# Prokaryotic genome

-Dr Ekta Khare

https://biosiva.50webs.org/rep1.htm

https://www.sciencefacts.net/operon.html

https://biologyinsights.com/operon-structure-and-function-in-prokaryotic-gene-regulation/

# Genome

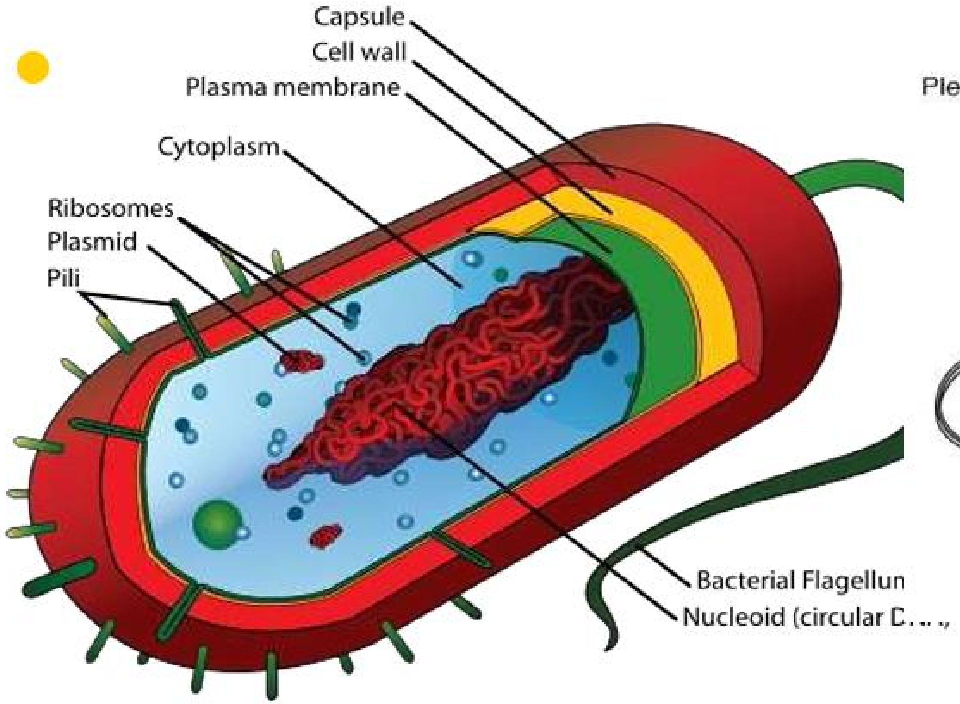
- A genome is the complete set of genetic material in an organism, including all of its DNA. The genome carries the hereditary information of an organism and is passed from one generation to the next.

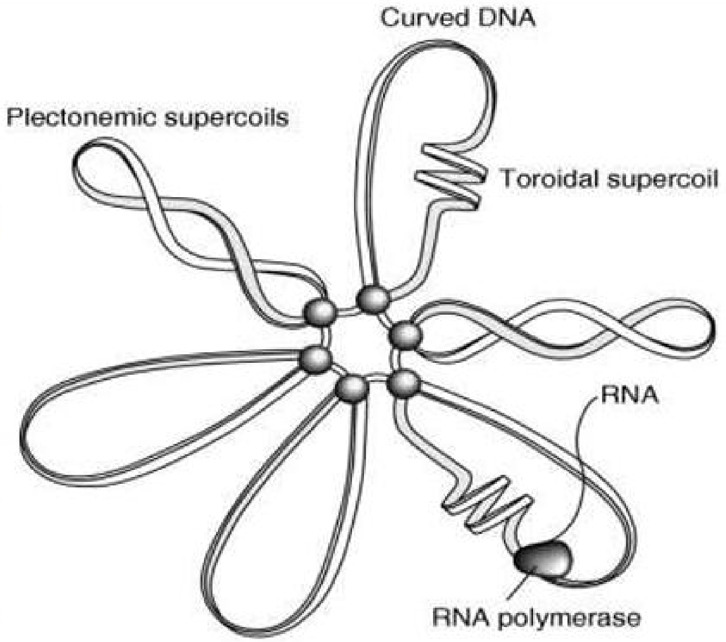
- This includes:
- Genes: Segments of DNA that encode instructions for synthesizing proteins or functional RNA.
- Gene : A gene is the basic physical and functional unit of heredity. Genes are made up of DNA.
- Non-coding DNA: Regulatory sequences, introns, and other DNA segments with non-coding functions.
- In some viruses, the genome is made of RNA instead of DNA.

