Introduction to Bacteria

Classification, Morphology and Structures

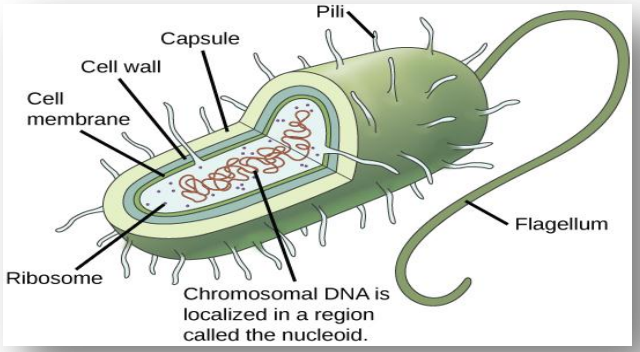
Introduction

- Microbial taxonomy
- Identification methods
  - Characteristics for microbial classification
    - Phenotypic
    - Genotypic

Bacterial Structure

A. The envelope:

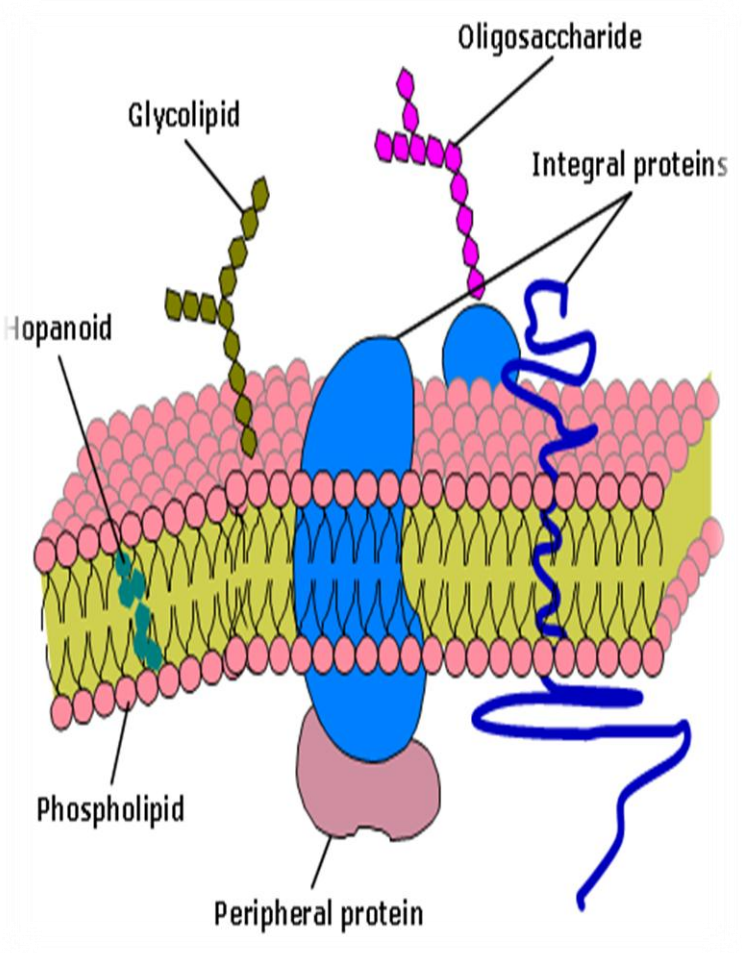
1. Cytoplasmic membrane
2. Cell wall (Peptidoglycan)
3. Extracellular polysaccharides: capsules, microcapsules and loose slime
4. Appendages
5. Antigenic variation



