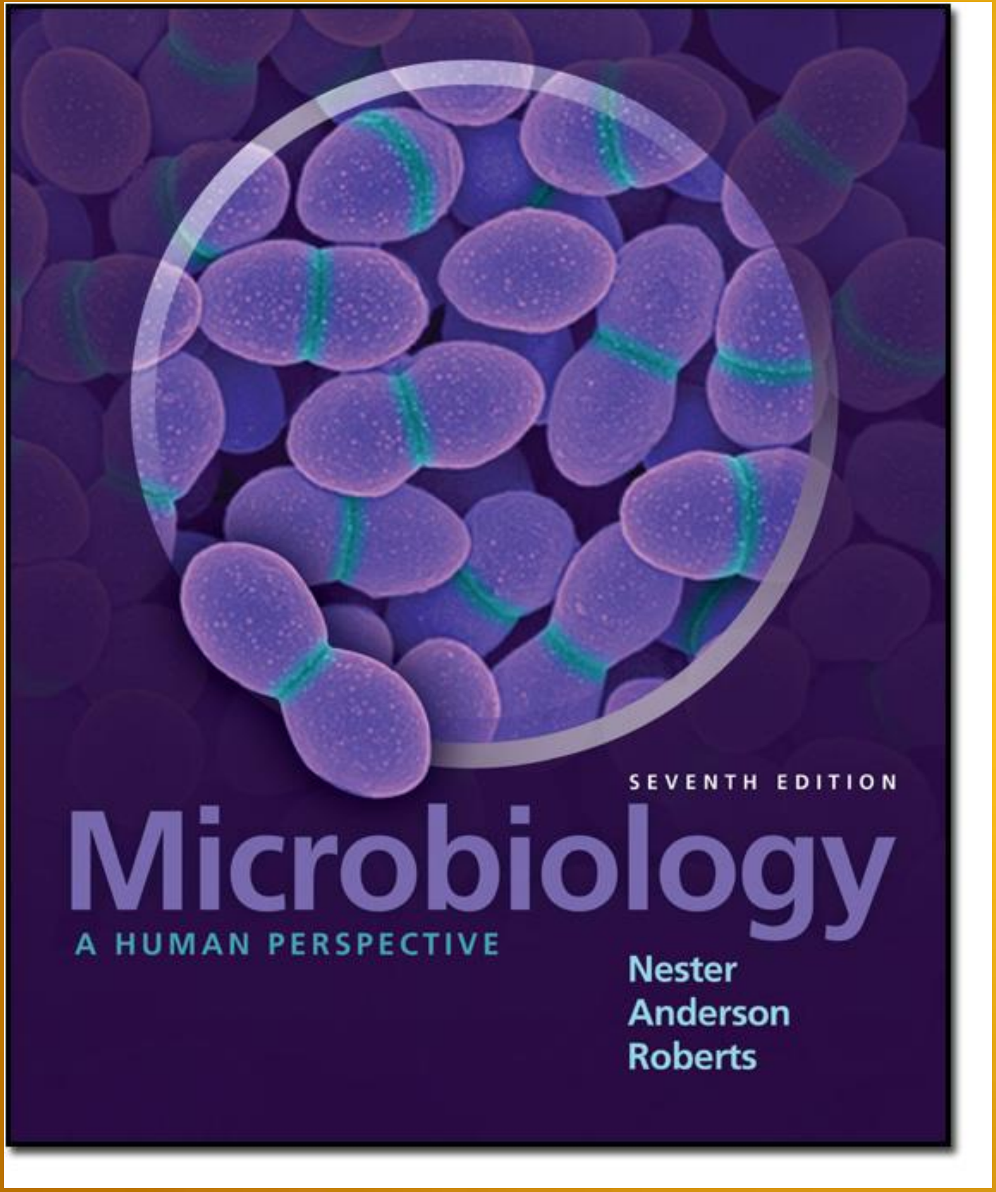


Chapter 08
Lecture

Antibiotic Resistance

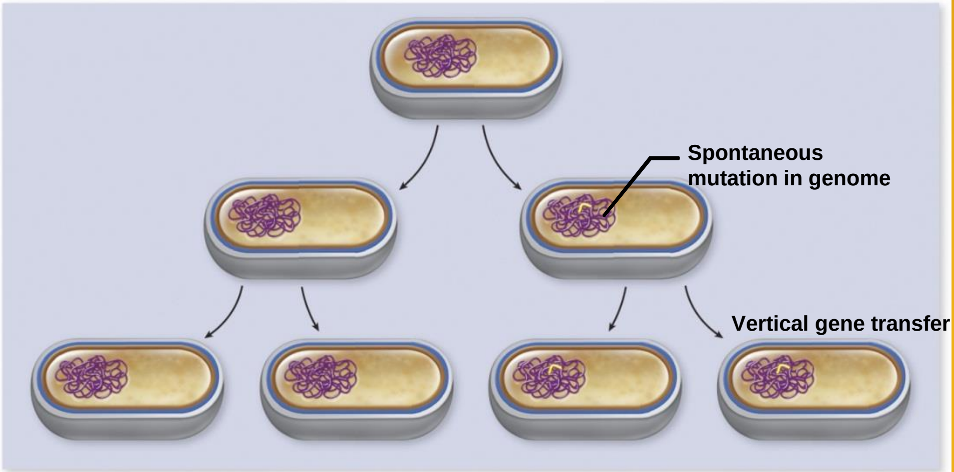
- *Staphylococcus aureus*
  - Gram-positive coccus; commonly called Staph
  - Frequent cause of skin and wound infections
  - Since 1970s, treated with penicillin-like antibiotics
    - E.g., methicillin
  - In 2004, over 60% of *S. aureus* strains from hospitalized patients were resistant to methicillin
  - ~2.3 million healthy people in U.S. harbor methicillin-resistant *S. aureus* (MRSA)
  - Healthcare-associated MRSA (HA-MRSA) resistant to other antibiotics, including vancomycin
    - Vancomycin considered drug of last resort

