16

Mechanisms of Genetic Variation

16.1 Mutations

1. Distinguish spontaneous from induced mutations, and list the most common ways each arises
2. Construct a table, concept map, or picture to summarize how base analogues, DNA-modifying agents, and intercalating agents cause mutations
3. Discuss the possible effects of mutations

Mutations: Their Chemical Basis and Effects

- Stable, heritable changes in sequence of bases in DNA
  - point mutations most common
    - from alteration of single pairs of nucleotide
    - from the addition or deletion of nucleotide pairs
  - larger mutations are less common
    - insertions, deletions, inversions, duplication, and translocations of nucleotide sequences
- Mutations can be spontaneous or induced

16.4 Creating Additional Genetic Variability

1. Describe in general terms how recombinant eukaryotic organisms arise
2. Distinguish vertical gene transfer from horizontal gene transfer
3. Summarize the four possible outcomes of horizontal gene transfer
4. Compare and contrast homologous recombination and site-specific recombination

Creating Additional Genetic Variability

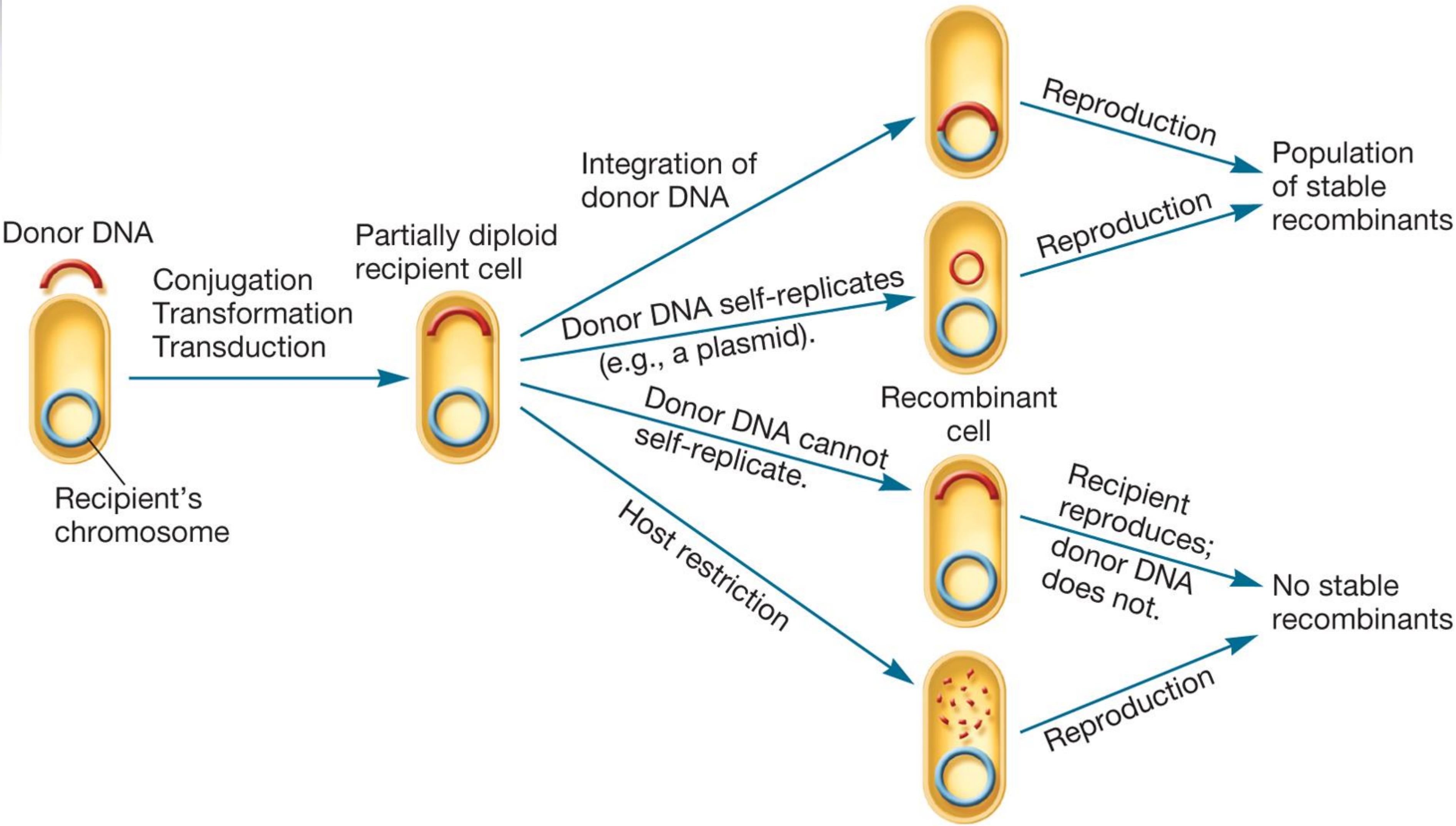
- Mutations are subject to selective pressure
  - each mutant form that survives becomes an allele, an alternate form of a gene
- Recombination is the process in which one or more nucleic acids are rearranged or combined to produce a new nucleotide sequence (recombinants)

Sexual Reproduction and Genetic Variability

- Vertical gene transfer = transfer of genes from parents to progeny
- In eukaryotes
  - sexual reproduction is accompanied by genetic recombination due to
    - crossing over between sister chromatids during meiosis
    - fusion of gametes

Horizontal Gene Transfer (HGT) in *Bacteria* and *Archaea*

- HGT differs from vertical gene transfer
  - transfer of genes from one independent, mature organism to another
    - stable recombinant has characteristics of donor and recipient
- Important in evolution of many species
  - expansion of ecological niche, increased virulence
  - occurs in the three mechanisms evolved by bacteria to create recombinants
  - genes can be transferred to the same or different species


Donor DNA
Conjugation
Transformation
Transduction
Recipient's
chromosome
Partially diploid
recipient cell
Integration of
donor DNA
Donor DNA self-replicates
(e.g., a plasmid).
Donor DNA cannot
self-replicate.
Host restriction
Recombinant
cell
Reproduction
Reproduction
Population
of stable
recombinants
Recipient
reproduces;
donor DNA
does not.
Reproduction
No stable
recombinants

16.5 Transposable Elements

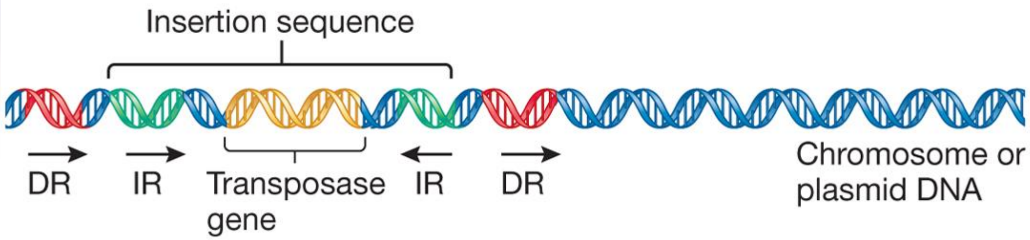
1. Differentiate insertion sequences from transposons
2. Distinguish simple transposition from replicative transposition
3. Defend this statement: “Transposable elements are important factors in the evolution of bacteria and archaea.”

Transposable Elements

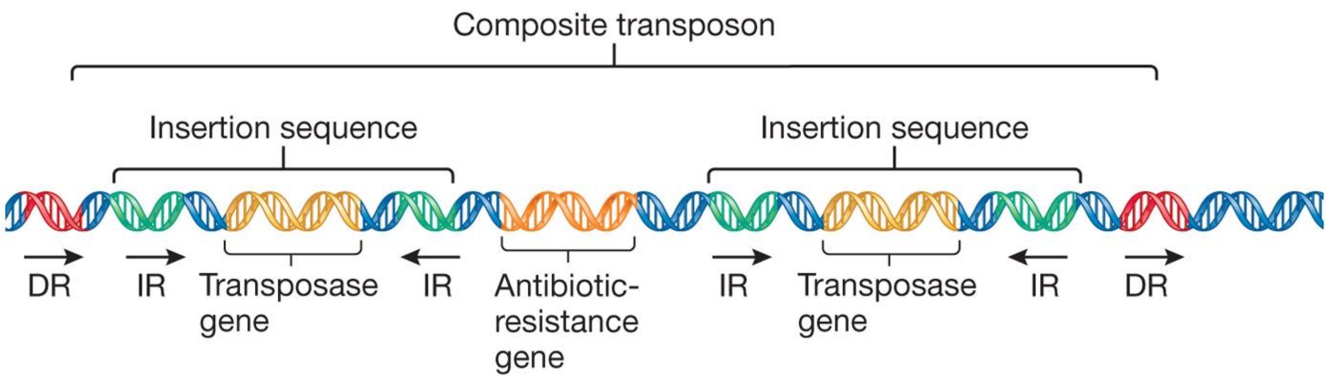
- Segments of DNA that move about the genome in a process called transposition
  - can be integrated into different sites in the chromosome
- Are sometimes called “jumping genes”
- Simplest transposable elements = insertion sequences
- Transposable elements which contain ‘extra’ genes are called composite transposons

Table 16.3 Some Types of Transposable Elements

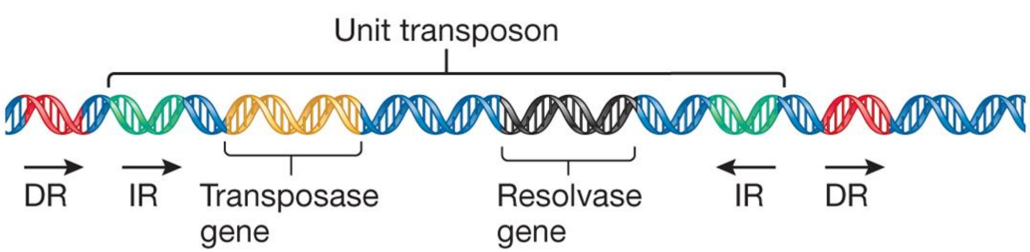
Transposable Element	Description
Insertion sequences (IS)	Small genetic elements consisting of a transposase gene bracketed by inverted repeats
Composite transposons	Contain genes unrelated to transposition bounded at each end by IS elements, which supply the transposase activity needed for transposition
Unit transposons	Contain one or more genes encoding enzymes needed for transposition, as well as other genes unrelated to transposition (e.g., antibiotic-resistance genes). They are not associated with an IS element.
Integrative conjugative elements	In addition to transposition functions, contain genes for conjugative transfer to a new host cell and other genes (e.g., antibiotic-resistance genes)