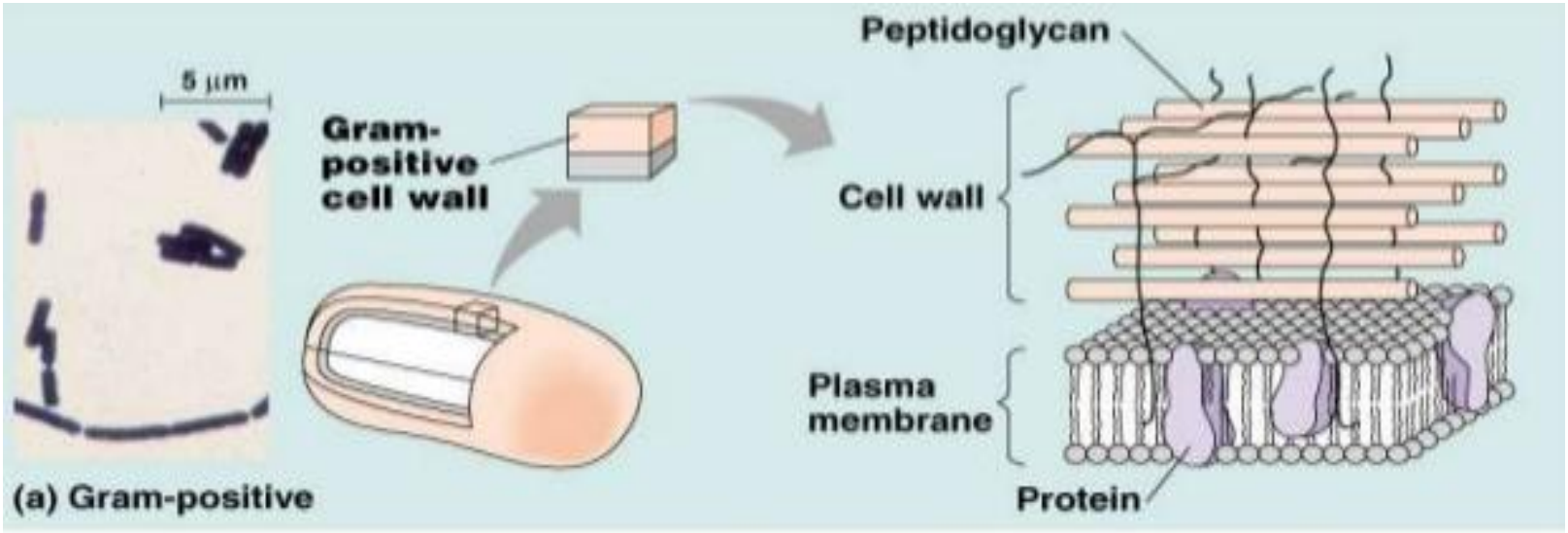
B. Cytoplasmic components

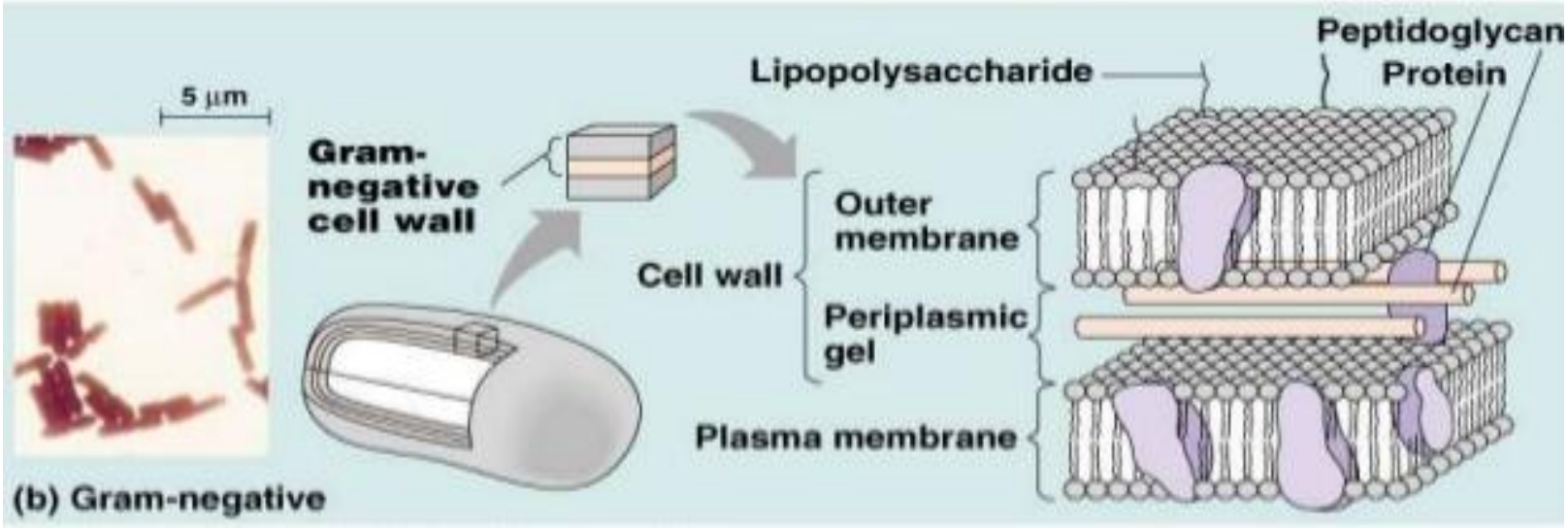
Bacterial Structure

1. **Cytoplasmic membrane**
2. **Cell wall**



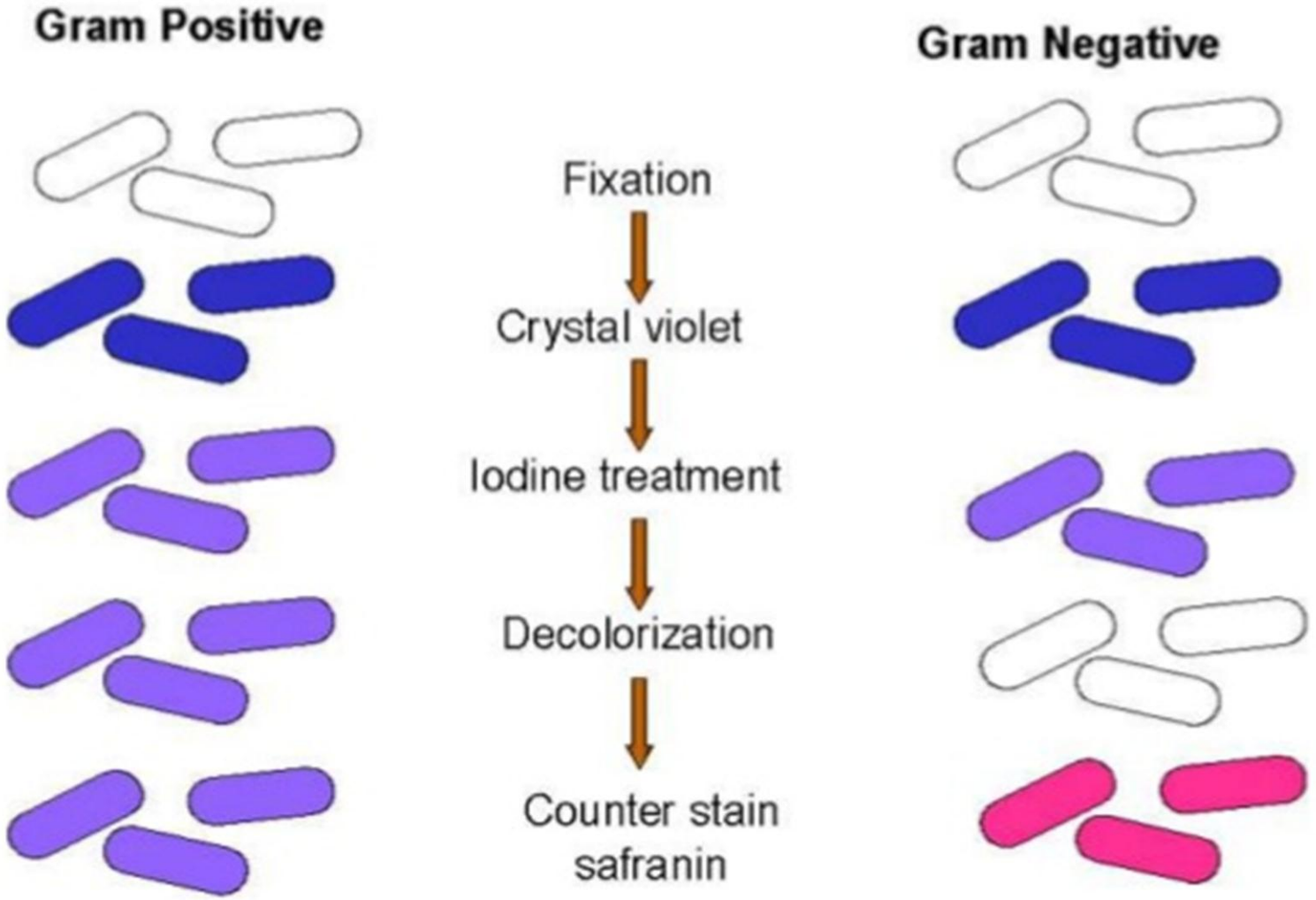
Bacterial Structure





Characteristic	Gram-negative Bacteria	Gram-positive Bacteria
Wall Structure	They have a thin lipopolysaccharide exterior cell wall.	The peptidoglycan layer is thick
Effect of Dye	do not retain the crystal violet dye, and react only with a counter-stain, generally stain pink.	retain the crystal violet dye, and change into purple during staining identification.
Effect of Antibiotics	• resistant to penicillin • contain an endotoxin called LPS	susceptible to the enzyme lysozyme and to penicillin
Flagellum	If present, the flagellum has four supporting rings, namely 'L' ring, 'P' ring, 'M' ring, and 'S' ring.	The flagellum has two supporting rings, in the peptidoglycan layer, and in the plasma membrane.
Teichoic Acids	absent.	present.
Liproproteins	They are attached to the polysaccharide backbone.	absent.
Periplasmic Space	present.	absent.

Bacterial Structure



Bacterial Structure

3. **Extracellular polysaccharides:**
   - **Capsules**
   - **Microcapsules**
   - **Loose slime**

4. **Appendages**
   - **Flagella**
   - **Pili**

5. **Antigenic variation**
   - important in virulence & immunity

Bacterial Structure

2. Cytoplasmic components:

1. Cytoplasm
2. Nuclear material (nucleoid or nuclear body)
3. Ribosomes
4. Cytoplasmic inclusion

SPORES AND SPORULATION

- Highly resistant resting stages formed during adverse environment (depletion of nutrients)
- Formed inside the parent cell, hence called Endospores
- Very resistant to heat, radiation and drying and can remain dormant for hundreds of years
- This makes them difficult to kill during sterilization

– **Spore germination**

SPORES AND SPORULATION

- **Medical significance of sporulation**
  - Bacillus species including anthracis (anthrax) and cereus (endotoxin causes ~5% of food poisoning)
  - Clostridium species including tetani (tetanus), perfringens (gangrene), and botulinum (botulism: food poisoning from improperly canned food)

Bacterial classification

- Cell wall
- Staining characteristic
- Ability to grow in the presence of O_2
- Ability to form spores
- Ability to form filamentous
- Diseases related organisms

Bacterial classification

A. Rigid, thick-walled cells

1. Free-living

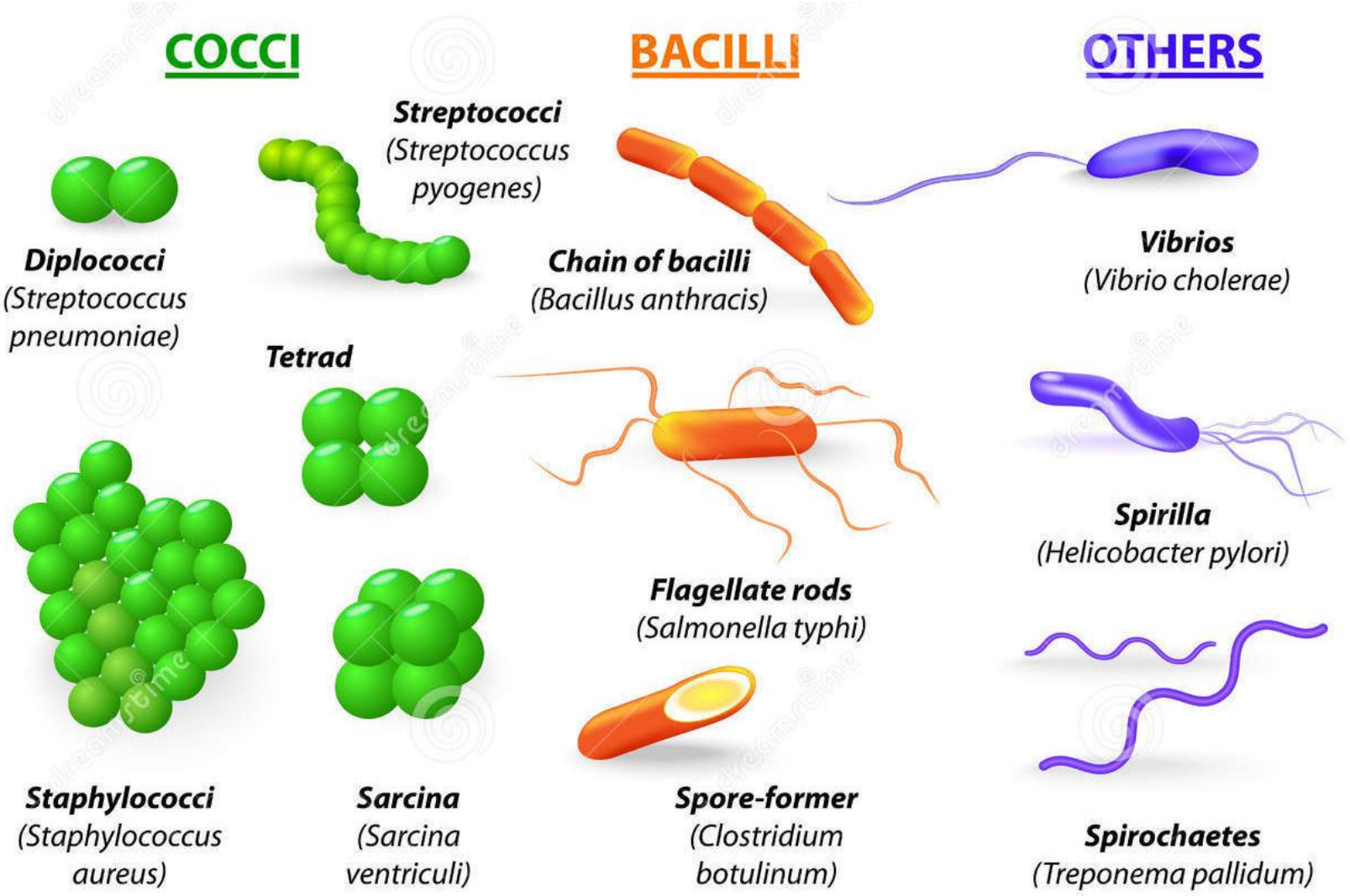
- Gram +
- Gram –
- Acid-fast

2. Non-free-living

B. Flexible, thin-walled cells

C. Wall-less cells

Bacterial Morphology



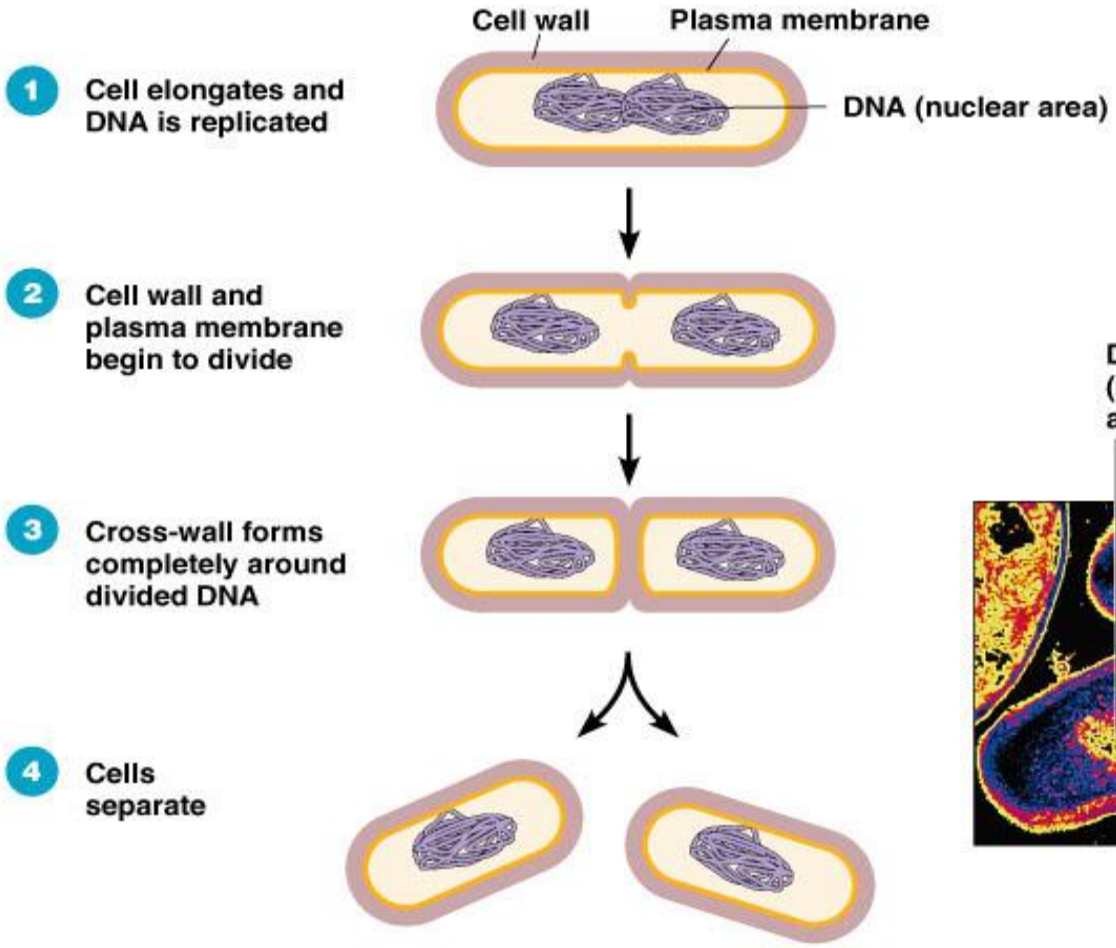
Microbial Growth requirements

Microbial growth

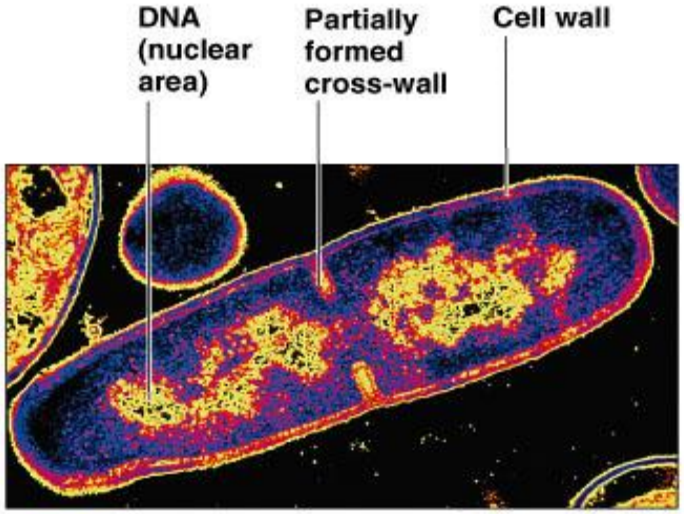
- Microbial growth involves both an increase in the size of organisms and an increase in their number ----------> *biomass*

- The importance:
  - The course and outcome of infections
  - The measurement of antibiotic effect

Microbial multiplication



(a) A diagram of the sequence of cell division.

