

# **Genetics**

*for*

## **M.Sc. Semester II**

**(Paper 1)**

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# UNIT 1

## Inheritance (Mendelian and Non-Mendelian), Fine Structure of Gene and Gene Regulation

### Introduction

Classical Mendelian genetics, based on Gregor Mendel's principles of segregation and independent assortment, explains inheritance of traits controlled by single genes with dominant/recessive alleles. However, the genomic era has revealed that inheritance is often more complex. Non-Mendelian inheritance encompasses patterns where traits do not segregate in typical Mendelian ratios due to factors like cytoplasmic genes, genomic imprinting, or multi-gene interactions. Quantitative genetics studies traits controlled by many genes (polygenic) and influenced by the environment, while population genetics focuses on genetic variation within and between populations and how it changes over evolutionary time. Recent technological advances in genomics, epigenomics, and bioinformatics have dramatically refined our understanding of all these fields.

### RECENT ADVANCES IN MENDELIAN AND NON-MENDELIAN INHERITANCE

#### Recent Advances in Mendelian Genetics

**Definition:** Mendelian inheritance refers to the transmission of traits governed by single nuclear genes following Mendel's laws. Advances have come in identifying genes, understanding gene function, and diagnosing disorders.

#### Recent Advances & Examples:

- **Precision in Gene Mapping & Diagnosis:** Next-Generation Sequencing (NGS) has revolutionized the identification of Mendelian disorder genes (e.g., genes for Huntington's disease, cystic fibrosis). Whole Exome/Genome Sequencing allows for rapid diagnosis of even rare single-gene disorders in clinical settings.
- **CRISPR-Cas9 and Gene Therapy:** Technologies like CRISPR enable precise editing of mutant alleles responsible for Mendelian conditions. Successful clinical trials for blood disorders like sickle cell anemia (caused by a point mutation in the HBB gene) represent a pinnacle of applied Mendelian genetics.
- **Modifier Genes and Variable Expressivity:** Advanced studies show that even "simple" Mendelian traits can be influenced by genetic background. Modifier genes can affect the severity (expressivity) or whether a disease manifests at all (penetrance), blurring the line between Mendelian and complex traits.

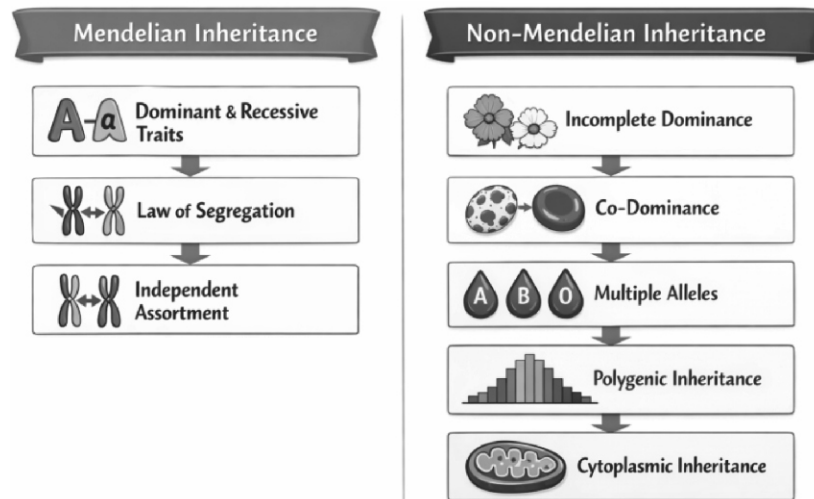


**Fig. Mendelian vs Non-mendelian inheritance**

## Recent Advances in Non-Mendelian Inheritance

**Definition:** Inheritance patterns that deviate from Mendel's laws, involving mechanisms outside the nuclear DNA sequence or interactions that alter expected phenotypic ratios.

### Cytoplasmic (Extranuclear) Inheritance:

- **Definition:** Transmission of genetic material via organelles like mitochondria and chloroplasts, typically maternal in most eukaryotes.

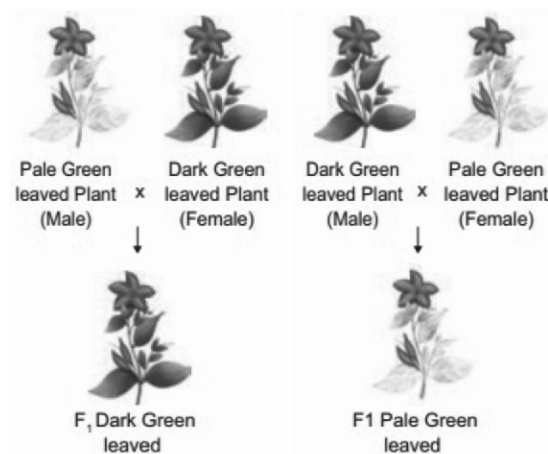


**Fig. Chloroplast inheritance**

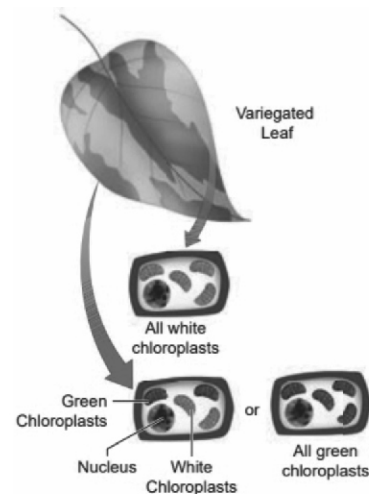


**Fig. Variegated phenotype of the leaves in *Mirabilis jalapa***

- **Advances:** Recognition of mitochondrial diseases (e.g., MELAS) and their role in aging, cancer, and neurodegeneration. Techniques like mitochondrial replacement therapy (MRT) or "three-parent IVF" to prevent transmission of severe mitochondrial disorders.
- **Plant Example:** Variegation in *Mirabilis jalapa* (due to chloroplast DNA mutations) is a classic model now studied via organelle genomics.

### 👉 Genomic Imprinting:

- **Definition:** Epigenetic silencing where expression of an allele depends on its parental origin, mediated by DNA methylation and histone modifications.
- **Advances:** Discovery of imprint control regions (ICRs) and their regulation. Link to human disorders: Prader-Willi syndrome (paternal deletion/maternal imprint) and Angelman syndrome (maternal deletion/paternal imprint). Environmental factors can disrupt imprinting (loss of imprinting linked to cancers).

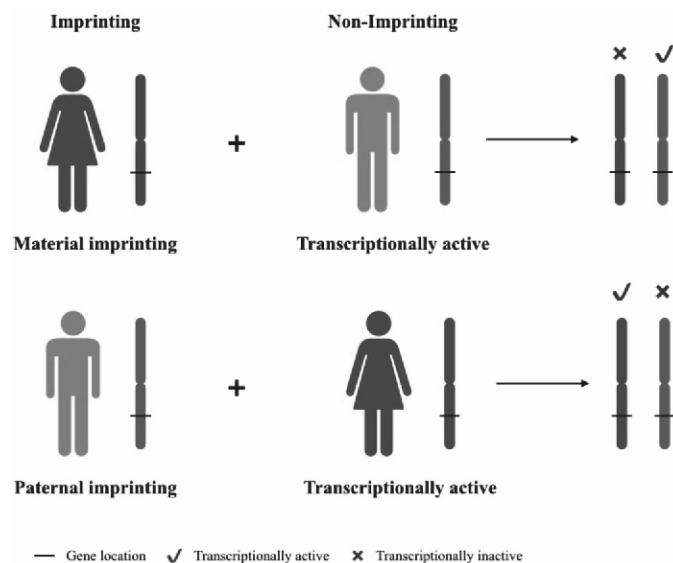


**Fig. Genomic Imprinting**

### 👉 Tri-Nucleotide Repeat Expansions:

- **Definition:** Dynamic mutations where increasing number of repeats (e.g., CAG) across generations leads to earlier onset and severity (anticipation).
- **Advances:** Understanding of RNA toxicity and protein gain-of-function mechanisms in disorders like Huntington's, Fragile X syndrome, and Friedreich's ataxia. Development of antisense oligonucleotide therapies.

### Epigenetic Inheritance:

- **Definition:** Heritable changes in gene expression not due to DNA sequence change, involving chromatin marks (DNA methylation, histone modifications, non-coding RNAs).
- **Advances:** Evidence for transgenerational epigenetic inheritance in plants and animals. In plants, epigenetic states (e.g., paramutation) can be stable over many generations and influence traits like flowering time and stress response. Role of small RNAs in guiding these changes.

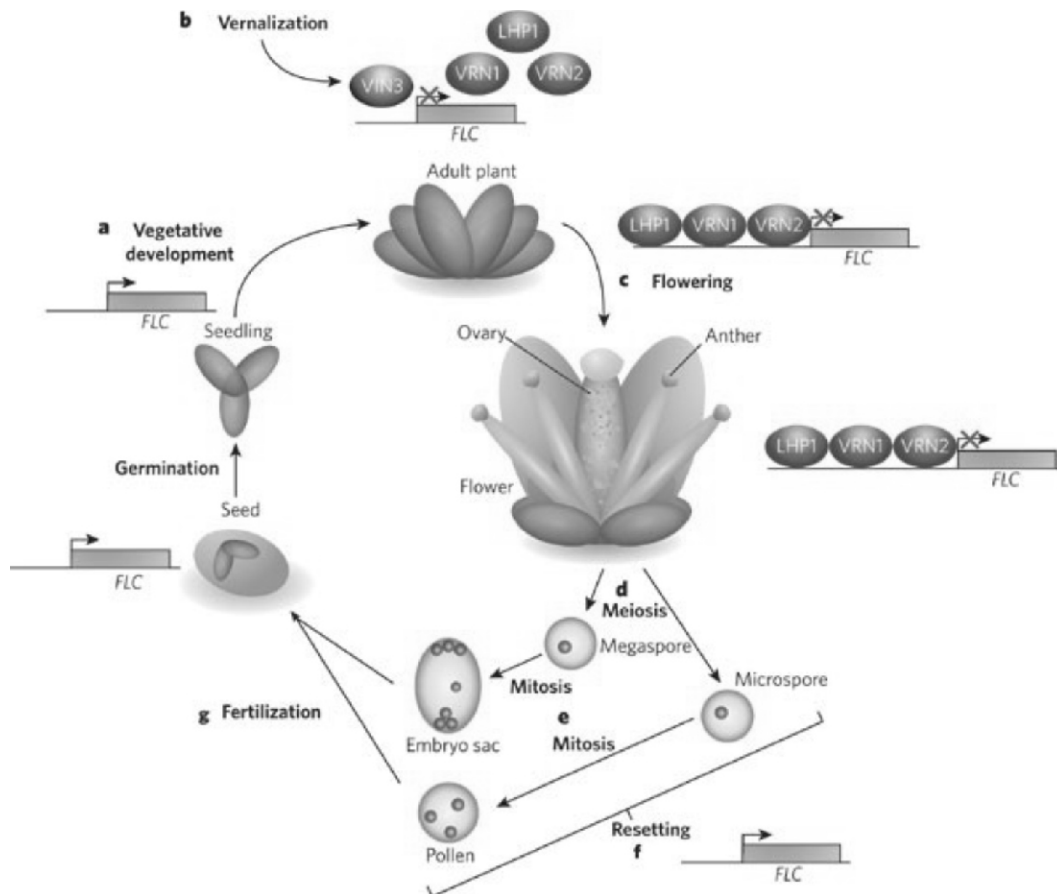


**Fig. Epigenetic Inheritance in plants**

### Multi-Gene Interactions:

- **Definition:** Phenotypes resulting from interaction between alleles of two or more genes.
- **Examples:** Complementary genes (9:7 ratio), duplicate genes (15:1 ratio), epistasis (where one gene masks another, e.g., 9:3:4 ratio in coat color of mice).