8.1. Genetic Change in Bacteria

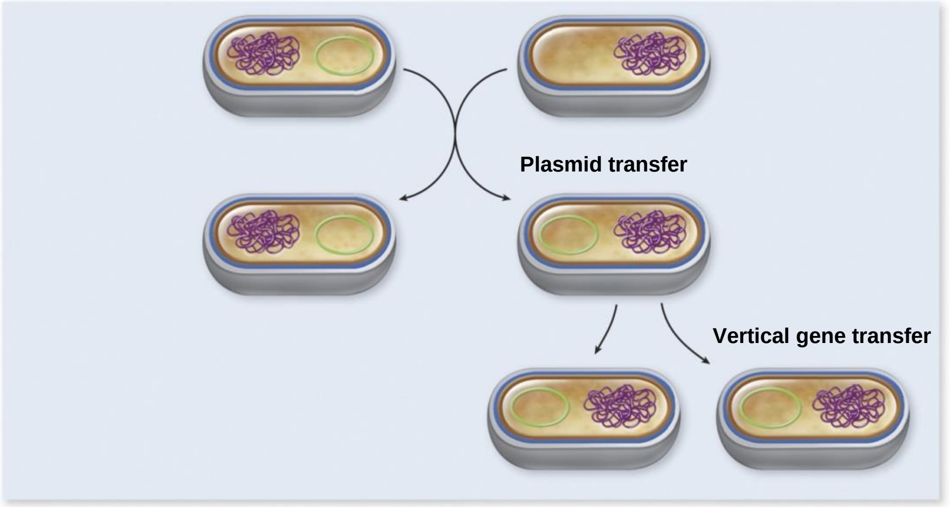
- Organisms adapt to changing environments
  - <u>Natural selection</u> favors those with greater fitness
  - Bacteria adjust to new circumstances
    - Regulation of gene expression (Chapter 7)
    - Genetic change (Chapter 8)
  - Bacteria excellent system for genetic studies
    - Rapid growth, large numbers
    - More known about *E. coli* genetics than any other
  - Change in organism's DNA alters <u>genotype</u>
    - Sequence of nucleotides in DNA
    - Bacteria are haploid, so only one copy, no backup
  - May change observable characteristics, or <u>phenotype</u>
    - Also influenced by environmental conditions

8.1. Genetic Change in Bacteria

- Mutation and horizontal gene transfer



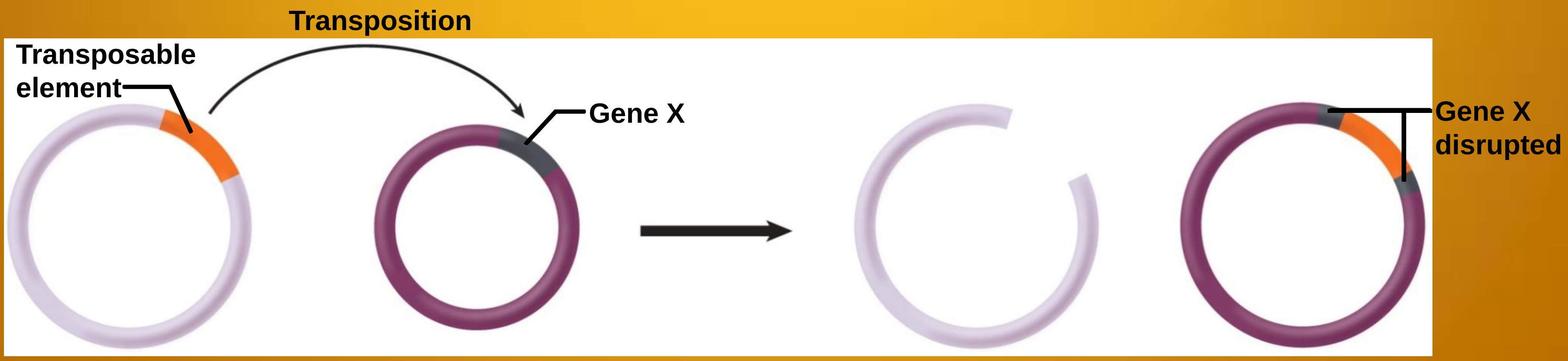
(a) Mutation



(b) Horizontal gene transfer

8.2. Spontaneous Mutations

- Transposons (jumping genes)
  - Can move from one location to another
  - Process is <u>transposition</u>
  - Gene insertionally inactivated
    - Function destroyed
  - Most transposons have transcriptional terminators
    - Blocks expression of downstream genes in operon



8.2. Spontaneous Mutations

- Transposons (jumping genes) (continued…)
  - Classic studies carried out by Barbara McClintock
    - Observed color variation in corn kernels resulting from transposons moving into and out of genes controlling pigment synthesis