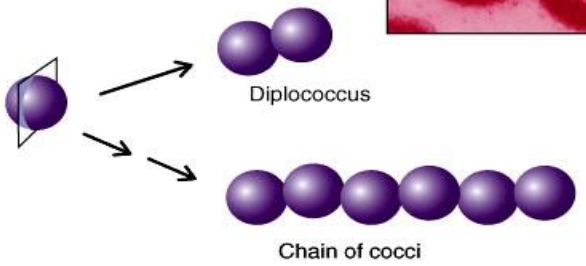


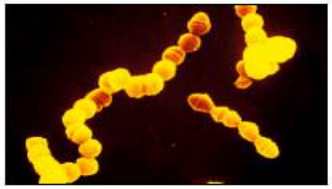
(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Reproduction

(a) Chains

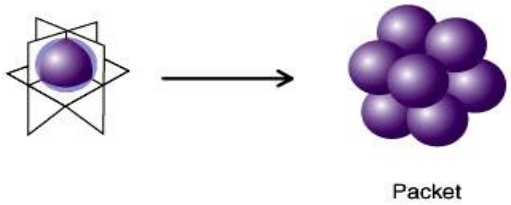
Cell divides
in one plane





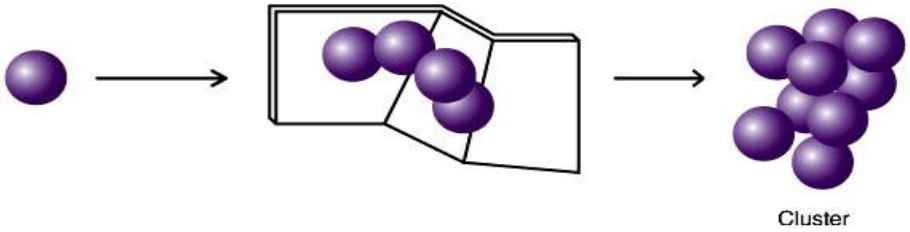
(b) Packets

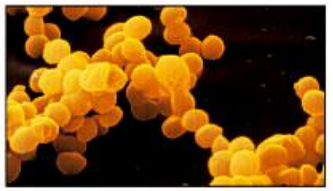
Cell divides
in two or more planes
perpendicular
to one another



(c) Clusters

Cell divides in several
planes at random





Bacterial growth

- Forms of growth:
  1. Development of colonies
  2. Transformation of a clear broth to a turbid one
  3. Biofilm formation

Generation (doubling) time

– The time for a cell to divide
  - Between 13 min for *vibrio cholerae* and 18-24 h for *M. tuberculosis*

Nutritional requirements

1. **The energy source**
   - ***Phototrophic*** --------- light
   - ***Chemotrophic*** --------- chemical oxidations

2. **The hydrogen donors**
   - ***Organotroph*** -------- use organic source of Hydrogen
   - ***Lithotroph*** --------- use inorganic sources

3. **Carbon source**
   - ***Autotrophs*** -------- fix CO_2 directly
   - ***Heterotrophs*** ----- requires organic source

Physical requirements

1. Growth atmosphere

- **Oxygen**

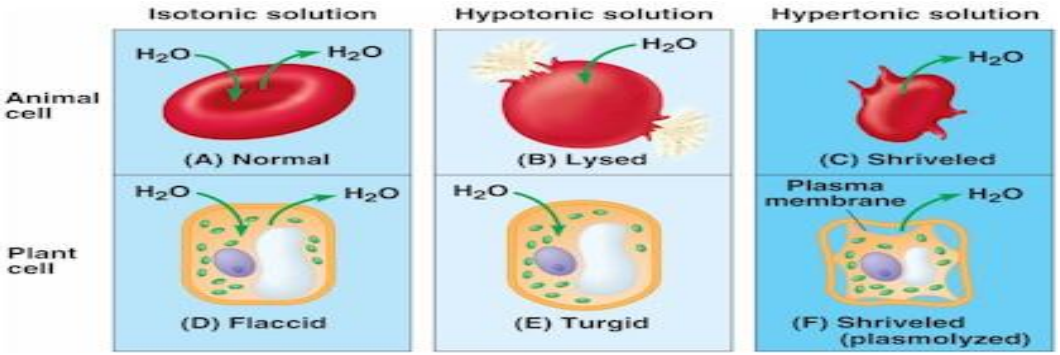
  Types of bacteria according to energy production

  - ***Strict or Obligate aerobe***
  - ***Strict or Obligate anaerobe***
  - ***Facultative anaerobes***
  - ***Microaerophilic***
  - ***Aerotolerant anaerobes***
  - ***Capnophiles***

Physical requirements

1. Growth atmosphere

- **Moisture**
  - Only bacterial endospores and protozoan cysts can exist in a dormant state in a dry environment (desiccation)
- **Osmotic pressure**
  - **Hypertonic**
  - **hypotonic**
  - ***Extremophiles***
    - Require enormous pressures
  - ***Halophilic***
  - ***haloduric organisms***



Physical requirements

2. Growth temperature

- *Psychrophiles* (< 20°C)
- *Mesophiles* (20-40°C)
- *Thermophiles* (> 45°C)
- *Hyperthermophiles* (or extreme thermophiles) (> 100°C)

3. Growth pH

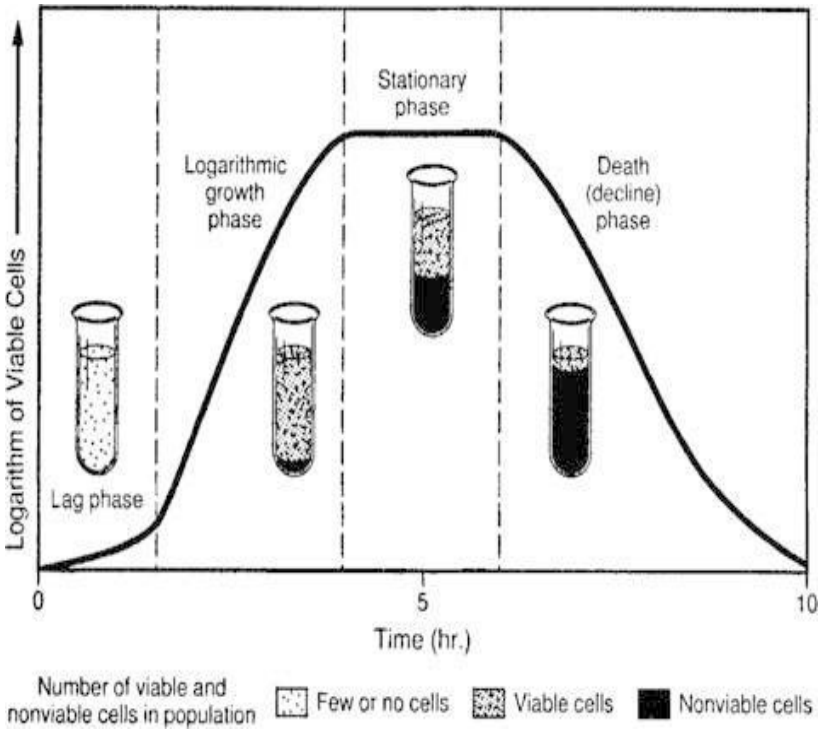
- *Neutrophiles* (6.2- 7.6)
- *Acidophiles* (pH < 6.0)
- *Alkaphiles* (pH > 8.0)

Chemical requirements

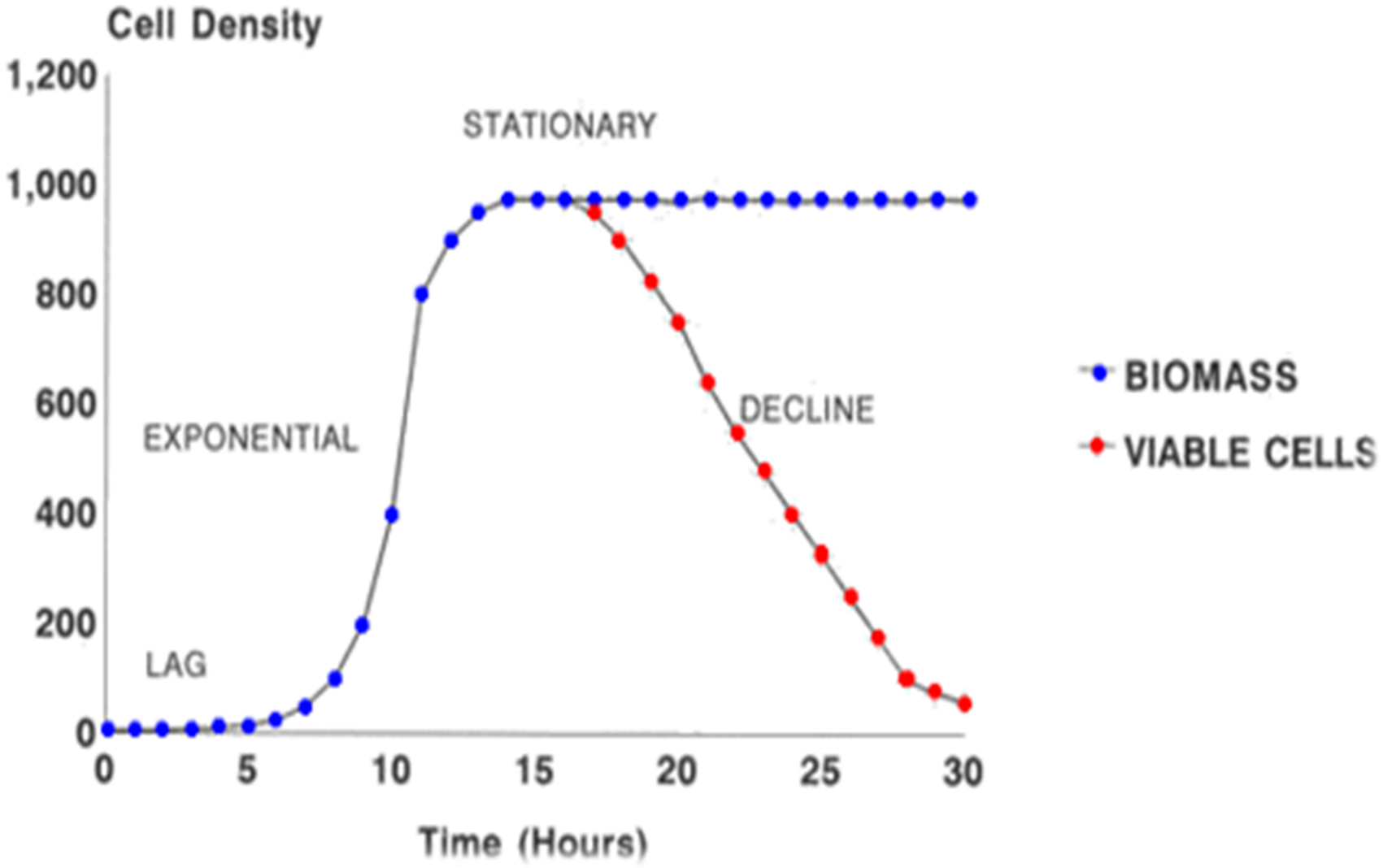
- Carbon
  - Carbon is the structural backbone of all living matter
  - Organic compounds
- Nitrogen, Sulfur, Phosphorus
  - N2 and P required for synthesis of DNA and ATP
  - N2 required for protein synthesis
- Trace Elements
  - Needed for enzymatic functions
  - Can be added to media to culture microbes
  - Fe, Cu, Zn