**Comparison Table: Mendelian vs. Non-Mendelian Inheritance**

| Feature                 | Mendelian Inheritance                                            | Non-Mendelian Inheritance                                                                                        |
|-------------------------|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| Genetic Basis           | Single nuclear genes, alleles follow dominance/recessiveness.    | Involves cytoplasmic genes, epigenetic marks, multi-gene interactions, repeat expansions.                        |
| Prediction              | Phenotypic ratios predictable from pedigree (3:1, 1:1, 9:3:3:1). | Ratios are distorted or unpredictable from standard crosses.                                                     |
| Parental Influence      | Generally none (except sex-linkage).                             | Often present (e.g., maternal inheritance in organelles, parental origin in imprinting).                         |
| Environmental Influence | Usually minimal on inheritance pattern.                          | Can be significant (e.g., epigenetic marks altered by environment).                                              |
| Molecular Mechanism     | Based on DNA sequence variation.                                 | Often based on regulatory mechanisms (epigenetic, dosage, RNA interference).                                     |
| Example                 | Seed shape in peas, ABO blood groups in humans.                  | Leaf variegation in plants (chloroplast), Prader-Willi syndrome (imprinting), kernel color in maize (epistasis). |

## QUANTITATIVE AND POPULATION GENETICS



### Quantitative Genetics

**Definition:** Study of inheritance of complex (quantitative) traits influenced by multiple genes (polygenes/QTLs) and the environment, showing continuous variation (e.g., height, yield, drought tolerance).

**Core Concepts:** Polygenic Inheritance, Quantitative Trait Loci (QTL), Heritability (Broad-sense  $H^2 = \frac{V_G}{V_P}$ ; Narrow-sense  $h^2 = \frac{V_A}{V_P}$ ), Mean, Variance, Selection Response

( $R = h^2 * S$ ).

#### Recent Advances:

- **High-Resolution QTL Mapping:** Using dense molecular markers (SNPs) and advanced populations (Nested Association Mapping, Multiparent Advanced Generation Inter-Cross - MAGIC) to pinpoint QTLs to candidate gene levels.
- **Genome-Wide Association Studies (GWAS):** Scanning genomes of many individuals to find SNPs statistically associated with complex traits. Revolutionized plant breeding for disease resistance and climate-resilient crops.
- **Genomic Selection (GS):** A form of marker-assisted selection that uses genome-wide marker data to predict the breeding value of individuals, accelerating crop and livestock improvement.

- **Integration with Omics:** Transcriptomics, Metabolomics, and Proteomics data are integrated with genetic maps (-QTL analyses) to understand the pathways from genotype to phenotype (Systems Genetics).

## Recent Advances in Population Genetics

**Definition:** Study of genetic composition of populations and how allele frequencies change over time and space under evolutionary forces.

**Core Principles:** Gene Pool, Allele & Genotype Frequencies, Hardy-Weinberg Equilibrium (HWE), Evolutionary Forces (Mutation, Gene Flow, Genetic Drift, Non-Random Mating, Natural Selection).

### 📖 Gene Pool

**Definition:** The complete set of genetic information (all alleles) present in all individuals of a population at a given time.

#### Key Points:

- Represents the total genetic diversity of a population.
- Only includes heritable traits (genes passed to offspring).
- Changes in the gene pool drive evolution.

### 📖 Allele & Genotype Frequencies

#### Allele Frequency:

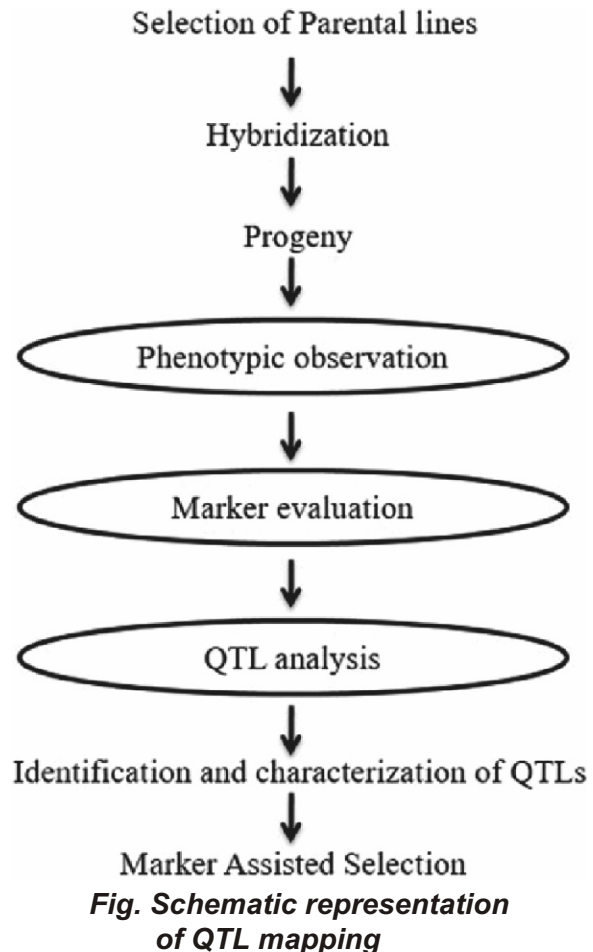
The proportion of a specific allele in the gene pool relative to all alleles for that gene.

$$p = \frac{\text{Number of copies of allele A}}{\text{Total number of alleles in population}}$$

#### Genotype Frequency:

The proportion of individuals in a population with a specific genotype.

$$\text{Frequency of AA} = \frac{\text{Number of AA individuals}}{\text{Total number of individuals}}$$



## Example:

In a gene with two alleles (A and a):

- Allele frequencies:  $p$  (for A),  $q$  (for a)  $p + q = 1$
- Genotype frequencies: AA, Aa, aa

## Hardy-Weinberg Equilibrium (HWE)

**Definition:** A theoretical model that predicts that allele and genotype frequencies will remain constant across generations if certain conditions are met.

### Conditions (No evolution occurring):

- No mutation
- No gene flow (migration)
- Large population size (no genetic drift)
- Random mating
- No natural selection

### HWE Equations:

For two alleles (A and a):

- $p^2$  frequency of AA
- $2pq$  = frequency of Aa
- $q^2$  frequency of aa
- $p^2 + 2pq + q^2 = 1$

### Use:

Serves as a null model to detect evolutionary change.

## Evolutionary Forces (Five Mechanisms)

### a. Mutation

- Source of new alleles increases genetic diversity.
- Rare and random; rates are low.
- Ultimate source of all genetic variation.

### b. Gene Flow (Migration)

- Movement of alleles between populations via migration.
- Can increase or decrease genetic variation depending on direction.
- Reduces genetic differences between populations.

### c. Genetic Drift

- Random change in allele frequencies due to sampling error in small populations.
- Effects are stronger in small populations.
- Can lead to loss of alleles or fixation (100% frequency).
- **Examples:** Founder effect, bottleneck effect.

## THE HARDY-WEINBERG PRINCIPLE

The frequencies of different alleles and genotypes within a population will remain constant through time, as long as there are no evolutionary influences present.

### This means:

- the population is very large
- there are no new mutations
- there is no migration
- there is no natural selection
- there is no sexual selection (mating is random)

## THE HARDY-WEINBERG EQUATIONS

$p$  = frequency of allele A in population

$q$  = frequency of allele a in population

If there are only 2 alleles for a gene then:

$$p + q = 1$$

Allele frequencies can be used to determine genotype frequencies too!

$$p^2 + 2pq + q^2 = 1$$

EXAMPLE: If there are 200 M alleles in the gene pool and 800 m alleles, then  $\frac{0.2}{M} + \frac{0.8}{m} = 1$

and  $\frac{(0.2)^2}{MM} + 2\frac{(0.2 \times 0.8)}{Mm} + \frac{(0.8)^2}{mm} = 1$

0.04      0.32      0.64

The Hardy-Weinberg equilibrium is never achieved in nature, but it's very useful in studying populations.

**Fig. Hardy-Weinberg Equilibrium (HWE)**

**d. Non-Random Mating**

- Mating based on phenotype or relatedness.
- Does not change allele frequencies directly, but changes genotype frequencies.
- **Types:** Assortative mating (similar individuals mate), disassortative mating, inbreeding.

**e. Natural Selection**

- Differential survival and reproduction of individuals due to phenotypic differences.
- Increases frequency of advantageous alleles.
- **Modes:** Directional, stabilizing, disruptive, balancing, sexual selection.

 **Recent Advances:**

- **Landscape Genomics:** Combining population genomics with environmental data to identify loci under selection due to local adaptation (e.g., to altitude, temperature, soil pH).
- **Environmental DNA (eDNA):** Using genetic material from soil or water to assess biodiversity and population genetic parameters without direct sampling of organisms.
- **Ancient DNA (aDNA) Analysis:** Sequencing DNA from historical specimens or fossils to track temporal changes in allele frequencies, document selection, and understand past population dynamics (e.g., crop domestication).
- **Detection of Selection Signatures:** Using statistical methods on large-scale genomic data to detect patterns left by positive or balancing selection (e.g., through Tajima's D,  $F_{ST}$  outlier tests).

