الـجـمـهـوريـة الجـزائـرية الديـمـقـراطيـة الشعـبـيـة
People's Democratic Republic of Algeria
Ministry of higher education and scientific research
University of Ibn Khaldoun – Tiaret
Faculty of Natural and Life Sciences
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Course Manual

# Elements of Molecular Genetic of Microorganisms

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# Preface

Microbial genetics is an ever-evolving field that bridges the gap between molecular biology, biotechnology, and medicine. This course, *Elements of Molecular Genetics of Microorganisms*, is specifically designed for third-year undergraduate students in *Molecular Biology* and *Microbial Biotechnology*. It provides a structured and comprehensive exploration of bacterial, yeast, and viral genetics, highlighting their fundamental principles and applications.

This course has been carefully prepared according to the official academic framework, ensuring that it aligns with educational requirements and provides students with a solid foundation in microbial genetics. The topics covered include bacterial plasmids, gene regulation, horizontal gene transfer, yeast as a eukaryotic model, and the molecular biology of viruses. The selection of topics has been made to equip students with both theoretical knowledge and practical skills relevant to research and industry.

The development of this course is based on the contributions of numerous researchers, scientists, and educators whose work has shaped our current understanding of microbial genetics. Special appreciation is extended to those whose research and discoveries continue to advance the field.

It is our hope that this course serves as a valuable resource for students, fostering curiosity and inspiring further exploration into the fascinating world of microorganisms and their genetic mechanisms.

**Wassim YEZLI**

# Tribute to Pr. Mebrouk Kihal

This work is dedicated to the memory of Professor Mebrouk Kihal, a remarkable scientist, mentor, and educator whose contributions to microbiology and biotechnology have left a lasting impact. His dedication to research, his passion for teaching, and his unwavering support for his students have shaped many academic and professional careers, including my own.

Professor Kihal was not only an exceptional scholar but also a generous and inspiring mentor. His guidance, encouragement, and invaluable advice have profoundly influenced my scientific journey. His legacy will continue to inspire future generations of researchers and students in the field of microbiology.

With deep gratitude and respect, I dedicate this course to his memory, hoping that it reflects, in some way, the excellence and rigor he always upheld in his work.

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# Course syllabus according to the official framework

## Elements of Molecular Genetics of Microorganisms

**Semester:** 5.

**Fundamental Teaching Unit 2 (UEF 2):** Elements of Molecular Genetics of Microorganisms.

**Subject 1:** Elements of Molecular Genetics of Microorganisms.

**Credits:** 8.

**Coefficient:** 4.

**Course Objectives**

This unit complements the previous one. It focuses on the structural aspects and genetic and molecular mechanisms involved in gene expression in bacteria, eukaryotic microorganisms, and viruses. Fundamental knowledge will be acquired regarding the organization and functioning of the microbial genome, with the ability to compare it to that of higher eukaryotes (humans).

**Recommended Prerequisites**

This unit particularly requires knowledge of general microbiology, as well as basic understanding in genetics, structural biochemistry, and virology.

- ❖ **Course Content**
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    2. Mobile genetic elements.
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            2. Plasmid classification :
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                - R plasmids
                - Col plasmids

        - Degradative plasmids
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2. Transposons
    1. General structure of transposons.
    2. Different types of transposons.
    3. Transposition mechanisms in bacteria:
        - Replicative transposition
        - Conservative transposition
        - Consequences of transposition on bacterial genome expression



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3. Replication of the Bacterial Genome
4. Alterations and Repair Mechanisms of the Bacterial Genome

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    2. Structure and function of the ribosome
    3. Translation initiation
    4. Elongation
    5. Termination

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2. Inducible operons: Lactose operon

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8. Transposable elements
9. Tools and methods for yeast genetic transformation: Practical applications
10. Cell division and cell cycle
11. Sexual reproduction in yeast (haplodiplobiontic cycle)

➢ **Part 3: Viruses**

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2. Viral nucleic acids
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    2. RNA genomes
    3. Case of bacteriophages
3. Viral cycle
    1. Lytic cycle
    2. Lysogenic cycle
4. Replication of viral genetic material
    1. Replication of DNA viruses (Study model: bacteriophage T4)
    2. Replication of RNA viruses

❖ **Evaluation Method**

- **Continuous assessment /20**
    - Evaluation (average) of lab reports (/20)
    - Lab exam (/10)

    - One test on tutorial sessions (/10)
    - Article analysis (presentation /5, written summary /5, test /10)
- **ETLD /20**

**Bibliographic References**


- *Introduction to Microbiology.* Gerard J. Tortora, Berdell R. Funke, Christine L. Case. Editions du Renouveau Pédagogique Inc., 2003.
- *Introduction to Genetic Analysis.* Anthony J. F. Griffiths, Jeffrey H. Miller, David T. Suzuki, Richard C. Lewontin, William M. Gelbart. De Boeck University Editions, 2002.
- *Genetics of Bacteria.* Sheela Srivastava. Springer, 2013.
- *Genetics: Principles and Analysis.* Daniel L. Hartl, Elisabeth W. Jones. Dunod Editions, 2003.
- *Genetics.* William S. Klug, Michael R. Cummings, Charlotte A. Spencer. Pearson Education France, 2006.
- *Bacterial Transposable Elements.* Christophe Merlin, Ariane Toussaint. *m/s* No. 8-9, Vol. 15, 1999.

# Figures and Table

## Figures

## Table

# Abbreviations

ATP: Adenosine Triphosphate

bp: Base Pair

cDNA: Complementary DNA

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

dsDNA: Double-stranded DNA

dsRNA: Double-stranded RNA

EF-G: Elongation Factor G

fMet: N-Formylmethionine

gDNA: Genomic DNA

GTP: Guanosine Triphosphate

HGT: Horizontal Gene Transfer

IF: Initiation Factor

IS: Insertion Sequence

kb: Kilobase

mRNA: Messenger RNA

NCBI: National Center for Biotechnology Information

nt: Nucleotide

ORF: Open Reading Frame

PCR: Polymerase Chain Reaction

RBS: Ribosome Binding Site

RdRp: RNA-dependent RNA Polymerase

RF: Release Factor

RNA: Ribonucleic Acid

rRNA: Ribosomal RNA

RRF: Ribosome Recycling Factor

SAGE: Serial Analysis of Gene Expression

SNP: Single Nucleotide Polymorphism

ssRNA: Single-stranded RNA

tRNA: Transfer RNA

WGD: Whole Genome Duplication

WPI: Whey Protein Isolate

# General Introduction

Molecular microbial genetics is a fundamental field of molecular biology that explores the genetic mechanisms of microorganisms, particularly bacteria, fungi (yeast), and viruses. This discipline provides essential insights into gene structure, function, regulation, and horizontal gene transfer, all of which contribute to microbial adaptation and evolution. The study of microbial genomes has significant implications in biotechnology, medicine, and industrial microbiology.

This course covers three major domains: bacterial genetics, yeast genetics, and virology. The bacterial genetics section delves into the structure and organization of the bacterial genome, including chromosomes, plasmids, and mobile genetic elements such as transposons. It also examines DNA replication, genetic mutations, repair mechanisms, and horizontal gene transfer (transformation, conjugation, and transduction), which play a crucial role in microbial evolution and antibiotic resistance.

The yeast genetics section introduces *Saccharomyces cerevisiae* as a model organism for genetic studies, highlighting its genome organization, transcription, and proteome analysis. It also covers essential topics such as genetic mutations, tetrad analysis, transposable elements, and the yeast cell cycle. This part provides a deeper understanding of eukaryotic gene regulation and genetic engineering applications.

Finally, the virology section focuses on virus structure, classification, and replication strategies. It differentiates between DNA and RNA viruses, with particular emphasis on bacteriophages as models for studying viral genetic processes. The course also examines viral life cycles, including lytic and lysogenic cycles, as well as mechanisms of viral genome replication.

Understanding viruses is critical in medical microbiology, vaccine development, and emerging infectious disease research.

By integrating knowledge from bacterial, fungal, and viral genetics, this course equips students with the theoretical and practical foundations necessary to understand genetic mechanisms in microorganisms. These insights are essential for applications in biotechnology, genetic engineering, and molecular diagnostics.

# Part 1: Bacteria

# Chapter 1: Bacterial Genome

# *Part 1: Bacteria*

## Chapter 1: Bacterial Genome

### Introduction



Bacteria are unicellular organisms that contain the essential components for cellular life. Their size ranges from 1 to 10 microns (µm), making them visible only under an optical or electron microscope. They can be broken down through various physical and chemical processes, allowing the study of their released cellular components.

Here are some key figures regarding a model bacterium, *Escherichia coli*:

- Cell weight: $10^{-12}$ g
- Water content: 70%
- Dry weight of a cell: $3 \times 10^{-13}$ g
- Composition of the dry weight:
- Proteins: 55%
- Lipids: 10%
- Lipopolysaccharides (LPS): 3%
- Peptidoglycan: 3%
- Ribosomes: 40%
- RNA: 20%
- DNA: 3%

### 1. Structure of the Bacterial Genome

#### 1.1. Bacterial chromosome

Bacteria contain a nuclear structure composed of deoxyribonucleic acid (DNA), which serves as the carrier of genetic information. The bacterial chromosome is located in an irregularly shaped region known as the **nucleoid**. While most bacteria have a single chromosome, some species, such as *Vibrio cholerae*, possess multiple chromosomes.

Bacterial chromosomal DNA is typically circular and double-stranded. This helical structure is tightly coiled and supercoiled within the cytoplasm, a process regulated by topoisomerases (four types are present in bacteria). If fully extended, the bacterial chromosome would

measure about 1 mm in length, approximately 1,000 times the length of the bacterial cell, while its width ranges from 3 to 5 nm.

During replication, the two nucleotide strands follow the **Watson and Crick model**, in which each strand serves as a template for synthesizing a complementary strand. This process follows a **semi-conservative mechanism**, meaning that one of the original strands is retained in each newly synthesized DNA molecule (Figure 1).



DNA is composed of a chain of nucleotides, which are the fundamental units of genetic material. Each nucleotide consists of :

- A phosphate group (phosphoric acid)
- A sugar molecule (deoxyribose)
- A nitrogenous base, which can be:
    - Adenine (A)
    - Thymine (T)
    - Cytosine (C)
    - Guanine (G)

The DNA backbone is formed by alternating sugar-phosphate units (Figure 2).

Nitrogenous bases fall into two categories:

- Purines (adenine and guanine), which have a two-ring structure.
- Pyrimidines (thymine and cytosine), which have a single-ring structure (Figure 3).

Base pairing occurs through hydrogen bonds:

- Adenine (A) pairs with thymine (T) via two hydrogen bonds.
- Guanine (G) pairs with cytosine (C) via three hydrogen bonds.

A chemical analysis of the bacterial nuclear apparatus shows that the chromosome is composed of:

- 80% DNA
- 10% RNA, which plays a structural role
- 10% proteins, including:
    - DNA polymerases, which replicate DNA
    - Topoisomerases, particularly DNA gyrases, which unwind the DNA to facilitate replication
    - RNA polymerases, which synthesize various types of RNA

Many antibiotics specifically target bacterial nuclear components:

- Quinolones inhibit topoisomerases, preventing DNA supercoiling.
- Rifamycins inhibit RNA polymerases, blocking transcription.

- Nitroimidazoles cause DNA fragmentation in strictly anaerobic bacteria, leading to cell death.

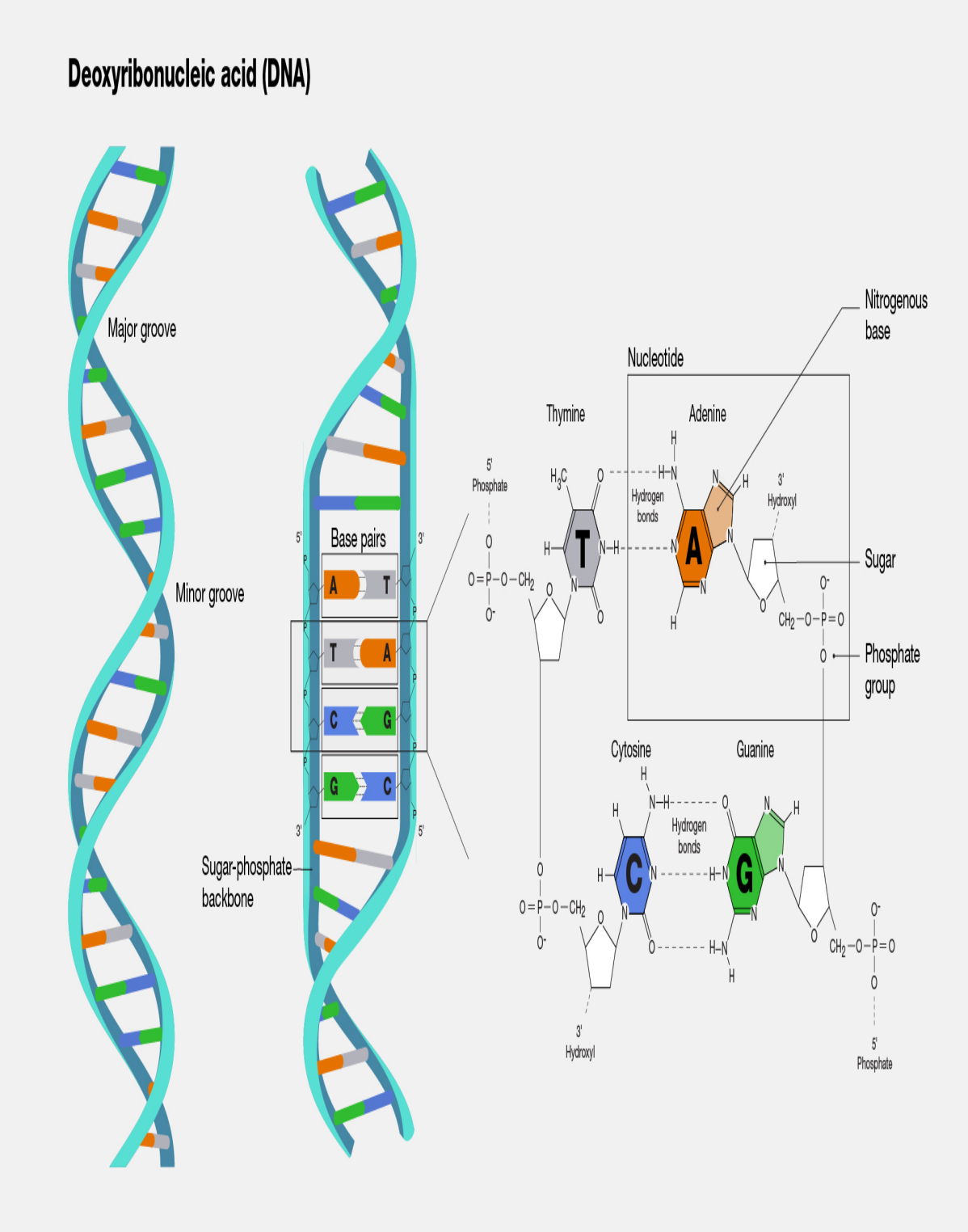



**Figure 1. The Double Helix Structure of DNA.**

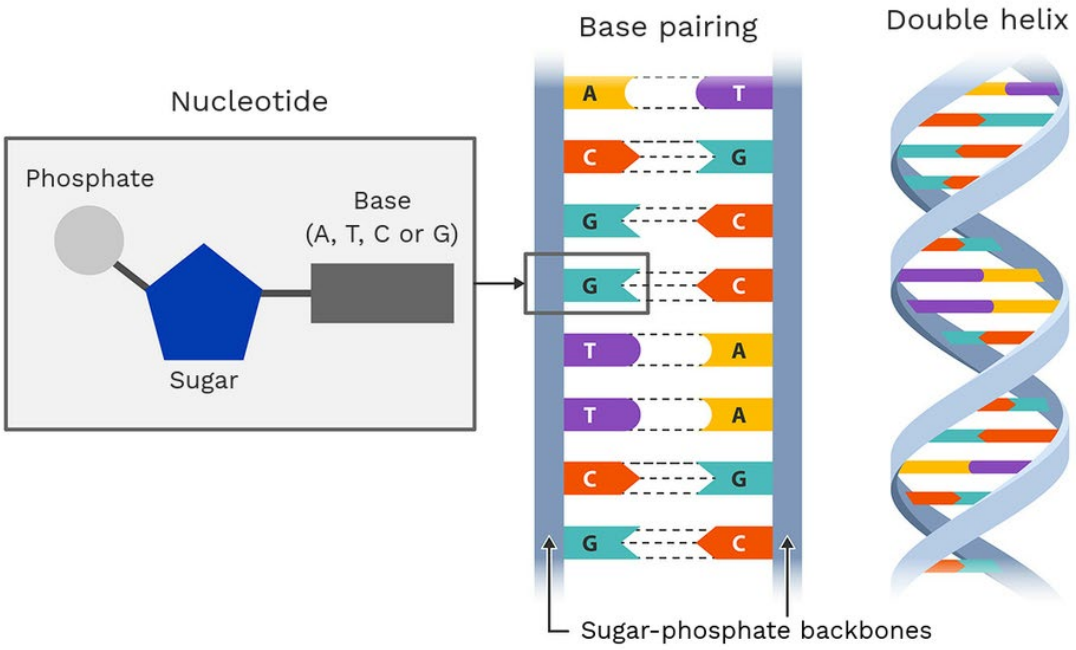
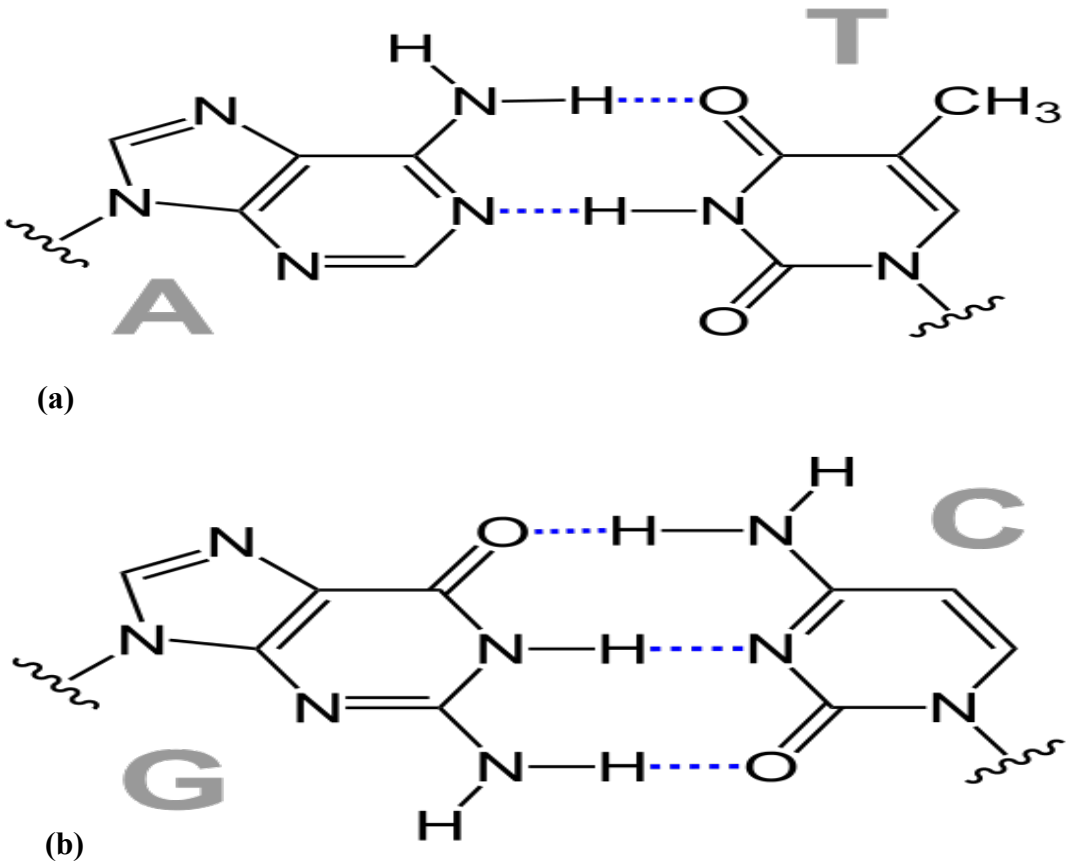



**Figure 2. Composition and Structural Organization of the Nucleotide**




**(a)**

**(b)**

**Figure 3. Structure of Purine and Pyrimidine Bases**

## 1.2. Mobile genetic elements

### 1.2.1. Plasmids

#### 1.2.1.1. General organization of plasmids



In addition to the chromosome, which serves as the primary carrier of genetic information, bacteria can contain small extrachromosomal DNA elements that make up 0.5% to 5% of the bacterial chromosome. These elements, called plasmids, are not essential for bacterial survival under normal growth conditions but can provide advantageous traits.

Plasmids replicate autonomously and generally faster than the bacterial chromosome. Their presence is typically detected when they confer new functional properties to the bacterium through the genes they encode.

#### 1.2.1.2. Classification of plasmids

**A. Conjugative plasmids (Fertility Factor or F Factor)**

Conjugative plasmids were the first plasmids discovered in *Escherichia coli* in the 1950s. Also known as fertility factors (F plasmids), these plasmids enable the synthesis of specialized pili, known as sex pili, which facilitate the transfer of genetic material. Through these pili, a bacterium carrying the F plasmid (donor) can transfer a copy of the plasmid to a recipient cell via bacterial conjugation.

F plasmids contain at least a replication origin and all the genes required for pilus formation and plasmid transfer. Some F plasmids function as episomes, meaning they can integrate into the bacterial chromosome.

**B. Antibiotic resistance plasmids (R Factors)**

These plasmids carry genes that provide resistance to various antibiotics. Unlike chromosomal mutations that confer resistance to a single antibiotic, R plasmids can carry multiple resistance genes, affecting several antibiotic families (Figure 4).

The resistance encoded by plasmid-borne genes is often associated with the production of enzymes that inactivate antibiotics. For instance, resistance plasmids commonly found in *Staphylococcus* species carry a gene encoding penicillinase, an enzyme that deactivates penicillin G and group A penicillins (e.g., ampicillin). This mechanism is also observed in *E.*

*coli* and *Neisseria gonorrhoeae*. In many cases, resistance genes are organized within transposons on the plasmid.

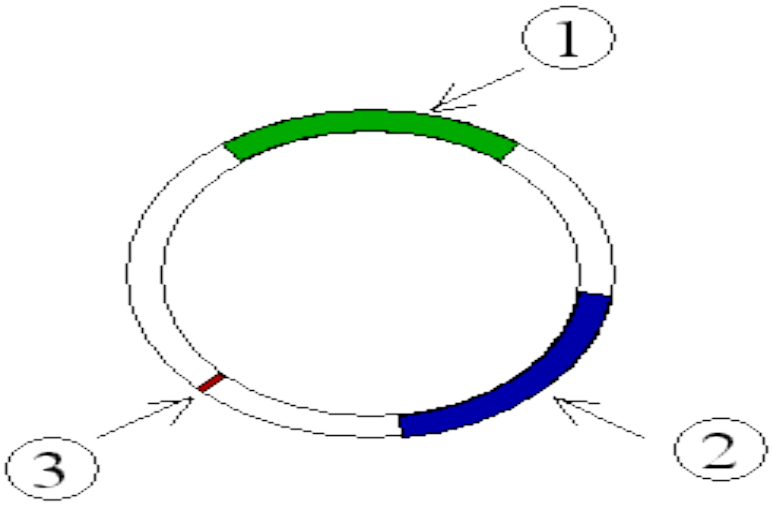



**Figure 4. Diagram of a plasmid encoding resistance to a given antibiotic.**

① Gene encoding resistance to an antibiotic (ATB); ② Transfer gene (Tra); ③ Origin of replication.

### C. Col Plasmids

Col plasmids encode the synthesis of bacteriocins, extracellular proteins that are lethal to both the producer bacterium and surrounding non-producing bacteria. However, these plasmids also encode a second intracellular protein that provides resistance to the bacteriocin.

Bacteriocins target essential bacterial functions. In *E. coli*, various types of colicins are encoded by Col plasmids. For example:

- The colE1 gene encodes an endonuclease.
- The colE3 gene encodes a ribonuclease that inactivates ribosomes.

### D. Degradative (Metabolic) Plasmids

Metabolic plasmids carry genes that enable bacteria to utilize specific nutrients. In *E. coli*, these plasmids may encode:

- The ability to use citrate as a carbon source.
- The production of sulfur-containing compounds.
- The hydrolysis of urea.

In *Salmonella*, some plasmids have been observed to enable lactose degradation, which is highly unusual for this bacterial genus. Most of these plasmids encode one or more metabolic enzymes.

**E. Virulence Plasmids**



Pathogenic bacteria frequently harbor conjugative plasmids that contribute to their virulence. These plasmids carry genes encoding virulence factors, which enhance bacterial pathogenic potential.

For example:

- Enterotoxigenic *E. coli* (ETEC), responsible for traveler's diarrhea (tourist diarrhea), contains at least two plasmids:
    - One encodes colonization factors.
    - Another encodes enterotoxins.
- Shigella's invasive properties are linked to the pInv plasmid.
- In *Salmonella* and other pathogens, virulence plasmids encode a protein complex (pili-adhesin complex) that allows the bacterium to adhere to carbohydrate receptors on the surface of epithelial cells (e.g., enterocytes).

Some plasmids encode tumor-inducing factors, such as the Ti plasmid (tumor-inducing plasmid) in *Agrobacterium*, which causes crown gall disease in plants.

### 1.2.1.3. Properties of plasmids

- Plasmids possess several properties that enhance bacterial adaptation to their environment.
- They carry non-essential but beneficial genes, which are not required for the normal metabolism of the host cell.
- Their natural transmission during cell division is stable and typically occurs via bacterial conjugation.

### 1.2.1.4. Replication and Transmission of plasmids

Each plasmid is a double-stranded DNA molecule containing at least one origin of replication (ori), a specific DNA sequence that serves as the starting point for replication. This allows plasmid DNA to replicate independently of the bacterial or yeast chromosome.

Plasmids can exist in circular or, in some cases, linear forms. They are transferred between bacteria through bacterial conjugation, mediated by sex pili.

During cell division, plasmids are distributed randomly between daughter cells, unlike chromosomes. Consequently, while rare, it is possible for one daughter cell to lack plasmids entirely. This likelihood increases as the number of plasmids in the mother cell decreases (Figure 5).



The origin of replication (oriC) in *E. coli* spans 245 base pairs (bp) and contains:

- Upstream: Three repeated 13 bp sequences (GATCTNTTNTTTT).
- Downstream: Four 9 bp sequences (9 mers) arranged in varying orientations.

Replication begins when DnaA proteins bind to the repeated 9-bp sequences, causing the DNA to coil around the DnaA complex. This conformational change opens the double helix at the A=T rich 13 bp repeats, allowing replication enzymes and other factors to bind to the single-stranded DNA and initiate replication.

The transfer of plasmids from a donor ($F^+$) to a recipient ($F^-$) cell occurs in four major steps (Figure 6):

1. Recognition and attachment – The donor and recipient recognize each other through the formation of a sex pilus (a hollow tube).
2. Single-strand transfer – One of the two plasmid DNA strands is transferred to the recipient.
3. Complementary strand synthesis – The recipient cell synthesizes the complementary DNA strand, restoring a double-stranded plasmid.
4. Plasmid circularization – The transferred plasmid recircularizes in the recipient cell, completing the process.

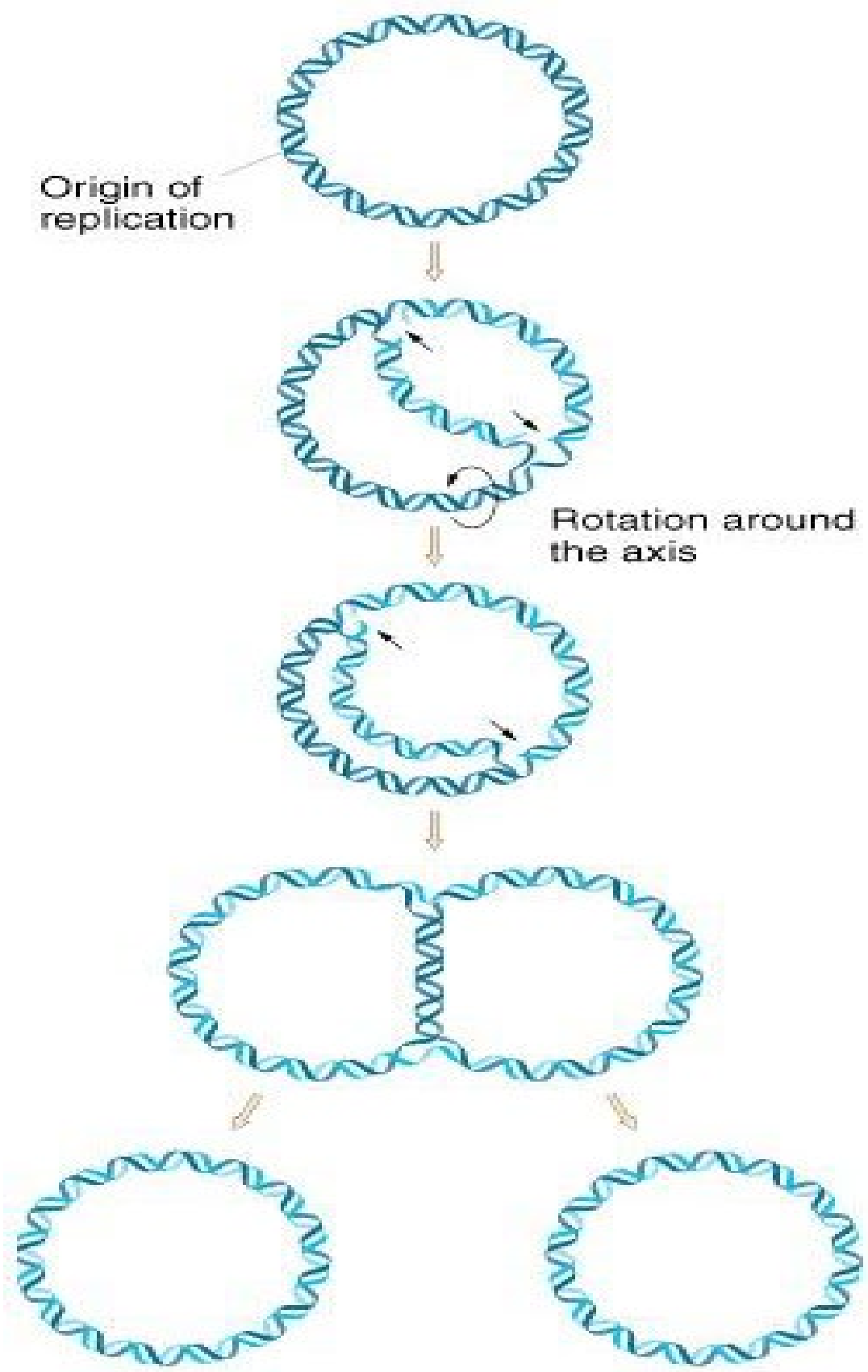



**Figure 5. Plasmid replication diagram.**

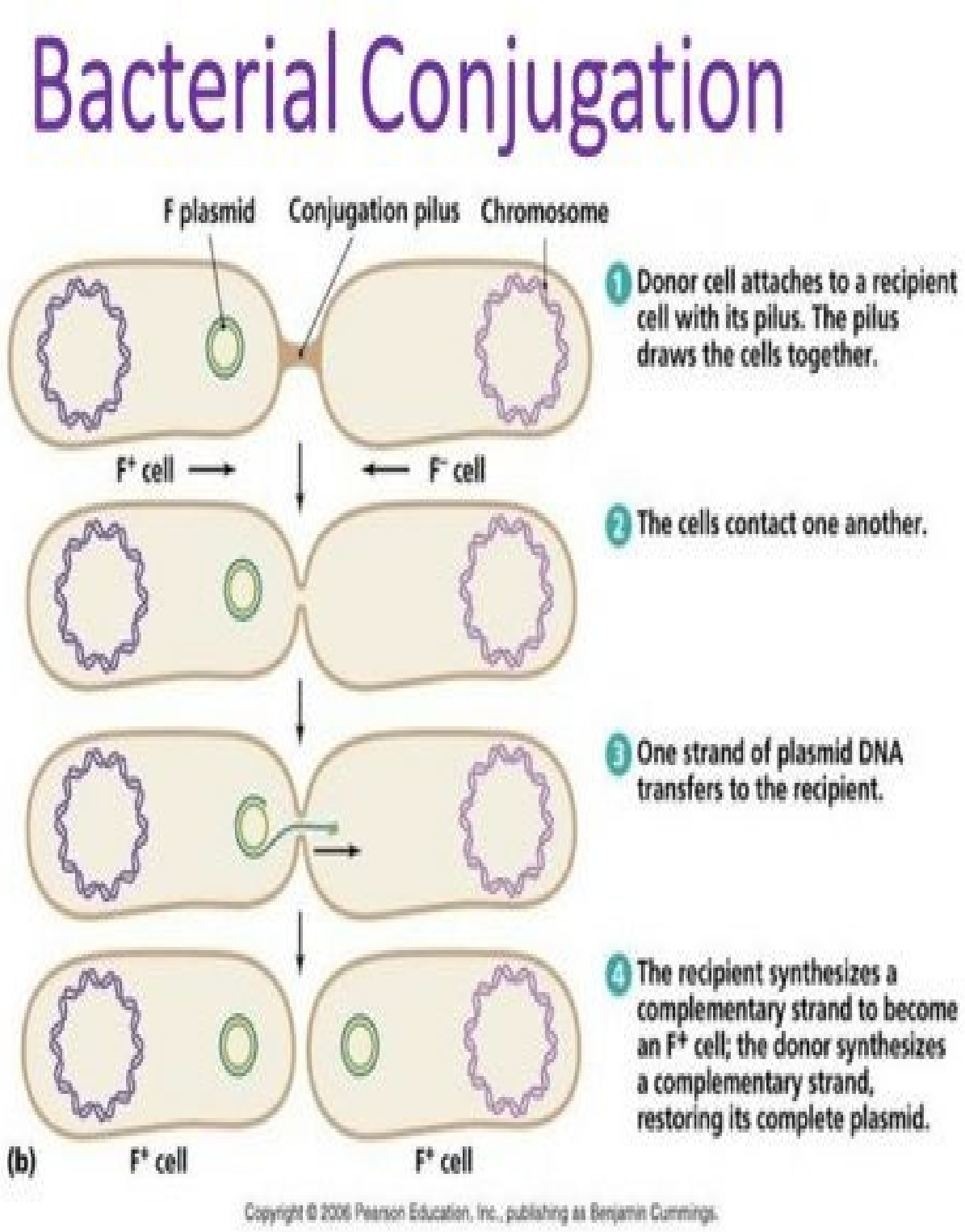



**Figure 6. Steps of bacterial conjugation**

### 1.2.2. Transposons (Jumping Genes)

Transposons, also known as "jumping genes," are DNA sequences capable of moving from one location to another within a genome. This process, called transposition, allows transposons to insert themselves into different positions, sometimes disrupting or modifying gene function. They are found in both prokaryotic and eukaryotic genomes and play a crucial role in genome evolution, genetic diversity, and horizontal gene transfer.



#### 1.2.2.1. General structure of transposons

Most transposons share a common structure:

- **Inverted Repeat Sequences (IRs)** – Short sequences at both ends that are essential for transposase recognition.
- **Transposase Gene** – Encodes the enzyme responsible for catalyzing transposition.

**Other Functional Genes** – Some transposons carry additional genes, such as antibiotic resistance genes in bacteria.

#### 1.2.2.2. Different types of transposons

Transposons are generally classified into two main categories based on their mechanism of movement:

**A. Class I: Retrotransposons (Copy-and-Paste Mechanism)**

- Move via an RNA intermediate.
- The transposon is first transcribed into RNA, then reverse transcribed into DNA by reverse transcriptase, and finally inserted into a new genomic location.
- Common in eukaryotes but rarely found in prokaryotes.
- Example: Long Interspersed Nuclear Elements (LINEs), Short Interspersed Nuclear Elements (SINEs), and LTR retrotransposons.

**B. Class II: DNA Transposons (Cut-and-Paste Mechanism)**

- Move directly as DNA without an RNA intermediate.
- A transposase enzyme cuts the transposon from its original site and inserts it into a new site.
- Found in both prokaryotes and eukaryotes.
- Example: Insertion Sequences (IS elements) and Composite Transposons in bacteria.

### 1.2.2.3. Bacterial transposons

Bacteria contain various types of transposons that contribute to genetic variability and antibiotic resistance. The main types include:

#### A. Insertion sequences (IS Elements)



Insertion sequences (IS elements) are the simplest form of bacterial transposable sequences, capable of integrating into various locations within the bacterial chromosome or plasmids through illegitimate recombination.

These elements are generally short and contain a single gene encoding the transposase enzyme, which facilitates their movement.

IS elements were first discovered as spontaneous insertions in specific *lac* operon mutations of *E. coli*, leading to gene inactivation and disruption of transcription and translation. The mutations in the *lac* operon were found to be unstable, and molecular analysis revealed the presence of additional DNA sequences near the *lac* gene. When *E. coli* underwent reverse mutation, these extra DNA sequences were lost.

A bacterial chromosome can harbor multiple copies of the same IS element. For instance, *E. coli* contains approximately 6 to 10 copies of IS1 in its genome. Similarly, plasmids may also carry IS elements (Figure 7).

- IS elements are compactly organized and containing about 1000 nucleotide pairs and contain only genes (open reading frame) which encode for enzyme for regulating transposition.
- Many distinct types of IS elements have been identified. The smallest IS element is IS*1* which is 768 nucleotide pairs long.

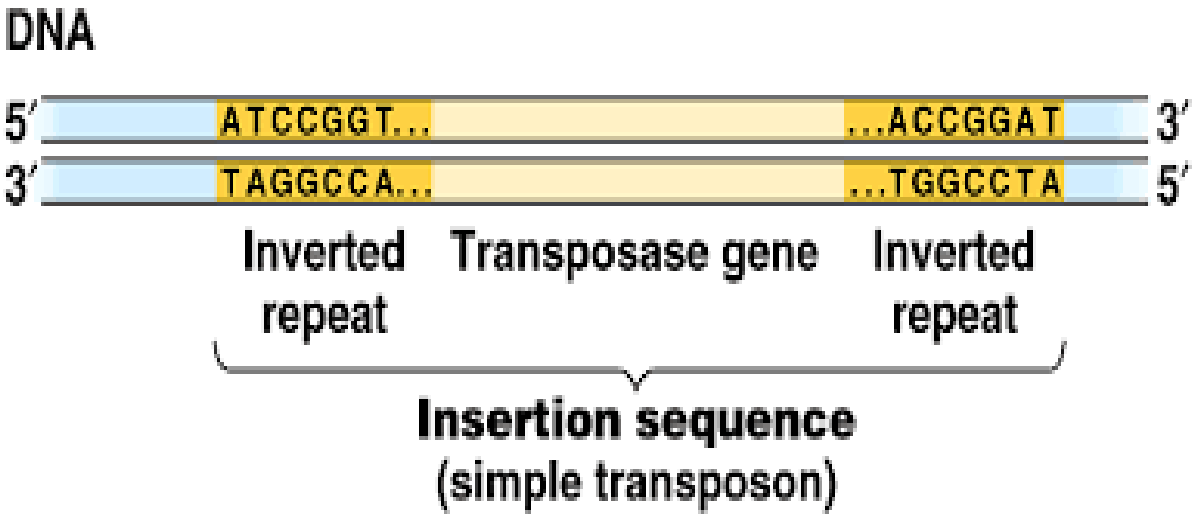



**Figure 7. Diagram of Insertion Sequence (IS Elements)**

### B. Composite transposons

Composite transposons are formed when two IS elements integrate near each other, allowing the entire intervening region to be mobilized as a single unit when the IS elements function together.

For instance, Tn10 is a 9.3 kb composite transposon that includes 1.4 kb terminal inverted repeats. Between these repeats, it carries a transposase gene along with a gene conferring antibiotic resistance (carries tetracycline resistance gene) (Figure 8).