Growth phases in broth culture

1. **Lag phase** Adapt to nutrients
2. **Logarithmic phase** Active Growth
3. **Stationary phase** Growth equals death
4. **Decline phase(Death phase)** Nutrients Consumed



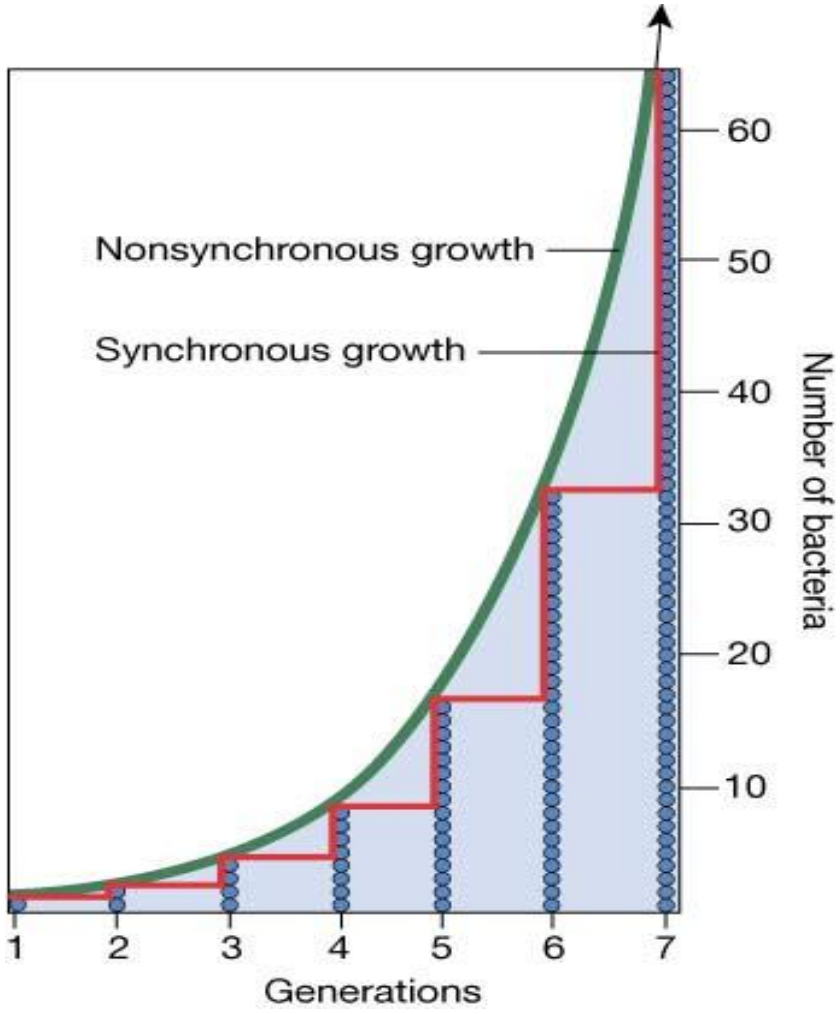
Growth phases in broth culture



Bacterial growth

Synchronous growth

all bacteria in the population divide at the same moment. This is not what normally happens. Grown in media, bacteria divide non-synchronously.



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Measuring Bacterial Growth

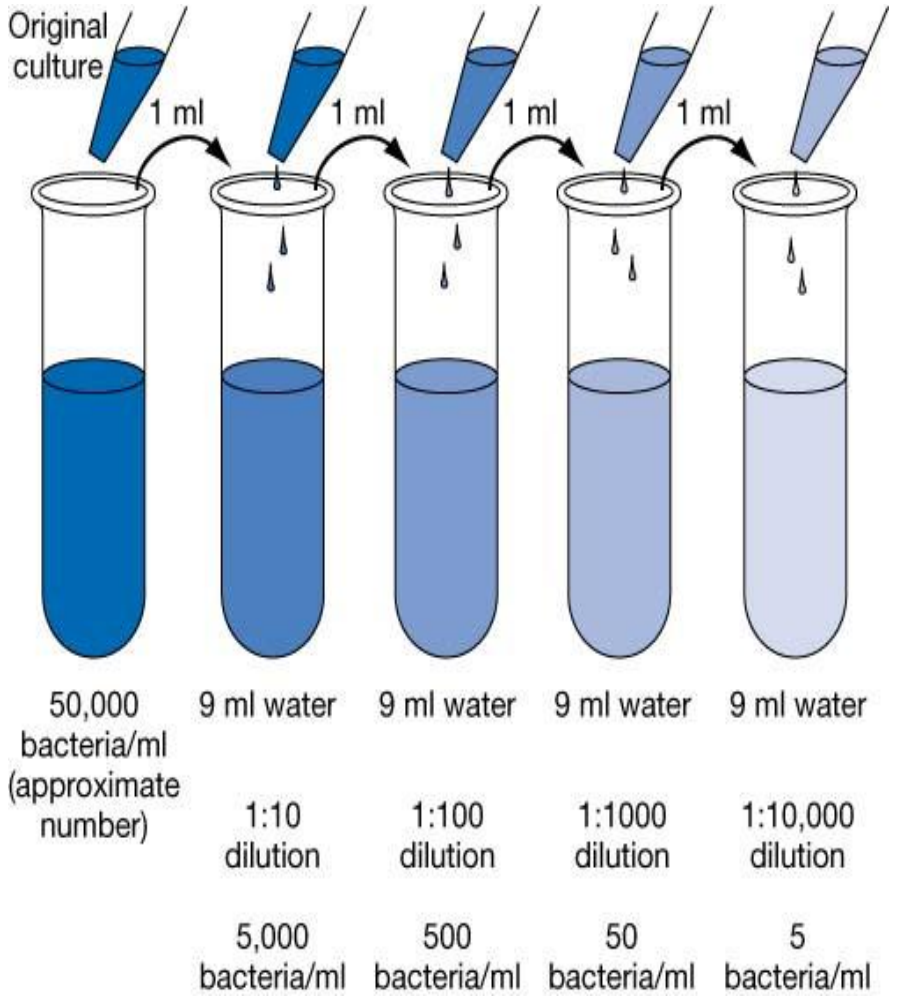
Measuring Bacterial Growth = number of live(viable) organisms per milliliter

Serial dilution

Dilute the original bacterial culture

standard plate counts

Transfer a known volume onto a solid plate (agar).



Bacterial Metabolism

Bacterial Metabolism

- Metabolism The sum total of all chemical reactions & physical workings occurring in a cell
    - Fueling
    - Biosynthesis
    - Polymerization
    - Assembly

Bacterial Metabolism

- Advantages of bacterial metabolism to our environment
  - Original development of O_2 in atmosphere
  - Availability of elemental Sulphur
  - Flow of Nitrogen
- Commercial exploitation of bacterial metabolism has given us
  - Ethanol
  - Other alcohols
  - Acids resulting from fermentation

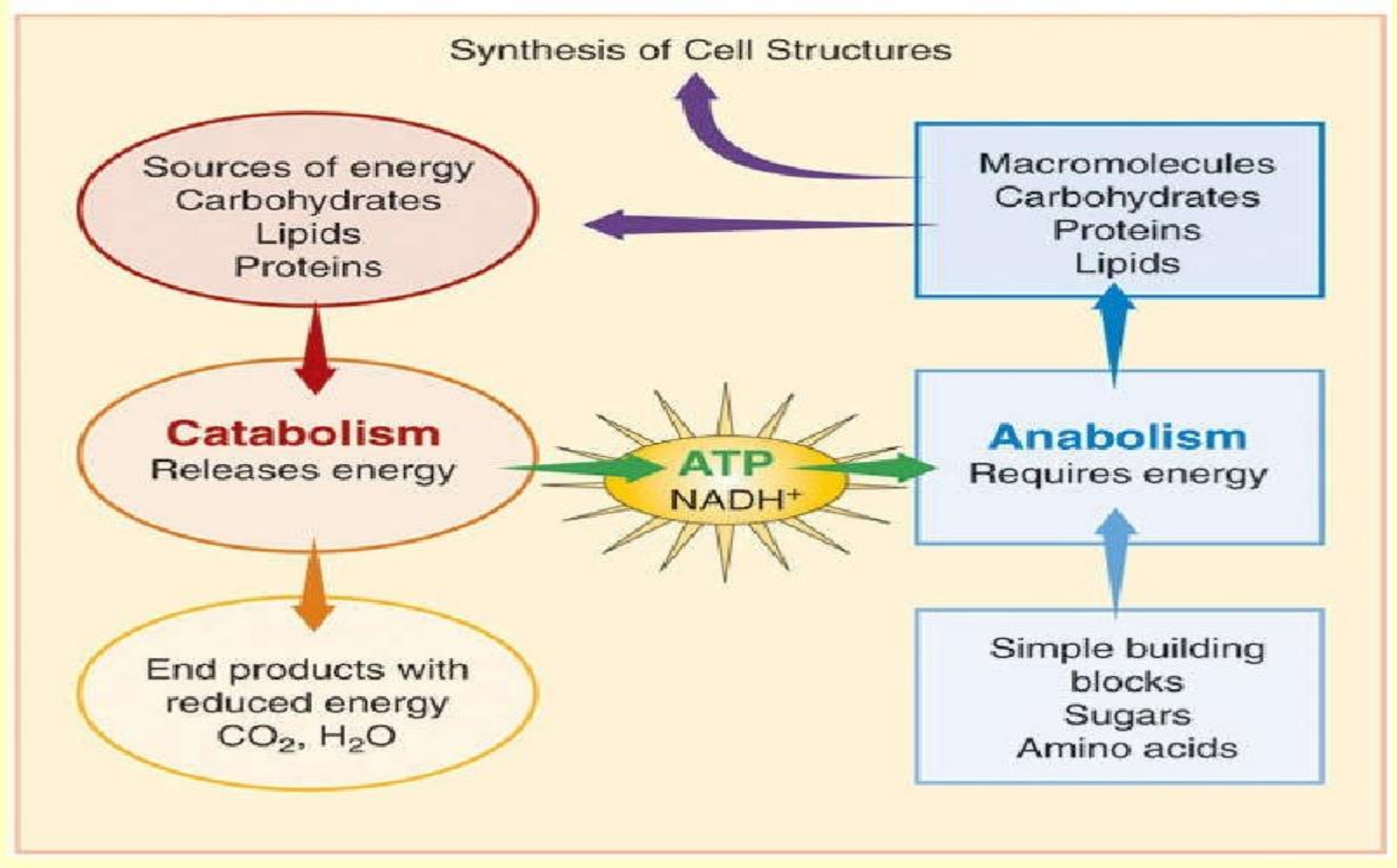
Bacterial Metabolism

- **Basic chemical reactions**
    A. Anabolism and Catabolism
    B. Oxidation and Reduction reactions
    C. ATP production and Energy storage