8.3. Induced Mutations

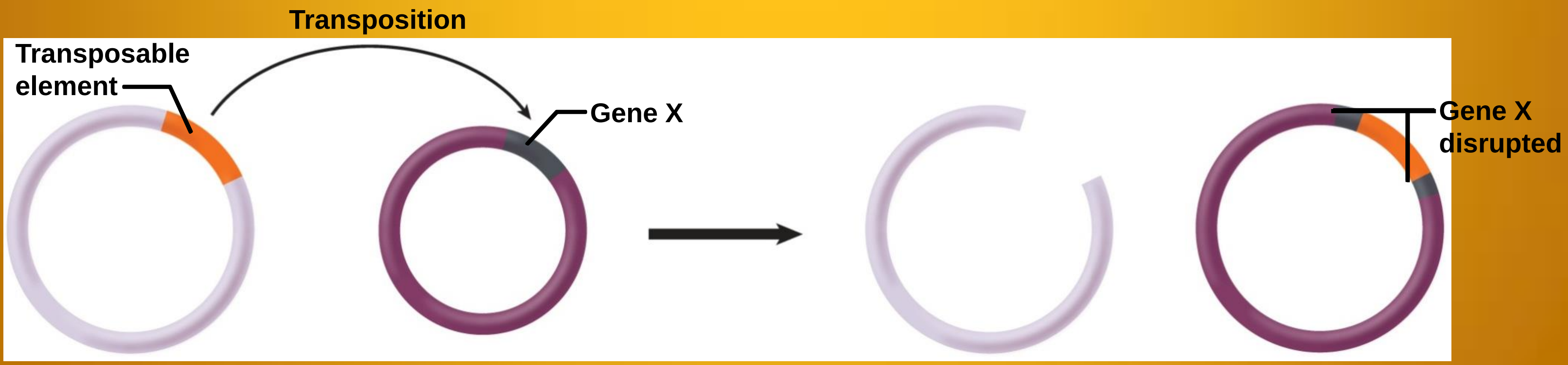
- Induced mutations result from outside influence
  - Agent that induces change is <u>mutagen</u>
  - Geneticists may use mutagens to increase mutation rate
  - Two general types: <u>chemical</u>, <u>radiation</u>

TABLE 8.1 Common Mutagens

Agent	Action	Result
Chemical Agent		
Chemicals that modify nucleobases		
Nitrous acid	Converts cytosine to uracil	Nucleotide substitution
Alkylating agents	Adds alkyl groups (CH_3 and others) to nucleobases	Nucleotide substitution
Base analogs 5-Bromouracil	Used in place of normal nucleobases in DNA	Nucleotide substitution
Intercalating agents Ethidium bromide	Inserts between base pairs	Addition or subtraction of nucleotides
Transposons	Randomly insert into DNA	Insertional inactivation
Radiation		
Ultraviolet (UV) light	Causes intrastrand thymine dimer to form	Errors during repair process
X rays	Cause single- and double-strand breaks in DNA	Deletions

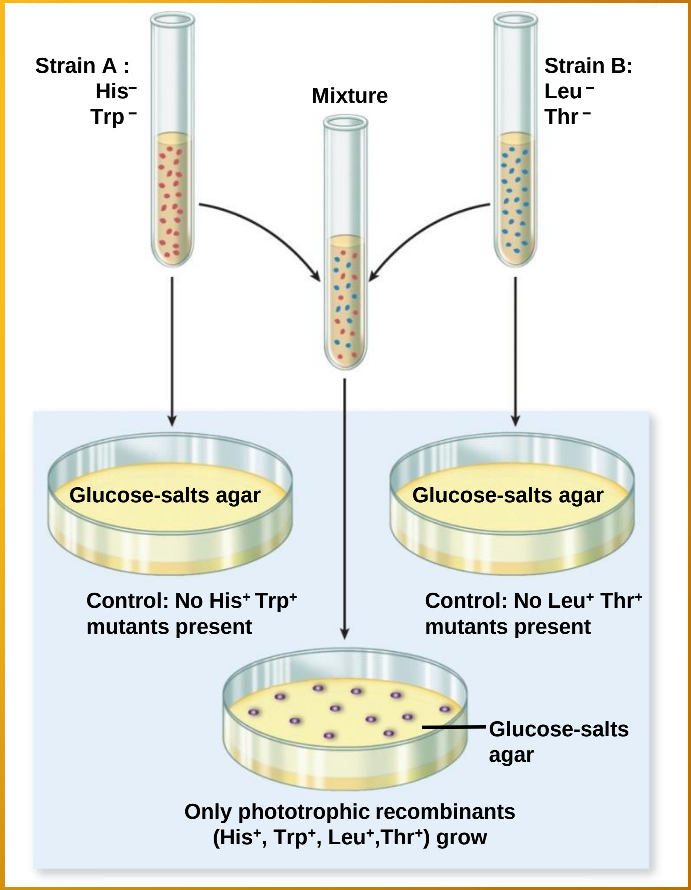
8.3. Induced Mutations

- <u>Transposition</u>
  - Transposons can be used to generate mutations
  - Transposon inserts into cell's genome
  - Generally inactivates gene into which it inserts



Horizontal Gene Transfer as a Mechanism of Genetic Change

- Microorganisms commonly acquire genes from other cells: <u>horizontal gene transfer</u>
  - Can demonstrate recombinants with auxotrophs
    - Combine two strains
      - E.g., His^-, Trp^- with Leu^-, Thr^-
    - Spontaneous mutants unlikely
    - Colonies that can grow on glucose-salts medium most likely acquired genes from other strain



Horizontal Gene Transfer as a Mechanism of Genetic Change

- Genes naturally transferred by three mechanisms
  - Transformation: naked DNA uptake by bacteria
  - Transduction: bacterial DNA transfer by viruses
  - Conjugation: DNA transfer between bacterial cells

