Bacterial genetics

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Bacterial genetics

- Introduction to:

1: Mutations

2: Genetic exchange

The bacterial genome:

- **chromosome**
- **plasmids**
- **bacteriophage**
- **insertion sequences and transposons**

haploid - one copy of chromosome

- **Mutation:**

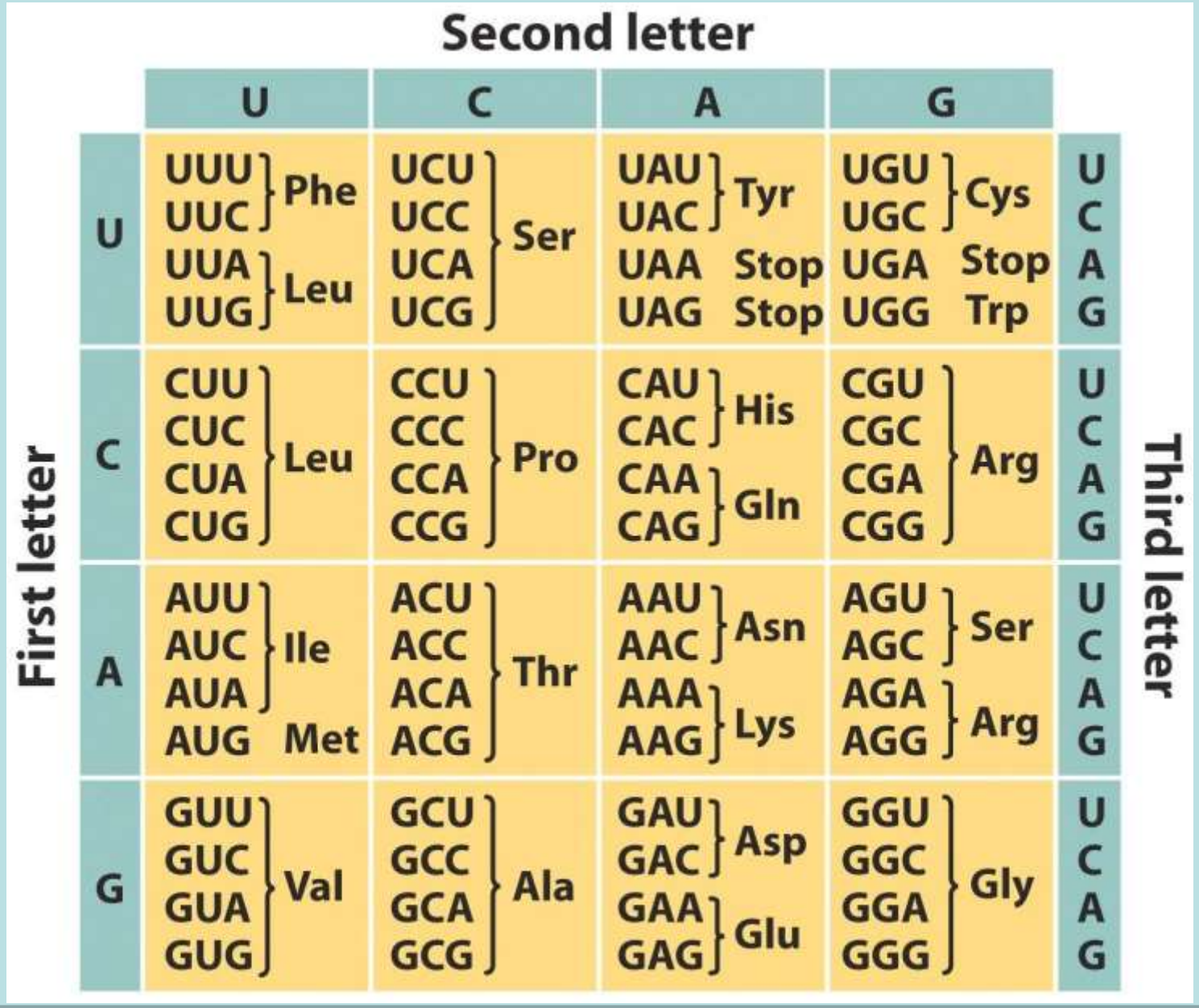
- Change in the base sequence of DNA
- Happen all of the time, regardless of growth conditions: Spontaneous, pressure..

- **Effects of genotype on phenotype**

i. Silent

ii. Loss of function

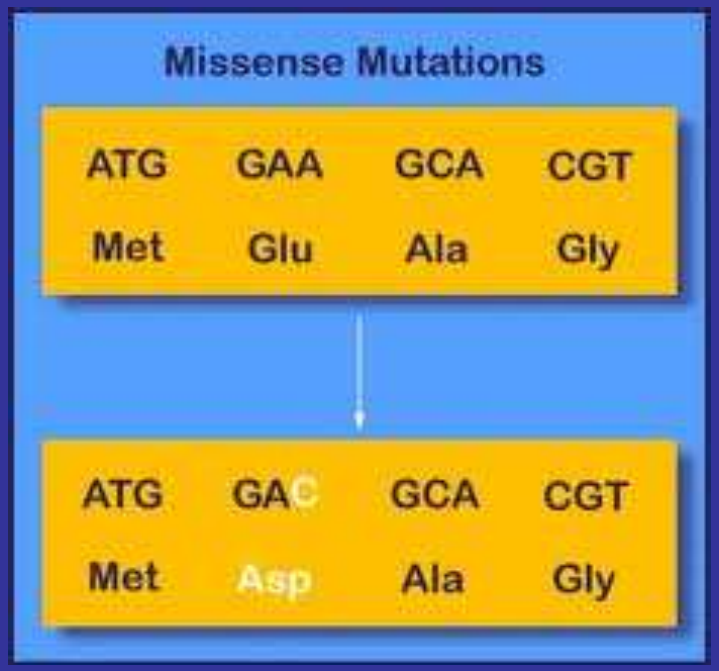
iii. Altered function


Second letter
U
C
A
G
First letter
Third letter
U
UUU Phe
UUC
UUA Leu
UUG
UCU
UCC
UCA Ser
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
U C A G
C
CUU
CUC
CUA Leu
CUG
CCU
CCC
CCA Pro
CCG
CAU His
CAC
CAA Gln
CAG
CGU
CGC
CGA Arg
CGG
U C A G
A
AUU
AUC Ile
AUA
AUG Met
ACU
ACC
ACA Thr
ACG
AAU Asn
AAC
AAA Lys
AAG
AGU Ser
AGC
AGA Arg
AGG
U C A G
G
GUU
GUC
GUA Val
GUG
GCU
GCC
GCA Ala
GCG
GAU Asp
GAC
GAA Glu
GAG
GGU
GGC
GGA Gly
GGG
U C A G

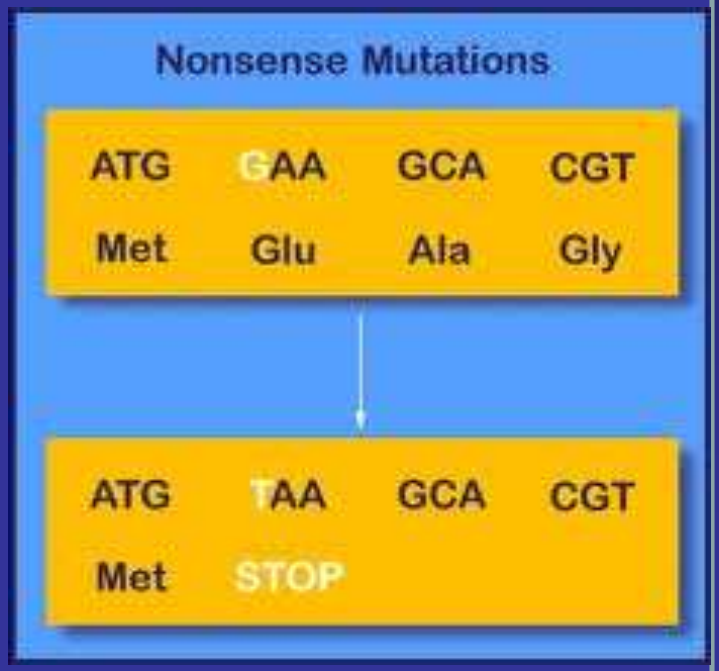
- Types of mutation:

1. base substitution:

- DNA polymerase error or due to mutagens
- Missense vs nonsense mutations



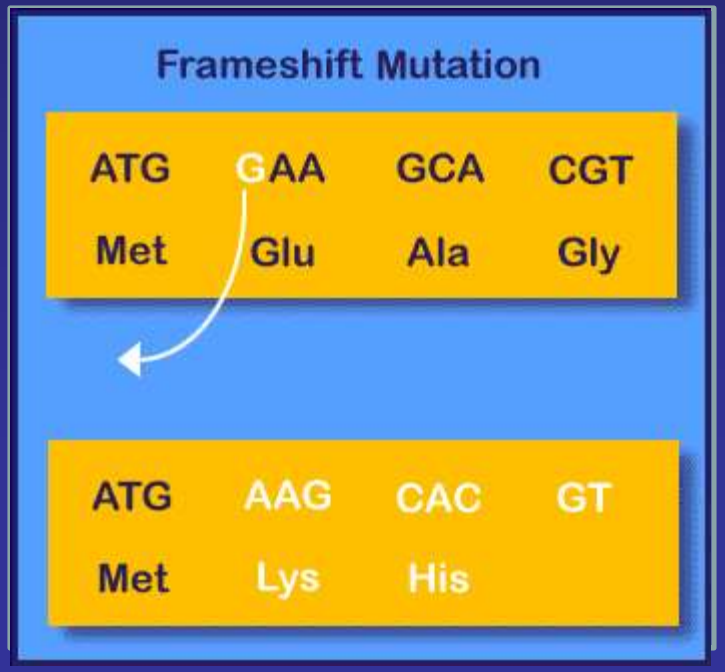
Mis-sense mutation



Non-sense mutation

2. Frame shift mutation:

- One or more base are added or deleted
- Shift in the reading frame
- Corrupting the reading codons downstream mutations leading to inactive protein