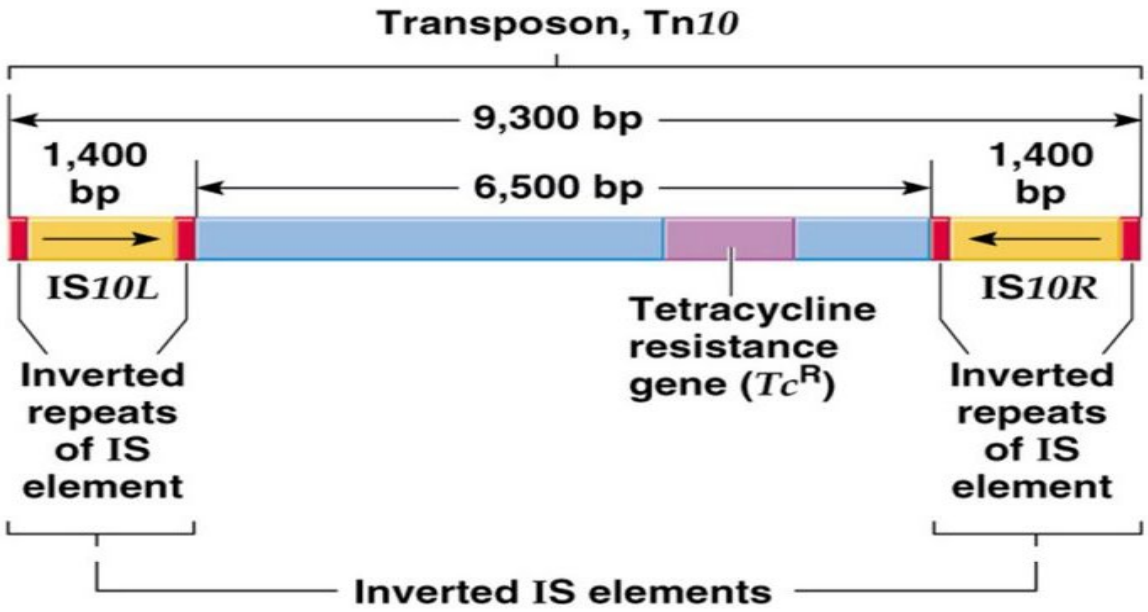



**Figure 8. Diagram of Tn10 composite transposon.**

### C. Non-composite transposons

Non-composite transposons are DNA sequences that contain a transposase gene along with multiple other genes, flanked by terminal inverted repeats.

Unlike composite transposons, they do not have IS elements at their ends but instead possess simple inverted repeats of 38–40 nucleotide pairs.



For example, Tn3 is a 5 kb non-composite transposon that carries three genes: *bla* (encoding β-lactamase gene for ampicillin resistance), *tnpA* (encoding transposase), and *tnpB* (encoding resolvase). The β-lactamase enzyme confers resistance to ampicillin, while transposase and resolvase are essential for transposition and recombination (Figure 9).

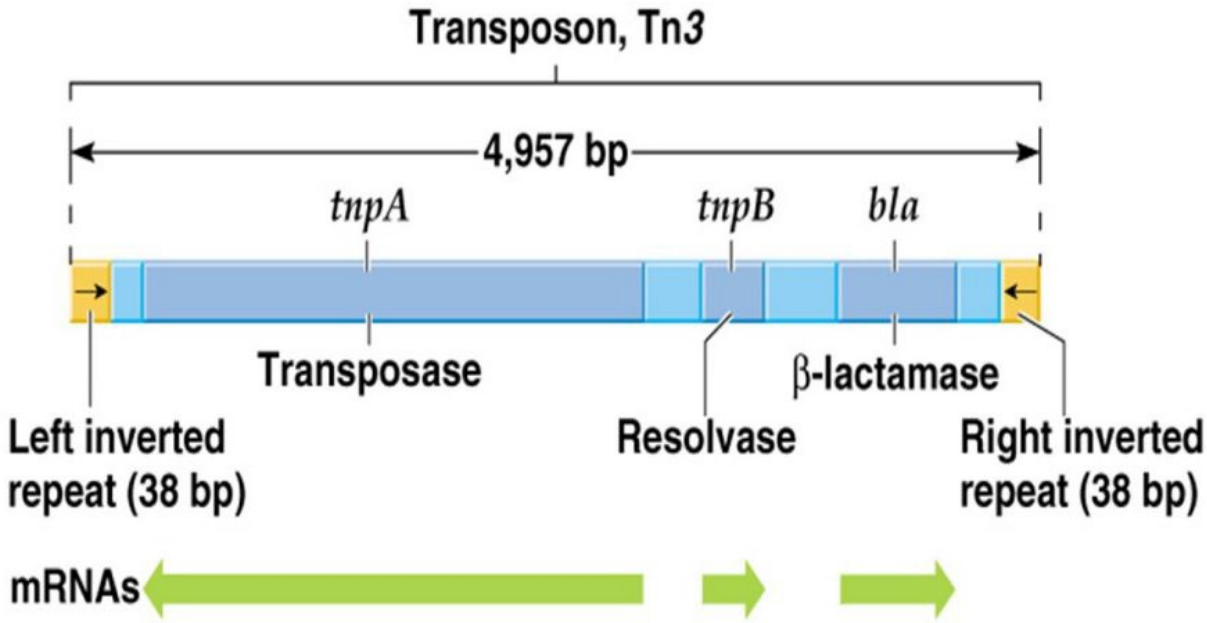



**Figure 9. Diagram of Tn3 Non-composite transposon.**

### 1.2.2.4. Bacterial mechanism of transposition

Transposition occurs through two main mechanisms:

### A. Replicative transposition (Copy-and-Paste)

The transposon is copied, and the new copy is inserted into a different site, leaving the original intact. Tis transposition increases the number of transposon copies in the genome (Figure 10).

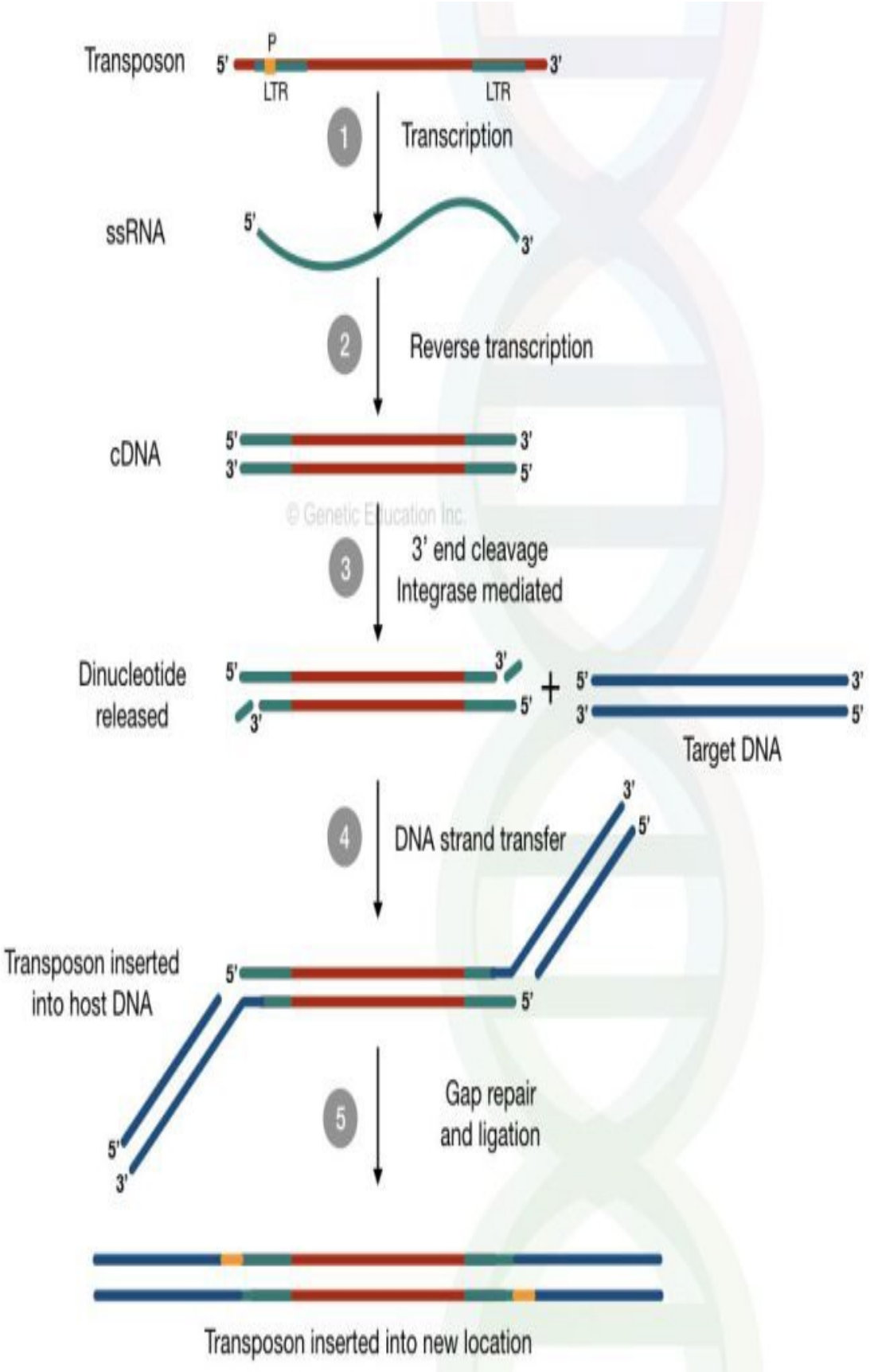



**Figure 10. Process of replicative transposition.**

**B. Conservative transposition (Cut-and-Paste)**

- The transposon is excised from its original location and inserted into a new site without leaving a copy behind (Figure 11).

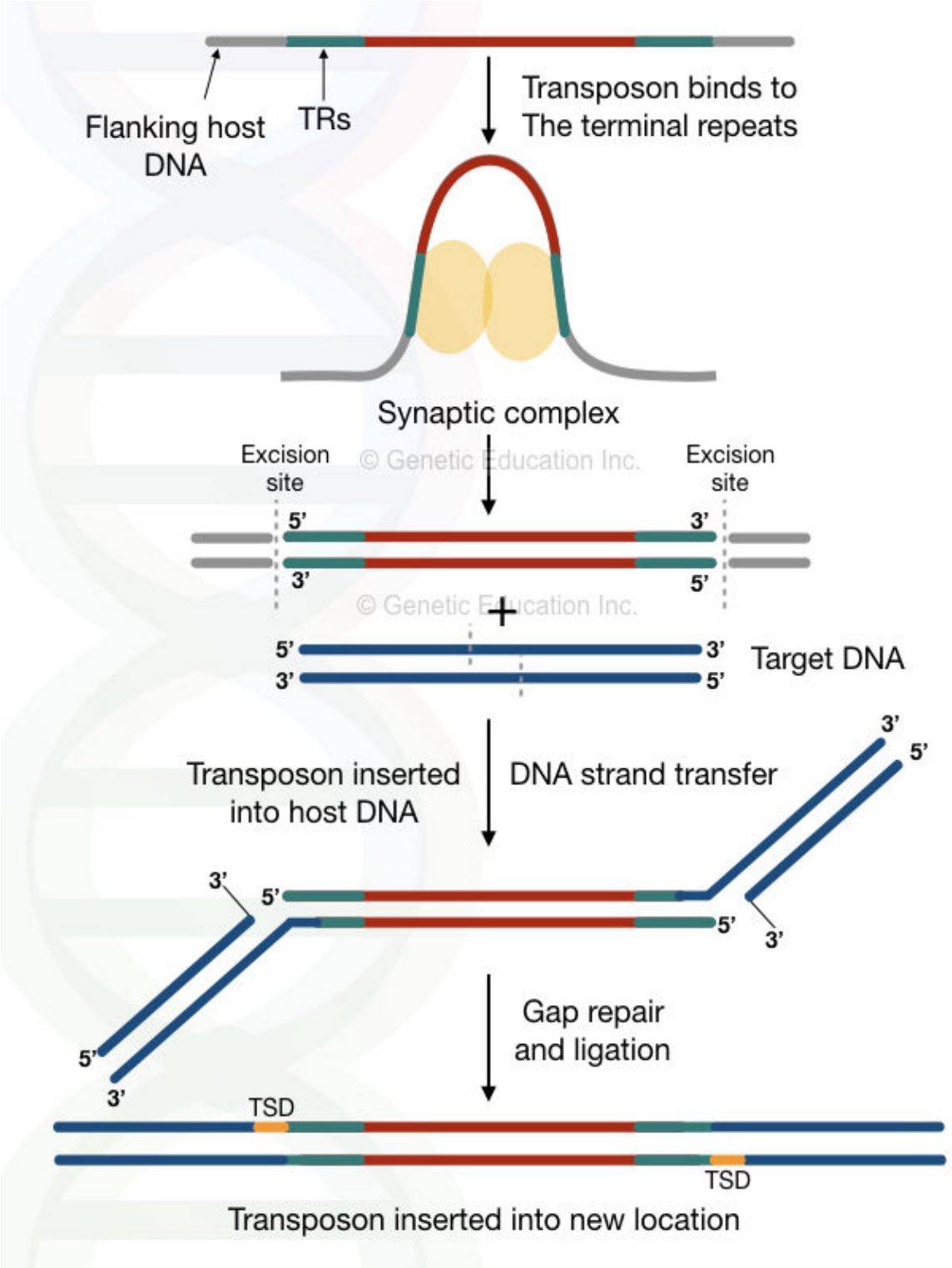



**Figure 11. The mechanism of conservatrice transposition.**

### 1.2.2.5. Biological significance of transposons

Transposons play an essential role in genome evolution and adaptation:

- **Genetic variation** – Introduce mutations by inserting into genes or regulatory regions.
- **Antibiotic resistance** – Many bacterial transposons carry resistance genes that spread through horizontal gene transfer.
- **Genome evolution** – Drive rearrangements, duplications, and deletions.
- **Horizontal gene transfer** – Facilitate the exchange of genetic material between organisms.



### 1.2.2.6. Applications of transposons in biotechnology

- **Genetic engineering** – Used as tools for gene delivery and mutagenesis (e.g., Sleeping Beauty transposon system).
- **Gene therapy** – Potential use in correcting genetic defects.
- **Antibiotic resistance studies** – Help in understanding the spread of resistance genes.

### 1.2.2.7. Consequences of Transposition on Bacterial Genome Expression

Transposition, the process by which a mobile genetic element (transposon) relocates within the bacterial genome, can significantly impact gene expression. When a transposon inserts into a coding region, it may disrupt the target gene, leading to its inactivation. Additionally, insertion into a regulatory region can alter the expression of adjacent genes by modifying transcription factor binding sites or introducing new promoters or terminators. Some transposons also carry antibiotic resistance genes or virulence factors, which can enhance bacterial adaptability and pathogenicity. Furthermore, transposition events can contribute to genome plasticity, facilitating genetic variability and evolution in bacterial populations.

### 1.2.2.8. Organization of prokaryotic genes

In prokaryotes, genes are typically organized in operons, which are clusters of functionally related genes transcribed as a single mRNA molecule. This polycistronic arrangement allows coordinated regulation of genes encoding proteins involved in the same metabolic pathway or cellular function. Each operon is controlled by a promoter and an operator, where regulatory proteins such as activators or repressors modulate transcription. In addition to operons, prokaryotic genomes contain regulatory elements like riboswitches, small RNAs, and global transcription factors that fine-tune gene expression in response to environmental signals. Unlike eukaryotic genomes, prokaryotic DNA is not compartmentalized within a nucleus but

is organized into a single, circular chromosome located in the nucleoid region, often accompanied by plasmids that carry accessory genes, including those for antibiotic resistance and virulence.

### 1.2.2.9. Replication of the bacterial genome



Bacterial genome replication follows a semiconservative mechanism, ensuring the faithful transmission of genetic material to daughter cells. It begins at a specific origin of replication (*oriC*) and proceeds bidirectionally with the help of the replisome, a complex of enzymes that includes DNA helicase, primase, DNA polymerase III, and ligase. DNA gyrase and topoisomerase IV alleviate supercoiling stress during replication. The replication forks continue until they reach the *ter* region, where terminator sequences and the *Tus* protein ensure proper completion of the process. Since bacteria divide rapidly, some species initiate a new round of replication before completing the previous one, allowing rapid genome duplication under favorable conditions (Figure 12).

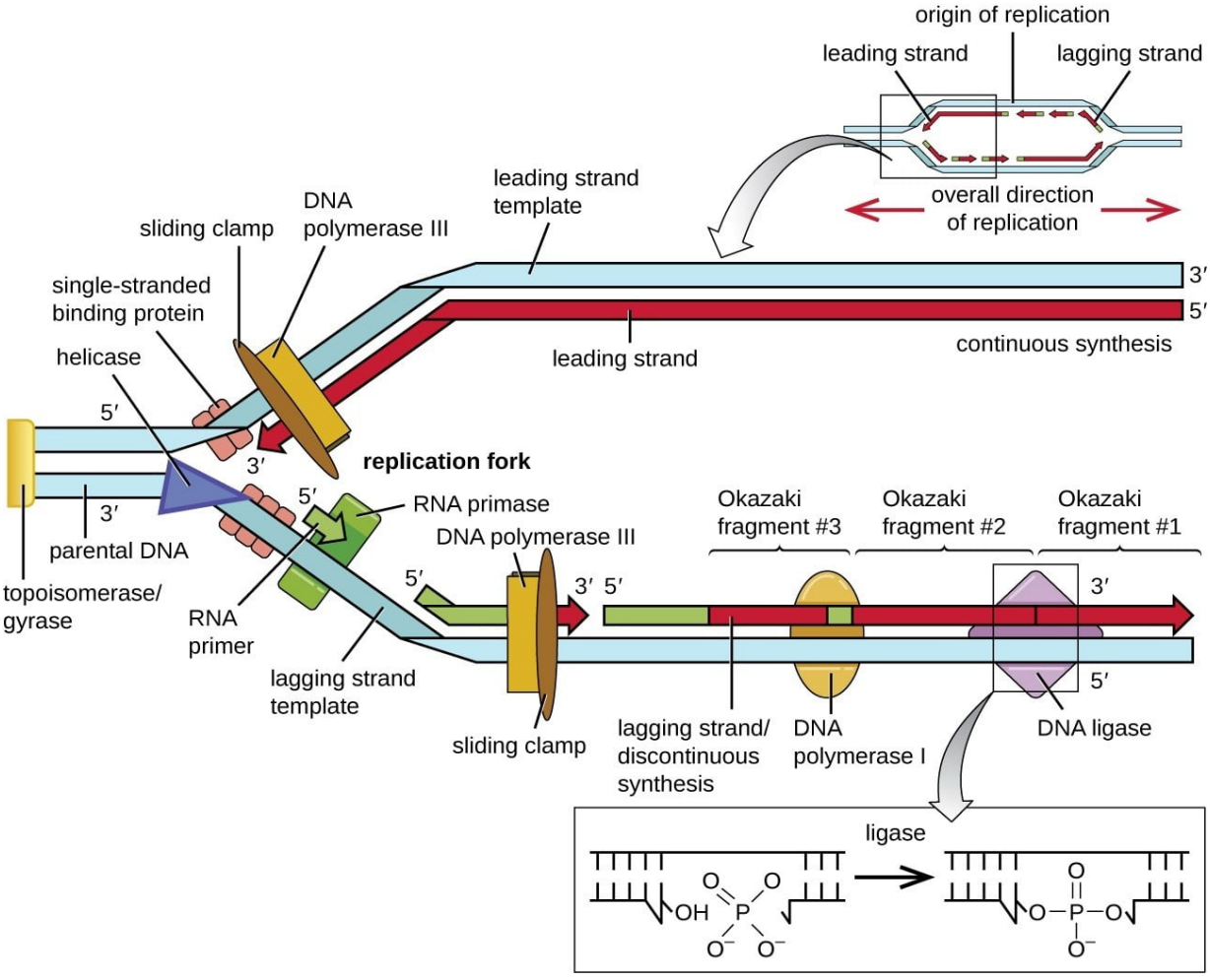



**Figure 12. Bacterial DNA Replication.**

### 1.2.2.10. Alterations and repair mechanisms of the bacterial genome

Bacterial genomes are constantly exposed to mutations caused by DNA replication errors, environmental stress, and mobile genetic elements. These alterations can lead to point mutations, insertions, deletions, or large-scale genomic rearrangements. To maintain genome integrity, bacteria have evolved several DNA repair mechanisms:



- **Mismatch Repair (MMR):** Recognizes and corrects replication errors by distinguishing between newly synthesized and template strands.
- **Base Excision Repair (BER):** Removes damaged bases, such as those caused by oxidative stress or alkylation, and replaces them with the correct nucleotides.
- **Nucleotide Excision Repair (NER):** Eliminates bulky DNA lesions, including UV-induced pyrimidine dimers, by excising a short DNA segment and filling the gap with DNA polymerase I.
- **Recombinational Repair:** Uses homologous recombination to repair double-strand breaks and collapsed replication forks, mediated by the RecA protein.
- **SOS Response:** An inducible repair system activated under extreme DNA damage, involving error-prone polymerases (*Pol IV* and *Pol V*) that introduce mutations but allow cell survival.

Despite these repair mechanisms, some mutations persist, contributing to bacterial evolution, antibiotic resistance, and adaptation to changing environments (Figure 13).

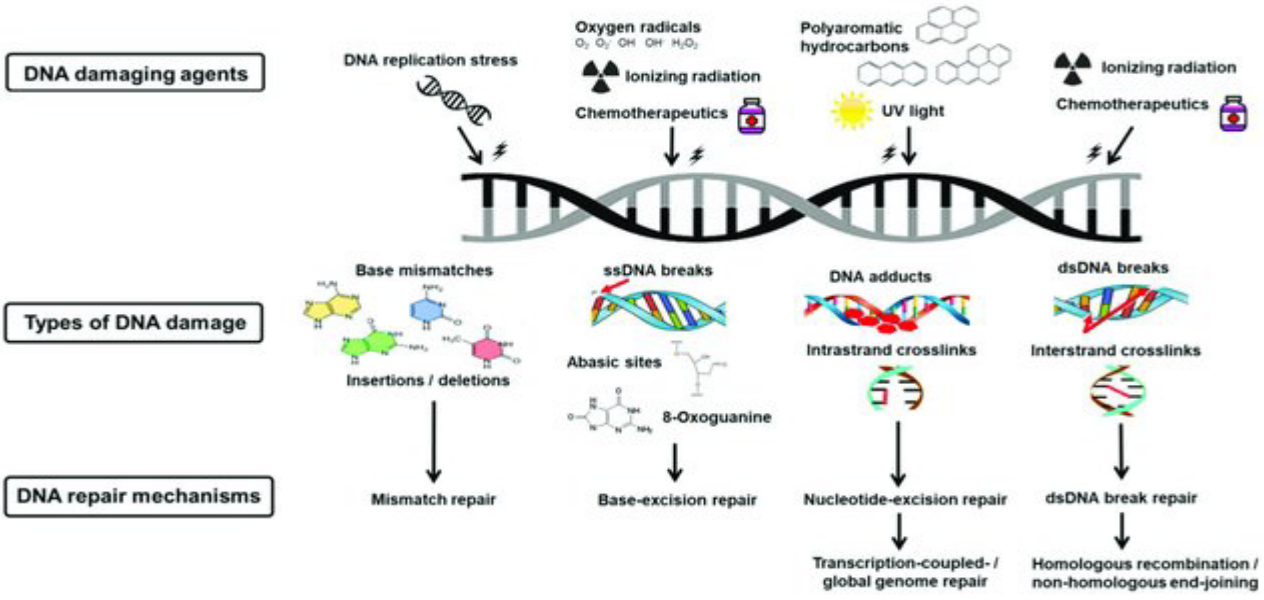



**Figure 13. DNA damage and repair mechanisms.**

# Part 1: Bacteria

# Chapter 2: Horizontal Gene Transfer

# Chapter 2: Horizontal Gene Transfer

## Introduction

Horizontal gene transfer (HGT) is also widespread among Archaea and between bacteria. This process is considered one of the main factors contributing to the increasing resistance of bacteria to antibiotics. Once resistance is acquired by a cell, it can be transmitted between bacteria of different species and even different genera. There are three primary systems of genetic material exchange in prokaryotes: conjugation, transformation, and transduction.



## 1. Bacterial conjugation

Conjugation is a form of genetic exchange in which conjugative plasmids are transferred from a donor bacterium to a recipient bacterium via sex pili. The transfer of the plasmid between donor and recipient organisms occurs in four main steps (Figure 14):

### A. Recognition between donor (F+) and recipient (F-)

Recognition is mediated by the pilus, a proteinaceous structure that links the donor and recipient. The donor synthesizes the pilus using genetic information present on the plasmid. The pilus is a tubular structure composed of protein filaments (a polymer of pilin) (Figure 15).

The pilus is essential for initiating the donor-receptor aggregation. It interacts with OmpA proteins on the outer membrane of the recipient bacterium (F-). Once established, the pilus brings the two bacteria into contact by depolymerizing in the middle, pulling the cells together. Upon contact, the pilus reorganizes into a secretion pore, with part of it penetrating the recipient membrane to facilitate plasmid transfer.

### B. Transfer of a single DNA strand

Once the two bacteria are stably aggregated, a single strand of the plasmid is transferred. A set of proteins known as the relaxosome facilitates this process. The relaxosome contains proteins responsible for DNA cleavage, strand unwinding (via helicases), and strand transport to the secretion pore (via the transferasome). A single-stranded nick is introduced at the origin of transfer (*oriT*), and the strand is transferred to the recipient bacterium in a 5' to 3' direction. Simultaneously, in the donor cell, the complementary strand is resynthesized by DNA polymerase to maintain a double-stranded plasmid.

### C. Synthesis of the Complementary Strand

Once inside the recipient bacterium, the complementary strand is synthesized by DNA polymerase, forming a new double-stranded plasmid.

### D. Recircularization of the New Plasmid

Recircularization occurs via a protein (relaxase) that joins the two ends of the newly synthesized strand.

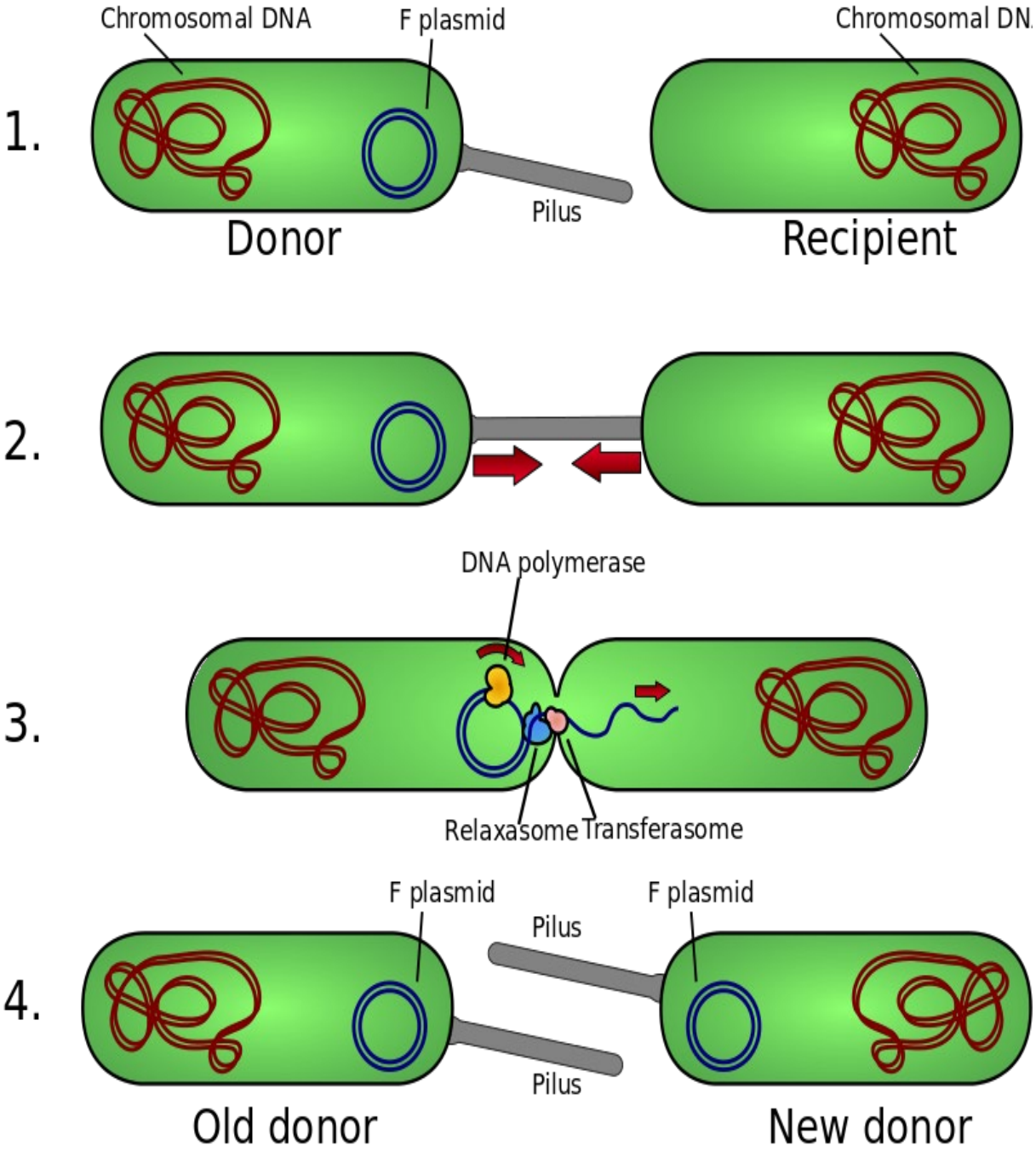



**Figure 14. Bacterial conjugation steps.**

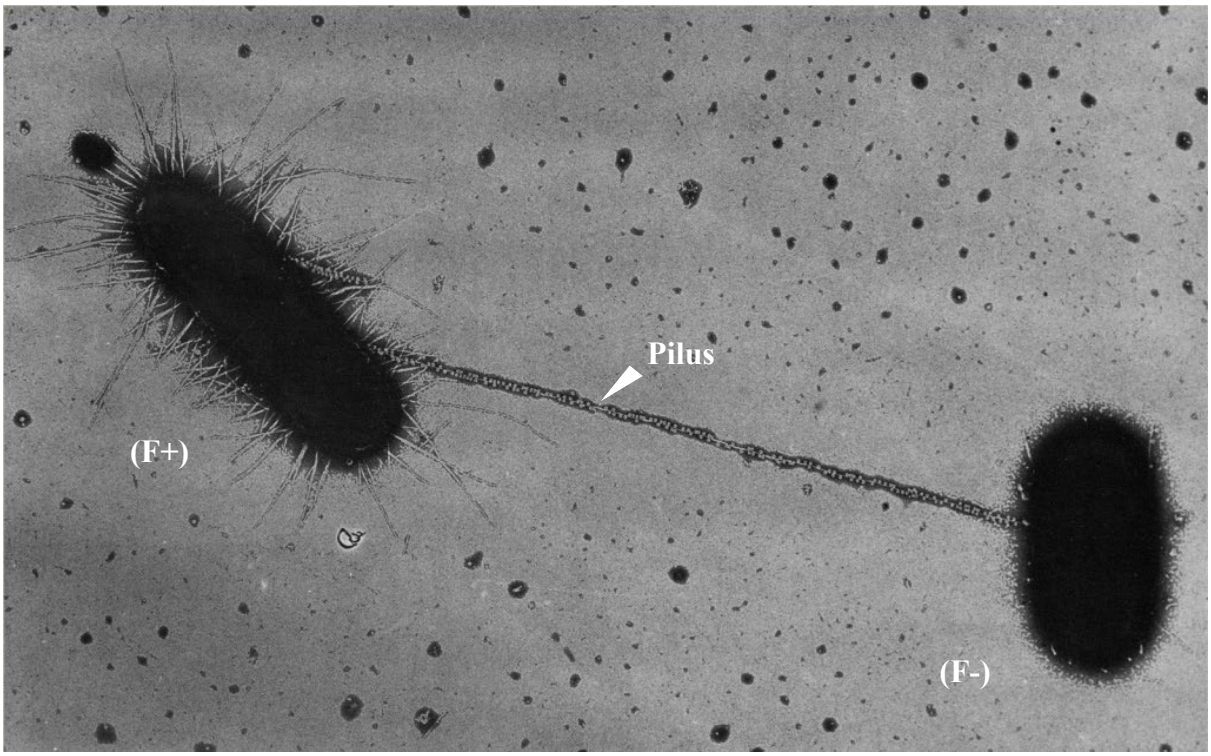





**Figure 15. Bacteria exchanging genes (*E. coli*).**

## 2. Genetic transformation

Genetic transformation is the uptake and integration of foreign DNA fragments into a bacterial cell, potentially altering the recipient organism's phenotype. It is a natural and common phenomenon among bacteria. This process was first discovered in 1928 by the English physician Frederick Griffith.

Natural genetic transformations contribute to genetic recombination and introduce new traits into the genome, playing a role in species evolution. Artificial transformations, on the other hand, are performed through genetic engineering techniques, allowing gene insertion or replacement (transgenesis) (Figure 16).

Bacterial transformation is a widely used technique in molecular biology laboratories. It enables the introduction of genes of interest into bacteria. A well-known experiment in bacterial transformation is Griffith's experiment, which demonstrated transformation when non-virulent (R) bacterial strains became virulent (S) due to the uptake of genetic material from previously killed (S) bacteria (Figure 17).

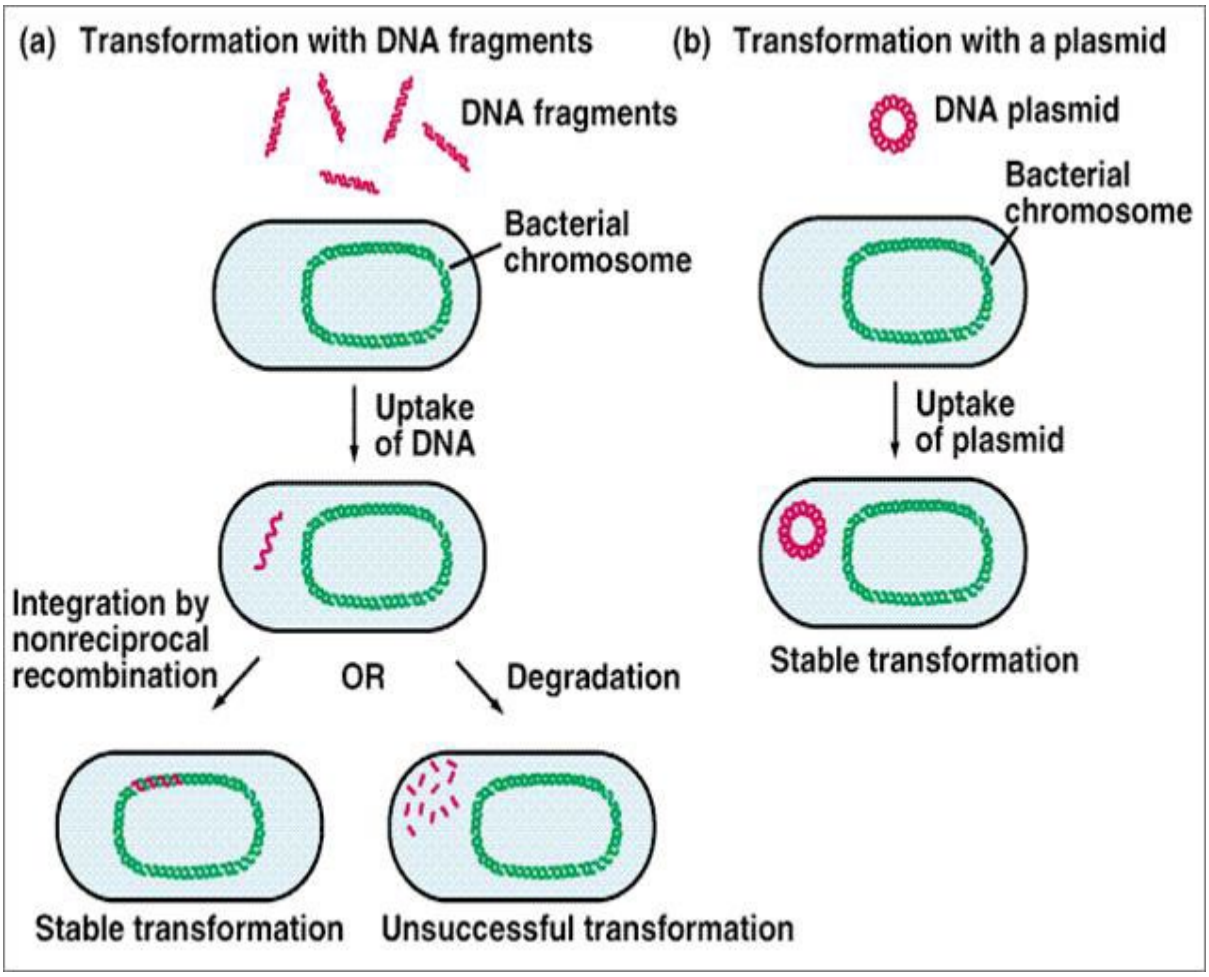



**Figure 16. Mecanism of bacterial transformation.**

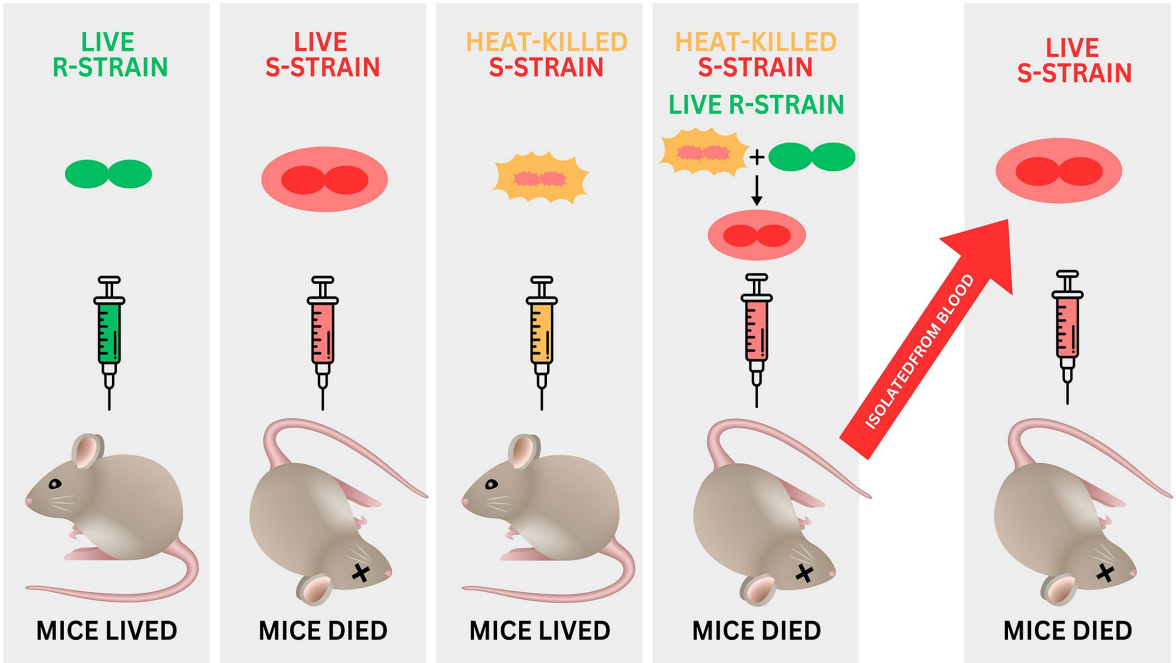



**Figure 17. Griffith's experiment.**

### 3. Transduction

In genetics, transduction is a process by which bacterial DNA is transferred from a donor bacterium to a recipient bacterium via a viral vector (bacteriophage). A genetic marker is transduced when it has been packaged into a phage particle and integrated into the genome by recombination.

There are two types of transduction: generalized and specialized. Transduction, as well as the bacteriophage lambda, was discovered by Esther Lederberg.



### 3.1. Bacteriophage

A bacteriophage infects a donor bacterium and injects its viral DNA through the bacterial cell wall. New phages develop inside the host, some incorporating bacterial DNA into their capsids. Upon release, these phages infect other bacteria, transferring bacterial DNA into the new recipient cell (Figure 18).

