

Bacterial Genetics

- Impact on medicine
 - **Antibiotic resistance** through mutation, plasmids, genetic exchange
 - Genetics of **virulence** (bacteriophages encoding virulence traits)
 - **Plasmids** encoding virulence traits and antibiotic resistance

The bacterial genome:

- chromosome
- plasmids
- bacteriophage
- insertion sequences
- transposons

haploid - one copy of chromosome, mutations are homozygous (exception – *Neisseria*)

Mutation

- **change in the DNA sequence**
- 10^{-9} /base or 10^{-6} /gene per replication
- happen all of the time, regardless of growth conditions - are they selected for to become enriched in the bacterial population?
- **point mutations** - single bases (insertion, deletion, missense, nonsense)
- **macro-mutation** - affect >1 base (insertion, deletion, inversion, duplication)

- **can change a gene so that protein is no longer affected by an antibiotic, yet still retains its function**
- **effects of genotype on phenotype**
 - silent
 - loss of function
 - altered function
 - completely new genes are not constructed by a single mutation

Genetic exchange

- **Importance**

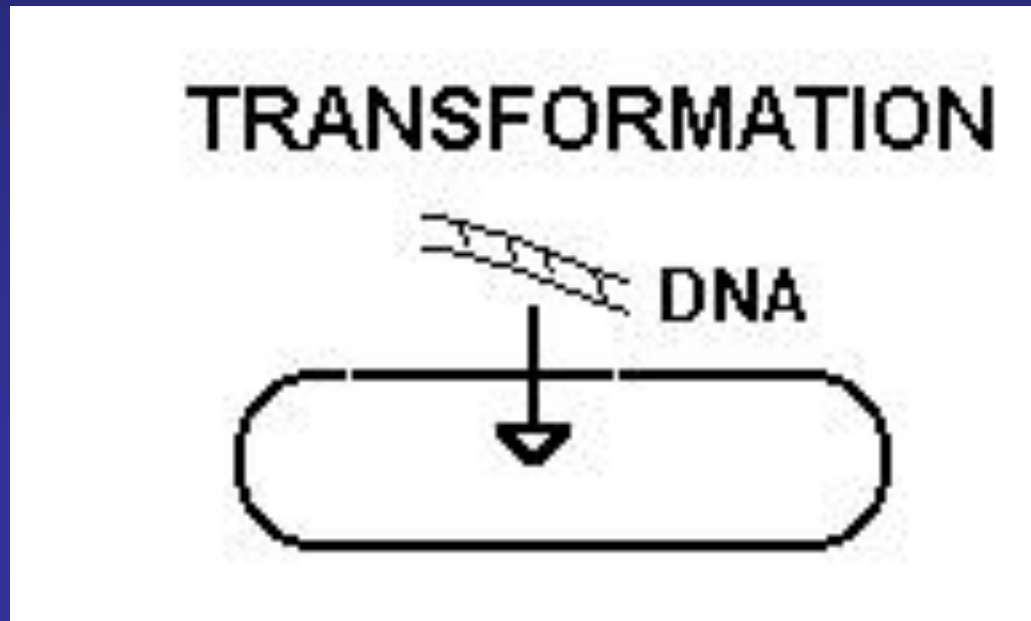
- moving **antibiotic resistance** genes among bacteria
- moving **virulence** gene among bacteria
- changing the **antigenic make-up** to avoid immunity

- **Mechanisms**

- **transformation** - uptake of naked DNA
- **transduction** - bacteriophage as vectors
- **conjugation** - plasmids moved by cell-cell contact

Transformation

- recipient cell must be **competent** for uptake of DNA
- **natural competence** versus **artificial competence**
- only certain bacteria are naturally transformable - *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Vibrio*



Transduction

- **bacteriophage (phage)** are viruses of bacteria
 - **lytic** - always lyse (kill) host bacterial cell
 - **temperate** - can stably infect and coexist within bacterial cell (**lysogeny**) until a **lytic phase** is induced
- **lysogeny**
 - phage genome = **prophage**
 - bacterial cell = **lysogen**
 - **lysogenic conversion**
 - phage encodes observable function
 - (e.g., diphtheria toxin in *Corynebacterium diphtheriae*)

Lysogenic Conversion



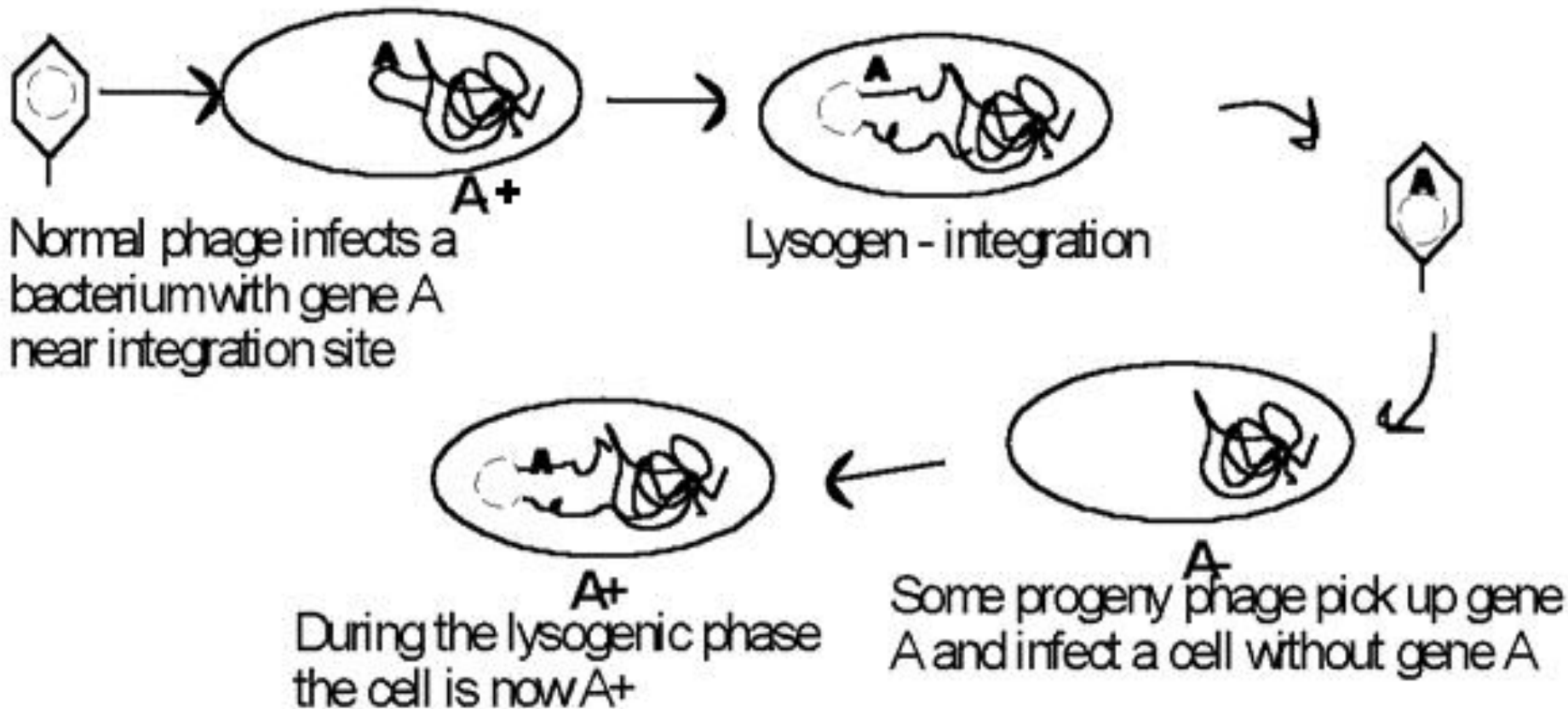
phage with toxin gene
as part of its genome
infects a bacterium