**Comparison Table: Quantitative vs. Population Genetics**

| Feature         | Quantitative Genetics                                                            | Population Genetics                                                                    |
|-----------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Primary Focus   | Within-population variation of complex traits; partitioning phenotypic variance. | Between and within-population genetic variation; changes in allele frequencies.        |
| Key Question    | How is a trait inherited? What is its genetic architecture and breeding value?   | How is genetic diversity distributed? What evolutionary forces are acting?             |
| Main Parameters | Heritability, Breeding Value, Genetic Correlation, QTL.                          | Allele Frequency, HWE, F-statistics ( $F_{ST}$ ), Effective Population Size ( $N_e$ ). |
| Scale           | Often focuses on families/lineages in controlled crosses or breeding programs.   | Focuses on natural or experimental populations, often at the species level.            |
| Application     | Plant/Animal breeding, improving agricultural traits.                            | Conservation biology, understanding evolution, epidemiology, forensic science.         |
| Modern Tool     | Genomic Selection, Multi-omics integration.                                      | Landscape Genomics, aDNA analysis, Detection of Selection Signscapes.                  |

## Conclusion

The boundaries between Mendelian, non-Mendelian, quantitative, and population genetics are increasingly fluid. Modern genomics reveals that most traits exist on a spectrum, influenced by varying degrees of genetic complexity, epigenetic regulation, and environmental interaction. Understanding these integrated frameworks is essential for advancements in medicine, agriculture, and conservation biology.

## FINE STRUCTURE OF GENE

### Fine Structure of the Gene

#### Definition and Historical Context

The fine structure of a gene refers to the detailed molecular organization of a gene, including the identification of functional subunits within a gene (mutational sites, recombination units) that are smaller than the gene itself. This concept emerged from studies showing that genes were not indivisible "beads on a string" but had internal structure. Key work by Seymour Benzer using bacteriophage T4 rII system revolutionized our understanding.

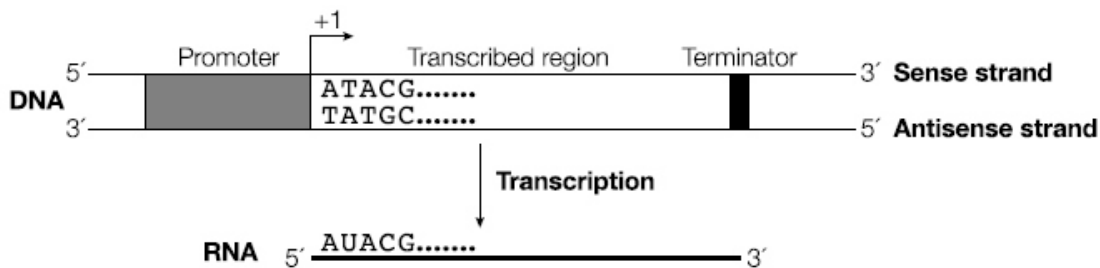


**Fig. Structure of a typical transcription unit showing promoter and terminator sequences, and the RNA product.**

#### Key Concepts and Advances

##### 1. Recon, Muton, and Cistron:

- **Cistron:** The functional unit of heredity, defined by the cis-trans complementation test. It is essentially equivalent to a gene. A cistron is the DNA sequence that codes for a single polypeptide chain.
- **Recon:** The smallest unit of DNA capable of recombination. It can be as small as a single nucleotide pair.
- **Muton:** The smallest unit of DNA that can undergo mutation. This is also a single nucleotide pair.

##### 2. Modern Refinement of Gene Structure:

Today, the fine structure is understood in terms of specific DNA sequences with precise functions within and around the coding region.

- **Coding Sequences (Exons):** Segments of DNA that are transcribed and translated into amino acid sequences. In prokaryotes, usually contiguous; in eukaryotes, interrupted by introns.
- **Non-Coding Regulatory Sequences:**
  - Promoter:** Recognized by RNA polymerase to initiate transcription. Contains consensus sequences (-10 Pribnow box and -35 box in prokaryotes).
  - Operator:** Specific DNA sequence where a repressor protein binds to inhibit transcription (key in operon regulation).
  - Regulatory Binding Sites:** For activators, inducers, etc.
  - 5' and 3' Untranslated Regions (UTRs):** Important for translation initiation, stability, and regulation of mRNA.

### Intragenic Recombination and Complementation:

- **Intragenic Recombination:** Recombination that occurs within a gene, between different mutant sites (alleles). This proved genes had linear, internal structure.
- **Complementation Test (cis-trans test):** Determines if two mutations are in the same gene (cistron) or different genes.
  - If two recessive mutations in trans configuration (on different chromosomes) produce a wild-type phenotype mutations are in different cistrons (they complement).
  - If they produce a mutant phenotype mutations are in the same cistron (no complementation).

**Table: Benzer's Functional Units vs. Modern Molecular Units**

| Benzer's Term | Definition (Functional)                                                                | Modern Molecular Equivalent                                   |
|---------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------|
| Cistron       | Unit of function; defined by complementation test.                                     | A gene; DNA sequence encoding a single polypeptide/rRNA/tRNA. |
| Recon         | Unit of recombination; smallest element interchangeable by recombination.              | A single nucleotide base pair (theoretically).                |
| Muton         | Unit of mutation; smallest element alterable by mutation to yield a phenotypic effect. | A single nucleotide base pair.                                |

## PROKARYOTIC GENE REGULATION: THE OPERON MODEL

### Definition and General Principle

Gene regulation in prokaryotes primarily occurs at the level of transcription initiation, allowing rapid adaptation to environmental changes. The operon model, proposed by Jacob and Monod (1961), is the paradigm for coordinated regulation of functionally related genes. An operon is a cluster of structural genes under the control of a single promoter, transcribed as a single polycistronic mRNA, along with its adjacent regulatory elements.

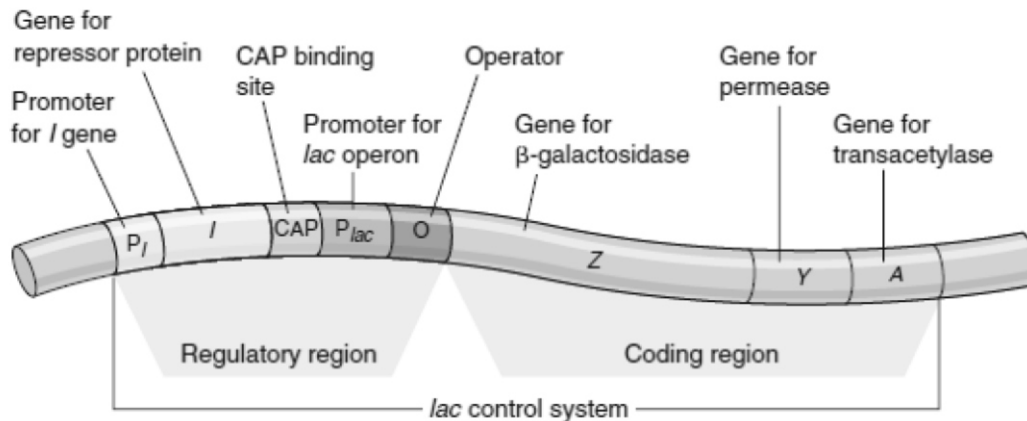


**Fig.** The *lac* region of the *Escherichia coli* chromosome. The *lac* operon consists of a promoter, an operator, and three genes that code for proteins required for the metabolism of lactose. In addition, there is a binding site for the catabolite activator protein (CAP), which affects whether or not RNA polymerase will bind to the promoter. Gene *I* codes for a repressor protein, which will bind to the operator and block transcription of the *lac* genes. The genes *Z*, *Y*, and *A* encode the two enzymes and the permease involved in the metabolism of lactose.

### 🔗 Core Components of an Operon

1. **Structural Genes:** Genes coding for enzymes/proteins of a metabolic pathway (e.g., *lacZ*, *lacY*, *lacA* in *lac* operon).
2. **Promoter (P):** Site where RNA polymerase binds.
3. **Operator (O):** A DNA sequence between the promoter and structural genes where a repressor protein binds.
4. **Regulator Gene:** Located outside the operon, it codes for the repressor protein (e.g., *lacI* gene).
5. **Inducer/Corepressor:** Small effector molecules that regulate operon activity by interacting with the repressor protein.

### 🔗 Types of Operon Regulation

#### 1. Inducible Operon System (Negative Control)

- **Default State:** OFF. The repressor is active and bound to the operator, blocking transcription.
- **Induction:** Presence of a specific inducer molecule (often a substrate) inactivates the repressor by binding to it, causing it to detach from the operator. This allows RNA polymerase to transcribe the genes.
- **Logic:** "The enzyme is synthesized only when its substrate is present."
- **Classic Example:** The *lac* Operon  
**Function:** Metabolism of lactose.

**Genes:** lacZ ( -galactosidase), lacY (permease), lacA (transacetylase).

**Regulator:** lacI gene produces Lac repressor.

**Inducer:** Allolactose (isomer of lactose). Binds to repressor, inactivating it.

**Additional Layer:** Catabolite Repression (Positive Control): When glucose is present, cAMP levels are low. cAMP binds to CAP (Catabolite Activator Protein). The cAMP-CAP complex is an activator that binds near the lac promoter, enhancing RNA polymerase binding. Thus, the lac operon is maximally expressed only when lactose is present AND glucose is absent.

## 2. Repressible Operon System (Negative Control)

- **Default State:** ON. The repressor is inactive and cannot bind the operator. Transcription proceeds.
- **Repression:** Presence of a corepressor (often an end-product of the pathway) activates the repressor. The active repressor-corepressor complex binds the operator and shuts down transcription.
- **Logic:** "The enzyme synthesis is halted when the end-product is abundant."
- **Classic Example:** The trp Operon
  - Function:** Biosynthesis of tryptophan (an amino acid).
  - Genes:** trpE, trpD, trpC, trpB, trpA (encode enzymes for Trp synthesis).
  - Regulator:** trpR gene produces an inactive apo-repressor.

