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DEPARTMENT OF PG MICROBIOLOGY

STUDY MATERIAL

SEMESTER-II

MBY 203 MOLECULAR AND MICROBIAL GENETICS

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MOLECULAR ORGANISATION OF CHROMOSOMES IN EUKARYOTES AND PROKARYOES

The genomes of prokaryotes are contained in single chromosomes, which are usually circular DNA molecules. In contrast, the genomes of eukaryotes are composed of multiple chromosomes, each containing a linear molecule of DNA.

The molecular organization of chromosomes in eukaryotes and prokaryotes differs significantly due to their distinct structural and organizational characteristics.

Prokaryotes (e.g., bacteria):

Circular DNA: Prokaryotic chromosomes typically consist of a single circular DNA molecule.

Nucleoid Region: The DNA is located in the nucleoid region, which is a condensed area of the bacterial cytoplasm where the chromosome is found.

No Histones: Prokaryotic DNA is not associated with histone proteins.

Plasmids: In addition to the main chromosome, prokaryotes may contain smaller circular DNA molecules called plasmids, which often carry accessory genes.

Eukaryotes (e.g., plants, animals, fungi):

Linear DNA: Eukaryotic chromosomes are typically linear DNA molecules.

Nucleus: Chromosomes are housed within the nucleus of the cell, separated from the cytoplasm by a nuclear membrane.

Histones: Eukaryotic DNA is tightly wound around histone proteins to form nucleosomes, which are further coiled and packed into chromatin fibers.

Multiple Chromosomes: Eukaryotic cells typically have multiple linear chromosomes, varying in number depending on the species.

Comparison summary:

- **DNA Form**: Prokaryotes have a single circular chromosome, while eukaryotes have multiple linear chromosomes.
- **Location**: Prokaryotic DNA is in the nucleoid region of the cytoplasm, whereas eukaryotic DNA is in the nucleus.
- **Structural Proteins**: Prokaryotes lack histones, while eukaryotes use histones to organize DNA into chromatin
- **Additional DNA Elements**: Prokaryotes may have plasmids in addition to their main chromosome, whereas eukaryotes do not have plasmids as part of their chromosomal structure.

MOLECULAR MARKERS

Molecular markers are specific DNA sequences that can be used to identify particular regions within a genome. They are widely used in various fields of biology and genetics for different purposes, including mapping genes, determining genetic diversity, and studying evolutionary relationships.

- RFLP
- RAPD
- Polymorphism

RFLP (Restriction Fragment Length Polymorphism)

RFLP is a technique that detects variation in DNA sequence by digesting DNA with specific enzymes (restriction enzymes) that cut the DNA at specific recognition sites. Variations in these recognition sites result in different fragment lengths, which can be visualized using gel electrophoresis.

Principle:

- Different individuals or species may have different patterns of fragment lengths due to variations (polymorphisms) in the DNA sequence.
- RFLPs were historically significant in genetic mapping and early studies of genetic diversity.

Applications:

- **Genetic Mapping**: Used to map genes and genetic markers on chromosomes.
- **Disease Association Studies**: Investigating the association of certain RFLP patterns with diseases.
- **Forensic Analysis**: Used in forensic science for DNA profiling.

RAPD (Random Amplified Polymorphic DNA)

RAPD is a PCR-based molecular marker technique that detects polymorphisms in DNA sequences using short, arbitrary primers.

Principle:

- RAPD involves using short (usually 10 nucleotides) arbitrary primers that bind to random regions of the genome.
- Variations in DNA sequence at the primer-binding sites result in different patterns of amplified DNA fragments.

Applications:

- **Genetic Diversity Studies**: Assessing genetic variation within and between populations.
- **Species Identification**: Distinguishing between closely related species.

- **Forensic Applications**: Used in DNA fingerprinting and criminal investigations.

Polymorphism

Polymorphism refers to the occurrence of multiple forms (alleles) of a gene or DNA sequence within a population. Molecular markers such as RFLP and RAPD detect these polymorphisms.