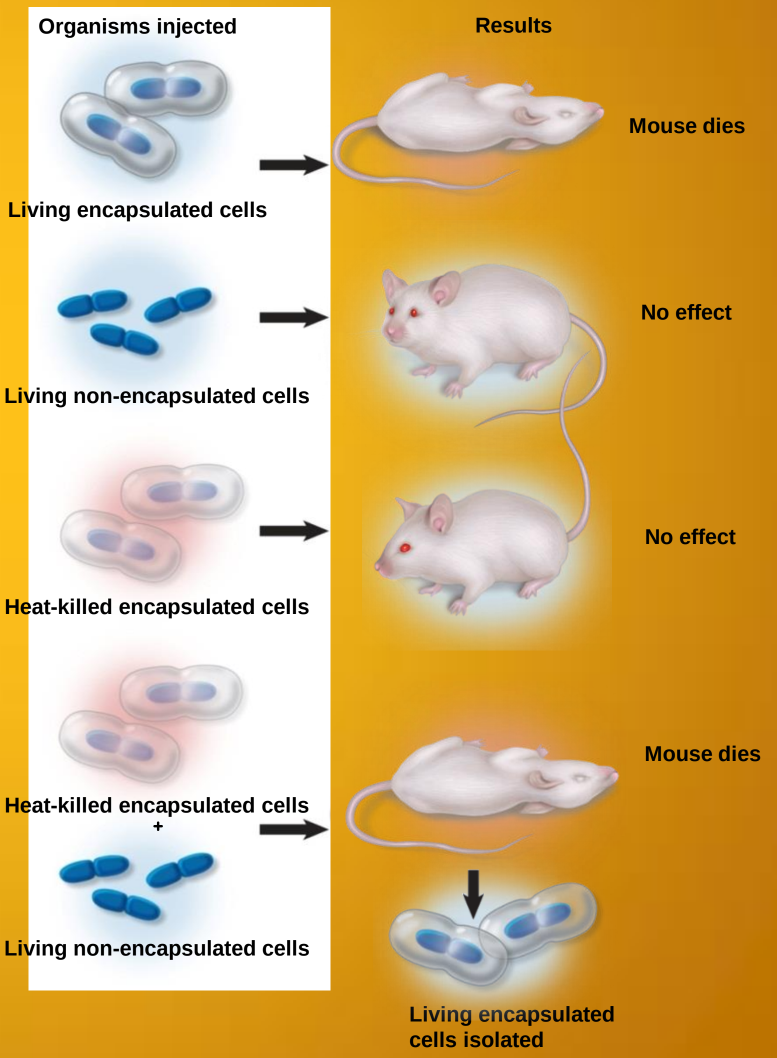
TABLE 8.3 Mechanisms of DNA Transfer

Mechanism	Main Feature	Size of DNA Transferred	Sensitivity to DNase Addition*
Transformation	Naked DNA transferred	About 20 genes	Yes
Transduction	DNA enclosed in a bacteriophage coat	Small fraction of the chromosome	No
Conjugation			
Plasmid transfer	Cell-to-cell contact required	Entire plasmid	No
Chromosome transfer	Cell-to-cell contact required; only Hfr cells can be donors	Variable fraction of chromosome	No

*DNase is an abbreviation of deoxyribonuclease, an enzyme that degrades DNA.

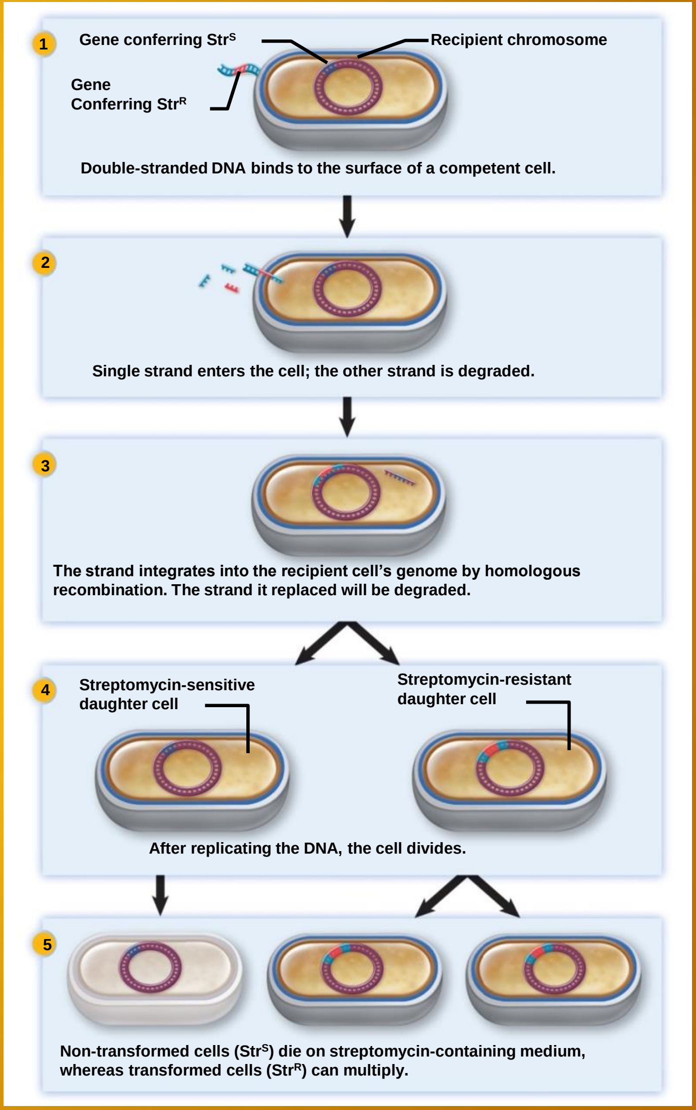
8.6. DNA-Mediated Transformation

- Naked DNA is not within cell or virus
- Cells release when lysed
- Addition of DNase prevents transformation
- Demonstration of transformation in pneumococci
    - Only encapsulated cells pathogenic