Lysogeny and integration cause
conversion of cell to Tox+

Specialized transduction

- Some **prophages integrate** into the bacterial genome at a specific location.
- When a prophage is induced to **lytic phase**, it may drag along a piece of the **bacterial genome next to the integration site** and move that bacterial sequence into the new recipient host cell, **changing the recipient's genome**.
- Not very important medically since only selected genes can be transferred.

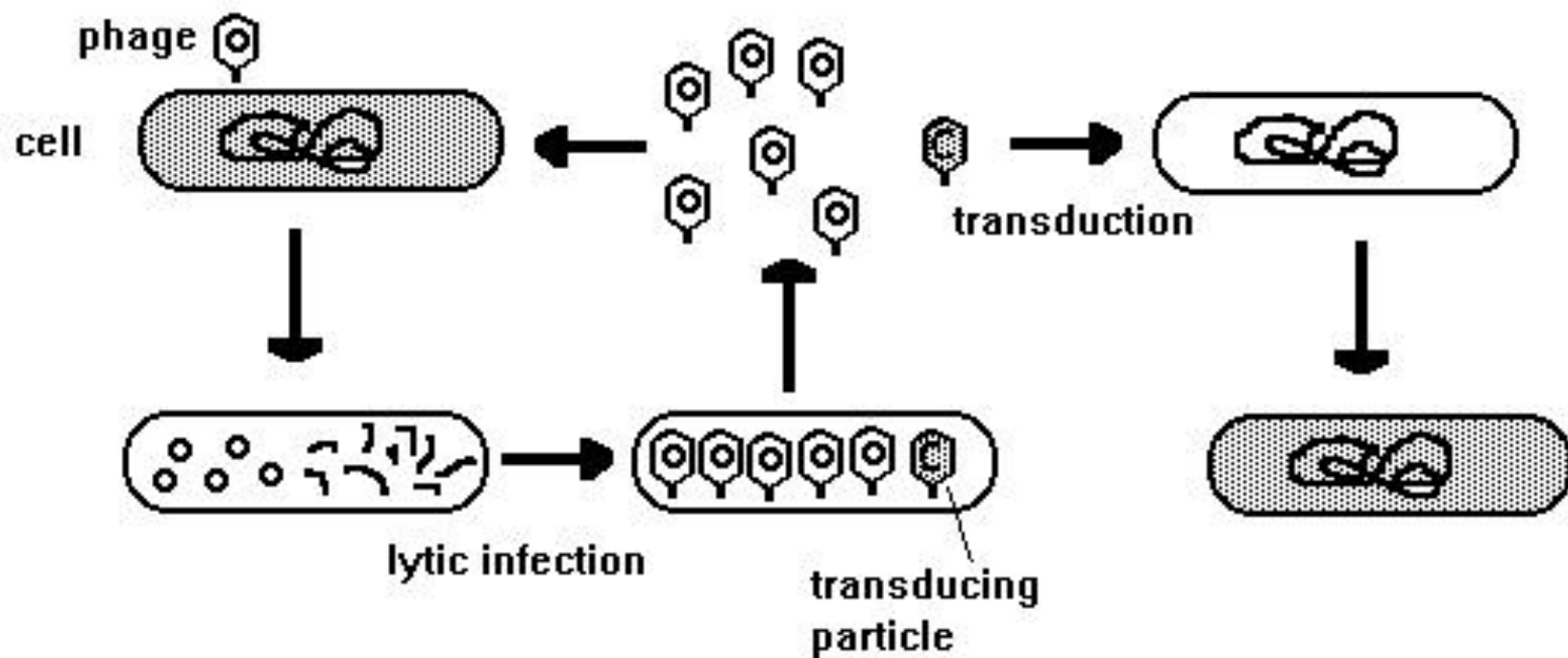
Specialized Transduction



Generalized transduction

- When a phage lyses the host bacterial cell, it normally **packages phage genome** into the capsid.
- Sometimes the **capsid is accidentally** filled with random pieces of **bacterial genome**, possibly including plasmids.
- When the capsid injects the host genes into a new recipient, the new gene can **recombine** into the recipient genome and cause a change.
- **Virulence** and **antibiotic resistance** genes can be moved by generalized transduction.

GENERALIZED TRANSDUCTION



NOTE:

- **lysogeny**
 - **phage gene** = lysogenic conversion
 - **host gene** = specialized transduction.
- **generalized transduction**
 - phage particle = **vehicle** to move **bacterial genes** from one cell to another
 - phage genome is not moved