Types:

- **SNPs (Single Nucleotide Polymorphisms)**: Variations at single nucleotide positions in the DNA sequence.
- **Indels (Insertion/Deletion Polymorphisms)**: Variations involving the insertion or deletion of a small number of nucleotides.

Applications:

- **Marker-Assisted Selection (MAS)**: In agriculture and breeding, using polymorphic markers to select for desirable traits.
- **Population Genetics**: Studying population structure, genetic drift, and gene flow.
- **Medical Genetics**: Studying genetic predisposition to diseases.

PLASMIDS

Plasmids are small, circular DNA molecules that are separate from the chromosomal DNA in bacteria and some other organisms. They replicate independently and can confer various traits to their host cells.

Types of Plasmids;

- F plasmid
- R plasmid
- Ti plasmid

F Plasmid (Fertility Plasmid)

The F plasmid (or fertility factor) is responsible for the transfer of genetic material (often antibiotic resistance genes) between bacteria during conjugation. It allows bacteria to form a conjugation bridge and transfer the plasmid to a recipient cell.

Importance: F plasmids play a crucial role in the spread of antibiotic resistance genes among bacterial populations.

R Plasmid (Resistance Plasmid)

R plasmids carry genes that provide resistance to antibiotics or other toxic substances. These genes can encode enzymes that degrade antibiotics, alter antibiotic targets, or pump antibiotics out of the cell, thereby conferring resistance to the host bacterium.

Importance: R plasmids contribute significantly to the development and spread of antibiotic resistance in bacteria, posing a serious challenge in clinical settings and public health.

Ti Plasmid (Tumor-Inducing Plasmid)

Ti plasmids are found in certain strains of Agrobacterium, a soil bacterium. They are responsible for the bacterium's ability to transfer a segment of its DNA (T-DNA) into plant cells, inducing the formation of crown gall tumors. The Ti plasmid integrates its T-DNA into the plant genome, where it can be expressed by the plant cell.

Importance: Ti plasmids have been widely used in genetic engineering to introduce foreign genes into plants. They serve as vectors for creating genetically modified (GM) crops and studying gene function in plants.

Types of Ti Plasmids:

1. **Octopine-Type Ti Plasmids**: These Ti plasmids induce the synthesis and accumulation of octopine in plant cells.
2. **Nopaline-Type Ti Plasmids**: These Ti plasmids induce the synthesis and accumulation of nopaline in plant cells.

Both types of Ti plasmids have been extensively studied and utilized in plant genetic engineering and biotechnology.

TRANSPOSABLE ELEMENTS

Transposable elements (TEs), often referred to as transposons, are DNA sequences that have the ability to move (transpose) within the genome of an organism. They are found in both prokaryotic and eukaryotic genomes and can cause genetic variation and genome rearrangements.

Transposition refers to the process by which transposable elements (TEs) move within a genome, changing their location from one genomic position to another. This ability to transpose is a defining characteristic of TEs and distinguishes them from other stable genetic elements like genes.

Mechanisms of Transposition:

Transposable elements utilize different mechanisms to transpose within genomes:

1. **Replicative Transposition**:
   - **Copy-and-Paste Mechanism**: In this mechanism, the TE replicates itself, and one copy remains in the original location while the other copy inserts into a new genomic location.
   - **Process**:
     - The TE is transcribed into an RNA intermediate (in the case of retrotransposons) or directly recognized by transposase (in the case of DNA transposons).
     - The RNA (in retrotransposons) is reverse transcribed into cDNA, which integrates into a new site.
     - The original TE remains intact.
2. **Non-Replicative Transposition**:
   - **Cut-and-Paste Mechanism**: Here, the TE is excised from its original position and moves to a new location in the genome without creating a new copy.
   - **Process**:
     - The transposase enzyme recognizes specific sequences at the ends of the TE (inverted terminal repeats, or ITRs).
     - Transposase catalyzes the excision of the TE from its original location.
     - The TE is then inserted into a new genomic location, typically accompanied by small target site duplications (direct repeats).