Insertion sequence
DR
IR
Transposase gene
IR
DR
Chromosome or plasmid DNA

(a)


Composite transposon
Insertion sequence
Insertion sequence
DR
IR
Transposase gene
IR
Antibiotic-resistance gene
IR
Transposase gene
IR
DR

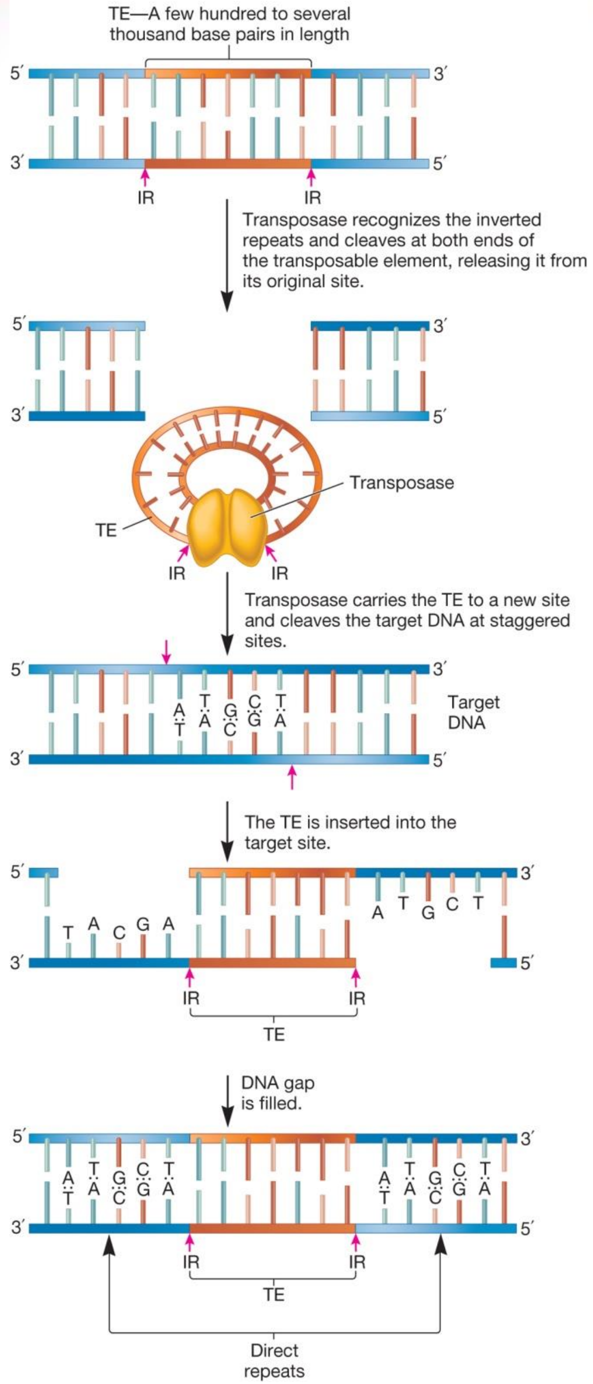
(b)


Unit transposon
DR
IR
Transposase gene
Resolvase gene
IR
DR

(c)

Simple Transposition

- Also called cut-and-paste transposition
- Transposase catalyzed excision
- Cleavage of new target site and ligation into site

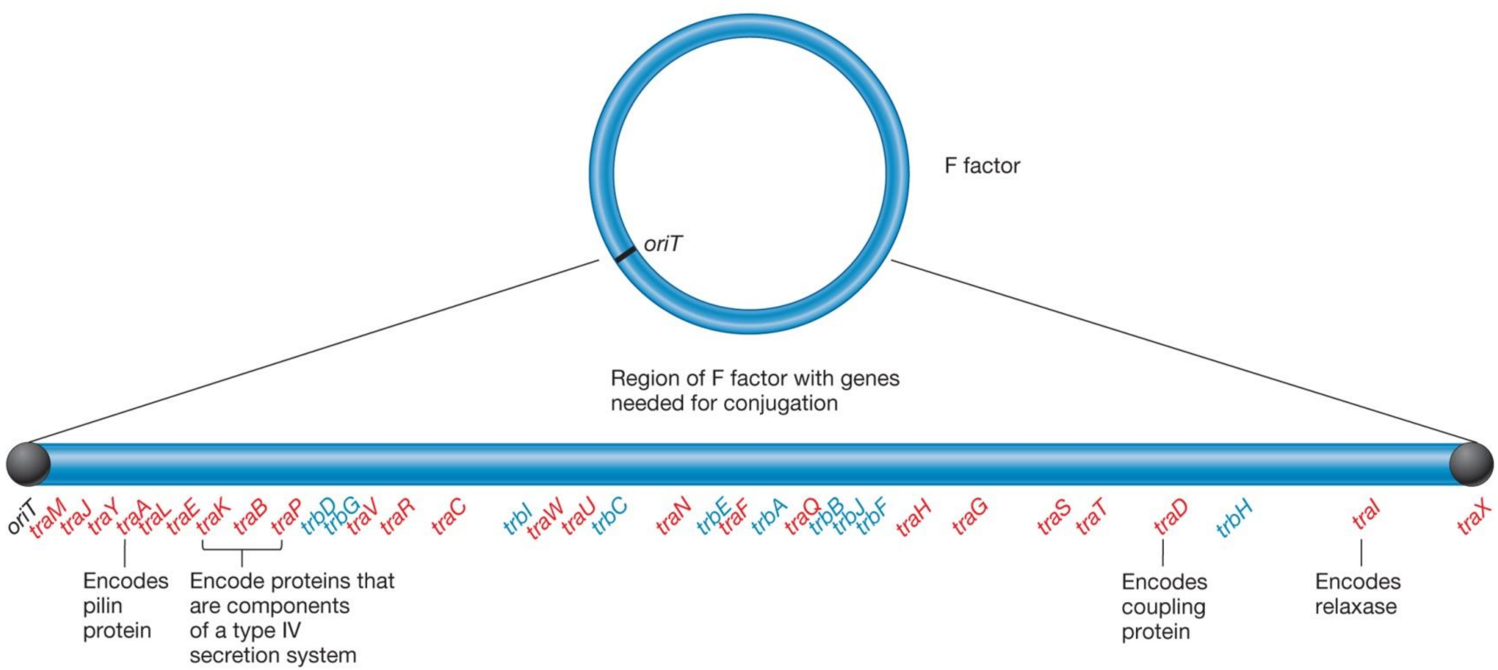


16.6 Bacterial Conjugation

1. Identify the types of plasmids that are important creators of genetic variation
2. Describe the features of the F factor that allow it to (1) transfer itself to a new host cell and (2) integrate into a host cell's chromosome
3. Outline the events that occur when an F^+ cell encounters an F^- cell
4. Distinguish F^+, Hfr, and F' cells from each other
5. Explain how Hfr cells arise
6. Outline the events that occur when an Hfr cell encounters an F^- cell