- **2. Genetic exchange:**

1. **Importance**

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

2. **Mechanisms**

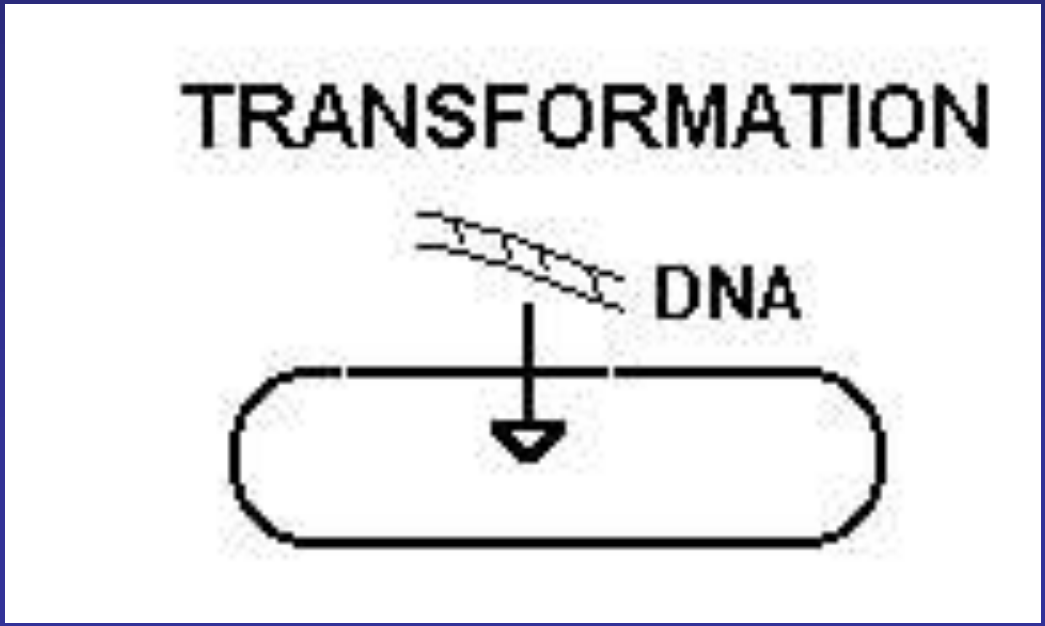
a. transformation - uptake of naked DNA
b. transduction - bacteriophage as vectors
c. conjugation - plasmids moved by cell-cell contact
d. Transposons

1. Transformation

a. recipient cell must be **competent for uptake of DNA**

b. **natural competence** versus **artificial competence**

c. only **certain bacteria** are naturally transformable - ***Streptococcus pneumoniae, Haemophilus influenzae, Neisseria gonorrhoeae, Vibrio***



2. Transduction

- **bacteriophage (phage)**: viruses infect bacteria - can be either **lytic** or **temperate (Lysogenic)**

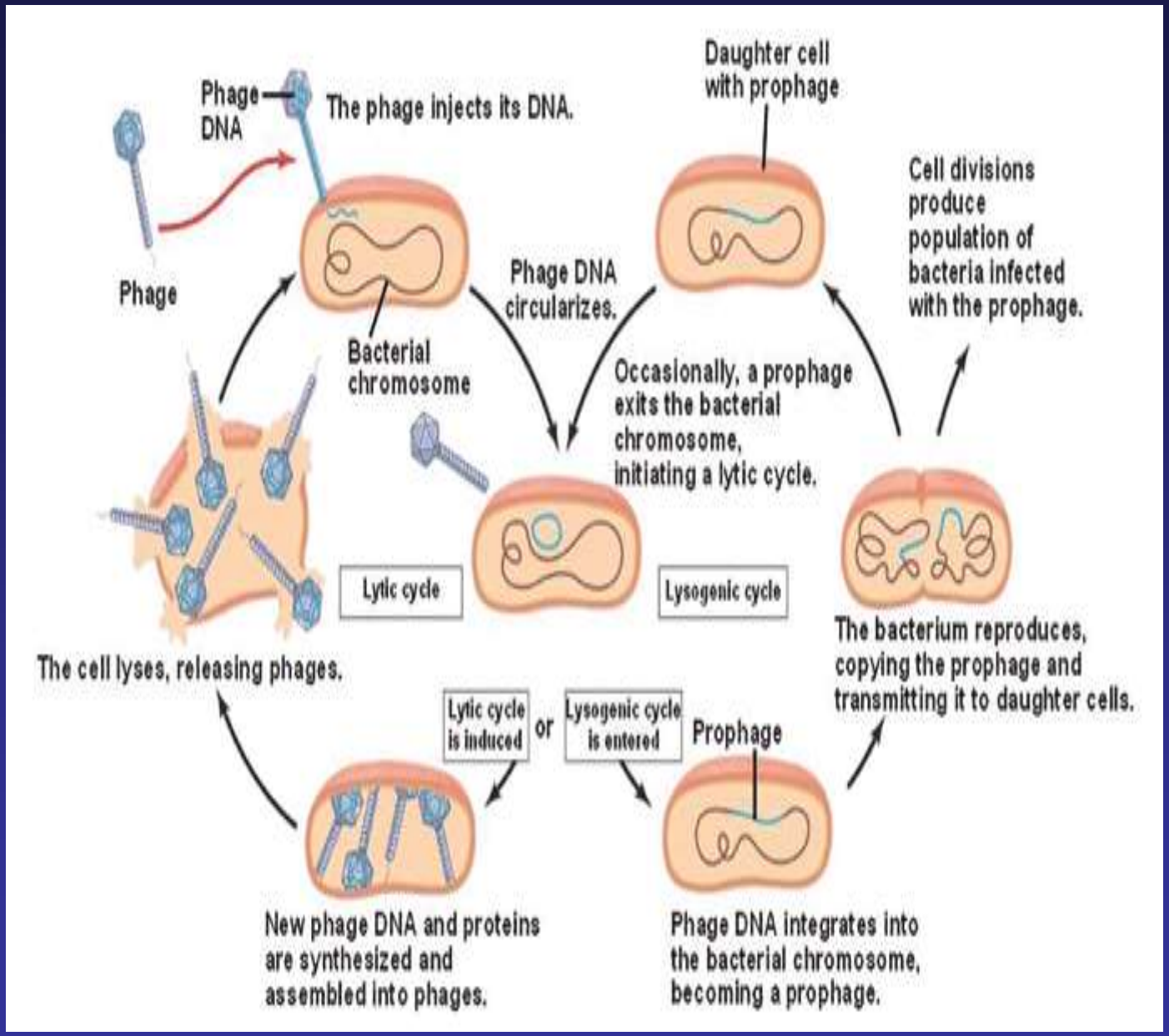
i. **lytic** - always lyse (kill) host bacterial cell

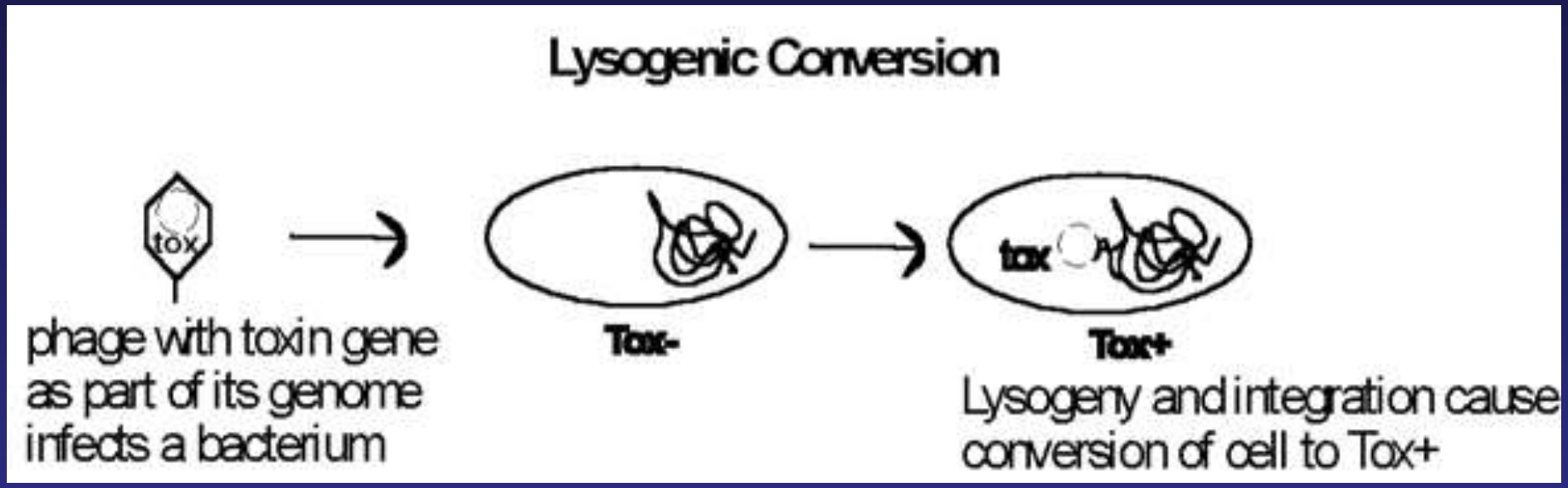
ii. **temperate** - can stably infect and coexist within bacterial cell (**lysogeny**) until a **lytic phase** is induced

- **lysogeny**

i. the phage genome during lysogeny is called the **prophage**, and the bacterial cell is called a **lysogen**

ii. if the phage genome encodes an **observable function**, the lysogen will be altered in its phenotype - **lysogenic conversion (e.g., diphtheria toxin in *Corynebacterium diphtheriae*)**


Phage
Phage DNA
The phage injects its DNA.
Bacterial chromosome
Phage DNA circularizes.
Daughter cell with prophage
Cell divisions produce population of bacteria infected with the prophage.
Occasionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.
Lytic cycle
Lysogenic cycle
The cell lyses, releasing phages.
The bacterium reproduces, copying the prophage and transmitting it to daughter cells.
Lytic cycle is induced
or
Lysogenic cycle is entered
Prophage
New phage DNA and proteins are synthesized and assembled into phages.
Phage DNA integrates into the bacterial chromosome, becoming a prophage.