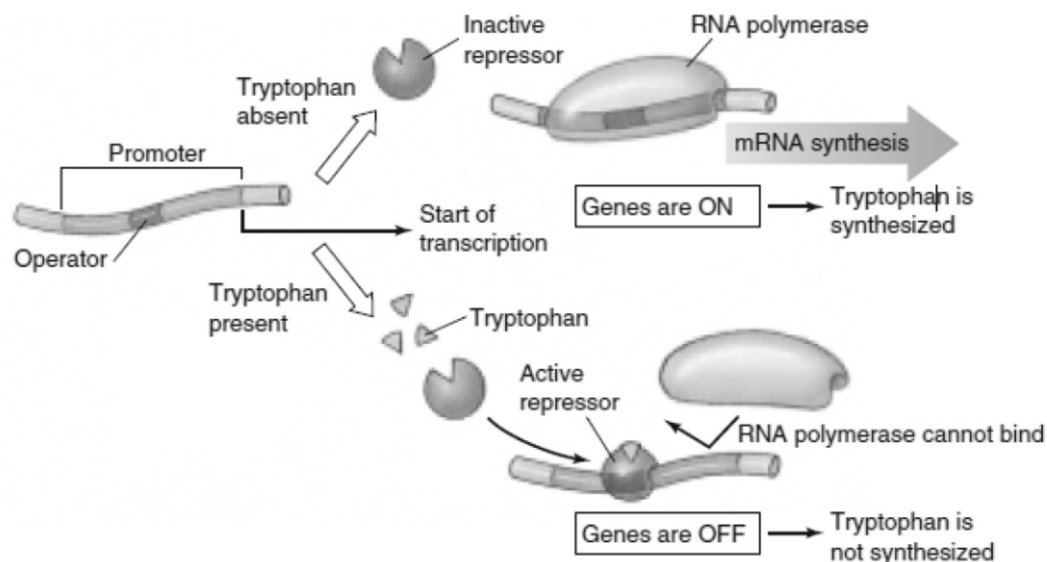


**Fig. How the trp operon is controlled. The tryptophan repressor cannot bind the operator (which is located within the promoter) unless tryptophan first binds to the repressor. Therefore, in the absence of tryptophan, the promoter is free to function and RNA polymerase transcribes the operon. In the presence of tryptophan, the tryptophan-repressor complex binds tightly to the operator, preventing RNA polymerase from initiating transcription.**

**Corepressor:** Tryptophan itself. It binds to the apo-repressor, activating it to block transcription.

**Additional Layer: Attenuation:** A fine-tuning mechanism based on translation of a leader peptide. Under conditions of high tryptophan, a terminator hairpin forms in the mRNA, causing premature transcription termination before the structural genes.

**Comparison Table: Inducible vs. Repressible Operons (Negative Control)**

| Feature            | Inducible Operon (e.g., lac)                         | Repressible Operon (e.g., trp)                       |
|--------------------|------------------------------------------------------|------------------------------------------------------|
| Default State      | Usually OFF (repressor bound).                       | Usually ON (repressor not bound).                    |
| Pathway Type       | Catabolic (breaks down a nutrient).                  | Anabolic (synthesizes a molecule).                   |
| Effector Molecule  | Inducer (e.g., allolactose, IPTG).                   | Corepressor (e.g., tryptophan).                      |
| Effect of Effector | Inactivates the repressor.                           | Activates the repressor.                             |
| Biological Logic   | "Make enzymes only when the substrate is available." | "Stop making enzymes when the product is plentiful." |
| Regulator Gene     | Produces an active repressor.                        | Produces an inactive apo-repressor                   |

## Positive vs. Negative Gene Regulation in Operons

- **Negative Regulation:** A repressor protein inhibits transcription. This is the primary mechanism in the classic lac and trp operons.
- **Positive Regulation:** An activator protein is required to stimulate transcription. This enhances the basal level of expression.

**Example:** CAP-cAMP complex in the lac operon is a positive regulator. RNA polymerase binds very inefficiently to the lac promoter unless CAP-cAMP is bound.

**Summary Table: Regulatory Mechanisms in Prokaryotes**

| Mechanism            | Key Protein        | Effector Molecule             | Effect on Transcription        | Example                         |
|----------------------|--------------------|-------------------------------|--------------------------------|---------------------------------|
| Negative Inducible   | Active Repressor   | Inducer (binds repressor)     | Derepression (Turns ON)        | lac operon (LacI + allolactose) |
| Negative Repressible | Inactive Repressor | Corepressor (binds repressor) | Repression (Turns OFF)         | trp operon (TrpR + tryptophan)  |
| Positive Regulation  | Activator Protein  | Inducer (often cAMP)          | Activation (Turns ON strongly) | lac operon (CAP + cAMP)         |
| Attenuation          | (RNA-based)        | Availability of charged tRNA  | Premature Termination          | trp, his, phe operons           |

## Recent Advances and Broader Context

- **Quorum Sensing:** A form of intercellular communication where bacteria regulate gene expression (often via operons) in response to cell population density, using autoinducer molecules.

- **Riboswitches:** RNA-based regulatory elements within the mRNA leader sequence that bind small metabolites directly, changing conformation to affect transcription or translation. They represent a mechanism independent of protein repressors.
- **Global Regulatory Networks:** Operons are often part of larger networks (regulons) controlled by a common regulator (e.g., CRP regulon in *E. coli*), allowing coordinated response to complex environmental signals like carbon source availability or stress.

### Conclusion:

The operon model elegantly explains the efficiency and economy of prokaryotic gene regulation. The fine structure concept revealed that genes are complex, divisible entities. Together, they form the foundation of molecular genetics, with principles that continue to inform our understanding of gene regulation in all organisms.

## CRISPR-CAS SYSTEMS

### Definition and Discovery

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR associated proteins) is an adaptive immune system in prokaryotes (bacteria and archaea) that provides resistance against foreign genetic elements like bacteriophages and plasmids. Discovered in *E. coli* in 1987, its function as an immune system was elucidated in the 2000s.

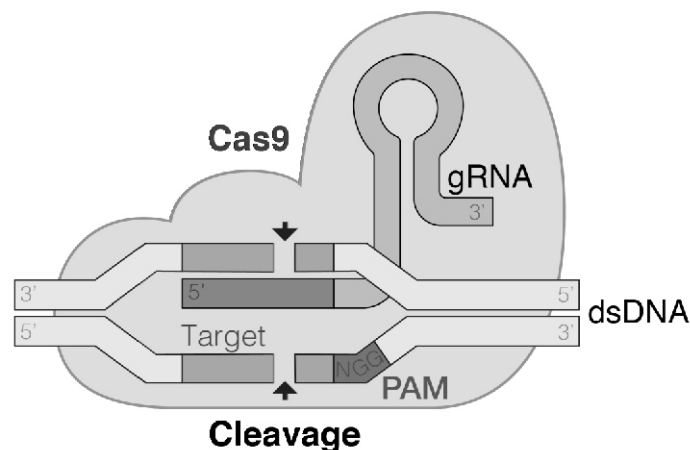
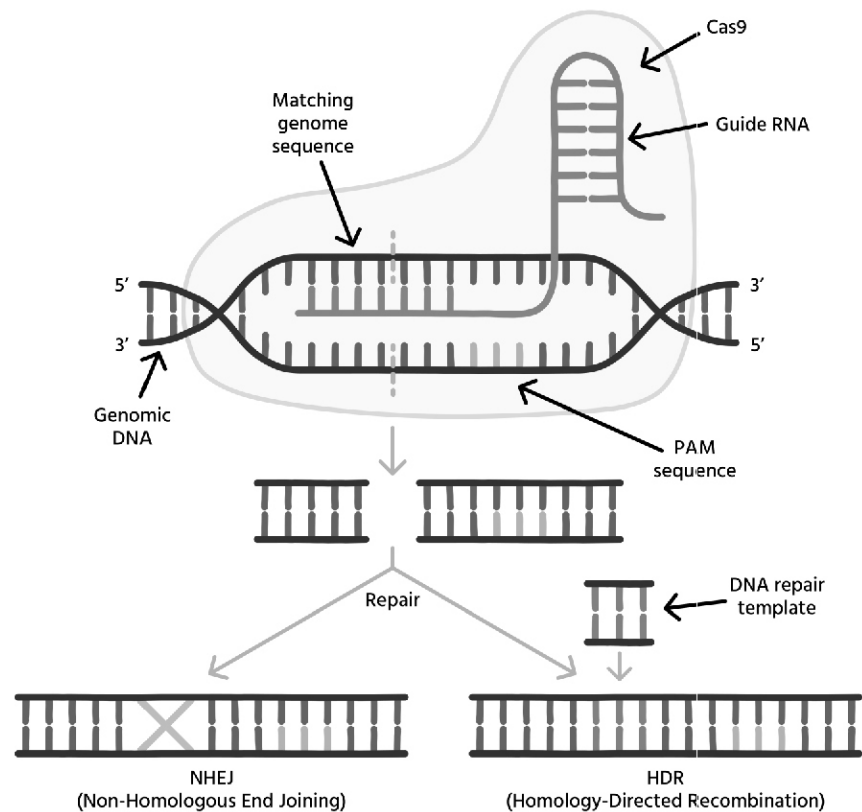


*Fig. CRISPR-Cas Systems*

### 🔑 Key Components and Mechanism

#### 1. Genetic Organization:

- **CRISPR Array:** A chromosomal locus containing:



**Schematic overview of CRISPR-Cas9 gene editing. In the absence of a repair template, cells will repair the break via error-prone non-homologous end joining (NHEJ), leading to functional gene disruption (gene/protein knock-out). Alternatively, a repair template can be used to introduce specific sequence changes via homologous-directed repair (HDR).**

**Repeats:** Short (21-48 bp), identical, palindromic sequences.

**Spacers:** Variable sequences (26-72 bp) between repeats, derived from past infections by viruses/plasmids. Serve as a "genetic memory."

- **Cas Genes:** Encode Cas proteins (nucleases, helicases) that execute the immune response.

## 2. Three-Stage Mechanism:

- **Adaptation (Spacer Acquisition):**

Foreign DNA (e.g., phage) is cleaved into small fragments.

New "protospacers" are integrated into the CRISPR array as novel spacers at the leader-proximal end.

Requires Cas1 and Cas2 proteins universally.

- **Expression (crRNA Biogenesis):**

The CRISPR array is transcribed into a long precursor CRISPR RNA (pre-crRNA). The pre-crRNA is processed into short, mature crRNAs, each containing a single spacer sequence.

