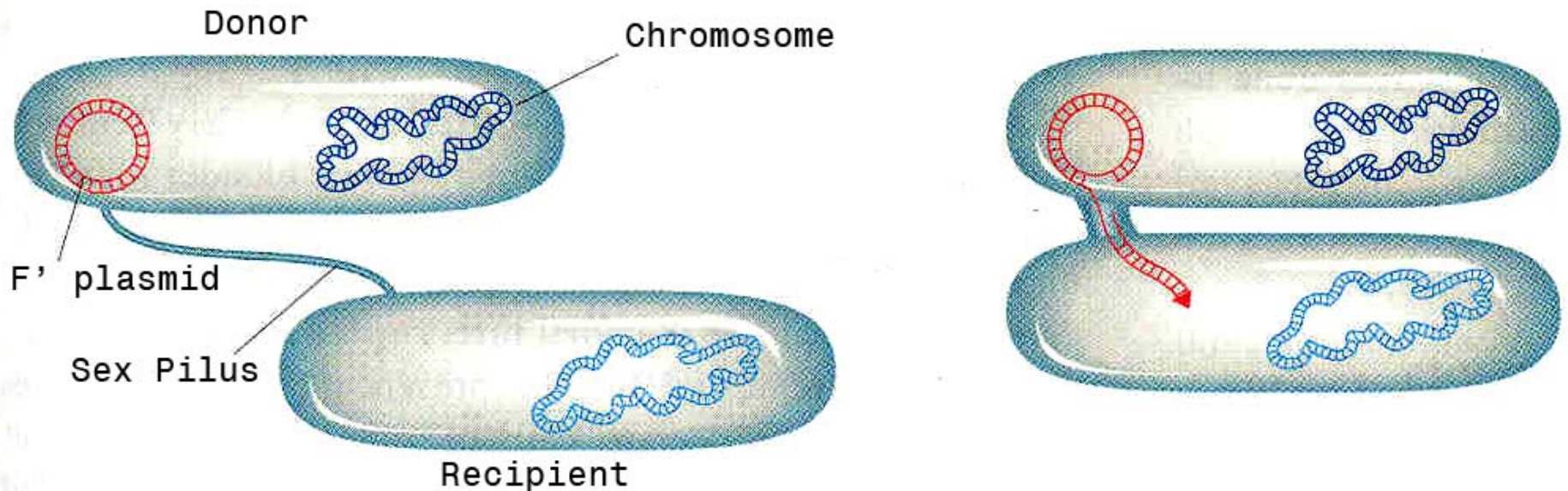


# Bacterial Genetics

- Bacteria acquire genetic diversity through the following mechanisms:
  - Mutation
  - Conjugation
  - Transduction
  - Transformation

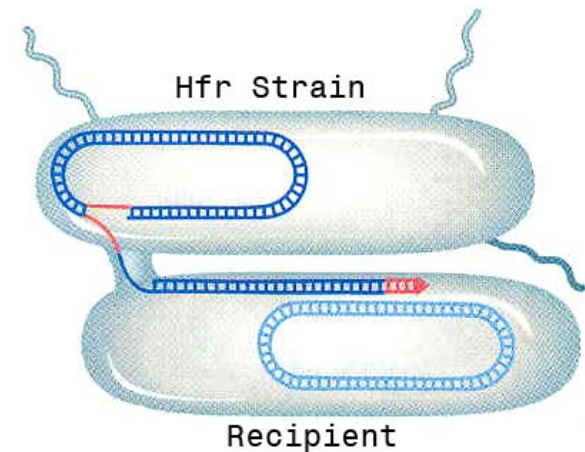
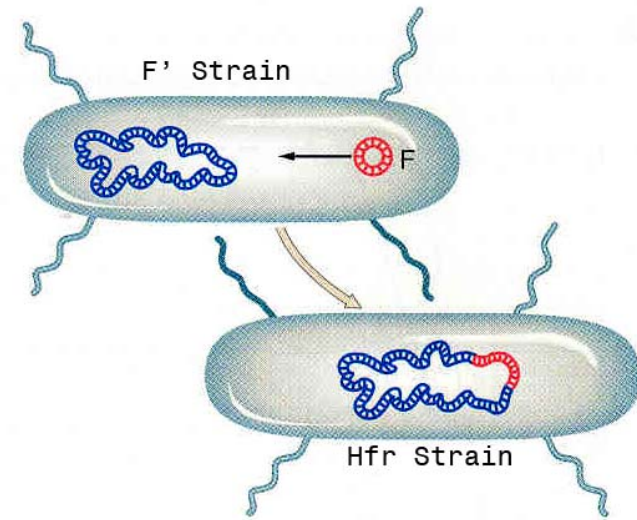
# Conjugation