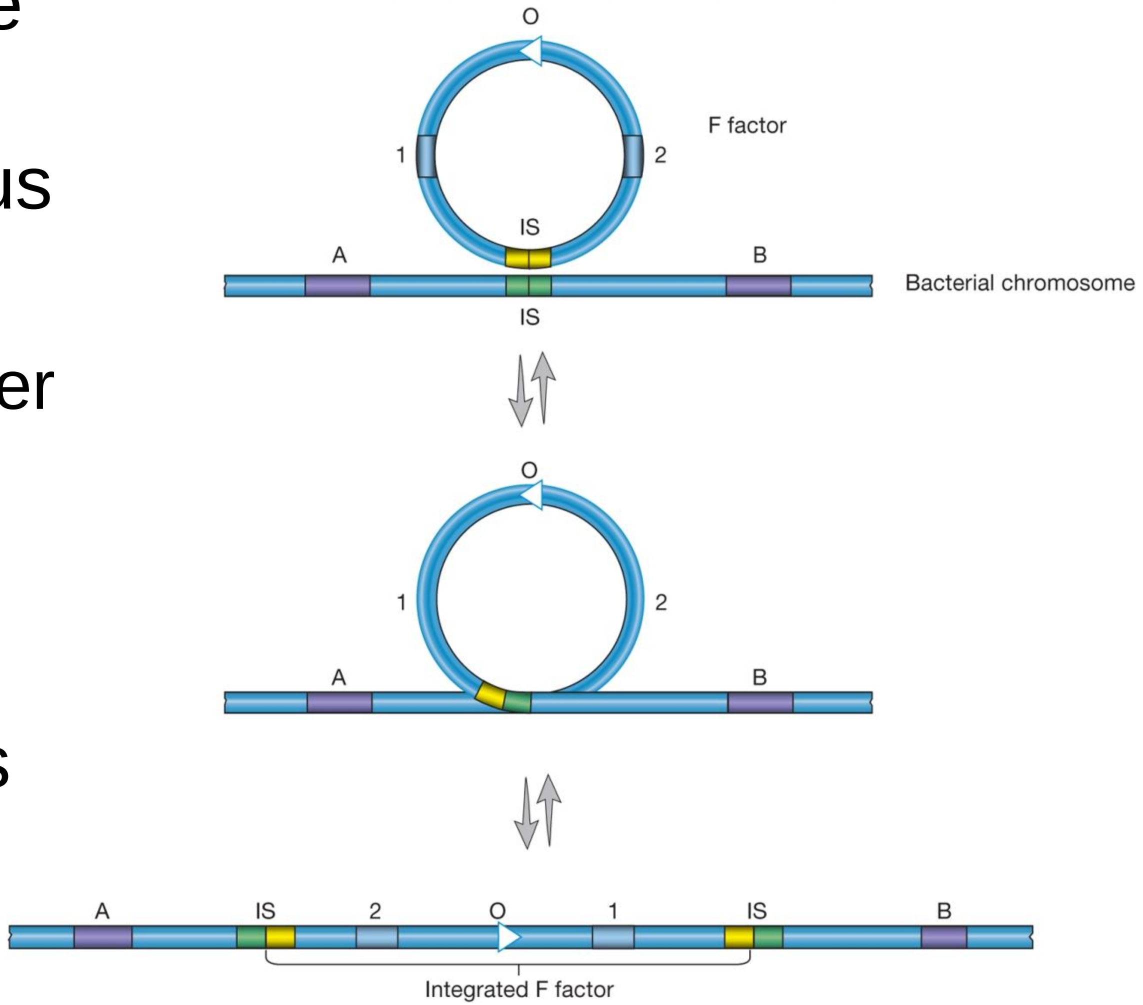
Bacterial Plasmids

- Small, autonomously replicating DNA molecules
  - can exist independently from host chromosome
  - can integrate reversibly into the host chromosome (episomes)
- Conjugative plasmids (F plasmid) can transfer copies of themselves to other bacteria during conjugation



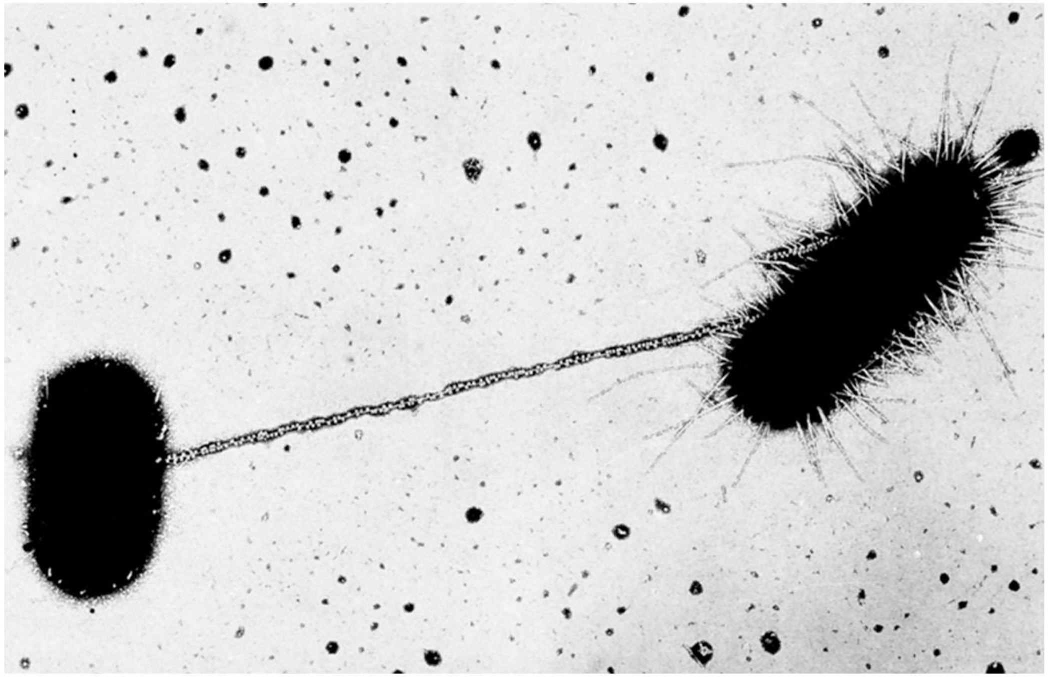
Bacterial Plasmids - 2

- F factors contain the information for formation of sex pilus
  - attach F^+ cell to F^- cell for DNA transfer during bacterial conjugation
- F factors have insertion sequences (IS)
  - assists in plasmid integration



Bacterial Conjugation

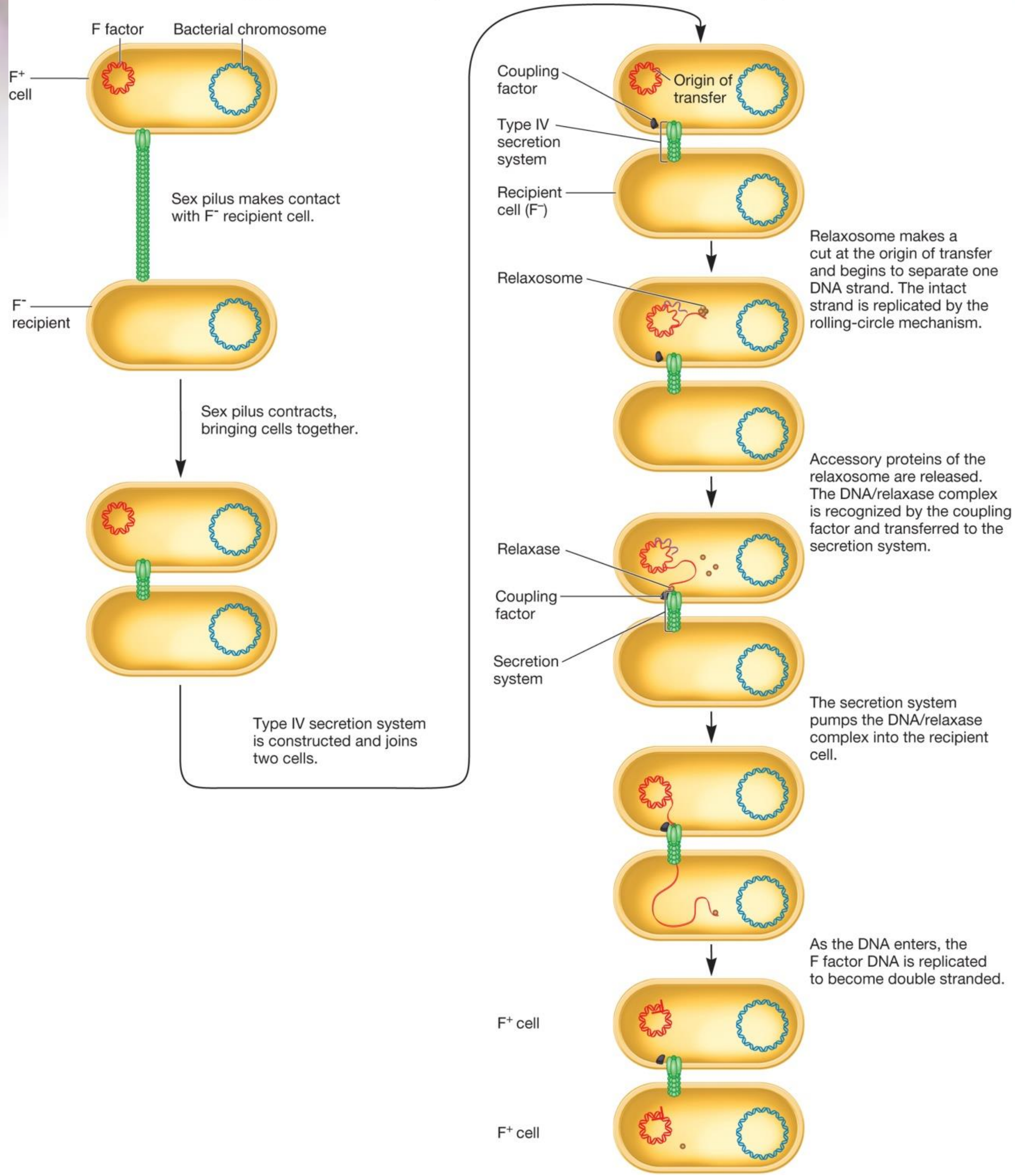
- J. Lederberg and E. Tatum demonstrated the transfer of genes between bacteria that depends on
  - direct cell to cell contact mediated by the F pilus
  - unidirectional DNA transfer from donor to recipient