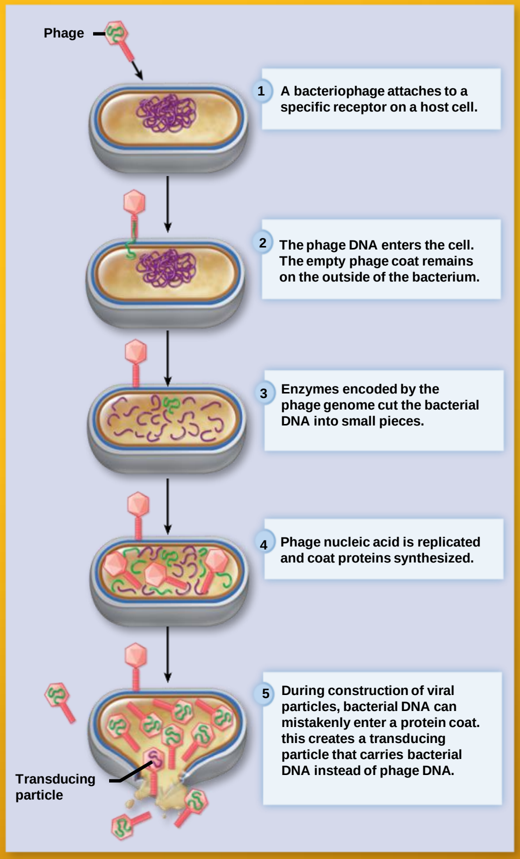
8.6. DNA-Mediated Transformation

- Transformation
  - Recipient cell must be <u>competent</u>
  - Most take up regardless of origin
    - Some accept only from closely related bacteria (DNA sequence)
  - Process tightly regulated
  - *Bacillus subtilis* has two-component regulatory system
    - Recognizes low nitrogen or carbon
    - High concentration of bacteria (quorum sensing)
    - Only a fraction of population becomes competent

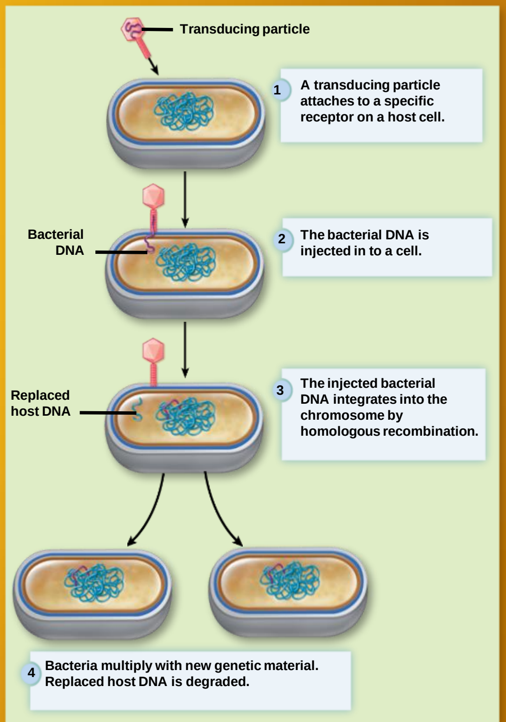


8.7. Transduction

- Transduction: transfer of genes by bacteriophages
  - Specialized transduction: specific genes (Chapter 13)
  - Generalized transduction: any genes of donor cell
    - Rare error during phage assembly
    - Transfer of DNA to new bacterial host