- The F' plasmid is a small, circular DNA molecule containing genes that encode a sex pilus.
- F+ donor strains can transfer a copy of their F' plasmid to F- recipient cells through the sex pilus, converting them to F+ strains



# Conjugation

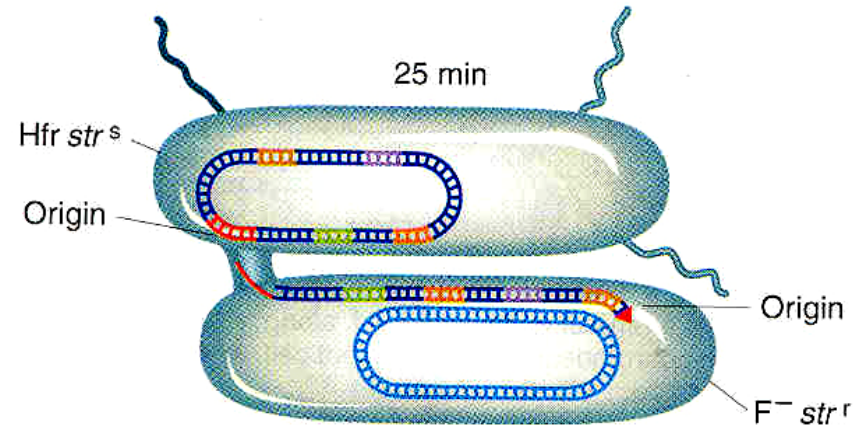
- In some bacteria the F' plasmid integrates into the chromosome, converting the bacterium into an Hfr (high frequency of recombination) strain. The F' plasmid can integrate into several sites in the E. coli chromosome.
- Hfr donor strains can efficiently transfer chromosomal DNA to F-recipient strains
- The first chromosomal genes to be transferred are those adjacent to the F' DNA. Genes are always transferred in the same linear sequence



# Mapping E. Coli genes by interrupted mating

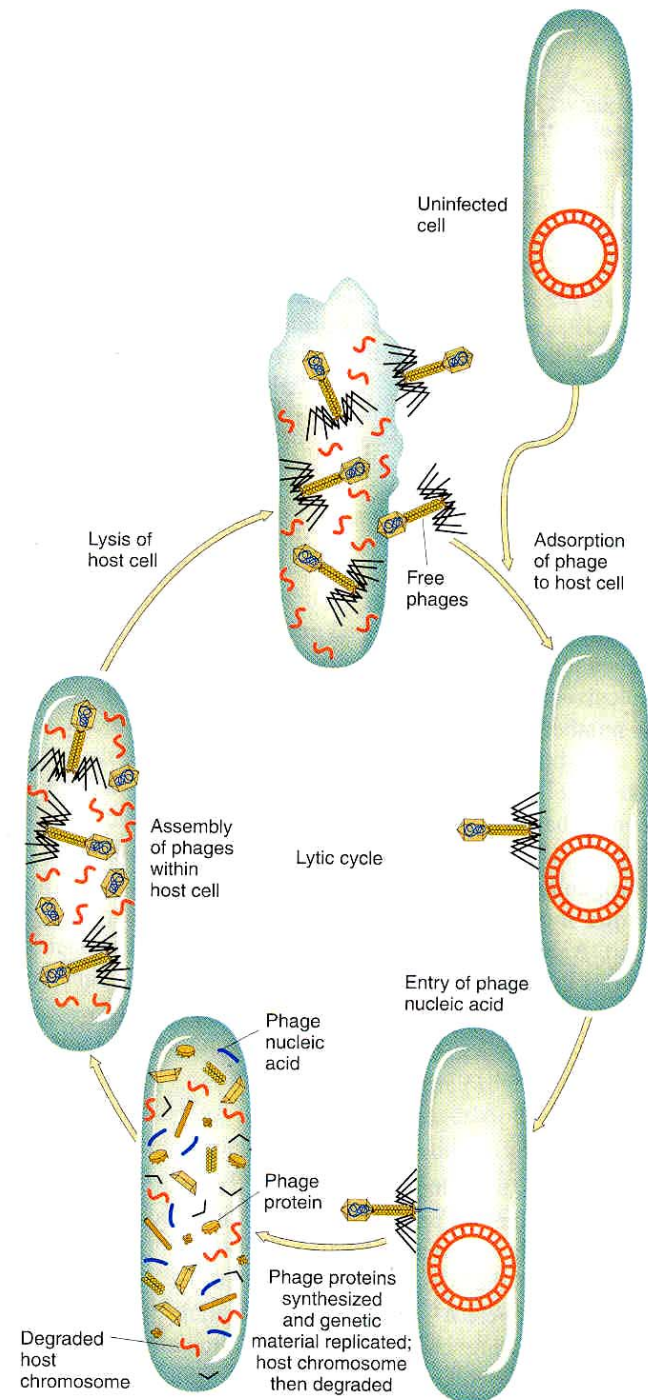
The linear order of E. coli genes can be determined by their order of transfer to the recipient.