# NUCLEOID

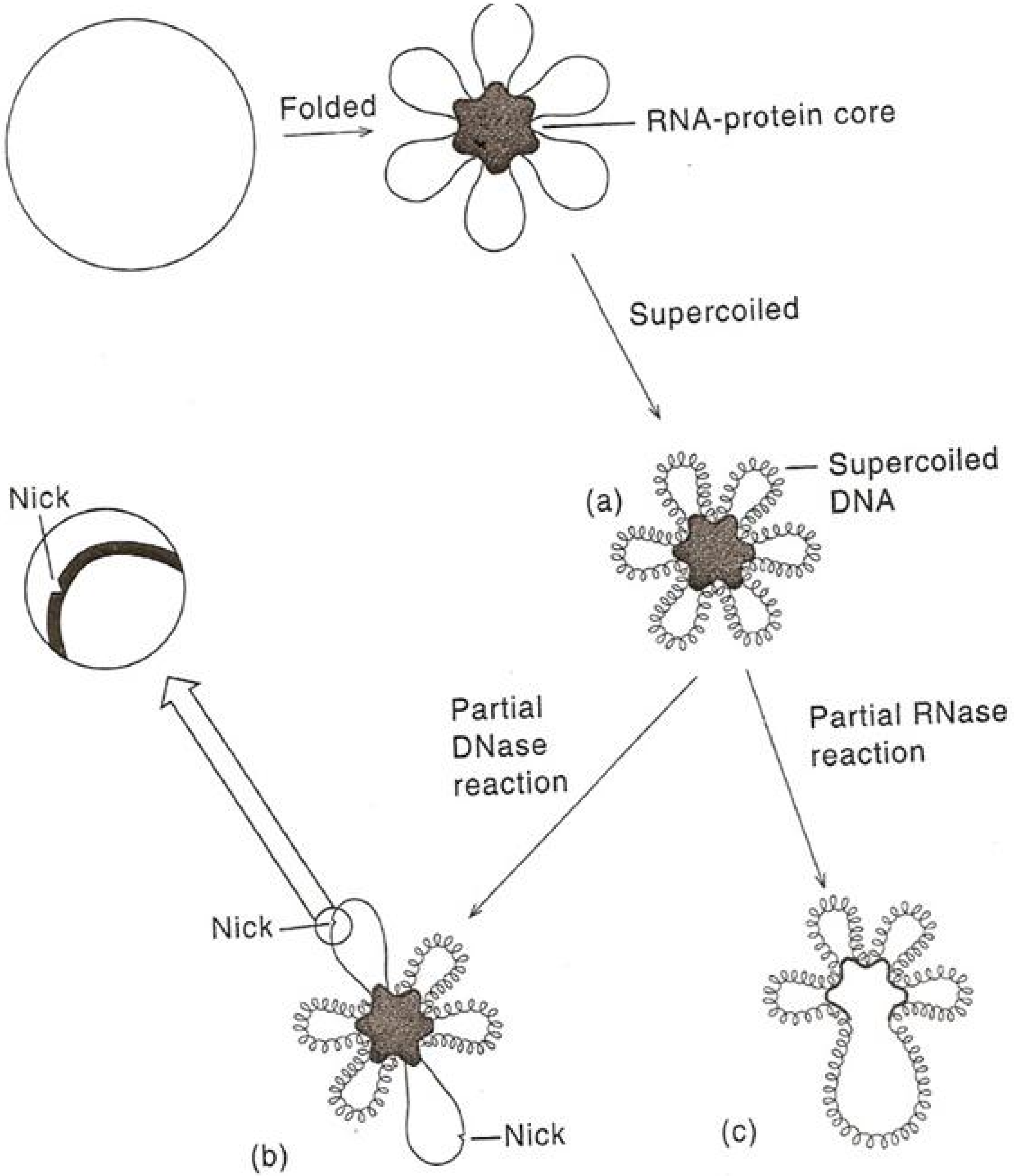
- Single-celled organisms that lack a membrane-bound nucleus. Commonly referred to as NUCLEOID.
- Genome: Circular DNA located in a nucleoid region.
- Plasmids: Small, circular, extrachromosomal DNA often carrying beneficial genes (e.g., antibiotic resistance).
- Rapid reproduction and gene transfer (horizontal gene transfer through conjugation, transformation, or transduction).
- Nucleoid is composed of 60% DNA and small amounts of RNA and protein.
- Nucleoid proteins help to maintain the supercoiled structure of DNA.
- DNA supercoiling refers to over or under winding of a DNA strand.
- It is important for DNA packing within all cells.
- In a “relaxed” double-helical segment of B-DNA, the two strands twist around the helical axis once every 10.4 to 10.5 base pairs of sequence.
- Adding or subtracting twists, as some enzymes can do, imposes strain. The circular DNA would contort into a new shape, such as a simple figure-eight. Such a contortion is a supercoil.
- Extra helical twists are positive and lead to positive supercoiling, while subtractive twisting causes negative supercoiling. Many topoisomerase enzymes sense supercoiling and either generate or dissipate it as they change DNA topology.