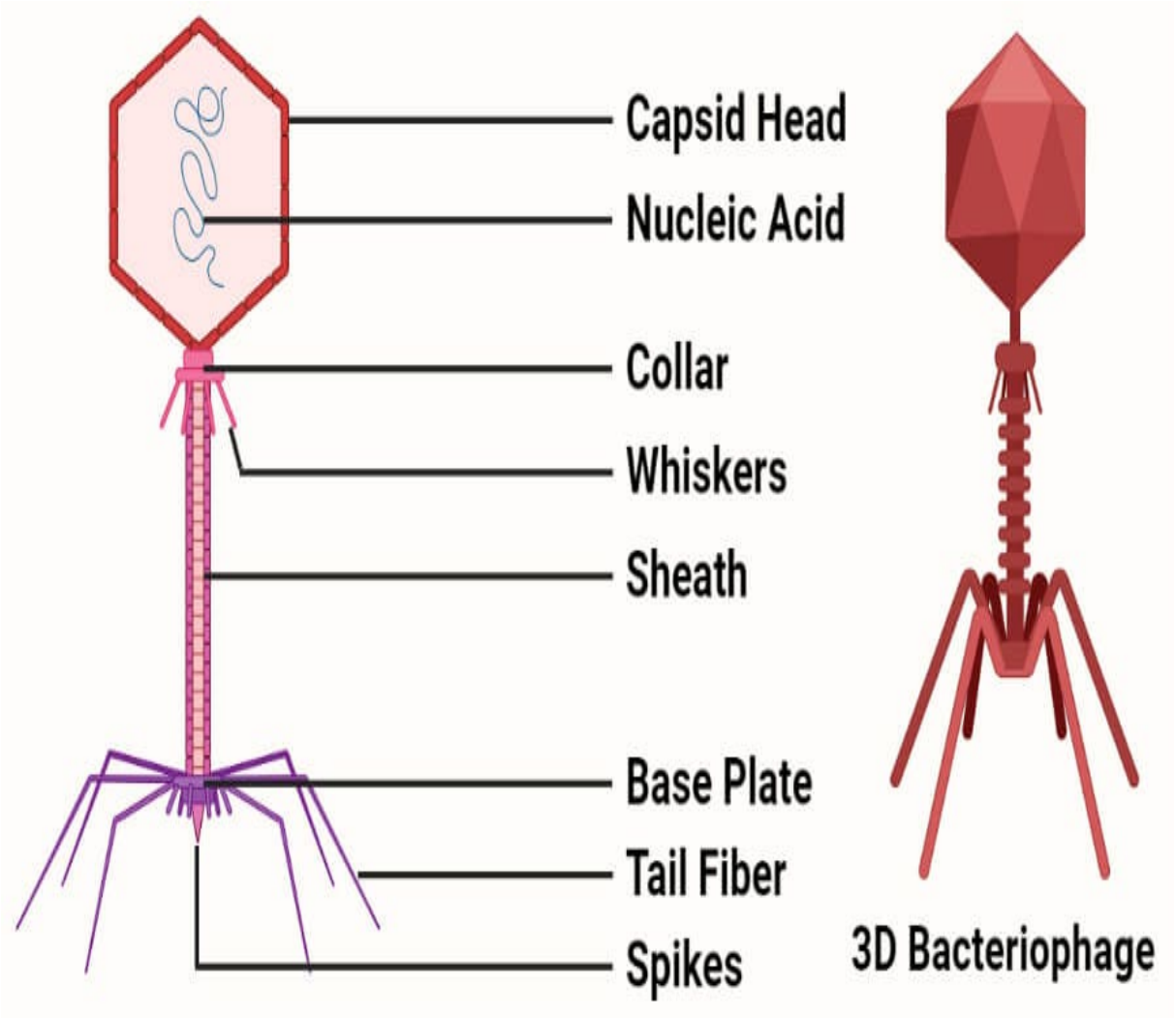



**Figure 18. Structure of bacteriophage.**

### 3.1.1. Infection process of bacteriophage

The infection process of a bacteriophage consists of several key steps (Figure 19):

- **Adsorption**

The bacteriophage recognizes and binds to specific receptors on the bacterial cell surface. This interaction is highly specific and depends on the type of bacteriophage and the host bacterium.



- **Penetration**

The phage injects its genetic material (DNA or RNA) into the bacterial cell. This is usually achieved by contracting its tail sheath, which acts as a syringe, puncturing the bacterial membrane and allowing the nucleic acid to enter. The empty capsid remains outside the host.

- **Biosynthesis**

Once inside the bacterium, the phage genome hijacks the host's cellular machinery to replicate its DNA/RNA and produce viral proteins. Host metabolism is redirected towards viral genome replication and protein synthesis.

- **Assembly**

New phage particles are assembled by combining replicated viral genomes with newly synthesized capsid proteins. This process leads to the formation of mature virions inside the host cell.

- **Release**

In the lytic cycle, the bacteriophage produces enzymes (e.g., endolysins) that break down the bacterial cell wall, leading to cell lysis and the release of newly formed phages, which can then infect new bacterial cells.

In the lysogenic cycle, the viral genome integrates into the bacterial chromosome as a prophage. The prophage is replicated along with the host genome until external factors trigger its excision, leading to the lytic cycle.

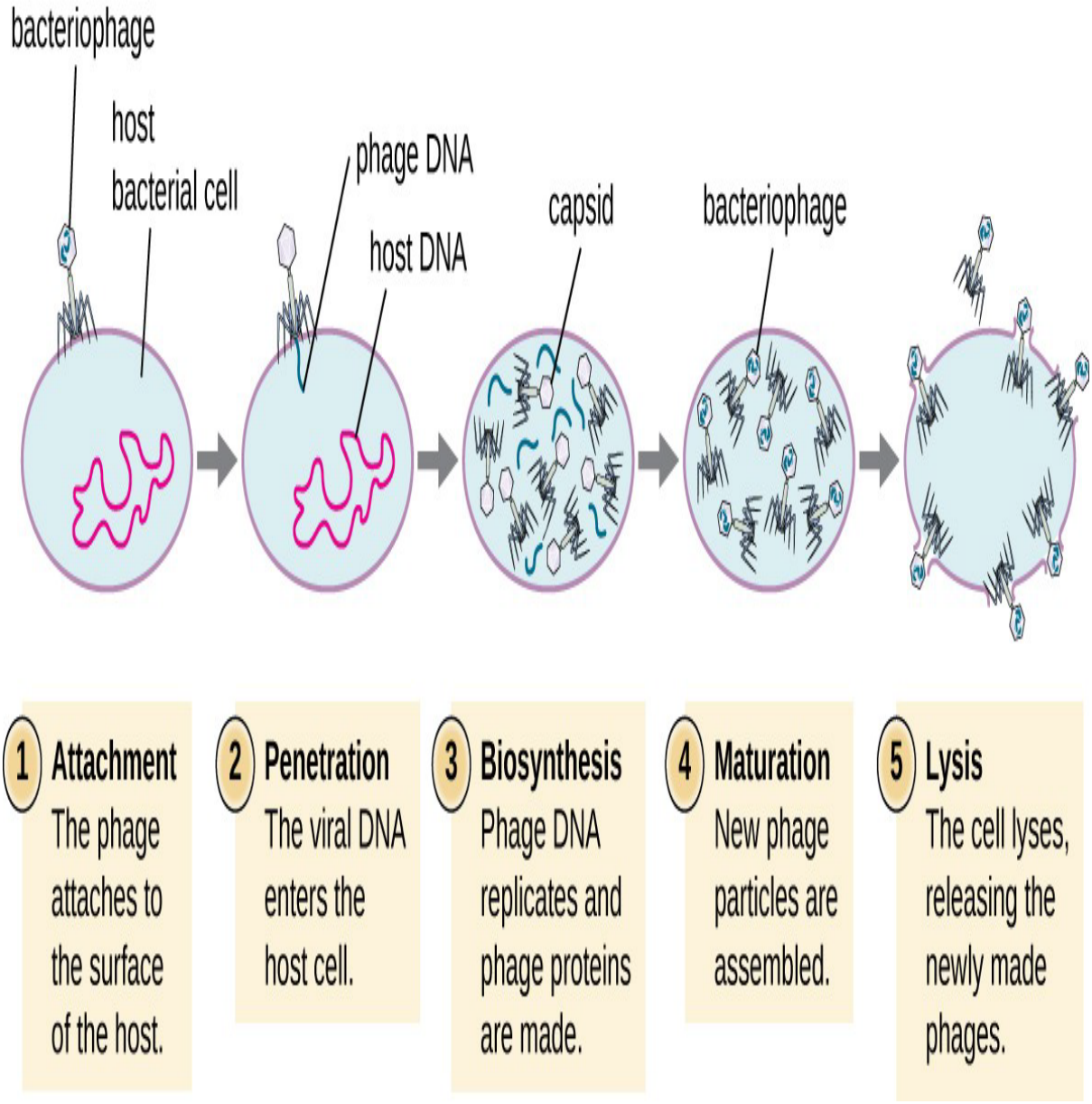



**Figure 19. The infection process of a bacteriophage.**

### 3.1.2. Lysogenic and lytic cycles

Once integrated, the viral DNA may follow either a lysogenic or lytic pathway (Figure 20).

- In the lysogenic cycle, the viral DNA remains dormant as a prophage, integrated into the bacterial genome without being expressed. The host bacterium, termed lysogenic, remains unaffected under normal conditions. However, under stress (e.g., UV radiation or environmental pressures), lysogenic phages may transition to a lytic cycle.
- The lytic cycle involves the production of numerous new phages, ultimately causing bacterial cell lysis and phage release.

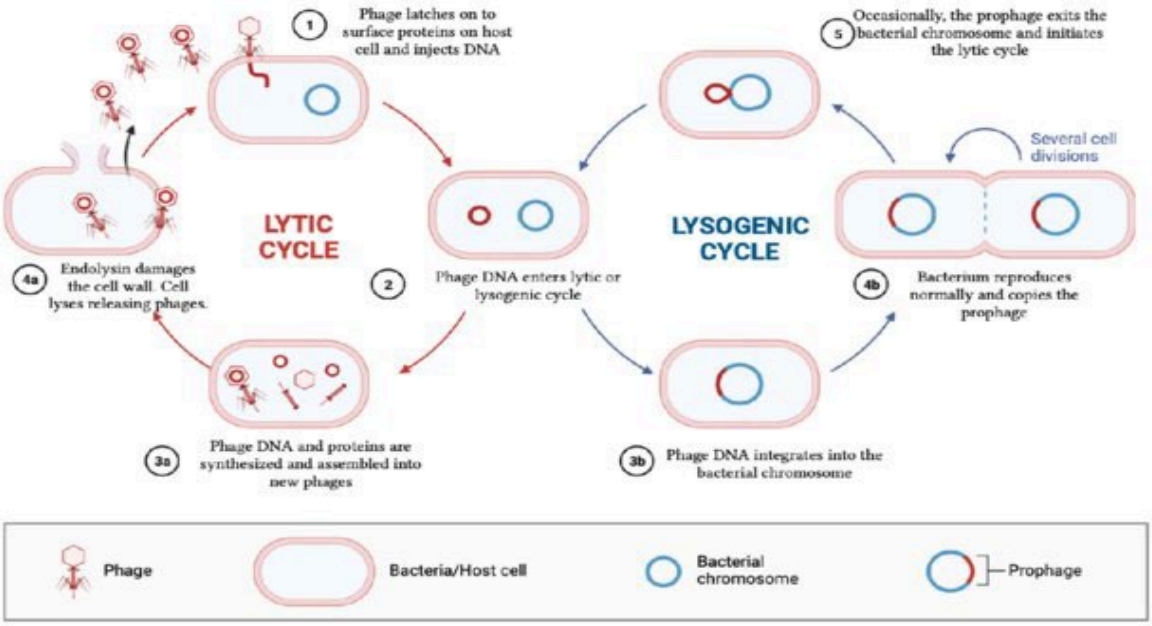



**Figure 20. Lysogenic and lytic cycles.**

### 3.2. Generalized transduction

Generalized transduction is mediated by lytic phages. These phages degrade the host genome to package their own viral DNA. Occasionally, host DNA fragments of the same size as the viral genome are mistakenly packaged into phage capsids. These host DNA fragments can then integrate into a new recipient bacterium via homologous recombination.

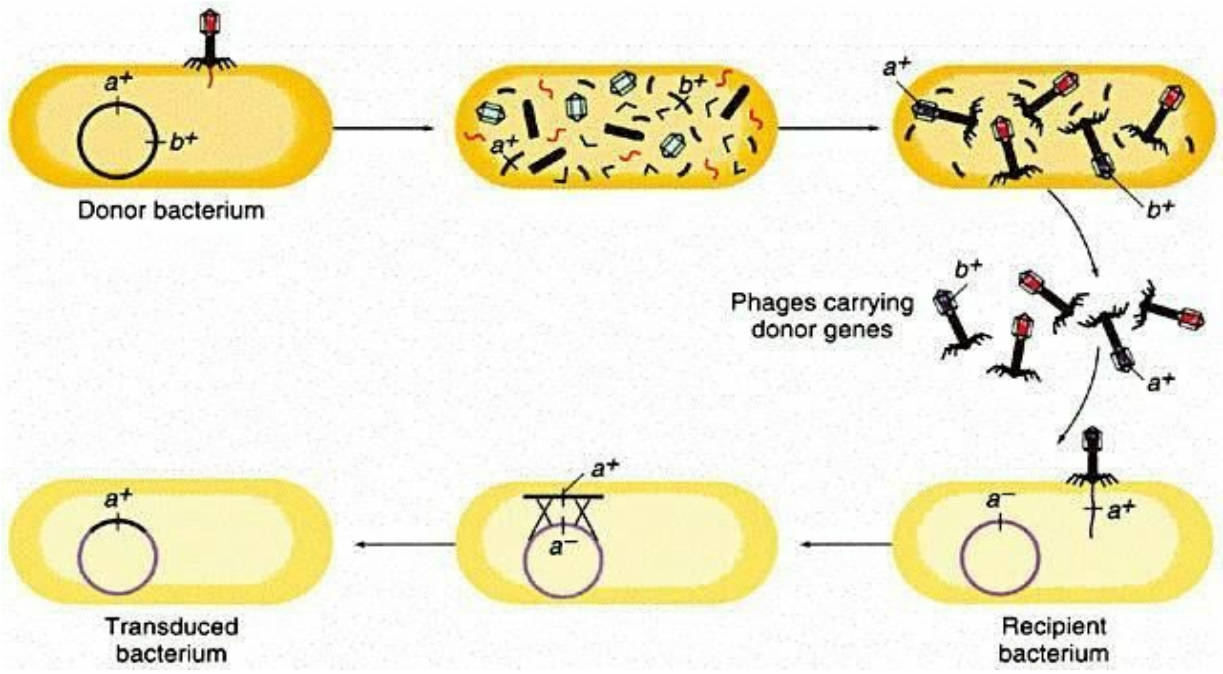



**Figure 21. The mechanism of generalized transduction.**

### 3.3. Specialized transduction

Specialized transduction is mediated by temperate phages, which can enter both lysogenic and lytic cycles. Only specific regions of bacterial DNA can be transduced. This process results from improper excision of the viral genome, producing a hybrid genome containing both phage and bacterial DNA.



Under normal conditions, phage DNA is fully excised from the bacterial genome. However, with a low frequency ($10^{-5}$ to $10^{-6}$), improper excision leads to the release of a hybrid DNA molecule composed of viral and bacterial DNA fragments. For example, in the case of **lambda phage**, only **gal** or **bio** genes can be transferred from a donor bacterium to a recipient bacterium (Figure 22).

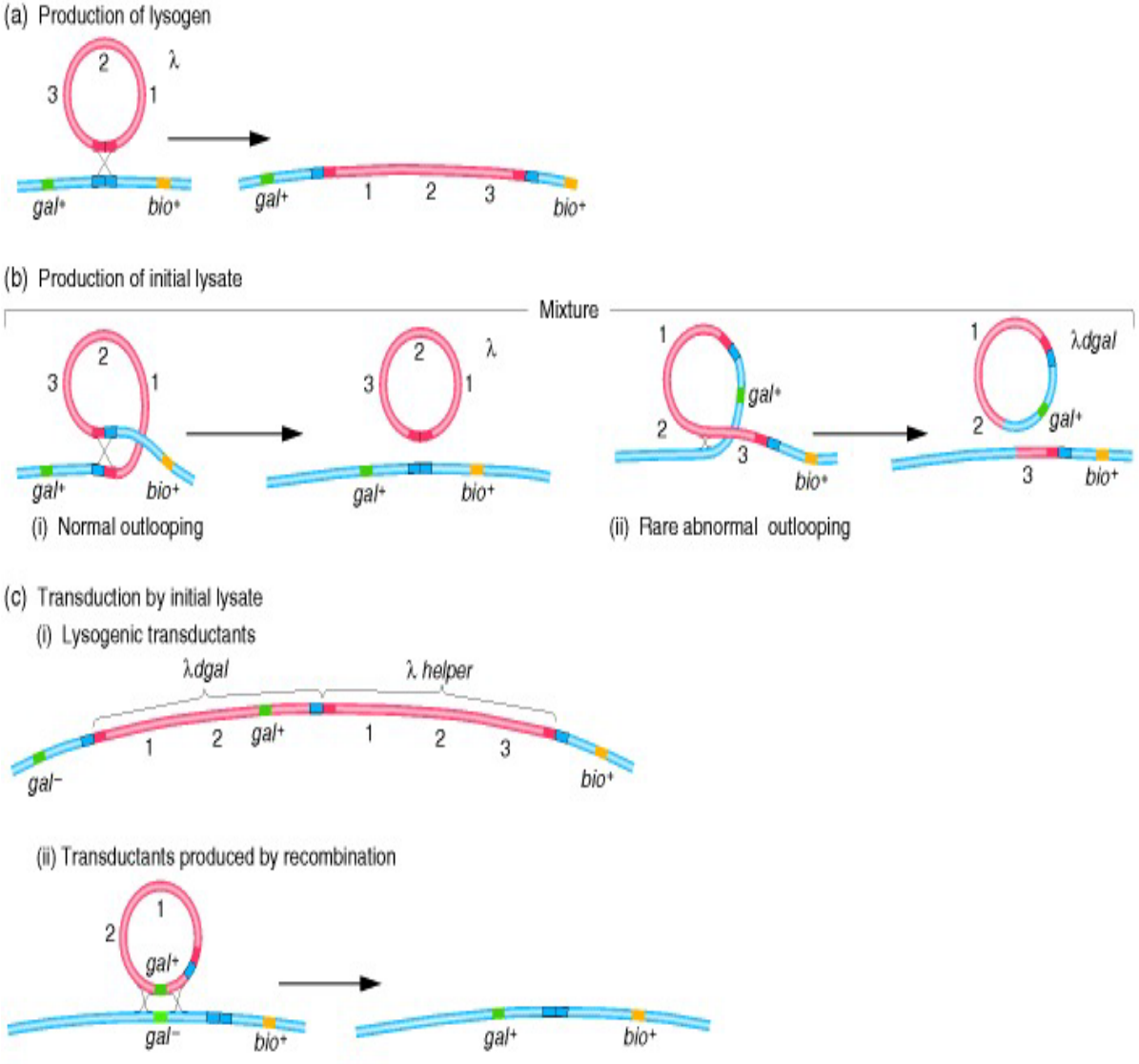



**Figure 22. The mechanism of specialized transduction.**

### 4. Genetic mapping in prokaryotes

Genetic mapping in prokaryotes refers to the determination of gene order and relative distances on the bacterial chromosome based on recombination frequencies. Unlike eukaryotic genetic maps, which rely on meiosis, bacterial genetic maps are constructed using three main methods: **conjugation, transformation, and transduction**. In conjugation-based mapping, the transfer of genes from an Hfr donor to an $F^-$ recipient is timed, allowing genes to be mapped in **minutes** based on their entry sequence. Transformation mapping relies on the uptake of foreign DNA and co-transformation frequency to determine gene proximity. Transduction mapping, performed using bacteriophages, measures the frequency of co-transduction to establish genetic linkage. These methods provide a functional and evolutionary understanding of bacterial genomes, helping to track gene transfer, antibiotic resistance spread, and genome organization (Figure 23).

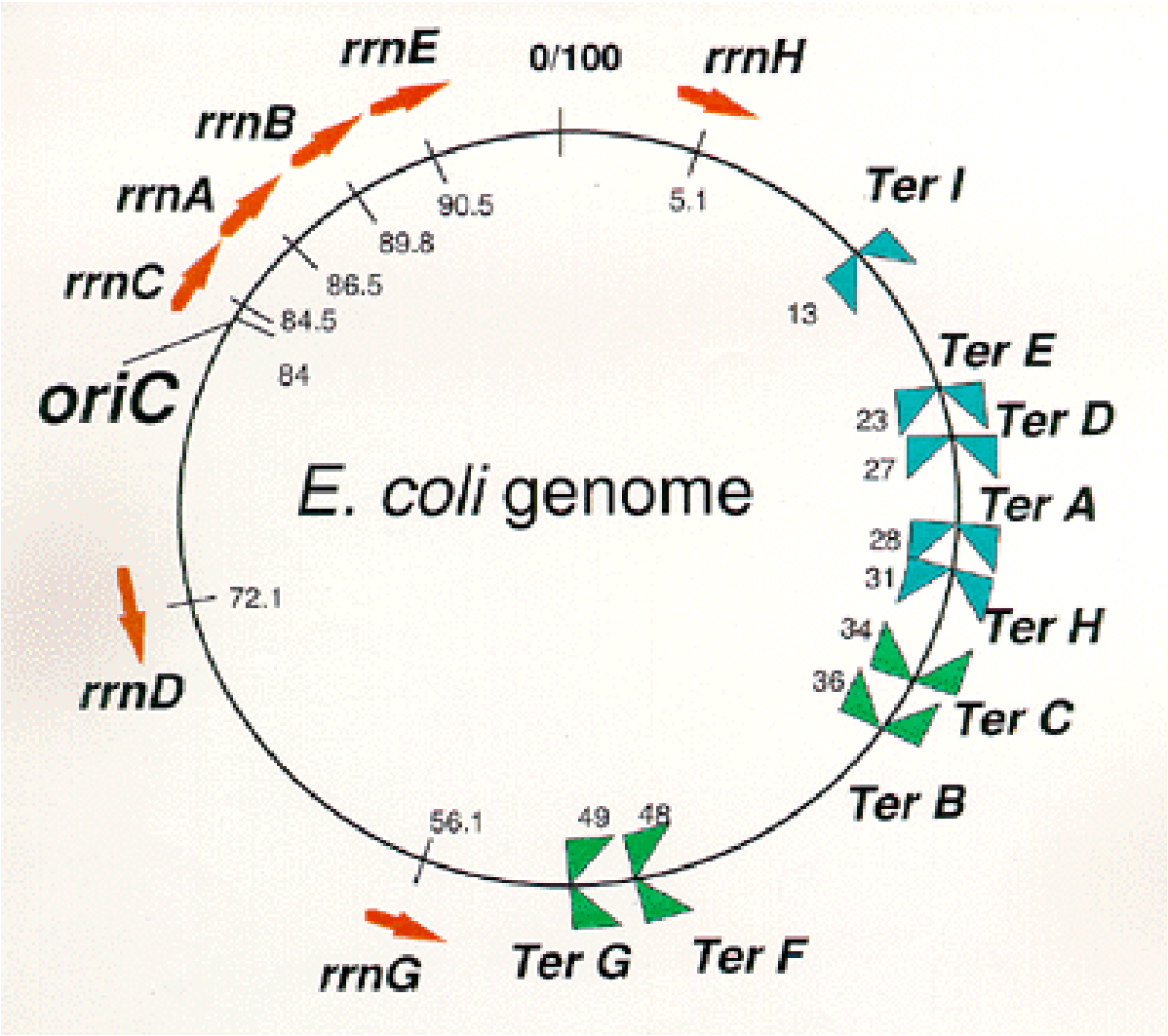



**Figure 23. Genetic mapping of *E. coli*.**

# Part 1: Bacteria

# Chapter 3: Protein Biosynthesis

# Chapter 3: Protein Biosynthesis

## Introduction

Protein biosynthesis is the complex set of biochemical processes that enable cells to produce proteins from their genes, compensating for protein loss due to secretion or degradation. This process includes DNA transcription into messenger RNA (mRNA), aminoacylation of transfer RNAs (tRNAs), and translation of mRNA into polypeptide chains. Protein synthesis is tightly regulated at multiple levels, mainly during transcription and translation, ensuring proper gene expression and cellular function.



## 1. Transcription

The first step of protein synthesis is transcription, during which a DNA gene is transcribed into an mRNA molecule. RNA has a structure similar to DNA but differs in key aspects: it is single-stranded, contains ribose instead of deoxyribose, and uses uracil (U) instead of thymine (T). In prokaryotic cells, transcription occurs in the cytoplasm and can be divided into three major stages: initiation, elongation, and termination. Each step is regulated by specific proteins to ensure the accurate transcription of the correct gene.

### 1.1. Initiation

Transcription begins at a promoter, a specific nucleotide sequence that includes conserved regions such as the -35 Pribnow box (5’ TTGACA 3’) and the -10 box (5’ TATAAT 3’) in prokaryotes. These adenine-thymine (A=T) rich sequences facilitate the unwinding of the DNA double helix by an RNA polymerase holoenzyme with the help of sigma factors. The RNA polymerase binds to the promoter, unwinds the DNA, and initiates RNA synthesis by reading the template strand in the 3’ → 5’ direction, while synthesizing mRNA in the 5’ → 3’ direction. Unlike in eukaryotes, the prokaryotic mRNA is immediately available for translation upon transcription (Figure 24).

### 1.2. Elongation

During elongation, RNA polymerase moves along the template DNA strand (3’ → 5’), unwinding the DNA and adding ribonucleotides (rNTPs) to the growing RNA strand in the 5’ → 3’ direction. The newly synthesized mRNA remains temporarily hybridized to the DNA before being released (Figure 25).

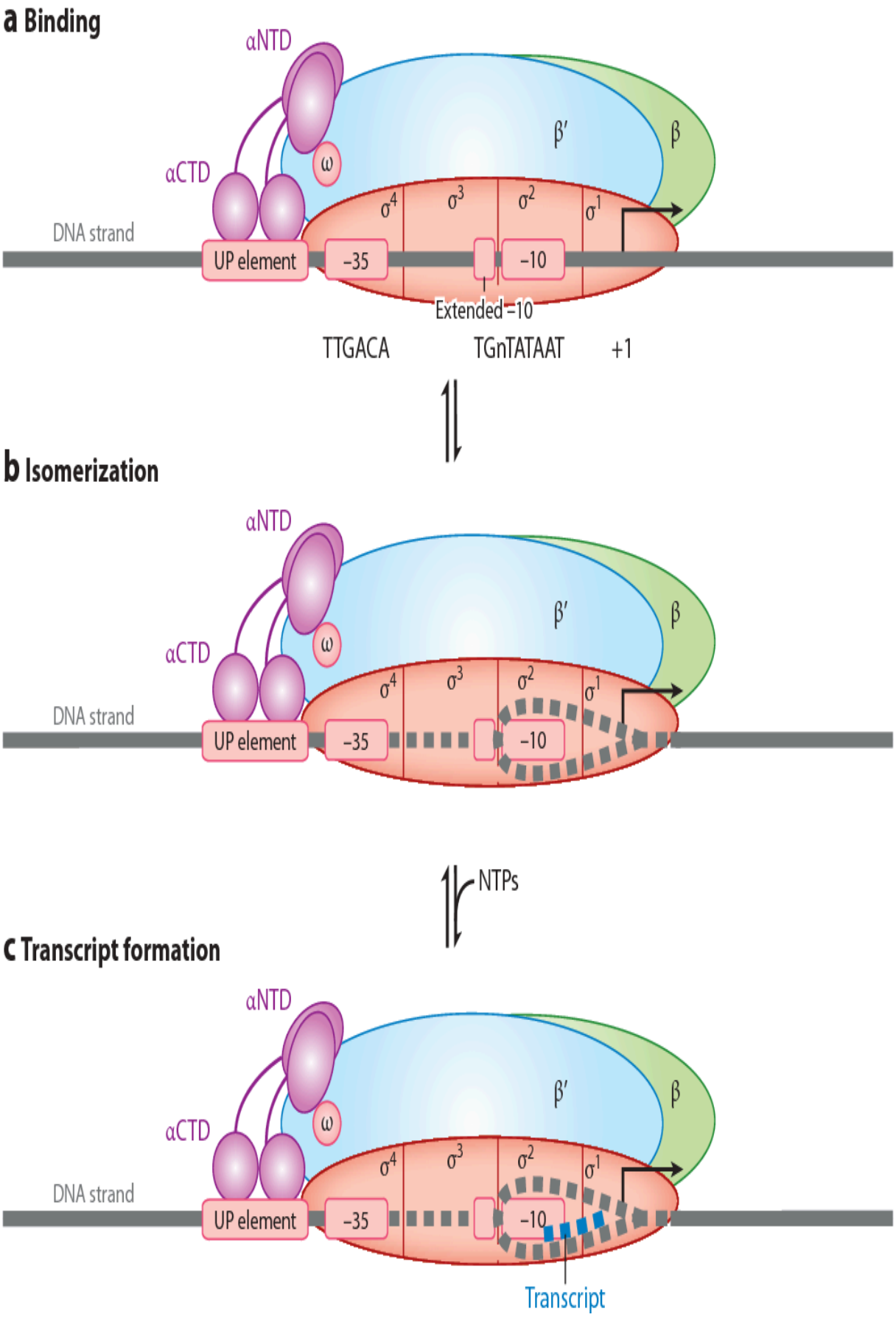



**Figure 24. Transcription initiation mechanism.**

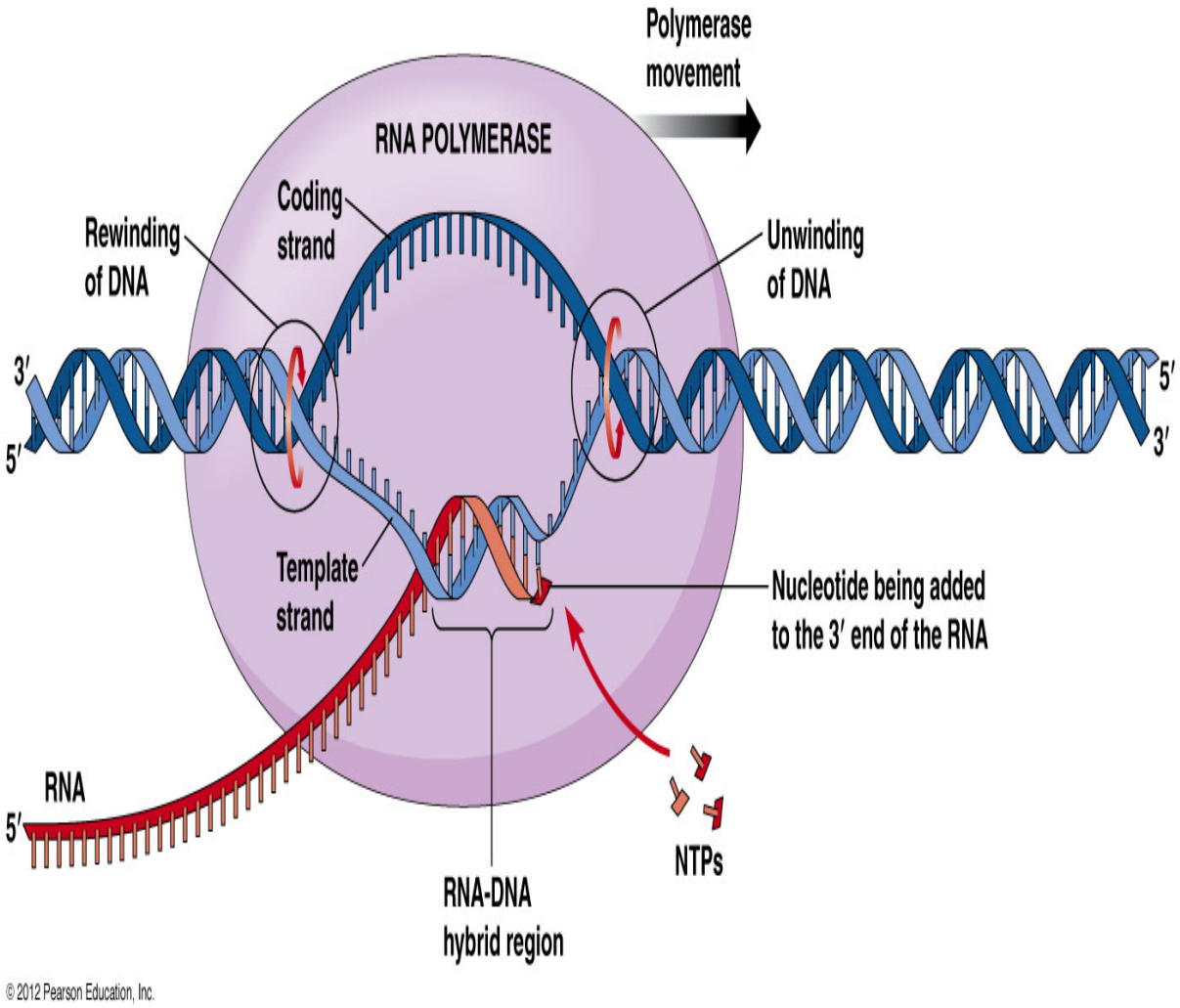



**Figure 25. Transcription elongation mechanism.**

### 1.3. Termination

Transcription stops when RNA polymerase reaches a termination signal. two main mechanisms exist in prokaryotes:

- **Rho independent**

Transcription termination occurs due to specific sequences present in the terminator DNA. These sequences include an inverted repeat, which allows complementary base pairing within the transcribed RNA, forming a hairpin structure. This structural change destabilizes the transcription complex. Following the inverted repeat, a poly-T stretch (~8 bp) in the template DNA results in a corresponding poly-U sequence in the RNA. Since A=U base pairs are weaker than G≡C pairs, the combination of the hairpin structure and the weak RNA-DNA hybrid causes the RNA transcript to dissociate, effectively terminating transcription (Figure 26).

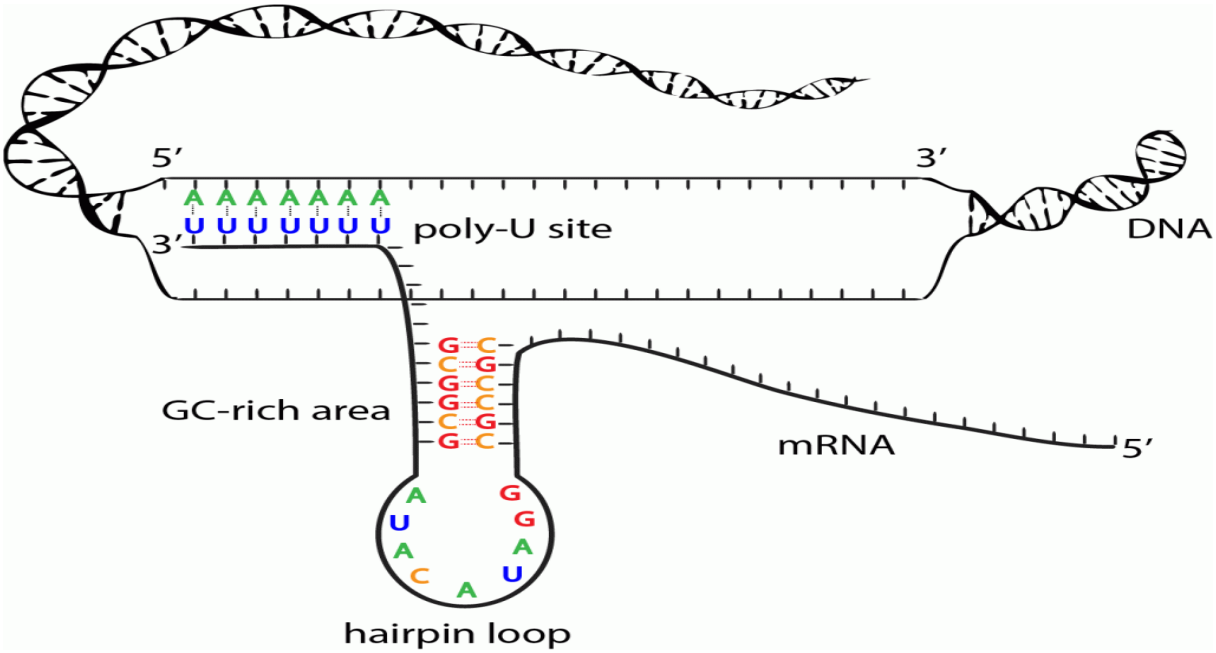



**Figure 26. Rho independent termination mechanism.**

- **Rho dependent**

In this mechanism, transcription termination is facilitated by the Rho (ρ) protein, a ring-shaped, single-stranded RNA-binding ATPase. As the RNA exits the polymerase enzyme complex, Rho binds to the untranslated RNA and hydrolyses it, leading to the release of the transcript from the enzyme complex. Notably, Rho does not bind to RNA that is actively undergoing translation. Since transcription and translation occur simultaneously in bacteria, Rho attaches to the RNA only after translation is complete, but while transcription is still in progress (Figure 27).

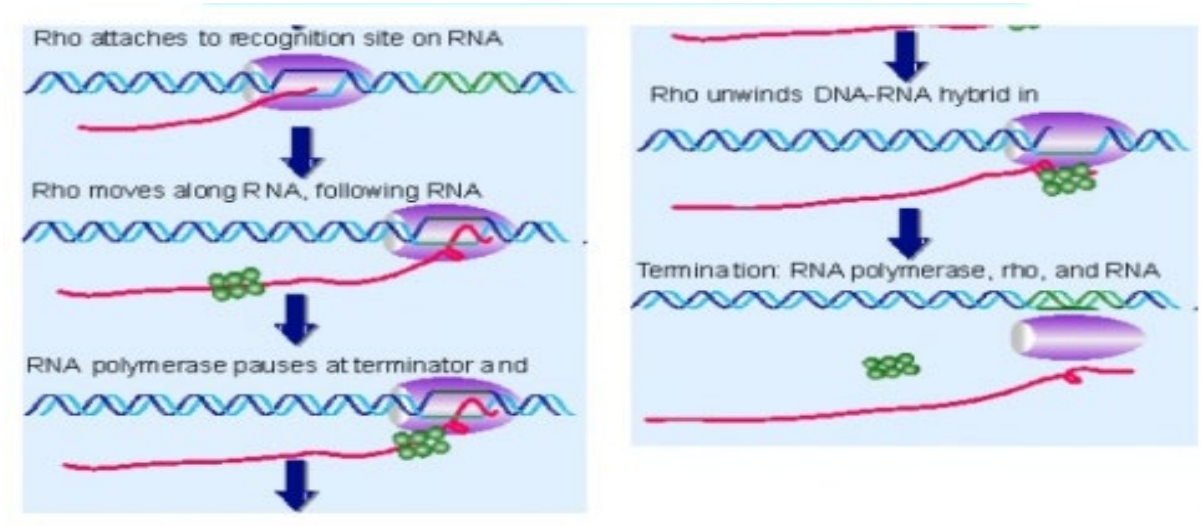



**Figure 27. Rho dependent termination mechanism.**

## 2. Translation

In molecular biology, translation is the process by which ribosomes synthesize proteins using the genetic information encoded in mRNA. The ribosome reads the mRNA sequence in triplets called codons, each corresponding to a specific amino acid. The relationship between codons and amino acids is defined by the genetic code (Table 1).

Translation occurs in three main stages: initiation, elongation, and termination.



**Table 1. The genetic code.**

| | U | | C | | A | | G | | |
|---|---|---|---|---|---|---|---|---|---|
| U | UUU | phénylalanine | UCU | sérine | UAU | tyrosine | UGU | cystéine | U |
| | UUC | | UCC | | UAC | | UGC | | C |
| | UUA | leucine | UCA | | UAA | stop | UGA | stop | A |
| | UUG | | UCG | | UAG | | UGG | tryptophane | G |
| C | CUU | leucine | CCU | proline | CAU | histidine | CGU | arginine | U |
| | CUC | | CCC | | CAC | | CGC | | C |
| | CUA | | CCA | | CAA | glutamine | CGA | | A |
| | CUG | | CCG | | CAG | | CGG | | G |
| A | AUU | isoleucine | ACU | thréonine | AAU | asparagine | AGU | sérine | U |
| | AUC | | ACC | | AAC | | AGC | | C |
| | AUA | | ACA | | AAA | lysine | AGA | arginine | A |
| | AUG | méthionine | ACG | | AAG | | AGG | | G |
| G | GUU | valine | GCU | alanine | GAU | acide aspartique | GGU | glycine | U |
| | GUC | | GCC | | GAC | | GGC | | C |
| | GUA | | GCA | | GAA | acide glutamique | GGA | | A |
| | GUG | | GCG | | GAG | | GGG | | G |

### 2.1. Synthesis of an aminoacyl-tRNA

Aminoacyl-tRNA synthesis is a crucial step in translation, ensuring that each transfer RNA (tRNA) is correctly charged with its corresponding amino acid. This process is catalyzed by a group of enzymes called aminoacyl-tRNA synthetases. Each aminoacyl-tRNA synthetase recognizes a specific amino acid and its corresponding tRNA, ensuring high specificity. The process occurs in two steps: first, the amino acid is activated by ATP, forming an aminoacyl-adenylate intermediate; second, the amino acid is transferred to the 3'-OH group of the tRNA, forming an aminoacyl-tRNA. This charged tRNA is then ready to participate in protein synthesis by delivering the correct amino acid to the ribosome during translation (Figure 28).

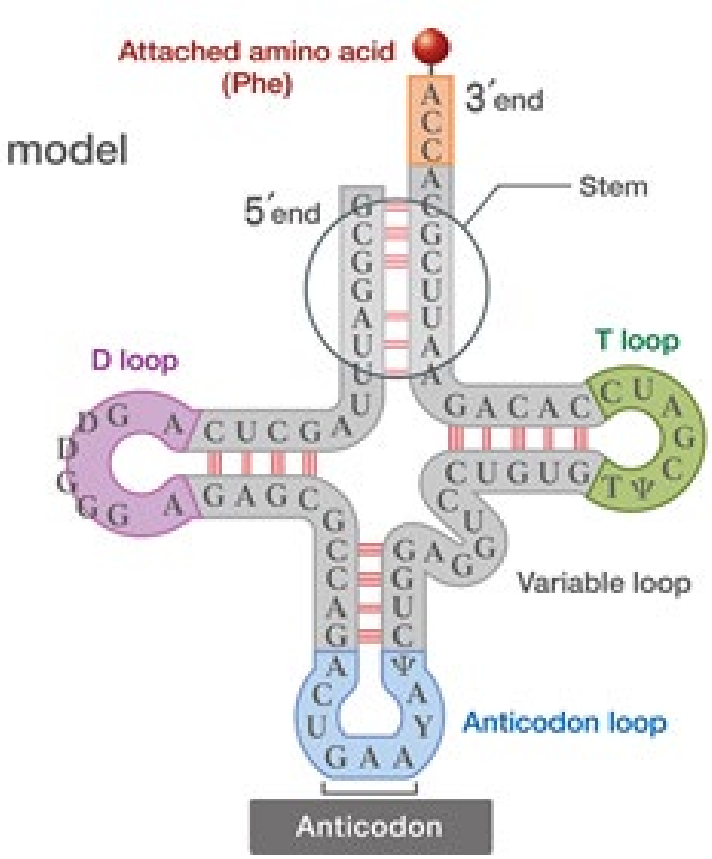



**Figure 28. Structure of an aminoacylated tRNA.**

### 2.2. Structure and function of ribosomes

Prokaryotic ribosomes are the molecular machines responsible for protein synthesis. They are composed of two subunits: the small 30S subunit and the large 50S subunit, forming the functional 70S ribosome. The 30S subunit contains the 16S rRNA, which plays a key role in mRNA decoding and interaction with the Shine-Dalgarno sequence during translation initiation. The 50S subunit contains the 23S and 5S rRNAs and is involved in catalyzing peptide bond formation. Ribosomes have three functional sites: the A (aminoacyl) site, where incoming aminoacyl-tRNAs bind; the P (peptidyl) site, where the growing polypeptide chain is held; and the E (exit) site, where uncharged tRNAs are released. These ribosomal components work together to ensure the accurate and efficient synthesis of proteins in prokaryotic cells (Figure 29).