- Consider the following cross. The Hfr donor ( $met^+$ ,  $leu^+$ ,  $thr^+$ ,  $str^s$ ) is mated with an  $F^-$  recipient ( $met^-$ ,  $leu^-$ ,  $thr^-$ ,  $str^r$ ).
- Mating is disrupted every 10 minutes by stirring the culture
- Bacteria are plated on media that contains streptomycin and that lacks either methionine, leucine or threonine amino acids.
- It is found that recombinants from early time points are always  $met^+$ , those from later time points are  $met^+$ ,  $leu^+$ , and those from even later time points are  $met^+$ ,  $leu^+$ ,  $thr^+$
- This result shows the order of genes on the chromosome as  $met - leu - thr$



# Lytic cycle of Bacteriophage

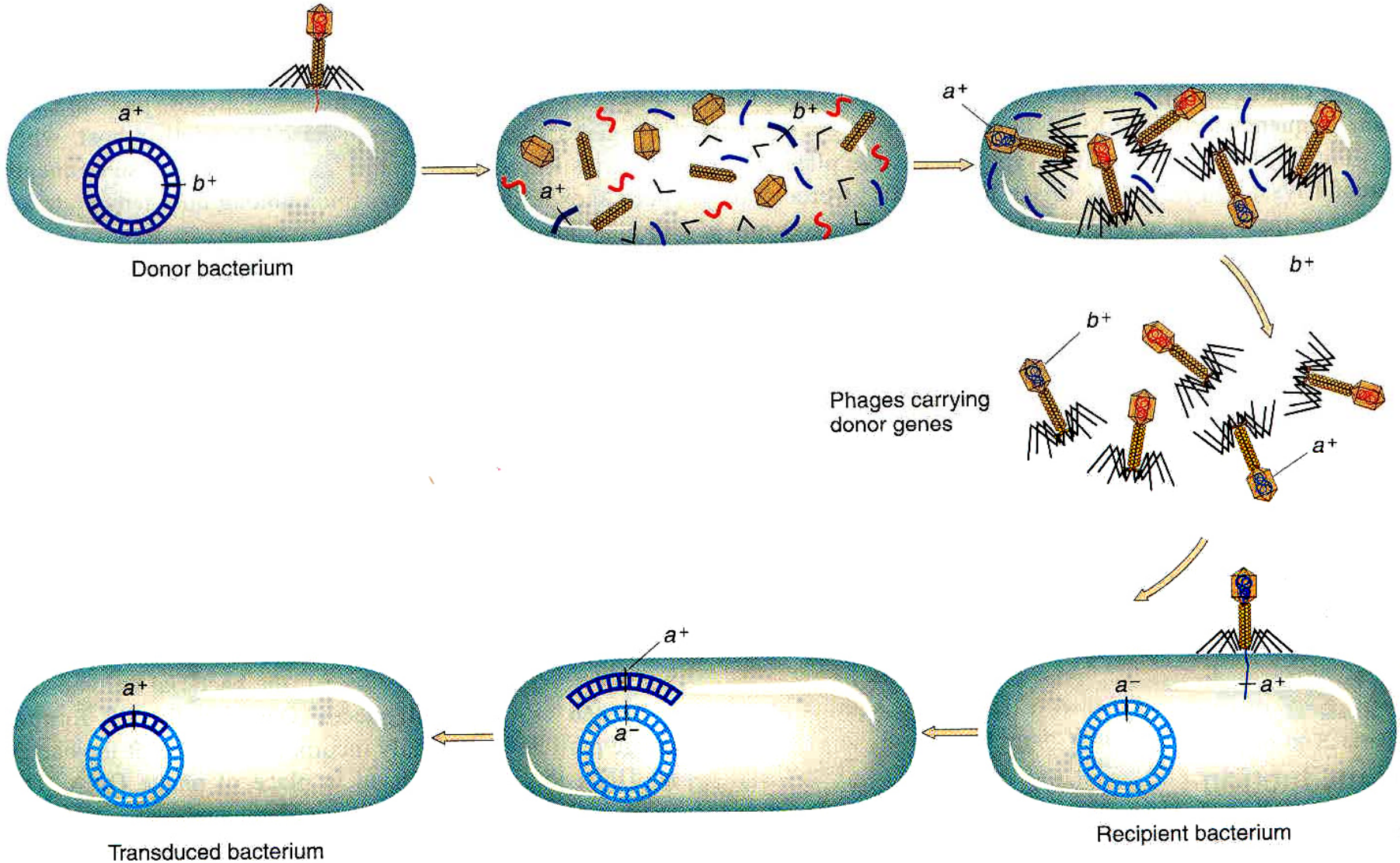
- Bacteriophage (phage) are viruses that infect bacteria. Phage have a head and tail made of protein. The head is filled with viral DNA.
- Phage bind to bacteria and then inject their DNA into the host bacterium.
- Expression of viral genes results in the formation of new phage, fragmentation of the host chromosome, and lysis of the host membrane.
- The new phage go on to infect neighboring bacteria and the cycle continues.



# Transduction

- Refer to the figure on the next slide while reading this slide.
- Transduction occurs when phage transfer bacterial DNA from one bacterium (donor) to another (recipient).
- Occasionally phage package bacterial chromosomal DNA into their head rather than viral DNA (as in the a<sup>+</sup> gene in the figure)
- In the figure, this phage infects a bacterium that happens to be defective for the a gene (a<sup>-</sup>). The phage injects its DNA into the host.
- If homologous recombination occurs then the a<sup>-</sup> bacterium is converted to a<sup>+</sup>