Regulation of Transposition:

The activity of transposable elements is regulated by the host genome to maintain genomic stability and integrity. Regulation mechanisms include:

- **Epigenetic Regulation**: DNA methylation, histone modifications, and small RNA pathways can control TE activity by silencing or activating transposition.
- **Host Factors**: Cellular proteins and pathways may facilitate or restrict TE transposition.
- **Environmental Factors**: Stress and environmental changes can influence TE activity and genome dynamics.

Applications:

- **Genetic Engineering**: TEs have been adapted as tools for gene delivery, mutagenesis, and gene tagging in genetic studies and biotechnology.
- **Evolutionary Studies**: Studying TE dynamics provides insights into genome evolution and species divergence.
- **Biomedical Research**: Understanding TE impact on gene regulation and disease susceptibility aids in medical research.

MOLECULAR BASIS OF MUTATIONS

Mutations are changes in the DNA sequence of an organism's genome. They are the basis of genetic variability and play a crucial role in evolution, development of diseases, and genetic diversity within populations.

Mutations are alterations in the DNA sequence that can arise spontaneously or be induced by external factors.

Spontaneous Mutations:

Spontaneous mutations occur naturally during DNA replication or due to intrinsic cellular processes. They arise from various sources, including:

1. **Replication Errors**:

   - DNA polymerase occasionally makes mistakes during DNA replication, leading to incorporation of incorrect nucleotides (point mutations).
   - These errors can result in substitutions (transition or transversion), insertions, or deletions in the DNA sequence.
2. **DNA Damage**:
   - Endogenous factors such as reactive oxygen species (ROS), which are byproducts of cellular metabolism, can damage DNA bases.
   - Spontaneous hydrolysis or deamination of bases can also lead to altered nucleotides that are not recognized correctly during replication.
3. **Replication Slippage**:
   - During replication, DNA polymerase may slip or stutter, causing the addition or deletion of repeat units (e.g., microsatellites), leading to frameshift mutations.
4. **Transposable Elements**:
   - Transposons can move within the genome spontaneously, inserting into new locations and potentially disrupting genes or regulatory sequences.

Induced Mutations:

Induced mutations are caused by external factors or agents that increase the rate of DNA damage or replication errors. Common sources of induced mutations include:

 Chemical Mutagens:

- Chemicals that directly modify DNA bases (e.g., alkylating agents), mimic nucleotides (base analogs), or cause intercalation between DNA base pairs (e.g., ethidium bromide).
- Examples include nitrous acid, EMS (ethyl methanesulfonate), and aflatoxins.

 Physical Mutagens:

- Environmental factors such as:
  - **UV Radiation**: Causes thymine dimers and other DNA lesions that can lead to point mutations or other forms of damage.
  - **Ionizing Radiation**: Generates free radicals that damage DNA strands and can lead to double-strand breaks or base modifications.

 Biological Agents:

- Certain viruses and bacteria can insert their DNA into host genomes (insertional mutagenesis), potentially disrupting gene function.

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UV DAMAGE OF DNA AND REPAIR

UV (ultraviolet) radiation can damage DNA by inducing structural changes and chemical modifications in the nucleotide bases. Here's how UV damage occurs and how cells repair it:

UV Damage to DNA:

1. **Formation of Thymine Dimers**:
   - UV radiation, particularly UV-B (280-315 nm) and UV-C (100-280 nm), can directly induce covalent bonds between adjacent thymine bases on the same DNA strand.
   - This leads to the formation of thymine dimers, such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts.
2. **DNA Structural Distortions**:
   - Thymine dimers cause structural distortions in the DNA helix, disrupting normal base pairing and DNA replication.
3. **UV-Induced DNA Lesions**:
   - Besides thymine dimers, UV radiation can induce other types of DNA lesions, including oxidative damage and base modifications.