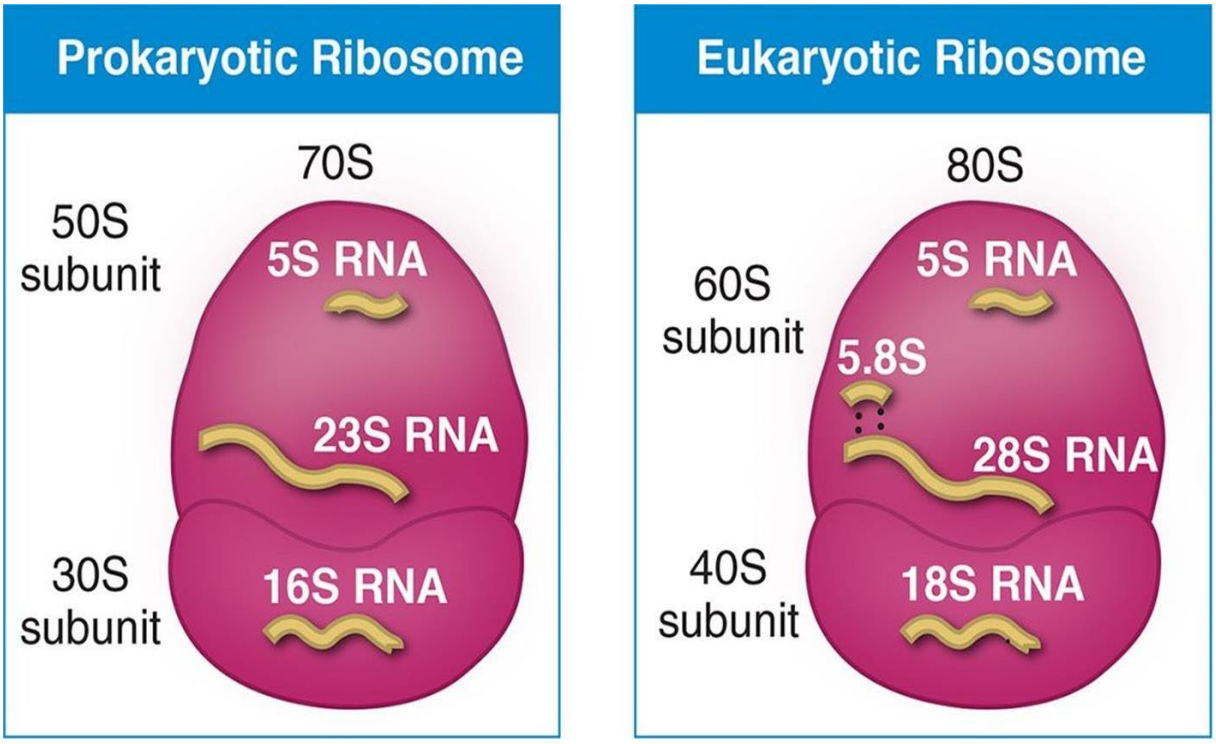



**Figure 29. Structure of procaryotic and eucaryotic ribosomes.**

### 2.3. Translation steps

#### 2.3.1. Initiation

The initiation phase involves the assembly of the ribosome on the start codon (AUG) of the mRNA (Figure 28). In prokaryotes, this process requires the small ribosomal subunit (30S), which must be dissociated from the large subunit (50S) by initiation factors IF1 and IF3.

The start codon (AUG) is recognized by base-pairing between the 3' end of the 16S ribosomal RNA (rRNA) and a complementary sequence on the mRNA known as the Shine-Dalgarno sequence (5' AGGAGGU 3'), located 6-12 nucleotides upstream of the start codon. This interaction positions the ribosome at the correct translation start site.

The first amino acid incorporated into the growing polypeptide is N-formylmethionine (fMet), carried by a specialized initiator tRNA (fMet-tRNA$^{fMet}$). This tRNA has an anticodon UAC, complementary to the AUG start codon. The binding of fMet-tRNA$^{fMet}$ to the ribosome requires the initiation factors IF1, IF2 (GTP-bound), and IF3.

Once the mRNA/tRNA/ribosome complex is correctly assembled, GTP is hydrolyzed, IF2 is released, and the large 50S ribosomal subunit joins the complex, completing ribosome assembly and marking the beginning of elongation (Figure 30).

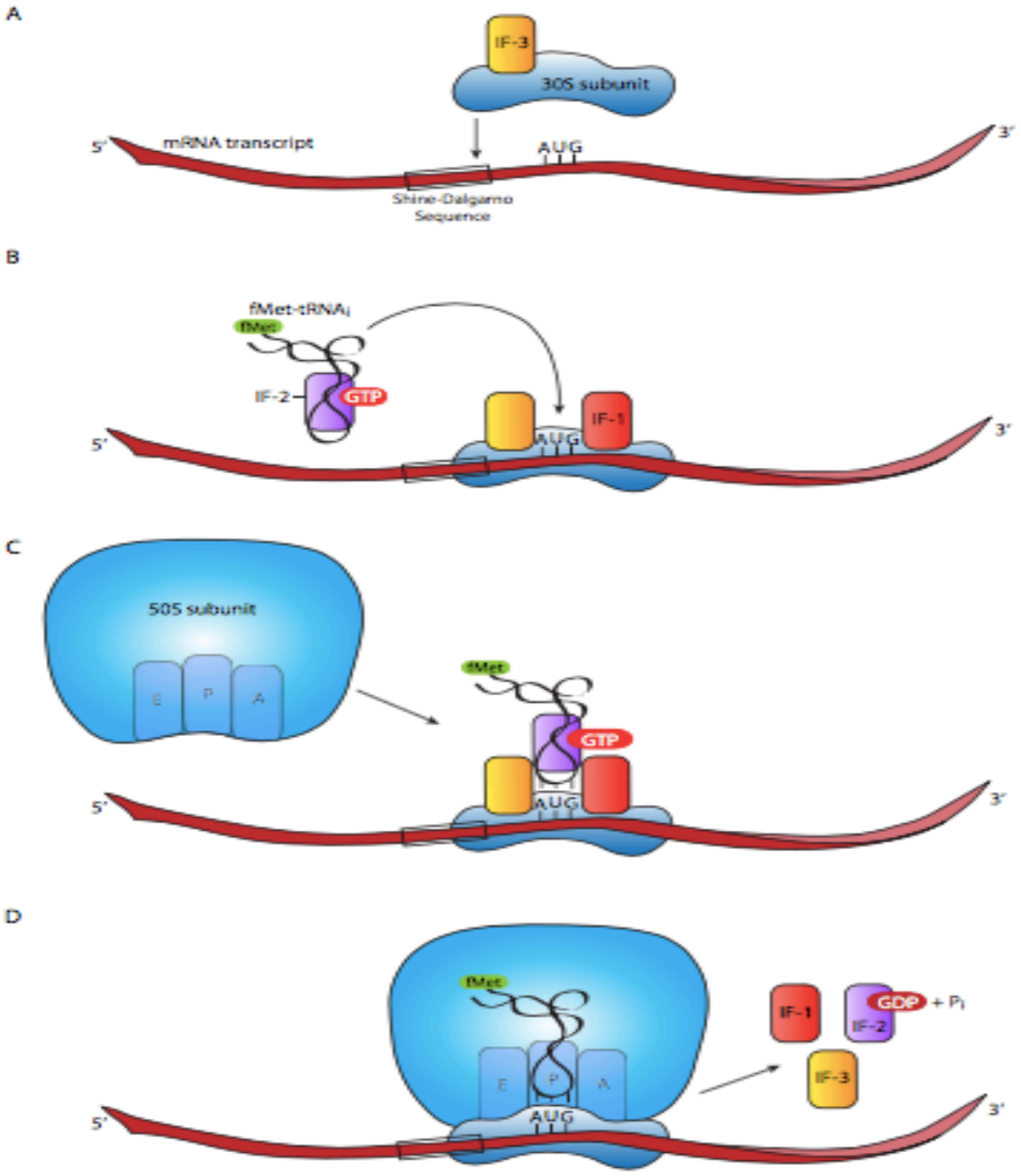



**Figure 30. Translation initiation mechanism.**

### 2.3.2. Elongation

During elongation, the ribosome moves along the mRNA in a 5' → 3' direction, adding amino acids one by one to the growing polypeptide chain. Each cycle involves:

- **Aminoacyl-tRNA selection**

Incoming aminoacyl-tRNAs diffuse into the A (aminoacyl) site of the ribosome. If the tRNA's anticodon is complementary to the mRNA codon, it binds through base-pairing.

- **Peptide bond formation**

The large ribosomal subunit (50S) catalyses the formation of a peptide bond between the carboxyl group of the amino acid in the P (peptidyl) site and the amino group of the amino acid in the A site. This reaction transfers the growing peptide chain onto the new amino acid.



- **Translocation**

The ribosome shifts three nucleotides (one codon) forward on the mRNA, requiring the elongation factor EF-G and GTP hydrolysis. The deacylated tRNA moves to the E (exit) site and is released, while the peptidyl-tRNA moves from the A site to the P site, allowing a new aminoacyl-tRNA to enter the A site.

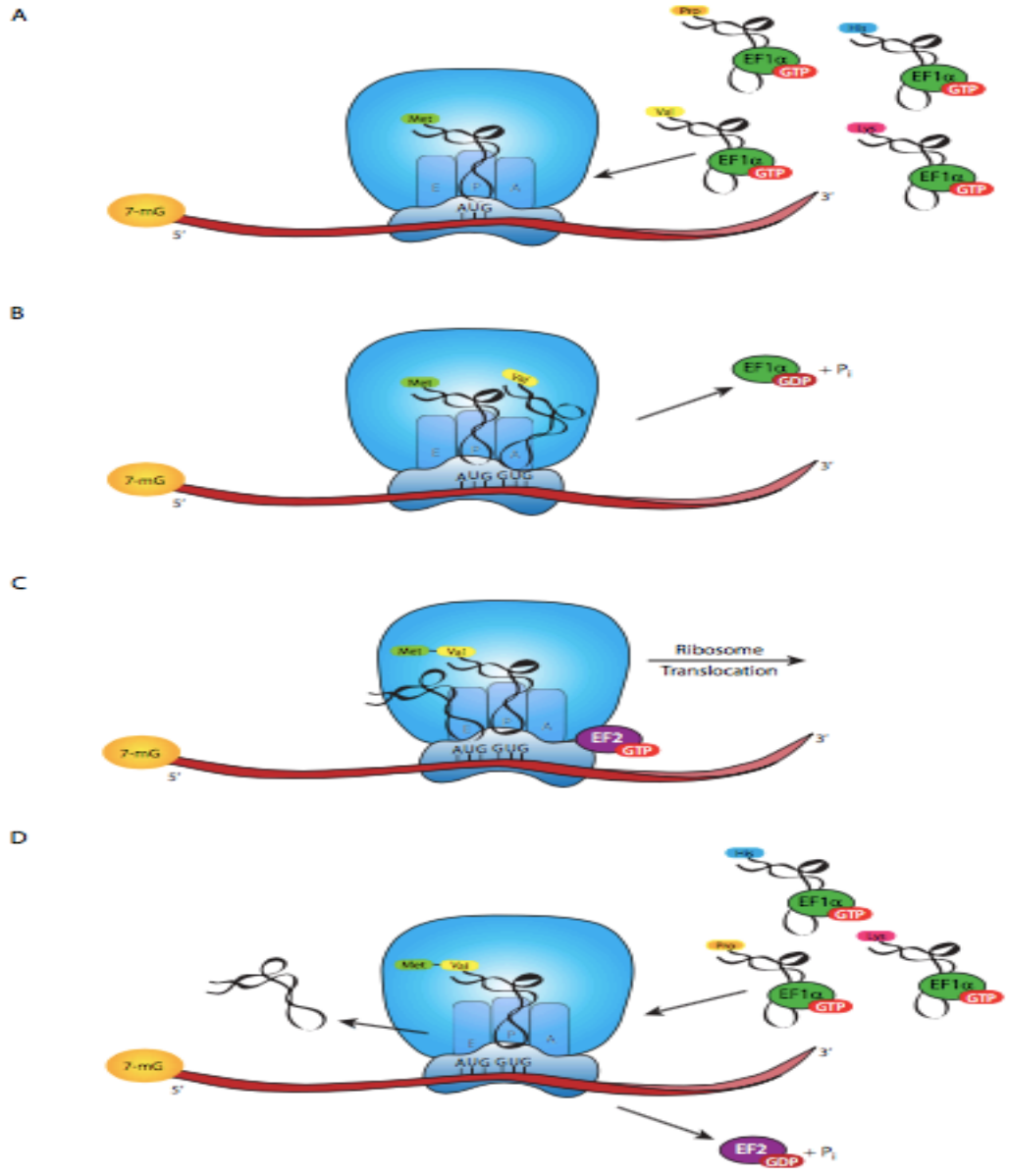



**Figure 31. Translation elongation mechanism.**

### 2.3.3. Termination

Termination occurs when the ribosome reaches a stop codon (UAA, UAG, or UGA), which does not code for any amino acid. Instead, release factors (RFs) bind to the ribosome and promote peptide release:

- RF1 recognizes UAG and UAA.
- RF2 recognizes UGA and UAA.
- RF3 facilitates RF1/RF2 activity.

In contrast, eukaryotic release factor eRF1 recognizes all three stop codons.

After peptide release, the ribosomal subunits dissociate with the help of a ribosome recycling factor (RRF), IF3, and EF-G, resetting the system for the next round of translation (Figure 32).

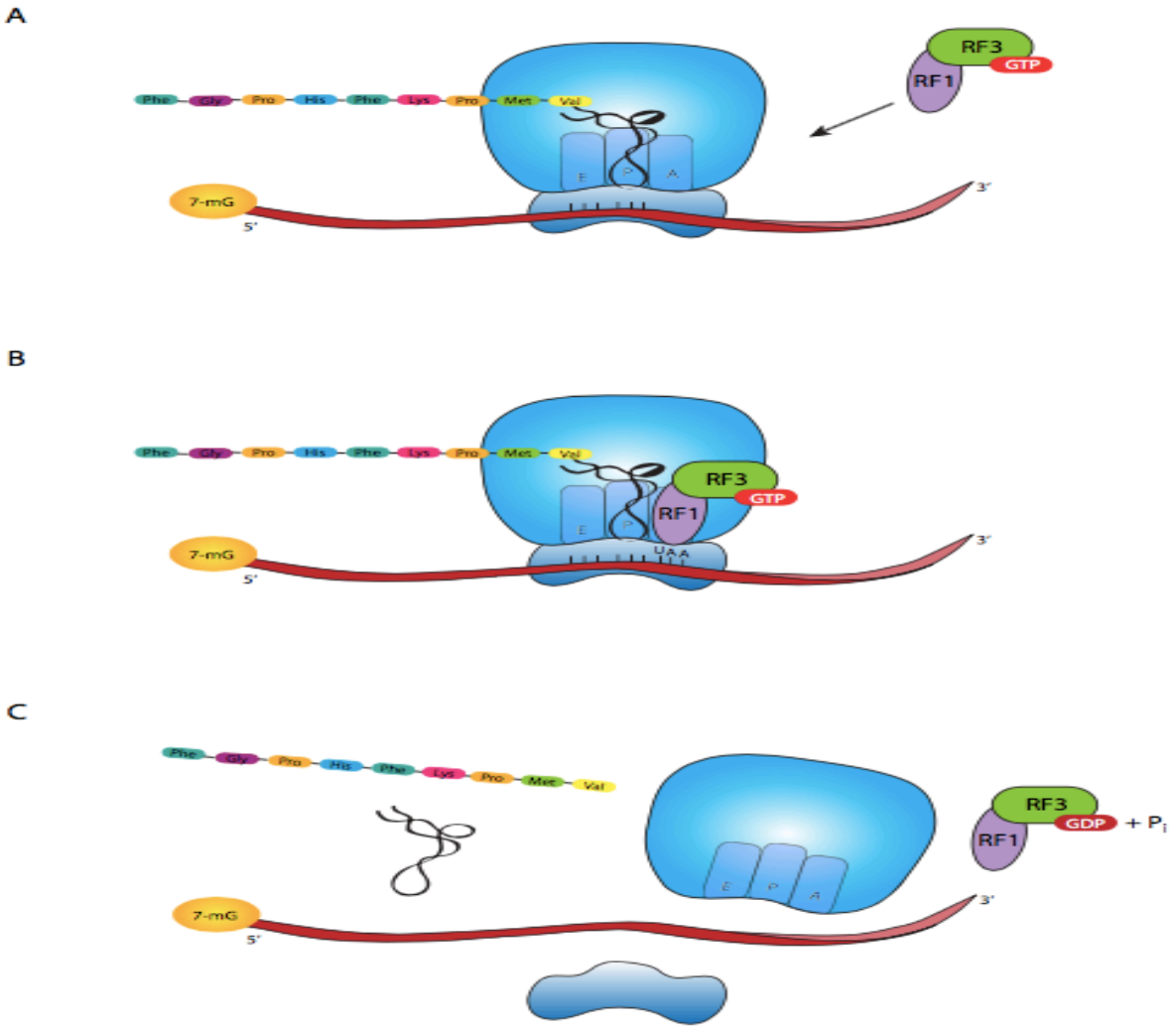



**Figure 32. Termination of translation.**

# Part 1: Bacteria

# Chapter 4: Regulation of Gene Expression

# Chapter 4: Regulation of Gene Expression

## 1. Definition and concept of the operon

### 1.1. General concept of the operon



In prokaryotic cells, genes are often organized into operons, which are clusters of genes transcribed together under the control of a single promoter. This allows bacteria to regulate gene expression efficiently, ensuring that proteins are only produced when necessary.

An operon consists of several key components:

- **The promoter**: A DNA sequence where RNA polymerase binds to initiate transcription. It is the starting point of gene expression regulation.
- **The operator**: A regulatory DNA sequence adjacent to the promoter, which interacts with regulatory proteins to either activate or repress transcription.
- **Structural genes**: These are the coding sequences for proteins that perform a specific function. In an operon, these genes are transcribed together into a single polycistronic mRNA (i.e., an mRNA containing multiple gene sequences).

Additionally, an operon is regulated by a regulatory gene, which encodes a regulatory protein (either a repressor or an activator). This regulatory gene is often located elsewhere in the genome but is essential for operon function.

### 1.2. Types of operons

Operons can be classified into two major categories based on their regulatory mechanisms:

#### 1.2.1. Inducible operons

- These operons are normally inactive because a repressor protein binds to the operator, preventing transcription.
- A specific molecule, called an inducer, binds to the repressor, causing a conformational change that detaches it from the operator. This allows RNA polymerase to transcribe the genes.

Example: The lac operon, responsible for lactose metabolism in *Escherichia coli*.

### 1.2.2. Repressible operons

- These operons are normally active and allow continuous transcription of structural genes.
- A specific molecule, called a corepressor, binds to the repressor protein, activating it. The active repressor then binds to the operator and blocks transcription.



Example: The trp operon, which regulates the synthesis of the amino acid tryptophan in *E. coli*.

## 2. Inducible operons: The Lac Operon

### 2.1. Function of the Lac Operon

The lac operon in *E. coli* is an example of an inducible operon that allows bacteria to metabolize lactose when glucose is unavailable. It consists of three structural genes:

- ***lacZ***: Encodes **β-galactosidase**, an enzyme that hydrolyzes lactose into glucose and galactose.
- ***lacY***: Encodes **lactose permease**, a membrane protein that facilitates lactose transport into the cell.
- ***lacA***: Encodes **thiogalactoside transacetylase**, whose function is less critical but may help detoxify certain metabolites.
- Upstream of these genes, there are two regulatory sites:
- **The promoter (*lacP*)**: Where RNA polymerase binds to initiate transcription.
- **The operator (*lacO*)**: Where the *lac* repressor protein binds to block transcription when lactose is absent.

Additionally, a regulatory gene (*lacI*), located outside the operon, encodes the lac repressor, which controls the expression of the lac operon.

### 2.2. Mechanism of lac operon regulation

#### 2.2.1. Regulation by lactose (induction mechanism)

* **In the absence of lactose**:
  - The *lacI* repressor is continuously expressed and binds to the operator.

- This prevents RNA polymerase from transcribing the lac operon genes, blocking lactose metabolism.

* **In the presence of lactose**:

- A small amount of lactose is converted into allolactose (an isomer of lactose).
- Allolactose binds to the repressor, inducing a conformational change that prevents it from binding to the operator.
- RNA polymerase is now free to transcribe the lac operon, leading to the production of β-galactosidase, permease, and transacetylase (Figure 33).



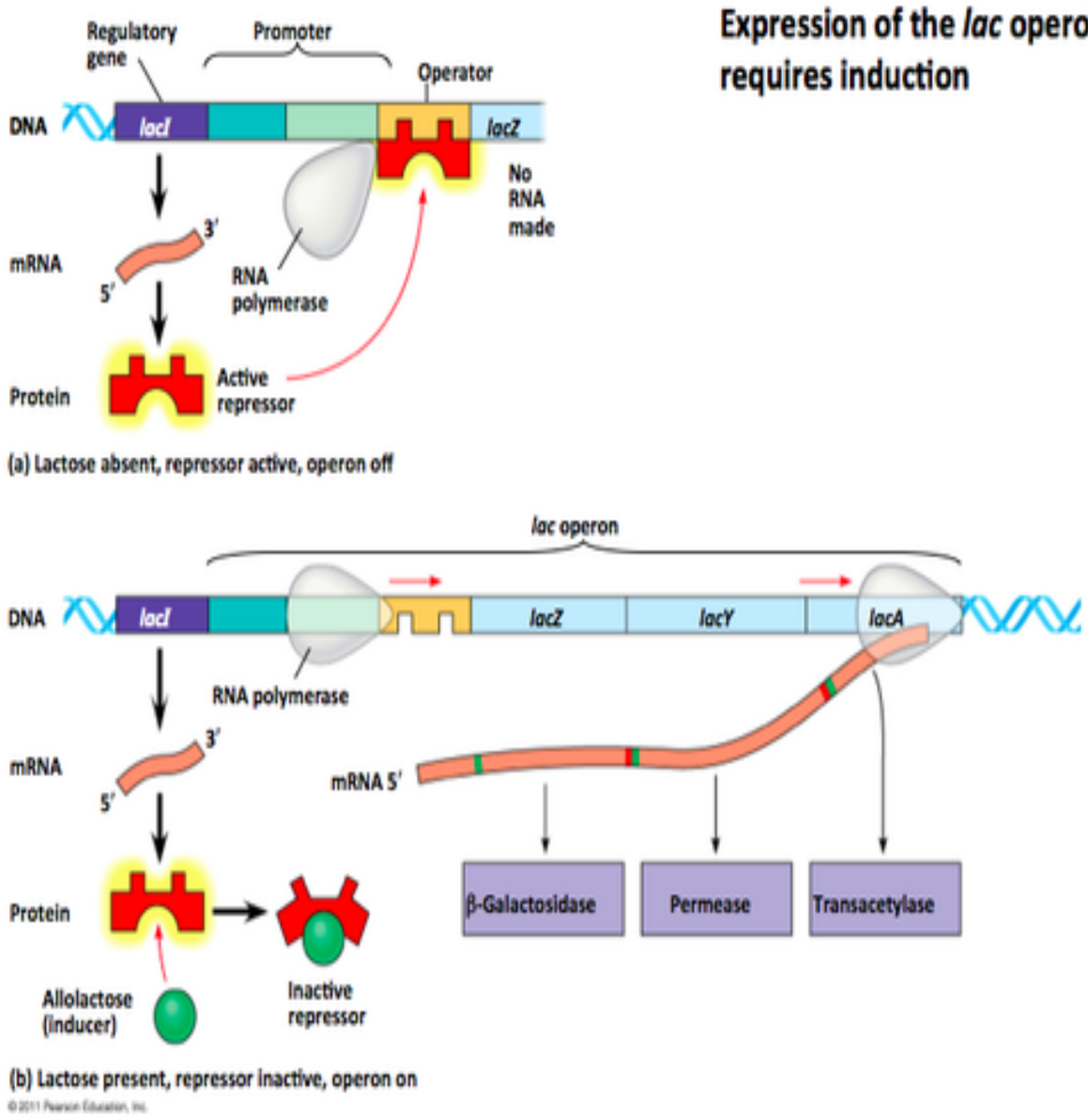



**Figure 33. Mechanism of lac operon regulation.**

### 2.2.2. Regulation by glucose (catabolite repression)

The lac operon is also regulated by glucose levels, through a mechanism called catabolite repression.

- When glucose is available, the cell prefers to use it as an energy source, so it reduces lac operon transcription.
- When glucose is scarce, cyclic AMP (cAMP) levels increase. cAMP binds to the catabolite activator protein (CAP), forming a CAP-cAMP complex.
- The CAP-cAMP complex binds to the lac promoter, enhancing RNA polymerase binding and increasing transcription.
- Thus, when both lactose and glucose are present, transcription is low. The operon is fully activated only when glucose is absent and lactose is available (Figure 34).

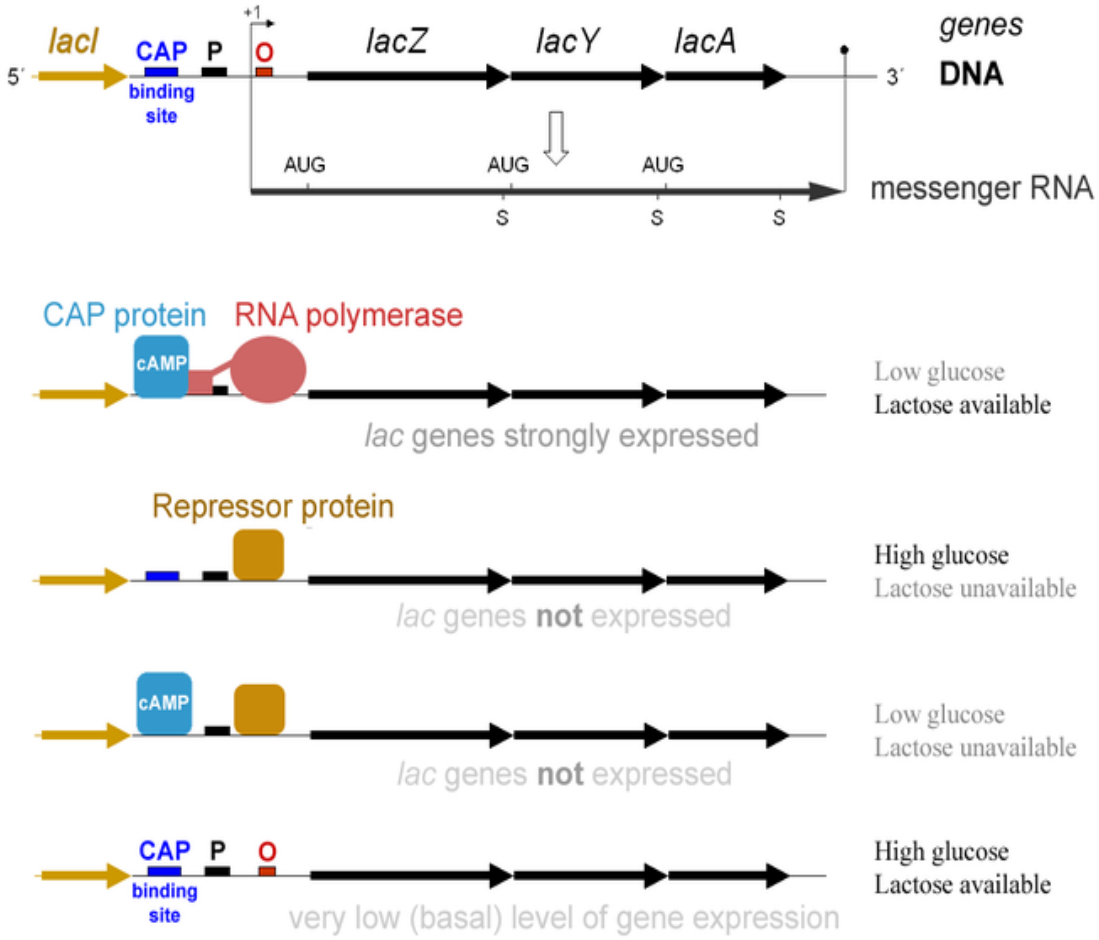



**Figure 34. Regulation of lac operon by Glucose.**

## 3. Repressible Operons: The Trp Operon

### 3.1. Function of the Trp Operon

The trp operon in *E. coli* is an example of a repressible operon that regulates the synthesis of the amino acid tryptophan. This operon consists of five structural genes (trpE, trpD, trpC, trpB, and trpA), which encode enzymes responsible for the biosynthesis of tryptophan from chorismate.



Upstream, there are two regulatory regions:

- **The promoter (*trpP*)**: The RNA polymerase binding site.
- **The operator (*trpO*)**: The binding site for the trp repressor.

A regulatory gene (*trpR*), located elsewhere in the genome, encodes the trp repressor protein, which controls operon activity.

### 3.2. Mechanism of Trp operon regulation

#### 3.2.1. Regulation by Tryptophan (repression mechanism)

* **In the absence of tryptophan**:
  - The **trp repressor is inactive** and cannot bind to the operator.
  - RNA polymerase binds to the promoter and transcribes the operon, allowing the synthesis of tryptophan-producing enzymes.
* **In the presence of tryptophan**:
  - Tryptophan acts as a corepressor by binding to the trp repressor protein.
  - This activates the repressor, which then binds to the operator, blocking transcription.
  - This ensures that tryptophan synthesis stops when sufficient amounts are available (Figure 35).

#### 3.2.2. Modulatory expression system: Attenuation

Attenuation is a regulatory mechanism that controls gene expression by prematurely terminating transcription based on the availability of specific metabolites, particularly amino acids. This process is best exemplified by the trp operon in *E. coli*, where transcription termination depends on the formation of alternative secondary structures in the leader mRNA sequence (*trpL*). The presence of high tryptophan levels allows ribosomes to quickly translate

the leader peptide, promoting the formation of a terminator hairpin, which halts transcription. In contrast, low tryptophan levels cause ribosome stalling, leading to the formation of an antiterminator structure, allowing transcription to continue.

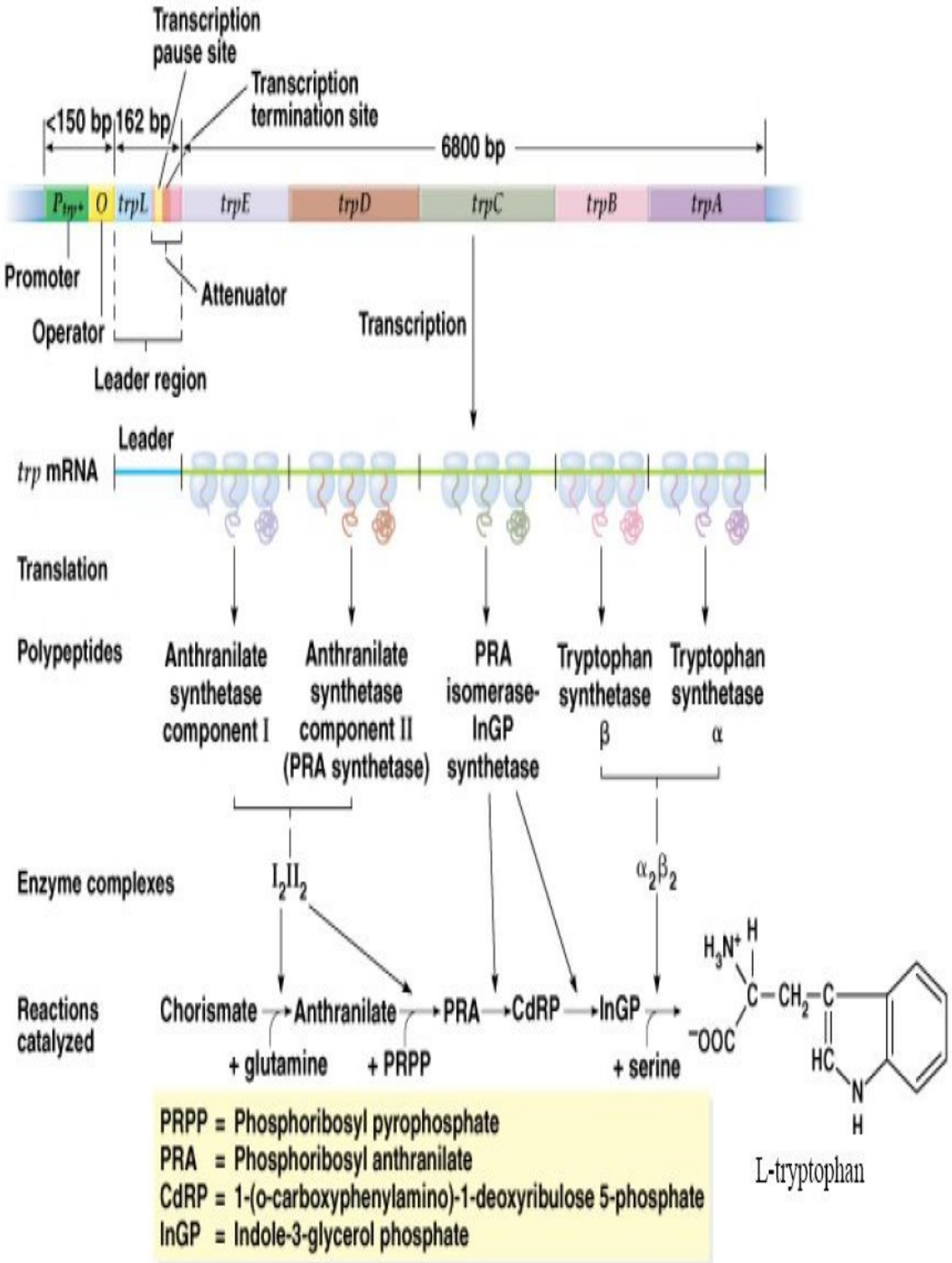



**Figure 35. Mechanism of trp operon regulation.**

## 4. Regulation by DNA sequence inversion

DNA sequence inversion is a regulatory mechanism that allows bacteria to reversibly switch gene expression by flipping a specific segment of DNA. This process plays a crucial role in phase variation, antigenic variation, and adaptation to environmental changes. The inversion of DNA sequences is mediated by site-specific recombinases, which recognize specific inverted repeat sequences flanking the invertible segment and catalyze the recombination event.



### 4.1. Mechanism of DNA inversion

The process of DNA sequence inversion involves three main components:

- **Inverted Repeats (IRs):** Short palindromic sequences that define the boundaries of the invertible DNA segment.
- **Recombinase Enzymes:** Specialized site-specific recombinases, such as **Hin** in *Salmonella* or **FimB/FimE** in *E. coli*, that catalyze the inversion.
- **Regulatory Factors:** Additional proteins that may modulate recombinase activity, ensuring that inversion occurs under specific environmental conditions.

The recombinase binds to the IRs and introduces a double-strand break, followed by a 180° rotation of the DNA fragment and re-ligation. This results in an on/off switching of gene expression, depending on the orientation of the regulatory elements relative to the promoter (Figure 36).

### 4.2. Biological significance of DNA inversion

#### A. Phase variation

Phase variation is a strategy used by bacteria to reversibly switch between different phenotypes without altering the genetic sequence. This helps them evade immune detection and adapt to changing environmental conditions.

- Example: *Salmonella flagellar* phase variation
    - *Salmonella enterica* alternates between two flagellin proteins (**FliC and FljB**) using the **Hin recombinase**.

The inversion of the hix region controls the expression of either FljB (phase 1) or FliC (phase 2), allowing the bacterium to present different antigens.

**B. Antigenic Variation**

DNA inversion contributes to antigenic variation by modifying the expression of surface proteins that interact with the host immune system.

- Example: *Neisseria gonorrhoeae* modifies its pili structure through recombination, altering antigenic properties and evading immune recognition.



**C. Regulation of adhesion factors in *E. coli***

In *Escherichia coli*, the Fim system controls the expression of type 1 fimbriae, which mediate bacterial adhesion to host tissues.

- **FimB** and **FimE** recombinases regulate the inversion of the **FimS** promoter, switching between fimbriae production (ON) and repression (OFF).
- This regulation helps *E. coli* attach to host cells during infection and detach when dispersal is needed.

**4.3. Environmental and evolutionary implications**

- **Host adaptation:** Pathogens use DNA inversion to dynamically regulate virulence factors, increasing their survival in different host environments.
- **Antibiotic resistance:** Some bacteria regulate efflux pumps or resistance genes through DNA inversion, contributing to antibiotic resistance.
- **Bacteriophage interactions:** Some temperate phages utilize DNA inversion to control lysogenic or lytic cycles, influencing bacterial population dynamics.

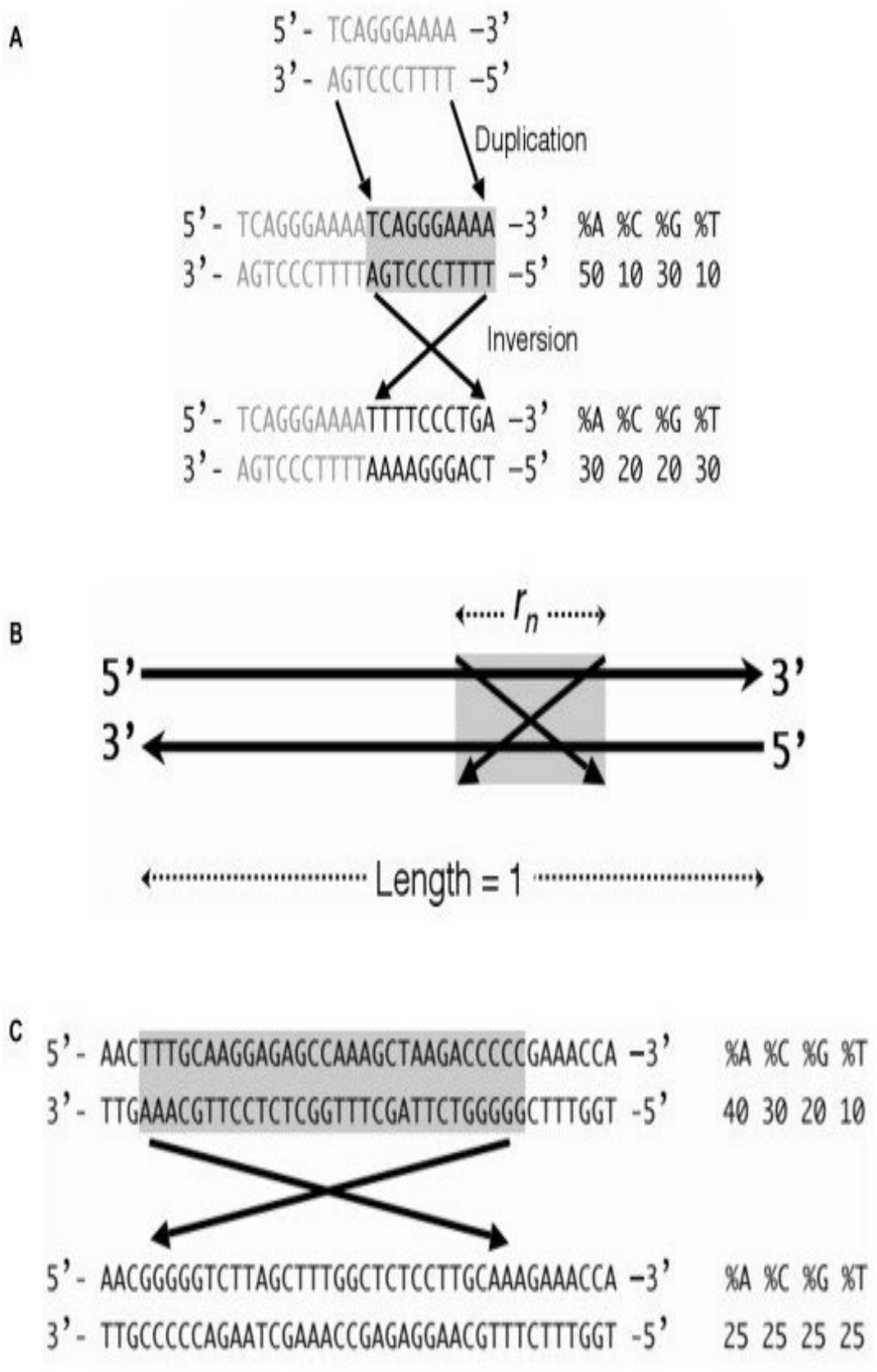



**Figure 36. Mechanism of regulation by DNA sequence inversion.**

# Part 2: Fungi
# (Yeast as a model system)

# *Part 2: Fungi (Yeast as a model system)*

## 1. Overview of yeast biology

### 1.1. General principles



Yeasts are unicellular fungi (eukaryotes) that belong mainly to the phylum *Ascomycota*, with some species classified under *Basidiomycota*. The most studied yeast species is *Saccharomyces cerevisiae*, which has been widely used as a model organism in molecular and cellular biology (Figure 37). Unlike filamentous fungi, yeasts do not form true mycelia, although some species exhibit dimorphic growth, switching between yeast-like and hyphal forms under certain conditions like pathogenic form of *Candida albicans*.

