Game plan

Lecture

Antibiotics
Antibiotic resistance
Gene transfer
- Transformation
- Transduction
- Conjugation

Lab

Review temp and UV labs
Growth control: alcohol, antiseptics and antibiotics

Pre-lab
Transformation and DNA fingerprinting

Discovery of Antimicrobial Drugs

- **1928**: Fleming discovered penicillin, produced by *Penicillium*

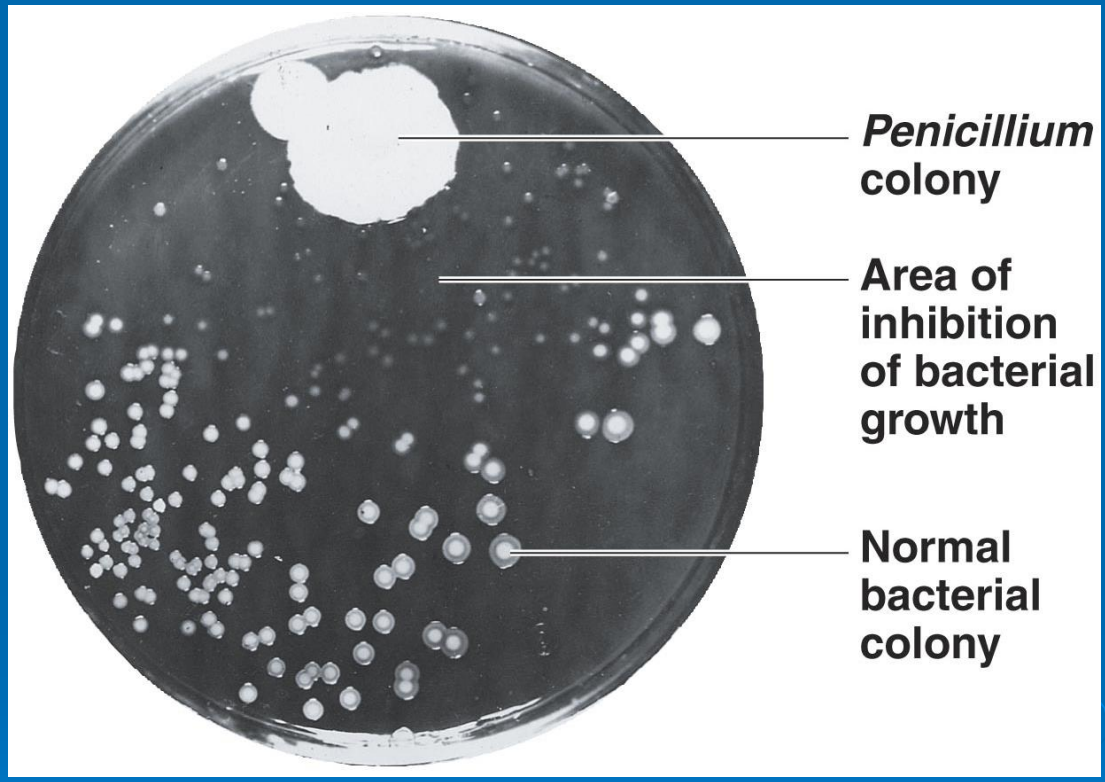


Figure 1.5

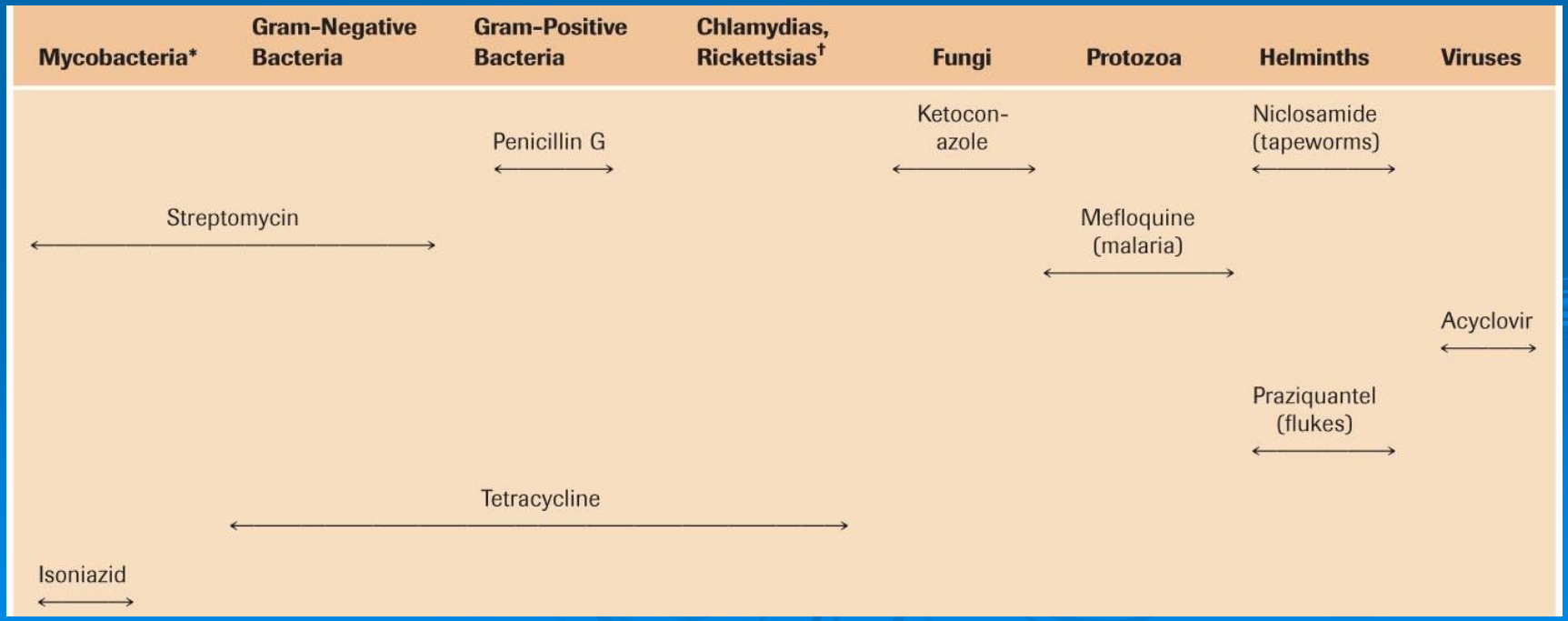
Where to antimicrobials come from?

Gram-Positive Rods	
Bacillus subtilis	Bacitracin
Paenibacillus polymyxa	Polymyxin
Actinomycetes	
Streptomyces nodosus	Amphotericin B
Streptomycems venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Saccharopolyspora erythraea	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpurea	Gentamicin
Fungi	
Cephalosporium spp.	Cephalothin
Penicillium griseofulvum	Griseofulvin
Penicillium chrysogenum	Penicillin

Spectrum of activity

Broad spectrum- affect a broad range of gram-positive and gram-negative bacteria

Narrow spectrum- affects a narrow range of bacteria



Pit Stop

This bacterium is lysing because an antibiotic disrupted its cell wall. Why doesn't the antibiotic lyse human cells?