Cellular Response to UV Damage:

Cells have evolved several mechanisms to detect and repair UV-induced DNA damage, ensuring genomic stability:

1. **Photoreactivation Repair (Light Repair)**:
    - In some organisms, including bacteria and certain vertebrates, photolyases are enzymes that use visible light energy to directly reverse thymine dimers.
    - Photolyases bind to DNA containing thymine dimers and break the covalent bonds, restoring the original DNA structure.
2. **Nucleotide Excision Repair (NER)**:
    - NER is the primary mechanism in most organisms for repairing UV-induced DNA damage.
    - **Recognition**: Proteins such as XPC-RAD23B complex recognize distortions caused by thymine dimers.
    - **Excision**: A complex of proteins including TFIIH, XPA, XPG, and XPF-ERCC1 sequentially excises a short single-stranded DNA segment containing the damage.
    - **Resynthesis**: DNA polymerase fills in the gap, using the undamaged strand as a template.
    - **Ligation**: DNA ligase seals the nick to complete repair.
3. **Global Genomic Repair (GGR) and Transcription-Coupled Repair (TCR)**:
    - **GGR**: Targets damage throughout the genome.
    - **TCR**: Prioritizes repair of lesions that block RNA polymerase during transcription, ensuring rapid repair of actively transcribed genes.

Importance of Repair:

- **Maintaining Genomic Integrity**: Effective repair of UV-induced DNA damage prevents mutations that could lead to cancer and other diseases.

- **Environmental Adaptation**: UV-induced DNA repair mechanisms are crucial for organisms exposed to sunlight, adapting to UV exposure and preventing UV-induced mutations.
- **Clinical Relevance**: Defects in DNA repair pathways can lead to disorders such as xeroderma pigmentosum (XP), characterized by extreme sensitivity to UV radiation and a high risk of skin cancer.

UV radiation damages DNA primarily by inducing thymine dimers, which distort the DNA helix and interfere with replication and transcription. Cells respond with specialized repair mechanisms like photoreactivation (in some organisms) and nucleotide excision repair (NER), which identify and correct UV-induced DNA damage to maintain genome stability and ensure proper cellular function.

BACTERIAL RECOMBINATION

Bacterial recombination refers to the exchange of genetic material between bacteria, which can occur through several mechanisms including conjugation, transformation, and transduction.

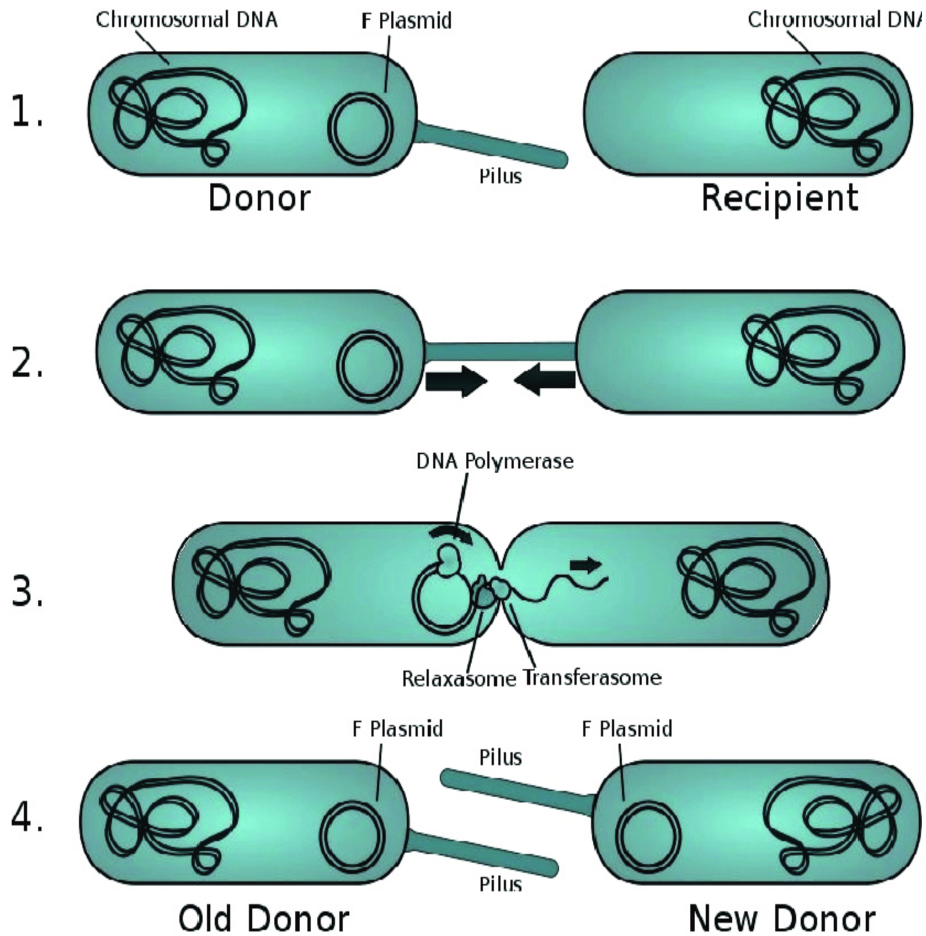
Conjugation:

Conjugation is a process of genetic transfer that involves direct cell-to-cell contact between two bacteria.

 Mechanism:

- The donor bacterium contains a conjugative plasmid (such as F plasmid or fertility factor) that carries genes necessary for conjugation.
- A conjugative pilus forms between the donor (F^+) and recipient (F^-) bacteria, bringing them into contact.

- The plasmid DNA is transferred from the donor to the recipient through the pilus.
- Recombination may occur if homologous sequences are present on the plasmid and recipient chromosome, leading to genetic exchange.

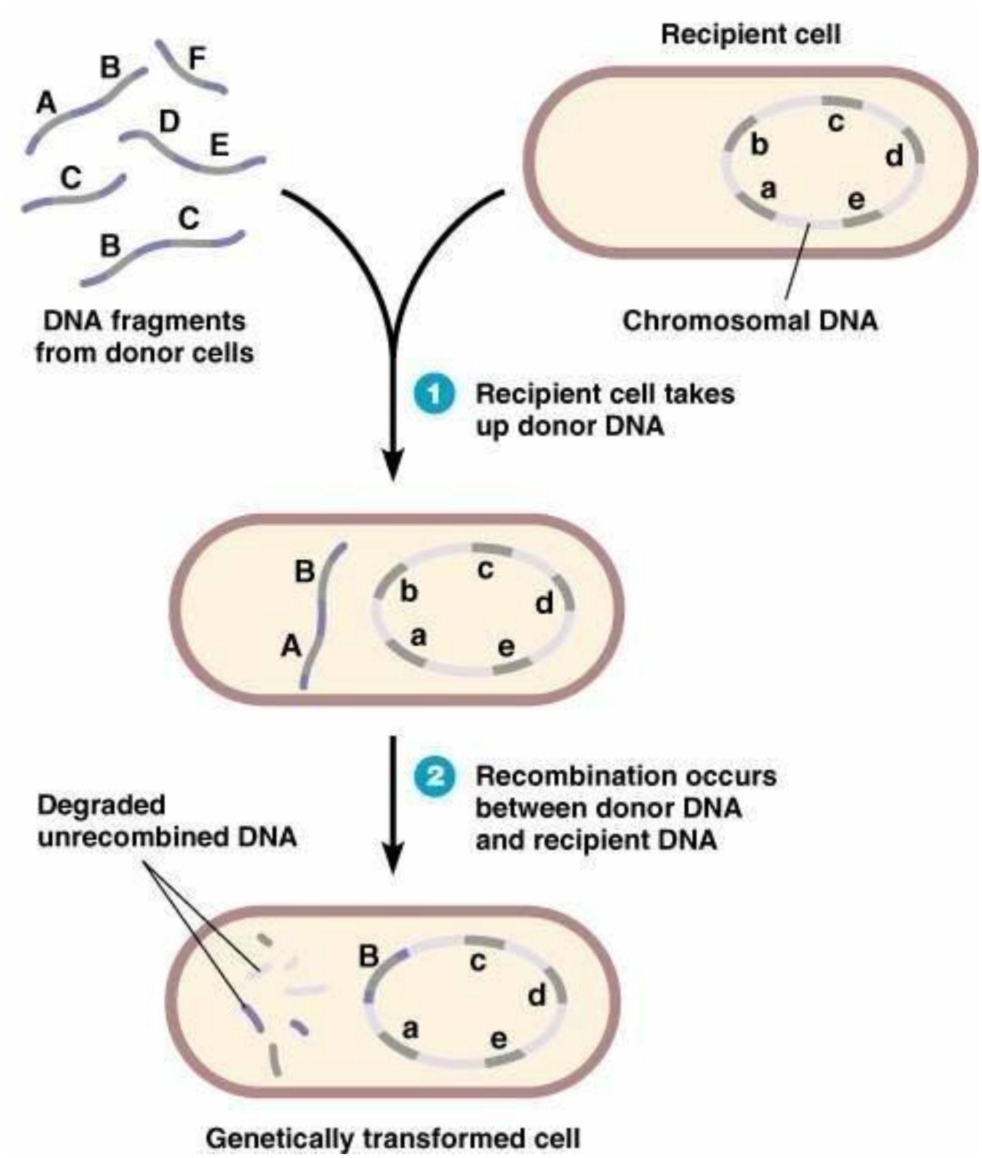


Importance: Conjugation allows for the transfer of antibiotic resistance genes, virulence factors, and other beneficial traits among bacteria.

Transformation:

 Transformation is the uptake and incorporation of exogenous DNA (usually free-floating in the environment) by a bacterial cell.

- **Mechanism**:
  - Bacteria become competent (able to take up DNA) through physiological changes or artificial induction.
  - Naked DNA from the environment is bound by competence proteins and transported into the bacterial cell.
  - Once inside, the exogenous DNA may integrate into the recipient bacterium's genome via recombination.