- **Interference (Target Destruction):**

The mature crRNA guides a Cas protein complex to complementary foreign DNA/RNA.

The complex cleaves and degrades the invading nucleic acid, neutralizing the threat.

**Comparison Table: Major CRISPR-Cas Systems**

| Type     | Signature Proteins                                     | Mechanism & Function                           | Target        | Example Application          |
|----------|--------------------------------------------------------|------------------------------------------------|---------------|------------------------------|
| Type I   | Cas3 (helicase-nuclease), Cascade complex              | Multi-protein complex, cleaves DNA             | dsDNA         | Bacterial immunity           |
| Type II  | Cas9 (single multidomain protein), tracrRNA, RNase III | Simpler system; uses dual RNA (crRNA:tracrRNA) | dsDNA         | CRISPR-Cas9 genome editing   |
| Type III | Cas10, Csm/Cmr complexes                               | Targets both DNA and RNA                       | dsDNA & ssRNA | Broad antiviral defense      |
| Type V   | Cas12a/Cpf1 (single protein)                           | Creates staggered cuts; no tracrRNA needed     | dsDNA         | Genome editing, diagnostics  |
| Type VI  | Cas13a (single protein)                                | Targets and cleaves RNA                        | ssRNA         | RNA editing, viral detection |

🔧 **CRISPR-Cas9 as a Genome Engineering Tool (Revolutionary Application)**

- **Simplification:** In Type II systems, the dual guide RNA can be engineered into a single-guide RNA (sgRNA).
- **Mechanism:** The sgRNA guides Cas9 to a specific genomic locus complementary to its 20-nucleotide spacer.
- **Cleavage:** Cas9 creates a double-strand break (DSB) 3 bp upstream of a required Protospacer Adjacent Motif (PAM) (e.g., 5'-NGG-3' for *S. pyogenes* Cas9).
- **DNA Repair & Editing:**
  - Non-Homologous End Joining (NHEJ):** Error-prone, leads to indels (insertions/deletions), often causing gene knockout.
  - Homology-Directed Repair (HDR):** With a donor DNA template, allows precise gene knock-in or correction.

🔧 **Applications Beyond Genome Editing**

1. **CRISPR Interference/Activation (CRISPRi/a):** Using catalytically dead Cas9 (dCas9) fused to repressors/activators for transcriptional control.

2. **Base Editing:** dCas9 fused to deaminase enzymes for direct C-to-T or A-to-G conversions without DSBs.
3. **Prime Editing:** A more precise "search-and-replace" system using Cas9 nickase and reverse transcriptase.
4. **Diagnostics:** SHERLOCK/DETECTR: Using Cas13/Cas12 collateral RNA/DNA cleavage for sensitive pathogen detection (e.g., SARS-CoV-2).

## SIGMA ( ) FACTORS



### Definition and Core Function

A sigma factor is a dissociable subunit of bacterial RNA polymerase essential for promoter recognition and transcription initiation. It confers promoter specificity, directing the core RNA polymerase to initiate transcription at appropriate genes.

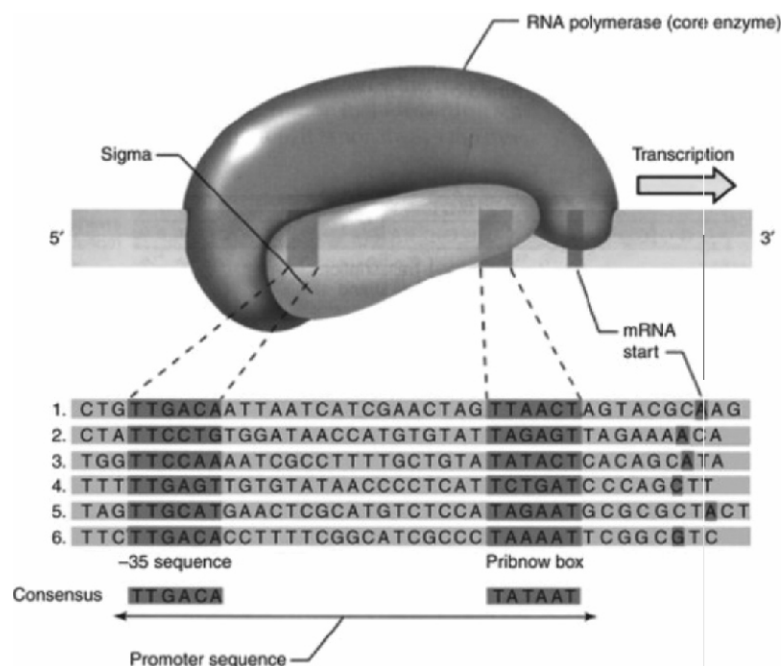


**Fig. Sigma ( ) Factors**

### Mechanism of Action

1. **Holoenzyme Formation:** Core RNA polymerase (  $\sigma_2$  ) binds to a sigma factor forms the RNA polymerase holoenzyme.
2. **Promoter Recognition:** The sigma factor recognizes and binds to specific consensus sequences in the promoter.
  - **-10 Box (Pribnow Box):** TATAAT (variations)

- **-35 Box:** TTGACA (variations)
  - Spacing between -10 and -35 is critical (~17 bp).
3. **Initiation & Release:** After transcription initiation (formation of ~9-nt RNA), the sigma factor often dissociates ( -cycle), and core polymerase proceeds with elongation.

### 🔗 Types, Roles, and Regulation

#### 1. Primary/Housekeeping Sigma Factor ( <sup>70</sup>/RpoD in *E. coli*):

- **Function:** Transcribes most genes required for growth and metabolism under optimal conditions.
- **Consensus:** Recognizes standard -10 and -35 sequences.

#### 2. Alternative Sigma Factors:

- Induced in response to specific environmental stresses or developmental cues.
- Recognize different promoter sequences, redirecting RNA polymerase to specific gene subsets.

**Table: Major Sigma Factors in *E. coli* and Their Roles**

| Sigma Factor         | Gene | Primary Role/Regulation                                      | Promoter Specificity | Target Gene Examples                         |
|----------------------|------|--------------------------------------------------------------|----------------------|----------------------------------------------|
| <sup>70</sup> (RpoD) | rpoD | Housekeeping, exponential growth                             | Consensus -10/-35    | Metabolic enzymes, ribosomal proteins        |
| <sup>54</sup> (RpoN) | rpoN | Nitrogen metabolism, flagellar synthesis                     | -24 (GG) / -12 (GC)  | GlnA (glutamine synthetase), flagellar genes |
| <sup>32</sup> (RpoH) | rpoH | Heat shock response ( at >37°C)                              | Different -10/-35    | Chaperones (DnaK, GroEL), proteases          |
| <sup>5</sup> (RpoS)  | rpoS | Stationary phase & general stress (osmotic, acid, oxidative) | Altered -10 (no -35) | Stress resistance genes, virulence factors   |
| <sup>D</sup> (FliA)  | fliA | Flagellar biosynthesis, chemotaxis                           | Specific -10/-35     | Flagellin (fliC), chemotaxis genes           |
| <sup>7E</sup> (RpoE) | rpoE | Extreme heat shock, envelope stress                          | Specific -10/-35     | Periplasmic chaperones, proteases            |

### Regulation of Sigma Factor Activity

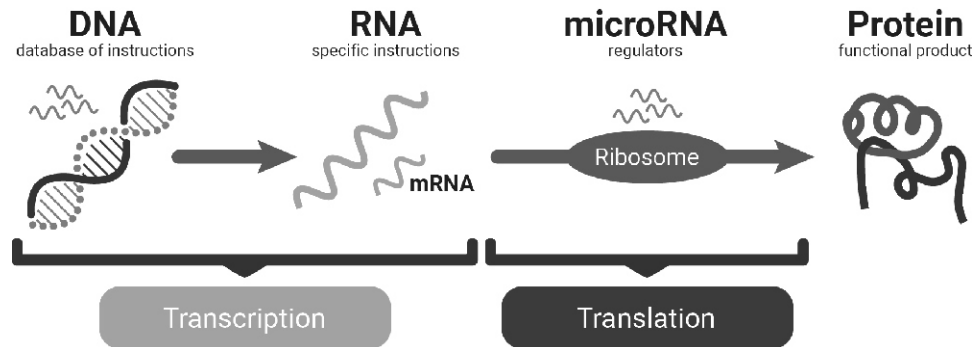
- **Transcriptional Control:** Expression of sigma factor genes (e.g., rpoH induced by heat).
- **Post-Translational Control:** Anti-sigma factors bind and inhibit sigma factors; anti-anti-sigma factors release them (e.g., control of <sup>D</sup> in flagellar synthesis).

- **Protein Stability:** is rapidly degraded at normal temperatures but stabilized upon heat shock.
- **Competition:** Different sigma factors compete for binding to a limited pool of core RNA polymerase.

## MICRORNAS (MIRNAS)

### Definition and Discovery

microRNAs (miRNAs) are a class of small (20-24 nt), single-stranded, non-coding RNAs that regulate gene expression post-transcriptionally, primarily by translational repression or mRNA degradation. Discovered in *C. elegans* (\*lin-4\* and \*let-7\*), they are now known to be ubiquitous in eukaryotes and crucial for development, physiology, and disease.



### Biogenesis and Mechanism: A Two-Step Process

#### 1. Nuclear Biogenesis:

- **Transcription:** RNA polymerase II transcribes miRNA genes into long primary miRNA (pri-miRNA) transcripts, often forming hairpin structures.
- **Cleavage:** The Drosha enzyme (with DGCR8/Pasha) processes pri-miRNA into ~70-nt precursor miRNA (pre-miRNA) hairpins in the nucleus.

#### 2. Cytoplasmic Processing & Action:

- **Export:** Exportin-5 transports pre-miRNA to the cytoplasm.
- **Dicing:** The Dicer enzyme cleaves pre-miRNA into a \*\*~22-nt miRNA:miRNA\* duplex\*\*.
- **Loading:** One strand (the guide strand) is loaded into the RNA-Induced Silencing Complex (RISC), whose core component is an Argonaute (Ago) protein. The other strand (passenger) is degraded.
- **Target Recognition & Silencing:** The miRNA guides RISC to target mRNAs via imperfect base-pairing, primarily with the 3' Untranslated Region (3' UTR).  
 "Seed Sequence" (nt 2-8): Critical for target specificity.