A. Anabolism and Catabolism

- **2 types of metabolism**
  - **Anabolism - biosynthesis**
    - **building complex molecules from simple ones**
    - **requires energy (ATP)**
  - **Catabolism - degradation**
    - **breaking down complex molecules into simple ones**
    - **generates energy (ATP)**

A. Anabolism and Catabolism

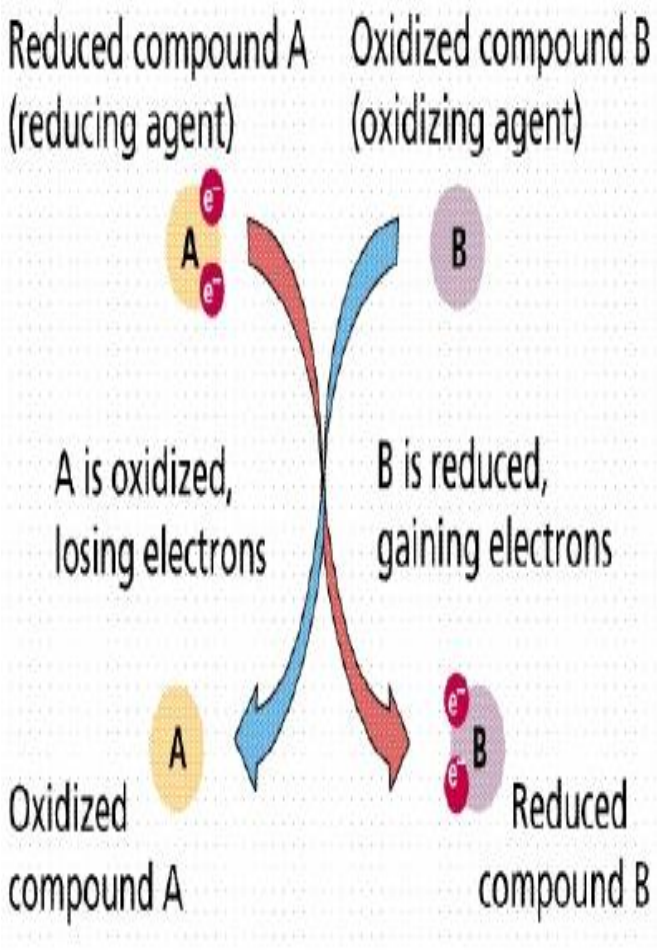


A. Anabolism and Catabolism

- **Anabolism**
  - Organisms catabolize carbohydrates as the primary energy source for anabolic reactions
- **Catabolism**
  - Glucose catabolized by
    - Aerobic cellular respiration -> Results in complete breakdown of glucose to carbon dioxide, water and a lot of ATP
    - Anaerobic respiration and Fermentation -> Only partially breaks down glucose, into pyruvic acid and organic waste products and a little ATP

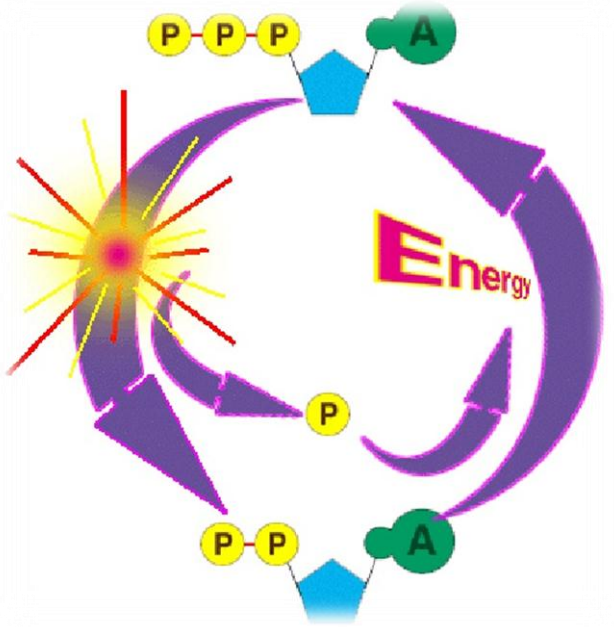
B. Oxidation and Reduction reactions

- **Redox reaction**
  - A chemical reaction in which electrons are gained, lost or shared
- **Oxidation**
  - The loss of electrons by a molecule, atom or ion
- **Reduction**
  - The gain of electrons by a molecule, atom or ion



C. ATP production and Energy storage

- Phosphorylation An organic phosphate is added to substrate
- Energy storing nucleotide