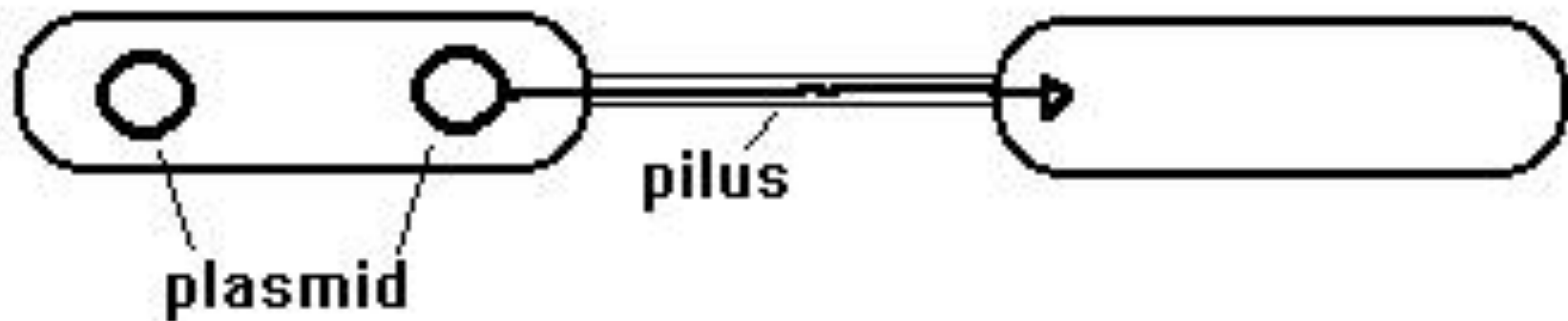
Conjugation

- 3 possible states for conjugation
 - **conjugative** - plasmid encodes all of the functions for conjugation and **can move itself** from the donor cell to the recipient cell
 - **mobilizable** - plasmid cannot move itself, but **can be moved with help** from a conjugative plasmid
 - **non-transmissible** - **can't move by conjugation**
- conjugation functions
 - synthesis of sex **pilus**
 - **cell to cell contact** via pilus
 - **copying plasmid DNA and transfer** of copy into recipient cell

CONJUGATION

Donor

Recipient



- bacteria containing a **conjugative plasmid** are called donor, **male, (F+)**
- bacteria **receiving** the plasmid are called recipient, **female, (F-)**
- F plasmid is a specific *E. coli* plasmid that has nothing to do with medicine other than its historical and laboratory use (and standardized exams)

- other terms of interest for standardized exams
 - **Hfr** - a plasmid **integrates into the chromosome**, conjugation will **move part of the chromosome** into the recipient (not of medical relevance)
 - **F'** - plasmid integrates into the chromosome, excision drags along **piece of the chromosome**. F' can move host DNA between cells (not of medical relevance) (similar to specialized transduction)
 - **phenocopy**
 - when **phenotype** does not match **genotype**
 - a cell with the F plasmid, but **lacking pili**

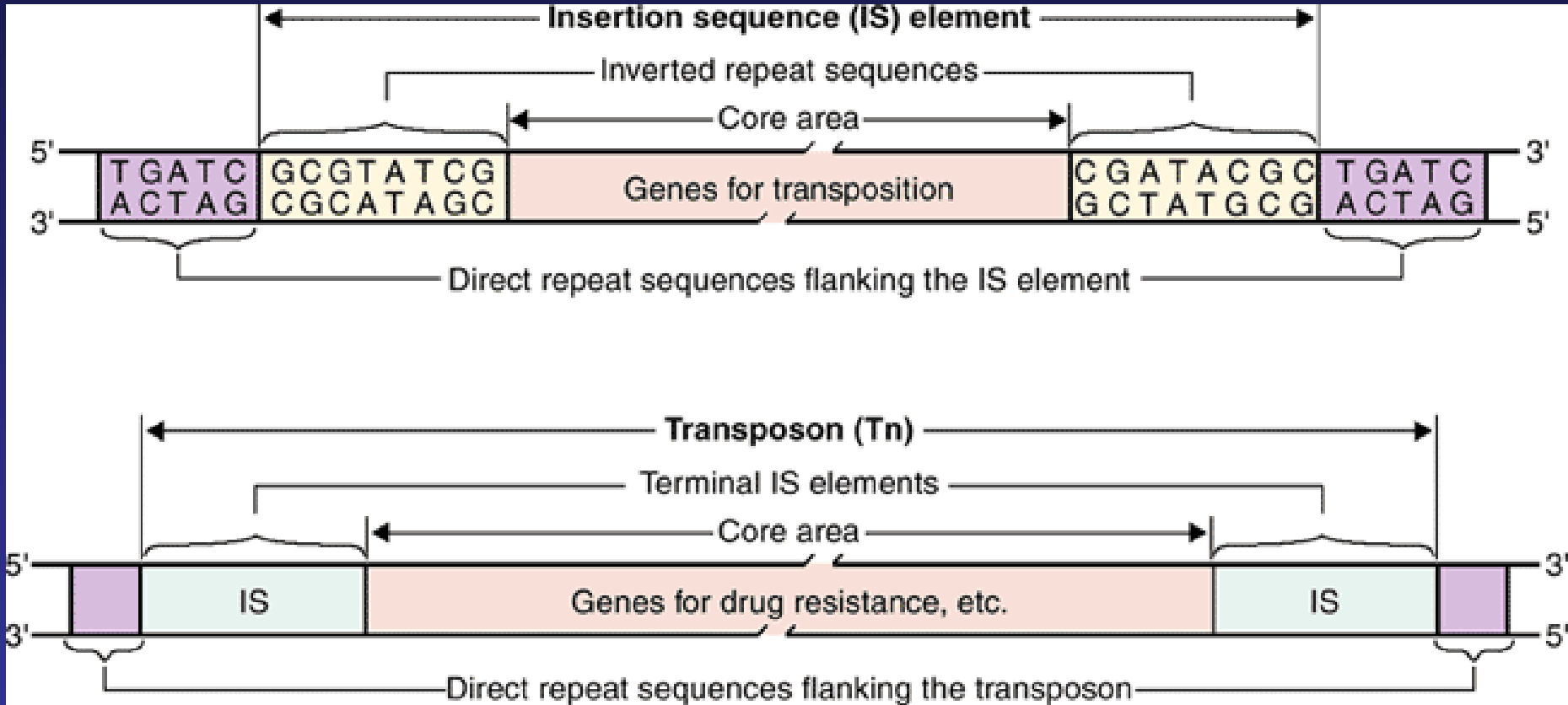
- Unlike transduction by phages, **most plasmids are more promiscuous in their host range.**
- **importance of conjugation - moving plasmids encoding multiple antibiotic resistance genes (R plasmids)** among diverse bacterial

Other mobile DNA elements

- **Insertion Sequences and Transposons**
- **Part of other genetic elements** - chromosome, plasmid. They move from one site in DNA to another **WITHIN the same cell (transposition)**

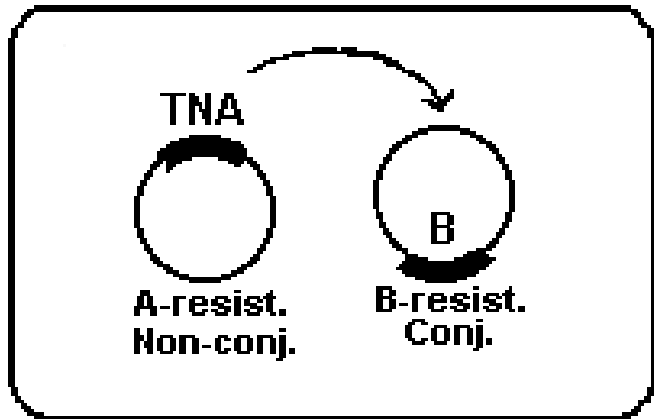
Insertion sequence (IS)