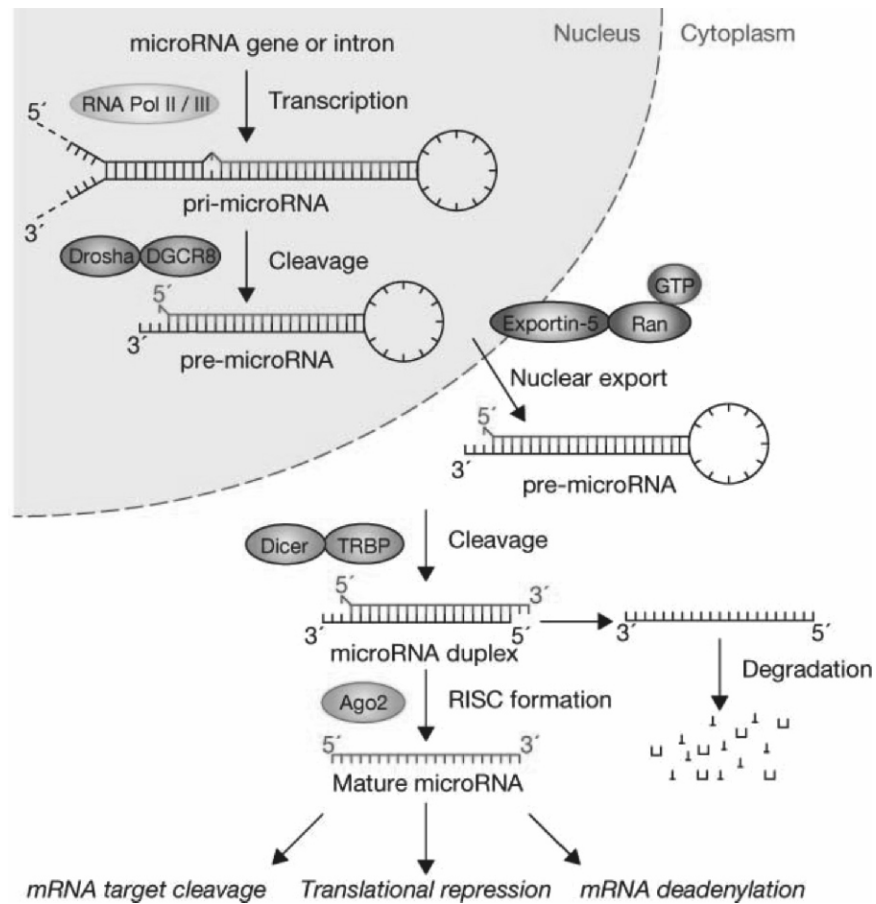


**Fig. Biogenesis of micro RNA**

- **Mode of Action:**

**Perfect complementarity (common in plants):** Ago cleaves mRNA (slicer activity).

**Imperfect complementarity (common in animals):** RISC inhibits translation initiation/elongation and promotes mRNA deadenylation and decay.

- **Biological Functions and Significance**

- **Developmental Timing:** \*lin-4\* and \*let-7\* control larval stage transitions in *C. elegans*.
- **Cell Differentiation & Proliferation:** Regulate key genes in stem cell maintenance, apoptosis, and tissue development.
- **Stress Response:** Expression changes in response to biotic/abiotic stress in plants and animals.

- **Disease Biomarkers & Therapeutics:** Dysregulation of miRNAs is linked to cancer, neurological disorders, and cardiovascular diseases. Circulating miRNAs are potential diagnostic markers. miRNA mimics (to restore function) and antagomiRs (to inhibit overactive miRNAs) are in therapeutic development.

#### Comparison of Small Regulatory RNAs

| Feature             | microRNA (miRNA)                                     | siRNA (Small Interfering RNA)                             | piRNA (Piwi-interacting RNA)                            |
|---------------------|------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------------------|
| Origin              | Endogenous (genome-encoded)                          | Exogenous (viruses, transposons) or endogenous (dsRNA)    | Endogenous (transposons, repetitive elements)           |
| Length              | 20-24 nt                                             | 21-23 nt                                                  | 26-31 nt                                                |
| Biogenesis          | Processed from hairpin precursors by Drosha/Dicer    | Processed from long dsRNA by Dicer                        | Dicer-independent, from long single-stranded precursors |
| Completeness        | Imperfect (animals) / Perfect (plants)               | Perfect to target                                         | Perfect to target                                       |
| Primary Function    | Endogenous gene regulation (development, physiology) | Defense against viruses/transposons, viral gene silencing | Silencing transposons in germline, genome defense       |
| Key Protein Partner | Argonaute (Ago) proteins                             | Argonaute (Ago) proteins                                  | Piwi clade of Argonaute proteins                        |

#### Integration and Interdisciplinary Connections

- **CRISPR-Cas & miRNA:** Both use RNA-guided systems for specific targeting (DNA/RNA vs. mRNA). CRISPR technology can be used to edit miRNA genes.
- **Sigma Factors & miRNA:** Both are master regulators, but at different levels (transcriptional initiation vs. post-transcriptional) and in different domains of life (prokaryotes vs. eukaryotes).
- **Agricultural Importance:** In plants, miRNAs are key regulators of development, nutrient homeostasis, and stress responses. CRISPR is used to create crops with improved yield, disease resistance, and nutritional quality.
- **Medical Importance:** miRNAs are drug targets. CRISPR-Cas enables gene therapy for Mendelian disorders and functional genomics screens to identify disease genes.

#### Conclusion

CRISPR-Cas represents a revolutionary prokaryotic defense system turned universal tool; sigma factors exemplify elegant transcriptional reprogramming in prokaryotes; and miRNAs illustrate the complexity of post-transcriptional gene regulation in eukaryotes. Together, they highlight the diverse and sophisticated molecular strategies life employs to control genetic information flow, offering powerful technologies for research, medicine, and biotechnology.

# QUESTIONS



## Section A: Very Short Answer Type Questions

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**1. What is the main difference between Mendelian and non-Mendelian inheritance?**

**Ans.** Mendelian inheritance follows predictable patterns of dominant/recessive alleles segregating independently, while non-Mendelian inheritance involves deviations from these patterns due to factors like cytoplasmic inheritance, genomic imprinting, or polygenic traits.

**2. Name one example of non-Mendelian inheritance in plants.**

**Ans.** Cytoplasmic male sterility (CMS) in maize or variegation in *Mirabilis jalapa* (due to chloroplast inheritance).

**3. What is a quantitative trait locus (QTL)?**

**Ans.** A QTL is a region of DNA associated with a quantitative trait (polygenic trait) that shows continuous variation, such as height or yield.

**4. Define the term "cistron" in the context of gene fine structure.**

**Ans.** A cistron is the smallest unit of genetic material that encodes a single polypeptide chain or functional RNA, determined by the cis-trans complementation test.

**5. What is the function of the operator region in an operon?**

**Ans.** The operator is a DNA sequence where a repressor protein binds to regulate transcription of the operon by blocking RNA polymerase.

**6. Which CRISPR component acts as a guide to target specific DNA sequences?**

**Ans.** The guide RNA (gRNA), which combines tracrRNA and crRNA, directs the Cas nuclease to the target DNA.

**7. What is the role of alternative sigma factors in prokaryotes?**

**Ans.** Alternative sigma factors direct RNA polymerase to specific sets of promoters, allowing coordinated expression of genes in response to environmental stresses (e.g., heat shock, sporulation).

**8. How do microRNAs (miRNAs) regulate gene expression in eukaryotes?**

**Ans.** miRNAs bind to complementary sequences on target mRNAs, leading to translational repression or mRNA degradation.

**9. What does the Hardy-Weinberg equilibrium describe?**

**Ans.** It describes the theoretical state in which allele and genotype frequencies in a population remain constant from generation to generation in the absence of evolutionary forces.

**10. Which type of gene regulation involves attenuation in prokaryotes?**

**Ans.** Attenuation is a transcriptional regulatory mechanism in amino acid biosynthesis operons (e.g., trp operon), where translation of a leader peptide affects RNA polymerase termination.

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Section B: Short Answer Type Questions

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**11. Explain genomic imprinting as an example of non-Mendelian inheritance.**

**Ans.** Genomic imprinting is an epigenetic phenomenon where genes are expressed in a parent-of-origin-specific manner. Certain alleles are silenced when inherited from one parent but active when inherited from the other. For example, in plants, imprinting occurs in endosperm development, where paternal alleles of some genes are silenced, ensuring proper seed development.

**12. Differentiate between penetrance and expressivity in quantitative genetics.**

**Ans.** Penetrance is the proportion of individuals carrying a specific genotype who express the associated phenotype. Expressivity is the degree or range of phenotypic expression among individuals with the same genotype. A trait can have complete penetrance but variable expressivity.

**13. Describe the concept of "recombination mapping" in determining the fine structure of a gene.**

**Ans.** Recombination mapping involves analyzing crossover frequencies within a gene (intragenic recombination) to construct a detailed genetic map. By crossing mutants with different mutations within the same gene and scoring wild-type recombinants, the relative positions and distances between mutation sites can be determined, revealing the linear order of mutational sites within a cistron.

**14. Briefly explain the lac operon model of negative and positive regulation.**

**Ans.** In the lac operon, negative regulation occurs via the lac repressor protein, which binds to the operator in the absence of lactose, blocking transcription. Positive regulation occurs via CAP-cAMP complex: when glucose is low, cAMP levels rise, cAMP binds to CAP, and the CAP-cAMP complex binds to the promoter, enhancing RNA polymerase binding and transcription initiation.

**15. What are the key components of the CRISPR-Cas9 system and their functions?**

**Ans.** 1. Cas9 nuclease: Creates double-strand breaks at the target DNA site. 2. Guide RNA (gRNA): A chimeric RNA (crRNA + tracrRNA) that directs Cas9 to the specific DNA sequence via base pairing. 3. PAM sequence: A short adjacent motif (e.g., 5'-NGG-3') required for Cas9 recognition and cleavage.

**16. How do sigma factors contribute to global changes in gene expression in bacteria?**

**Ans.** Sigma factors confer promoter specificity to RNA polymerase. The primary sigma factor (<sup>70</sup> in *E. coli*) transcribes housekeeping genes. Alternative sigma factors (e.g., <sup>32</sup> for heat shock, <sup>54</sup> for nitrogen metabolism) are activated under specific stresses and redirect RNA polymerase to transcribe distinct sets of genes, enabling a rapid, coordinated response to environmental changes.