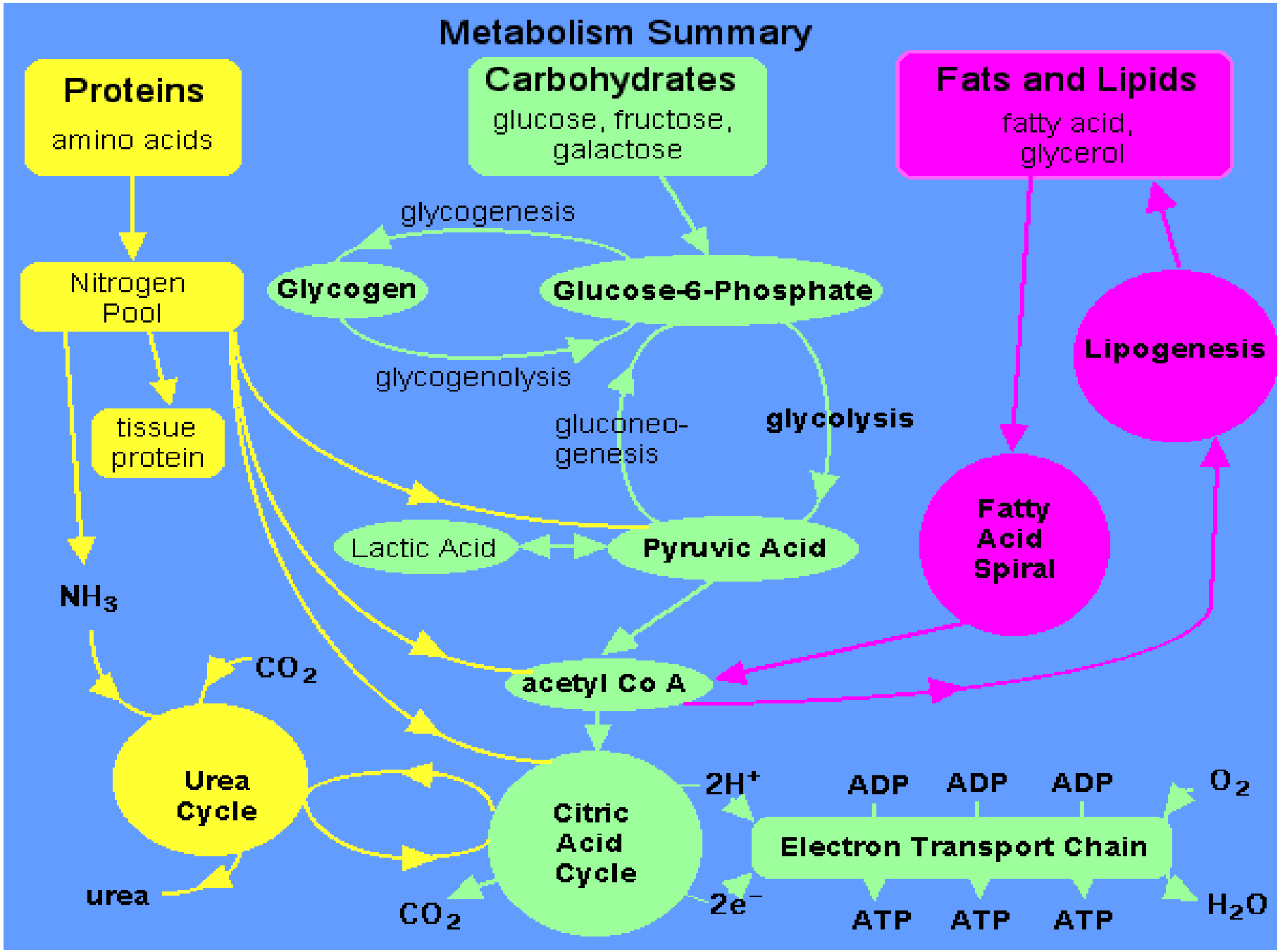


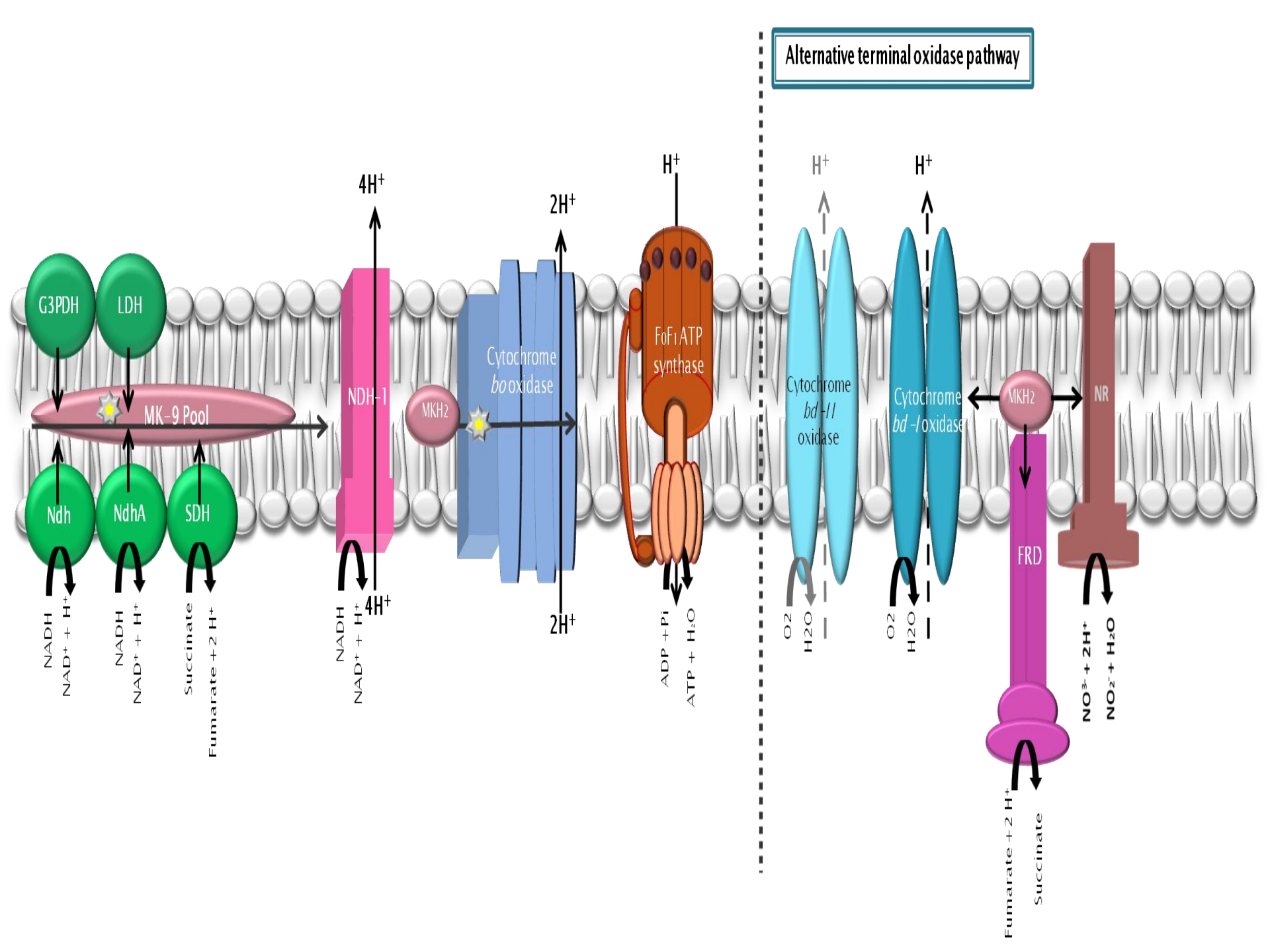
Bacterial respiration

- **Aerobic**
  - Final electron recipient in oxidation process is molecular oxygen
- **Anaerobic**
  - Final electron recipient is an organic molecule in the absence of oxygen, this oxidative process is referred to as ‘Fermentation’
  - All bacteria in evolution were anaerobes

Bacterial respiration

- **Aerobic cellular respiration**
  - Utilizes glycolysis, synthesis of acetyl CoA, Kreb ' s cycle, and electron transport chain; results in complete breakdown of glucose to carbon dioxide, water and ATP
  - A total of 38 molecules of ATP are formed from one molecule of glucose


Metabolism Summary
Proteins
amino acids
Carbohydrates
glucose, fructose, galactose
Fats and Lipids
fatty acid, glycerol
glycogenesis
Nitrogen Pool
Glycogen
Glucose-6-Phosphate
glycogenolysis
Lipogenesis
tissue protein
gluconeo-genesis
glycolysis
Fatty Acid Spiral
Lactic Acid
Pyruvic Acid
NH3
CO2
acetyl Co A
Urea Cycle
Citric Acid Cycle
2H+
ADP ADP ADP
O2
Electron Transport Chain
urea
CO2
2e−
ATP ATP ATP
H2O


Alternative terminal oxidase pathway
G3PDH
LDH
MK-9 Pool
Ndh
NdhA
SDH
NADH
NAD+ + H+
NADH
NAD+ + H+
Succinate
Fumarate + 2 H+
4H+
NDH-1
NADH
NAD+ + H+
4H+
MKH2
Cytochrome bo oxidase
2H+
2H+
H+
FoF1 ATP synthase
ADP +Pi
ATP + H2O
H+
Cytochrome bd-II oxidase
O2
H2O
H+
Cytochrome bd-I oxidase
O2
H2O
MKH2
NR
FRD
NO3- + 2H+
NO2- + H2O
Fumarate + 2 H+
Succinate

Bacterial respiration

- Using oxygen in metabolism creates toxic waste

  Superoxide (O_2 -), Hydroxyl (OH -) and Hydrogen peroxide (H_2O_2)

  – Microbes produce two enzymes to detoxify
    - Catalase: H_2O_2 -> H_2O and O_2
    - Superoxide dismutase (SOD):
    - Superoxide (O_2 -) -> H_2O and O_2
  – Microbes that don't make these enzymes cannot exist in the presence of oxygen
  – Also protect the pathogenic organisms against Superoxide of the phagocytic cells

Bacterial viability

- The ability of a particular cell to grow and undergo binary fission, and its progeny would have the same potential
- **VNC (viable but non-culturable)**
  - The organisms which are viable, but in a (reversible) physiological state in which they cannot be induced to form colonies, e.g; in TB that have latent phases

THE BACTERIAL GENOME

Understanding Genetics

- We resemble and differ because of Genetic configurations
- They breed true from Generation to Generation But vary in small proportions in progeny.
- Bacteria too obey the laws of Genetics

THE BACTERIAL GENOME

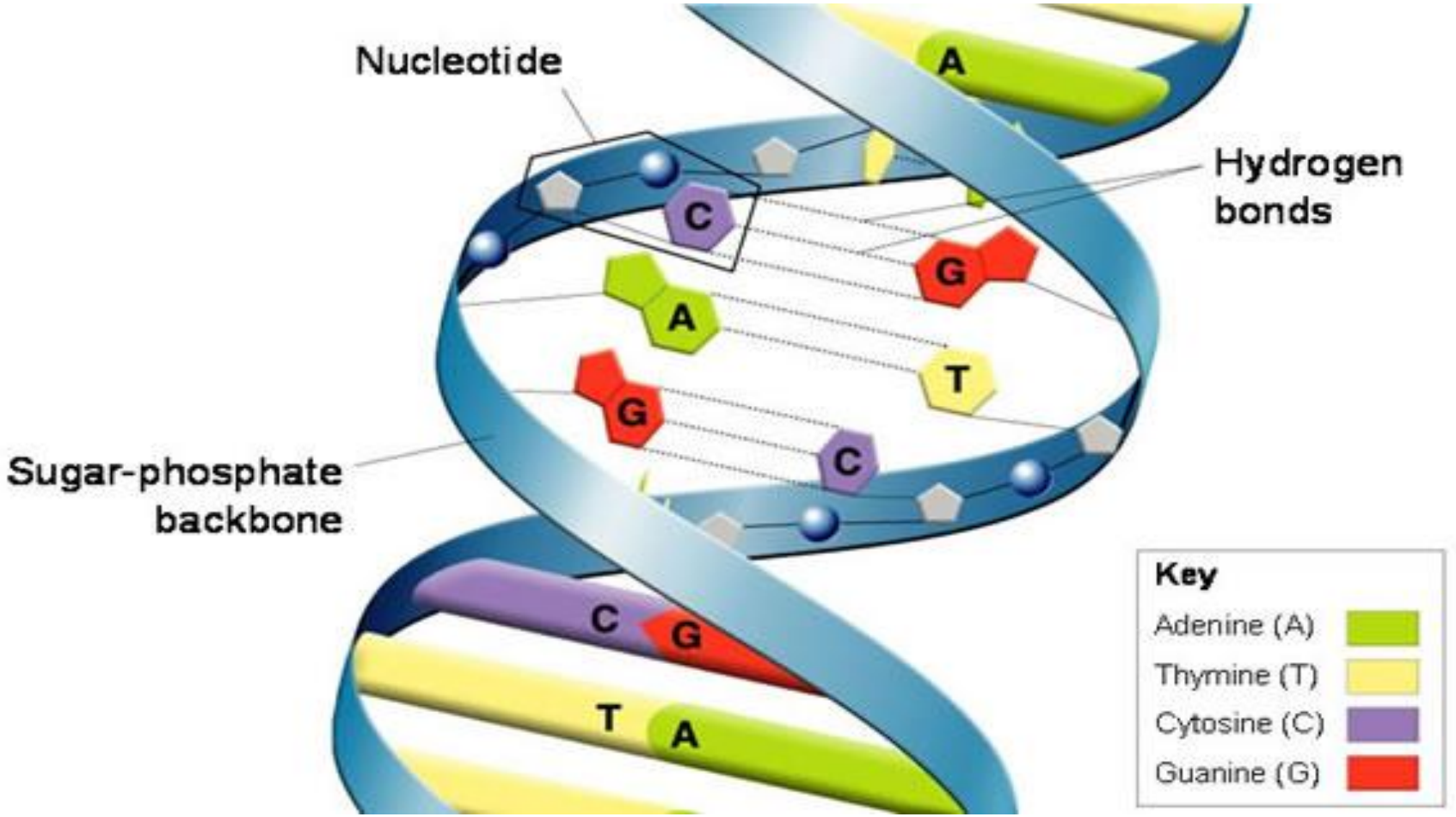
A. The chromosome

B. Pathogenicity islands

C. Plasmids

THE BACTERIAL GENOME

A. The chromosome



THE BACTERIAL GENOME

A. The chromosome

Genetic code:

- Code is triplet A-T- C
- A code can make single Amino acid
- Some Codons UAA don’t code for any Amino acid (Nonsense codon)

Gene:

- A sequence of DNA carrying codons specifying for particular polypeptide
- DNA contains many genes (A combinations of hundreds and thousands of Nucleotides)
- Constitutive genes
- Inducible genes

Bacterial Chromosome:

- Contains a Double stranded molecules of DNA arranged in circular form
- Bacterial DNA contains about 4,000 genes

THE BACTERIAL GENOME

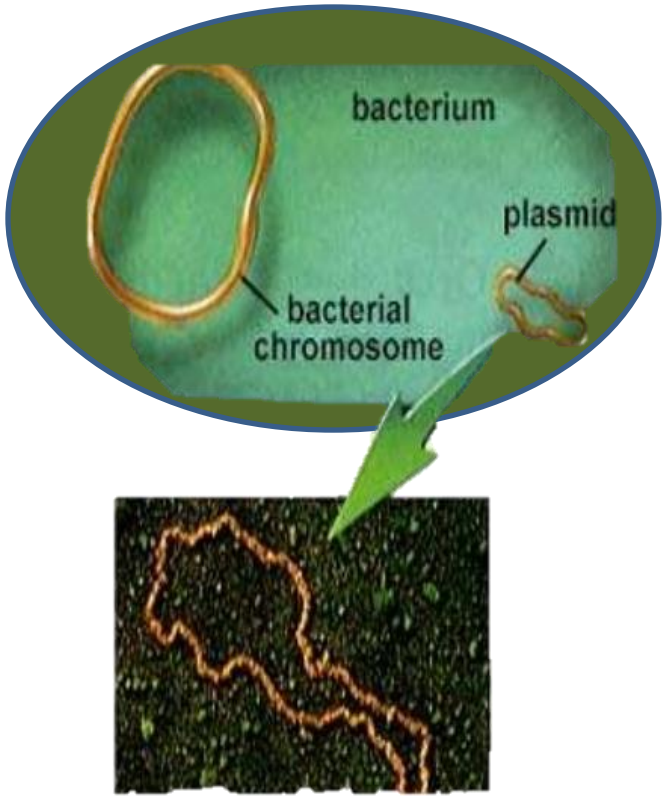
B. Pathogenicity islands

- Horizontal gene transfer
- G+C content
- PAIs are discrete genetic units flanked by direct repeats, insertion sequences or tRNA genes
- Encode virulence factors, such as toxins, adhesins, secretion systems, and iron transport proteins.