- gene encoding transposition enzyme (**transposase**) flanked by **inverted repeats** of DNA sequence
- can **interrupt** genes if they insert into them

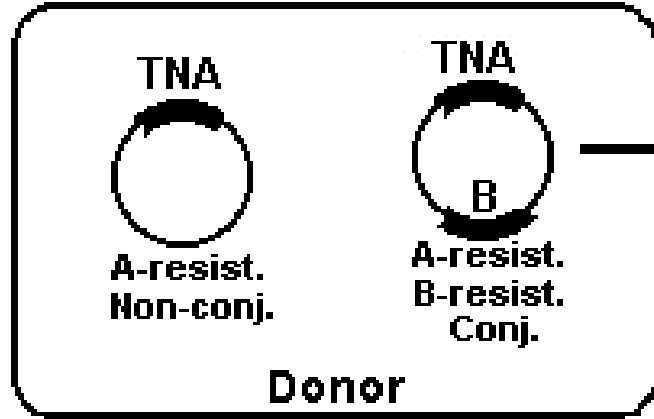


Transposon (Tn)

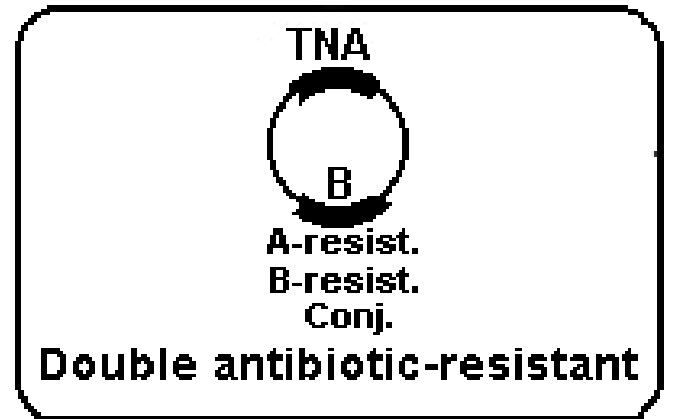
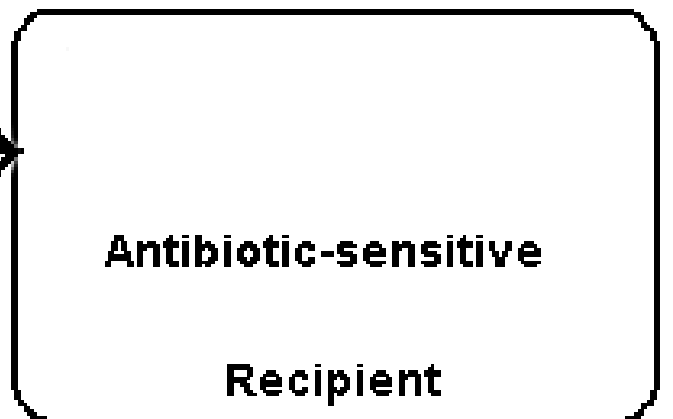
- composite insertion sequence
- gene usually encoding an observable function (e.g., **antibiotic resistance**) flanked by **two copies of an insertion sequence**
- insertion sequences = transposition engine
- move genes between chromosome and plasmids or between different plasmids
- **medical importance** - **antibiotic resistance genes** in transposons in antibiotic resistance plasmids



Transposition



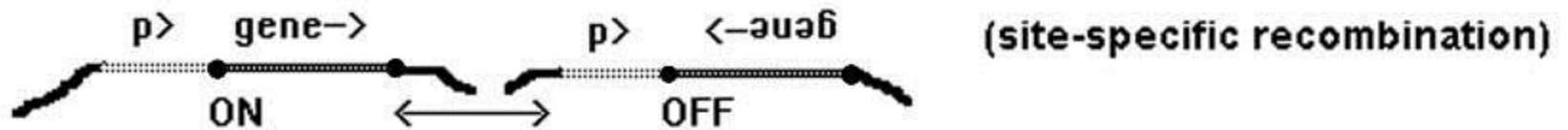
Conjugation



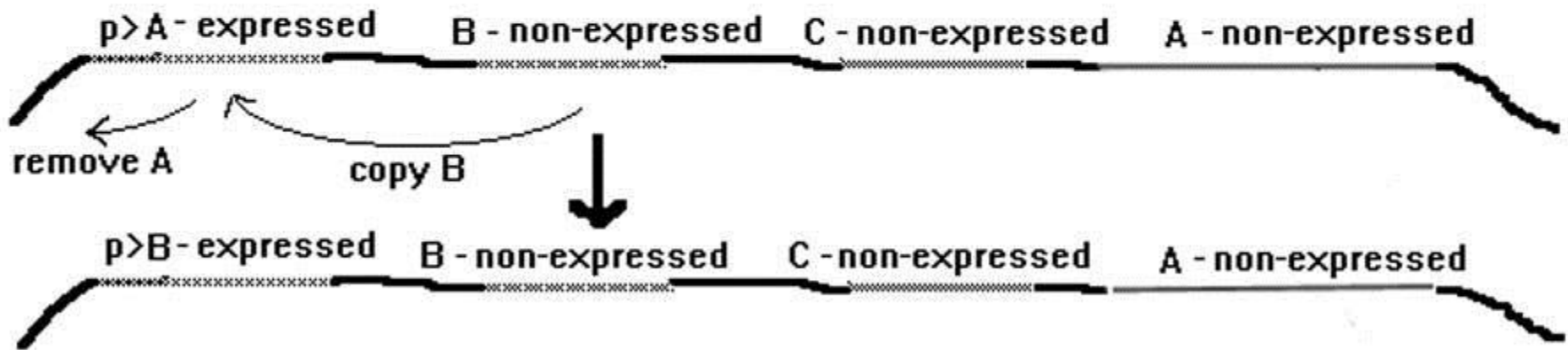
Phase variation and Antigenic variation

- **Phase variation**
 - change in DNA sequence leading to an **ON-OFF switch** of a gene or **A-B switch** of two different genes
 - caused by **inversion of a DNA** sequence
- **Antigenic variation**
 - change in DNA sequence leading to switch of expression among **multiple possible genes**
 - most common mechanism is the **cassette model**
 - non-expressed copies (**silent**) of a gene (the cassettes) are copied or recombined into a site where the cassettes can be **expressed**

PHASE VARIATION - INVERSION MODEL



ANTIGENIC VARIATION - CASSETTE MODEL (RecA-mediated recombination)



