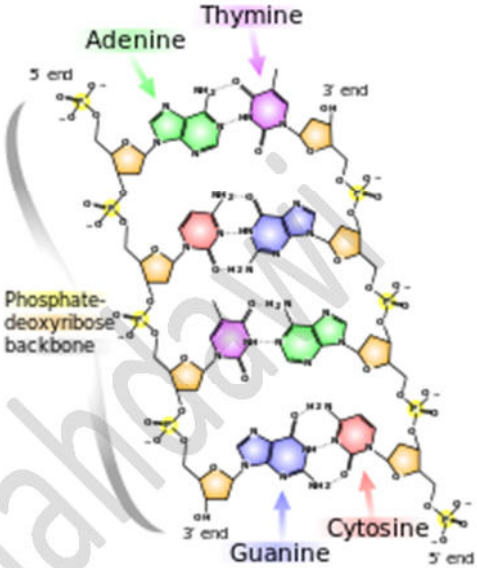
Bacterial Genetics

I. Organization of Genetic Information and General Concepts

A. Deoxyribonucleic acid (DNA)

- Stores genetic information as a sequence of nucleotide bases (adenine, thymine, guanosine, cytosine)
- Is generally double stranded, composed of complementary base pairs (A=T or G≡C) joined by hydrogen bonds.



B. Ribonucleic acid (RNA)

- Transcribes and translates DNA-bound genetic instructions for protein synthesis.
- Is generally single stranded.
- Substitutes uracil for the thymine base used by DNA; the complementary base pairs for RNA are A=U or G≡C.
- Is found in three main types:
  - **Messenger RNA (mRNA):** is the template that carries DNA gene sequences to ribosomes (the site of protein synthesis).
  - **Ribosomal RNA (rRNA):** is a structural component of ribosomes.
  - **Transfer RNA (tRNA):** carries specific amino acids to the triplet-encoded, mRNA-borne message that translates the message into the amino acid structure of proteins.
  - **microRNA:** regulation roles.

II. Comparison of Prokaryotic and Eukaryotic Genomes

A. Eukaryotic genomes

i. **Structure**

1. **Except in some fungi,** eukaryotes are **diploid** with **two homologous copies** of each chromosome.
2. Virtually all genetic information is contained in two or more **linear chromosomes** located in a membrane-bound nucleus.
3. Unlike prokaryotes and viruses, eukaryotic genomes **contain introns** (DNA sequences not translated into gene products) and **redundant genetic information**.
4. Certain **eukaryotic organelles (mitochondria, chloroplasts) contain** a self-replicating, **circular, double-stranded DNA molecule** relating to their intracellular function.

ii. **Replication**

1. Begins **at several points** along the linear DNA molecule.
2. Is regulated by **specific gene inducer or repressor** substances.
3. **Involves** a specialized structure, the **spindle that pulls newly formed chromosomes** into separate nuclei during mitosis.

B. Prokaryotic genomes

i. **Structure**

1. Most prokaryotes are **haploid (single chromosome).**
2. Genes essential for bacterial growth are carried on a **single, circular chromosome** encoding generally several thousand genes; they are **not enclosed in a membrane-circumscribed nucleus**.
3. Many bacteria **contain additional, specialized genes on smaller extrachromosomal plasmids**. Prokaryotic plasmids **exist in transmissible** and **nontransmissible** forms and **may be integrated** into the bacterial chromosome.

4. **Specialized information may also be carried on transposons (Tn)**, moveable genetic elements that cannot self-replicate. Transposons **contain insertion sequences** (**IS**) and **can transfer their information by inserting themselves into other loci** in the same or other genetic elements (e.g., plasmids, chromosomes, viral DNA).

ii. **Replication**

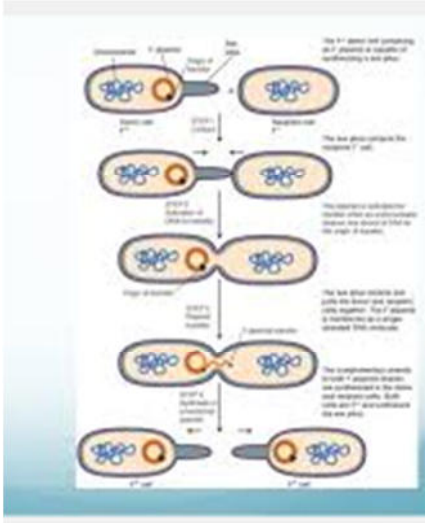
1. **Replicons:** Is a general term for **double-stranded DNA circles** (chromosomes, plasmids) capable of self-replication. Plasmid replication is independent of chromosome replication.
2. Replicate **bidirectionally** (5'-PO_4 —to→ 3'-OH) from a **fixed origin**.
3. The replicon **attaches to a projection of the cell membrane** (**mesosome**), which acts as the **replication origin site**, and one of the DNA strands is broken.
4. The 5' end of the broken strand attaches to a new membrane site.
5. Elongation of the cell membrane via localized membrane synthesis pulls the broken strand through the **mesosomal** attachment site, where replication takes place.
6. Replication is completed, and the free ends of the new replicon are joined.
7. **Transposons:** Are replicated, along with the code of the host, after insertion into a replicon.