- Supercoiled DNA forms two structures; a plectoneme or a toroid, or a combination of both.
- A negatively supercoiled DNA molecule will produce either a left-handed helix, the toroid, or a right-handed helix with terminal loops, the plectoneme.
- Plectonemes are typically more common in nature, and this is the shape most bacterial plasmids will take.



- The nucleoid is a folded structure, containing many supercoiled loops having many independent domains.
- There are about 100 domains per genome and each domain consists of about 40kb of DNA.
- The prokaryotic genome is a single replicon.
- A single chromosome of *E.coli* contains about $3x10^9$ Daltons or about $4.5 \times 10^6$bp of DNA.
- If all of this DNA were in a duplex structure stretched end to end, it would be 1.5mm long, which is about 75 cell diameter. But inside the cell, the chromosome is coiled to the size of 2 micrometer.
- Electron micrographs of *E.col*i chromosome suggest a folded circular structure containing 40-100 super coiled loops.
- This Structure is formed by the following steps i.e. initially circular DNA binds with the RNA-Protein core and forms loops.
- Secondly, the loops form super coiled structure.
- The interaction between the loops and RNA - Protein core is not understood. D.E. Pettijohn and his co-workers have provided evidence of such a core.
- They first showed that the individual supercoiled loops maintained their supercoiling independently of one another.
- Thus, if a single nick is introduced into one of the loops by limited DNase action. That loops adopts an expanded relaxed conformation, but supercoiling in the other loops is maintained.
- Limited RNase or protease treatment causes the partial breakdown of the looped structures without interfering with the supercoiling.
- These results have lead to the conclusion that each of the loops is a domain, the lateral motion of which is restricted by an RNA-Protein core complex.


Folded
RNA-protein core
Supercoiled
(a)
Supercoiled DNA
Nick
Partial DNase reaction
Partial RNase reaction
Nick
Nick
(b)
(c)

**Plasmids:**

- In addition to the main chromosome, prokaryotes often carry plasmids:
- Small, circular, extrachromosomal DNA molecules.
- Plasmids often contain genes that provide selective advantages, such as antibiotic resistance or the ability to metabolize unusual compounds.
- Plasmids are transferable between cells via horizontal gene transfer, enhancing adaptability.

**Gene Density:**

- Prokaryotic genomes are highly efficient, with around 85–90% of the genome coding for proteins or functional RNA.
- Minimal intergenic regions (non-coding DNA between genes) and regulatory sequences.

**Presence of Operons:**

- Many genes in prokaryotes are organized into operons:
  - Definition: A cluster of functionally related genes transcribed together as a single mRNA molecule.
  - Operons enable coordinated expression of genes required for a specific function.
  - Example: The lac operon in E. coli controls genes for lactose metabolism.
- Operons reduce redundancy and streamline gene regulation.

- **Lack of Introns:**
- Unlike eukaryotes, prokaryotic genes generally lack introns.
- This means transcription and translation can occur simultaneously, a process known as coupled transcription-translation.
- **Regulatory Elements:**
- Regulation of gene expression in prokaryotes is simpler than in eukaryotes.
- Key regulatory elements include:
  - Promoters: Specific DNA sequences where RNA polymerase binds to initiate transcription.
  - Operators: DNA segments where regulatory proteins (e.g., repressors) bind to control transcription of operons.