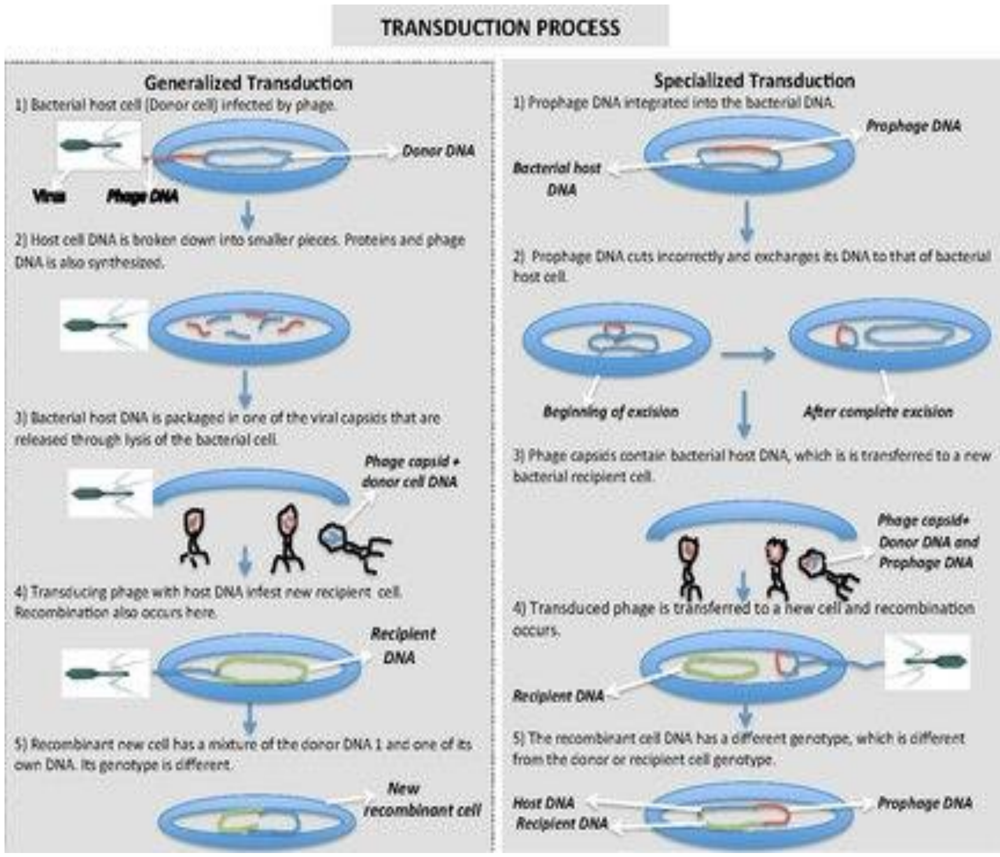


Importance: Transformation is a natural process in some bacteria and a crucial technique in genetic engineering and molecular biology.

Transduction:

- Transduction is the process by which bacterial DNA is transferred from one bacterium to another by a bacteriophage (virus that infects bacteria).

- **Mechanism**:
  - During viral replication, some bacteriophages accidentally package bacterial DNA instead of their own.
  - When this phage infects a new bacterial host, it injects the packaged bacterial DNA along with its own genetic material.
  - The transferred bacterial DNA may integrate into the recipient bacterium's genome through recombination.



- **Importance**: Transduction contributes to horizontal gene transfer in bacteria, aiding in the spread of virulence factors, antibiotic resistance genes, and other traits.

COMPLEMENTATION TEST

The complementation test, also known as the complementation assay or complementation analysis, is a fundamental genetic technique used to determine whether two mutations are in the same or different genes based on their phenotypic effects.

Principle of Complementation Test:

MBY 203 MOLECULAR AND MICROBIAL GENETICS

1. **Definition**: The complementation test assesses whether two different mutations that result in similar phenotypic traits (such as a mutant phenotype) can "complement" each other when they occur together in a diploid organism.
2. **Assay Setup**:
   - Typically performed in haploid organisms like bacteria or yeast or in diploid organisms where mutations are studied in a haploid condition.
   - Mutants with different phenotypes (e.g., inability to produce a certain enzyme) are crossed to produce diploid progeny.
3. **Expected Outcomes**:
   - **Complementation**: If the two mutations are in different genes (non-allelic), the diploid organism will display the wild-type phenotype (no mutant phenotype) because each copy of the gene can produce enough functional enzyme or protein to compensate for the deficiency caused by the other mutation.