Yeasts play a crucial role in various biological and industrial applications. Their fast growth, ease of genetic manipulation, and similarities with higher eukaryotes make them valuable in research on fundamental cellular processes such as DNA replication, transcription, and metabolism.

Yeasts play a fundamental role in research, biotechnology, and medicine:

- Research applications: Used in genetics, cell biology, and systems biology. Many discoveries, such as the cell cycle and gene regulation, were first elucidated in yeast.
- Industrial applications: Essential in food and beverage fermentation (bread, beer, and wine) and in bioethanol production.
- Medical applications: Used for recombinant protein production, vaccine development, and studies on pathogenic yeasts like *Candida albicans*.

### 1.2. Culture and nutrition

Yeasts can be grown in both liquid and solid media under aerobic or anaerobic conditions. The choice of medium depends on the experimental goal.

- Rich media (e.g., YPD - Yeast Peptone Dextrose) provide all necessary nutrients for optimal growth.
- Minimal media (e.g., SD - Synthetic Defined Medium) contain only essential nutrients and allow researchers to study auxotrophic mutants.

- Selective media are used to isolate yeast strains based on genetic markers, such as antibiotic resistance or nutrient utilization.

Yeasts metabolize a variety of carbon and nitrogen sources:

- Carbon sources: Glucose, galactose, ethanol, and glycerol.
- Nitrogen sources: Ammonium sulfate, amino acids, and urea.
- Vitamins and minerals: Essential cofactors for enzymatic reactions.



Environmental factors such as temperature, pH, osmotic pressure, and oxygen availability influence yeast growth. Most yeasts thrive at temperatures between 25–30°C and in slightly acidic conditions (pH 4–6).

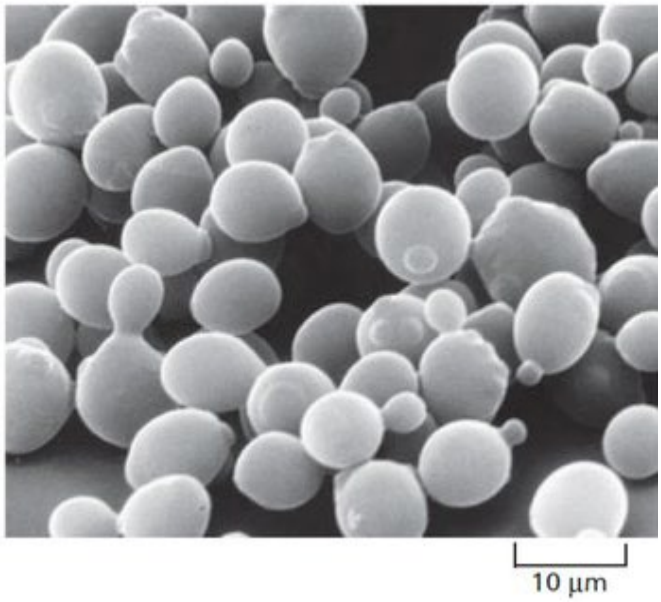



**Figure 37. Electron micrograph of *Saccharomyces cerevisiae* yeast cells.**

## 2. Yeast genome

The yeast genome is compact and well-organized, making it an ideal model for genetic studies. *S. cerevisiae* has 16 chromosomes with a total genome size of approximately 12 million base pairs (Mb). The yeast has around 6,000 genes, many of which have human homologs (Figure 38).

Unlike bacterial genomes, yeast chromosomes are linear and contain introns, but far fewer than in mammals (~5% of genes contain introns).

- The *Saccharomyces cerevisiae* genome was the first eukaryotic genome to be fully sequenced (1996), providing key insights into eukaryotic gene organization and regulation.
- It shares many conserved genes with humans, particularly those involved in cell cycle control, metabolism, and DNA repair.
- Yeast genetic studies have contributed to the understanding of fundamental biological processes, including the discovery of cyclins and CDKs (Cyclin-Dependent Kinases), which regulate the eukaryotic cell cycle.



Advancements in genome sequencing technologies (next-generation sequencing, whole-genome CRISPR screens) have allowed researchers to study yeast genetics at an unprecedented level, aiding in functional genomics and synthetic biology applications.

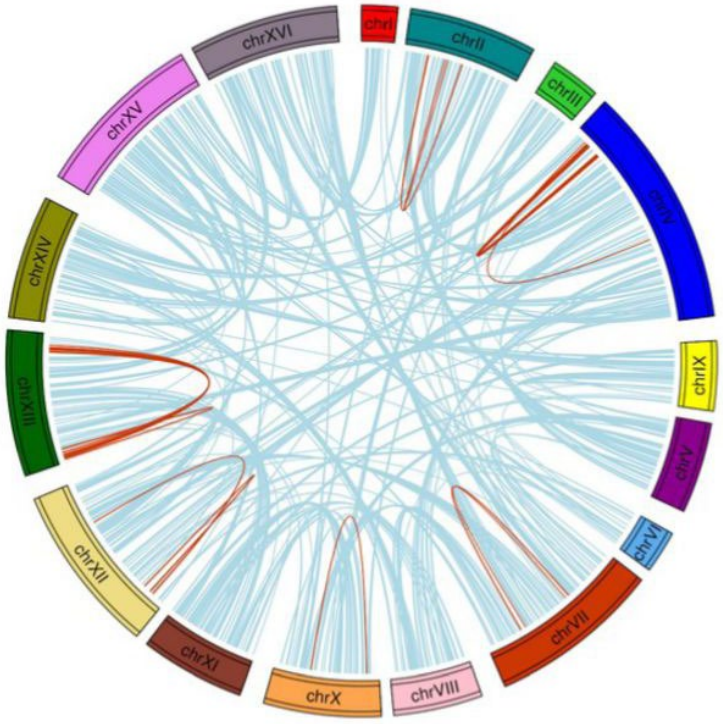



**Figure 38. Whole Genome Duplicates (WGD).**

## 3. Yeast Transcriptome

While the genome is identical in every cell of a given organism, gene expression can vary depending on specific conditions, such as developmental time (specific developmental stages), space (specific to cell, tissue, or organ types), or physiological state (normal, pathological, or in response to external stimuli). Gene expression analysis involves characterizing and quantifying the products of DNA expression (messenger RNAs – the

transcriptome) to identify active sequences at a specific time and under particular conditions, thereby revealing the level of expression of the corresponding genes.

The transcriptome reflects the active genes in a given tissue, developmental stage, or physiological condition. Currently, two dominant strategies are used for qualitative and quantitative gene expression analysis: **SAGE (Serial Analysis of Gene Expression)** and **DNA microarray hybridization** techniques.



### 3.1. Serial Analysis of Gene Expression (SAGE Method)

#### 3.1.1. Definition

SAGE is a high-throughput method for both qualitative and quantitative analysis of gene expression. It involves cataloging the transcripts (mRNA from the sample, converted to cDNA) by isolating short, unique fragments (tags) of 9-14 base pairs from each cDNA. These fragments, known as expressed sequence tags (ESTs), are then concatenated into a long sequence, which is subsequently sequenced. This process allows identification of each tag and its corresponding gene. The number of times each tag appears is quantified, offering a precise measure of gene expression levels.

By generating a large number of sequences from a sample, SAGE enables researchers to assess the frequency of various transcripts, providing valuable insights into gene expression patterns. This approach is beneficial for identifying genes that are actively transcribed in specific tissues or conditions. However, SAGE is less effective when dealing with rare transcripts or numerous samples, as it requires a comprehensive genomic database for comparison, and its application to less-characterized species can be limited.

#### 3.1.2. Principle

In SAGE, a pool of cDNA is synthesized from the mRNA extracted from a sample (whether cells, tissues, or organs). A subsequent enzymatic treatment isolates short sequence tags from the 3' ends of each cDNA molecule. These tags are concatenated to form a long DNA fragment that can be sequenced. The genes from which the tags originate are identified by comparing the sequences with a genomic database. By analyzing numerous sequences, the relative abundance of different tags in the sample can be quantified, providing an accurate expression profile (Figure 39).

SAGE has been applied in various organisms, including humans, yeast, and plants, and has provided valuable insights into gene expression across different biological systems.

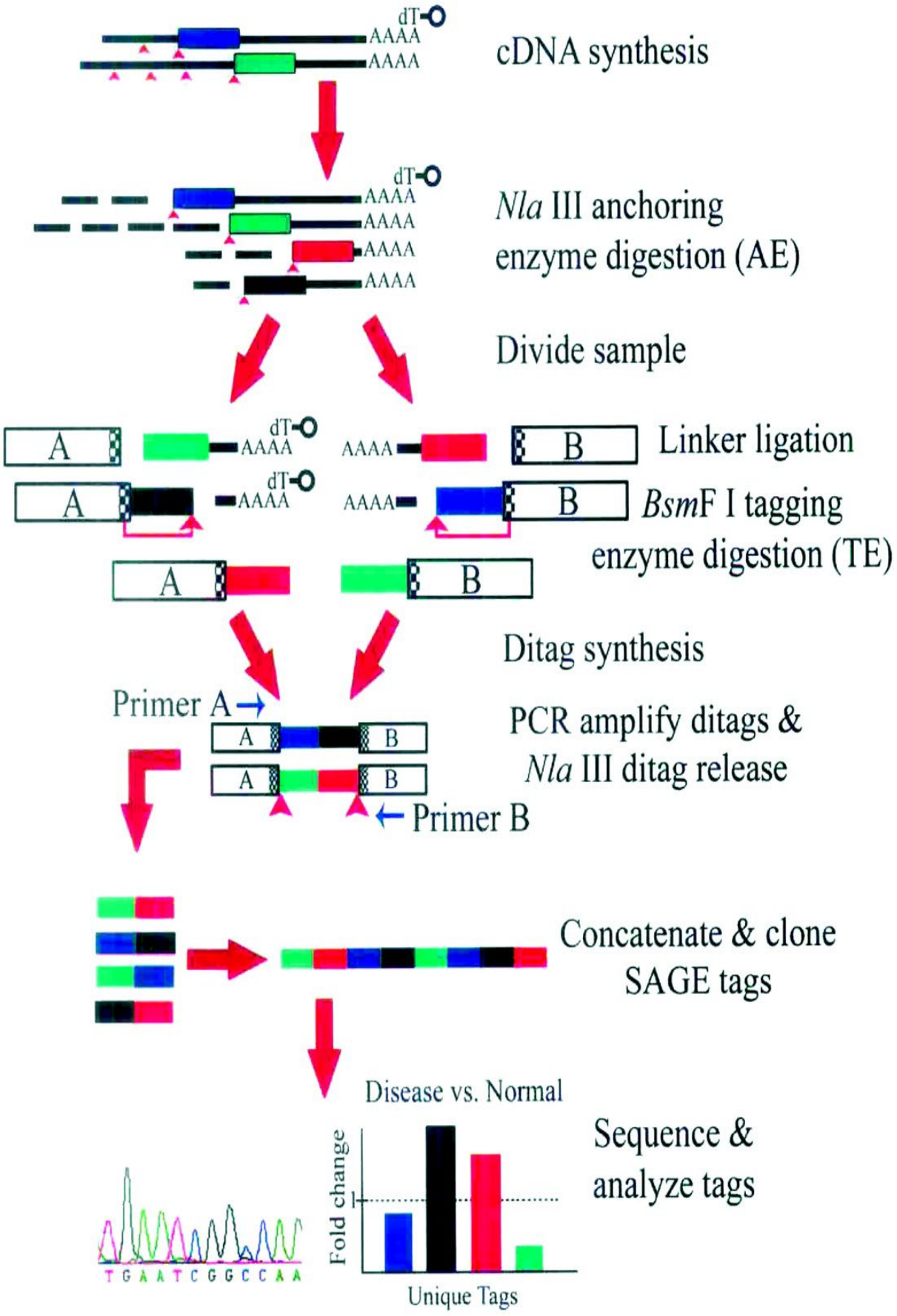



**Figure 39. Serial Analysis of Gene Expression method.**

## 3.2. DNA Microarrays

### 3.2.1. Definition

DNA microarrays (also known as biochips) are a hybridization-based technique for the comparative genomic analysis of gene expression. They consist of oligonucleotides (single-stranded DNA), which are immobilized on a solid support (a matrix). These oligonucleotides correspond to known genes or cDNAs, and their role is to act as probes to detect complementary sequences (targets) in the sample being analyzed (mRNA converted to cDNA). The probes can either be pre-attached to the solid support or synthesized *in situ*.



Once the sample has been hybridized to the microarray, the hybridization signals are detected using various labeling methods, such as fluorescence or radioactivity, which are then quantified. This allows researchers to measure the relative expression of thousands of genes simultaneously.

### 3.2.2. Principle

The concept of the DNA microarray emerged in the 1990s and integrates microelectronics, nucleic acid chemistry, image analysis, and bioinformatics. The microarray technique is based on the principle of hybridization: the target DNA (sample cDNA) is labeled with a fluorochrome, which becomes fluorescent when exposed to light. The sample is then applied to the microarray, where the probes (specific to various genes or cDNAs) recognize and bind to their complementary sequences.

The hybridization process occurs in a controlled incubator, followed by washing to remove any unbound material. The hybridization signals are detected and quantified, either by fluorescence or radiography, depending on the labeling method used (Figure 40).

### 3.2.3. Support

The microarray support typically consists of a small surface area (less than 1 $cm^2$) made from materials such as glass, polymers, silicon, gold, or platinum. The matrix is either flat or porous, containing wells that allow for the attachment of probes. The choice of material depends on the experimental requirements, as it must support efficient immobilization of the probes and allow clear signal detection.

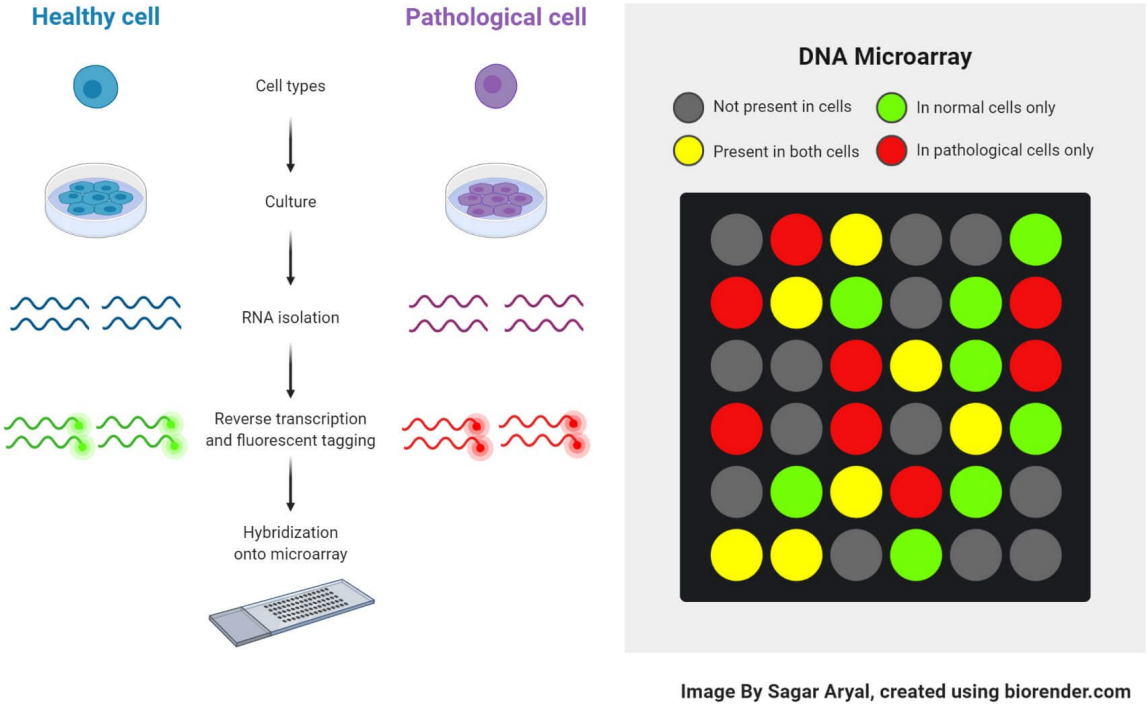



**Figure 40. DNA Microarrays principle.**

### 3.2.4. Hybridization process

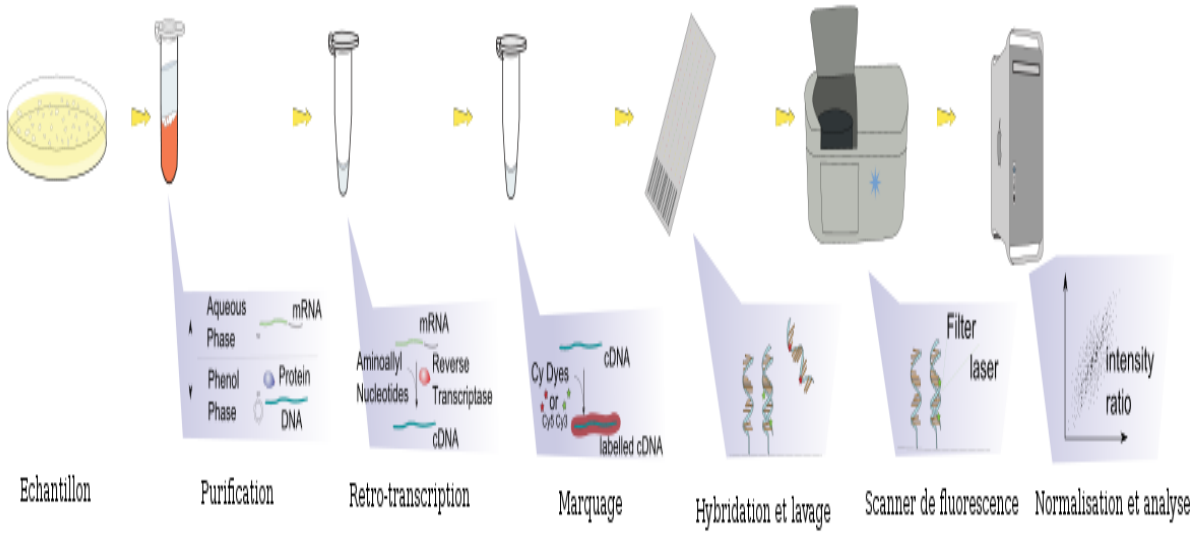
During hybridization, the target sequence (cDNA from the sample) is marked with a fluorochrome, which becomes fluorescent under a specific light. This marked cDNA is incubated with the microarray, where the probes specifically bind to their complementary sequences. The hybridization process is followed by a washing step to remove unbound material, leaving only the sequences that have bound to the probes. This allows for highly specific and sensitive detection.

### 3.2.5. Detection of hybridization

Once hybridization is complete, the goal is to identify which probes have successfully hybridized with their target sequences. The hybridization signals are detected through optical reading, which can reveal fluorescent spots on the microarray, indicating successful binding. The data is then analyzed using software tools that process the image and quantify the fluorescence, providing a clear picture of the gene expression profile.

For more detailed experiments, dual fluorochrome labeling allows the detection of signals from two different probes in a single experiment. For example, Cyanine 3 (Cy3) fluoresces

green, while Cyanine 5 (Cy5) fluoresces red, making it possible to compare expression levels between two conditions in the same experiment (Figure 41).






### 3.2.6. Applications of DNA Microarrays

- Mutation Analysis: By comparing wild-type sequences and potential mutant sequences, DNA microarrays can be used to detect mutations, including substitutions, deletions, and insertions.
- Sequencing by Hybridization: Microarrays can be employed to read overlapping sequences from short fragments, which are then reassembled using computational tools to form a complete sequence.
- Genetic Disease Diagnosis: Microarrays can identify genetic mutations responsible for diseases, detect specific hormones or cancer markers, and study oncogenes.
- Infectious Disease Diagnosis: Microarrays are used to detect the presence of microorganisms, their mutations, and resistance to specific antibiotics or enzymes.
- Pharmacogenomics: By identifying gene targets, microarrays are valuable tools for drug development.
- Toxicogenomics: In toxicological studies, microarrays analyze the differential expression of genes in response to toxic substances, helping understand the molecular mechanisms behind toxic effects.

- Agro-Food Industry: DNA microarrays are used to monitor microorganisms involved in food production, detect genetically modified organisms (GMOs) in seeds or foods, and identify foodborne pathogens like *Salmonella* and *Listeria*.
- Environmental Analysis: Microarrays help monitor water quality, detecting bacterial contamination, and analyzing air and water samples for infectious agents.



## 4. Yeast Proteome

Studying and analyzing gene expression regulation at the cellular level requires more than just information on mRNA levels. Proteome data, which reflect the actual expression of proteins, are often more informative. What truly matters in a cell is the activity of proteins, which are frequently modulated and regulated by post-translational modifications (PTMs).

The challenge of proteomics is to develop methodologies to measure active proteins on a proteome-wide scale and determine their interactions with various cellular components, including DNA, RNA, other proteins, and molecules. Unlike the genome, which remains constant (except for somatic mutations) across all cells of an organism, the proteome, like the transcriptome, varies according to cell type, physiological state, and developmental stage. Consequently, an individual does not have a single proteome but multiple proteomes.

A significant portion of cellular function variability is attributed not only to the presence or absence of specific proteins but also to the modulation of their abundance and activity. Initially, cellular differences were explained through the binary "on/off" regulation of specific genes among the estimated 100,000-120,000 genes. However, with the revised estimate of approximately 30,000 human genes, this explanation is insufficient. Instead, differences between cells are better explained by variations in protein abundance and post-translational modifications.

### 4.1. Classical methodologies in proteomics

The methods currently used for protein analysis generally lack high throughput, reproducibility, and automation required for large-scale genomic studies. Several techniques are employed to analyze and identify proteins, including:

#### 4.1.1 Two-Dimensional gel electrophoresis (2D-PAGE)

Two-dimensional electrophoresis is used to separate proteins from cellular extracts. It remains one of the most reliable methods for studying the abundance and post-translational

modifications of hundreds of proteins simultaneously. Recent advancements have improved resolution and reproducibility, with automated software available for quantifying protein spots. Several databases store results from 2D electrophoresis experiments, allowing for comparative proteomic studies (Figure 41).

One key application of proteomics is studying gene function using knock-out or overexpression mutants and analyzing changes in protein profiles via two-dimensional gel electrophoresis.



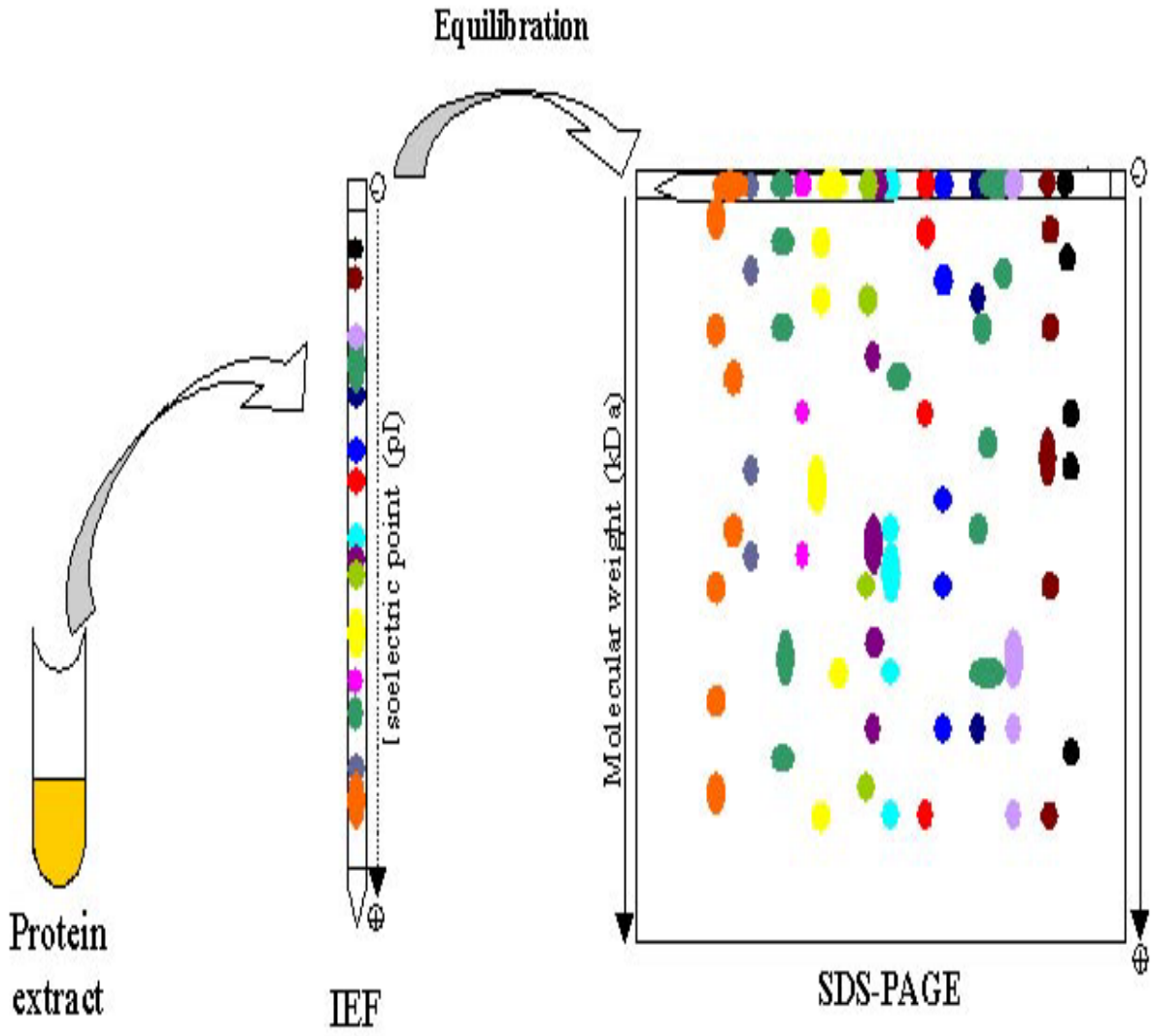



**Figure 41. Demonstration of Two-Dimensional gel electrophoresis (2D-PAGE).**

### 4.1.2. Enzymatic digestion

Enzymatic digestion involves treating proteins with specific proteases such as trypsin, which cleaves proteins into characteristic peptide fragments. This approach enables protein

identification by generating peptide mass fingerprints that can be analyzed using bioinformatics tools (Figure 42).

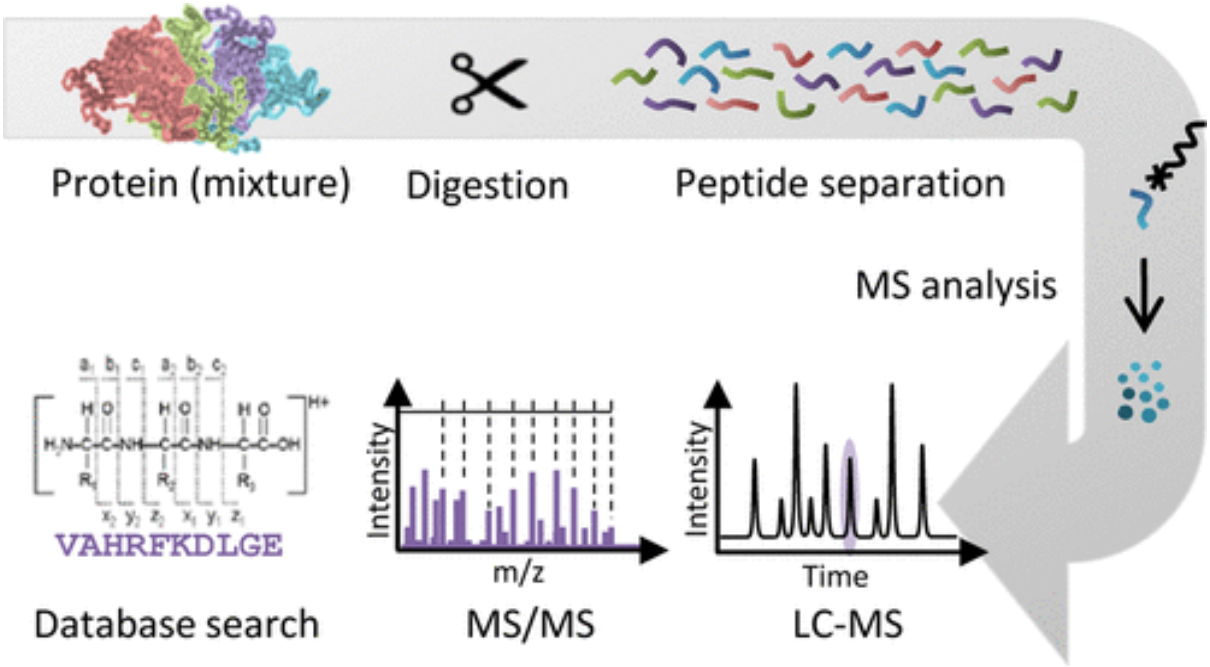



**Figure 42. Enzymatic digestion method.**

### 4.1.3. Mass Spectrometry (MS)

Mass spectrometry allows protein identification and characterization of modifications by measuring peptide fragment masses following enzymatic digestion. Databases store spectral data, which can be analyzed using specialized software (Figure 43).

Sequence databases predict the theoretical mass of proteolytic fragments, enabling protein identification when a complete organism's genome is available. This approach, known as "peptide mass fingerprinting," facilitates accurate protein identification from mass spectra.

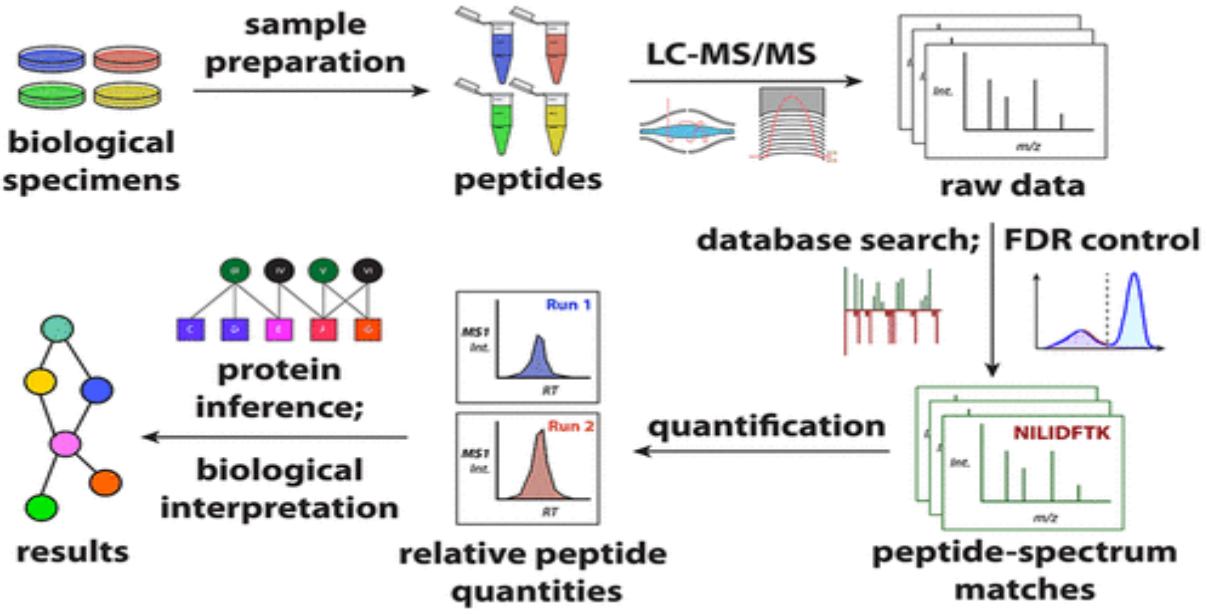



**Figure 43. Description of Mass Spectrometry method.**

### 4.1.4. N-terminal sequencing

- **Via Edman degradation**

Automated Edman degradation sequencing enables the determination of protein or peptide sequences from minute protein quantities. This method is crucial for identifying proteins and mapping functional domains within proteins (Figure 44).

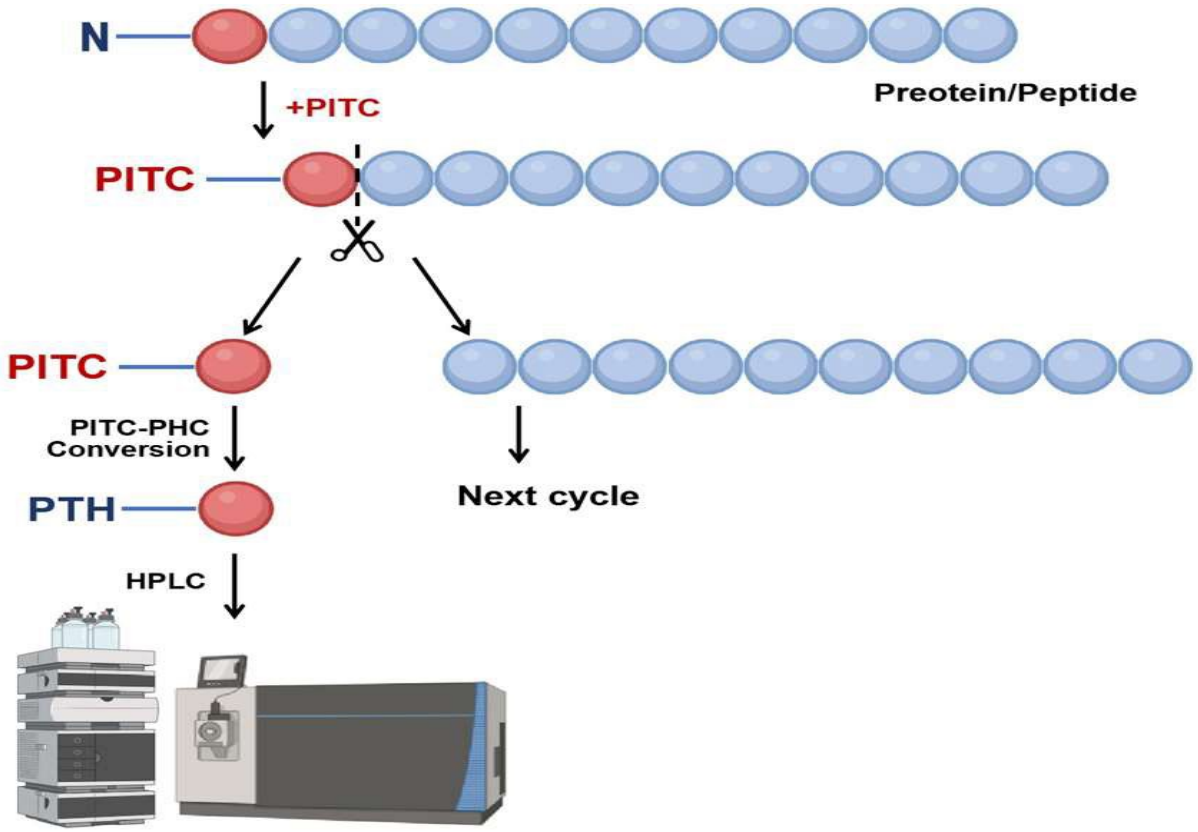



**Figure 44. N-terminal sequencing via Edman degradation.**

- ***De novo* N-terminal peptide sequencing by Tandem MS**

This technique utilizes fluorescent reagents to label the N-terminal α-amino group of proteins. Following enzymatic digestion with trypsin, the resulting peptides are analyzed using liquid chromatography Tandem mass spectrometry (LC-MS/MS) combined with fluorescence detection.

A differential peptide mapping approach is then applied to identify N-terminal peptides modified by pyroglutamate, enabling high-precision sequencing (Figure 45).

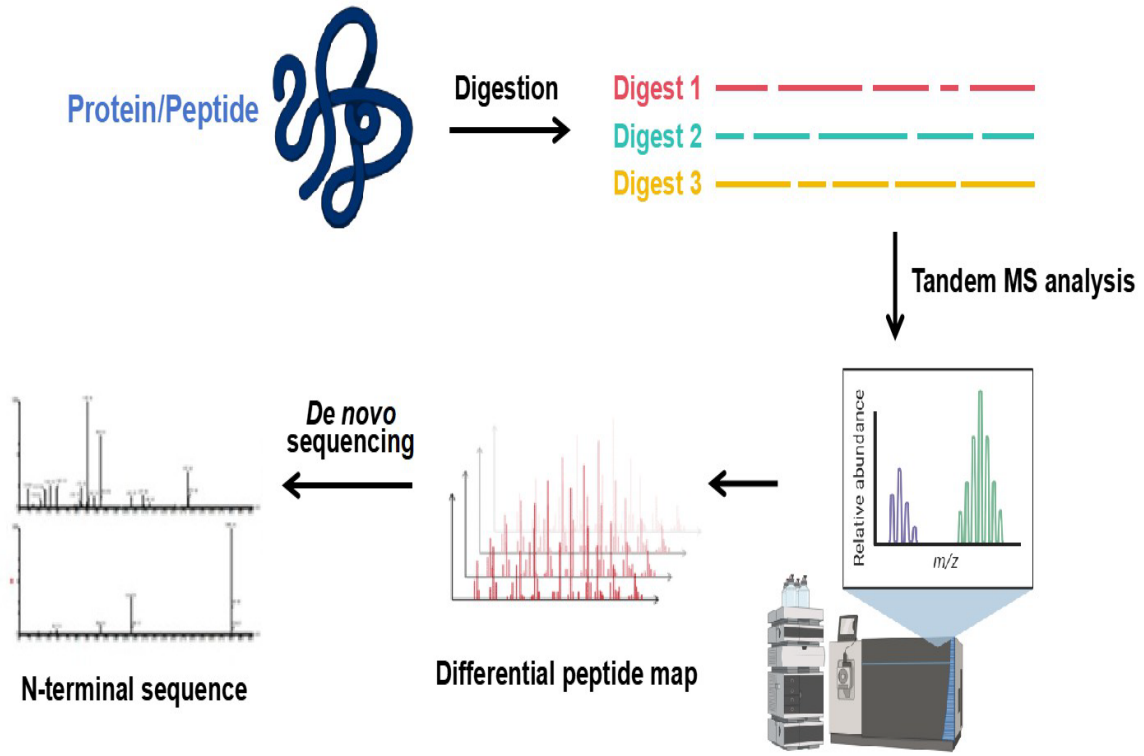



**Figure 45. *De novo* N-terminal peptide sequencing by Tandem MS.**

### 4.1.5. Protein microarrays

Protein microarrays aim to identify multiple proteins simultaneously within complex mixtures. These systems rely on antigen-antibody interactions, where specific antibodies immobilized on the microarray surface capture target proteins. This technology is valuable for high-throughput proteomic studies, biomarker discovery, and diagnostics (Figure 46).

### 4.1.6. Yeast two-hybrid system

One major proteomic strategy involves mapping protein-protein interaction networks to reconstruct metabolic pathways. Interaction maps provide insights into the function of unknown proteins if they associate with well-characterized proteins (Figure 47).

The yeast two-hybrid system is a widely used technique for detecting protein interactions in vivo. This method involves:

- Expressing a "bait" protein in yeast.
- Introducing a "prey" protein to test interaction.
- Detecting interaction via the activation of a reporter gene.

This approach helps identify potential therapeutic targets. The "prey" peptide competes with endogenous proteins without fulfilling their function, allowing researchers to study the effect of interaction inhibition on cellular processes. The ultimate goal is to develop molecules capable of modulating these interactions for therapeutic applications.