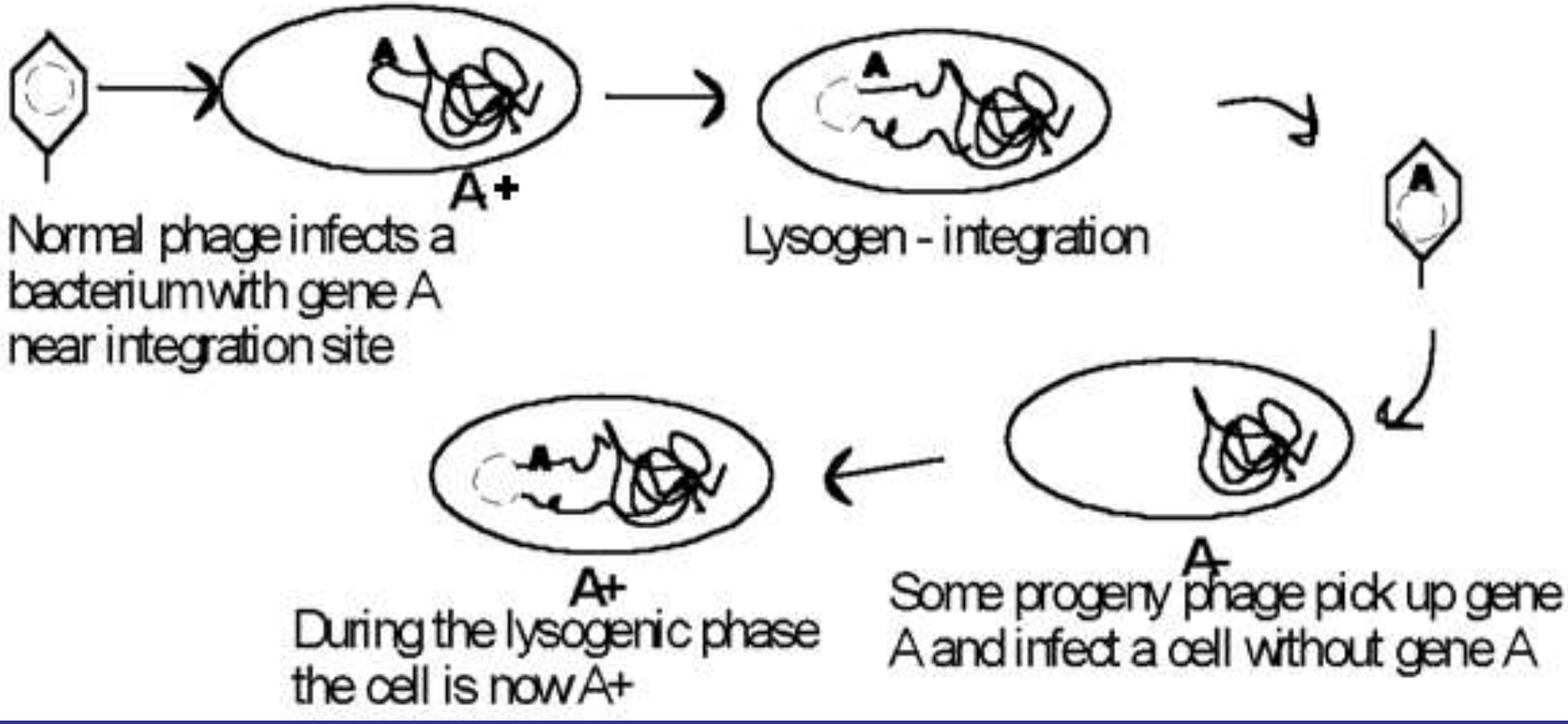
Diphtheria, Cholera, botulinum and erythrogenic toxins

A. specialized transduction

i. some prophages **integrate** into the bacterial genome at a specific location

ii. 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**

Specialized Transduction



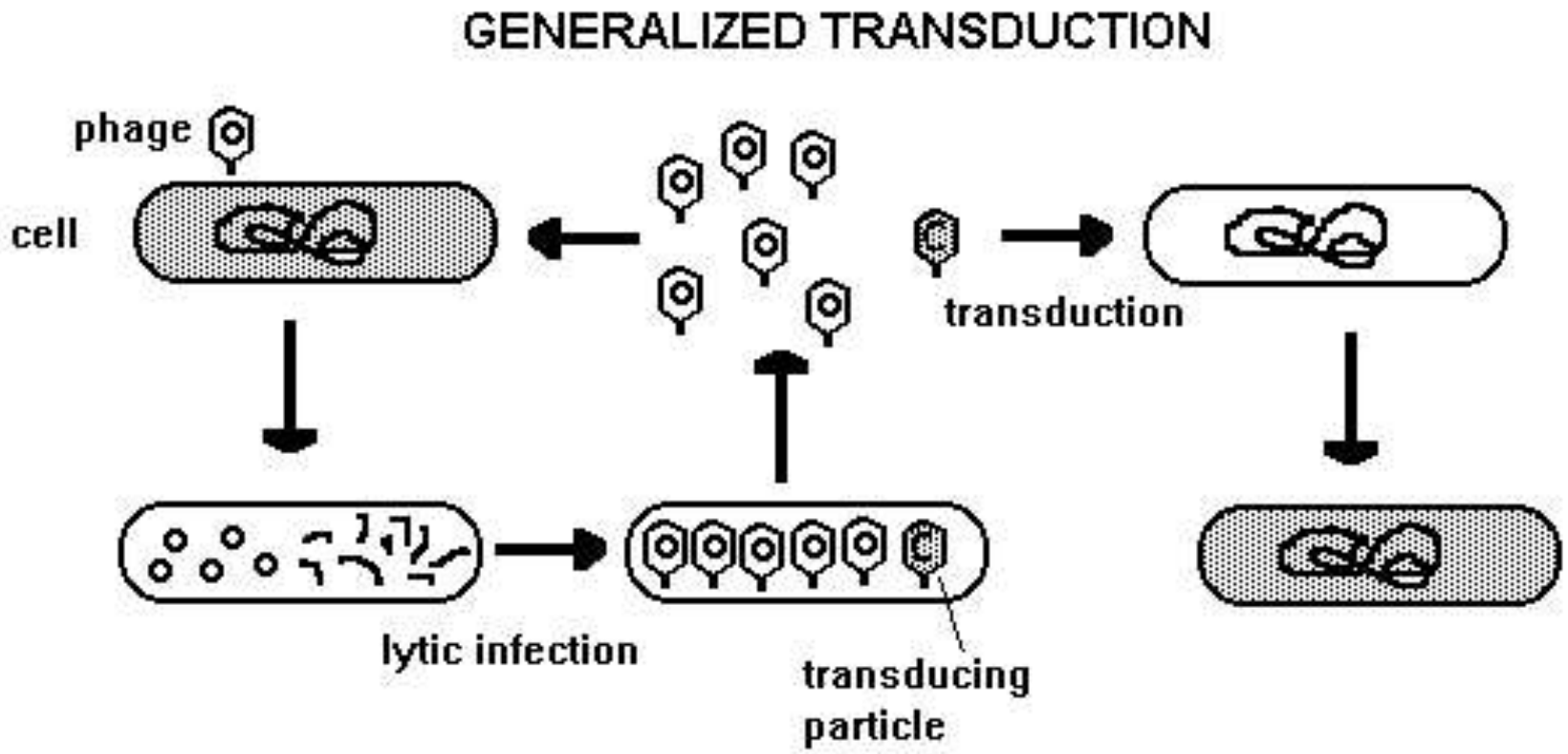
B. generalized transduction

i. when a phage lyses the host bacterial cell, it normally **packages phage genome** into the capsid

ii. sometimes the capsid is **accidentally** filled with random pieces of **bacterial genome**, possibly including plasmids

iii. when the capsid injects the host genes into a new recipient, the new gene can **recombine** into the recipient genome and cause a change

iv. **virulence and antibiotic resistance genes** can be moved by generalized transduction


GENERALIZED TRANSDUCTION
phage
cell
transduction
lytic infection
transducing particle

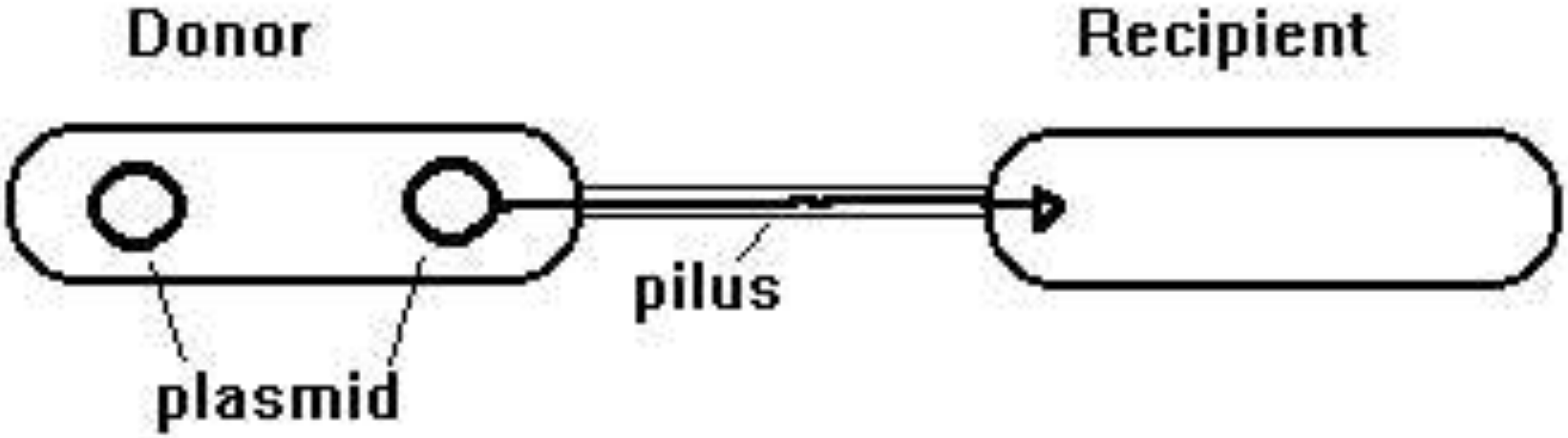
3. Conjugation

Conjugation process:

i. synthesis of sex **pilus**
ii. **cell to cell contact** via pilus
iii. **copying plasmid DNA and transfer** of copy into recipient cell

Importance of conjugation:
Moving plasmids encoding multiple antibiotic resistance genes (R plasmids) among diverse bacterial

CONJUGATION



Antimicrobials

Main Contents:

1. Introduction and history

2. Different classes of antibiotics and its Mechanism of action

3. Basic principles on usage

4. Resistance

Antibacterial therapy

- Antimicrobial chemotherapy
- What is an Antibiotics?