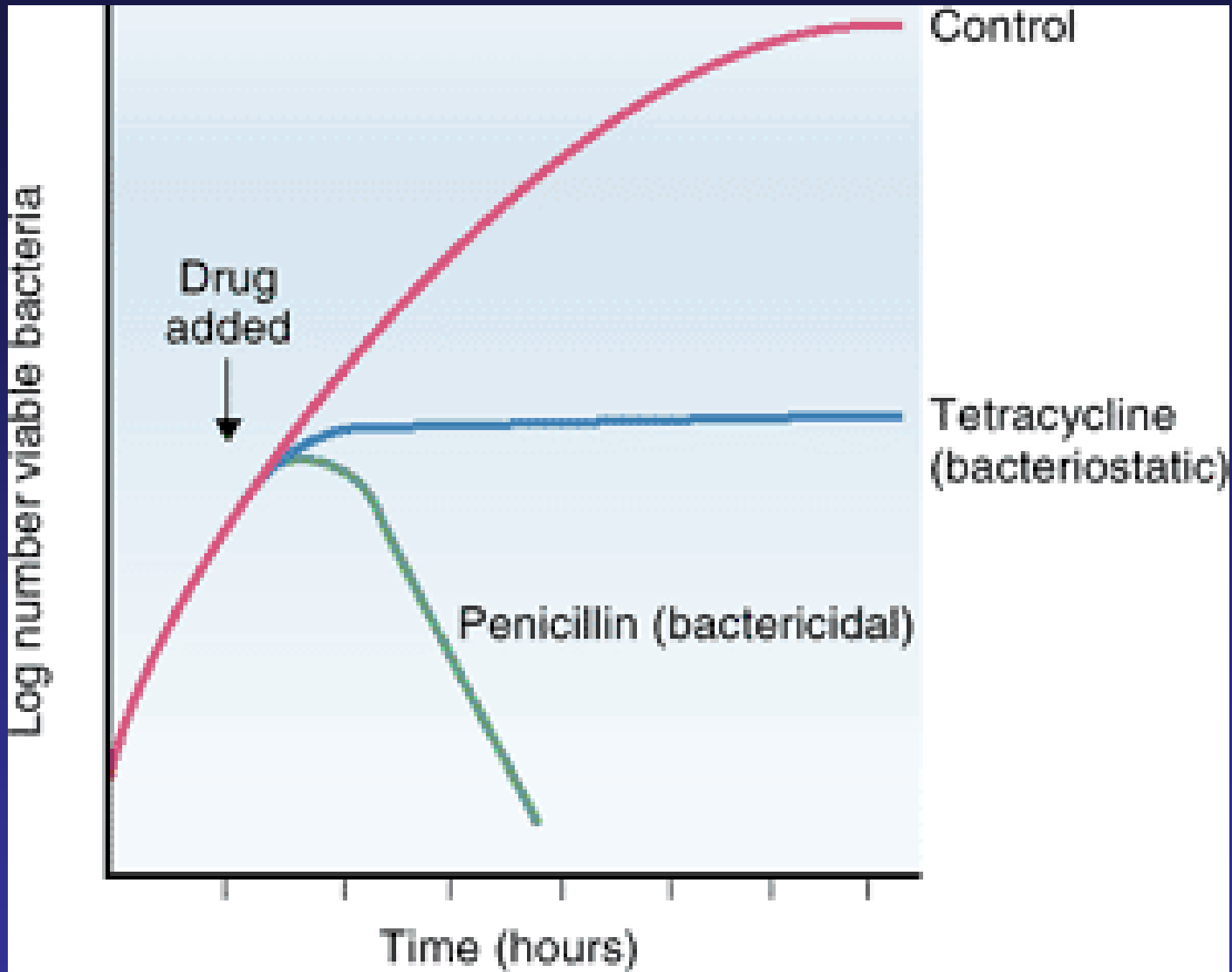
$p>$ promoter and direction of transcription

Antimicrobial chemotherapy

- **Selective toxicity**
 - toxic to bacteria, not bad for us
 - Selective toxicity based on **differences in physiology** between bacteria and us
 - **therapeutic index** = $\frac{\text{toxic dose to us}}{\text{therapeutic dose against bugs}}$
(high is good)
- **allergenicity**

Bactericidal vs. bacteriostatic

- **bactericidal - kills** bacteria (irreversible)
- **bacteriostatic - stops growth** (reversible)
- measured in lab as:
 - **Minimum Bactericidal Concentration (MBC)**
 - lowest dose for complete killing
 - **Minimum Inhibitory Concentration (MIC)**
 - lowest dose for stasis



Pharmacologic absorption and distribution in body

- oral vs. i.v.
- **penetration** to relevant site (e.g., blood brain barrier or inside of host cells)
- rate of **excretion**
- rate of **metabolism**

Broad spectrum vs. narrow spectrum

- broad good for unknown bacterial agent with serious effects
- narrow good for known bacterial agent

- Existing **antibiotic resistance** among the population
- **Combination** therapies
 - synergistic
 - antagonistic
- Other **practical considerations** for compliancy
 - **cost \$\$\$**
 - **frequency and length of administration**
 - for pediatrics - taste!