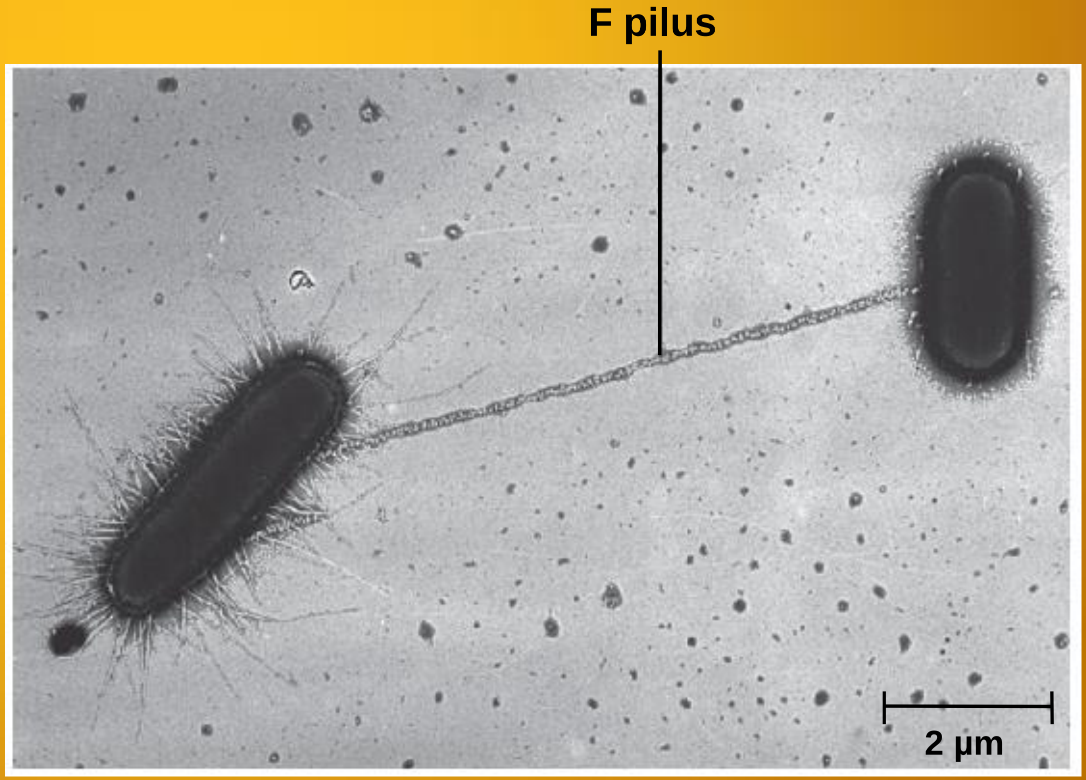
(a) Formation of a transducing particle



(b) The process of transduction

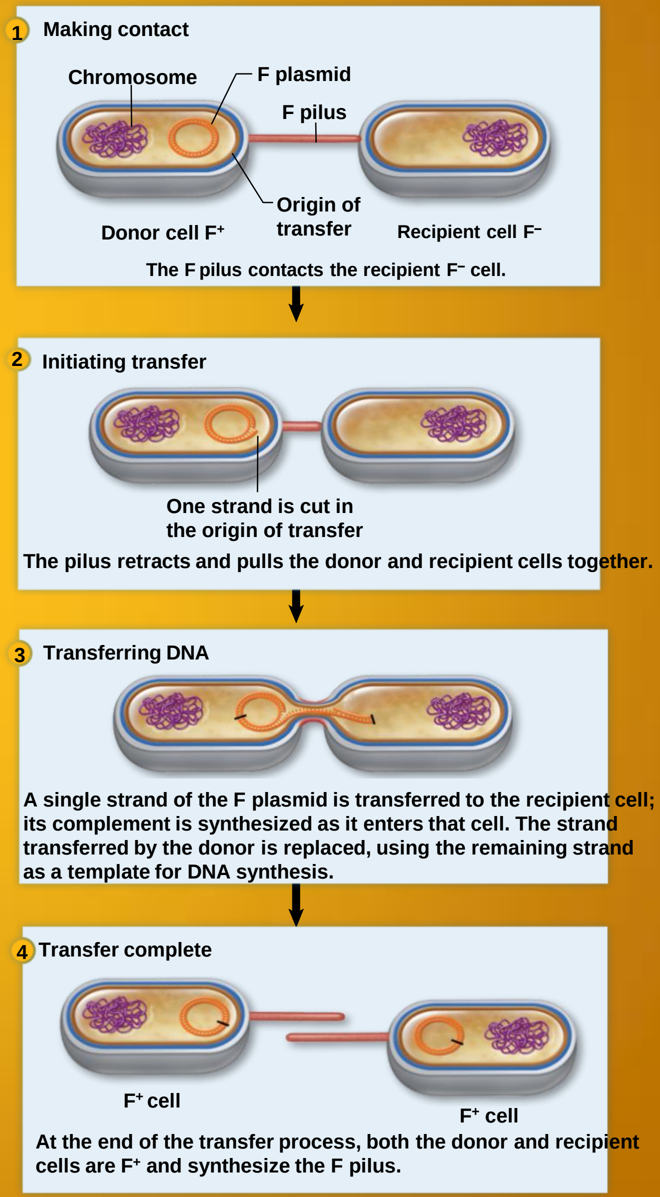
8.8. Conjugation

- Conjugation: DNA transfer between bacterial cells
  - Requires contact between donor, recipient cells
  - <u>Conjugative plasmids</u> direct their own transfer
    - Replicons
  - F plasmid (fertility) of *E. coli* most studied



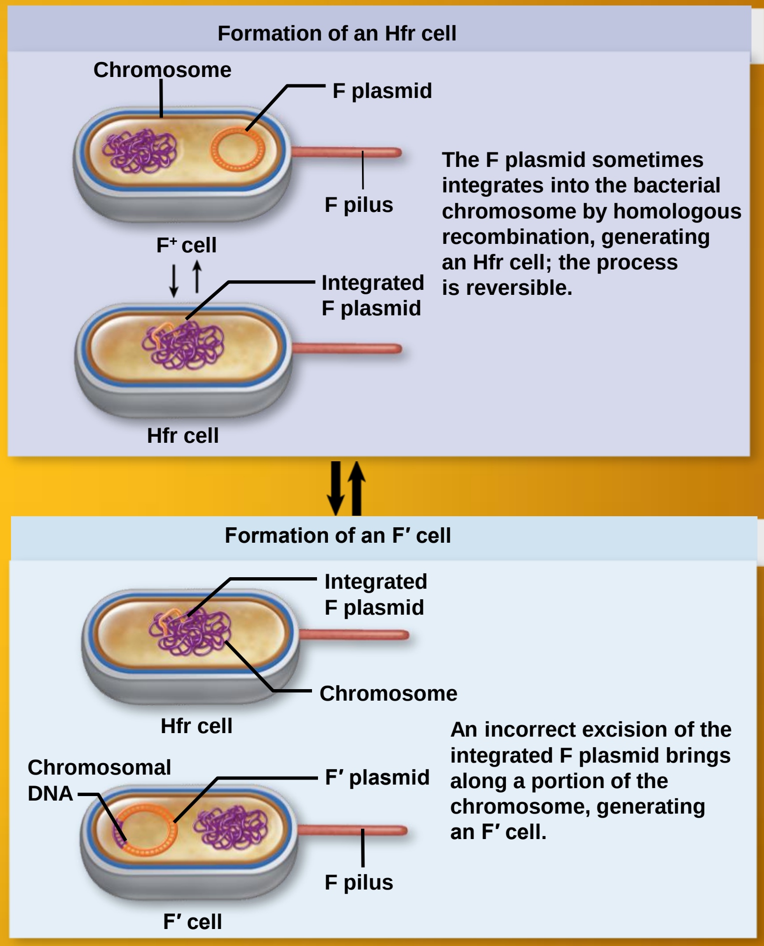
8.8. Conjugation

- Conjugation (continued…)
  - F plasmid of *E. coli*
    - F^+ cells have, F^- do not
  - Encodes proteins including F pilus
    - Sex pilus
    - Brings cells into contact
    - Enzyme cuts plasmid
    - Single strand transferred
    - Complementary strands synthesized
    - Both cells are now F^+