   - **No Complementation**: If the mutations are in the same gene (allelic), the diploid organism will exhibit the mutant phenotype because both copies of the gene are defective, and neither can produce the functional enzyme or protein.
4. **Interpretation**:
   - Complementation is indicated by the presence of a wild-type phenotype in the diploid organism.
   - No complementation (non-complementation) is indicated by the persistence of the mutant phenotype in the diploid organism.

Significance of Complementation Test:

- **Gene Identification**: Helps determine whether mutations are in the same gene (allelic) or different genes (non-allelic).
- **Functional Analysis**: Provides insights into the function of genes and proteins by assessing their ability to complement each other's deficiencies.
- **Genetic Mapping**: Used in conjunction with other genetic techniques to map genes and understand their interactions within cellular pathways.

- **Evolutionary Studies**: Complementation tests can reveal evolutionary relationships between genes and organisms based on their ability to complement mutations.

Example Application:

- **Enzyme Deficiency**: Suppose two different mutants in a bacterial strain are unable to produce a specific enzyme needed for growth on a particular substrate. By performing a complementation test, researchers can determine if the mutations affect the same gene or different genes involved in the enzyme's production.

The complementation test is a powerful tool in genetics for identifying gene loci, studying gene function, and understanding the genetic basis of phenotypic traits. It relies on the principle that mutations in different genes (non-allelic) can complement each other to restore wild-type function, whereas mutations in the same gene (allelic) cannot. This test is foundational in genetic research, especially in microbial genetics and model organism studies.