The Action of Antimicrobial Drugs

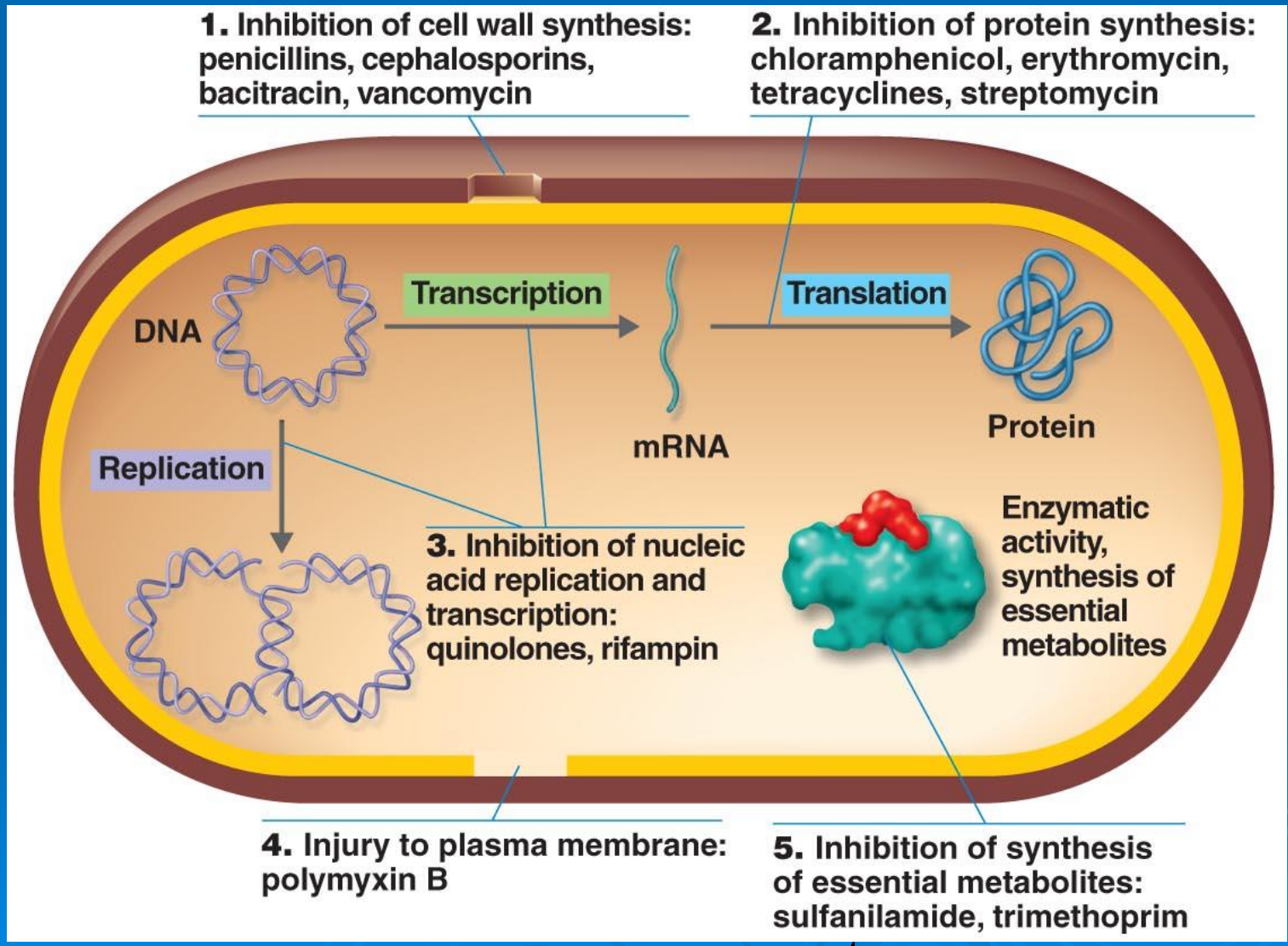
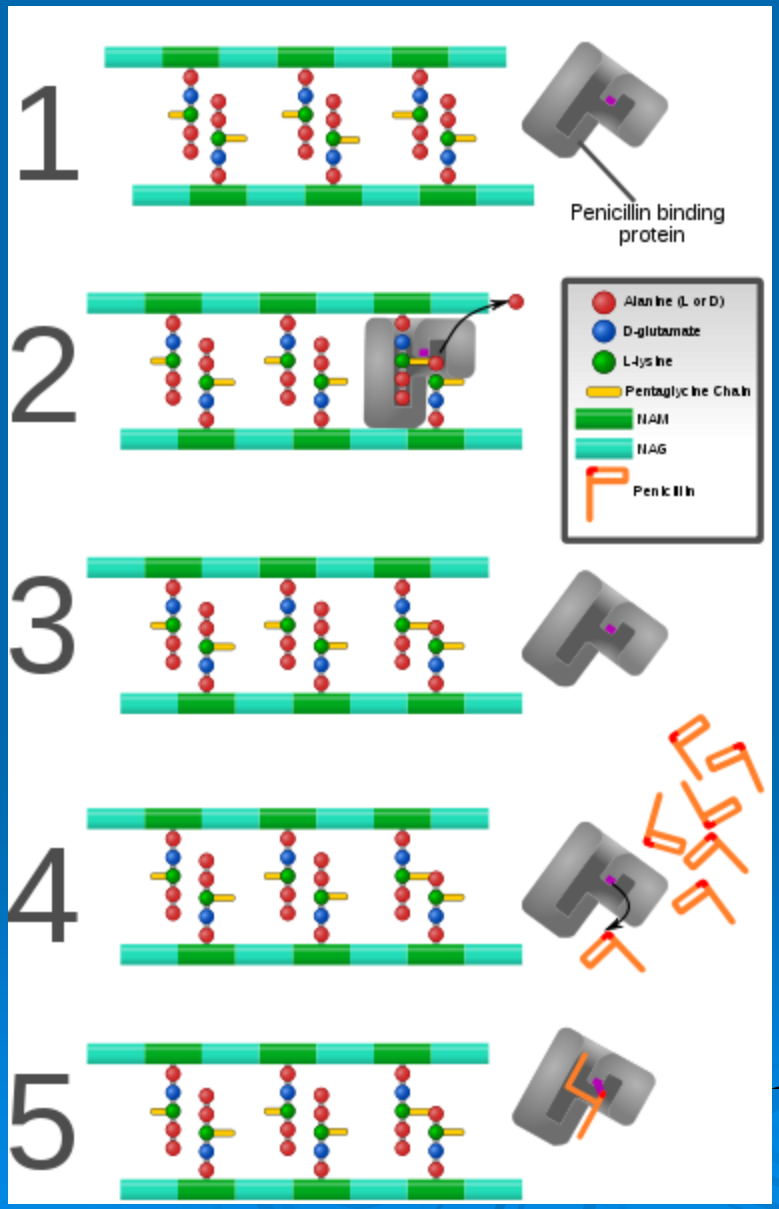


Figure 20.2

Case study in narrow spectrum antibiotics: Penicillin

http://ihm.nlm.nih.gov/images/A20824

Penicillin prevents cross-linking in cell wall



A peek at antibiotic resistance… penicillinase

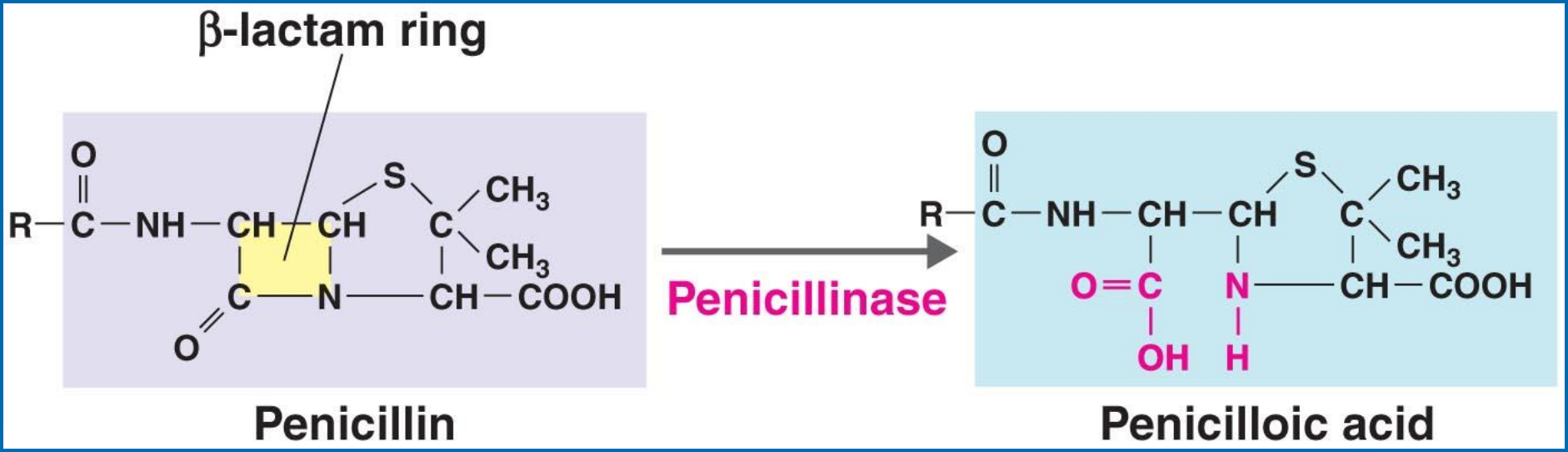
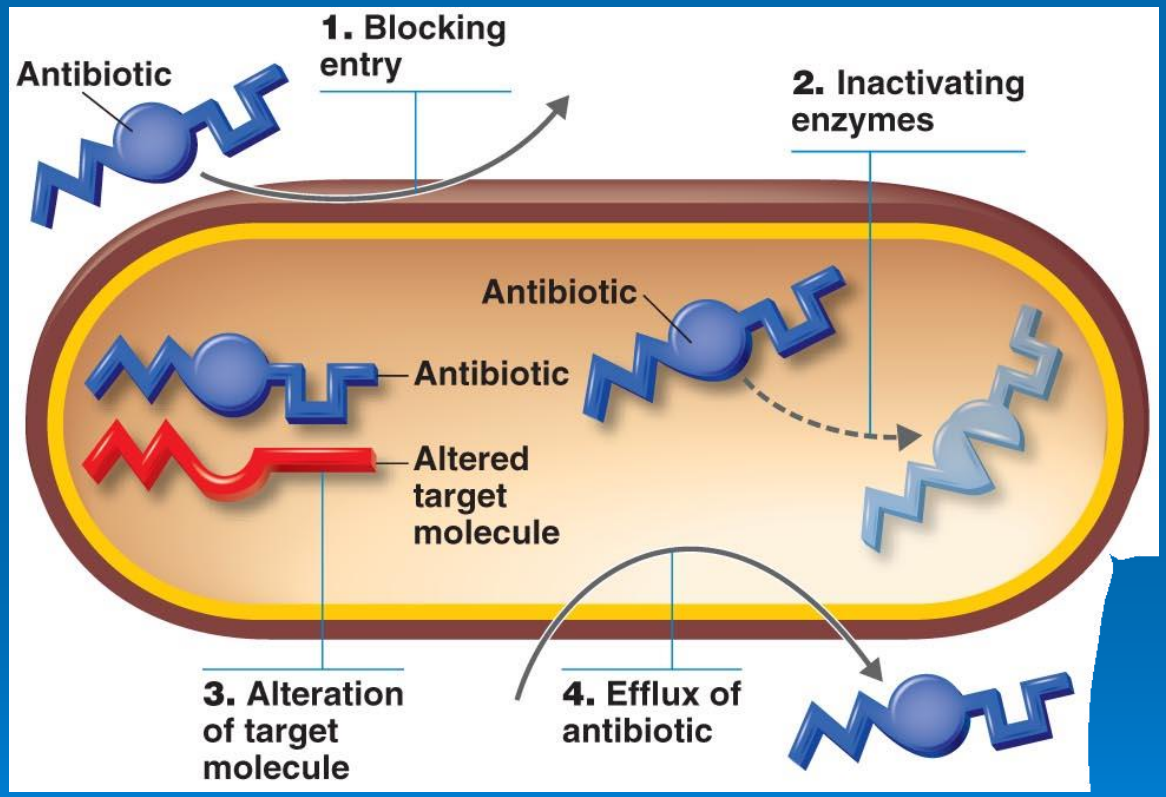