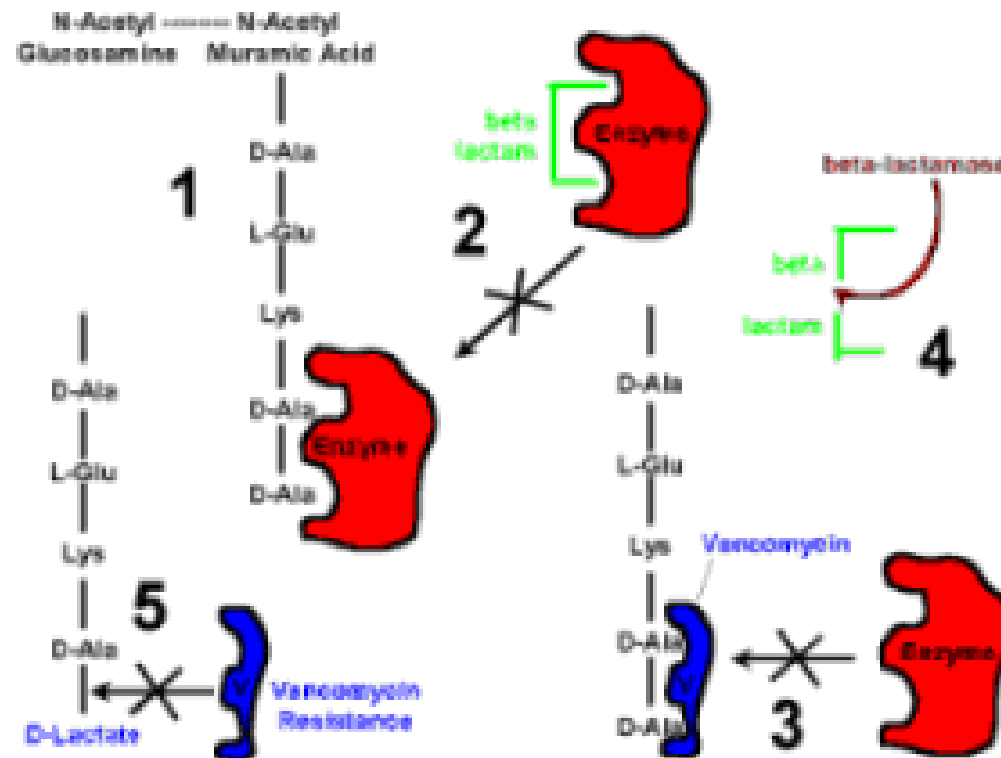
Summary of key antimicrobials

- **peptidoglycan**
 - **β -lactams**
 - **inhibit transpeptidation (D-Ala-D-Ala)**
 - **penicillins** (methicillin, amoxicillin)
 - **cephalosporins** (in third generation)
 - **carbapenems**
 - **monobactams** inhibit transpeptidation

peptidoglycan - continued

- **vancomycin** inhibits transpeptidation and transport (recognizes D-Ala-D-Ala, note difference with
- β -lactams)
- **bacitracin** transport of the subunits across membrane
- vancomycin and bacitracin are too big to fit through porins of gram-negative bacteria

Inhibition of peptidoglycan cross-linking by Beta-Lactams and Vancomycin and mechanisms of resistance.



1. Transpeptidase enzyme binds to D-Ala-D-Ala for cross-linking.
2. Beta-lactam antibiotic binds to transpeptidase inhibiting cross-linking.
3. Vancomycin binds to D-Ala-D-Ala preventing binding of enzyme.
4. Beta-lactamase cleaves beta-lactam antibiotic.
5. Changing terminal D-Ala to D-Lactate prevents vancomycin binding.

Membranes

- gram-negatives - **outer membrane** - **polymyxins** (similar to cationic detergents)
- gram-positives – **daptomycin** – K⁺ channels

Protein synthesis - ribosome

- **aminoglycosides**: gentamicin, kanamycin, neomycin, streptomycin
- **aminocyclitols**: spectinomycin
- **tetracyclines**
- **chloramphenicol**
- **macrolides**: erythromycin, azithromycin
- **clindamycin**: similar to macrolides
- **streptogramins** - similar to macrolides (Synercid)
- **oxazolidinones** (linezolid/ketolids)- synthetic, similar to macrolides, inhibits initiation of translation in gram-positives (Zyvox)
- **inhibit tRNA synthesis** (isoleucine) - **mupirocin**, topical

DNA synthesis

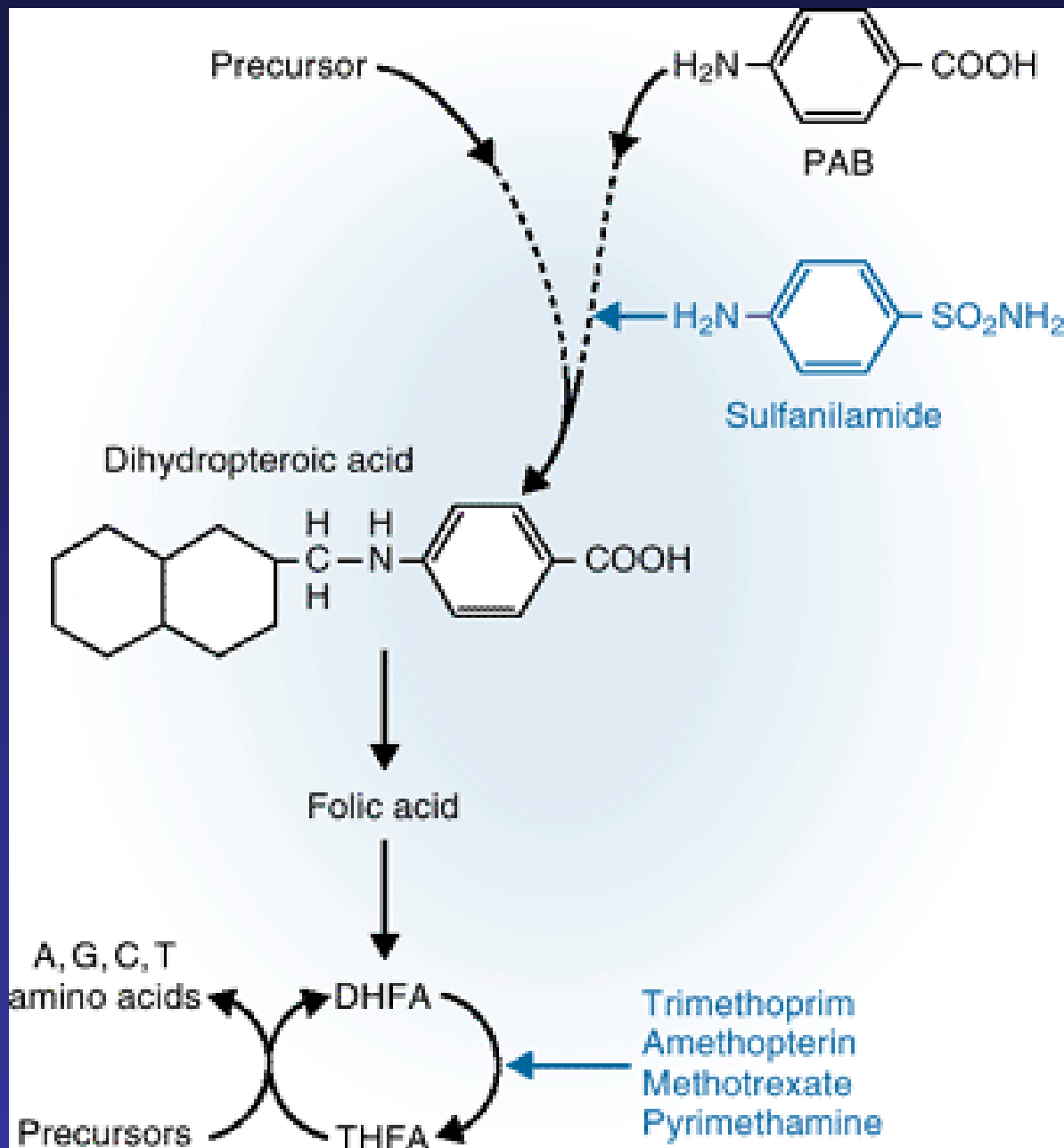
- **quinolones** inhibit gyrase (supercoiling)
 - **nalidixic acid, ciprofloxacin**
- **metronidazole** disrupts DNA after **reduction** in
 - **anaerobes** (and some protozoans)