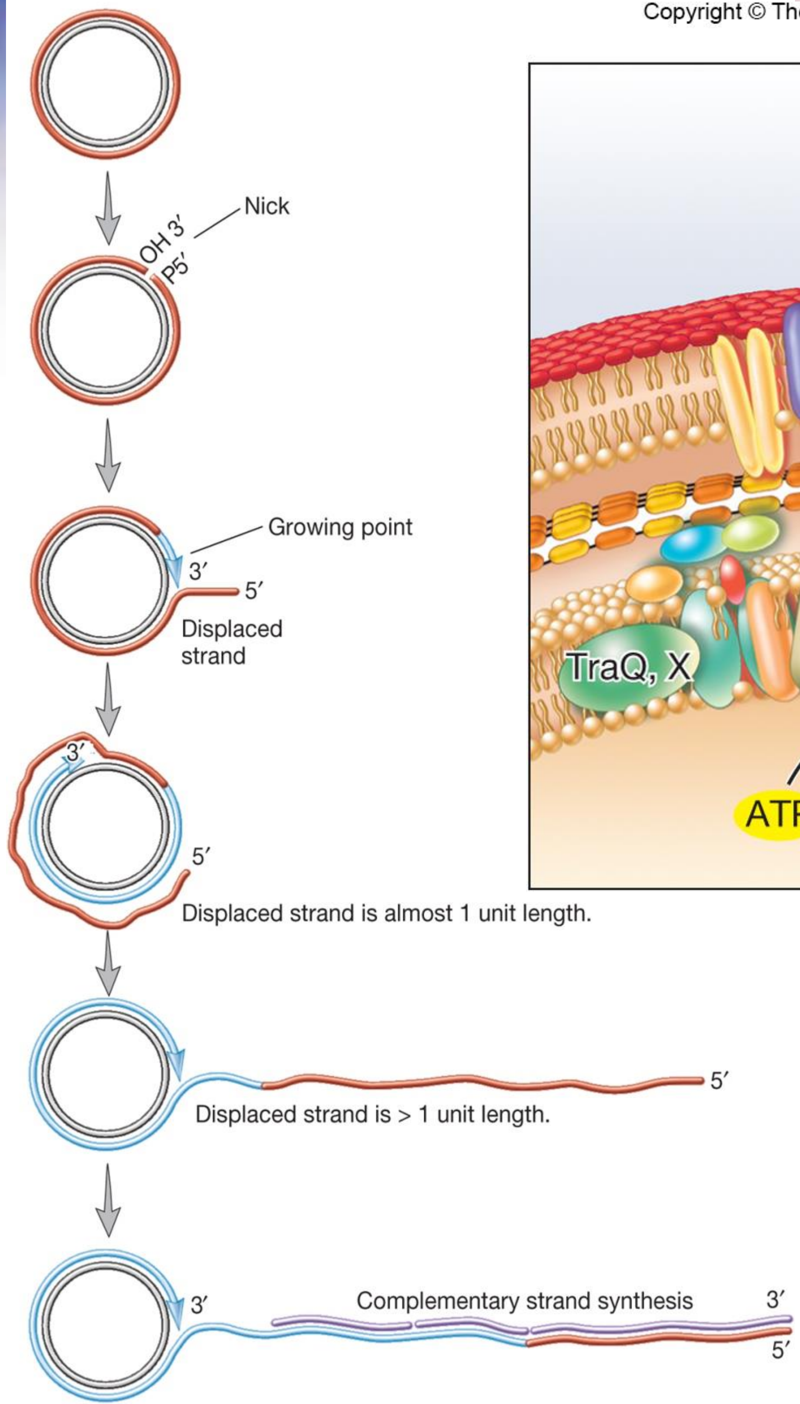


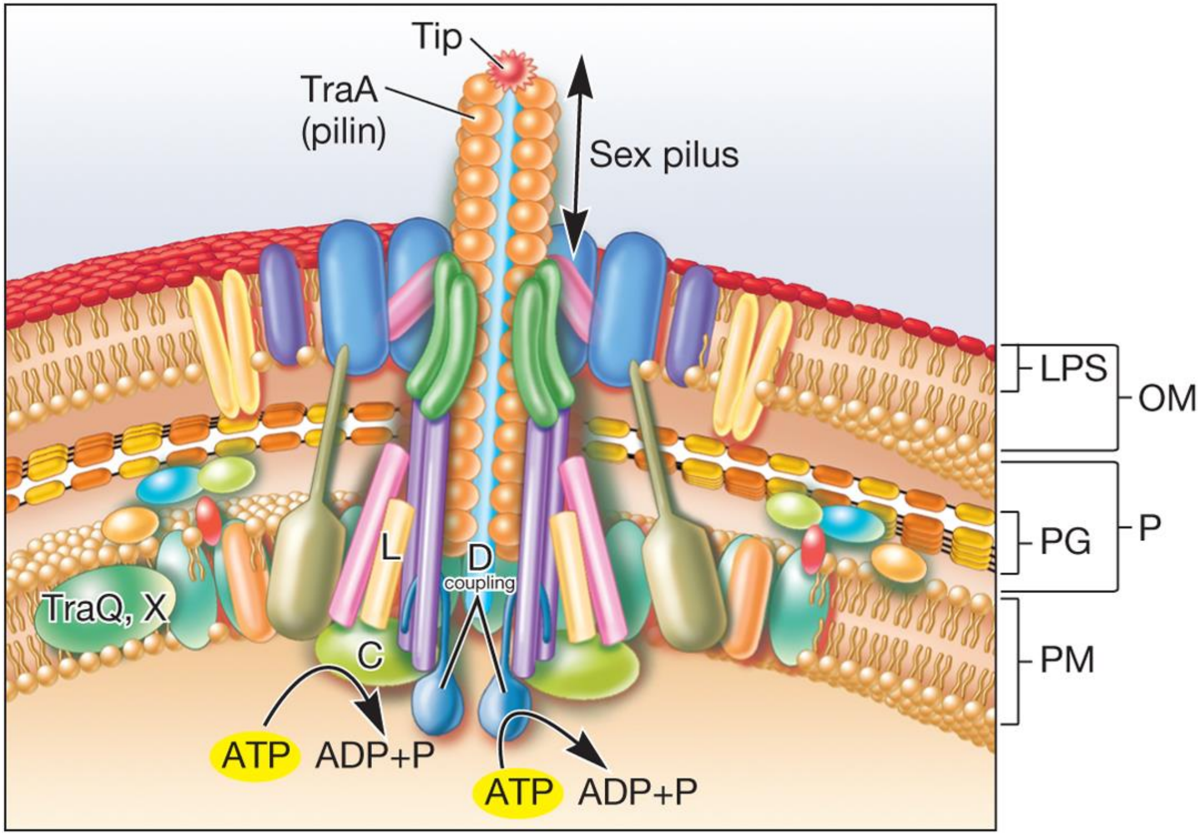
Courtesy of Charles C. Brinton, Jr. and Judith Carnahan

F^+ x F^- Mating

- A copy of the F factor is transferred to the recipient and does not integrate into the host chromosome
- Donor genes usually not transferred
- F factor codes for sex pilus
  - Type IV secretion system that makes contact between cells that DNA moves across
- Plasmid is replicated by rolling circle method

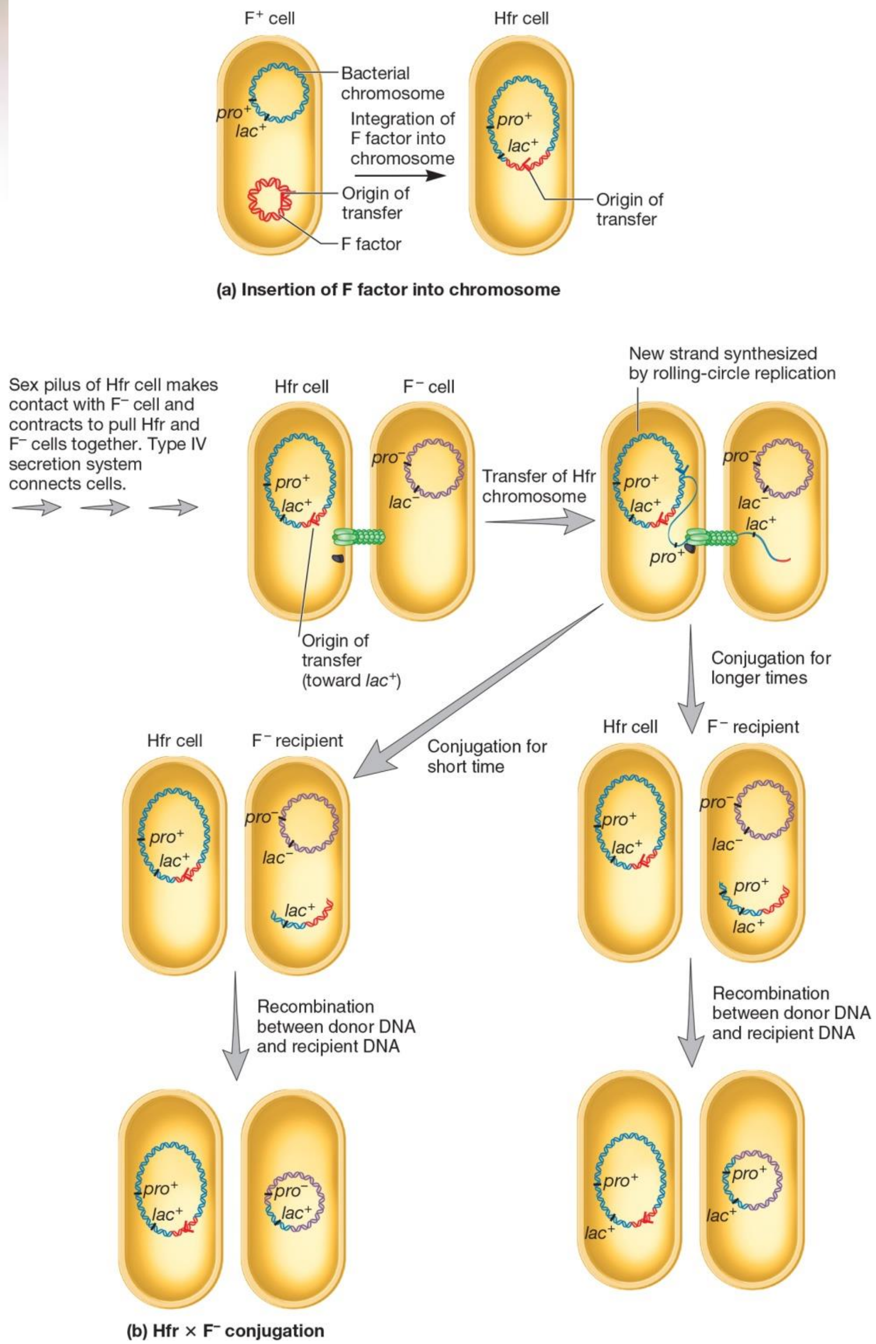

F factor
Bacterial chromosome
F⁺ cell
Sex pilus makes contact with F⁻ recipient cell.
F⁻ recipient
Sex pilus contracts, bringing cells together.
Type IV secretion system is constructed and joins two cells.
Coupling factor
Origin of transfer
Type IV secretion system
Recipient cell (F⁻)
Relaxosome makes a cut at the origin of transfer and begins to separate one DNA strand. The intact strand is replicated by the rolling-circle mechanism.
Relaxosome
Accessory proteins of the relaxosome are released. The DNA/relaxase complex is recognized by the coupling factor and transferred to the secretion system.
Relaxase
Coupling factor
Secretion system
The secretion system pumps the DNA/relaxase complex into the recipient cell.
As the DNA enters, the F factor DNA is replicated to become double stranded.
F⁺ cell
F⁺ cell