Figure 20.8

Resistance to Antibiotics



- Inactivating enzymes: (i.e. enzymatic destruction of drug)
  - e.g. Penicillin
- Blocking entry of drug
- Alteration of drug's target molecule
- Rapid efflux (ejection) of the drug

Figure 20.20

Mechanisms of resistance

- A variety of mutations can lead to antibiotic resistance

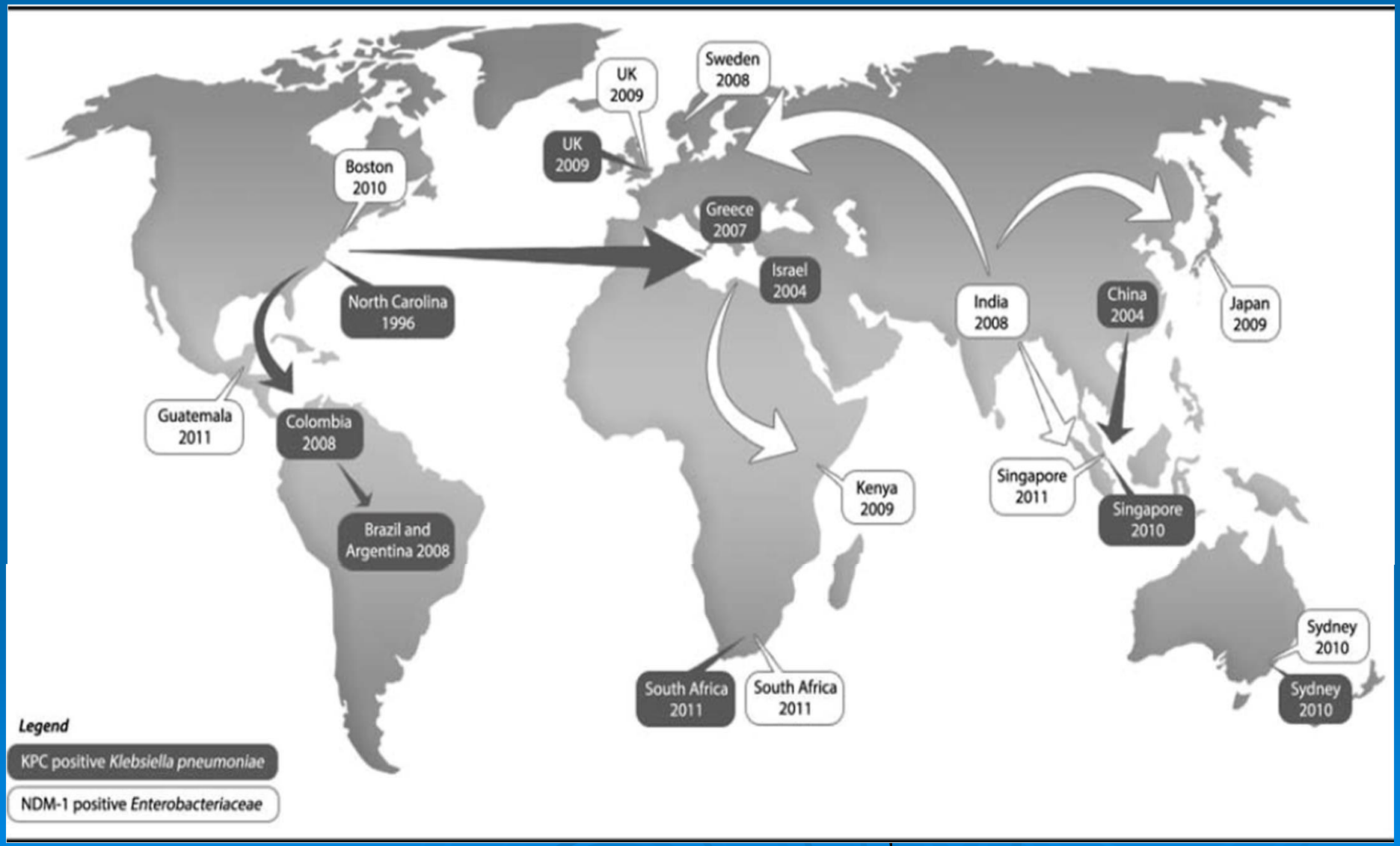
- Misuse of antibiotics selects for resistance mutants. Misuse includes:
  - Using outdated or weakened antibiotics
  - Using antibiotics for the common cold and other inappropriate conditions
  - Using antibiotics in animal feed
  - Failing complete the prescribed regimen
  - Using someone else's leftover prescription

The future of antibiotic resistance… Carbapenem-Resistant Enterobacteriaceae (CREs)

- **CRE definition by CDC:** resistant to large range of beta-lactam antibiotics
  - Nonsusceptible to meropenem, imipenem, doripenem
  - Resistant to 3rd generation cephalosporins
    - Ceftriaxone, cefotaxime, ceftazadime