**17. Explain the role of miRNAs in post-transcriptional gene silencing (PTGS).**

**Ans.** miRNAs are ~22 nt non-coding RNAs processed from precursor hairpins. They are incorporated into the RNA-induced silencing complex (RISC). The miRNA guides RISC to partially complementary sequences in the 3'UTR of target mRNAs, leading to either translational repression (in animals) or mRNA cleavage/degradation (common in plants), thereby fine-tuning gene expression.

**18. What is linkage disequilibrium (LD) and what causes it?**

**Ans.** Linkage disequilibrium (LD) is the non-random association of alleles at two or more loci in a population. It occurs when alleles are inherited together more often than expected by chance due to physical linkage on a chromosome, low recombination rates, population bottlenecks, founder effects, or selective sweeps.

**19. Compare constitutive, inducible, and repressible operons with one example each.**

**Ans.** Constitutive operons are always transcribed (e.g., genes for ribosomal RNA). Inducible operons are turned ON by an inducer (e.g., lac operon by lactose/allolactose). Repressible operons are turned OFF by a corepressor (e.g., trp operon by tryptophan).

**20. How does the concept of "one gene-one polypeptide" relate to the fine structure of a gene?**

**Ans.** The "one gene-one polypeptide" hypothesis (refined from "one gene-one enzyme") posits that a gene codes for a single polypeptide. Fine structure analysis through intragenic recombination and complementation tests revealed that a gene (cistron) is a linear sequence of nucleotides encoding a polypeptide, and mutations can occur at multiple sites within it, supporting this molecular definition.

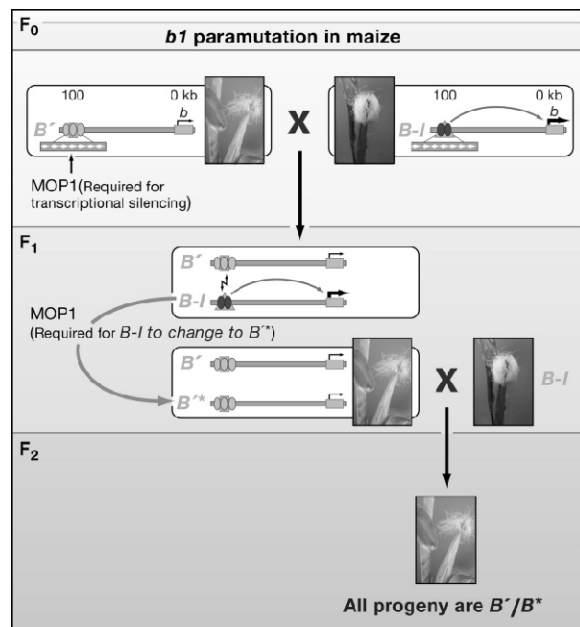
## Section C: Long Answer Type Questions

**21. Discuss recent advances in our understanding of non-Mendelian inheritance mechanisms, with emphasis on epigenetic inheritance (including paramutation and transgenerational epigenetic inheritance) and cytoplasmic inheritance in plants.**

**Ans.** Recent research has expanded our understanding of inheritance beyond DNA sequence, highlighting the role of epigenetic and cytoplasmic factors.

**1. Epigenetic Inheritance:**

- **Paramutation:** An interaction between alleles where one allele (paramutagenic) induces a heritable epigenetic change in another allele (paramutable), silencing it. In maize, alleles like *booster1* (*b1*) exhibit this. The silent state is mitotically and meiotically heritable and involves small RNA-directed DNA methylation and chromatin remodeling. It challenges Mendelian principles as the altered expression pattern is inherited even if the paramutagenic allele is no longer present.
- **Transgenerational Epigenetic Inheritance (TEI):** This refers to the transmission of epigenetic marks (DNA methylation, histone



modifications, small RNAs) across generations without changes in DNA sequence. In plants, TEI is well-documented. For example, stress-induced epigenetic changes (e.g., from drought or pathogen attack) can be transmitted to offspring, potentially providing adaptive benefits. Mechanisms involve the incomplete erasure of epigenetic marks during gametogenesis, especially in plants where the germline is not set aside early.

## 2. Cytoplasmic Inheritance:

- **Organelar Genomes:** Chloroplasts and mitochondria have their own genomes, inherited predominantly maternally in most angiosperms (with exceptions). This uniparental inheritance leads to non-Mendelian patterns. Mutations in chloroplast genes (e.g., causing variegation in *Mirabilis*) are transmitted only through the maternal line.
- **Cytoplasmic Male Sterility (CMS):** A classic example in crop plants like maize and rice. CMS is caused by mitochondrial genes that produce proteins disrupting pollen development. The trait is inherited maternally. Restoration of fertility is controlled by nuclear Rf (restorer-of-fertility) genes, demonstrating an intricate interplay between nuclear and cytoplasmic genomes.

### Recent Advances:

- **Role of Small RNAs:** 24 nt small interfering RNAs (siRNAs) are key mediators of paramutation and TEI. They guide de novo DNA methylation via the RdDM (RNA-directed DNA methylation) pathway, establishing silent epigenetic states that can be inherited.
- **Epigenetic Recombinants:** Studies show that epigenetic variants (epialleles) can recombine and segregate independently of genetic markers, adding a layer of complexity to inheritance patterns.
- **Environmental Modulation:** It is now clear that environmental factors can directly shape the epigenetic landscape, and these changes can be transmitted, providing a potential mechanism for rapid adaptation—a concept with Lamarckian overtones.

**Conclusion:** These advances reveal that inheritance is a complex interplay of genetic (Mendelian), epigenetic, and cytoplasmic information. Non-Mendelian mechanisms provide organisms, especially sessile plants, with additional layers of regulatory plasticity and adaptive potential, fundamentally enriching our understanding of heredity and evolution.

## 22. Explain the principles and applications of quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) in plant genetics. How have these approaches advanced crop improvement?

### Ans. QTL Mapping:

- **Principle:** QTL mapping identifies genomic regions associated with quantitative traits by correlating phenotypic variation with genetic markers in a segregating population (e.g.,

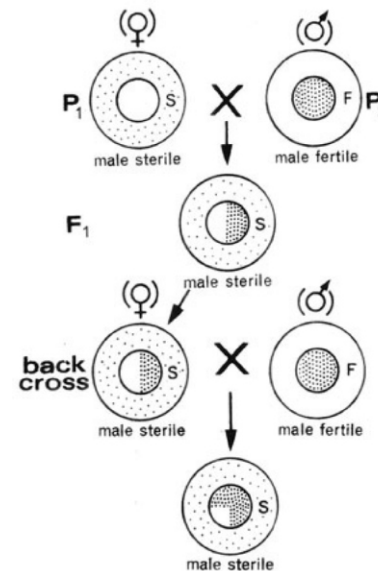


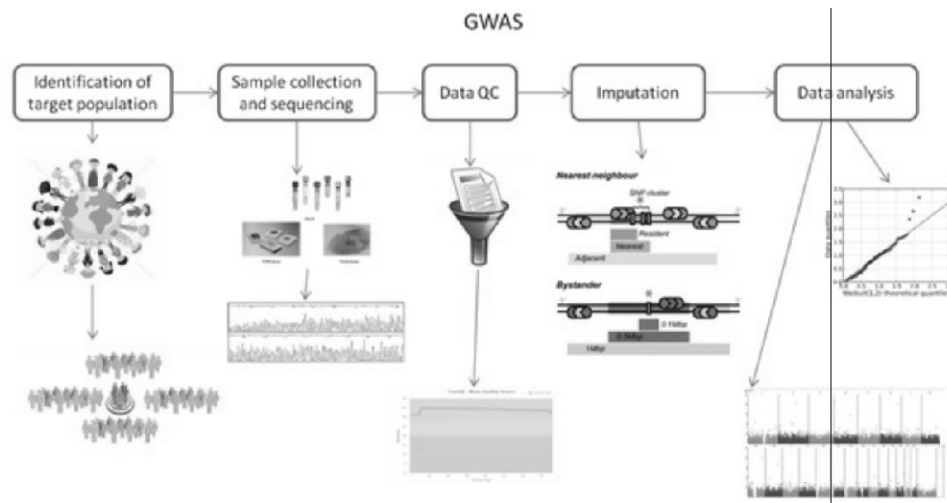
**Fig. Cytoplasmic Male Sterility (CMS)**

$F_2$ , RILs, NILs). It relies on genetic linkage. Statistical methods (interval mapping, composite interval mapping) calculate the likelihood of a QTL at each genomic position.

- **Strengths:** Effective for traits controlled by a few loci with large effects in controlled crosses. Allows dissection of complex traits into constituent loci.
- **Limitations:** Limited to alleles present in the two parents, low resolution (broad QTL intervals), and is population-specific.

#### Genome-Wide Association Studies (GWAS):

- **Principle:** GWAS scans for associations between genetic variants (typically SNPs) and traits across a diverse panel of individuals (a natural population). It relies on linkage disequilibrium (LD). Statistical tests identify markers significantly associated with the phenotype.



- **Strengths:** Explores a wider allele diversity from natural variation, provides higher resolution (with low LD), and does not require creating mapping populations.
- **Limitations:** Prone to false positives from population structure, requires large sample sizes, and detects mostly common alleles with moderate effects.

#### Applications and Advances in Crop Improvement:

1. **Gene Discovery:** Both methods have pinpointed genes underlying critical agronomic traits. QTL mapping identified major genes for rice blast resistance (Pi genes) and tomato fruit weight (fw2.2). GWAS has identified SNPs associated with flowering time in maize and drought tolerance in sorghum.
2. **Marker-Assisted Selection (MAS) and Genomic Selection (GS):** QTL-linked markers are used in MAS to introgress favorable alleles (e.g., for disease resistance) more efficiently. GS, an extension using genome-wide markers (often from GWAS panels), predicts breeding values by estimating effects of all markers simultaneously, accelerating breeding for complex polygenic traits like yield.

3. **Understanding Genetic Architecture:** Reveals if a trait is controlled by many small-effect loci (polygenic) or a few large-effect QTLs, guiding breeding strategy.
4. **Pyramiding Favorable Alleles:** Allows breeders to combine multiple QTLs/genes for traits like drought tolerance or nutrient efficiency into elite backgrounds.
5. **Cloning and Functional Analysis:** Fine-mapping of QTLs and GWAS hits leads to map-based cloning of causative genes (e.g., Ghd7 for yield in rice), opening avenues for genetic engineering.