# Transduction

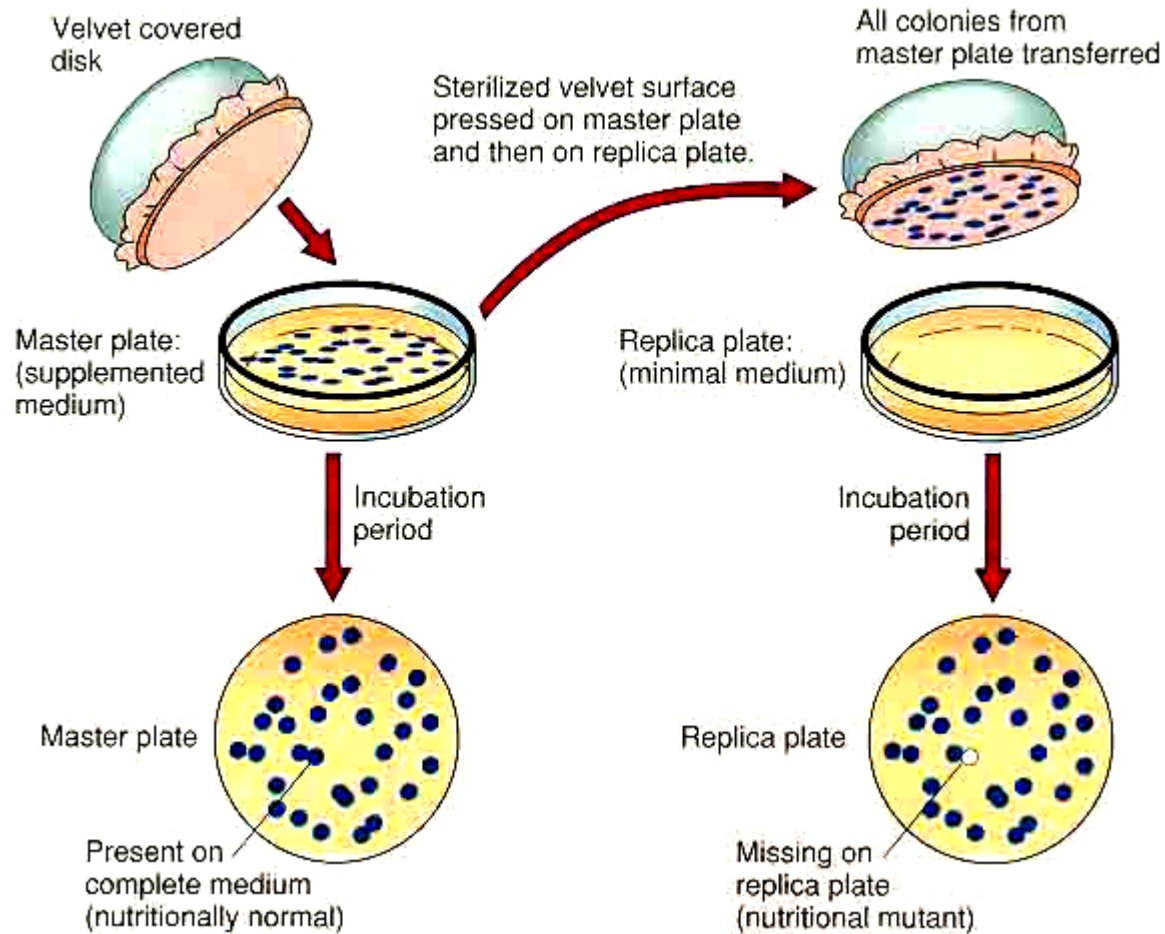


# Transduction mapping of E. coli genome

- Transducing phage can be used to map the linear order of genes on the E. coli chromosome.
- The premise is that only closely linked genes will be packaged together in one transducing phage.
- Consider the following experiment
  - The genotype of donor bacteria is Val<sup>+</sup>, Arg<sup>+</sup>, Cys<sup>+</sup>
  - The genotype of recipient bacteria is Val<sup>-</sup>, Arg<sup>-</sup>, Cys<sup>-</sup>. These bacteria can't synthesize the amino acids; valine, arginine, or cysteine.
  - Donor bacteria are infected with phage.
  - Progeny phage are purified and used to infect recipient bacteria.
  - Recipient bacteria are plated on to nutrient agar plates that lack arginine. 100 colonies grow. These cells must have obtained a functional Arg<sup>+</sup> gene from a transducing phage.
  - The Arg<sup>+</sup> recombinant colonies are replica plated onto nutrient agar that lacks both arginine and cysteine. 50 colonies grow. These colonies must have been infected with a transducing phage that carried both Arg<sup>+</sup> and Cys<sup>+</sup> genes.
  - The Arg<sup>+</sup> recombinant colonies are replica plated onto nutrient agar that lacks both arginine and valine. 20 colonies grow. These colonies must have been infected with a transducing phage that carried both Arg<sup>+</sup> and Val<sup>+</sup> genes.
  - These data tell us that the Arg gene is closer to Cys than to Val on the E. coli chromosome, but there are 2 possible orientations:
    - Val      Arg      Cys                      or                      Val                      Cys      Arg
  - 
  - see the next page for continued discussion



# Replica Plating



# Transformation

- Transformation is the process by which pure DNA (usually in the form of a plasmid) is taken up by a cell. Cells that take up the plasmid are called transformants.
- Typically a selectable marker such as an antibiotic resistance gene is incorporated into the plasmid.
- Only transformed cells will grow in the presence of the antibiotic
- By plating cells on nutrient media containing the antibiotic we can favor the growth of transformants and kill the untransformed cells.