- Egyptians 1500BC: Honey for wounds
- Alexander Fleming and Louis Pasteur

Antibacterial therapy

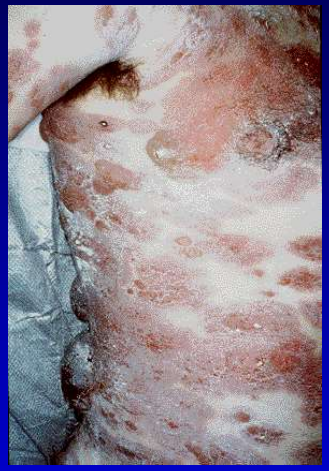
- 2000 B.C. - "Here, eat this root."
- 1000 B.C. - "That root is heathen, say this prayer."
- 1850 A.D. - "That prayer is superstition, drink this potion."
- 1940 A.D. - "That potion is snake oil, swallow this pill."
- 2000 A.D. - " That pill or antibiotic is ineffective. Here, eat this root."
  ~Author Unknown

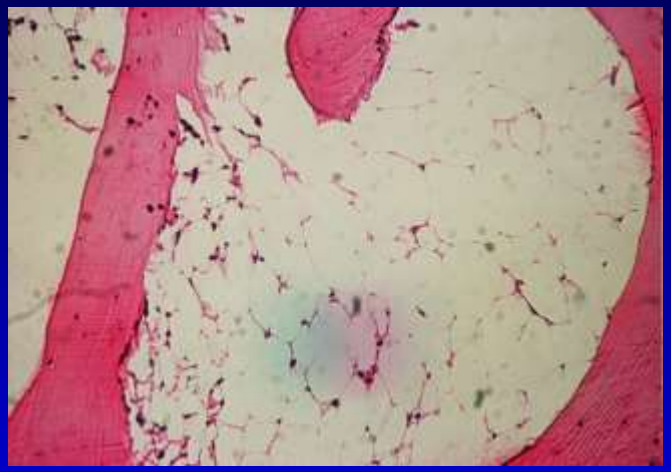
The Bright side

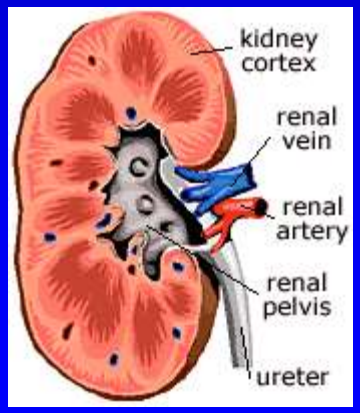
Yet …. even life savers may

- take life
- (remember! Antibiotics are DANGEROUS DRUGS!!)

Because antibiotics are DANGEROUS DRUGS







C deathicille (difficile)

- A UK Consultant Microbiologists nightmare !

Antibacterial therapy

> **Antibiotics**: natural products derived from soil bacteria and fungi

Examples:

Penicillin from penicillin notatum mould (Alexander Fleming)

> Semisynthetic agents:

Natural compounds that have been chemically modified to increase its activity and improve pharmacokinetics

Examples:

Cephalosporins and Carbapenems.

Antibacterial therapy

Synthetic chemicals:

Trimethoprim and linezolid, quinolones are examples

Antibiotics are loosely applied to all antibacterial agent

- **Terms related to antibiotics use:**

- Synergism
- Broad *vs* narrow spectrum
- Emperical use?
- Selective toxicity
- Static *vs* cidal (MIC *vs* MLC)

Antibacterial therapy

Basic principles:

- Selective toxicity:

-Kill or inhibit the growth of microorganism without harming human tissue.

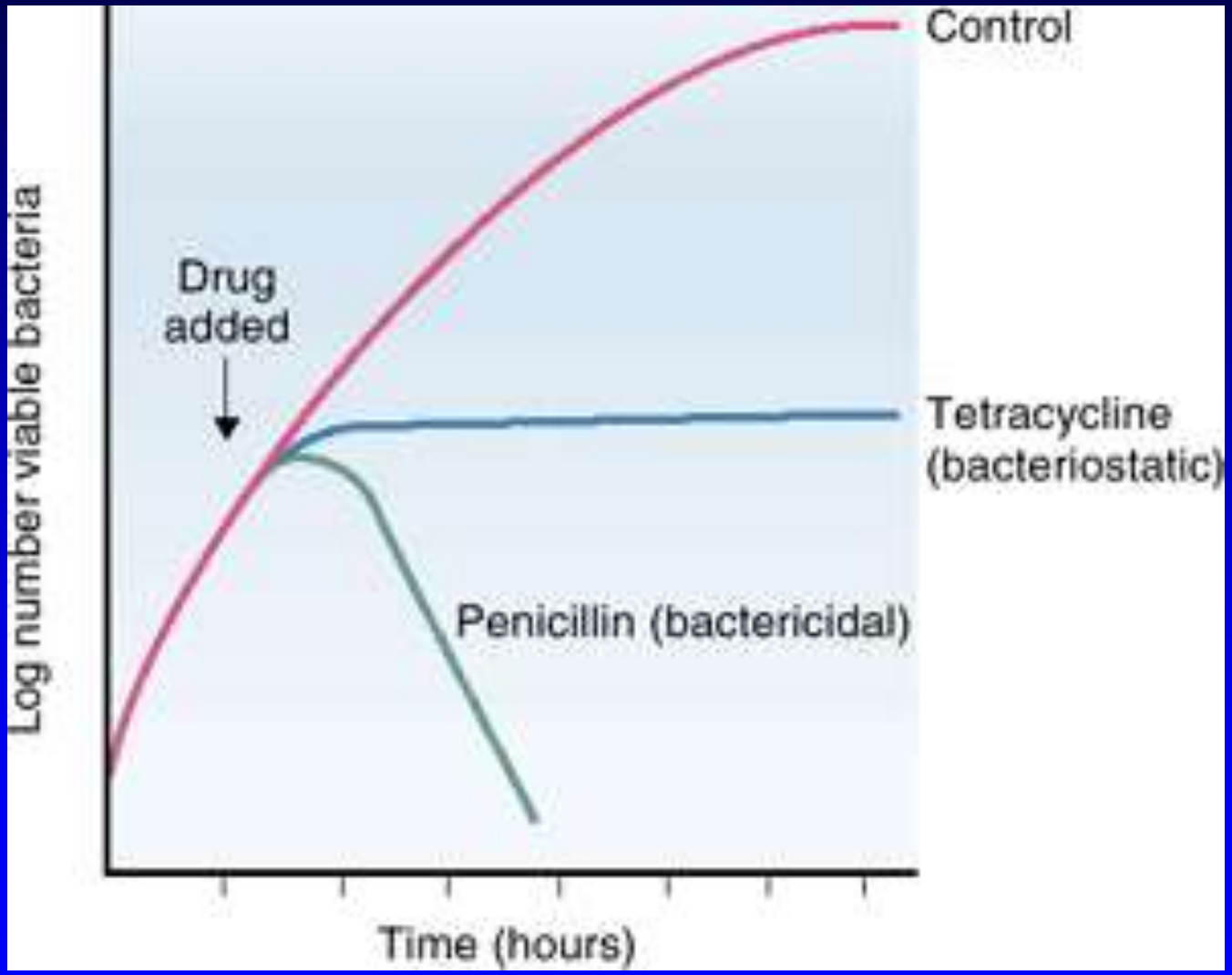
- Bactericidal versus bacteriostatic **FIGURE 1**

-Bactericidal: minimum lethal concentration (MLC)

-Bacteriostatic: minimum inhibitory concentration(MIC

- Some infections such as infective endocarditis or immunocompromised patients > Bactericidal is a must

Antibacterial therapy Figure 1



Antibacterial therapy/

Indications for use / to avoid abuse:

1. Treat infections empirically / culture sensitivity.

2. Prophylaxis/ limited situations.

Abuse:

Side effects, Resistance, Cost – effectiveness

Precautions:

>History of hypersensitivity

>Impaired liver and kidney functions

>Pregnancy, breastfeeding and children

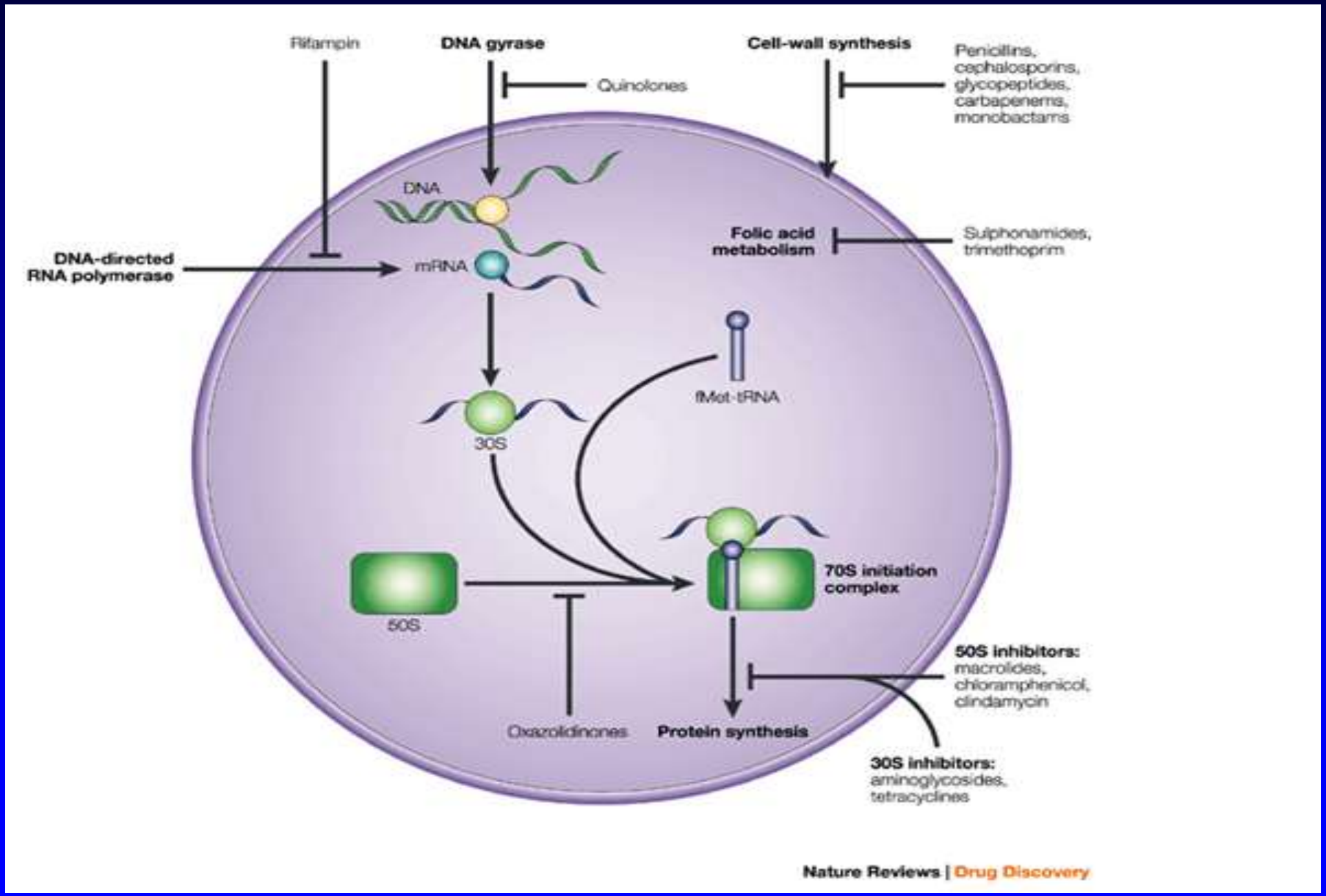
Antibacterial therapy

Target of antibacterial agents: Figure 2:

- Cell wall: Peptidoglycan?
- Protein synthesis: Ribosome 70S versus 80S
- Folate synthesis:

  Bacteria manufacture its own folates while human obtain it in food
- Nucleic acid synthesis
- Other sites such as bacterial cell membrane

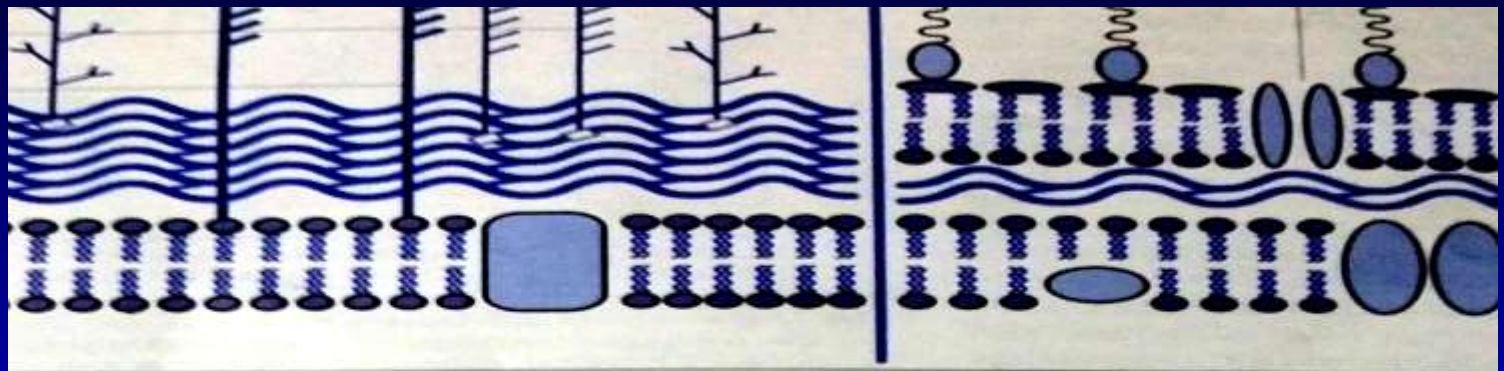
Antibacterial therapy Figure 2/ Antibiotics target



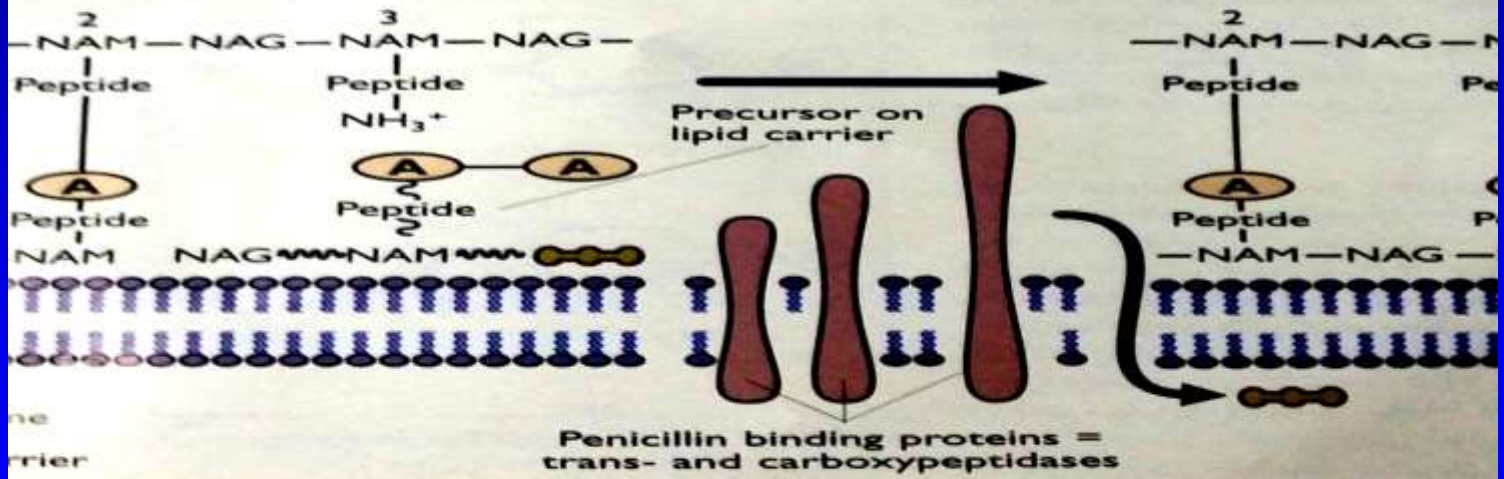
1- Antibacterial therapy/Inhibition of cell wall synthesis

- Inhibition of cell wall synthesis / Cell ruptures → Microbe death

- Eg. Penicillins cephalosporins, vancomycin and bacitracin



ell wall of gram-positive and gram-negative bacteria.