RNA synthesis

- **rifampin** (rifampicin, rifamycin)

Metabolic inhibitors - folic acid inhibitors

- **sulfonamides** - PABA analogs block dihydropteroate synthetase (we don't have this)
- **trimethoprim** blocks dihydrofolate reductase (ours is less sensitive)
- **dapsone** (acid fast)



Other **acid-fast** antibiotics

- **isoniazid** - inhibits **mycolic acid** production
- **ethambutol** - inhibits **arabinogalactan** production
- **pyrazinamide** - active in acidic environment (phagolysosome), mechanism unknown

Inhibitors of antibiotic resistance function

- **clavulanic acid** inhibits β -lactamase
- used in conjunction with amoxicillin (**Augmentin**)

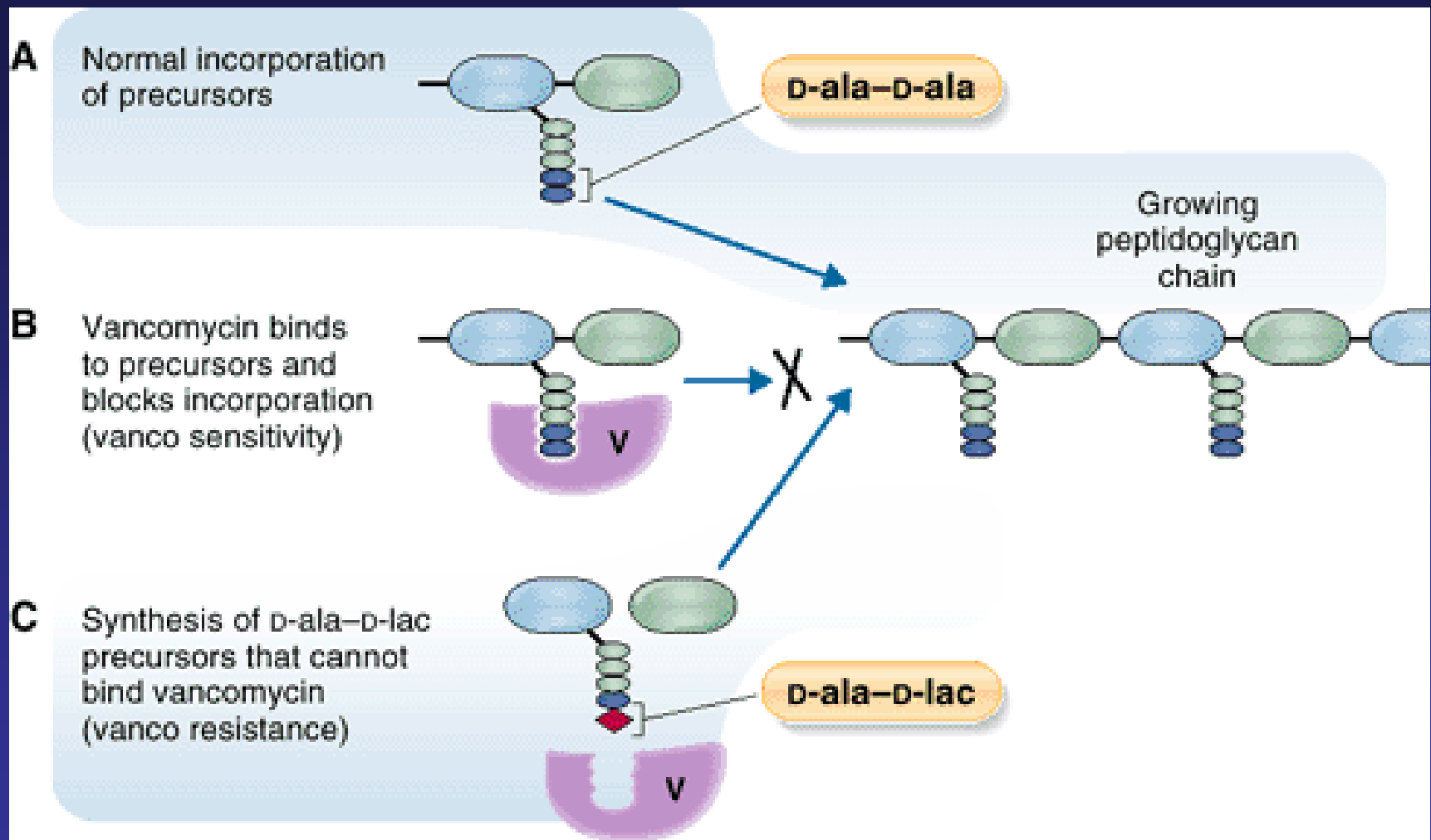
Antibiotic resistance

- Some bacteria are already resistant to all usable antibiotics.
- Proper and rational use of antimicrobials can help with the problem.
 - indiscriminate prescription and use
 - agricultural use

General mechanisms of resistance

- **enzymatically modify or degrade** the antibiotic
 - β -lactamase
 - chloramphenicol acetyl transferase
 - aminoglycoside phosphotransferase

- **alter the target of the antibiotic**
 - **spontaneous mutation** most common mechanism here (retain original function)
 - **enzymatic modification** – methylation of rRNA
 - **new biosynthetic pathway** yielding altered target (vancomycin)
 - acquire **new enzymes** that are resistant
 - methicillin-resistant *Staphylococcus aureus* - permanent change in genome - **penicillin-binding proteins**
 - **plasmid-encoded** enzymes - sulfa drugs



- **change flux** of antibiotic
 - **pump** the antibiotic out of the cell (tetracycline resistance, multiple antibiotic resistance (MAR))
 - **decreased uptake** - more specific pores - multiple antibiotic resistance (MAR)
- **innate** resistance
 - permeability barrier of **gram-negatives**
 - **lack of peptidoglycan** in *Mycoplasma*

Genetics of resistance (plasmid vs. chromosomal)

- antibiotic resistance **plasmid**:
 - new enzyme to modify/pump antibiotic
 - new enzyme to modify target
 - new biosynthetic pathway or target
 - (MRSA is an exception)
- chromosomal **point mutation**
 - target changed
 - not recognized by antibiotic
 - retains cellular function