C. Viral genomes

- **Structure**
  - Genetic information may be coded as DNA or RNA and in double-stranded or single-stranded form.
  - The viral genome may contain **exotic bases**.
- **Replication**
  - Takes place only after successful infection of an appropriate host.
  - Proceeds when the injected viral genome subverts normal replicative processes of the host, producing new virus particles.
- **Bacteriophage types:** May be discerned by their **mode of propagation**.
  - **Lytic phages** quickly produce many copies of themselves as they kill the host.
  - **Temperate phages** can lie seemingly **dormant** in the host (**prophage state**), timing replication of prophage genetic material to replication of the host cell. Various activation signals trigger the prophage to enter a **lytic cycle**, (host death and the release of new phages).

III. Gene Transfer Between Organisms

- Maintains **genetic variability** in microbes through the **exchange and recombination of allelic forms of genes**.
- Is **most efficient** between cells of the same species.
- May also occur as the **crossing over of homologous** chromosomes or by **nonhomologous** means (e.g., movement of plasmids or transposons, insertion of viral genes).
- Can **result in** the **acquisition of new characteristics** (e.g., antigens, toxins, antibiotic resistance).
- **Occurs via three mechanisms**: 1-conjugation, 2-transduction, and 3-transformation.

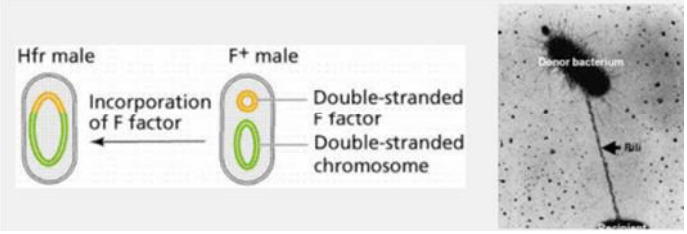


1. Conjugation

a) Is a one-way transfer of genetic material (usually plasmids) from donor to recipient by means of physical contact.

b) typically involves three types of plasmids:
   - **F^+ cell: (1)**Possesses a fertility (F) plasmid, mediating the creation of a sex pilus necessary for conjugal transfer of the F plasmid to the recipient. (2) Can integrate into chromosomal DNA, creating **high-frequency recombination** donors (**Hfr**) from which chromosomal DNA is readily transferred.

- **R factors: (1)** Contain genes conferring drug resistance. Frequently, the resistance genes are carried on transposons. **(2)**Express resistance phenotype through natural selection.
- **F′ cell and R factors:** Are recombinant fertility or resistance plasmids in which limited regions of chromosomal DNA can be replicated and transferred by conjugation independently of the chromosome**.**



B. Transduction

a) Is **phage-mediated transfer of host DNA sequences**
b) Can be **performed by temperate phages** and, under **special conditions,** by lytic phages.
c) **occurs in two forms:**
   - In **generalized transduction**, randomly packages host DNA in a bacteriophage coat and may transfer any gene. The transducing particle contains only host DNA.
   - In **specialized transduction**, the lysogenic phage favors the **transfer of host DNA segments near the site of prophage integration**. Specialized transducing phages **contain both viral and host genes.**

C. Transformation

- Is the **direct uptake and recombination of naked DNA fragments** through the cell wall by **competent** bacteria. Natural occurrence of this process is uncommon.
- Is sometimes mediated by surface competence factors (DNA receptor enzymes) produced only at a specific point in the bacterial growth cycle.
- Can sometimes be forced by treatment with **calcium chloride** and **temperature shock**.
- Is used in recombinant DNA research and commercially to introduce human genes via vectors into bacteria for rapid and large-scale production of human gene products.

IV. Gene Expression

A. Processes affecting expression

1) Transcription

- Is the transfer of protein synthesis instructions from DNA to mRNA.
- Is mediated by RNA polymerase.
- Is initiated by the binding of **sigma factor**, a subunit of RNA polymerase, to the **promoter region** of the DNA molecule.
- Involves the **unwinding** of a short sequence of DNA bases and **alignment of complementary ribonucleotide bases** onto the DNA template.
- Occurs in a 5′-PO_4 to 3′-OH direction

2) Translation

- **Occurs at the ribosomes**.
- Is accomplished by the tRNA-mediated linkage of amino acids, in accordance with the triplet-encoded mRNA transcript.
- Is **the assembly of polypeptide chains from the mRNA transcript**

B. Regulation of expression

(راجع محاضرة ٥ تنظيم فعالية الأيض/متعلق بنفس الموضوع)

- Occurs **primarily during transcription.**
- Is determined partly by the ability of the **DNA promoter region to bind with sigma factor**.
- Can be facilitated or blocked by regulator proteins binding to operator sequences near the promoter.