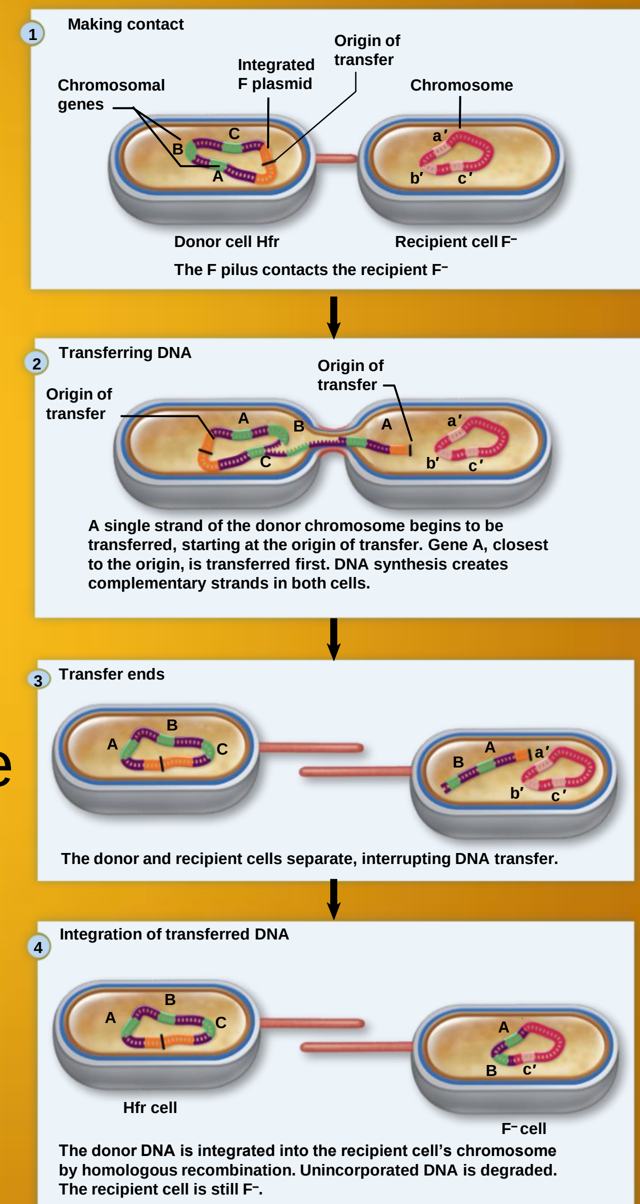
8.8. Conjugation

- Chromosomal DNA transfer less common
  - Involves Hfr cells (high frequency of recombination)
    - F plasmid is integrated into chromosome via homologous recombination
    - Process is reversible
    - F′ plasmid results when small piece of chromosome is removed with F plasmid DNA
    - F′ is replicon



8.8. Conjugation

- Chromosomal DNA transfer less common (continued…)
  - Hfr cell produces F pilus
  - Transfer begins with genes on one side of origin of transfer of plasmid (in chromosome)
  - Part of chromosome transferred to recipient cell
  - Chromosome usually breaks before complete transfer (full transfer would take ~100 minutes)
  - Recipient cell remains F⁻ since incomplete F plasmid transferred



8.9. The Mobile Gene Pool

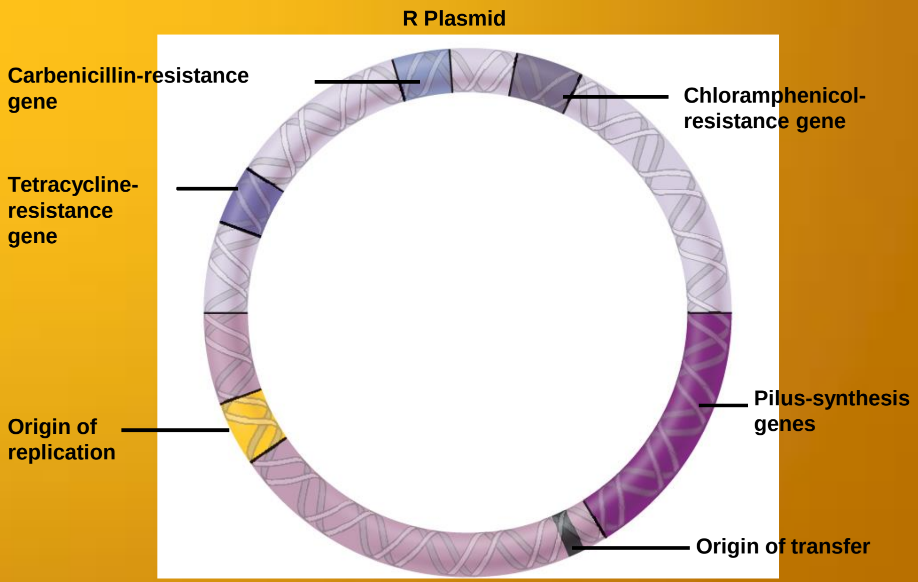
- Genomics reveals surprising variation in gene pool of even a single species
  - Perhaps 75% of *E. coli* genes found in all strains
    - Termed *core genome* of species
  - Remaining make up <u>mobile gene pool</u>
    - Plasmids, transposons, genomic islands, phage DNA

TABLE 8.4 The Mobile Gene Pool

	Composition	Property
Transposons		
Insertion sequences (ISs)	Transposase gene flanked by short repeat sequences	Move to different locations in DNA in same cell
Composite transposons	Recognizable gene flanked by insertion sequences	Same as insertion sequences, but encode additional information
Plasmids	Circular double-stranded DNA replicon; smaller than chromosomes	Generally code only for non-essential genetic information
Genomic Islands	Large fragment of DNA in a chromosome or plasmid	Code for genes that allow cell to occupy specific environmental locations
Phage DNA	Phage genome	May encode proteins important to bacteria