R plasmids

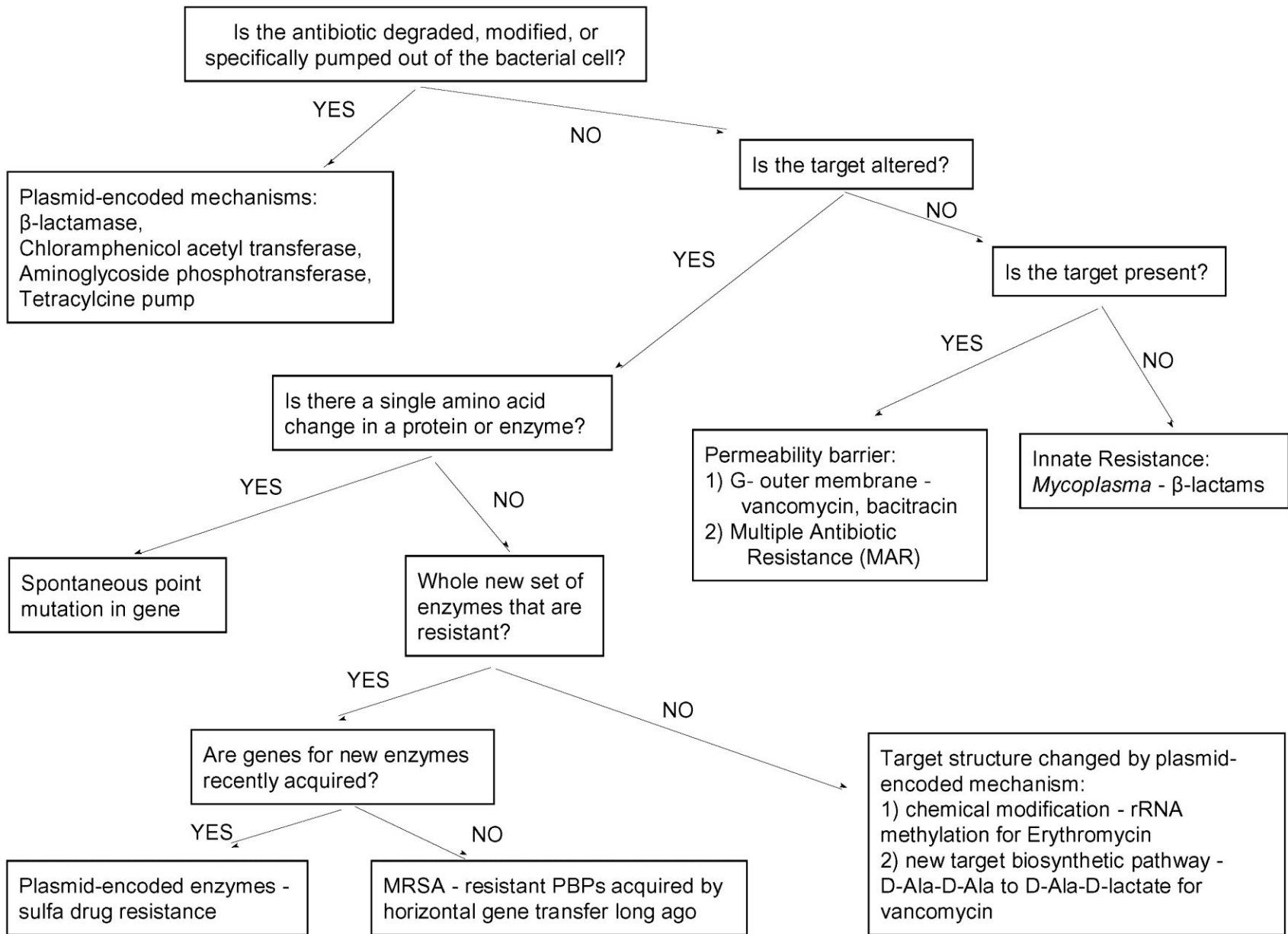
- **multiple resistances**
- **rapidly transferred to diverse bacteria**
- **Transposons**

Spontaneous point mutations

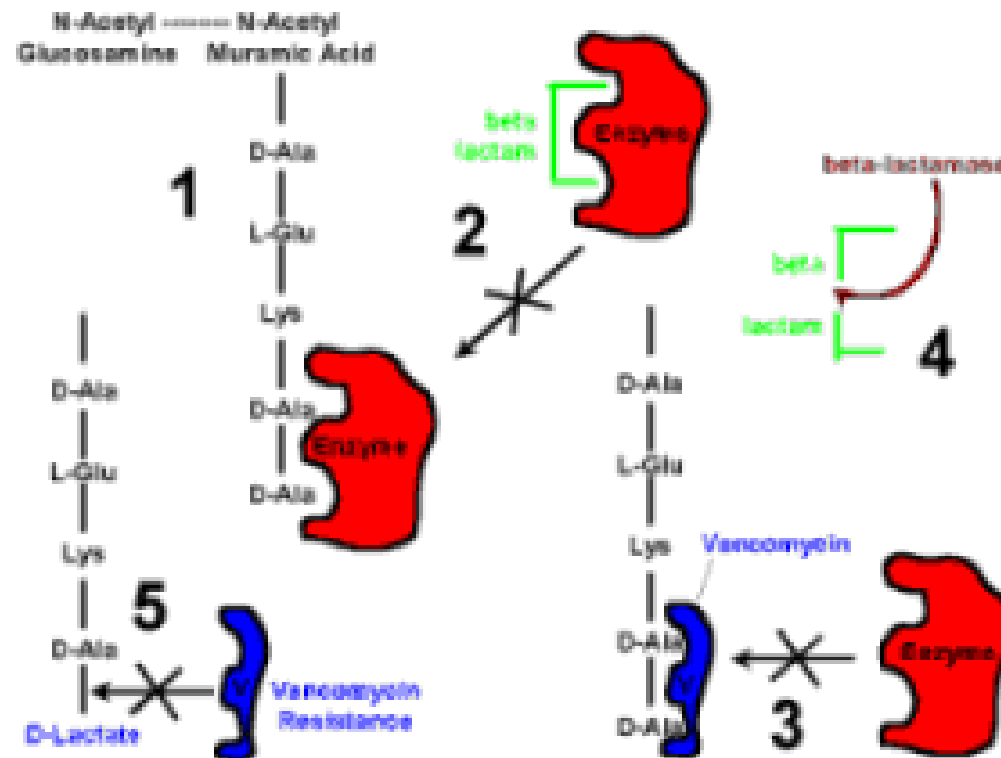
- less concern
- less spread, **resistant strain must spread**
- problem in hospital setting
- **Think about mutation rate and bacterial population size in patient (TB).**

Penicillin resistance of *Streptococcus pneumoniae*

- β -lactamase
- Exception - spontaneous mutations accumulating resistance in **penicillin-binding proteins**
- (contrast with MRSA)



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