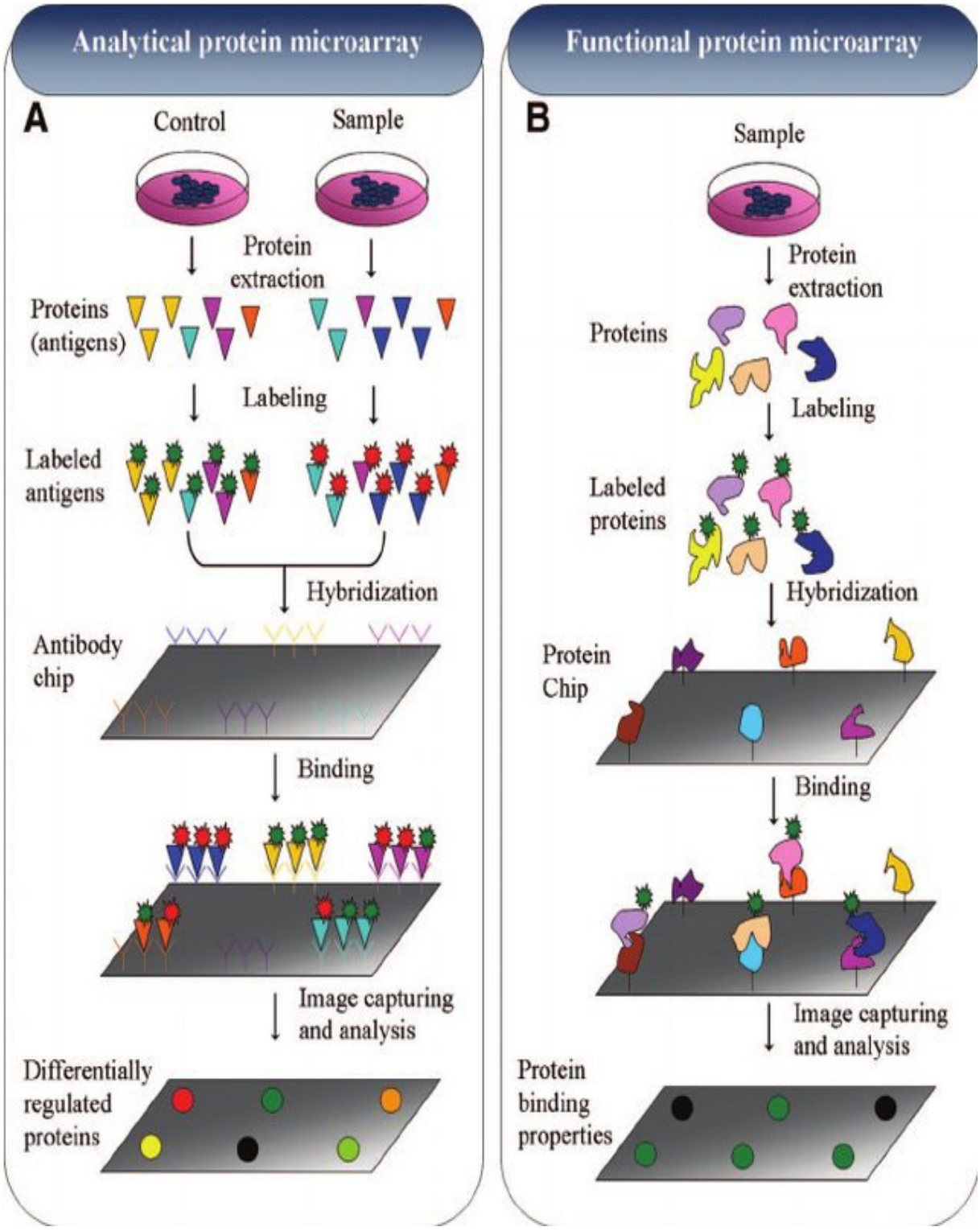



**Figure 46. Protein microarrays.**

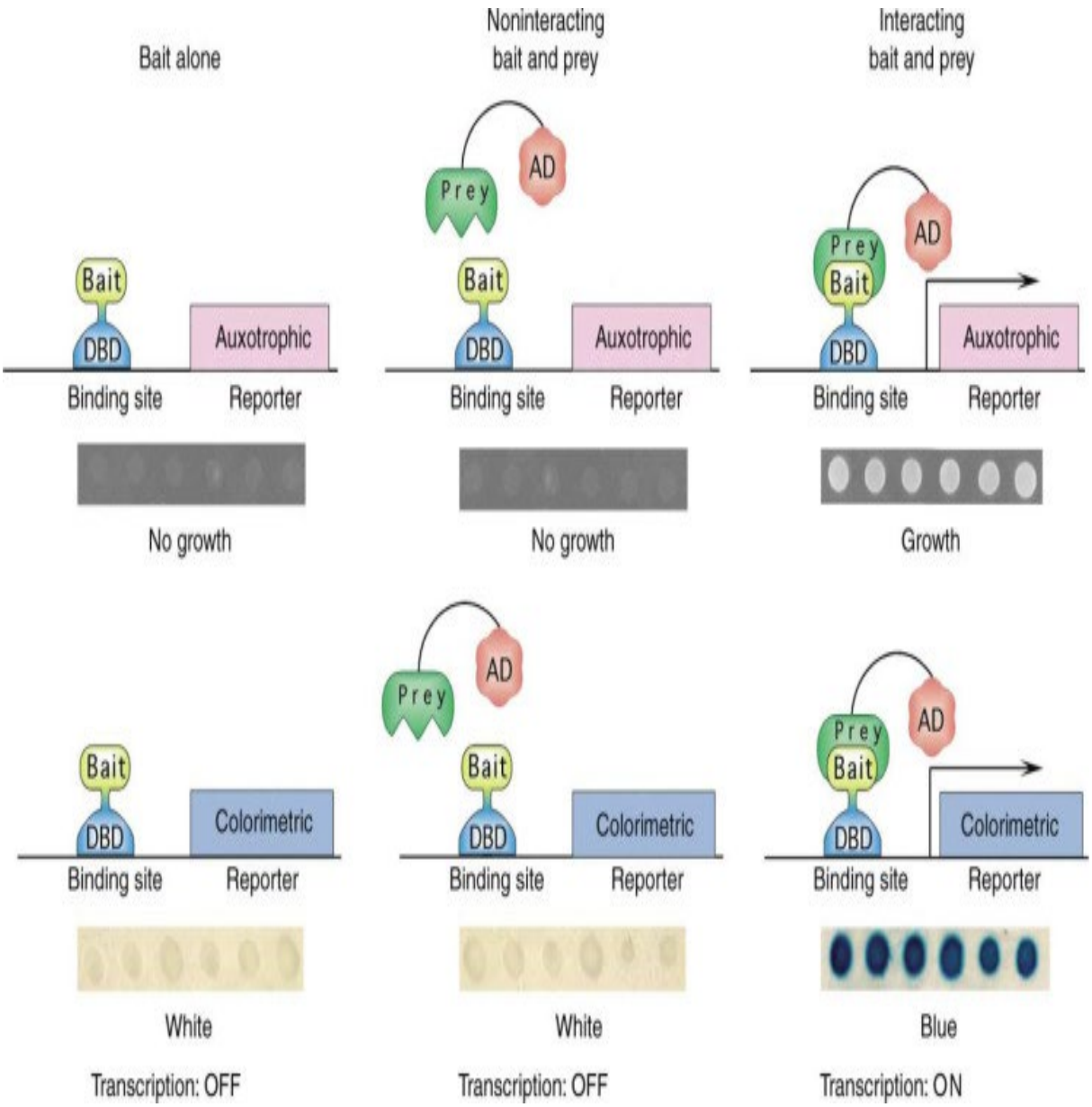



**Figure 47. Two-Hybrid Protein–Protein Interactions.**

### 4.2. Recent advances in proteomics

#### 4.2.1. Shotgun proteomics

Shotgun proteomics is a high-throughput technique that differs from traditional gel-based methods by digesting proteins into peptides before analysis. This approach employs liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), allowing for the comprehensive identification and quantification of proteins in complex biological samples. Its ability to analyze thousands of proteins simultaneously makes it a powerful tool in modern proteomics research (Figure 48).

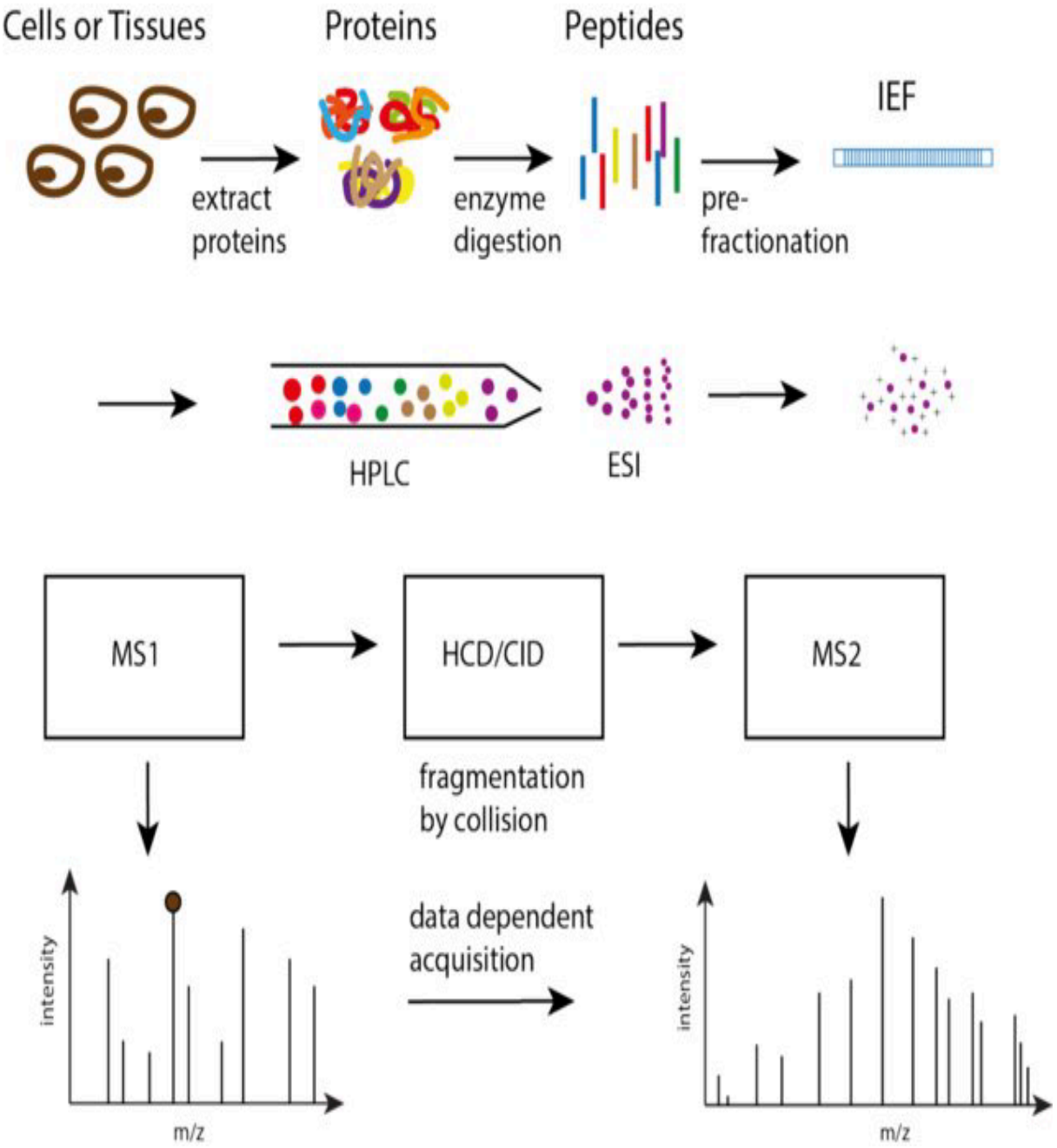



**Figure 48. Illustration of shotgun proteomics method.**

### 4.2.2. Targeted proteomics

Targeted proteomics focuses on the precise quantification of specific proteins using advanced mass spectrometry techniques such as Selected Reaction Monitoring (SRM) and Parallel Reaction Monitoring (PRM). These methods offer high sensitivity and reproducibility, making them valuable for applications such as biomarker validation and clinical diagnostics. By selectively analyzing predefined proteins of interest, targeted proteomics ensures accurate and reliable measurements, even in complex biological matrices (Figure 49).

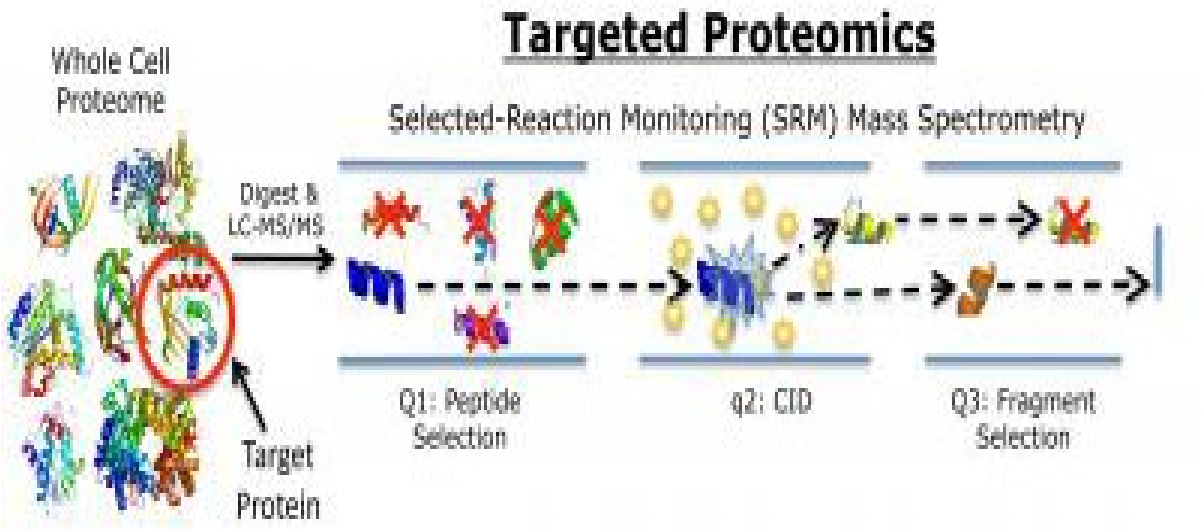





**Figure 49. Targeted proteomics.**

### 4.2.3. Isotope labeling techniques

Isotope labeling techniques enhance quantitative proteomics by incorporating stable isotopes into proteins or peptides, enabling accurate comparison between different biological samples. One widely used approach is Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC), where isotopically labeled amino acids are metabolically incorporated into proteins, allowing for direct quantitative comparisons. Another popular technique is Isobaric Tagging Methods (TMT/iTRAQ), which chemically labels peptides from different samples with isobaric tags, enabling multiplexed analysis in a single mass spectrometry run. These methods improve quantification accuracy and are particularly useful in large-scale proteomics studies (Figure 50).

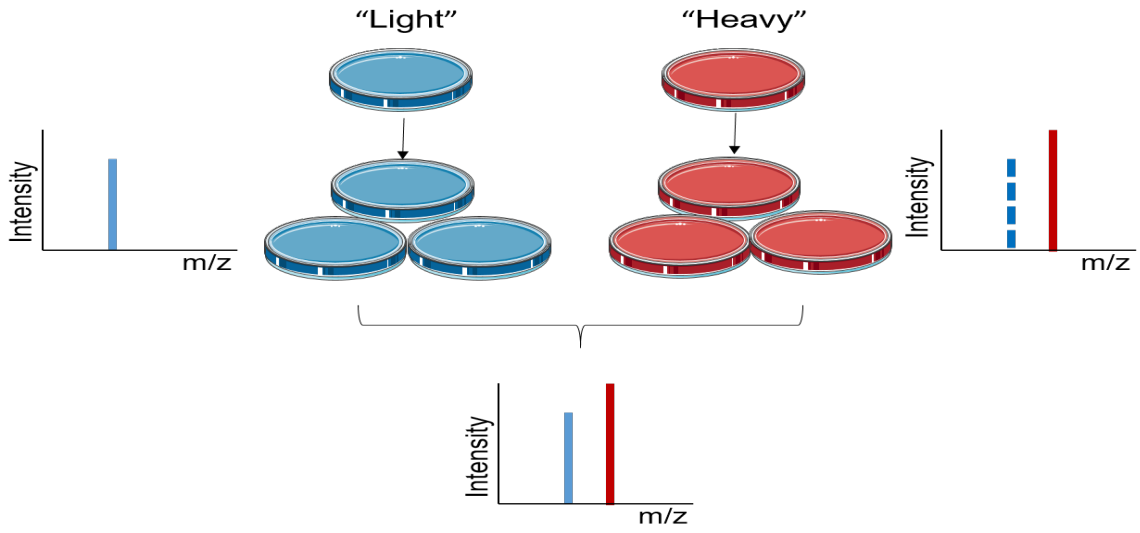



**Figure 50. Stable isotope labeling.**

### 4.2.4. Single-cell proteomics

Single-cell proteomics is an emerging field that aims to analyze protein expression at the individual cell level rather than in bulk cell populations. By employing advanced microfluidic systems and ultra-sensitive mass spectrometry techniques, researchers can capture the heterogeneity of cell populations and uncover rare subpopulations that would otherwise be masked in bulk analyses. This approach is crucial for studying complex biological systems, including cancer, stem cell differentiation, and immune responses, where cellular heterogeneity plays a significant role (Figure 51).



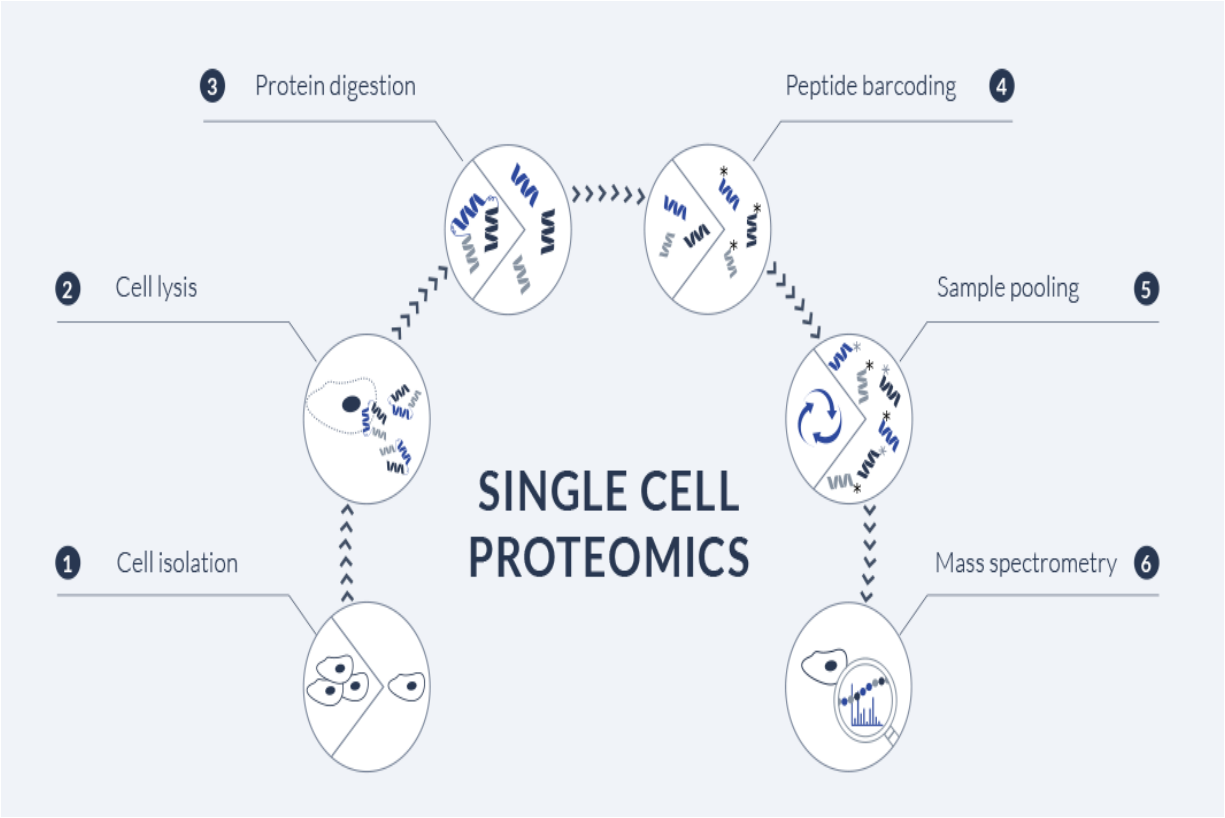



**Figure 51. Single-cell proteomics.**

### 4.2.5. Structural proteomics

Structural proteomics focuses on determining the three-dimensional structures of proteins and their interactions within cells. Techniques such as Cryo-Electron Microscopy (Cryo-EM) and Cross-linking Mass Spectrometry (XL-MS) enable the study of protein complexes at atomic resolution, providing insights into their functional mechanisms. These approaches are essential for understanding protein dynamics, interactions, and conformational changes, which are critical for drug discovery and the development of targeted therapies (Figure 52).

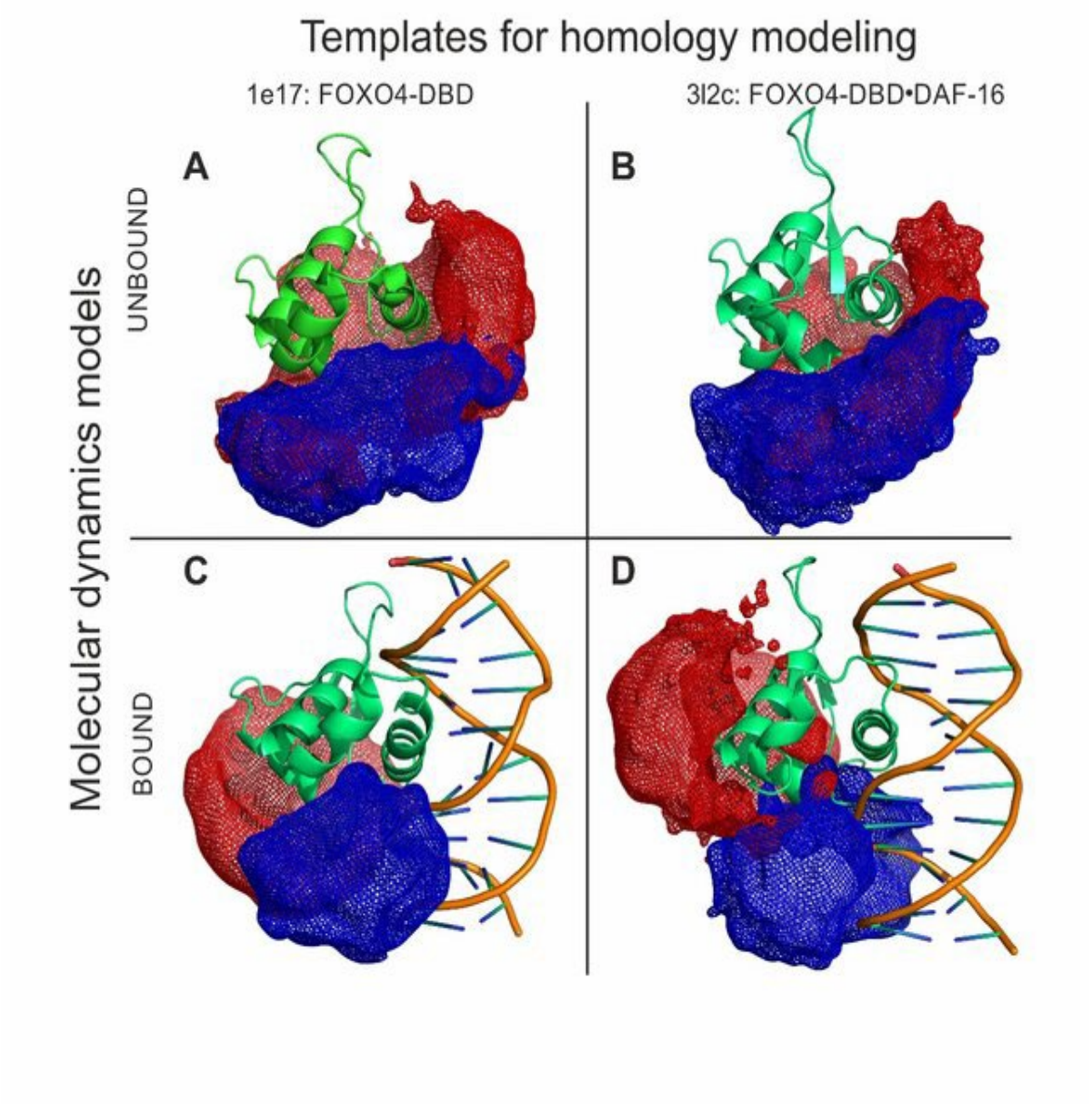



**Figure 52. Structural proteomics.**

### 4.2.6. Proximity labeling & interaction proteomics

Proximity labeling techniques are powerful tools for mapping protein-protein interactions in living cells. Methods such as BioID (Biotin Identification) and APEX (Ascorbate Peroxidase Proximity Labeling) utilize enzyme-mediated labeling to tag proteins in close proximity to a target protein. This approach enables the ide< ntification of interaction networks and subcellular localization of proteins, providing valuable insights into cellular signaling pathways and protein function. By allowing the capture of transient or weak protein interactions, proximity labeling techniques have become indispensable in studying dynamic cellular processes (Figure 53).

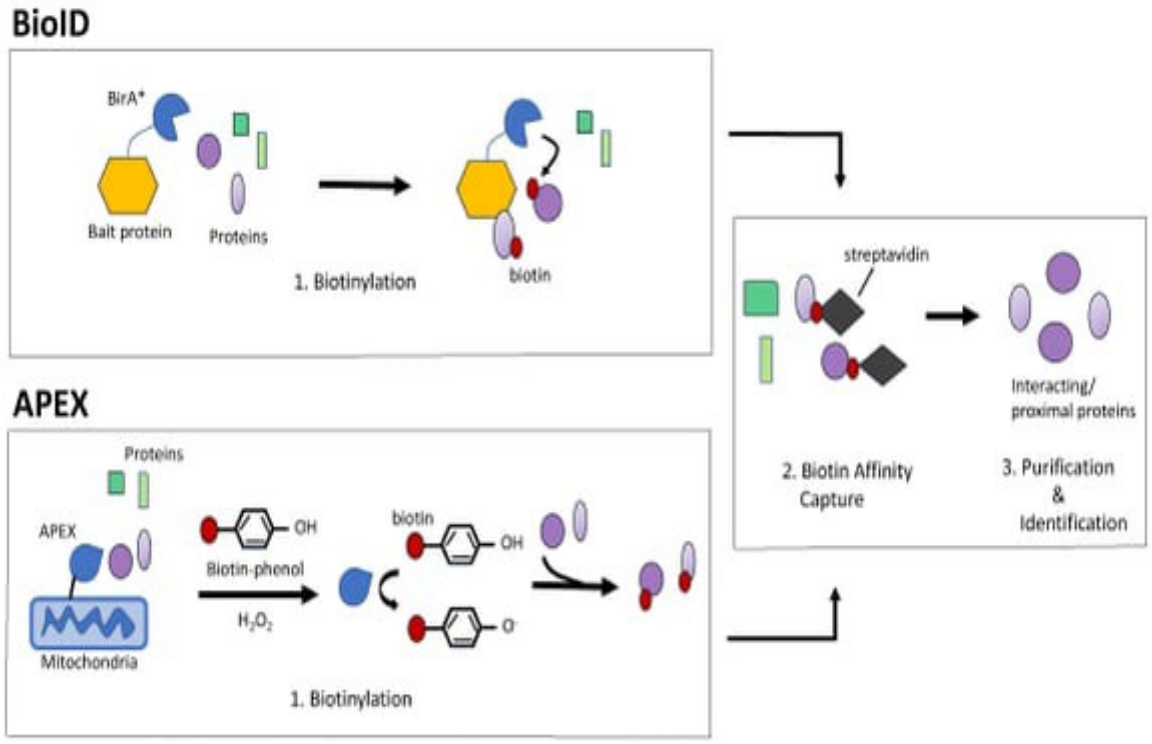



**Figure 53. Proximity labeling & interaction proteomics.**

## 5. Analysis of biochemical mutations and tetrads

Yeast genetics relies extensively on the study of biochemical mutations and tetrad dissection to understand genetic interactions and metabolic pathways. Biochemical mutants are yeast strains with specific metabolic deficiencies, often resulting from mutations in genes encoding enzymes of essential biosynthetic pathways. These mutants are typically identified using growth assays on selective media, where their ability or inability to synthesize specific metabolites reveals the affected biochemical pathway.

Tetrad analysis is a fundamental genetic technique in yeast research, primarily used to study meiotic recombination, gene linkage, and inheritance patterns. This method involves inducing sporulation in a diploid yeast strain to generate four haploid spores, which are carefully separated and analyzed for their genetic composition. By dissecting and analyzing individual tetrads, researchers can determine gene segregation patterns, assess recombination frequencies, and map genetic loci.

This approach is widely applied in functional genomics, enabling the characterization of gene function, interaction networks, and epistatic relationships. By combining tetrad dissection with modern molecular tools, yeast genetics continues to be a powerful system for unraveling complex biological mechanisms (Figure 54).

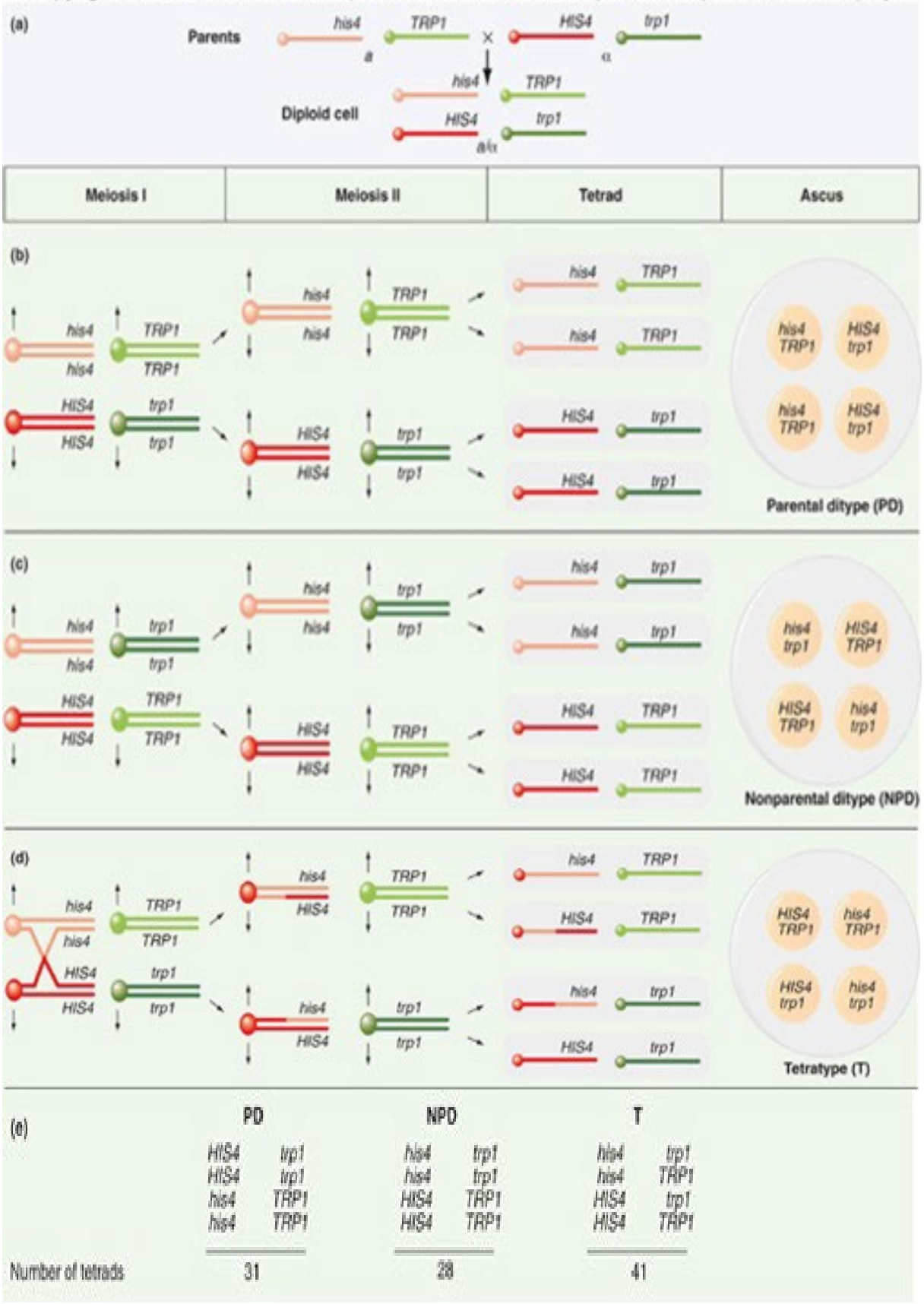



**Figure 54. Analysis of biochemical mutations and tetrads.**

## 6. Complementation and gene conversion

Genetic complementation is a technique used to determine whether two mutations affect the same gene or different genes.

- If two mutant strains with the same phenotype are crossed and the diploid progeny restores the wild-type function, the mutations are in different genes (complementation occurs).
- If the diploid remains mutant, the mutations are in the same gene (no complementation).



Gene conversion is a process of non-reciprocal genetic exchange during homologous recombination, often observed in meiosis. It plays a role in genome stability and evolution (Figure 55).

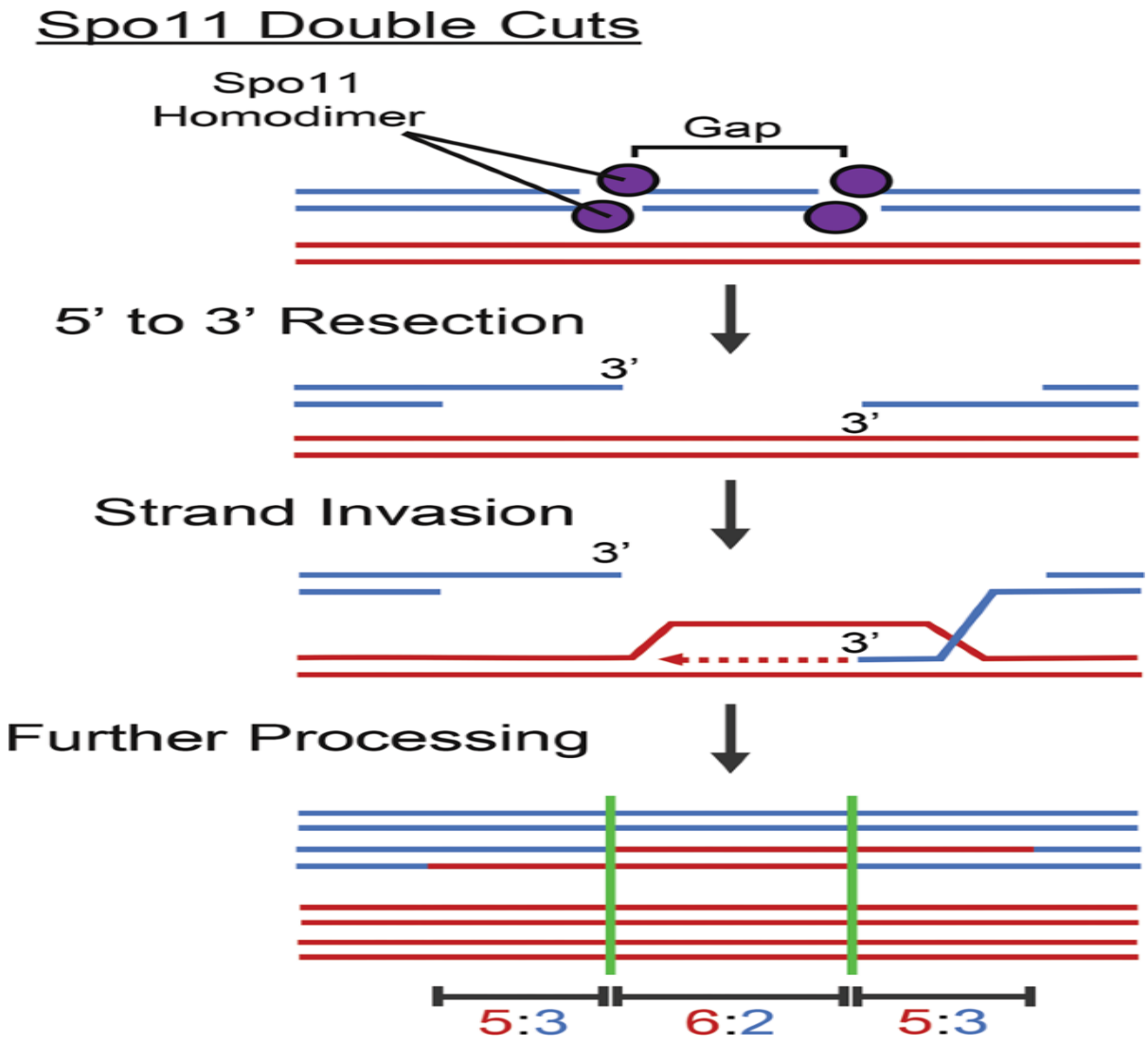



**Figure 55. Complementation and gene conversion.**

## 7. Mitochondrial genetics

Yeast mitochondrial DNA (mtDNA) is circular and contains essential genes for oxidative phosphorylation. Unlike nuclear DNA, mtDNA follows uniparental inheritance (mostly maternal in yeast).



- **Mitochondrial mutants (petite mutants)** are frequently used to study mitochondrial function and respiration.
- **Mitophagy and mitochondrial dynamics** regulate energy production and stress response in yeast cells.

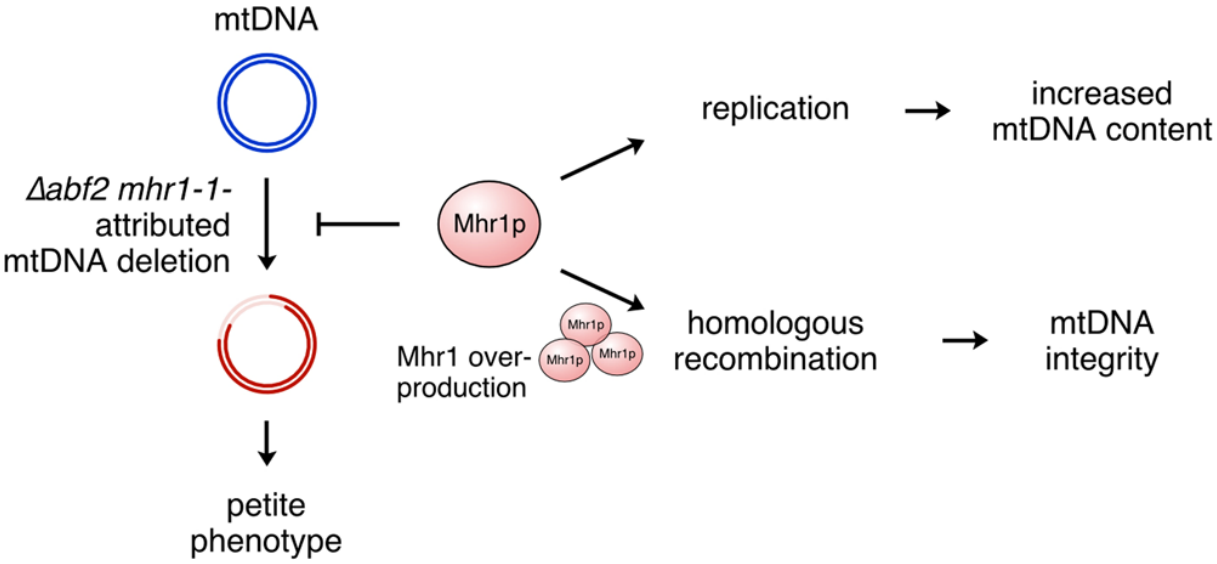



**Figure 56. Mitochondrial genetics.**

## 8. Transposable elements

Yeast genomes harbor mobile genetic elements known as transposons, which play a significant role in genome evolution and gene regulation.

- In *Saccharomyces cerevisiae*, the most well-characterized transposons are the Ty elements, which belong to the class of retrotransposons and share structural similarities with retroviruses. These elements replicate through an RNA intermediate and integrate into the genome via a "copy-and-paste" mechanism (Figure 57).
- Transposons contribute to genome variability by causing gene disruptions, chromosomal rearrangements, and alterations in gene expression. Their insertion can lead to mutations, gene duplications, or changes in regulatory sequences, shaping the genetic landscape of yeast populations.

- Transposon mutagenesis is a widely used technique in yeast genetics for functional genomics studies. By inserting transposable elements into different genomic locations, researchers can disrupt genes systematically, allowing the identification of essential genes, functional domains, and regulatory elements within the genome.

These mobile elements highlight the dynamic nature of the yeast genome and provide valuable tools for studying gene function, adaptation, and genome plasticity.