THE BACTERIAL GENOME

C. Plasmids

- ✓ Plasmids are circular DNA molecules present in the cytoplasm of the Bacteria
- ✓ Capable of Autonomous replication
- ✓ Can transfer genes from one cell to other
- ✓ Act as vectors in Genetic engineering
- ✓ Can also present in Yeasts
- ✓ Contain transposons



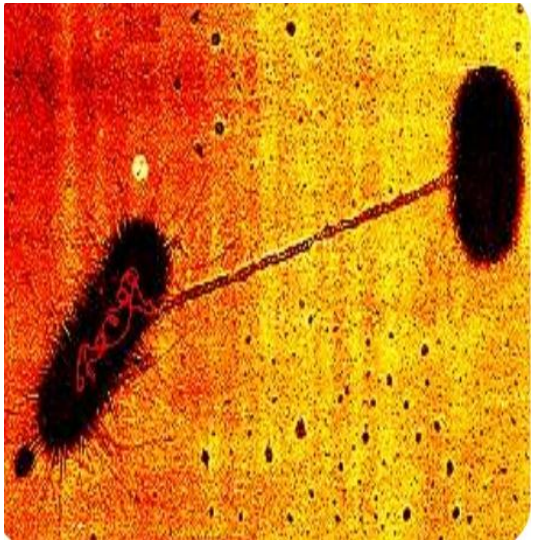
THE BACTERIAL GENOME

C. Plasmids functions

1. Resistance to Antibiotics
2. Bacteriocins production
3. Enterotoxin production
4. Enhanced pathogencity
5. Reduced Sensitivity to mutagens
6. Degrade complex organic molecules