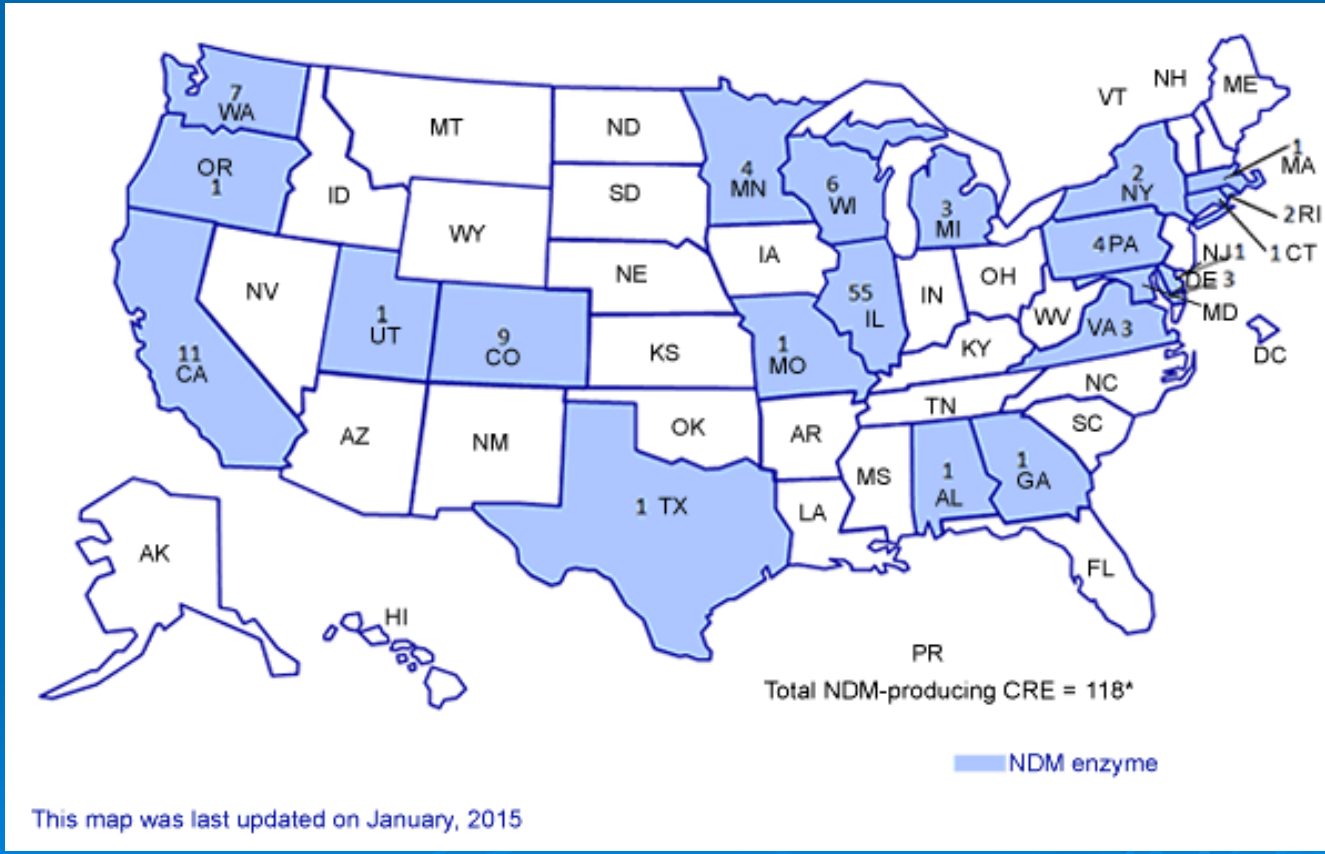
- **CREs** contain different enzymes that break down carbapenems:
  - **KPC** (***K****lebsiella* ***p****neumoniae* **C**arbapenemase)
    - Most common CRE in United States. First identified in 1996 in North Carolina. [Carbapenem resistant *Klebsiella pneumonia* (CRKP)]
  - **NDM-1** (**N**ew **D**elhi **M**etallo-beta-lactamase)- resistance to all antibiotics except colistin (affects membranes) and tigecycline (protein synthesis inhibitor ~ tetracylcine)
    - Originally identified in patient from New Delhi in 2008

Global spread of CREs

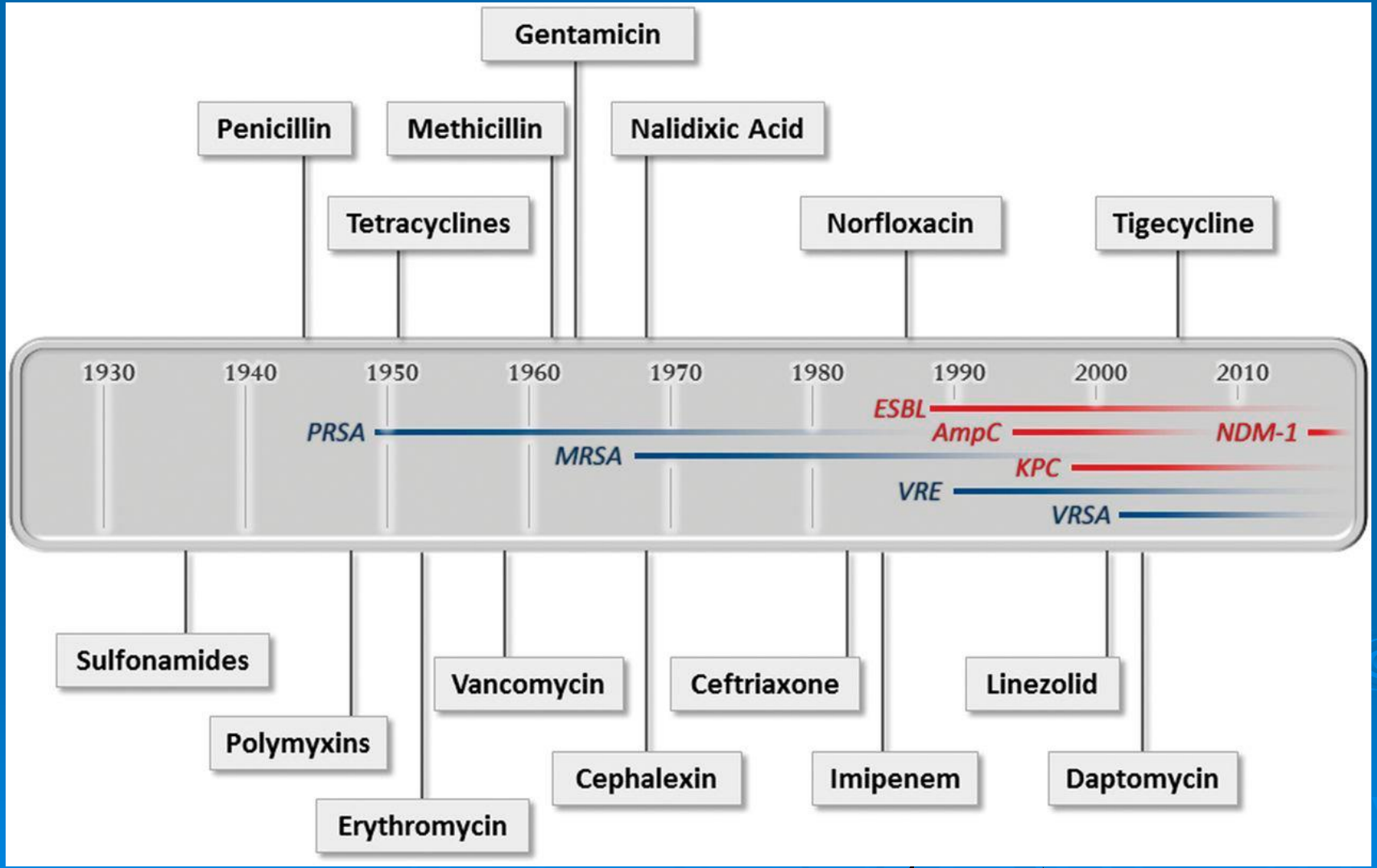


Molton et al CID 2013

Mapping of CREs in the US
(for updates, click on map below)