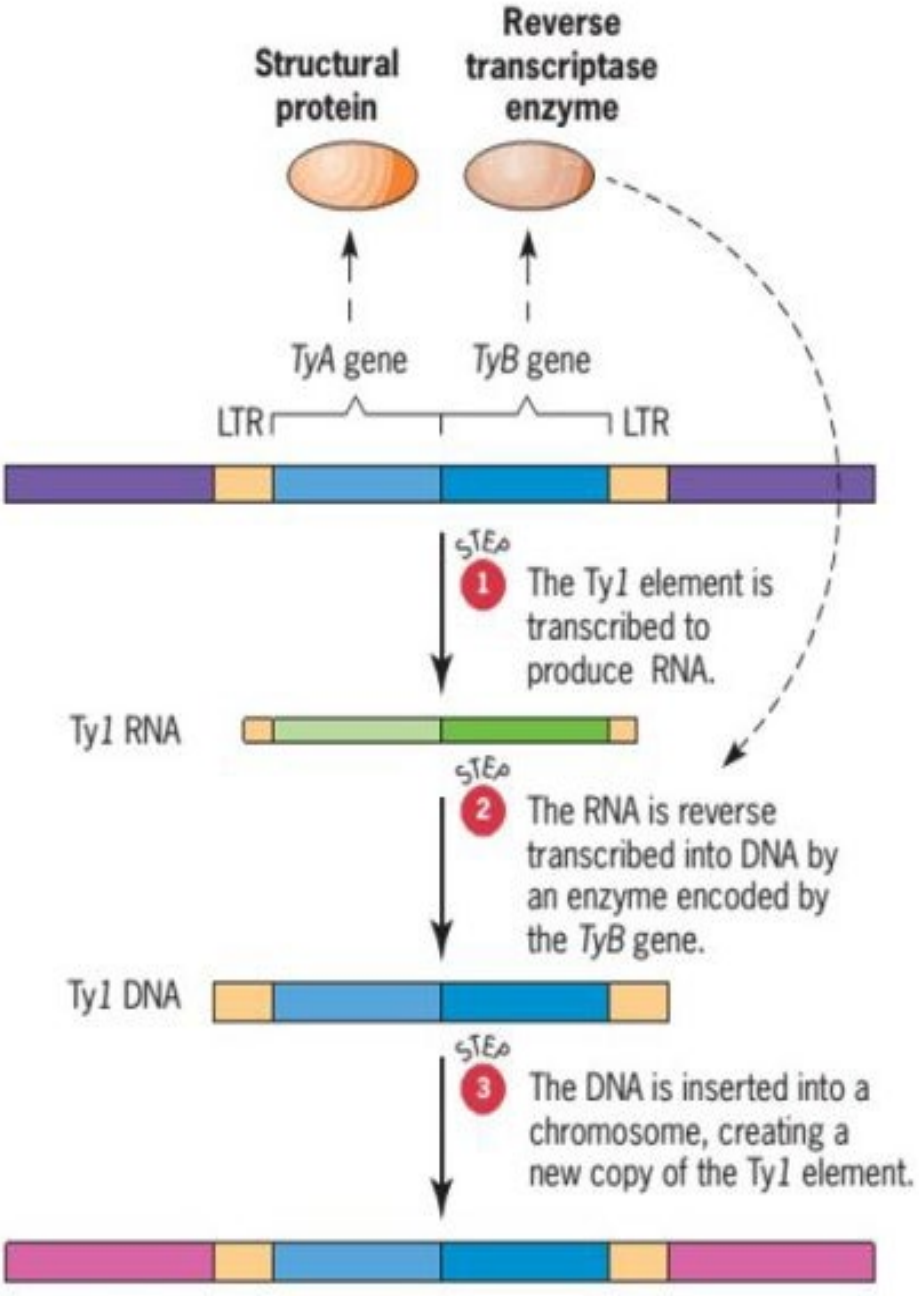



**Figure 57. Transposition of the yeast Ty1 element.**

## 9. Tools and methods for yeast genetic transformation: practical applications

Yeast transformation methods allow the introduction of foreign DNA for genetic studies and industrial applications (Figure 58).

- Common transformation techniques :
    - Lithium acetate method: Simple and widely used.
    - Electroporation: High-efficiency DNA uptake using electrical pulses.
    - Biolistic transformation : DNA delivery via microparticles.

Genetic transformation is applied in gene knockouts, reporter assays, and recombinant protein production in biotechnology.



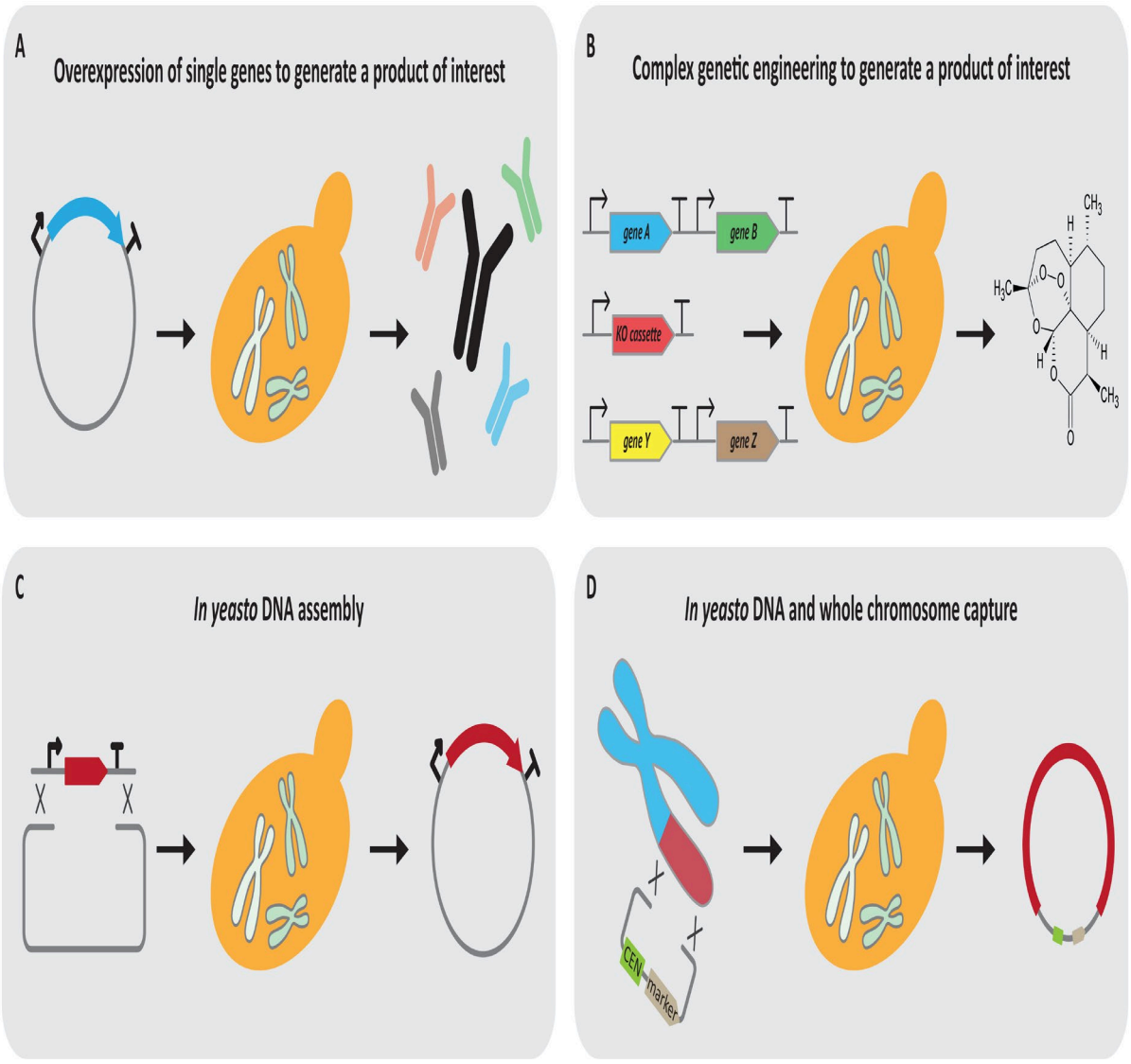



**Figure 58. Methods for yeast genetic transformation application.**

## 10. Cell division and cell cycle

Yeast undergoes both mitotic and meiotic cell division. The yeast cell cycle has four phases (G1, S, G2, and M), regulated by cyclins and CDKs (Figure 59).

- *S. cerevisiae* has been instrumental in discovering key regulators of the eukaryotic cell cycle, including cyclin-dependent kinases (CDKs).
- Cell cycle checkpoints ensure proper DNA replication and division.



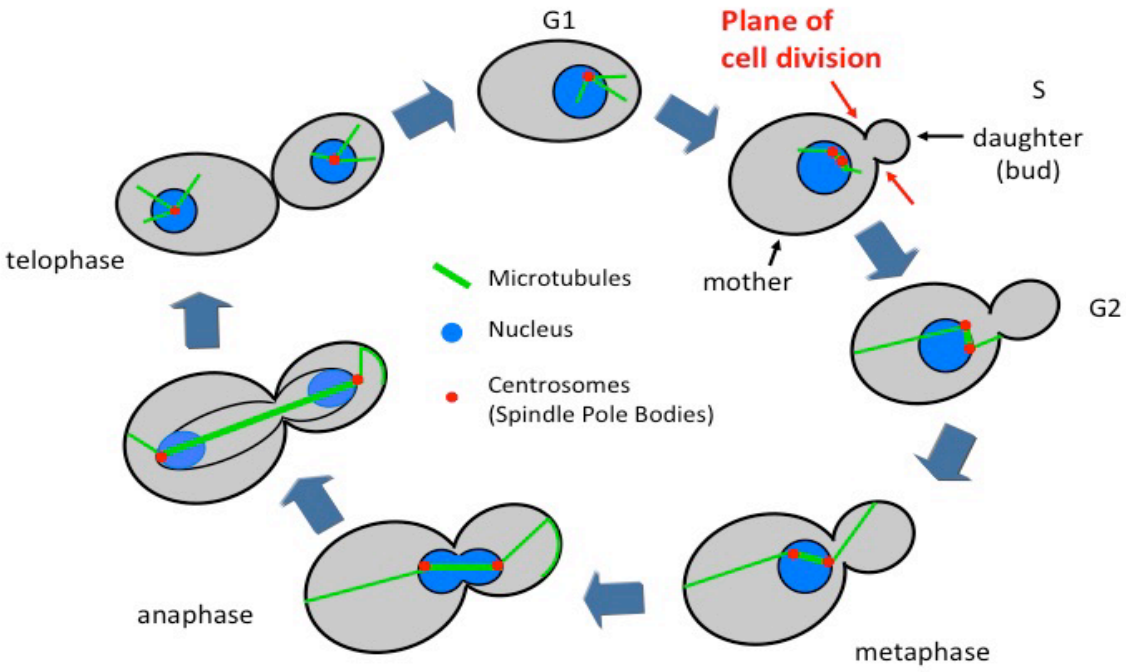



**Figure 59. Cell division and cell cycle**

## 11. Sexual reproduction in yeast (haplodiplobiontic cycle)

Yeast alternates between haploid and diploid states in a haplodiplobiontic life cycle (Figure 60).

- Haploid cells exist in two mating types (*a* and *α*), which fuse to form a diploid cell.
- Under nutrient starvation, diploid cells undergo sporulation, forming four haploid ascospores.
- The study of yeast mating and sporulation provides insights into eukaryotic development and differentiation.

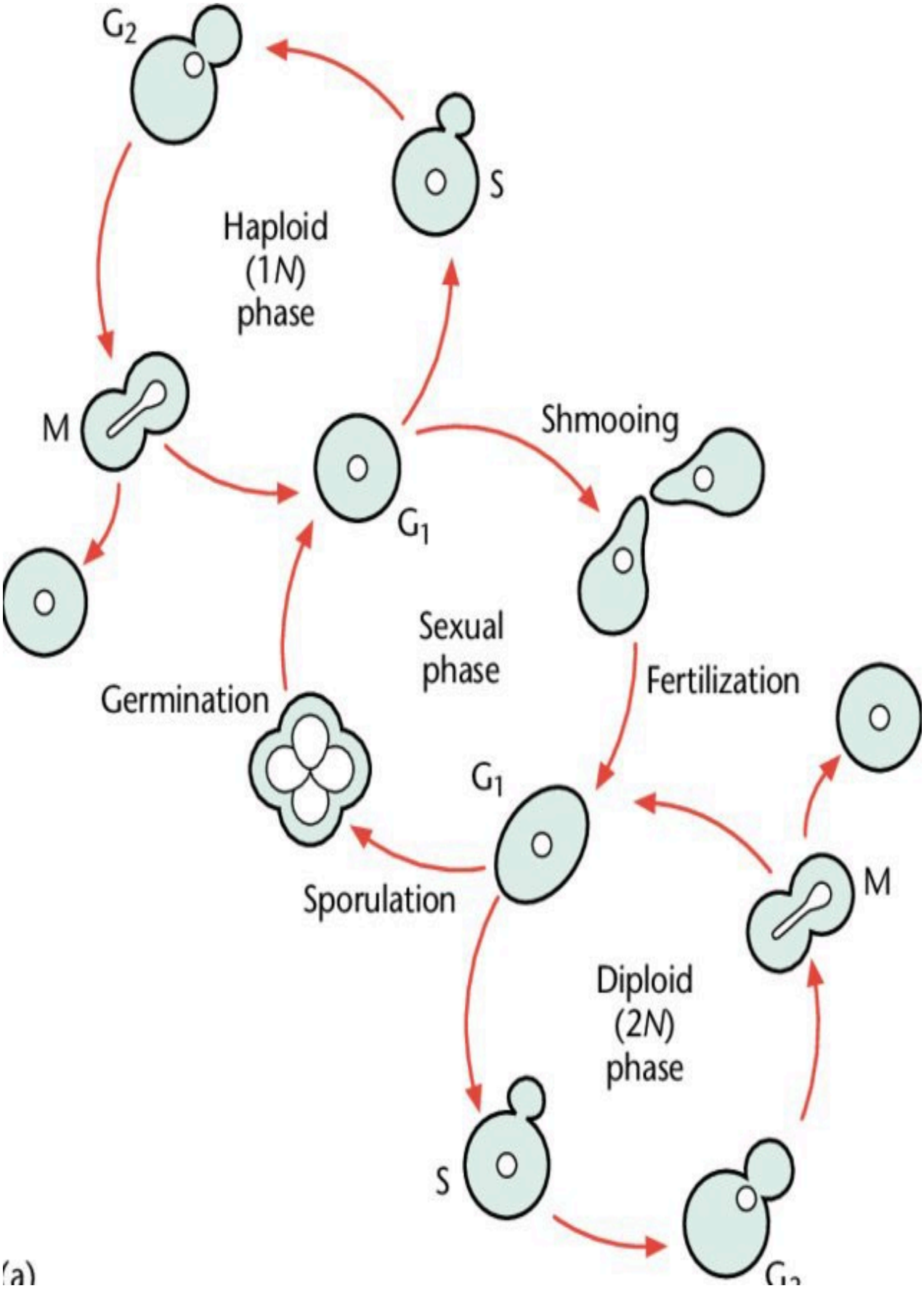



**Figure 60. The life cycle of *Saccharomyces cerevisiae*.**

# Part 3: Viruses

# *Part 3: Viruses*

Viruses are obligate intracellular parasites that require a host cell to replicate. They are submicroscopic infectious agents composed of genetic material (DNA or RNA) enclosed within a protein coat called a capsid, and in some cases, a lipid envelope derived from the host cell. Unlike living organisms, viruses lack cellular structures and metabolic machinery, making them dependent on host cells for replication.



Viruses infect all forms of life, including bacteria (bacteriophages), plants, animals, and humans. Their ability to hijack cellular processes allows them to propagate and spread within populations, sometimes causing diseases. However, viruses also play crucial roles in evolution, gene transfer, and biotechnology.

## 1. Virus structure and classification

Viruses are non-cellular infectious agents that require a host cell to replicate. They are composed of genetic material (DNA or RNA), a protein coat (capsid), and sometimes a lipid envelope. Based on their structure and genome composition, viruses can be regrouped as (Figure 61):

- Helical viruses: Capsid proteins assemble into a rod-like or filamentous structure (e.g., *Tobacco mosaic virus*).
- Icosahedral viruses: Capsid proteins form a symmetrical polyhedral structure (e.g., *Adenoviruses*).
- Complex viruses: Exhibit intricate structures with additional elements such as tail fibers and base plates (e.g., *Bacteriophage T4*).
- Enveloped viruses: Possess a lipid membrane derived from the host cell, which contains viral glycoproteins for host recognition (e.g., *Influenza virus, HIV*).

Viruses are also classified based on their genome type and replication strategy, following the Baltimore classification system (Groups I to VII), distinguishing DNA viruses, RNA viruses, and retrotranscribing viruses (Figure 62).

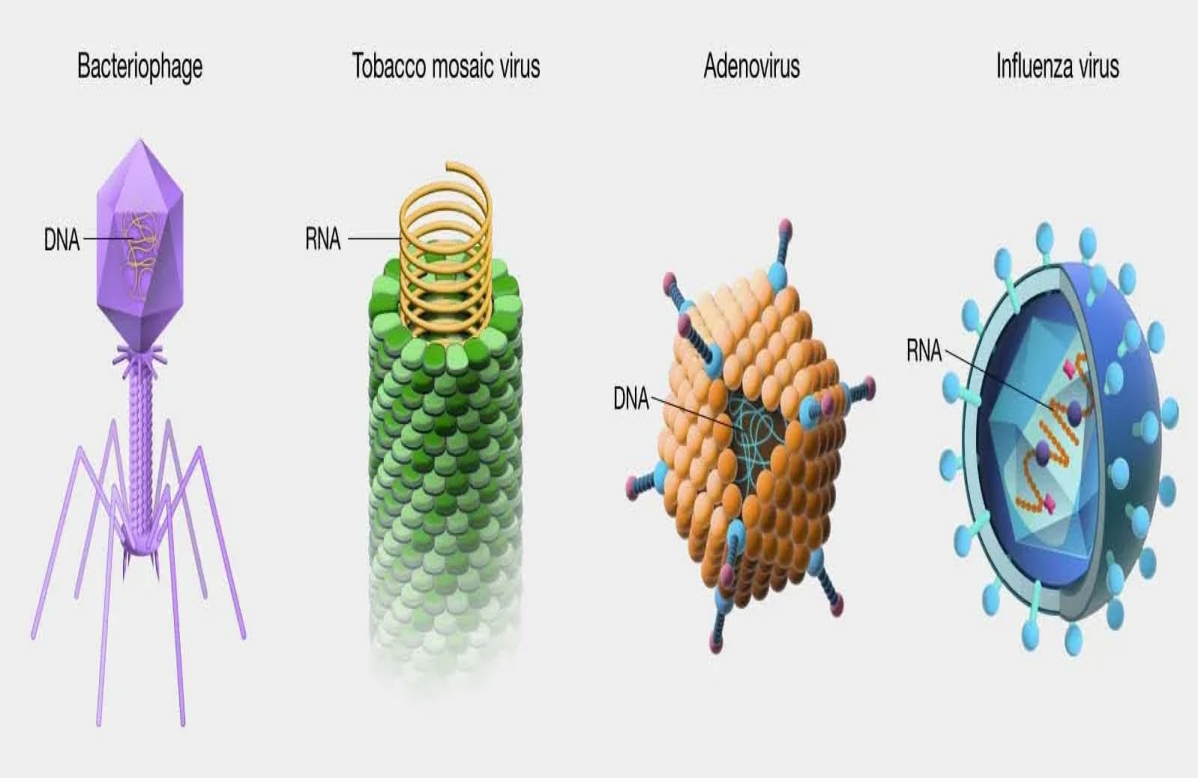





**Figure 61. Types of viruses.**

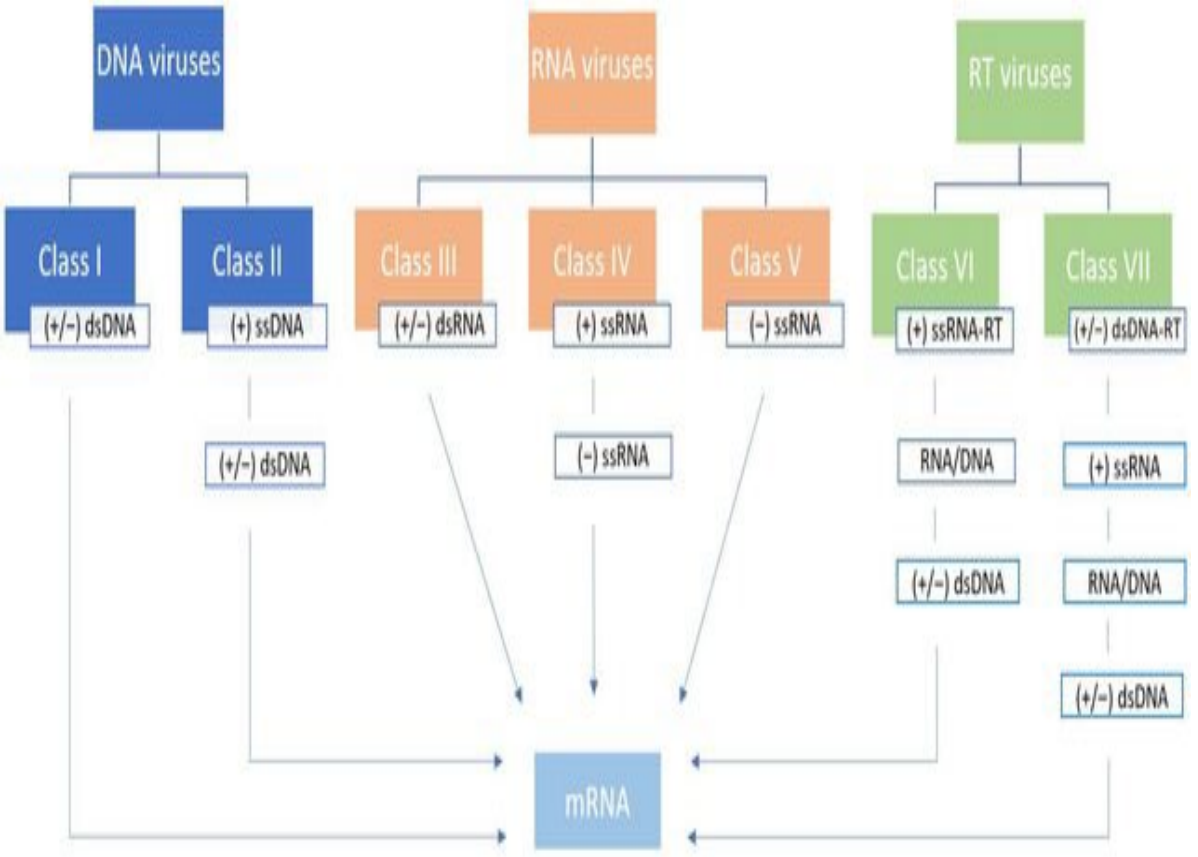



**Figure 62. Baltimore viruses classification.**

## 2. Viral nucleic acids

Viruses store their genetic information in DNA or RNA, varying in strandedness (single or double-stranded), sense (positive or negative), and structure (linear, circular, segmented, or non-segmented).

### 2.1 DNA genomes



DNA viruses have single-stranded (ssDNA) or double-stranded (dsDNA) genomes (Figure 63). Examples include:

- dsDNA viruses (*Herpesviruses, Adenoviruses*): Replicate in the nucleus using host DNA polymerases.
- ssDNA viruses (*Parvoviruses*): Must be converted into dsDNA before replication.

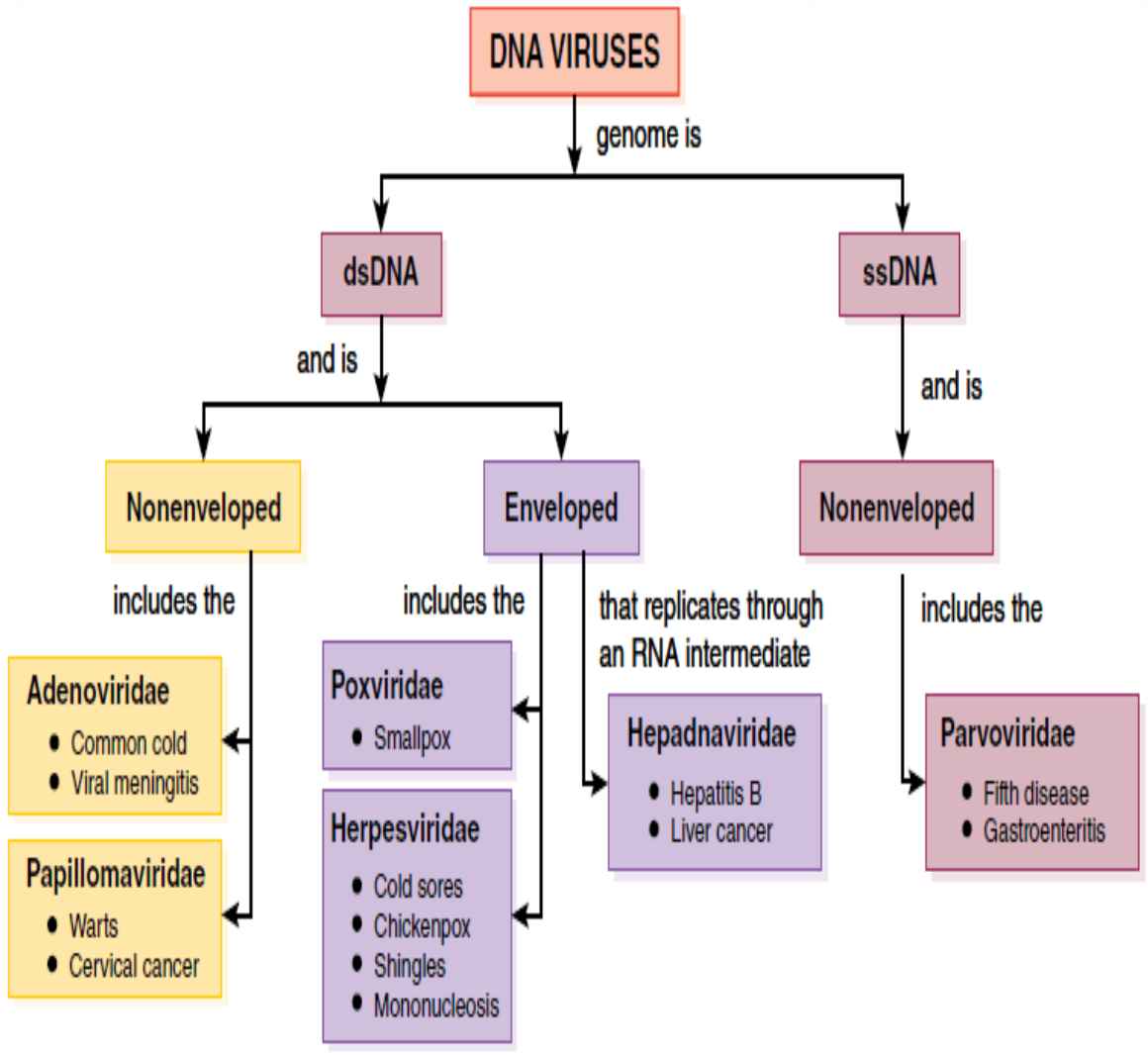



**Figure 63. DNA viruses.**

### 2.2 RNA genomes

RNA viruses use RNA-dependent RNA polymerase (RdRp) or reverse transcriptase for genome replication (Figure 64). Examples include:

- (+)ssRNA viruses (*Poliovirus, SARS-CoV-2*): Directly translated into viral proteins.
- (-)ssRNA viruses (*Influenza, Rabies virus*): Require RdRp to produce a complementary mRNA strand.
- dsRNA viruses (*Reoviruses*): Replicate in specialized compartments to avoid host immune detection.



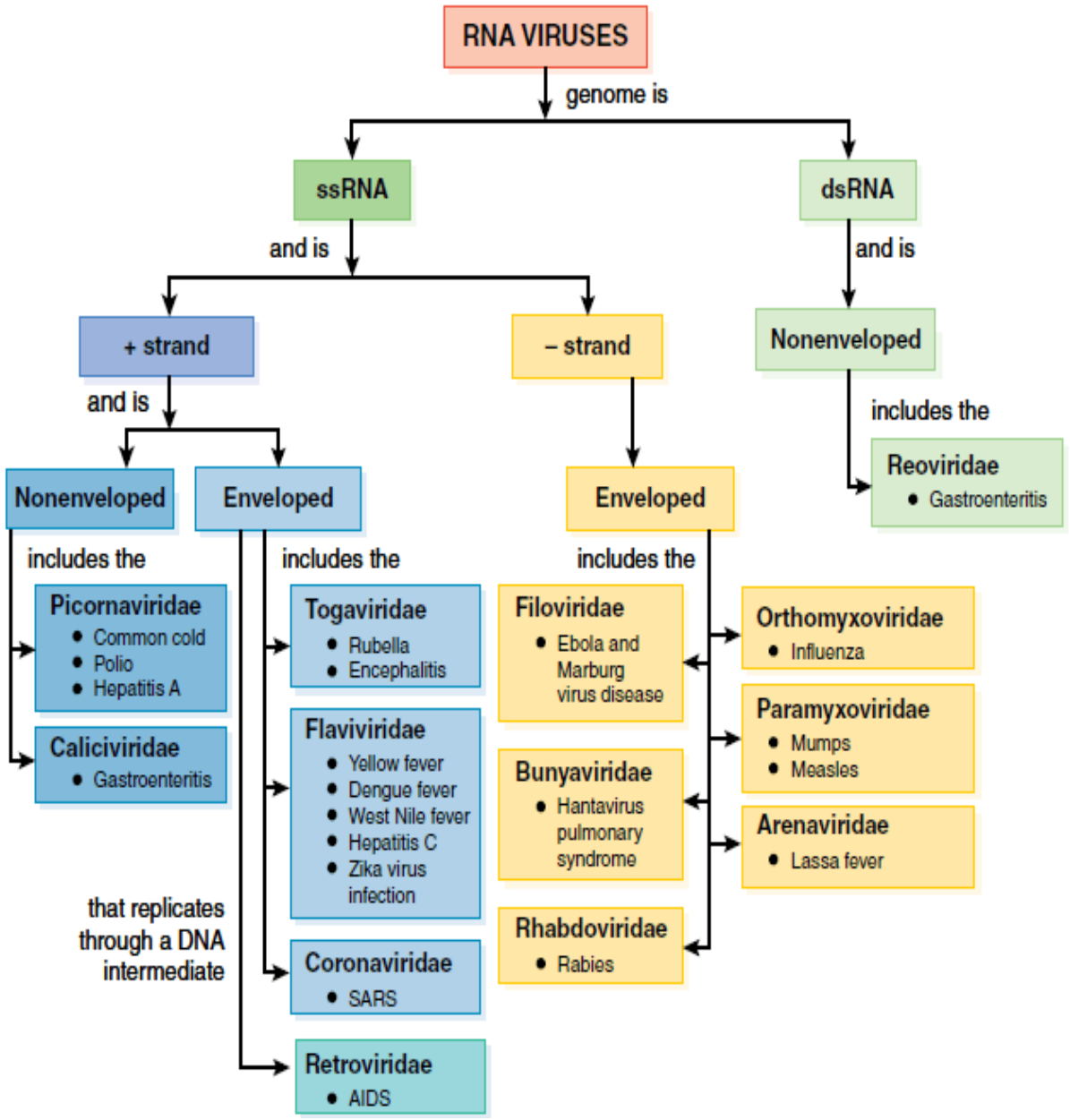



**Figure 64. RNA viruses.**

### 2.3 Case of bacteriophages

Bacteriophages (viruses that infect bacteria) exhibit both DNA and RNA genomes. They are widely used in molecular biology as models for studying virus-host interactions and gene regulation (Figure 65). Examples include:

- dsDNA phages (*T4, Lambda phage*): Follow lytic or lysogenic cycles.
- ssRNA phages (*MS2*): Infect bacteria via the F-pilus and directly translate viral RNA.

ds: double stranded / ss: simple stranded.

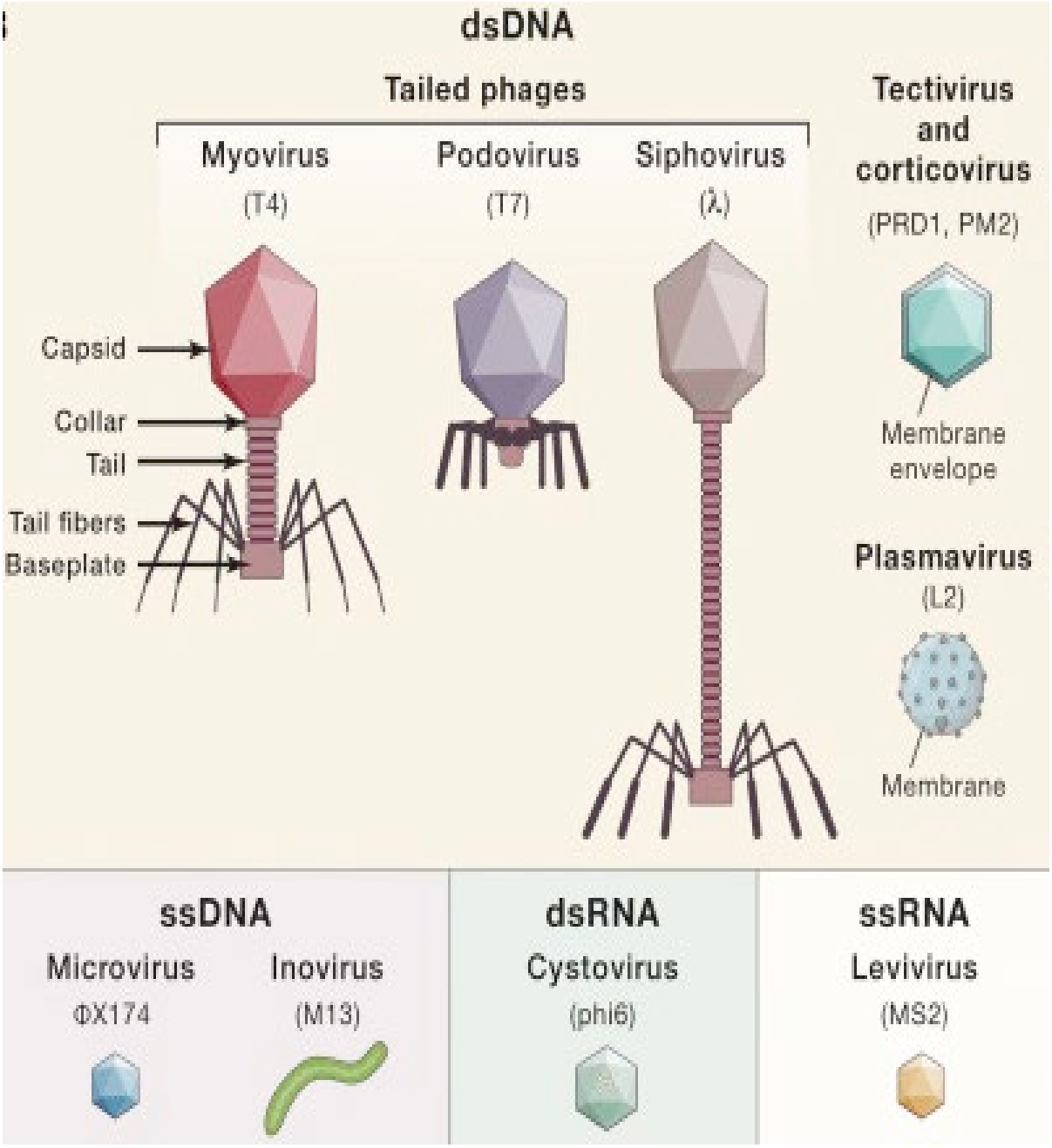



**Figure 65. Phage taxonomy based on morphology and genome composition.**

## 3. Viral cycle

The viral life cycle describes the process by which viruses infect host cells, replicate, and propagate. The two main cycles observed in bacteriophages and certain animal viruses are:

### 3.1 Lytic cycle



The lytic cycle is a rapid replication process where the virus hijacks the host's machinery, leading to cell lysis and virus release (Figure 66). The steps include:

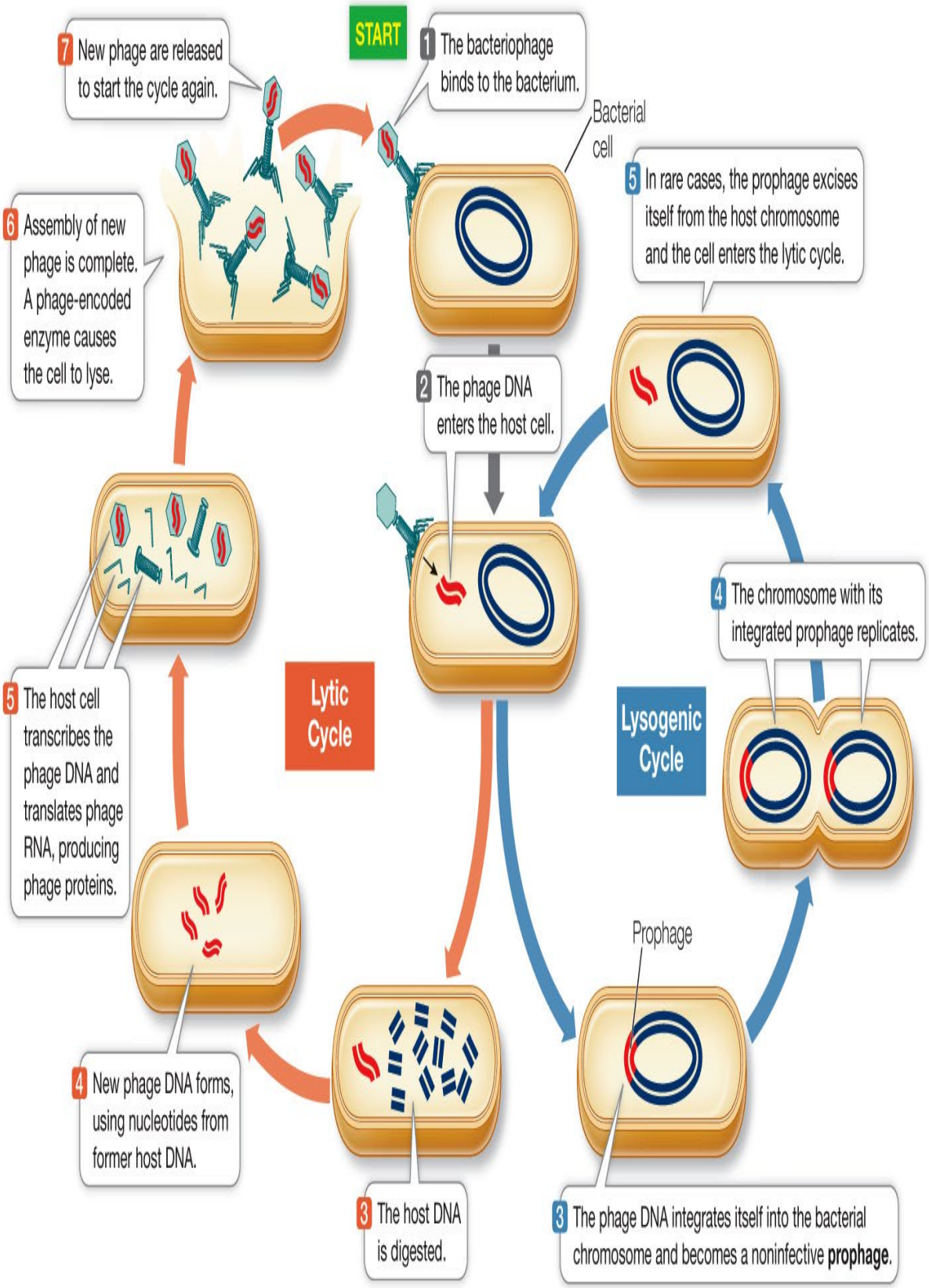
1. Attachment: The virus binds to specific receptors on the host cell membrane.
2. Penetration: The viral genome enters the host cell (injection for bacteriophages, endocytosis for animal viruses).
3. Biosynthesis: Viral genome is replicated, and proteins are synthesized using host enzymes.
4. Maturation: New viral particles are assembled from capsid proteins and genetic material.
5. Lysis: The host cell bursts, releasing new virions to infect other cells (*e.g., Bacteriophage T4*).

### 3.2 Lysogenic cycle

The lysogenic cycle allows the virus to integrate into the host genome without immediate destruction (Figure 66). The key steps are:

1. Attachment & Entry: The phage injects its genome into the bacterial cell.
2. Integration: The viral DNA incorporates into the bacterial chromosome as a prophage.
3. Dormancy: The viral genome replicates along with the host DNA without producing new virions.
4. Induction: Under stress conditions, the prophage exits the genome and switches to the lytic cycle (*e.g., Lambda phage*).

This strategy allows viruses to persist within host populations and activate replication when favorable.




LIFE: THE SCIENCE OF BIOLOGY 11e, Figure 16.12


**Figure 66. Lytic and lysogenic cycles.**

## 4. Replication of viral genetic material

Viruses rely on the host's cellular machinery to replicate their genetic material. The replication mechanism differs between DNA and RNA viruses.