# Plasmids

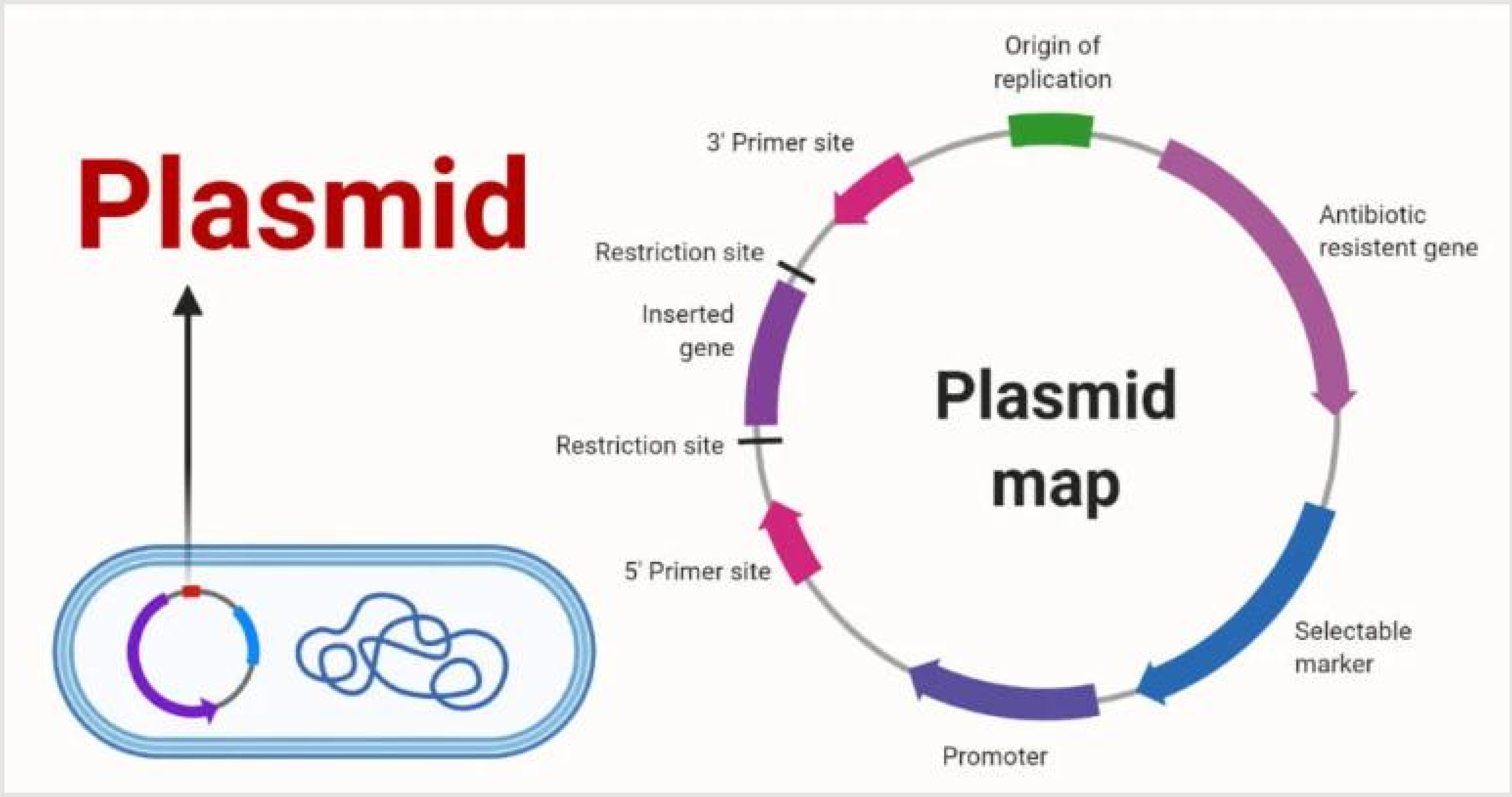
- Plasmids are small circular DNA fragments, double-stranded, self-replicating extra chromosomal structures found in many microorganisms.
- The term Plasmid was coined by Joshua Lederberg in 1952.
- Plasmids are important as genetic tools, which are used to introduce, manipulate or delete certain genes from the host cell.