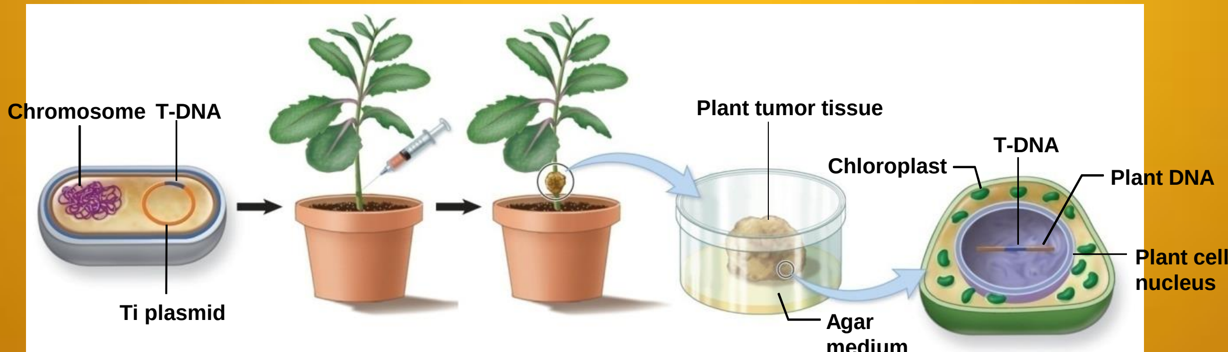
8.9. The Mobile Gene Pool

- Resistance plasmids (R plasmids)
  - Resistance to antimicrobial medications, heavy metals (mercury, arsenic)
    - Compounds found in hospital environments
  - Often two parts
    - R genes
    - RTF (resistance transfer factor)
      - Codes for conjugation
  - Often broad host range
  - Normal microbiota can transfer to pathogens



8.9. The Mobile Gene Pool

- Bacteria can conjugate with plants
  - Natural genetic engineering
  - *Agrobacterium tumefaciens* causes crown gall
    - Different properties, produces opine, plant hormones
    - Piece of tumor-inducing (Ti) plasmid called T-DNA (transferred DNA) is transferred to plant
    - Incorporated into plant chromosome via non-homologous recombination



Agrobacterium tumefacions cell

Agrobacterium cells inoculated into stem of plant

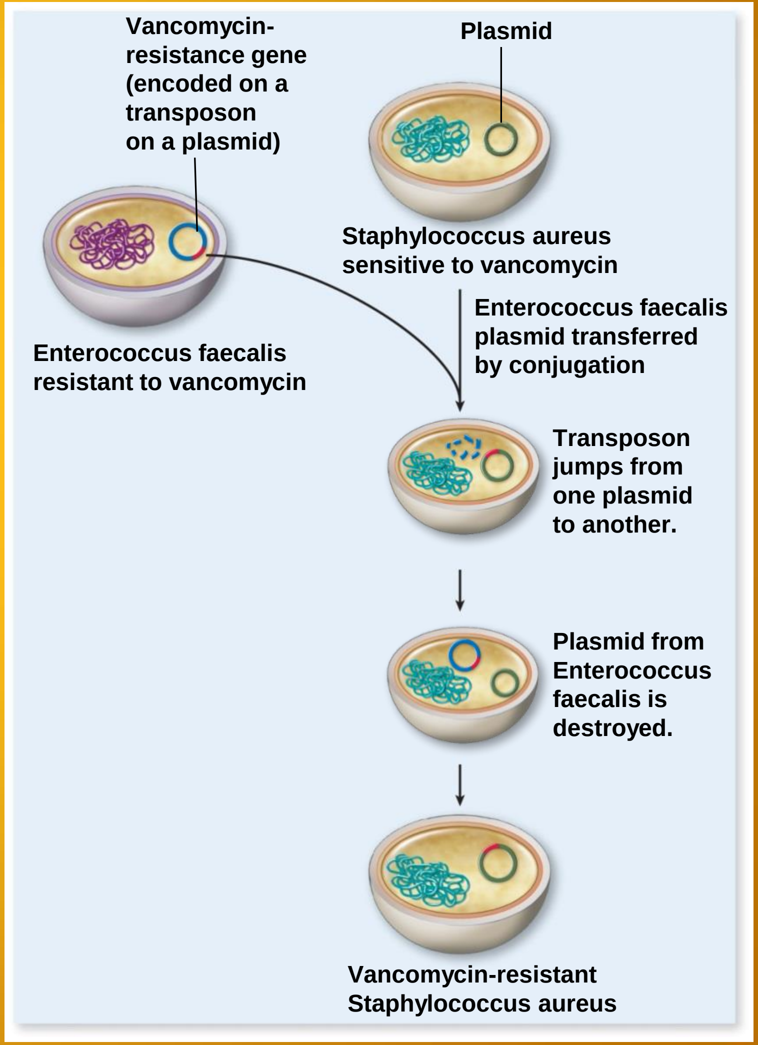
Crown gall tumor develops at site of inoculation.

Bacteria-free tumor tissue is hormone independent and synthesizes an opine.

Plant tumor cell contains T-DNA integrated into the plant chromosome.

8.9. The Mobile Gene Pool

- Transposons yielded vancomycin resistant *Staphylococcus aureus* strain
  - Patient infected with *S. aureus*
    - Susceptible to vancomycin
  - Also had vancomycin resistant strain of *Enterococcus faecalis*
    - Transferred transposon-containing plasmid to *S. aureus*
    - Transposon jumped to plasmid in *S. aureus*



8.9. The Mobile Gene Pool

- Genomic islands: large DNA segments in genome
  - Originated in other species
  - Nucleobase composition very different from genome
    - G-C base pair ratio characteristic for each species
  - May provide different characteristics
    - Utilization of energy sources
    - Acid tolerance
    - Development of symbiosis
    - Ability to cause disease
      - <u>Pathogenicity islands</u>