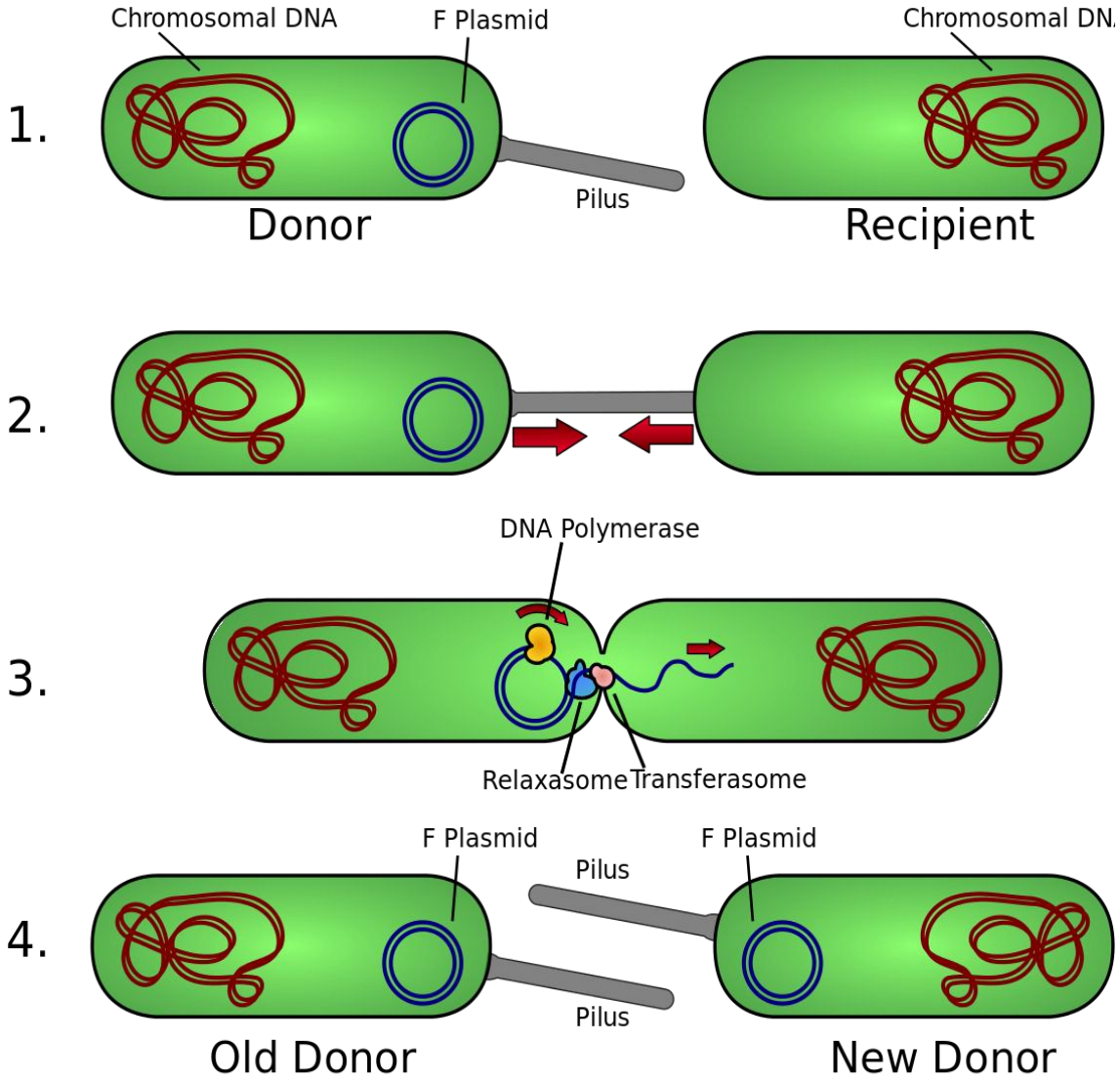
Gene transfer

1. Conjugation
2. Transduction
    A. Generalized transduction
    B. Specialized transduction
3. Transformation



Gene transfer

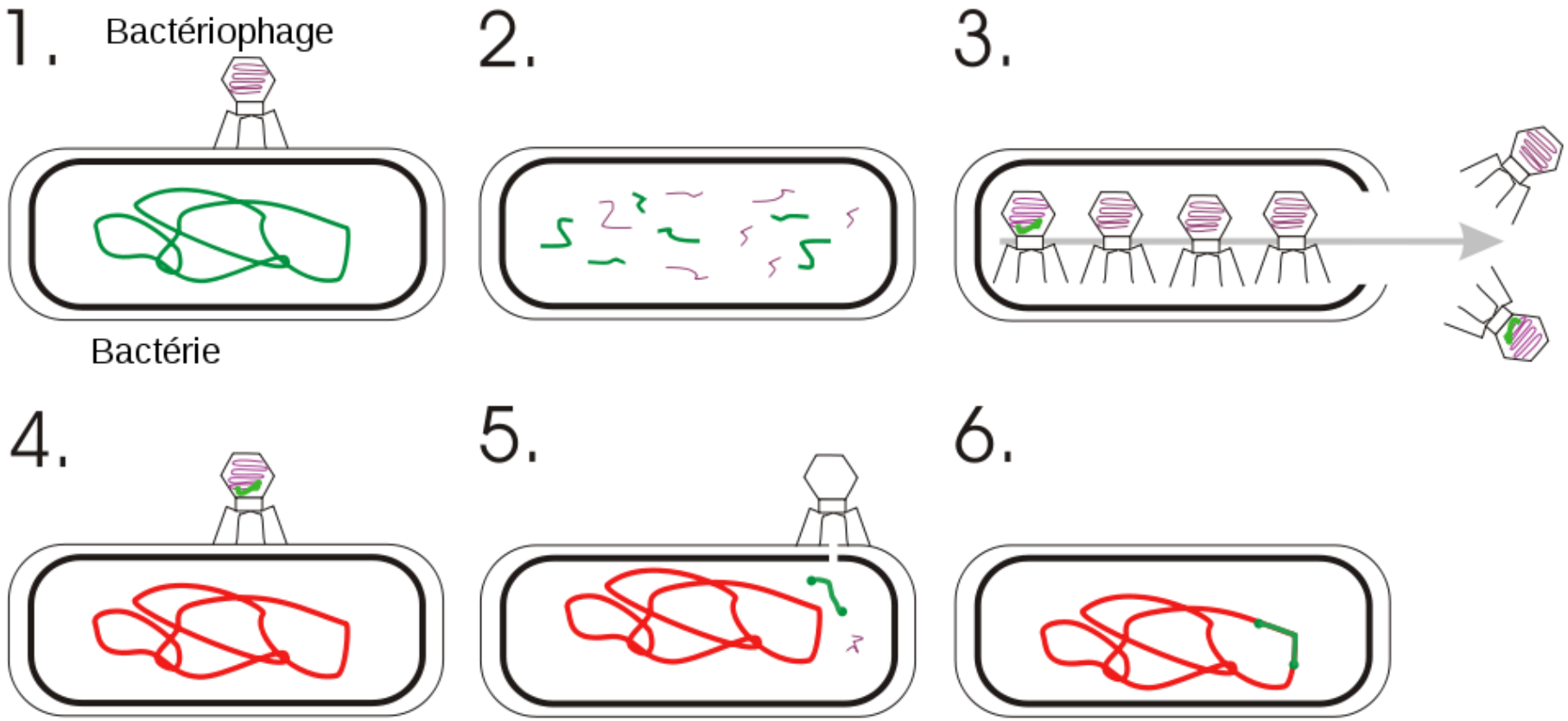
1. Conjugation



Gene transfer

2. Transduction

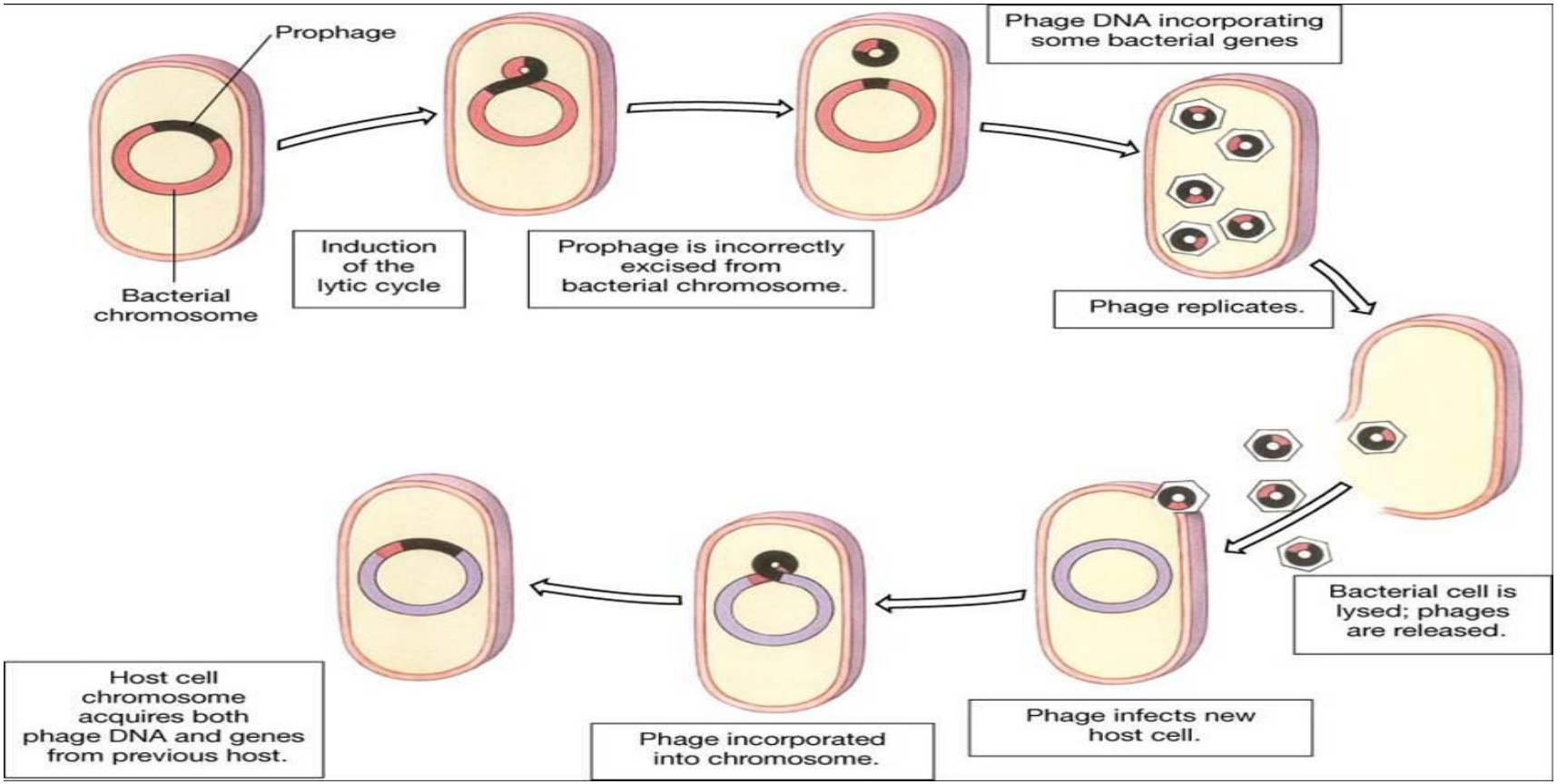
A. Generalized transduction



Gene transfer

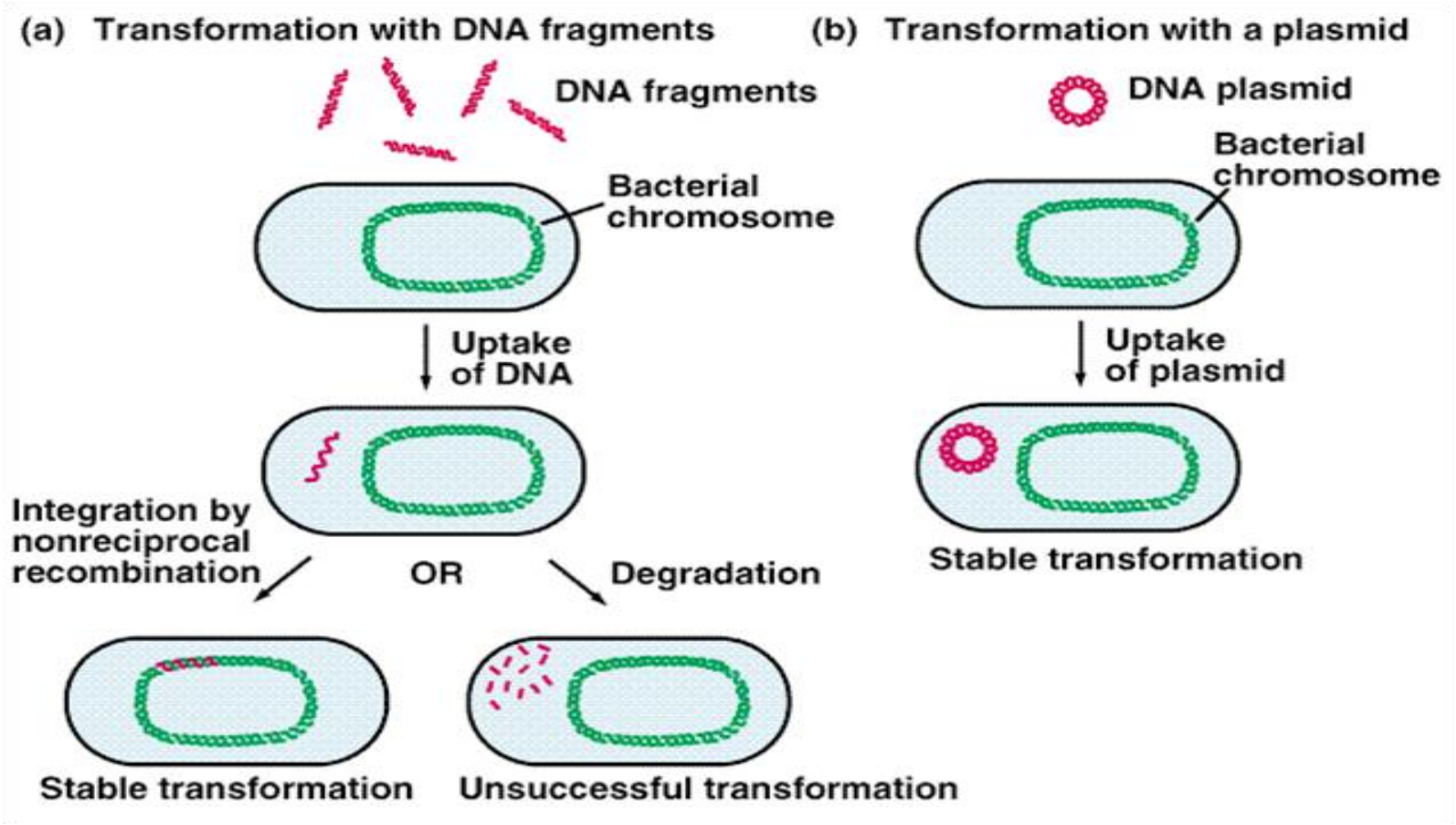
2. Transduction

B. Specialized transduction



Gene transfer

3. Transformation



GENETIC VARIATION

A. Mutation

B. Mobile genetic elements

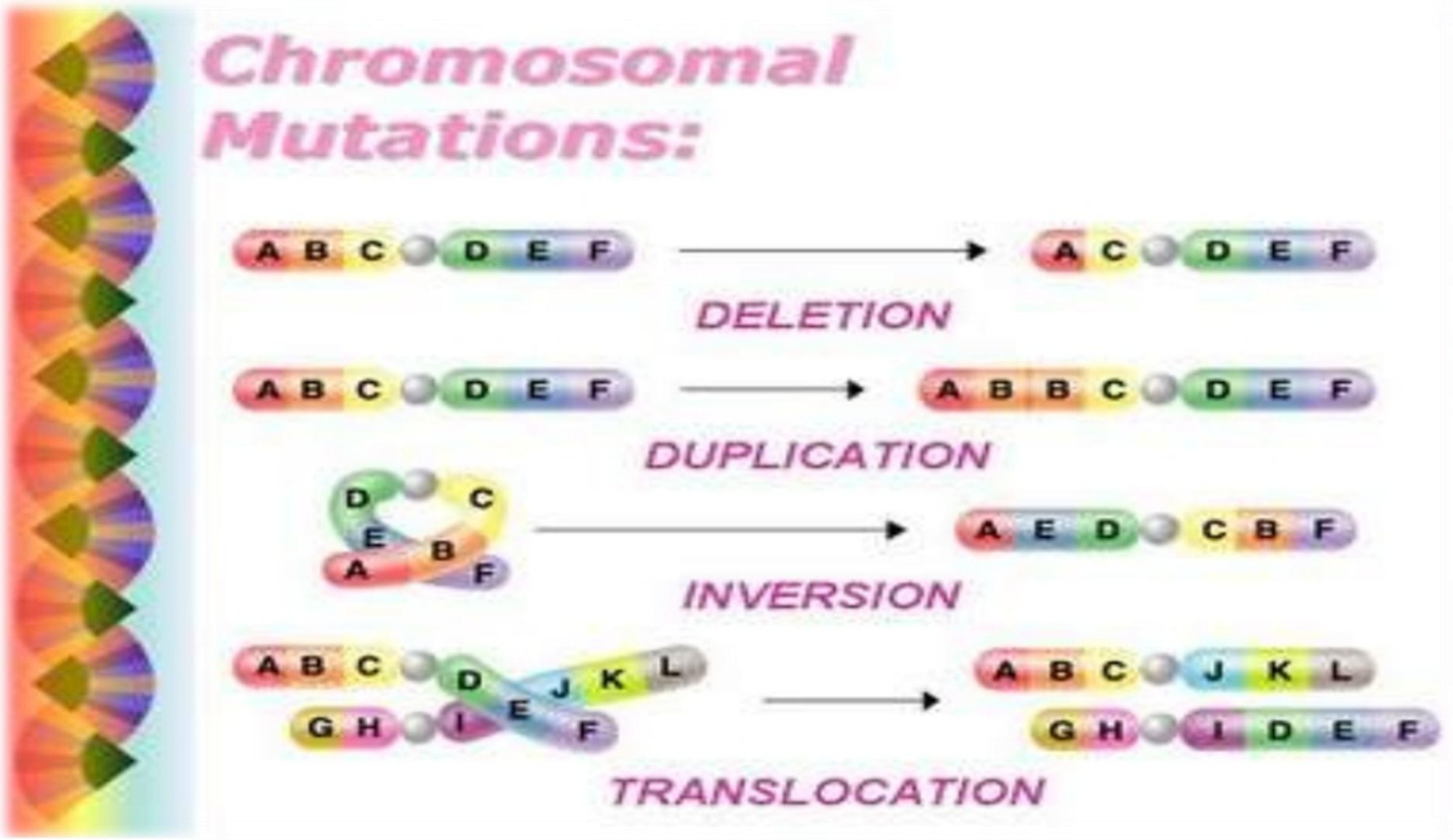
C. Mechanisms of acquired antibiotic resistance

GENETIC VARIATION

A. Mutation

- Mutation is a Random, Undirected, Heritable variation
- Caused by alteration in the Nucleotide sequence at some point of DNA which can occur due to Addition Deletion Substitution of one or more bases

GENETIC VARIATION



GENETIC VARIATION

B. Mobile genetic elements

Transposons (jumping genes)

- Replicative
- Non-replicative

✓ **The genetic variability bacterial populations**

✓ **The spread of antibiotic resistance genes**

GENETIC VARIATION

C. Mechanisms of acquired antibiotic resistance

1. Decreased uptake of antibiotic
2. Antibiotic efflux
3. Alteration of the target site for antibiotic
4. Acquisition of the ability to destroy or modify the antibiotic
5. Acquisition of a new target