# Properties of Plasmids

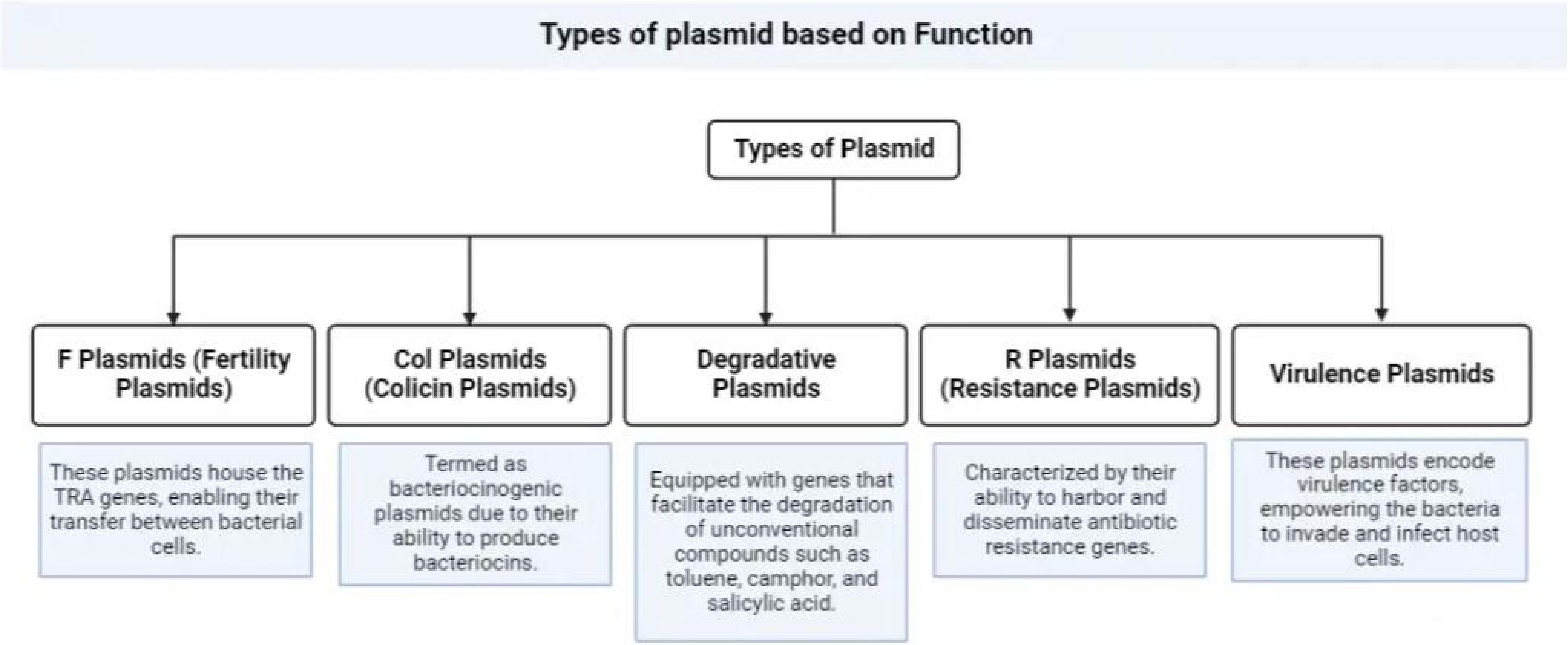
- They are extra chromosomal DNA fragments present in the cell.
- They are double stranded structures. Exceptions are the linear plasmids in bacteria Streptomyces spp and Borrelia spp.
- They can replicate independently.
- The absence of a plasmid in the cell does not affect cell functioning, but the presence of a plasmid in the cell is usually beneficial.
- Plasmids are also known as sex factors, conjugants, extra chromosomal replicons, or transfer factors.
- Copy number – the copy number refers to the number of copies of plasmid present in the bacterial cell. Usually, small plasmids are present in high numbers and large plasmids are present in few numbers.
- Compatibility of plasmids – this refers to the ability of two different plasmids to coexist in the same bacterial cell.

# Structure of Plasmids

- The size of the plasmid varies from 2 kb to 200 kb.
- 3. It is the extrachromosomal element of the cell which is not required for the growth and development of the cell.
- 4. Most of the plasmids contain the TRA gene, which is the transferred gene and is essential in transferring the plasmid from one cell to another.
- Every plasmid has certain essential elements. These are as follows –
- Origin of replication (OR) – This refers to a specific location in the strand where the replication process begins. In plasmids, this region is A=T rich region as it is easier to separate the strands during replication.
- Selectable marker site – This region consists of Antibiotic resistance genes which are useful in the identification and selection of bacteria that contain plasmids.
- Promoter region – this is the region where the transcriptional machinery is loaded.
- Primer binding site – this is the short sequence of single-strand DNA which is useful in DNA amplification and DNA sequencing.
- Multiple cloning sites – This site contains various sequences where the restriction enzymes can bind and cleave the double stranded structure.