### 4.1 Replication of DNA viruses (Study model: Bacteriophage T4)



The replication of bacteriophage T4 DNA follows a well-organized and tightly regulated process that ensures the efficient multiplication of the viral genome within the host *Escherichia coli* cell (Figure 67). The process involves several key steps:

- Initiation of replication: The replication of T4 DNA begins at multiple origins across the circularly permuted and terminally redundant genome. Specific replication proteins encoded by the phage, such as DNA polymerase, helicase, and primase, initiate the unwinding and synthesis of DNA. Unlike bacterial DNA, T4 replication does not follow a single defined origin but starts at various points along the genome.
- Bidirectional replication: The replication process proceeds in both directions from multiple initiation sites. T4 DNA polymerase synthesizes new strands using host nucleotides, while helicases and single-strand binding proteins stabilize the replication fork. Leading strand synthesis occurs continuously, while the lagging strand is synthesized discontinuously through Okazaki fragments.
- Formation of concatemers: Unlike most bacterial and eukaryotic DNA, T4 replication leads to the formation of large concatemeric DNA molecules. These long DNA strands consist of multiple copies of the phage genome joined end-to-end. Concatemers form through homologous recombination and rolling-circle replication mechanisms.
- Rolling-circle replication: As the infection progresses, replication shifts to a rolling-circle mechanism. In this process, a nick is introduced in one strand of the DNA, and the intact strand serves as a template for continuous synthesis, displacing the other strand, which is later copied. This method efficiently generates long, linear DNA molecules needed for packaging into new phage particles.
- DNA packaging into capsids: The concatemeric DNA is then cleaved by phage-encoded terminase enzymes, which cut the DNA at specific sequences, ensuring that each virion receives a full-length genome. The DNA is subsequently packaged into newly assembled capsids, completing the replication phase before viral assembly and lysis.

This process ensures the rapid and efficient replication of T4 DNA, leading to the production of numerous new virions, which are then released from the host cell through lysis.



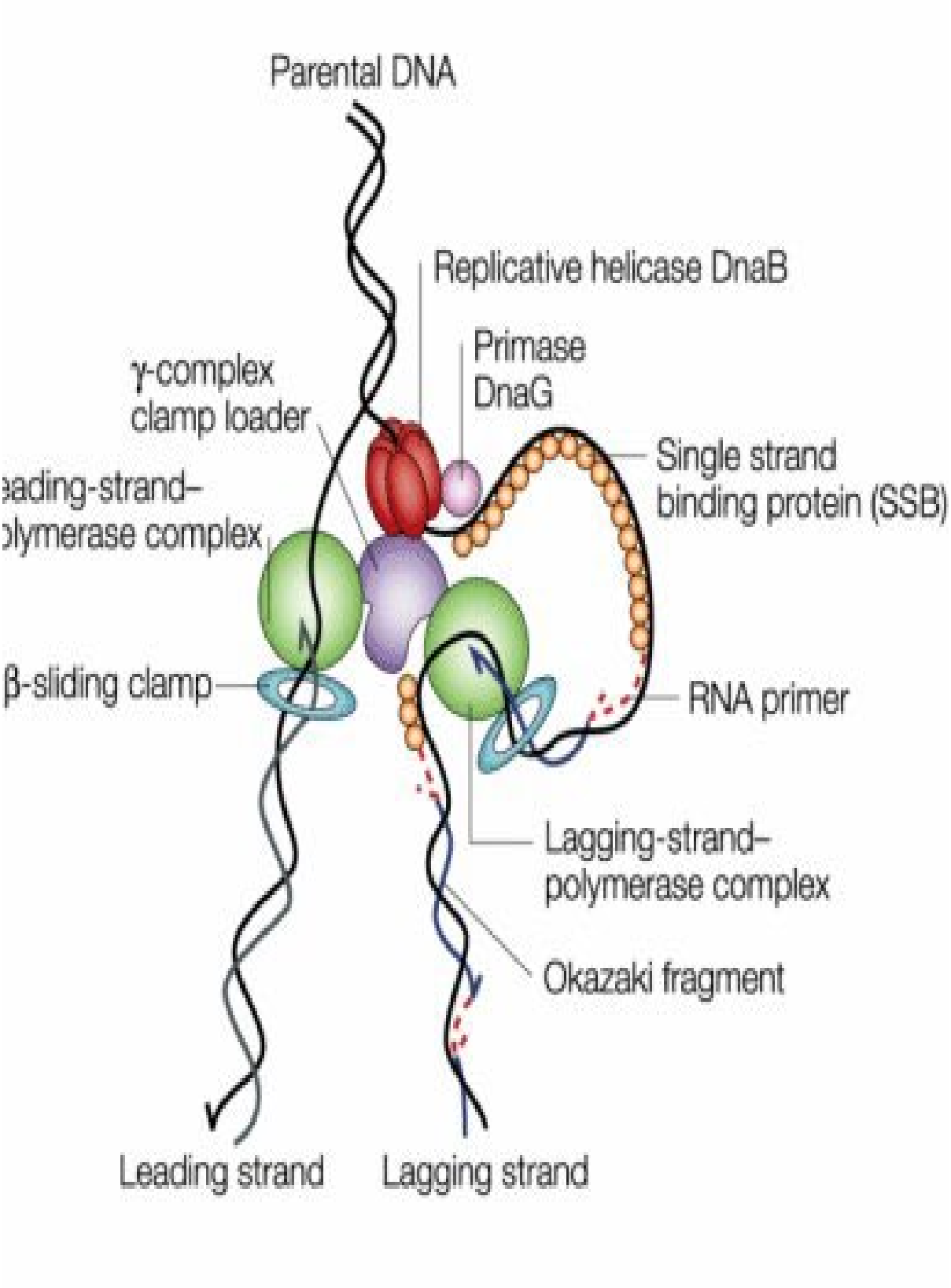



**Figure 67. Replication of Bacteriophage T4 DNA.**

### 4.2 Replication of RNA viruses

RNA viruses exhibit diverse replication strategies depending on their genome type. Unlike DNA viruses, they do not rely on host DNA polymerases but instead use viral RNA-dependent RNA polymerases (RdRp) or reverse transcriptase for genome replication. The replication process varies significantly between positive-sense, negative-sense, and retroviruses.



#### 4.2.1. Replication of positive-sense (+) ssRNA viruses

(*Example: Poliovirus - SARS-CoV-2*) (Figure 68).

- Genome as mRNA: The (+) ssRNA genome serves directly as messenger RNA (mRNA) for translation upon entering the host cell. Viral proteins, including RNA-dependent RNA polymerase (RdRp), are synthesized immediately.
- Replication Process: The viral RdRp synthesizes a complementary (-) ssRNA strand, which then serves as a template for generating multiple copies of the (+) ssRNA genome.
- Assembly and Release: Newly synthesized genomes are packaged into capsids and released by cell lysis or budding, depending on the virus.

#### 4.2.2. Replication of negative-sense (-) ssRNA viruses

(*Example: Influenza virus*) (Figure 68).

- Conversion to mRNA: The (-) ssRNA genome cannot be translated directly. The virus carries its own RNA-dependent RNA polymerase (RdRp), which synthesizes complementary (+) ssRNA strands to act as mRNA.
- Genome Replication: The newly synthesized (+) ssRNA is used as a template to produce more (-) ssRNA copies for packaging into new virions.
- Budding and Release: Most (-) ssRNA viruses, like Influenza, are enveloped and exit the host cell via budding, acquiring a lipid envelope from the host membrane.

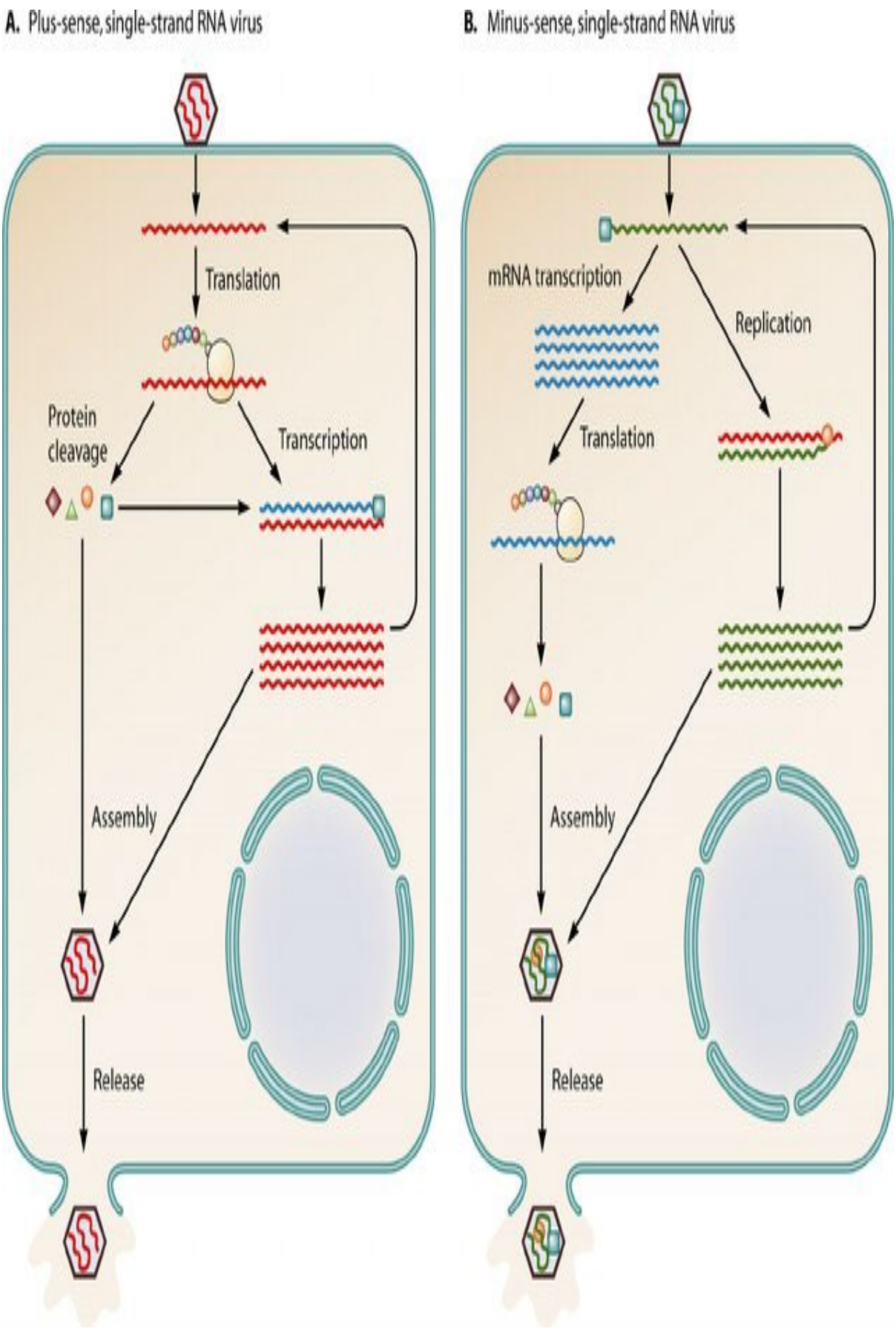



**Figure 68. Replication of (+) and (-) ssRNA viruses.**

### 4.2.3. Replication of double-stranded (dsRNA) viruses

(*Example: Rotavirus*) (Figure 69).

- Transcription of mRNA: The dsRNA genome remains inside the viral core, where viral RNA polymerase transcribes (+) ssRNA strands.
- Translation and Replication: These (+) ssRNA strands function as mRNA for protein synthesis and also serve as templates for synthesizing the complementary (-) RNA strands, forming new dsRNA genomes.
- Virus Assembly and Release: Newly formed dsRNA genomes are enclosed in capsids, and virions are released by cell lysis.



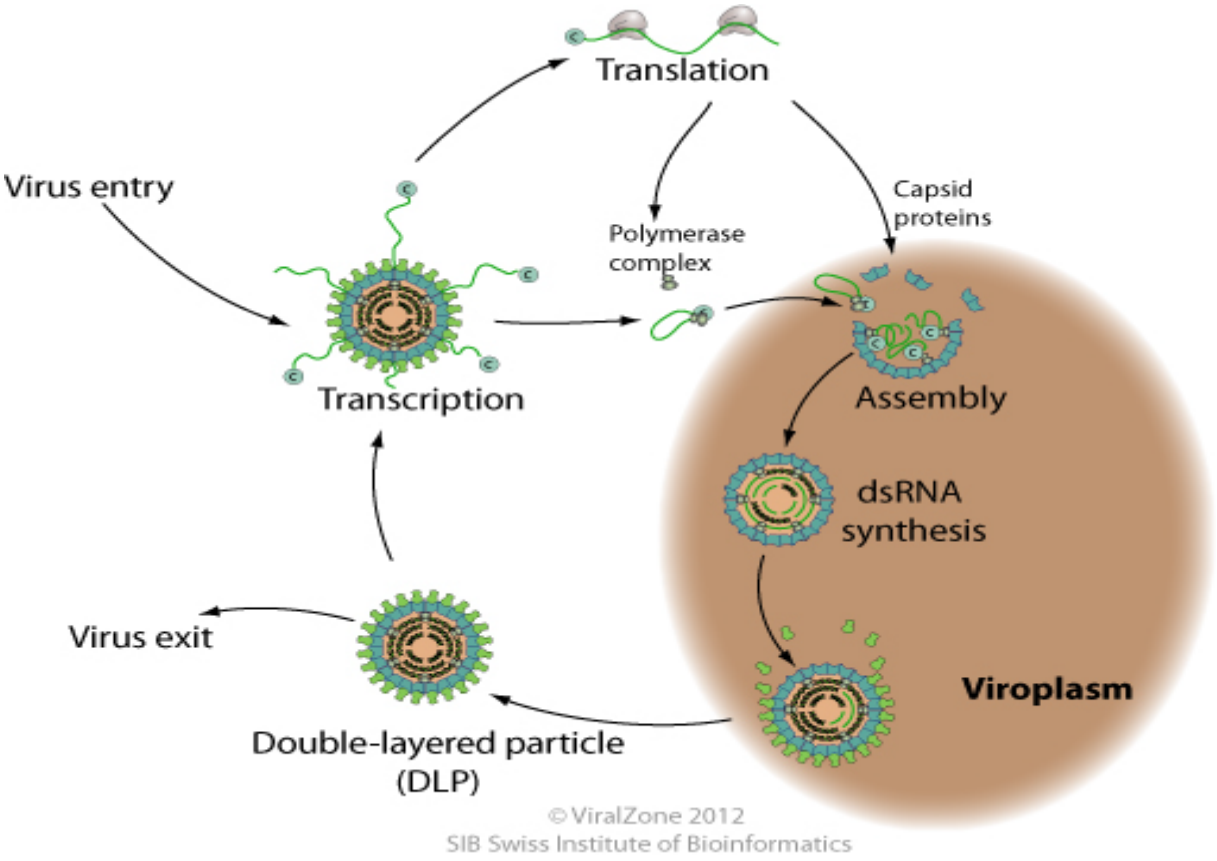



**Figure 69. Replication of double-stranded (dsRNA) viruses.**

### 4.2.4. Replication of retroviruses (reverse-transcribing viruses)

(*Example: HIV - Human Immunodeficiency Virus*) (Figure 70).

- Reverse Transcription: The viral RNA genome is reverse-transcribed into complementary DNA (cDNA) by reverse transcriptase.

- Integration into Host Genome: The cDNA integrates into the host cell's genome using the viral integrase enzyme, becoming a permanent part of the host DNA.
- Transcription and Translation: Host cell machinery transcribes viral DNA into new viral RNA, which serves both as mRNA for protein synthesis and as genomic RNA for new virions.
- Budding and Release: New virions bud from the host cell, acquiring an envelope derived from the host membrane.



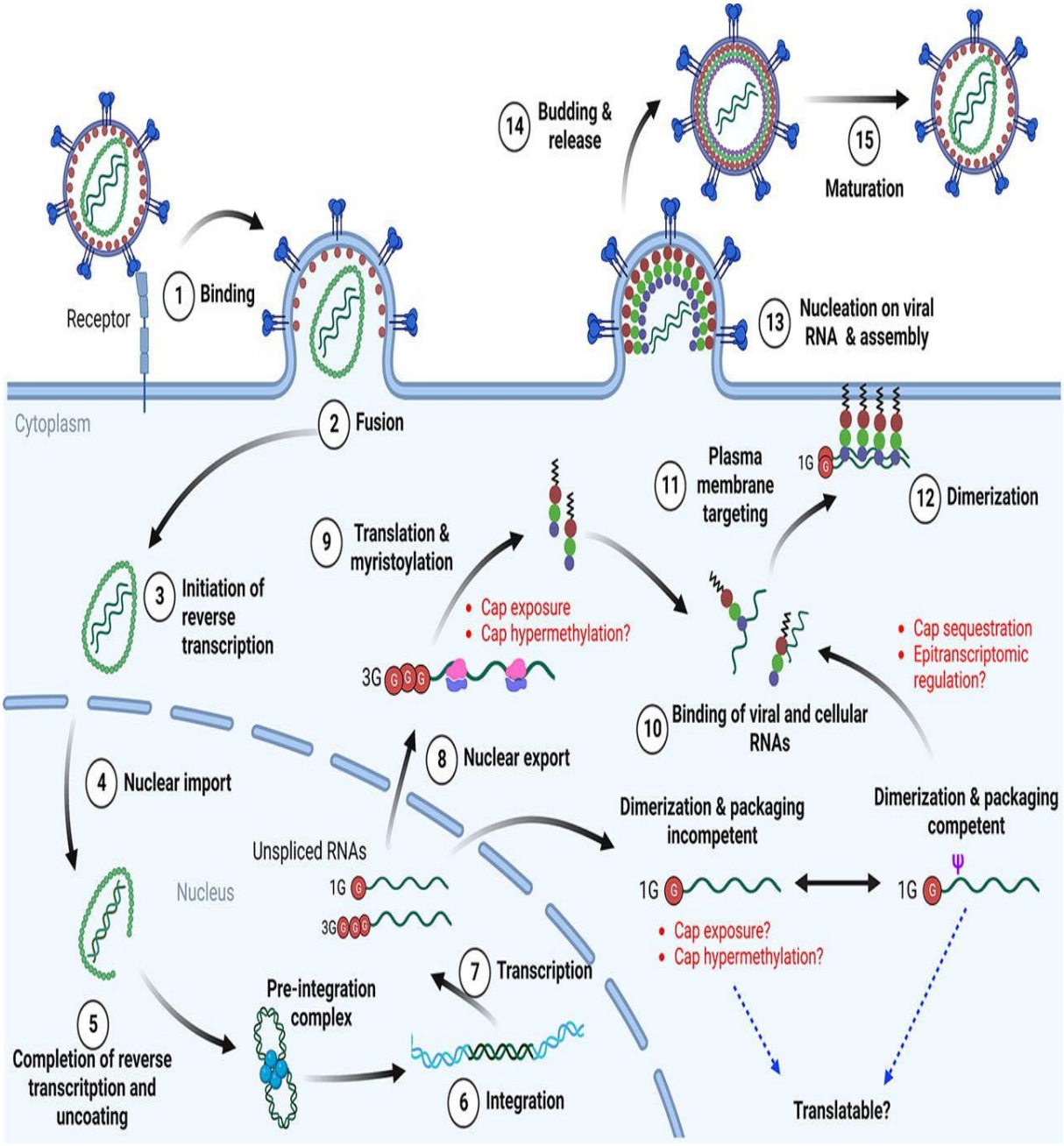



**Figure 70. Replication of retroviruses.**

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# 100 Questions QCM



1. Which of the following statements about the bacterial chromosome are true?
    a) It is usually circular.
    b) It is surrounded by a nuclear membrane.
    c) It is typically haploid.
    d) It is made of RNA.
    e) All answers are false.
2. Plasmids are characterized by:
    a) Their ability to replicate independently of the bacterial chromosome.
    b) Their essential role in bacterial survival under all conditions.
    c) Their involvement in antibiotic resistance.
    d) Their ability to transfer between bacteria through conjugation.
    e) All answers are false.
3. Which of the following are types of plasmids?
    a) Fertility (F) plasmids.
    b) R plasmids.
    c) Col plasmids.
    d) Lysogenic plasmids.
    e) All answers are false.
4. Mobile genetic elements in bacteria include:
    a) Plasmids.
    b) Transposons.
    c) Bacteriophages.
    d) Ribosomes.
    e) All answers are false.
5. Which are characteristics of transposons?
    a) They can move from one genomic location to another.
    b) They always replicate when they move.
    c) They can disrupt genes when inserted.
    d) They are only found in prokaryotes.
    e) All answers are false.
6. Bacterial genes are typically organized as:
    a) Operons.
    b) Introns and exons.
    c) Single, unlinked genes.
    d) Linear chromosomes.
    e) All answers are false.

7. DNA replication in bacteria requires:
    a) DNA polymerase III.
    b) RNA polymerase.
    c) DNA ligase.
    d) DNA helicase.
    e) All answers are false.
8. Which of the following statements about horizontal gene transfer are correct?
    a) It contributes to genetic diversity in bacteria.
    b) It includes transformation, conjugation, and transduction.
    c) It only occurs between closely related bacterial species.
    d) It requires the presence of a nucleus.
    e) All answers are false.
9. Which of the following is a correct description of transformation?
    a) It involves the uptake of free DNA from the environment.
    b) It requires a bacteriophage to mediate DNA transfer.
    c) It is the primary mechanism of antibiotic resistance spread.
    d) It occurs naturally in some bacterial species.
    e) All answers are false.
10. Conjugation is characterized by:
    a) The direct transfer of DNA from one bacterium to another.
    b) The use of a sex pilus in Gram-negative bacteria.
    c) The involvement of F plasmids.
    d) The packaging of bacterial DNA into phages.
    e) All answers are false.
11. Which of the following statements about transduction are true?
    a) It is mediated by bacteriophages.
    b) It allows the transfer of bacterial DNA between cells.
    c) It occurs through direct cell-to-cell contact.
    d) It includes generalized and specialized transduction.
    e) All answers are false.
12. In bacterial transcription, which enzyme synthesizes RNA?
    a) DNA polymerase.
    b) RNA polymerase.
    c) Reverse transcriptase.
    d) DNA ligase.
    e) All answers are false.
13. The stages of transcription include:
    a) Initiation.
    b) Elongation.
    c) Termination.

d) Transduction.

e) All answers are false.

14. Which factors play a role in bacterial translation initiation?

a) Ribosome binding site (Shine-Dalgarno sequence).

b) 5’ cap structure.

c) Initiator tRNA carrying N-formylmethionine (fMet).

d) RNA-dependent RNA polymerase.

e) All answers are false.



15. Which statements about ribosomes in bacteria are correct?

a) They are composed of 50S and 30S subunits.

b) They contain rRNA and proteins.

c) They are identical to eukaryotic ribosomes.

d) They catalyze peptide bond formation.

e) All answers are false.

16. During translation elongation, which molecule delivers amino acids to the ribosome?

a) mRNA.

b) tRNA.

c) DNA polymerase.

d) RNA helicase.

e) All answers are false.

17. Which statements about the lactose operon are correct?

a) It is an inducible operon.

b) It is activated when lactose is present.

c) It is controlled by a repressor protein.

d) It is always active in the presence of glucose.

e) All answers are false.

18. The tryptophan operon is an example of:

a) An inducible operon.

b) A repressible operon.

c) A transcription factor-independent system.

d) A viral genome.

e) All answers are false.

19. Which mechanisms regulate bacterial gene expression?

a) Operon model.

b) Attenuation.

c) DNA sequence inversion.

d) RNA interference.

e) All answers are false.

20. Which features are characteristic of yeast as a model system?
    a) It is a unicellular eukaryote.
    b) It has a well-characterized genome.
    c) It lacks mitochondria.
    d) It reproduces only asexually.
    e) All answers are false.
21. Which characteristics are specific to yeast genome organization?
    a) It is linear and divided into chromosomes.
    b) It contains introns.
    c) It lacks a nuclear membrane.
    d) It is similar in size to bacterial genomes.
    e) All answers are false.
22. Which methods are commonly used for yeast genetic transformation?
    a) Electroporation.
    b) Lithium acetate method.
    c) Heat shock treatment.
    d) Conjugation with bacteria.
    e) All answers are false.
23. What are the key functions of the yeast proteome?
    a) Enzyme catalysis.
    b) Signal transduction.
    c) Structural support.
    d) ATP synthesis in the cytoplasm only.
    e) All answers are false.
24. Tetrad analysis in yeast is used to:
    a) Study meiotic recombination.
    b) Determine gene linkage.
    c) Analyze sporulation defects.
    d) Identify bacteriophage mutations.
    e) All answers are false.
25. Which statements about yeast mitochondria are correct?
    a) They contain circular DNA.
    b) They are inherited maternally in most cases.
    c) They replicate independently from nuclear DNA.
    d) They use a genetic code identical to the universal code.
    e) All answers are false.
26. Transposable elements in yeast:
    a) Include Ty elements.
    b) Resemble retroviruses.
    c) Are always beneficial.

d) Do not integrate into the genome.

e) All answers are false.

27. Which proteins are involved in yeast cell cycle regulation?

a) Cyclins.

b) CDKs (Cyclin-Dependent Kinases).

c) DNA polymerase.

d) RNA helicase.

e) All answers are false.



28. Yeast sexual reproduction involves:

a) Alternation between haploid and diploid states.

b) Mating types a and α.

c) Binary fission.

d) Formation of ascospores.

e) All answers are false.

29. What are the main structural components of a virus?

a) Capsid.

b) Envelope (for some viruses).

c) Ribosomes.

d) Cytoplasm.

e) All answers are false.

30. How are viruses classified?

a) By genome type (DNA or RNA).

b) By capsid symmetry.

c) By presence or absence of an envelope.

d) By antibiotic sensitivity.

e) All answers are false.

31. Which viruses have a DNA genome?

a) Herpesviruses.

b) Adenoviruses.

c) Influenza viruses.

d) Retroviruses.

e) All answers are false.

32. Which statements about RNA viruses are true?

a) They can have positive-sense or negative-sense RNA.

b) They always integrate into the host genome.

c) They use an RNA-dependent RNA polymerase for replication.

d) They are never enveloped.

e) All answers are false.

33. Which bacteriophage replication cycle leads to cell lysis?
    a) Lytic cycle.
    b) Lysogenic cycle.
    c) Binary fission.
    d) Sporulation.
    e) All answers are false.
34. Which statements about the lysogenic cycle are correct?
    a) The viral genome integrates into the bacterial chromosome.
    b) The virus remains dormant as a prophage.
    c) It always leads to immediate cell destruction.
    d) It can be triggered into a lytic cycle under certain conditions.
    e) All answers are false.
35. Which enzymes are involved in bacteriophage T4 DNA replication?
    a) DNA polymerase.
    b) Helicase.
    c) Ligase.
    d) RNA polymerase.
    e) All answers are false.
36. Which viruses replicate via reverse transcription?
    a) Retroviruses (e.g., HIV).
    b) Hepadnaviruses (e.g., Hepatitis B virus).
    c) Herpesviruses.
    d) Picornaviruses.
    e) All answers are false.
37. Which RNA virus replication strategies exist?
    a) Direct translation of positive-sense RNA.
    b) Replication via an RNA-dependent RNA polymerase.
    c) Reverse transcription into DNA.
    d) DNA-dependent RNA synthesis.
    e) All answers are false.
38. Which structures are unique to bacteriophages?
    a) Tail fibers.
    b) Base plates.
    c) Peptidoglycan layer.
    d) Membrane-bound organelles.
    e) All answers are false.
39. What is the role of viral envelopes?
    a) Protection from the immune system.
    b) Attachment to host cells.
    c) Entry into the host cell.

d) Synthesis of viral capsid proteins.
e) All answers are false.

40. Which viral genome types exist?
a) Single-stranded DNA (ssDNA).
b) Double-stranded DNA (dsDNA).
c) Single-stranded RNA (ssRNA).
d) Double-stranded RNA (dsRNA).
e) All answers are false.



41. What is a characteristic of bacteriophage T4?
a) It has a double-stranded DNA genome.
b) It undergoes a lysogenic cycle only.
c) It infects eukaryotic cells.
d) It has a contractile tail.
e) All answers are false.

42. How do enveloped viruses enter host cells?
a) Endocytosis.
b) Fusion with the host membrane.
c) Injection of nucleic acid through tail fibers.
d) Direct penetration through the cell wall.
e) All answers are false.

43. What is the role of viral polymerases?
a) Replication of the viral genome.
b) Transcription of viral mRNA.
c) Degradation of host DNA.
d) Inhibition of host cell protein synthesis.
e) All answers are false.

44. Which of the following viruses have segmented genomes?
a) Influenza virus.
b) Rotavirus.
c) Poliovirus.
d) Rabies virus.
e) All answers are false.

45. What are key features of retroviruses?
a) They replicate through a DNA intermediate.
b) They use reverse transcriptase.
c) They have a double-stranded RNA genome.
d) Their replication occurs entirely in the cytoplasm.
e) All answers are false.

46. What is the main difference between positive-sense and negative-sense RNA viruses?
    a) Positive-sense RNA can be directly translated.
    b) Negative-sense RNA requires RNA-dependent RNA polymerase.
    c) Positive-sense RNA viruses must integrate into the host genome.
    d) Negative-sense RNA viruses have a DNA intermediate.
    e) All answers are false.
47. What factors influence bacteriophage host specificity?
    a) Presence of specific receptors on the bacterial surface.
    b) Host restriction-modification systems.
    c) Bacterial cell wall composition.
    d) The number of ribosomes in the host cell.
    e) All answers are false.
48. What are the main steps of the lytic cycle?
    a) Adsorption.
    b) Injection of genetic material.
    c) Viral genome integration.
    d) Assembly and lysis.
    e) All answers are false.
49. What characterizes viroids?
    a) They are composed only of RNA.
    b) They lack a protein coat.
    c) They infect only plants.
    d) They replicate through a rolling-circle mechanism.
    e) All answers are false.
50. Which features differentiate prions from viruses?
    a) Prions lack nucleic acids.
    b) Prions cause diseases by protein misfolding.
    c) Prions contain double-stranded RNA.
    d) Prions can be inactivated by heat.
    e) All answers are false.
51. How do lysogenic bacteriophages replicate?
    a) They integrate their genome into the bacterial chromosome.
    b) They remain dormant as a prophage.
    c) They continuously lyse bacterial cells.
    d) They carry out reverse transcription.
    e) All answers are false.
52. What happens during prophage induction?
    a) The viral genome is excised from the bacterial chromosome.
    b) The virus enters the lytic cycle.
    c) The host bacterium is immediately killed.

d) The bacterium gains resistance to further infections.

e) All answers are false.

53. What type of genome does the Influenza virus have?

a) Segmented RNA genome.

b) Single-stranded positive-sense RNA.

c) Double-stranded DNA.

d) Single-stranded DNA.

e) All answers are false.



54. What is a characteristic of double-stranded RNA viruses?

a) They require an RNA-dependent RNA polymerase for replication.

b) Their replication occurs in the nucleus.

c) They do not require a capsid.

d) They have a lipid envelope.

e) All answers are false.

55. How do bacteriophages contribute to horizontal gene transfer?

a) Through transduction.

b) By transferring genes between bacteria.

c) By degrading host bacterial DNA.

d) By preventing gene expression in bacteria.

e) All answers are false.

56. What is the purpose of the CRISPR-Cas system in bacteria?

a) To provide immunity against bacteriophages.

b) To facilitate horizontal gene transfer.

c) To promote bacterial conjugation.

d) To initiate the lysogenic cycle.

e) All answers are false.

57. What are the main steps in retrovirus replication?

a) Reverse transcription.

b) Integration into the host genome.

c) Direct translation of viral RNA.

d) Replication in the cytoplasm.

e) All answers are false.

58. What is the function of the T4 bacteriophage tail fibers?

a) Recognizing and binding to bacterial receptors.

b) Injecting viral genetic material.

c) Replicating viral DNA.

d) Producing viral enzymes.

e) All answers are false.

59. What are characteristics of virulent bacteriophages?
    a) They only undergo the lytic cycle.
    b) They kill their host bacteria upon replication.
    c) They integrate their genome into the host chromosome.
    d) They participate in generalized transduction.
    e) All answers are false.
60. What is the main function of RNA-dependent RNA polymerase?
    a) Synthesizing RNA from an RNA template.
    b) Synthesizing DNA from an RNA template.
    c) Replicating bacterial chromosomes.
    d) Producing proteins directly from viral RNA.
    e) All answers are false.
61. What happens during the attachment phase of viral infection?
    a) The virus recognizes specific receptors on the host cell.
    b) The viral genome is injected into the host.
    c) Viral proteins are immediately synthesized.
    d) The viral capsid fuses with the host membrane.
    e) All answers are false.
62. What is the function of hemagglutinin in Influenza viruses?
    a) It facilitates viral attachment to host cells.
    b) It enables viral genome replication.
    c) It allows viral release from infected cells.
    d) It protects the viral genome from degradation.
    e) All answers are false.
63. What is antigenic drift in viruses?
    a) Small mutations in viral surface proteins.
    b) Reassortment of viral genome segments.
    c) Incorporation of host DNA into the viral genome.
    d) The ability of a virus to integrate into the host genome.
    e) All answers are false.
64. What is antigenic shift in Influenza viruses?
    a) A major change in viral surface proteins due to reassortment.
    b) Accumulation of point mutations in viral genes.
    c) The transition from lysogenic to lytic cycle.
    d) The complete elimination of a viral infection.
    e) All answers are false.
65. What is the function of neuraminidase in Influenza viruses?
    a) It helps the virus release from infected cells.
    b) It facilitates viral entry into host cells.
    c) It integrates the viral genome into the host chromosome.

d) It degrades host ribosomal RNA.

e) All answers are false.

66. What is a major consequence of horizontal gene transfer in bacteria?

a) Increased genetic diversity.

b) Acquisition of antibiotic resistance.

c) Loss of bacterial plasmids.

d) Immediate cell death.

e) All answers are false.



67. What is the main function of bacterial plasmids?

a) They provide additional genetic traits, such as antibiotic resistance.

b) They encode essential bacterial metabolic enzymes.

c) They serve as the main bacterial chromosome.

d) They regulate bacterial protein synthesis.

e) All answers are false.

68. What is a common method used to study yeast genetics?

a) Tetrad analysis.

b) Complementation tests.

c) Gene knockout experiments.

d) Horizontal gene transfer.

e) All answers are false.

69. What are the stages of bacterial conjugation?

a) Formation of the mating pair.

b) DNA transfer through the conjugation pilus.

c) Integration of transferred DNA into the recipient genome.

d) Lysis of the recipient cell.

e) All answers are false.

70. What is the role of the F plasmid in bacterial conjugation?

a) It enables the donor cell to form a conjugation pilus.

b) It allows bacteria to produce antibiotics.

c) It prevents DNA replication in the recipient cell.

d) It destroys recipient bacterial DNA.

e) All answers are false.

71. What is the main difference between generalized and specialized transduction?

a) Generalized transduction transfers any bacterial genes, whereas specialized transduction transfers specific genes.

b) Generalized transduction is mediated by lysogenic phages only.

c) Specialized transduction occurs randomly during the lytic cycle.

d) Specialized transduction does not involve a bacteriophage.

e) All answers are false.

72. What is a characteristic of lysogenic bacteriophages?
    a) They integrate their genome into the host chromosome as a prophage.
    b) They immediately lyse the bacterial cell.
    c) They produce toxins to kill competitor bacteria.
    d) They contain RNA genomes.
    e) All answers are false.
73. What is the function of the ribosome in translation?
    a) Synthesizing proteins from mRNA.
    b) Transcribing DNA into RNA.
    c) Degrading misfolded proteins.
    d) Generating ATP for cellular energy.
    e) All answers are false.
74. What are the three stages of transcription?
    a) Initiation, elongation, termination.
    b) Replication, translation, transcription.
    c) Attachment, penetration, release.
    d) Conjugation, transduction, transformation.
    e) All answers are false.
75. What enzyme is responsible for synthesizing RNA during transcription?
    a) RNA polymerase.
    b) DNA polymerase.
    c) Reverse transcriptase.
    d) Ligase.
    e) All answers are false.
76. How is gene expression regulated in bacteria?
    a) Operon systems like the lac operon.
    b) Epigenetic modifications of DNA.
    c) RNA interference (RNAi).
    d) Alternative splicing of mRNA.
    e) All answers are false.
77. What is the role of lactose in the lac operon system?
    a) It acts as an inducer to activate gene expression.
    b) It inhibits RNA polymerase.
    c) It binds to ribosomes and promotes translation.
    d) It enhances DNA replication.
    e) All answers are false.
78. What is the role of the tryptophan operon?
    a) It is a repressible operon that regulates tryptophan biosynthesis.
    b) It is activated when tryptophan is absent.
    c) It is an inducible operon like the lac operon.

d) It controls antibiotic resistance.
e) All answers are false.

79. What happens during bacterial transformation?
a) Uptake of naked DNA from the environment.
b) Transfer of genetic material through a pilus.
c) Infection by a bacteriophage.
d) Exchange of genes between bacteria through a sex pilus.
e) All answers are false.



80. What is the importance of transposons in bacterial genomes?
a) They contribute to genetic variation.
b) They can transfer antibiotic resistance genes.
c) They stabilize bacterial chromosomes.
d) They only exist in eukaryotic cells.
e) All answers are false.

81. What is a characteristic of conservative transposition?
a) The transposon moves without leaving a copy behind.
b) The transposon duplicates itself before insertion.
c) It always integrates into the same genomic location.
d) It requires bacterial conjugation to occur.
e) All answers are false.

82. What are the main types of transposons in bacteria?
a) Insertion sequences (IS elements).
b) Composite transposons.
c) Non-composite transposons.
d) Retrotransposons.
e) All answers are false.

83. What is the function of the RecA protein in bacteria?
a) It facilitates homologous recombination.
b) It prevents mutations in bacterial DNA.
c) It degrades mRNA during translation.
d) It initiates transcription.
e) All answers are false.

84. Which of the following are examples of mobile genetic elements in bacteria?
a) Plasmids.
b) Transposons.
c) Bacteriophages.
d) Operons.
e) All answers are false.

85. What is the role of restriction enzymes in bacteria?
    a) They cut foreign DNA at specific sequences.
    b) They facilitate bacterial conjugation.
    c) They repair damaged bacterial DNA.
    d) They regulate bacterial transcription.
    e) All answers are false.
86. What is a feature of yeast mitochondrial genetics?
    a) Mitochondria have their own circular DNA.
    b) Mitochondria inherit genes only maternally.
    c) Yeast mitochondrial mutations can affect respiration.
    d) Mitochondria can replicate independently of the nucleus.
    e) All answers are false.
87. What is the main purpose of tetrad analysis in yeast genetics?
    a) To study meiotic recombination.
    b) To identify linkage between genes.
    c) To analyze segregation of genetic markers.
    d) To study chromosome duplication.
    e) All answers are false.
88. How does yeast sexual reproduction occur?
    a) Haploid cells of opposite mating types fuse.
    b) It follows a strict mitotic division cycle.
    c) Asexual spores give rise to genetically identical offspring.
    d) It occurs only under anaerobic conditions.
    e) All answers are false.
89. What is the role of Ty elements in yeast?
    a) They are transposable elements similar to retroviruses.
    b) They regulate gene expression.
    c) They function as bacterial plasmids.
    d) They produce ribosomal RNA.
    e) All answers are false.
90. What are the main structural components of a virus?
    a) Capsid.
    b) Nucleic acid genome.
    c) Envelope (in some viruses).
    d) Ribosomes.
    e) All answers are false.
91. What is a distinguishing feature of complex viruses like bacteriophage T4?
    a) They have additional structures like tail fibers.
    b) They contain both DNA and RNA genomes.
    c) They infect only eukaryotic cells.

d) They lack capsids.
e) All answers are false.

92. What is a characteristic of double-stranded RNA (dsRNA) viruses?
a) They use RNA-dependent RNA polymerase for replication.
b) They integrate into the host genome.
c) They use reverse transcriptase.
d) They produce mRNA directly from their genome.
e) All answers are false.



93. What are examples of dsRNA viruses?
a) Rotaviruses.
b) Reoviruses.
c) Influenza viruses.
d) HIV.
e) All answers are false.

94. How does the lysogenic cycle differ from the lytic cycle?
a) The viral genome integrates into the host genome in the lysogenic cycle.
b) The lysogenic cycle leads to immediate host cell lysis.
c) No new viral particles are produced in the lysogenic cycle.
d) Lysogenic viruses have only RNA genomes.
e) All answers are false.

95. What is a key feature of retroviruses?
a) They use reverse transcriptase to convert RNA into DNA.
b) They lack a capsid.
c) They do not integrate into the host genome.
d) They are all non-enveloped.
e) All answers are false.

96. What is a characteristic of bacteriophage T4 replication?
a) It follows a lytic cycle.
b) It uses host ribosomes for protein synthesis.
c) It injects DNA into the host cell.
d) It produces its own RNA polymerase.
e) All answers are false.

97. How do RNA viruses replicate their genetic material?
a) By using an RNA-dependent RNA polymerase.
b) By reverse transcription into DNA.
c) By direct integration into the host chromosome.
d) By using host DNA polymerase.
e) All answers are false.

98. What is a common feature of enveloped viruses?
    a) They acquire their lipid membrane from the host cell.
    b) They lack a protein capsid.
    c) They cannot infect eukaryotic cells.
    d) They use DNA as genetic material.
    e) All answers are false.
99. What is a property of virulence plasmids?
    a) They encode genes that enhance bacterial pathogenicity.
    b) They provide resistance to antibiotics.
    c) They facilitate horizontal gene transfer.
    d) They produce viral capsid proteins.
    e) All answers are false.
100. What is a function of bacterial operons?
    a) They allow coordinated gene regulation.
    b) They are found in eukaryotic cells only.
    c) They function in DNA replication.
    d) They are responsible for protein folding.
    e) All answers are false.