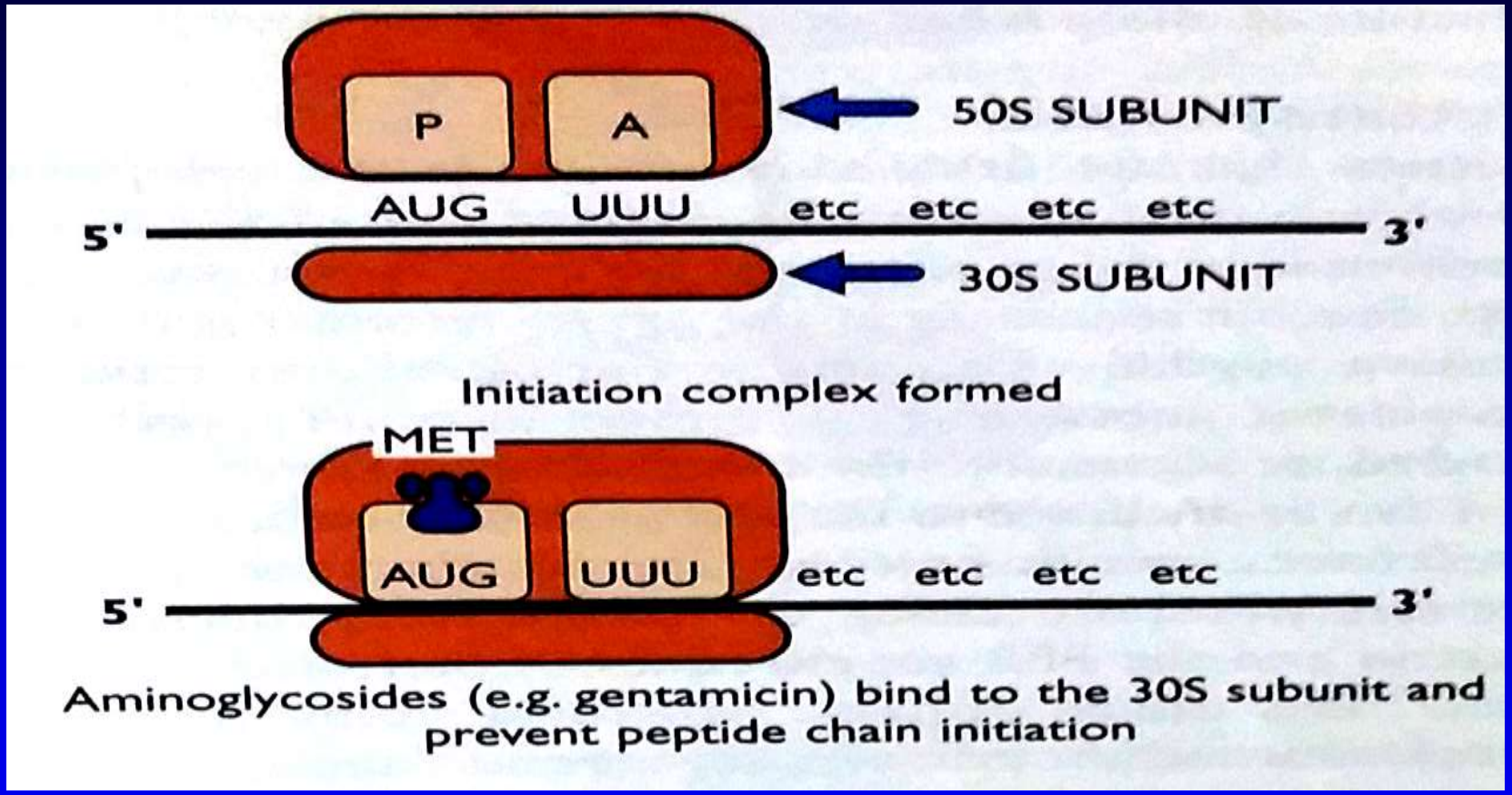


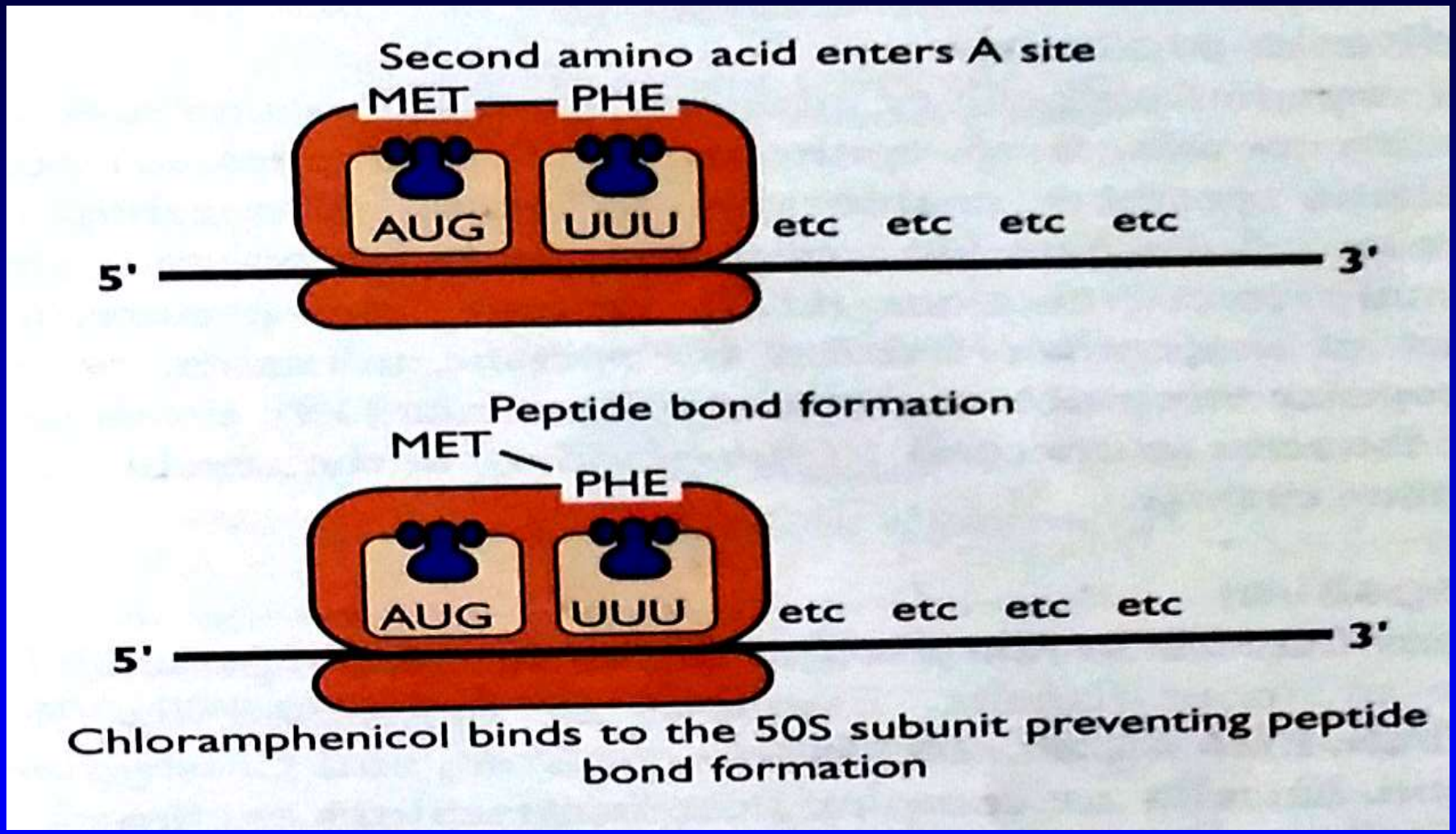
of repeating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid
teins (PBP) are responsible for cross-linking these peptide side chains.

2. Antibacterial therapy / inhibition of microbial protein synthesis

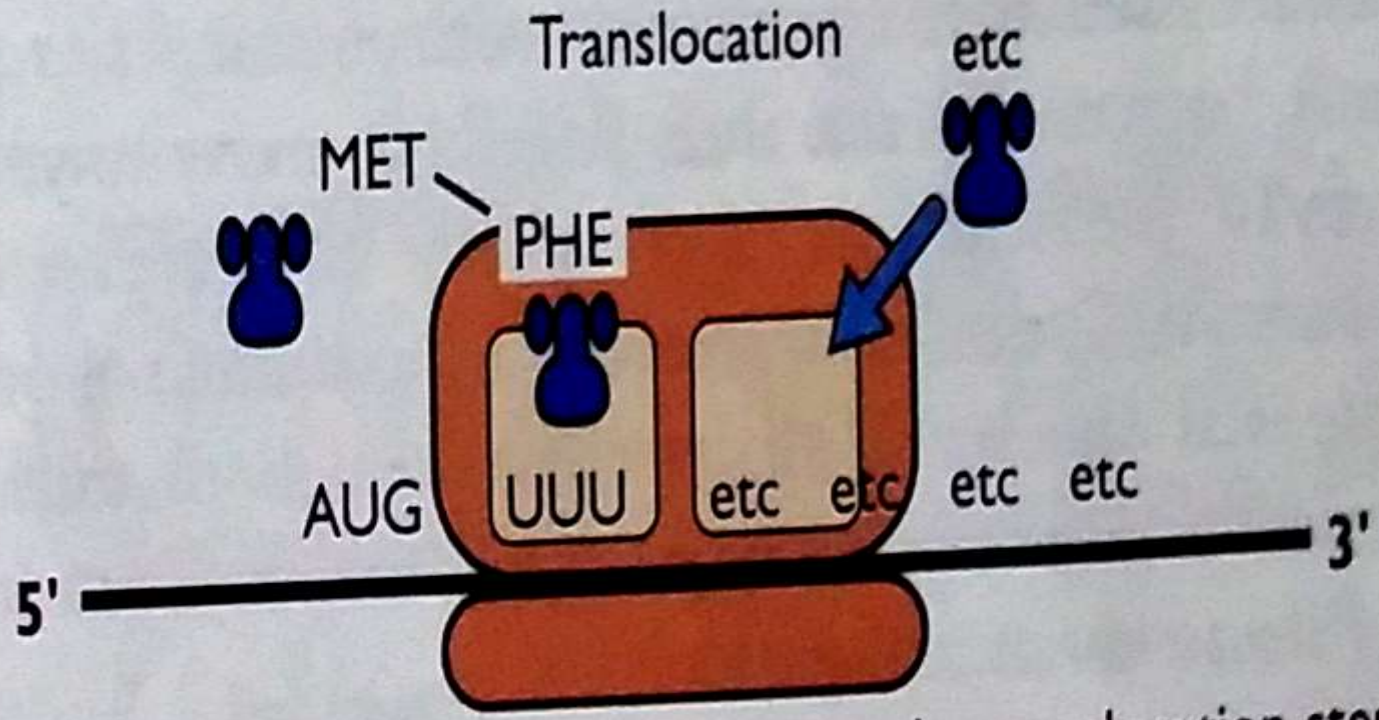
- Act at site of protein synthesis (ribosome):

- Aminoglycosides (cidal)
- Tetracyclines (static)
- Macrolides (static), e.g erythromycin
- Chloramphenicol (static)
- Clindamycin (static).