Nick
OH 3′
P5′
Growing point
3′
5′
Displaced strand
3′
5′
Displaced strand is almost 1 unit length.
5′
Displaced strand is > 1 unit length.
3′
Complementary strand synthesis
3′
5′


Tip
TraA (pilin)
Sex pilus
LPS
OM
PG
P
PM
L
D coupling
TraQ, X
C
ATP
ADP+P
ATP
ADP+P

HFr Conjugation

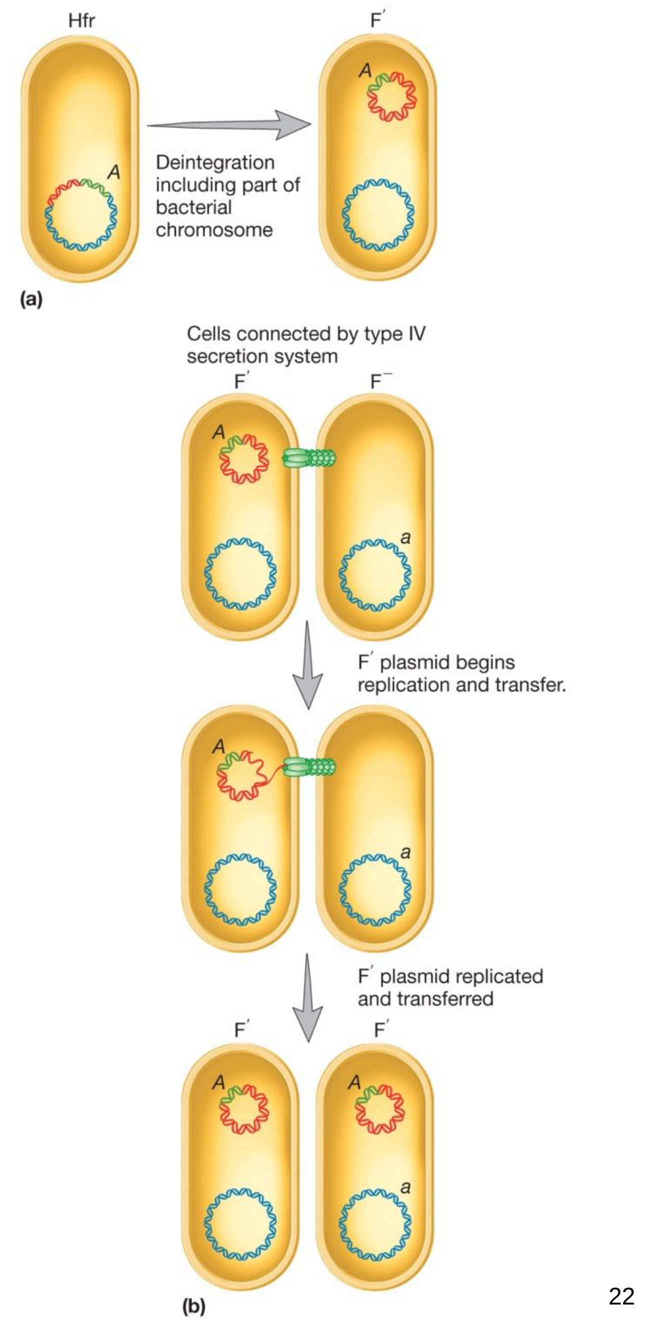
- Donor HFr cell has F factor integrated into its chromosome
- Donor genes are transferred to recipient cell
- A complete copy of the F factor is usually not transferred
- Gene transfer can be clockwise or counterclockwise



F⁺ cell
Hfr cell
Bacterial chromosome
pro⁺
lac⁺
Integration of F factor into chromosome
Origin of transfer
F factor
Origin of transfer
(a) Insertion of F factor into chromosome
Sex pilus of Hfr cell makes contact with F⁻ cell and contracts to pull Hfr and F⁻ cells together. Type IV secretion system connects cells.
Hfr cell
F⁻ cell
pro⁻
lac⁻
Transfer of Hfr chromosome
New strand synthesized by rolling-circle replication
Origin of transfer (toward lac⁺)
Conjugation for short time
Conjugation for longer times
Hfr cell
F⁻ recipient
Recombination between donor DNA and recipient DNA
Recombination between donor DNA and recipient DNA
(b) Hfr × F⁻ conjugation

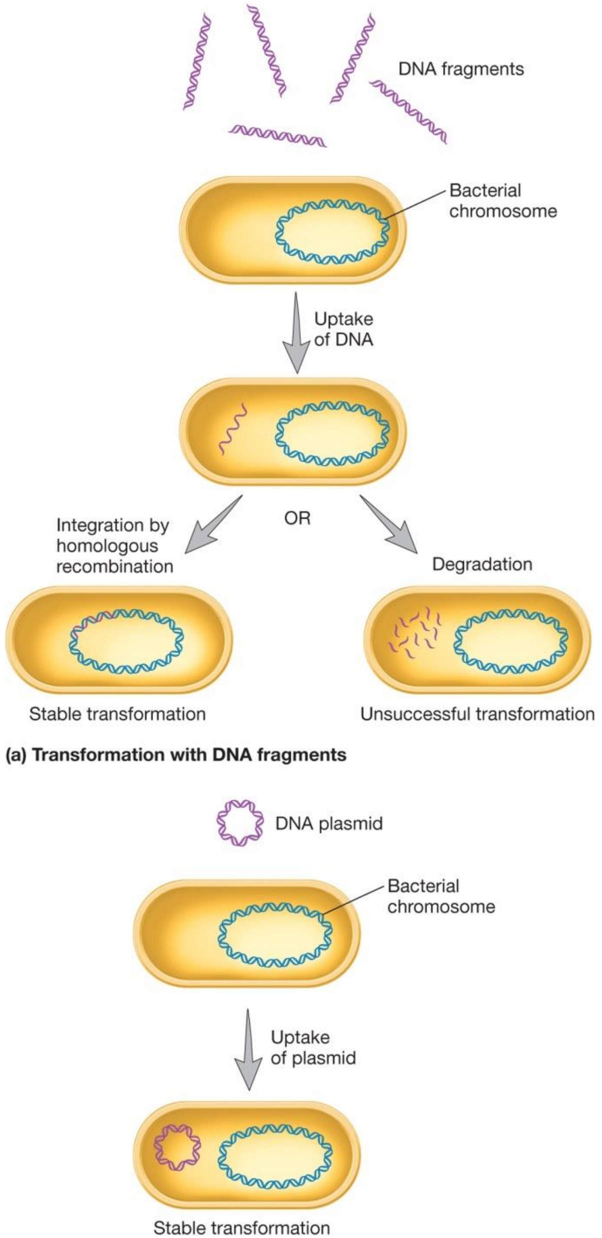
F’ Conjugation

- Result when the F factor incorrectly leaves the host chromosome
- Some of the F factor is left behind in the host chromosome
- Some host genes have been removed along with some of the F factor
  - these genes can be transferred to a second host cell by conjugation



16.7 Bacterial Transformation

1. Describe the factors that contribute to a bacterium being naturally transformation competent
2. Predict the outcomes of transformation using a DNA fragment versus using a plasmid
3. Design an experiment to transform bacteria that are not naturally competent with a plasmid that carries genes encoding ampicillin resistance and the protein that generates green fluorescence



(a) Transformation with DNA fragments

(b) Transformation with a plasmid

Bacterial Transformation

- F. Griffith demonstrated transformation
- Uptake of naked DNA by a competent cell followed by incorporation of the DNA into the recipient cell’s genome