Plasmid
Origin of replication
3' Primer site
Antibiotic resistent gene
Restriction site
Inserted gene
Plasmid map
Restriction site
5' Primer site
Selectable marker
Promoter

# Types of Plasmids

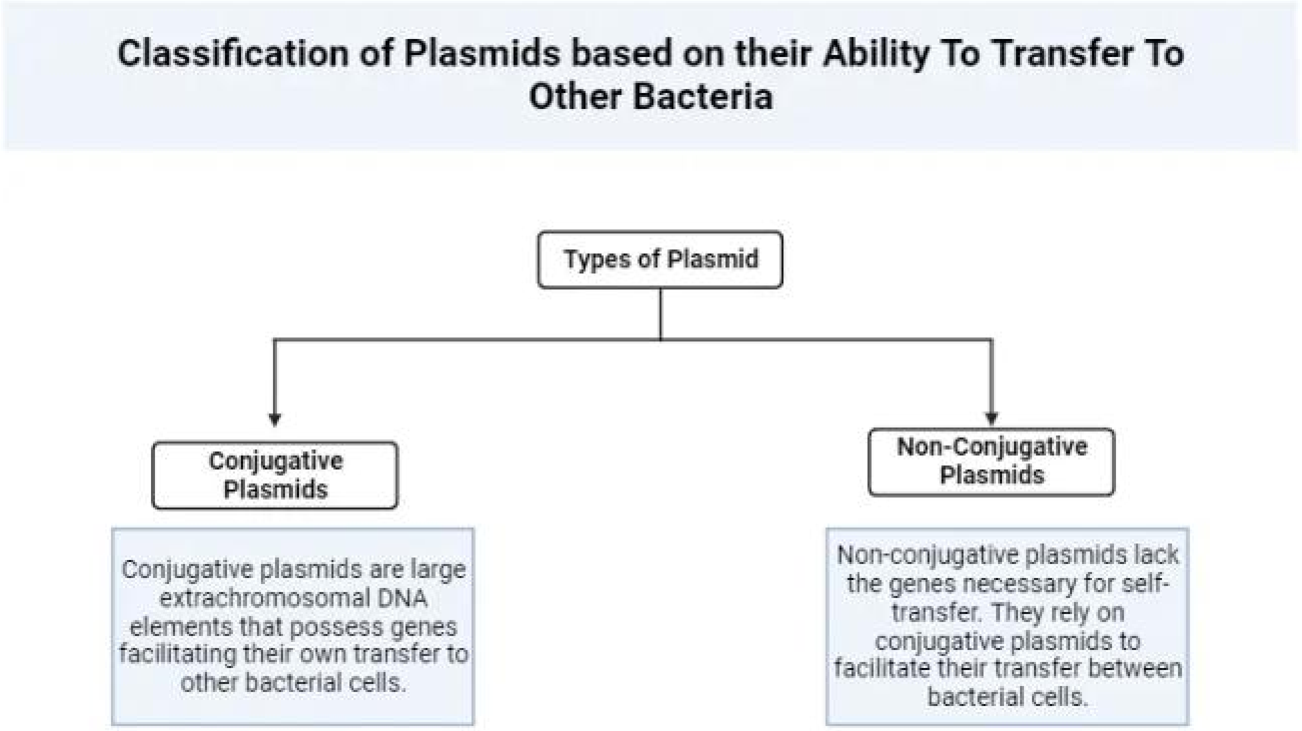


# Types of Plasmids

- Based on their functional attributes, plasmids can be systematically classified into the following categories:
- **F Plasmids (Fertility Plasmids):**
- These plasmids house the TRA genes, enabling their transfer between bacterial cells.
- Capable of autonomous replication within the bacterial cell.
- They orchestrate the synthesis of a pilus, a proteinaceous structure facilitating cell-to-cell interactions.
- Additionally, they possess sequences that determine incompatibility, preventing coexistence with similar plasmids.
- **R Plasmids (Resistance Plasmids):**
- Characterized by their ability to harbor and disseminate antibiotic resistance genes.
- These genes confer protection against both medically relevant antibiotics and naturally occurring antibiotics in the environment.
- Typically, R plasmids are larger and exist in fewer copies within the bacterial cell.
- **Col Plasmids (Colicin Plasmids):**
- Termed as bacteriocinogenic plasmids due to their ability to produce bacteriocins.
- These bacteriocins can target and eliminate closely related bacterial strains that lack the Col plasmid.
- E. coli is a notable bacterium where Col plasmids have been identified.