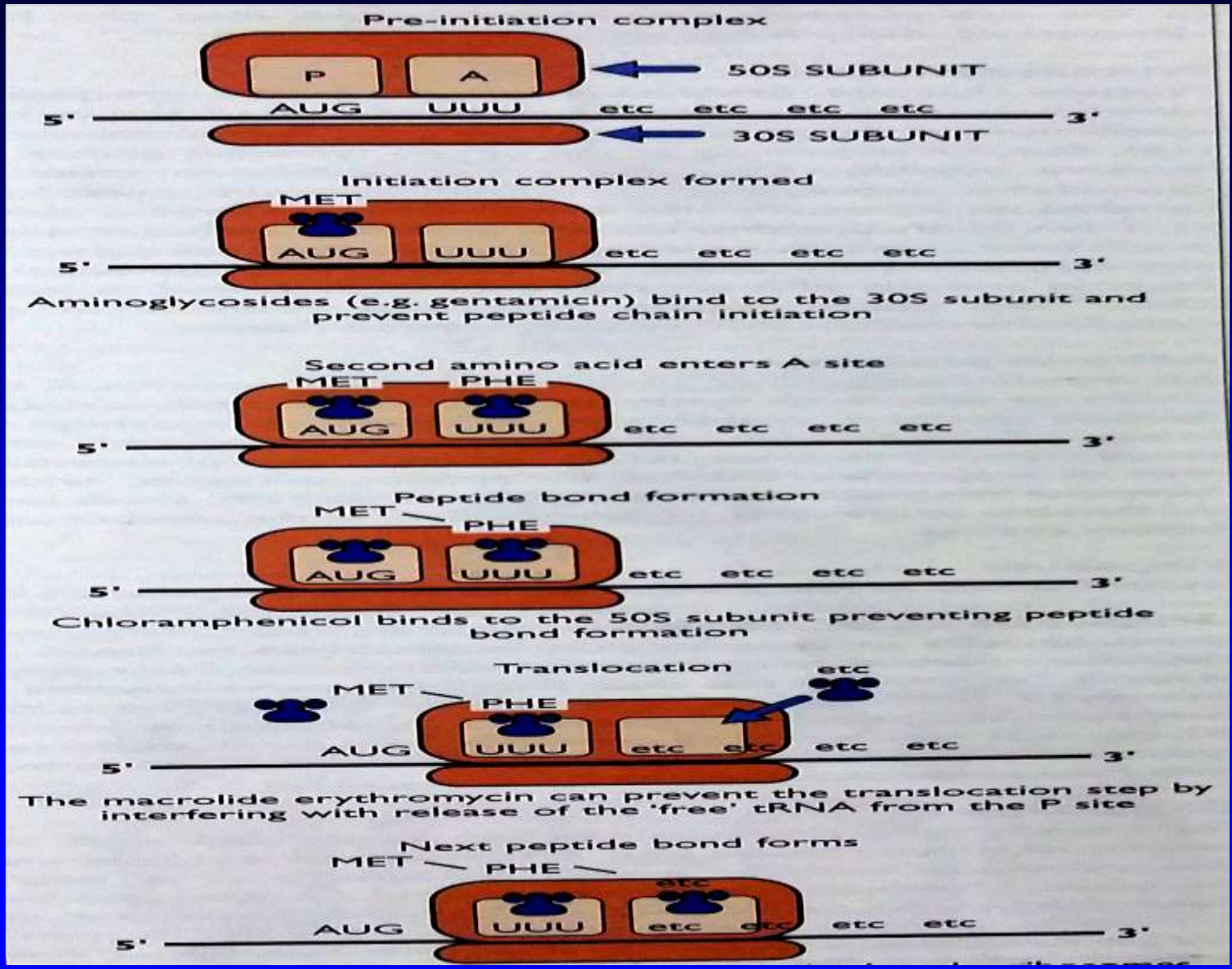

P
A
50S SUBUNIT
AUG
UUU
etc etc etc etc
5'
3'
30S SUBUNIT
Initiation complex formed
MET
AUG
UUU
etc etc etc etc
5'
3'
Aminoglycosides (e.g. gentamicin) bind to the 30S subunit and prevent peptide chain initiation



Chloramphenicol binds to the 50S subunit preventing peptide bond formation

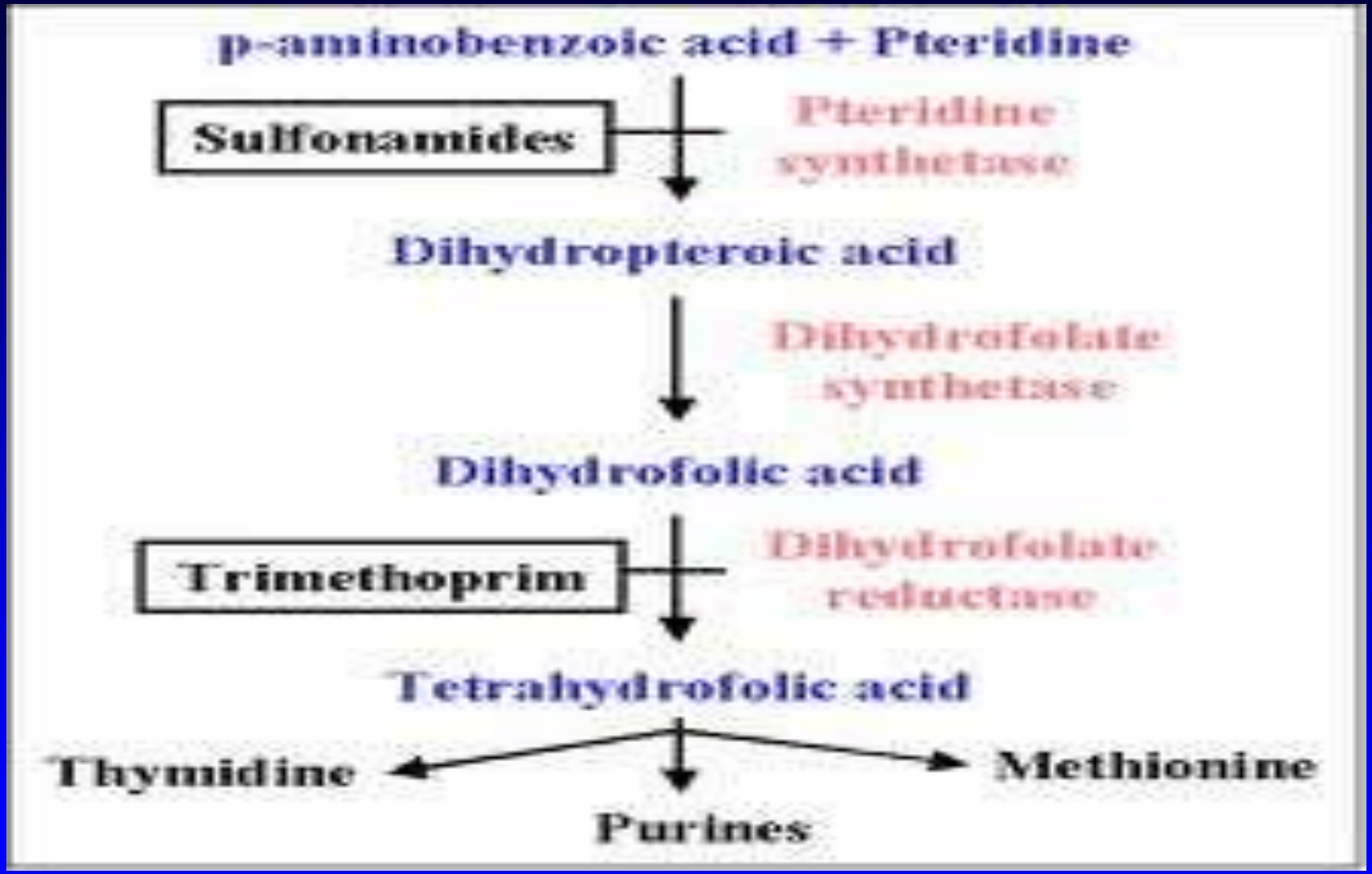


The macrolide erythromycin can prevent the translocation step by interfering with release of the 'free' tRNA from the P site


Pre-initiation complex
P
A
50S SUBUNIT
AUG
UUU
etc etc etc etc
5'
3'
30S SUBUNIT
Initiation complex formed
MET
AUG
UUU
etc etc etc etc
5'
3'
Aminoglycosides (e.g. gentamicin) bind to the 30S subunit and prevent peptide chain initiation
Second amino acid enters A site
MET
PHE
AUG
UUU
etc etc etc etc
5'
3'
Peptide bond formation
MET
PHE
AUG
UUU
etc etc etc etc
5'
3'
Chloramphenicol binds to the 50S subunit preventing peptide bond formation
Translocation
etc
MET
PHE
AUG
UUU
etc etc
etc etc
5'
3'
The macrolide erythromycin can prevent the translocation step by interfering with release of the 'free' tRNA from the P site
Next peptide bond forms
MET
PHE
etc
AUG
UUU
etc etc
etc etc
5'
3'

3. Antibacterial therapy/Inhibition of folates synthesis

- Eg Sulphonamides, Trimethoprim (static)
- Available as combination, Co-trimoxazole, or separately


p-aminobenzoic acid + Pteridine
Sulfonamides
Pteridine synthetase
Dihydropteroic acid
Dihydrofolate synthetase
Dihydrofolic acid
Trimethoprim
Dihydrofolate reductase
Tetrahydrofolic acid
Thymidine
Purines
Methionine

4. Antibacterial therapy/Inhibition of nucleic acid synthesis and function

- **A. INHIBITORS OF RNA SYNTHESIS AND FUNCTION**

Rifampicin (bactericidal)

- a. Mode of action

  These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of mRNA synthesis

- b. Spectrum of activity

  They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis and MRSA

  c. Combination therapy

  Since resistance is common, rifampin is usually used in combination therapy

Antibacterial therapy/Inhibition of nucleic acid synthesis and function

- **b. Inhibitors of DNA synthesis and function**

  Quinolones - nalidixic acid, ciprofloxacin, oxolinic acid (bactericidal)

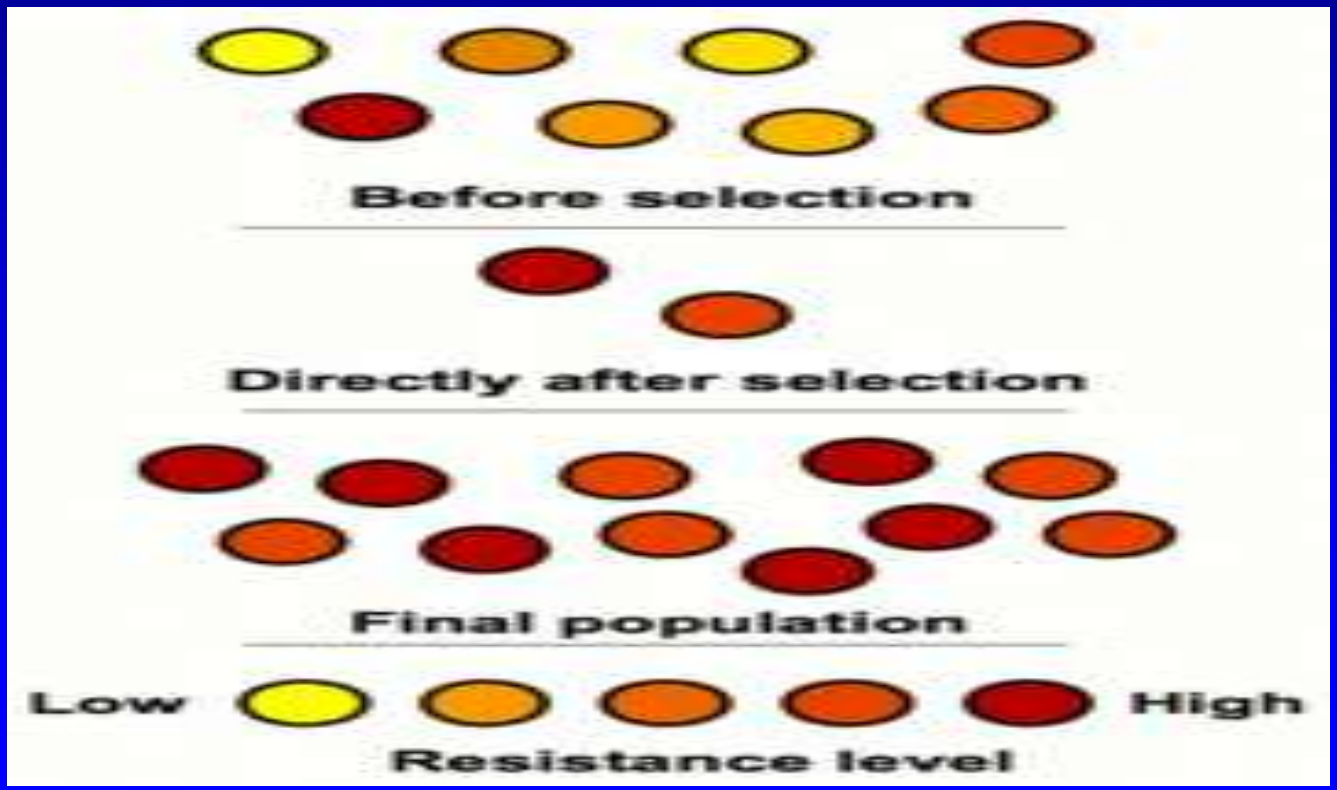
- Mode of action

  These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

Antibacterial therapy

Resistance: meaning?