- Typically affects an **operon:** a group of genes under the control of one operator controlled by the action of regulatory proteins.
- **May be negative or positive control**

a) **Negative control:** The inhibition of transcription by the binding of a repressor protein.
is exemplified by:
  - **The *lac* operon:** Controls expression of three structural genes for lactose metabolism via a **repressor protein**.(Transcription is **induced by the presence of lactose** which **binds to the repressor protein** and frees the lac operator).
  - **The *trp* operon:** controls tryptophan synthesis.(Synthesis of tryptophan is **halted** by the binding of a **repressor protein-tryptophan complex** to the trp operator when excess tryptophan is available).

b) **Positive control:** The initiation of transcription in response to the binding of an activator protein.
is exemplified by ***ara* operon**:
  - Expression of the *ara* operon proceeds only when arabinose binds to a special protein, forming an activator compound necessary for the transcription of the *ara* operon.
  - Cyclic adenosine monophosphate (cyclic AMP) binding protein, when bound to a specific DNA sequence near the promoter, enhances the expression of many genes associated with fermentation. Cyclic AMP enhances RNA polymerase activity.

V. Mutation

- Is an induced or spontaneous **heritable alteration** of the DNA sequence
- Occurs approximately once for any gene in every 1 million cells.
- Caused by various mutagens, including ultraviolet light, acridine dyes, base analogues, and nitrous acid.
- Introduces variability into the gene pool and changes in the phenotype.

A. Mutation types

- **Nucleotide substitutions**
  - Arise from mutagenic activity or the mispairing of complementary bases during DNA replication.
  - Often do not significantly disrupt the function of gene products.
- **Deletions:** Are usually large excisions of DNA, dramatically altering the sequence of coded proteins.
- **Insertions:** Change genes and their products by integration of new DNA via transposons.

B. Results of mutation

- **Missense mutations**
  - Result in the substitution of one amino acid for another.
  - May be without phenotypic effect (silent mutation).
- **Nonsense mutations**
  - Terminate protein synthesis and result in truncated gene products.
  - Usually result in inactive protein products.
- **Frame-shift mutations**
  - Result from the insertion or deletion of one or two base pairs, disrupting the phase of the triplet-encoded DNA message.

C. Reversions :Function lost to mutation may be regained in two ways:

  - **Genotypic (true) reversion:** Is restoration at the site of DNA alteration.
  - **Phenotypic (suppression) reversion:** Is restoration of an activity lost to mutation, often by a mutation at a second site (suppressor mutation).

Bacterial viruses (Bacteriophages)

Viruses Structure of a "Virus Particle"

- Non cellular Biological Entity
- Contains either DNA or RNA (not both)
- Nucleic Acid is surrounded or coated by a protein shell (capsid)
- Some viruses possess a membrane-like envelope surrounding the particle

Bacteriophages

A. General characteristics of bacteriophages

- Are bacterial viruses that are frequently called phages.
- Are obligate intracellular parasites.
- Are host-specific infectious agents for bacteria.
- Are called bacteriophage virions when they are complete infectious particles (genetic material and capsid).
- Contain protein and RNA or DNA as major components.

B. Morphologic classes of bacteriophages

- **Filamentous phages**
  - Have a filamentous protein capsid that surrounds a circular single-stranded DNA genome.
  - Are male bacteria specific in that infection occurs through the pili, which are only present on male bacteria
  - Do not lyses their host cells during the replication process.
- **Polyhedral phages**
  - Are usually composed of an outer polyhedral-shaped protein coat (capsid) that surrounds the nucleic acid.
  - May contain a lipid bilayer between two protein capsid layers (PM-2 phage).
  - Have either circular double-stranded or single-stranded DNA or linear single-stranded RNA as their genetic material, although one phage that has three pieces of double-stranded RNA has been described.
- **Complex phages**
  - Have a protein polyhedral head containing linear double-stranded DNA and a protein tail and other appendages.
  - Include the T and lambda (λ) phages of *E. coli*.

C. Genetic classes of bacteriophages

- **RNA phages**
  - Refer to all phages with RNA as their genetic material.
  - Are specific for bacteria with male pili (male-specific phages).
  - Contain single-stranded RNA which can act as polycistronic mRNA.
- **DNA phages**
  - Refer to all phages with DNA as their genetic material.
  - Contain nucleic acid bases that are frequently glycosylated or methylated.
  - May contain some unusual nucleic acid bases, such as 5-hydroxymethyl cytosine or 5-hydroxymethyl uracil.
  - Are classified as **virulent** or **temperate**, depending on whether their pattern of replication is strictly **lytic** (virulent) or alternates between lytic and **lysogenic** (temperate).

D. Viral Replication

- No independent metabolism or replication
- Replicate only inside an infected host cell

- Do not replicate via a process of cell division
- Replicate via a process of:
  - Attachment and Penetration
  - Disassembly (Uncoating)
  - Synthesis of Viral Protein and Nucleic Acid
  - Reassembly of new viral particles
  - Release of new viral particles