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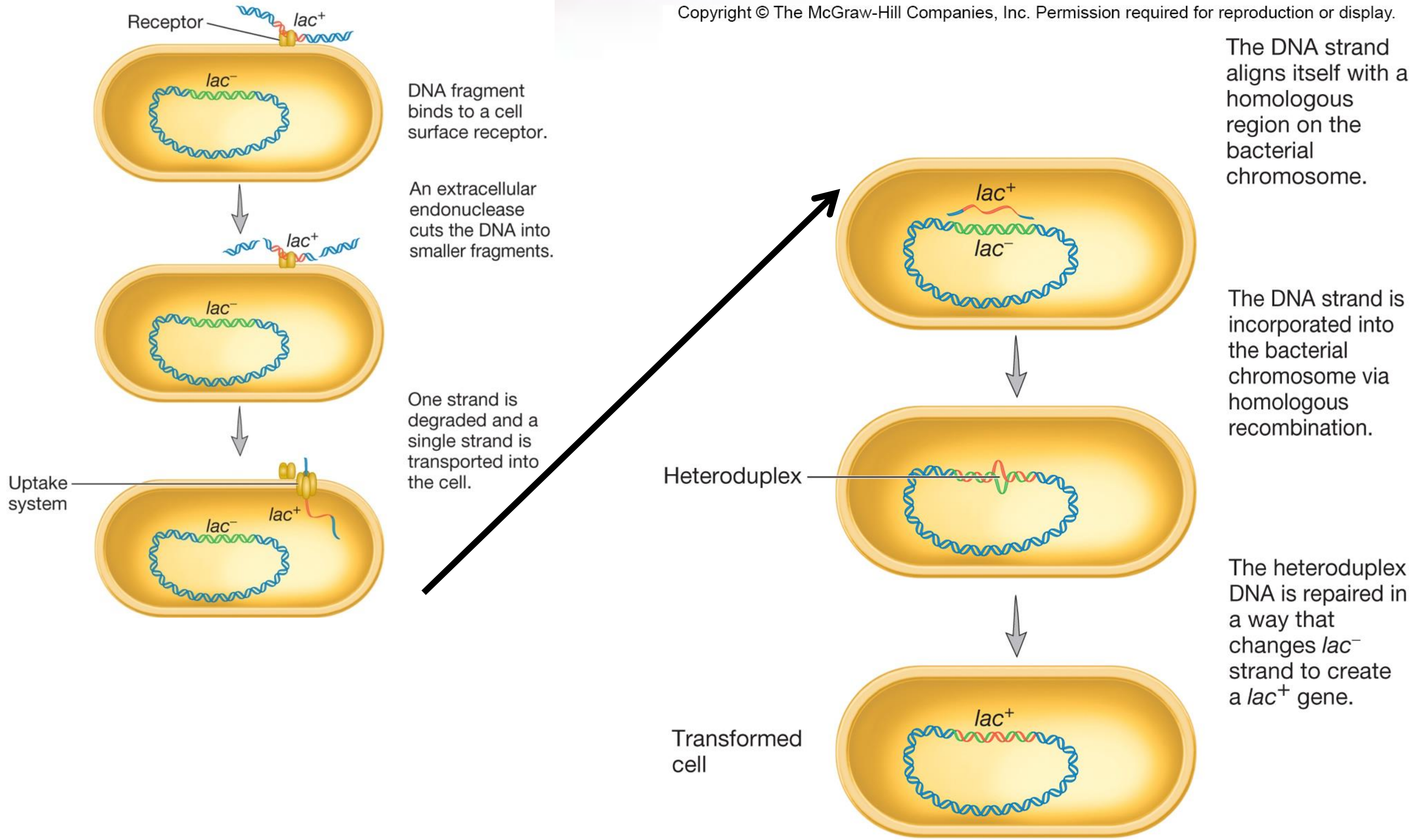
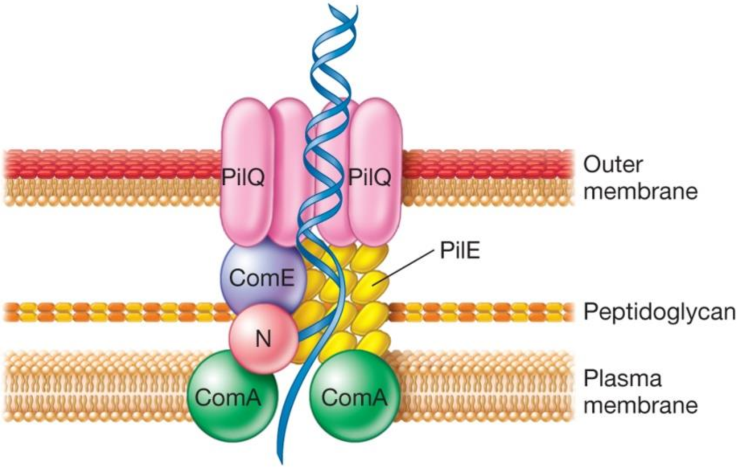
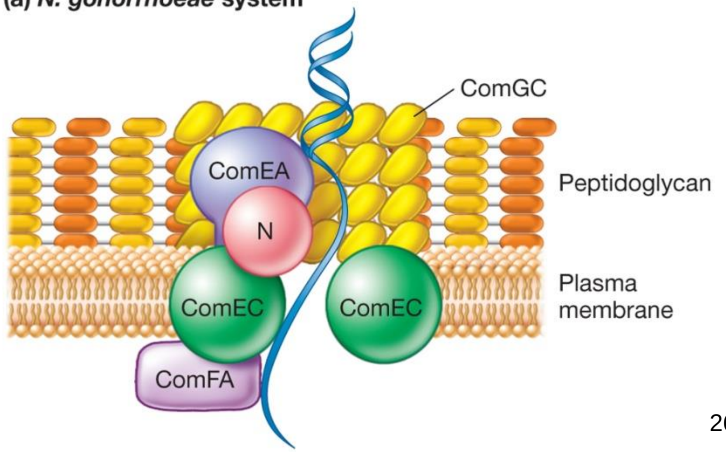


DNA Uptake in Bacterial Transformation

- Protein system allows DNA to move across cell walls
  - Gram-negatives
    - PilQ aids in movement across outer membrane
    - Pilin complex (PilE) moves DNA across periplasm and peptidoglycan
    - ComE is DNA binding protein
    - N is nuclease that degrades one strand
    - ComA forms transmembrane channel
  - Similar system in Gram-pos.



(a) *N. gonorrhoeae* system



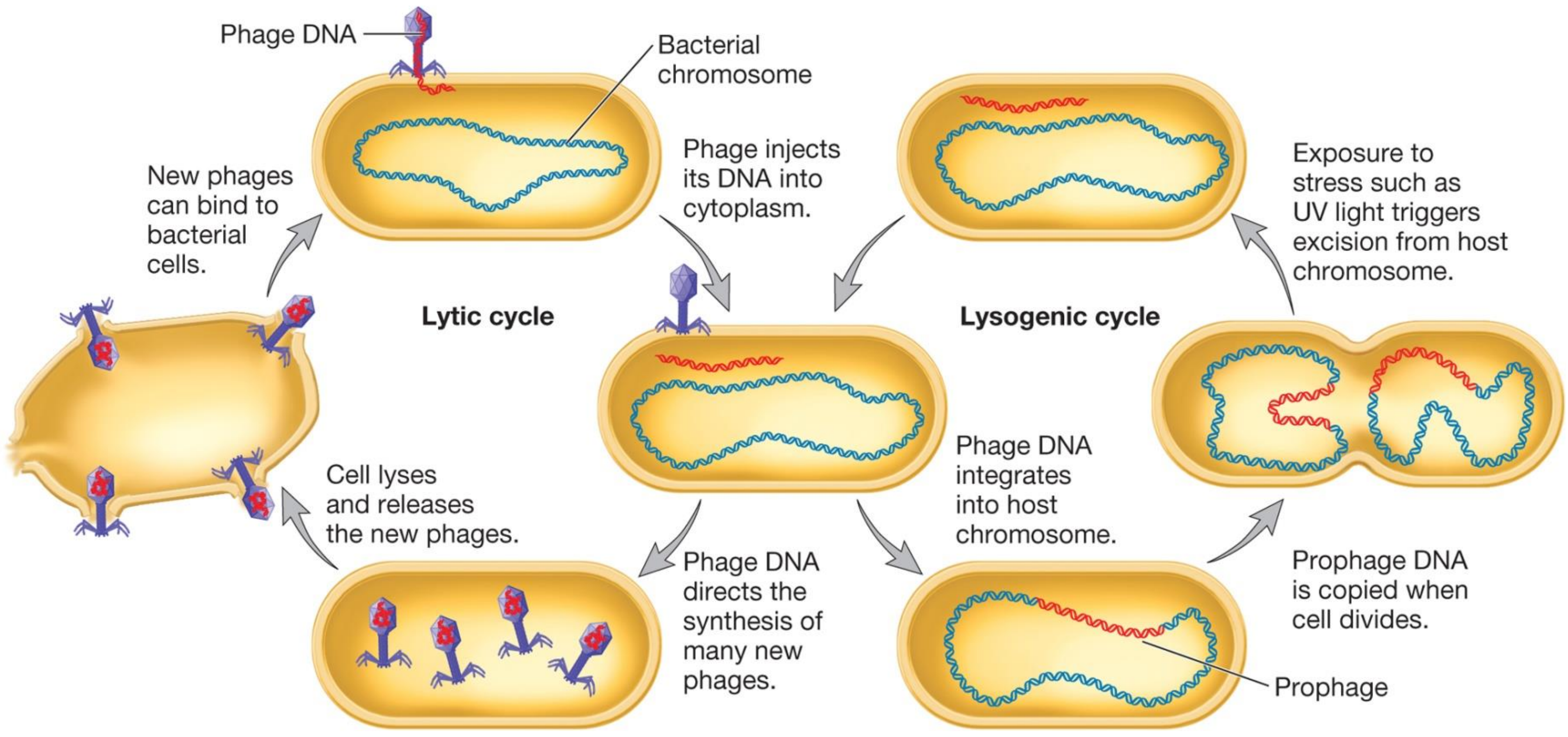
(b) *B. subtilis* system

16.8 Transduction

1. Differentiate generalized transduction from specialized transduction
2. Correlate a phage’s life cycle to its capacity to mediate generalized or specialized transduction
3. Draw a figure, create a concept map, or construct a table that distinguishes conjugation, transformation, and transduction