-The resistance is initially emerged by genetic process then selected by antibiotics



Extra wrinkle here

- Doctors/nurse precribers are not the only culprits!!!
- > examples of R bugs due to agricultural overuse/misuse??
- NB the food chain!!

Antibacterial therapy

Mechanism or resistance: FIGURE 4

1. Decreased accumulation:

Decreased permeability secondary to porins mutations

Increased efflux (pumping out the antibacterial using expressed efflux pump)

Antibacterial therapy

2. **Modification of the target:**

- Sequence mutation leading to target alteration

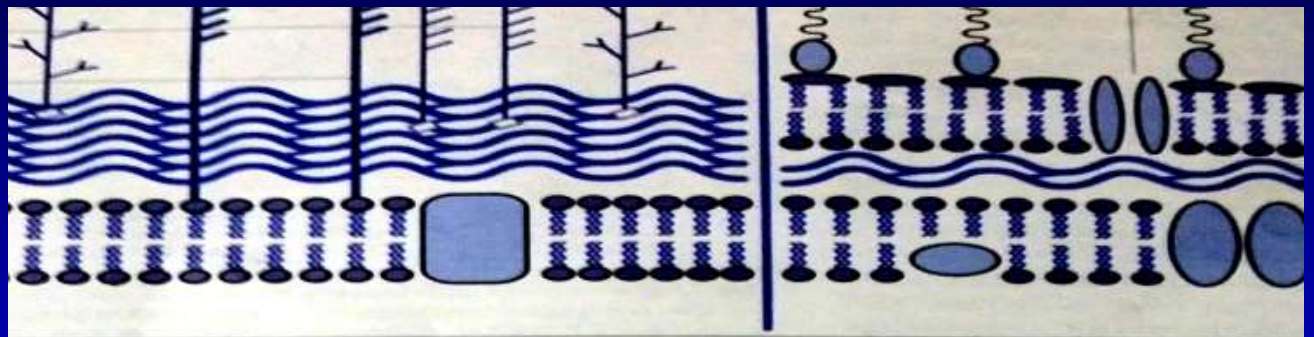
e.g in pneumocoocus resistance to penicillins >

- Target bypass:

Supplementary enzymes will do the same target function but without binding to the antibacterial agent e.g Meticillin resistant staph aureus MRSA (PBP2 coded by mec A gene)

- Target hyperproduction:

More drug is needed to inactivate the target



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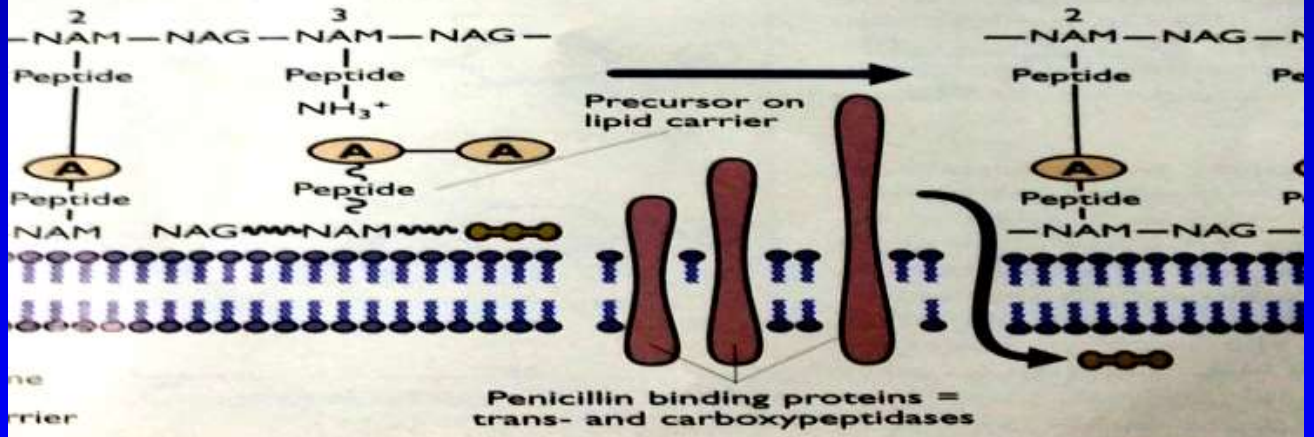


of repeating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid
teins (PBP) are responsible for cross-linking these peptide side chains.

Antibacterial therapy

3. Inactivation of the antibacterial agent:

- β lactamase is an enzyme produced by the bacteria

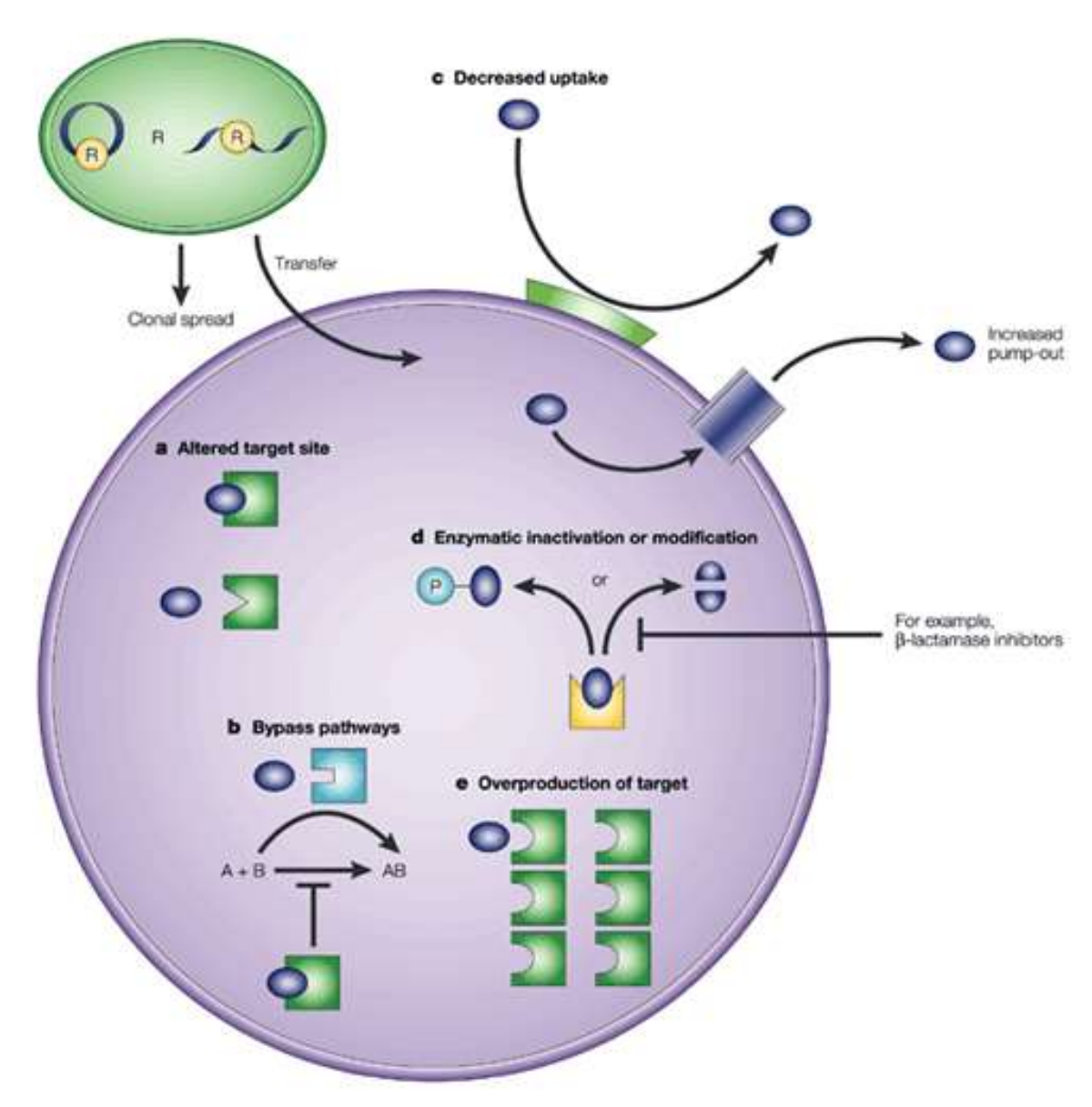
This enzyme will destroy the β- lactam ring (this is an essential ring in penicillins and cephalosporins) leading to inactivation of the antibacterial agent

- Some types of bacteria produce a β- lactamase with a wide range of activity (ESBLs)

Acetylating, adenylating and phosphorylating enzymes:

Produced by bacteria (gram negative bacteria) and cause resistance to aminoglycosides and chloramphenicol

Antibacterial therapy / Figure 4



The End