**Conclusion:** QTL mapping and GWAS are complementary tools that have moved plant breeding from a phenotype-only to a genotype-informed science. They have dramatically accelerated the rate of genetic gain, enabling the development of high-yielding, resilient crop varieties to meet the challenges of food security and climate change.

**23. Describe the fine structure of a gene as revealed by complementation tests and recombination analysis. How did Seymour Benzer's work with the rII locus of T4 phage contribute to this understanding?**

**Ans. Fine Structure of a Gene:** The concept that a gene is not an indivisible "bead on a string" but has an internal structure consisting of many mutable sites that can recombine.

**1. Complementation Test (cis-trans test):**

- **Purpose:** To determine if two mutations are in the same or different functional units (cistrons).
- **Method:** Two mutant alleles are tested in trans (on separate chromosomes) in a diploid or merodiploid condition. If the phenotype is wild-type (complementation), the mutations are in different cistrons (each provides the functional product the other lacks). If the phenotype is mutant (no complementation), the mutations are in the same cistron (both affect the same functional unit).
- **Outcome:** Defines the cistron as the unit of function.

**2. Recombination Analysis (Intragenic Recombination):**

- **Purpose:** To map mutations within a single cistron.
- **Method:** Crossing two mutants with different mutations within the same cistron. Despite both being mutant, rare wild-type recombinant progeny can arise via crossing-over between the two mutation sites. The frequency of these recombinants is proportional to the physical distance between the mutations.
- **Outcome:** Reveals that genes are linear arrays of nucleotide pairs, and recombination can occur between adjacent base pairs. Establishes the concept of mutons (smallest mutable unit, a nucleotide pair) and recons (smallest unit of recombination, can be as small as a single base pair).

**Seymour Benzer's Seminal Work with the rII Locus of T4 Bacteriophage:** Benzer exploited the unique properties of the rII region to dissect gene structure with unprecedented resolution.

**System Advantages:**

- (i) High phage numbers allowed detection of extremely rare recombinants ( $<10^{-8}$ ).
- (ii) rII mutants formed distinct plaques on E. coli B but not on E. coli K12(  $\lambda$  ), enabling powerful selection for wild-type recombinants.

**Experiments & Findings:**

1. **Complementation:** He crossed thousands of rII mutants in pairwise infections on E. coli K12( ). He found they fell into two complementation groups, rIIA and rIIB, defining two adjacent cistrons.
2. **High-Resolution Recombination Mapping:** Within each cistron (e.g., rIIA), he performed pairwise crosses between mutants. By scoring the frequency of wild-type recombinants, he constructed a detailed linear genetic map of over 300 distinct sites within the rIIA cistron.
3. **Key Conclusions:**
  - (a) A gene (cistron) is divisible by recombination and is a linear structure.
  - (b) The smallest unit of mutation and recombination is the nucleotide pair.
  - (c) Mutational "hotspots" exist within a gene.
  - (d) The gene is not the fundamental unit of mutation or recombination, but of function.

Benzer's work provided the first experimental proof of the linear arrangement of nucleotides within a gene and laid the foundation for modern molecular genetics, directly linking genetic maps to physical DNA sequences.

**24. Compare and contrast the regulation of gene expression in prokaryotes by the lac operon (inducible system) and the trp operon (repressible system). Include mechanisms of negative control, positive control, and attenuation.**

**Ans.**

| Aspect                        | lac Operon (Inducible System)                                                                                                                                                                                                                                                                                | trp Operon (Repressible System)                                                                                                                                                                       |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Function                      | Catabolism of lactose (disaccharide) as an alternative carbon source.                                                                                                                                                                                                                                        | Biosynthesis of the amino acid tryptophan.                                                                                                                                                            |
| Default State                 | OFF. The repressor is active and bound to the operator in the absence of lactose.                                                                                                                                                                                                                            | ON. The repressor is inactive and cannot bind the operator in the absence of tryptophan.                                                                                                              |
| Effector Molecule             | <b>Inducer:</b> Allolactose (isomer of lactose).                                                                                                                                                                                                                                                             | <b>Corepressor:</b> Tryptophan (end-product).                                                                                                                                                         |
| Mechanism of Negative Control | Active Repressor: LacI protein binds operator, blocks transcription. Induction: Allolactose binds LacI, causing a conformational change that releases it from the operator, allowing transcription.                                                                                                          | Inactive Repressor: TrpR protein alone cannot bind operator. Repression: When tryptophan levels are high, Trp binds TrpR, activating it. The TrpR-Trp complex binds operator, blocking transcription. |
| Mechanism of Positive Control | CAP-cAMP Activation: When glucose (preferred carbon source) is low, cAMP levels are high. cAMP binds CAP, and the CAP-cAMP complex binds a site upstream of the promoter, facilitating RNA polymerase binding and increasing transcription dramatically. This is a global response to carbon source quality. | Not a major component. The primary fine-tuning is via attenuation.                                                                                                                                    |

|                          |                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mechanism of Attenuation | Not present in the lac operon.                                                                                                                  | Critical fine-tuning mechanism. Occurs in the leader region (trpL) upstream of the structural genes.<br>1. Low Trp: Ribosome stalls at Trp codons in trpL, allowing formation of an anti-terminator hairpin in the mRNA, transcription continues.<br>2. High Trp: Ribosome rapidly translates trpL, allowing formation of a terminator hairpin (rho-independent), causing premature transcription termination. |
| Biological Logic         | Inducible: Express genes only when the substrate (lactose) is present and glucose is absent—a classic “use it when needed, save energy” system. | Repressible: Shut down biosynthesis when the end-product (tryptophan) is plentiful—a classic “don’t waste resources making what you already have” system.                                                                                                                                                                                                                                                      |
| Overall Regulation       | Dual-layered: Specific induction (by lactose) superimposed on global catabolite repression (by glucose via cAMP).                               | Dual-layered: Coarse repression (by TrpR-Trp) and fine-tuned attenuation (responding to charged tRNA <sup>Trp</sup> levels).                                                                                                                                                                                                                                                                                   |

4. **Conclusion:** Both operons exemplify elegant, economical bacterial gene regulation. The lac operon is an inducible system for catabolism, turned ON by the substrate. The trp operon is a repressible system for anabolism, turned OFF by the end-product. They both use negative control by repressors but with opposite logic (active vs. inactive repressor). Additionally, they incorporate higher-order controls (positive CAP activation for lac, attenuation for trp) that integrate specific signals with broader metabolic status, allowing for precise and adaptive gene expression.

**25. Explain the molecular mechanisms and biotechnological applications of the CRISPR-Cas system. How does it differ from earlier genome editing tools like Zinc Finger Nucleases (ZFNs) and TALENs?**

**Ans. Molecular Mechanism of CRISPR-Cas9 (Class 2, Type II):**

CRISPR-Cas is an adaptive immune system in prokaryotes. Its repurposing for genome editing involves:

1. **Adaptation:** A short piece of invading phage DNA is incorporated as a spacer into the CRISPR locus in the host genome.
2. **Expression:** The CRISPR locus is transcribed and processed to generate CRISPR RNAs (crRNAs).
3. **Interference:** In the engineered system, a single guide RNA (sgRNA) is designed, mimicking the natural crRNA-tracrRNA hybrid. The sgRNA-Cas9 ribonucleoprotein complex scans the genome.

4. **Target Recognition & Cleavage:** The sgRNA base-pairs with a complementary 20-nt DNA target sequence adjacent to a Protospacer Adjacent Motif (PAM, e.g., 5'-NGG-3' for SpCas9). Cas9 undergoes a conformational change and makes a double-strand break (DSB) 3 bp upstream of the PAM.

5. **DNA Repair & Editing: The cell repairs the DSB via:**

- **Non-Homologous End Joining (NHEJ):** Error-prone, often causes small insertions/deletions (indels), leading to gene knockouts.
- **Homology-Directed Repair (HDR):** In the presence of a donor DNA template with homology to the break site, precise edits (insertions, substitutions) can be introduced.

#### **Biotechnological Applications:**

- **Gene Knockouts:** Disrupt gene function via NHEJ-induced frameshifts.
- **Gene Knock-ins:** Insert genes or tags via HDR.
- **Gene Regulation:** Using catalytically dead Cas9 (dCas9) fused to activators/repressors for CRISPRa/CRISPRi.
- **Base Editing:** Using Cas9 nickase fused to deaminase enzymes for direct, precise single-base changes without DSBs.
- **Prime Editing:** A more precise "search-and-replace" system using a Cas9-reverse transcriptase fusion and a pegRNA.
- **Multiplex Editing:** Simultaneous editing of multiple loci using multiple gRNAs.
- **Applications in Plants:** Developing disease-resistant, drought-tolerant, and nutritionally enhanced crops (e.g., non-browning mushrooms, blast-resistant rice).

#### **Comparison with ZFNs and TALENs:**

- **Mechanism:** All three create DSBs at specific sites to harness cellular repair pathways.
- **Design & Assembly:**
  - **ZFNs & TALENs:** Require protein engineering for each new target. ZFNs use zinc finger domains (each recognizing 3 bp), TALENs use TALE repeats (each recognizing 1 bp). Both are modular but tedious and expensive to assemble, with potential off-target effects, especially for ZFNs.
  - **CRISPR-Cas9:** Requires only the synthesis of a short, programmable gRNA (20 nt) to guide the universal Cas9 nuclease to the target. This makes it radically simpler, faster, and cheaper to design and multiplex.
  - **Efficiency & Specificity:** CRISPR is generally more efficient. Off-target effects exist for all, but high-fidelity Cas9 variants and improved gRNA design have enhanced CRISPR specificity.
  - **Flexibility:** CRISPR's ease of retargeting with a new gRNA offers unparalleled flexibility for high-throughput screening and multiplexing.

**Conclusion:** CRISPR-Cas9 represents a paradigm shift in genome editing. Its simplicity, precision, versatility, and low cost have democratized genetic engineering, surpassing ZFNs and TALENs as the tool of choice. It has revolutionized basic research, synthetic biology, and holds immense promise for therapeutics, agriculture, and biotechnology, though ethical and regulatory considerations remain paramount.