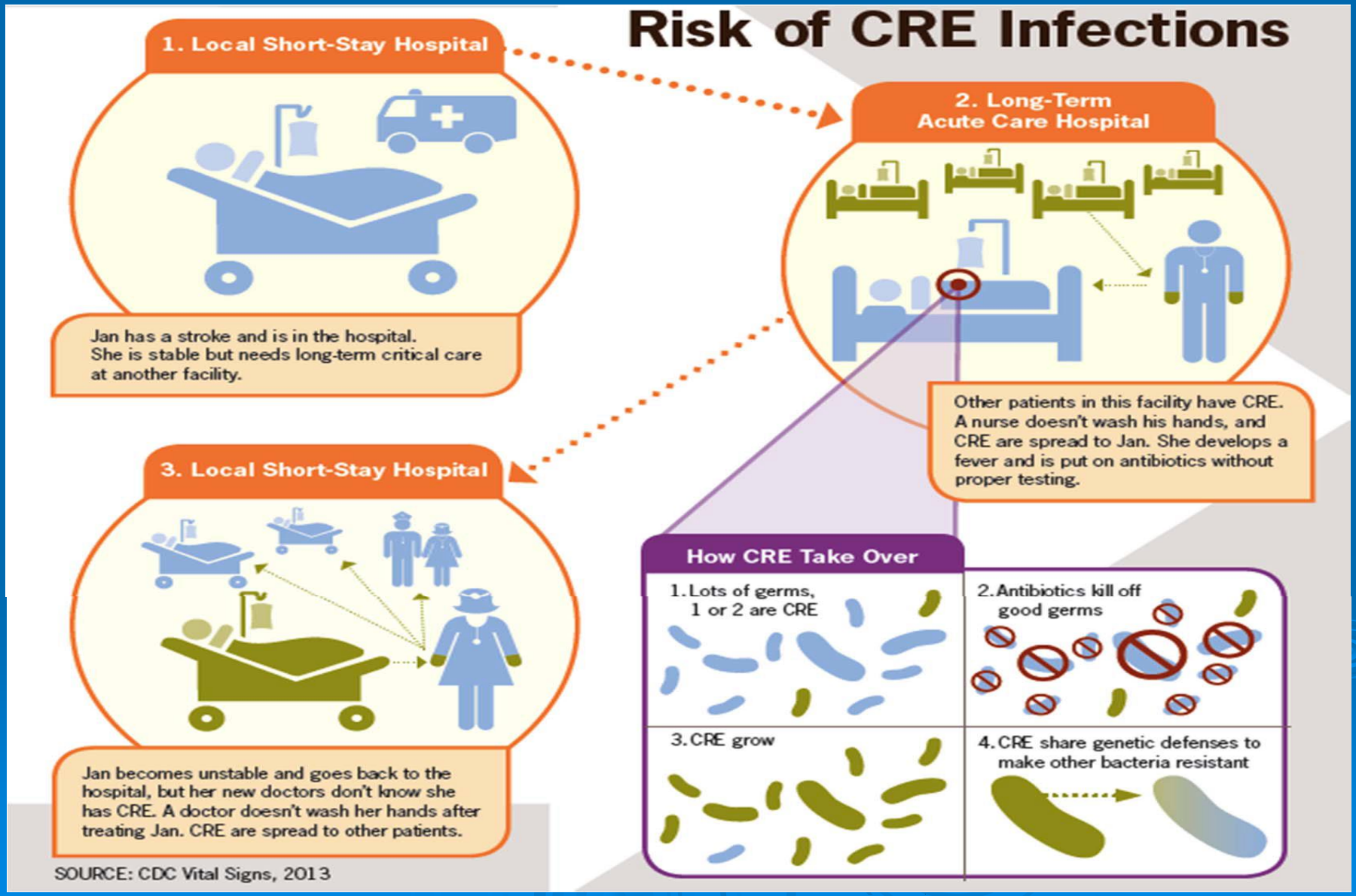


The antibiotic pipeline looks bleak

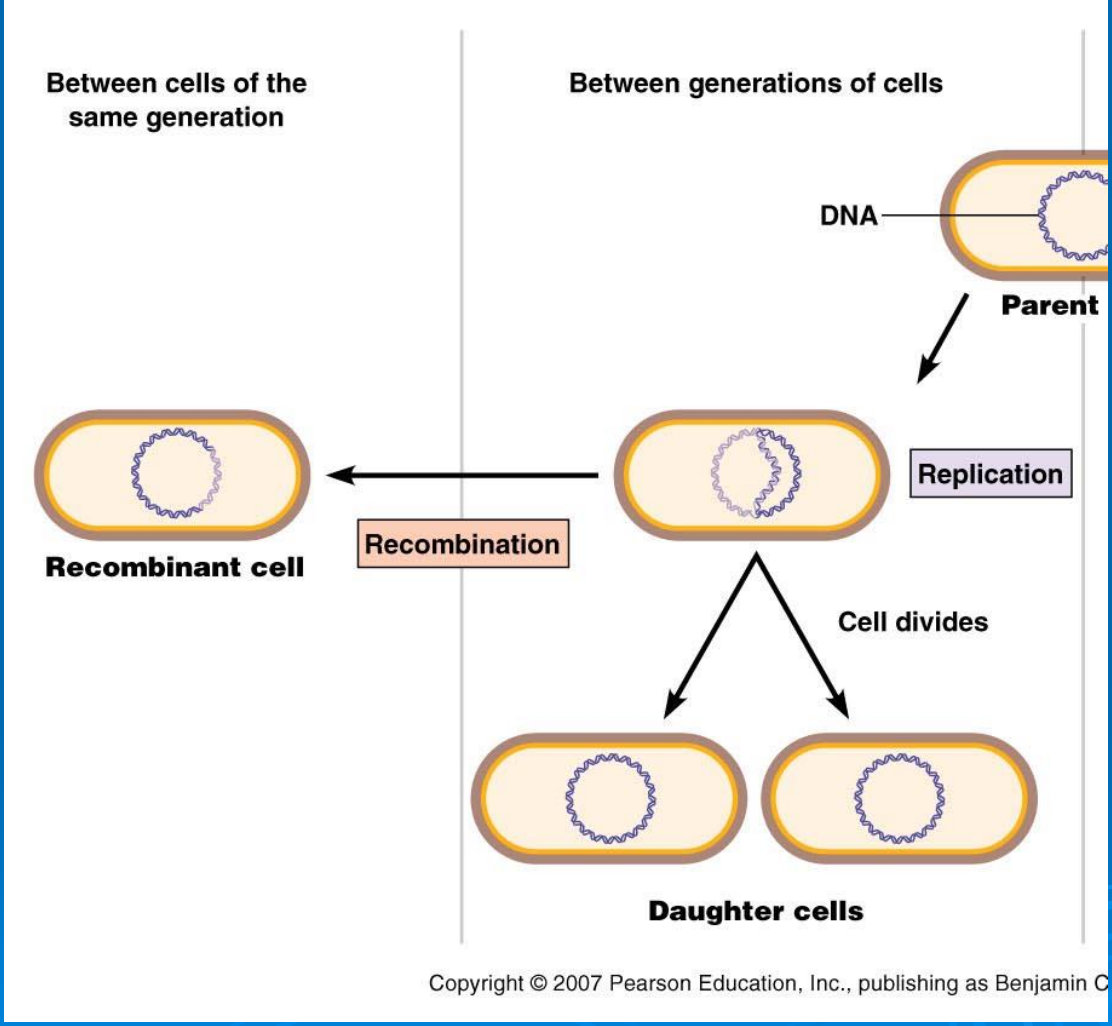


Molton *et al*. CID 2013

Resistance genes are spreading



CRE genes move through horizontal gene transfer



Horizontal gene transfer

Vertical gene transfer

Horizontal gene transfer: Transformation

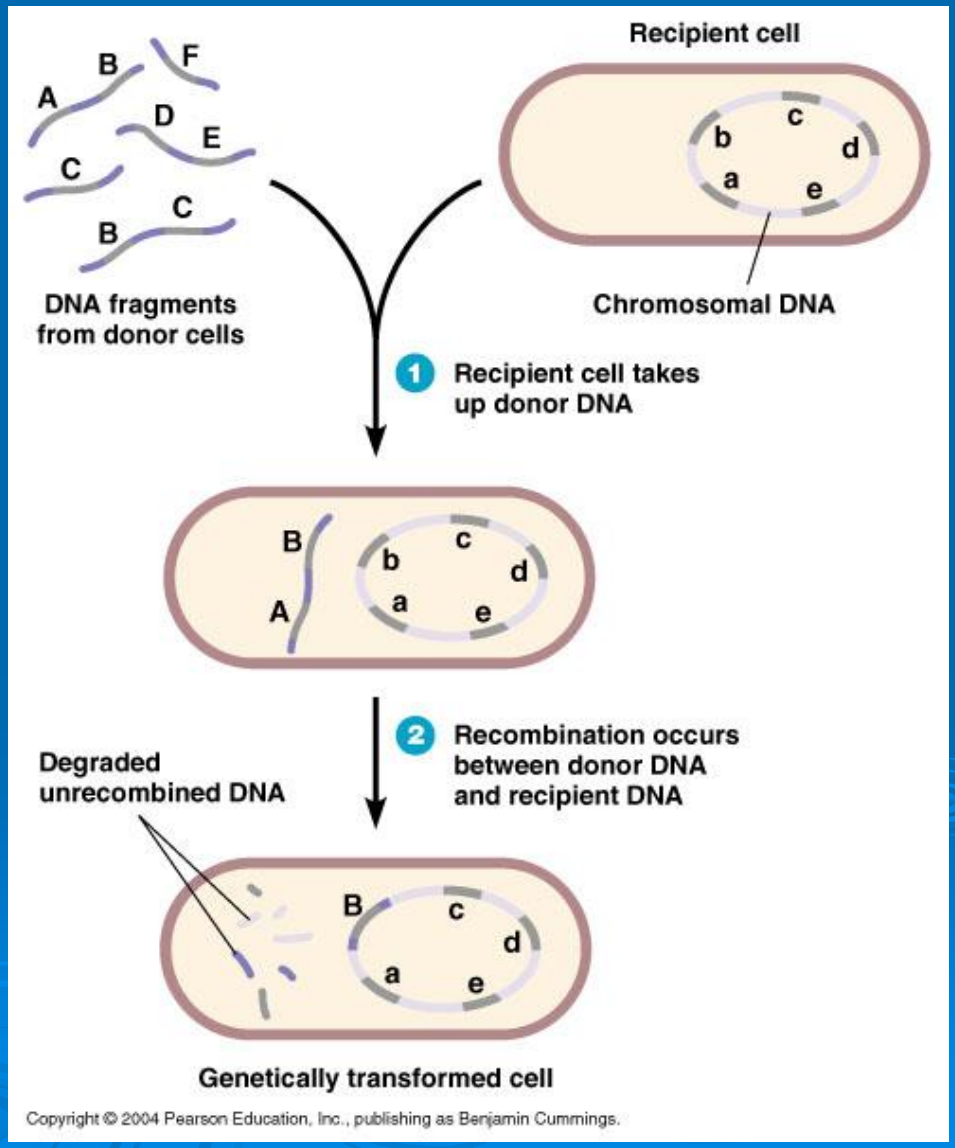
DNA transferred:

-Cell free or “naked” DNA

Requirements:

-<u>Competent cells</u> take up “naked DNA” from environment

-DNA recombines in host chromosome *if similar*



Horizontal gene transfer: Transformation

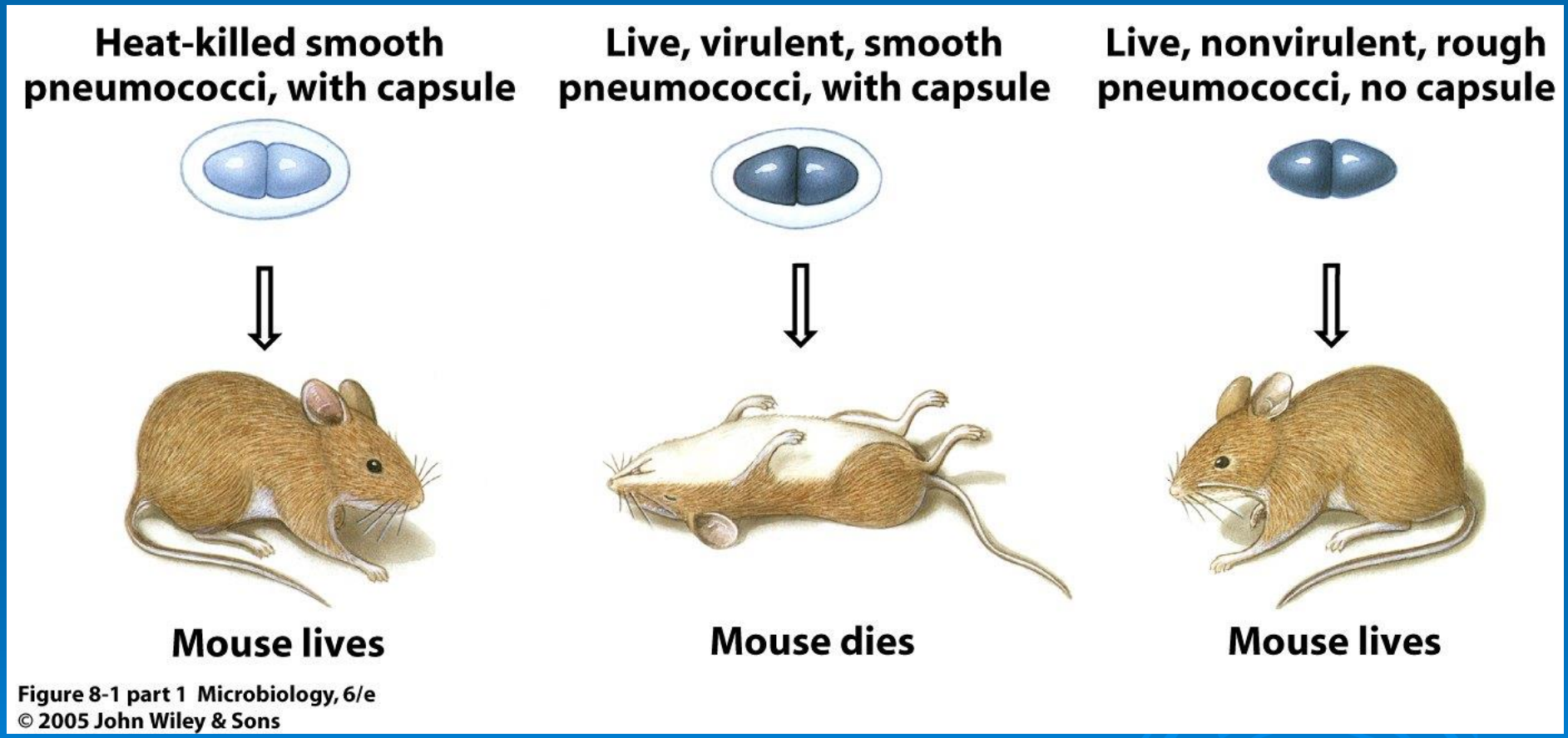


Figure 8-1 part 1 Microbiology, 6/e

1920s- Griffith experiment

Horizontal gene transfer: Transformation

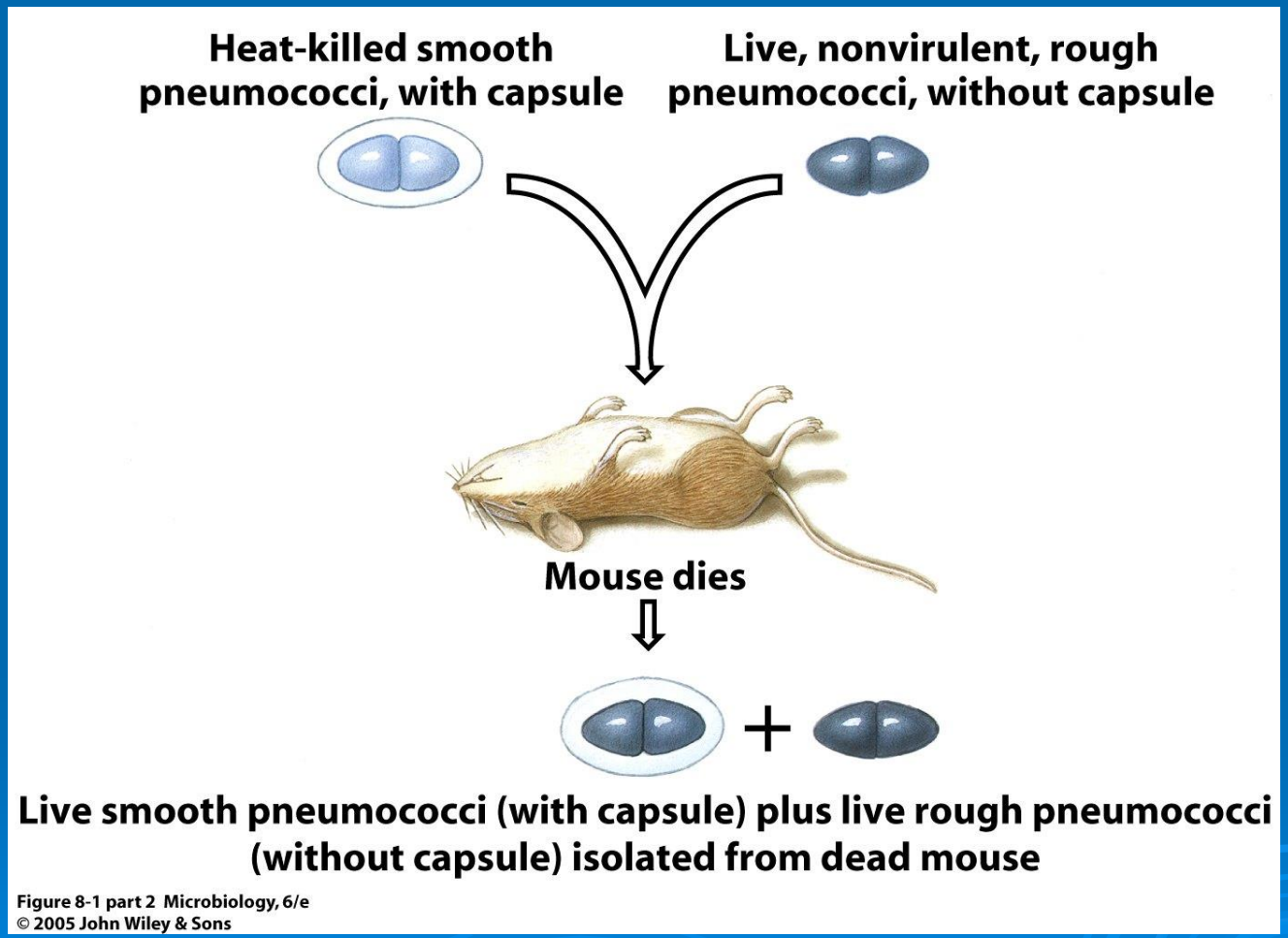


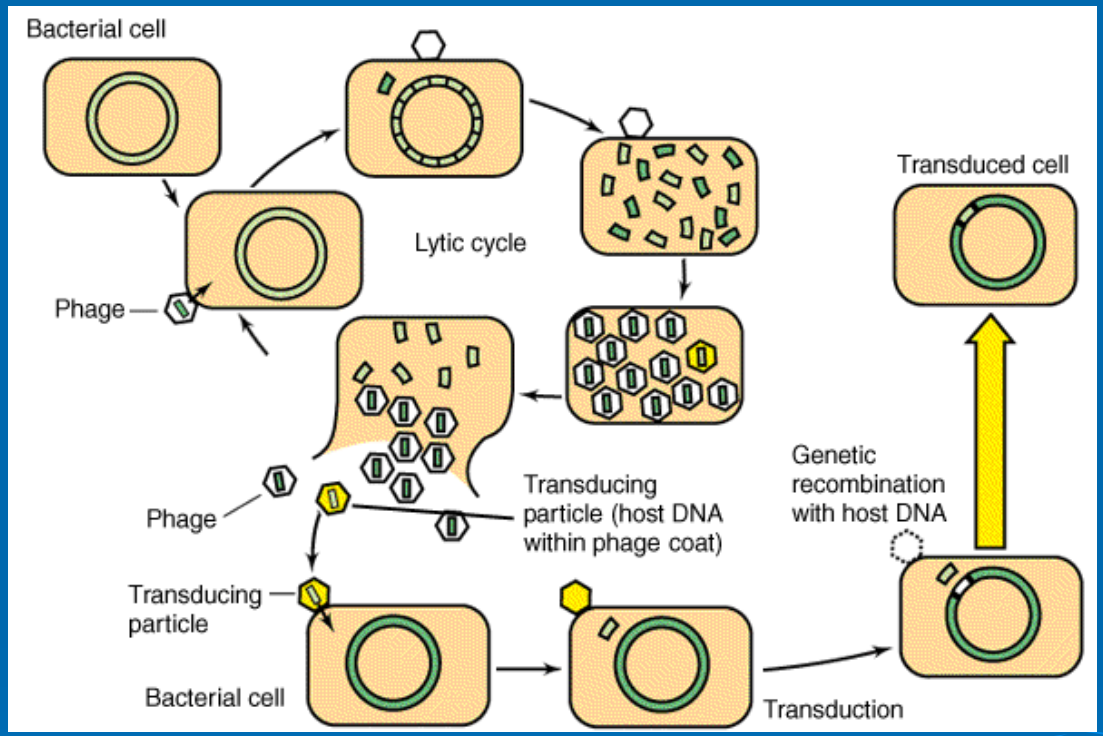
Figure 8-1 part 2 Microbiology, 6/e

1920s- Griffith experiment

Horizontal gene transfer: Generalized transduction

DNA transferred:

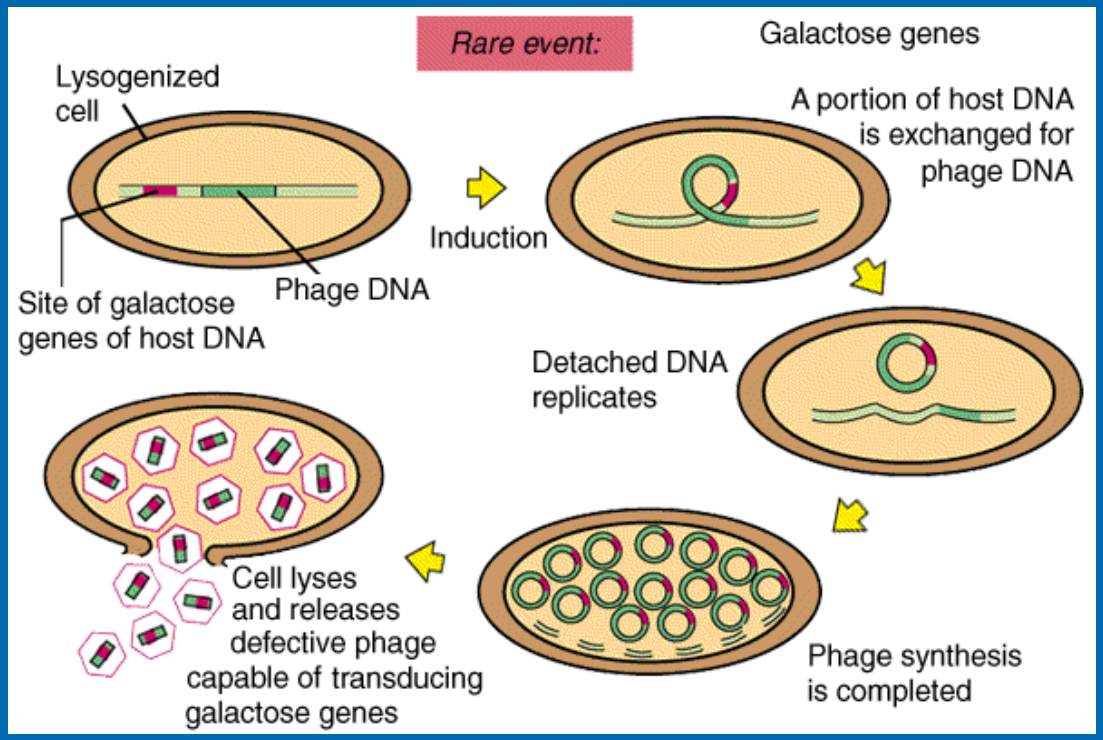
-Small chromosomal fragments from bacteria



Requirements:

-Lytic bacteriophage (phage) replicates in bacterial cell (lytic cycle)
-Imperfect packaging of new phage particles allows bacterial DNA to be included
- New phage inserts bacterial DNA into new bacterial cell
-DNA recombines into host chromosome *if similar*

Horizontal gene transfer: Specialized transduction



DNA:

-Bacterial chromosome fragment near prophage

Requirements:

-Lysogenic phage inserts phage DNA into host chromosome to become a prophage (lysogenic cycle)

-Once induced to lytic cycle, the prophage excises, including adjacent piece of host DNA, and makes new viral particles