# …Types of Plasmids

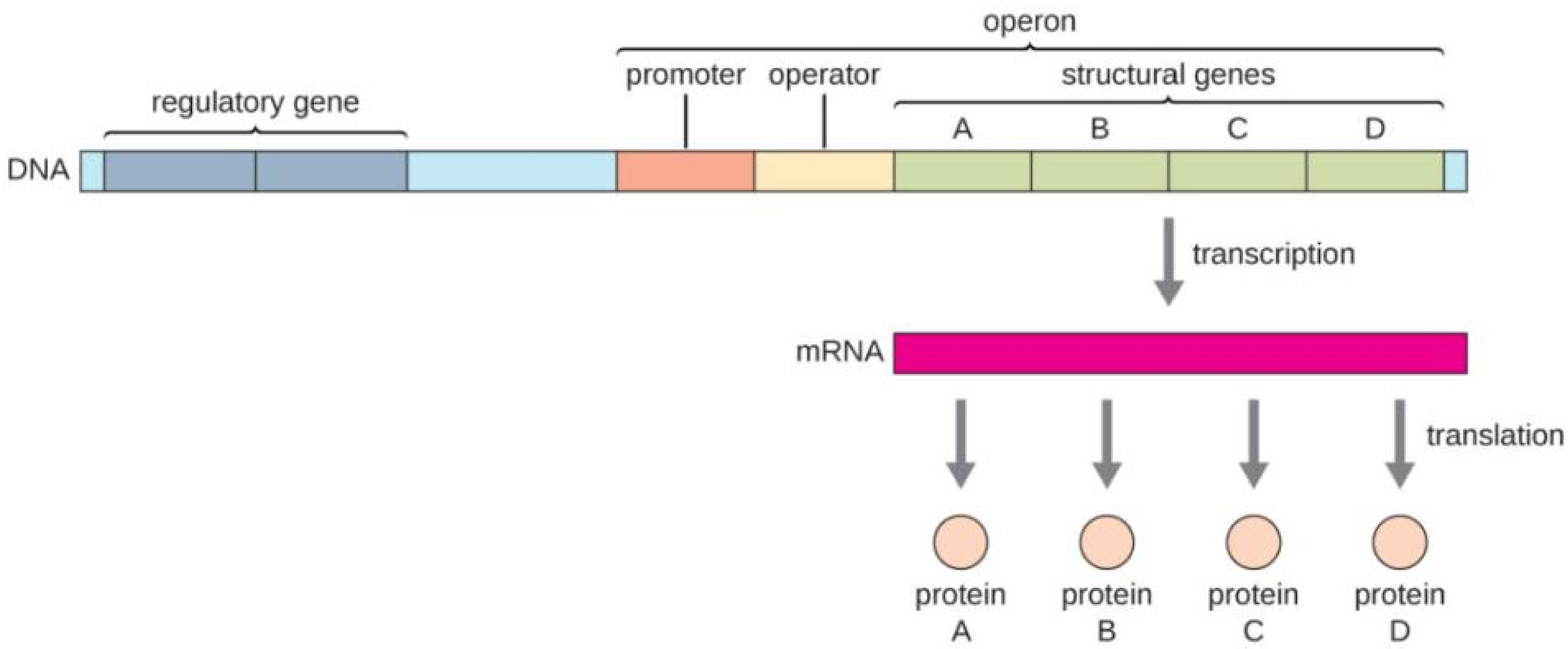
- **Degradative Plasmids:**
- Equipped with genes that facilitate the degradation of unconventional compounds such as toluene, camphor, and salicylic acid.
- Bacteria harboring these plasmids can metabolize a diverse range of chemicals, aiding in environmental detoxification and resource utilization.
- **Virulence Plasmids:**
- These plasmids encode virulence factors, empowering the bacteria to invade and infect host cells.
- Bacterial strains with virulence plasmids can target plant, animal, and human cells, leading to various diseases.
- A quintessential example is the Ti plasmid found in Agrobacterium tumefaciens, which induces crown gall disease in plants.
- **Episome**
- Episomes are an abacus-like plasmid or viral DNA that has the ability to incorporate itself in the DNA chromosomal an organism that hosts it .
- This is why it is able to remain in place for a lengthy period and be replicated in every cell division in its host. It can also eventually become an essential part of its genetic composition.