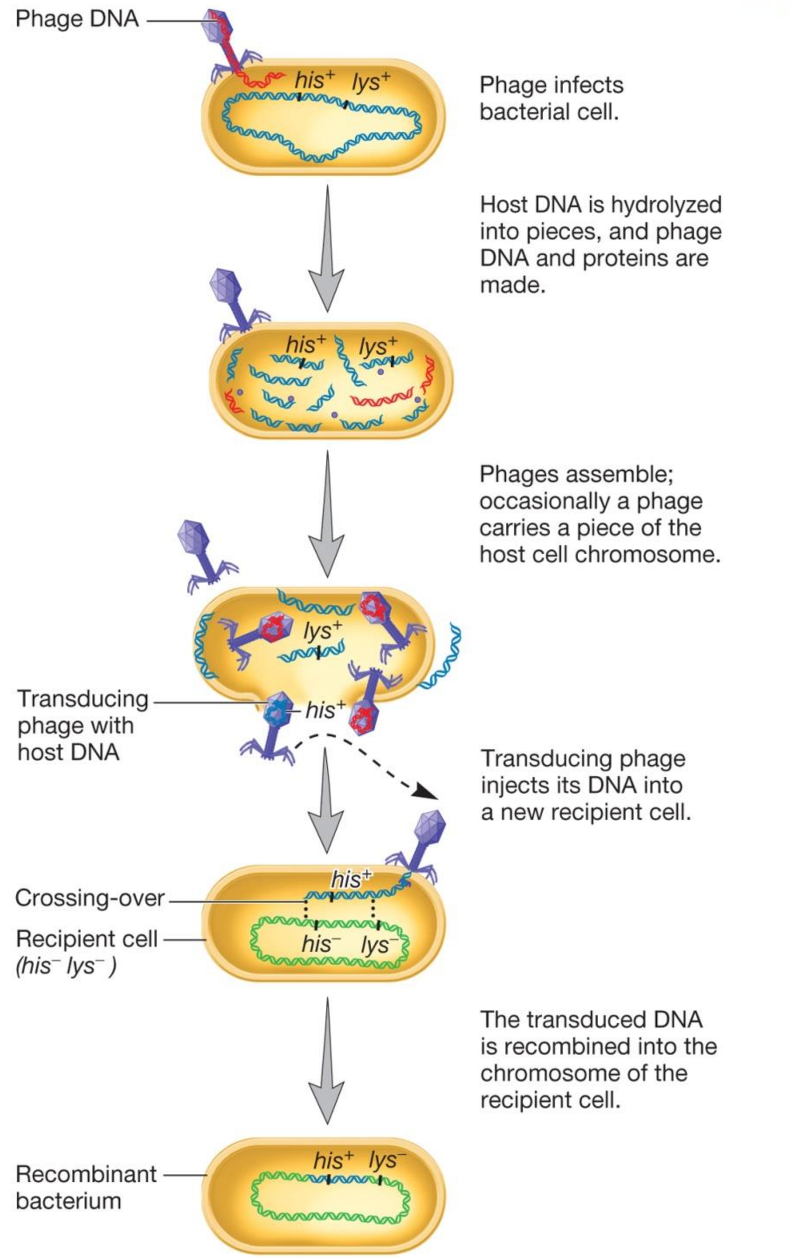
Transduction

- The transfer of bacterial genes by viruses
- Viruses (bacteriophages) can carry out the lytic cycle (host cell is destroyed) or viral DNA integrates into the host genome (becoming a latent prophage)

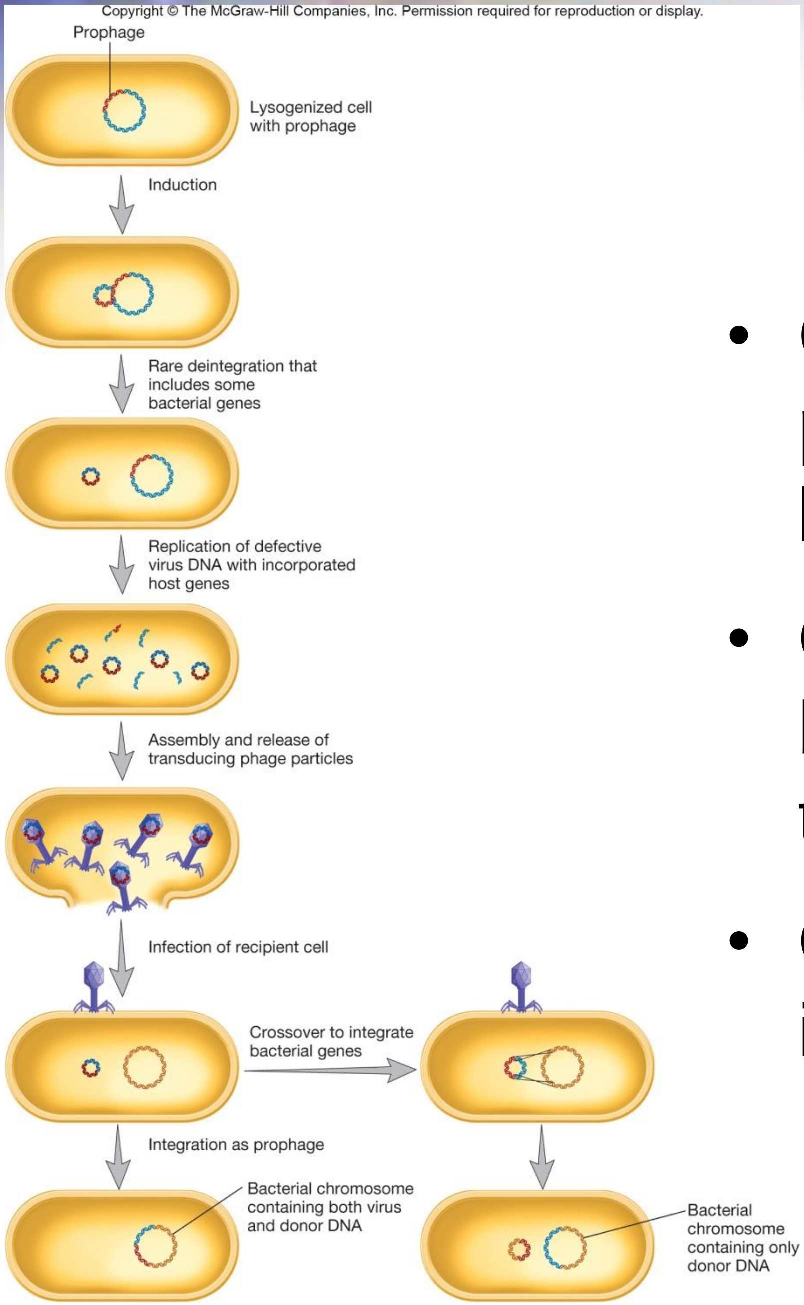


Generalized Transduction

- Any part of bacterial genome can be transferred
- Occurs during lytic cycle of virulent phage
- During viral assembly, fragments of host DNA mistakenly packaged into phage head
  - – generalized transducing particle

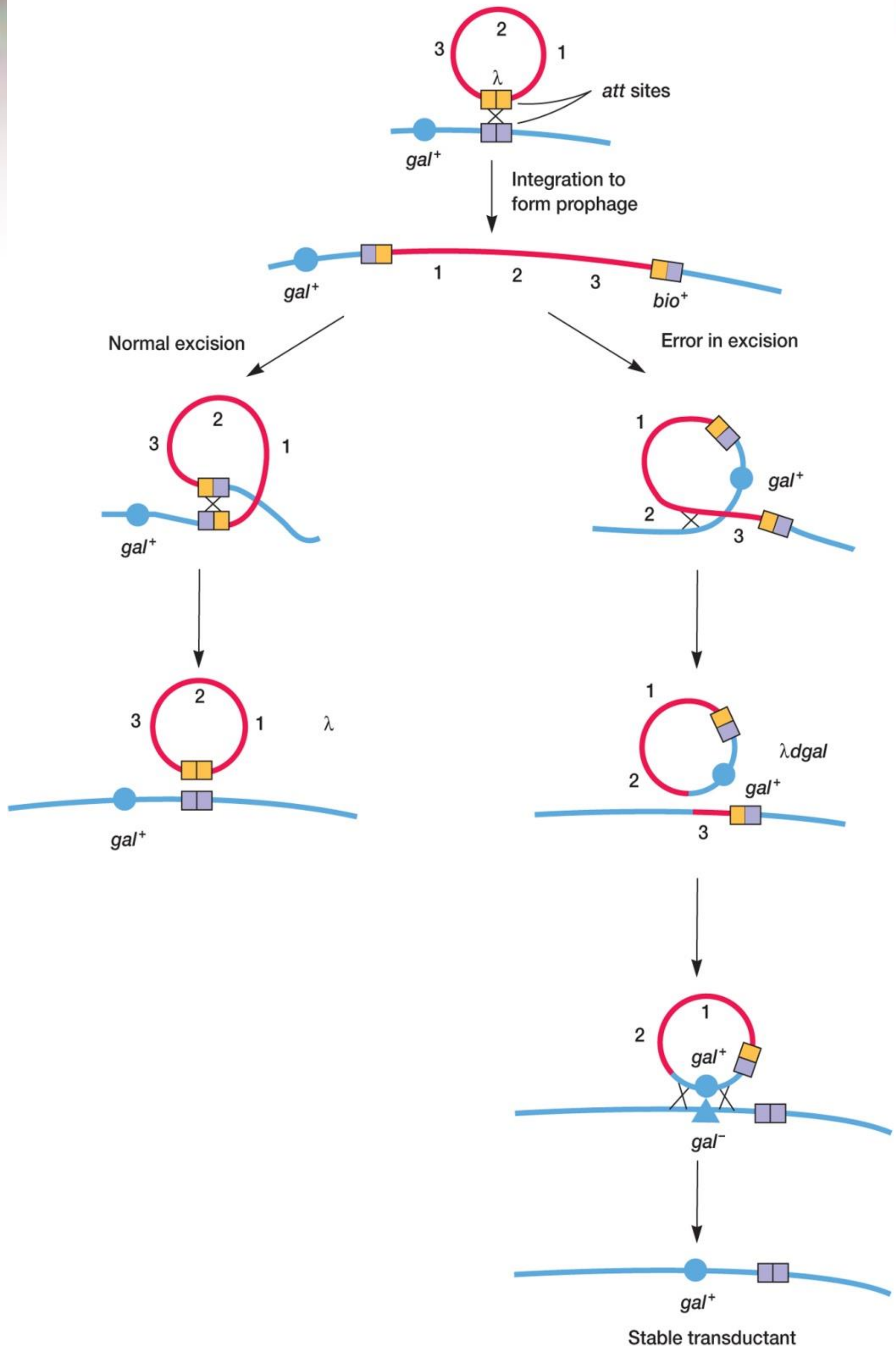


The recombinant bacterium has a genotype (his^+lys^-) that is different from recipient bacterial cell ($his^- lys^-$).