-New phage inserts bacterial DNA into new bacterial cell

-DNA recombines into host chromosome *if similar*

Comparison of Lytic and Lysogenic Phage/Cycles

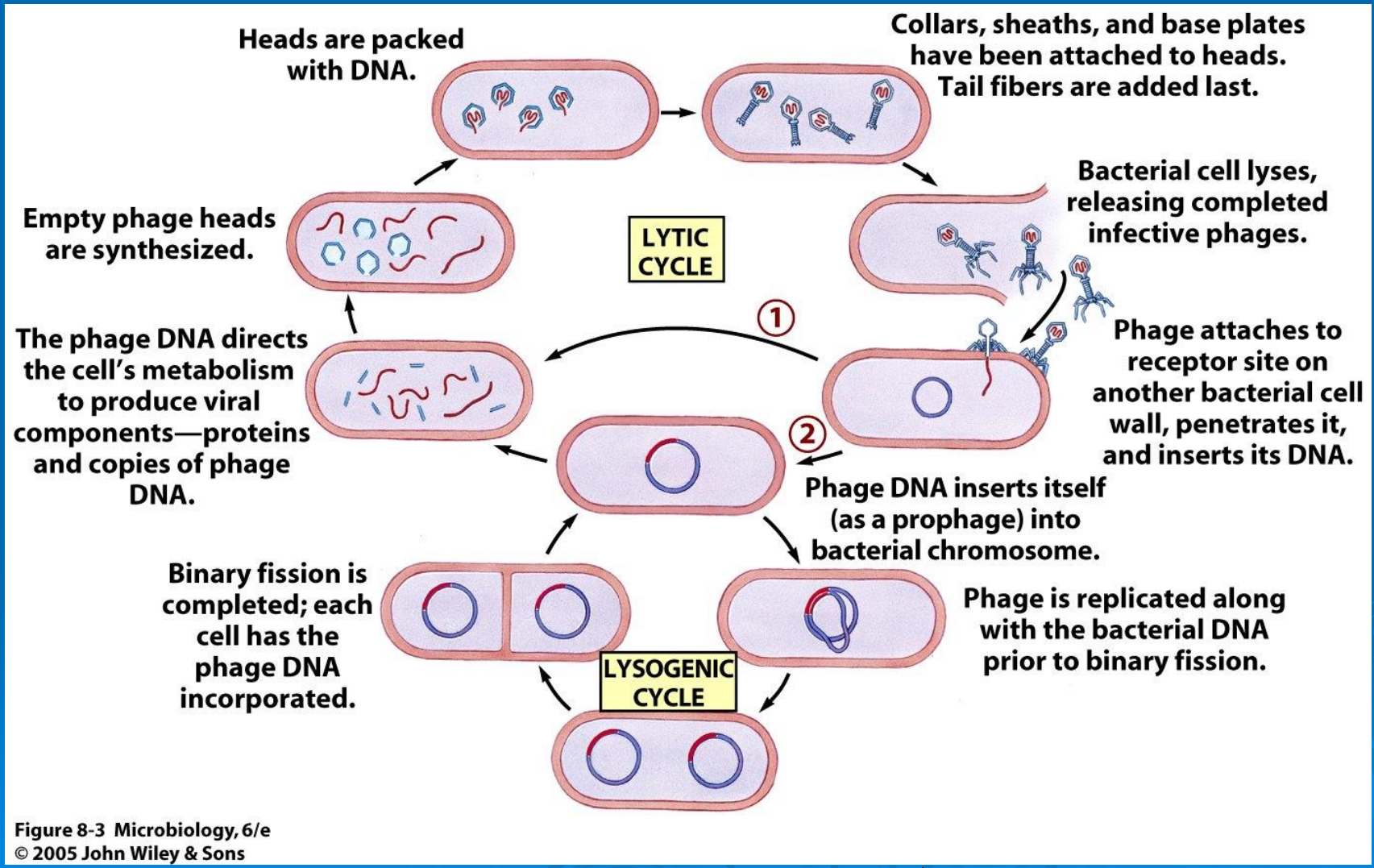


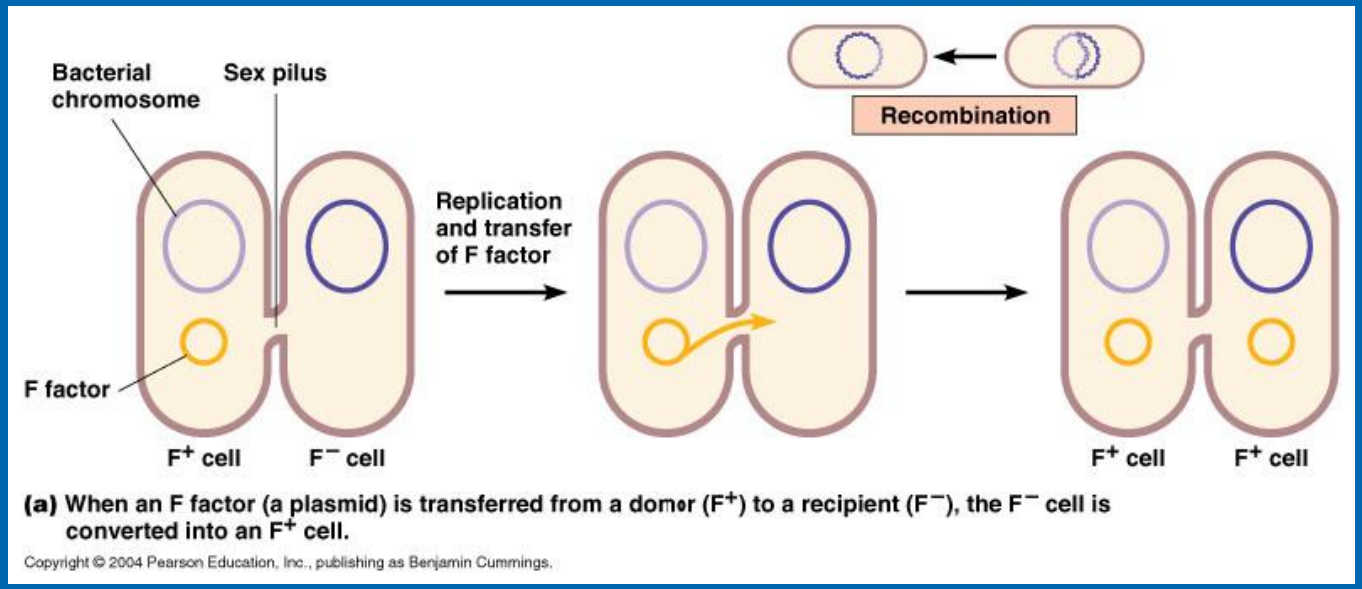
Figure 8-3 Microbiology, 6/e

Horizontal gene transfer: Conjugation

DNA transferred:

-F factor

Requirements:



-F + cell contains the F factor (i.e. plasmid containing conjugation and pili genes)

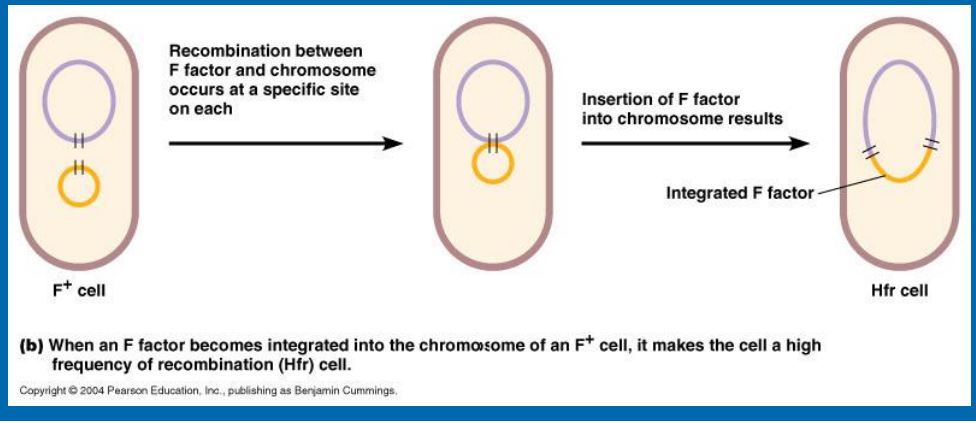
- F + cell will make conjugation pili and connect to F - cell that does NOT have the F factor

-F factor will replicate and pass to the F - cell

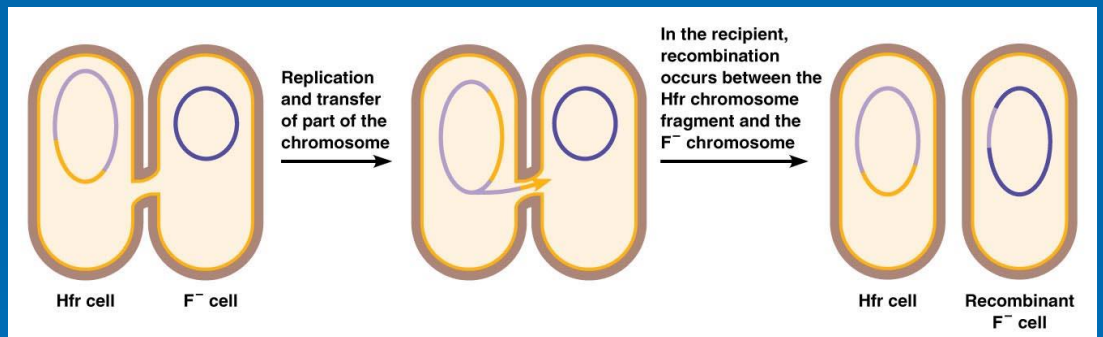
Horizontal gene transfer: Hfr Conjugation

DNA transferred:

-Partial F factor with bacterial chromosome DNA



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.



(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

Requirements:

-F factor will recombine with the bacterial chromosome and become a **h**igh-**f**requency of **r**ecombination cell (Hfr)

-Hfr will initiate conjugation with F - cell and transfer part of F factor combined with bacterial genes.

Horizontal gene transfer compared

TABLE 8.2

Summary of the Effects of Various Transfers of Genetic Information

Kind of Transfer	Effects
Transformation	Transfers less than 1 percent of cell's DNA. Requires competence factor. Changes certain characteristics of an organism, depending on which genes are transferred.
Transduction	Transfer is effected by a bacteriophage.
Specialized	Only genes near the prophage are transferred to another bacterium.
Generalized	Fragments of host bacterial DNA of variable length and number are packed into the head of a virus.
Conjugation	Transfer is effected by a plasmid.
F^+	A single plasmid is transferred.
Hfr	An initiating segment of a plasmid and a linear sequence of bacterial DNA that follows the initiating segment are transferred.
F′	A plasmid and whatever bacterial genes adhere to it when it leaves a bacterium are transferred.

Table 8-2 Microbiology, 6/e

Independent Study

Study for Exam 2