Specialized Transduction

- Carried out only by temperate phages that have established lysogeny
- Only specific portion of bacterial genome is transferred
- Occurs when prophage is incorrectly excised



2
3
1
λ
att sites
gal⁺
Integration to form prophage
gal⁺
1
2
3
bio⁺
Normal excision
Error in excision
2
3
1
gal⁺
1
gal⁺
2
3
2
3
1
λ
gal⁺
1
λdgal
2
gal⁺
3
1
2
gal⁺
gal⁻
gal⁺
Stable transductant

16.9 Evolution in Action: The Development of Antibiotic Resistance in Bacteria

1. Report the common reasons for increasing drug resistance
2. Describe common mechanisms by which antimicrobial drug resistance occurs
3. Suggest strategies to overcome drug resistance

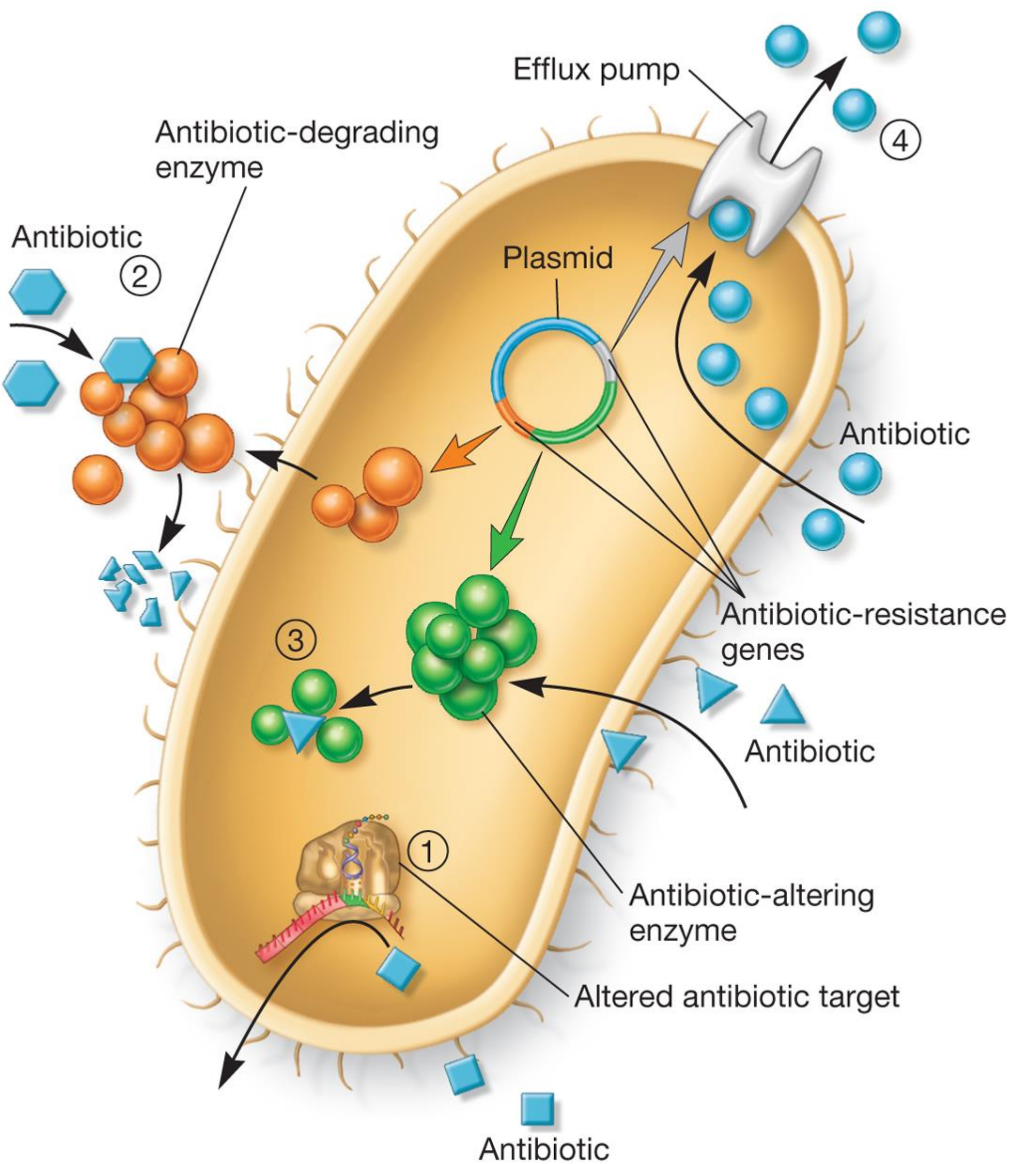
Drug Resistance

- An increasing problem
  - once resistance originates in a population it can be transmitted to other bacteria
  - a particular type of resistance mechanism is not confirmed to a single class of drugs
- Microbes in abscesses or biofilms may be growing slowly and not be susceptible
- Resistance mutants arise spontaneously and are then selected

Drug Resistant "Superbug"

- A methicillin-resistant *Staphylococcus aureus* (MRSA) that developed resistance to vancomycin
  - this new vancomycin-resistant *S. aureus* (VRSA) was also resistant to most other antibiotics
  - isolated from foot ulcers on a diabetic patient
  - Acquired from conjugation with vancomycin-resistant enterococci (VRE) were isolated from same patient
- These drug resistant organisms are a serious threat to human health

Mechanisms of Drug Resistance



- Prevent entrance of drug
- Drug efflux (pump drug out of cell)
- Inactivation of drug
  - chemical modification of drug by pathogen
- Modification of target enzyme or organelle
- Use of alternative pathways or increased production of target metabolite

The Origin and Transmission of Drug Resistance

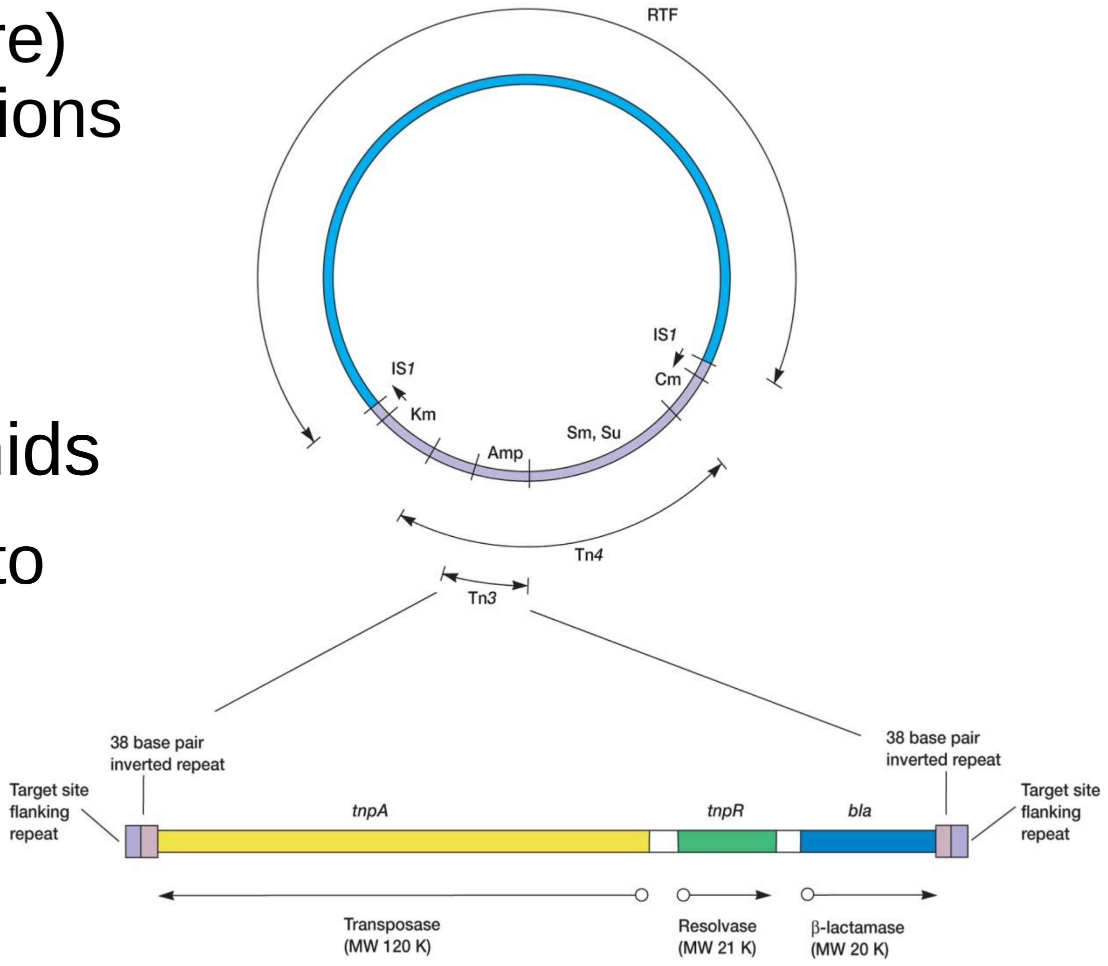
- Immunity genes
  - – resistance genes that exist in nature to protect antibiotic producing microbes from their own antibiotics
- Horizontal gene transfer
  - – transferred immunity genes from antibiotic producers to non-producing microbes

The Origin and Transmission of Drug Resistance

- Resistance genes can be found on
  - bacterial chromosomes
  - plasmids
  - transposons
  - integrons
- When found on mobile genetic elements they can be freely exchanged between bacteria

Origin and Transmission…

- Chromosomal genes
  - resistance from (rare) spontaneous mutations (usually result in a change in the drug target)
- R (resistance) plasmids
  - can be transferred to other cells by conjugation, transduction, and transformation
  - can carry multiple resistance genes



Origin and Transmission…

- Composite transposons
  - contain genes for antibiotic resistance – some have multiple resistance genes
    - can move rapidly between plasmids and through a bacterial population
- Gene cassettes
  - sets of resistance genes
  - can exist as separate genetic elements
  - can be part of transposon, integron, or chromosome