- In situations where a bacterium possesses both conjugative and non-conjugative plasmids, the non-conjugative plasmids can be “mobilized” for transfer.
- This means that while they cannot initiate the transfer process on their own, they can be co-transferred alongside conjugative plasmids during conjugation.

# Operons

- Prokaryotic organisms, such as bacteria, have evolved efficient mechanisms to regulate gene expression in response to environmental changes.
- One of the key systems they employ is the operon model, which allows for coordinated regulation of genes involved in similar pathways or functions.
- This system optimizes resource utilization and ensures rapid adaptation to fluctuating conditions.
- An operon is the functional unit of genetic regulation found in prokaryotic cells, such as bacteria. It consists of a cluster of genes that work together as a single unit to give a single messenger RNA (mRNA) molecule, which then encodes multiple proteins.
- The two most widely studied operons are the lactose (lac) operon and tryptophan (trp) operon in *Escherichia coli*.
- The promoter then has simultaneous control over the regulation of the transcription of these structural genes because they will either all be needed at the same time, or none will be needed.
- French scientists François Jacob (1920–2013) and Jacques Monod at the Pasteur Institute were the first to show the organization of bacterial genes into operons, through their studies on the lac operon of *E. coli*.
- They found that in E. coli, all of the structural genes that encode enzymes needed to use lactose as an energy source lie next to each other in the lactose (or lac) operon under the control of a single promoter, the lac promoter.
- For this work, they won the Nobel Prize in Physiology or Medicine in 1965.


operon
promoter
operator
structural genes
regulatory gene
A
B
C
D
DNA
transcription
mRNA
translation
protein
A
protein
B
protein
C
protein
D

# In prokaryotes, operons have several key components that work together to control gene expression efficiently. These components include:

- **Regulatory region**
- Each operon includes DNA sequences that influence its own transcription; these are located in a region called the regulatory region.
- The regulatory region includes the promoter and the region surrounding the promoter, to which transcription factors, proteins encoded by regulatory genes, can bind.
- **Promoter (P)**
- The promoter is a crucial region of DNA located upstream of the structural genes within the operon. It serves as the initiation site for transcription, providing a binding site for RNA polymerase.
- Transcription factors influence the binding of RNA polymerase to the promoter and allow its progression to transcribe structural genes.
- Promoters contain specific sequences, such as the -10 and -35 regions in bacteria, recognized by the sigma factor, a subunit of RNA polymerase.
- The strength of a promoter, which influences the frequency of transcription initiation, depends on how closely its sequences match the consensus sequences recognized by the sigma factor.

# Operator

- The operator is another critical DNA sequence within the operon, positioned between the promoter and the structural genes.
- The operator acts as a regulatory switch that can turn the expression of the operon on or off.
- Regulatory proteins, specifically repressors, bind to the operator to prevent RNA polymerase from proceeding with transcription.
- The presence or absence of specific molecules, such as inducers or corepressors, can influence the binding of these regulatory proteins to the operator.
- This interaction is a mechanism by which cells respond to environmental signals, allowing genes to be expressed only when needed.
- In some operons, the operator may overlap with the promoter region, allowing the binding of a repressor to directly obstruct RNA polymerase binding.

**Structural Genes**

- Structural genes within an operon encode proteins that typically participate in a common metabolic pathway or function.
- These genes are transcribed as a single mRNA strand, known as polycistronic mRNA, which is then translated into separate proteins.
- The arrangement of structural genes in an operon allows for coordinated expression, ensuring that all necessary components for a specific cellular function are produced simultaneously.

**Terminator**

- The terminator is a sequence downstream of the structural genes that signals the end of transcription.
- When RNA polymerase encounters this sequence, it dissociates from the DNA, releasing the newly synthesized mRNA.
- Terminators can be classified into two types: intrinsic and rho-dependent.
- Intrinsic terminators rely on a specific RNA sequence that forms a hairpin loop, causing the RNA polymerase to pause and dissociate.
- Rho-dependent terminators, on the other hand, require a protein called Rho, which binds to the mRNA and facilitates the dissociation of RNA polymerase from the DNA

# Regulatory Genes

- In addition to the components directly involved in transcription and translation, operons also involve regulatory genes located elsewhere in the bacterial genome.
- These regulatory genes code for regulatory proteins that influence the activity of the operon.
- There are two primary types of regulatory proteins: repressor and activator proteins.
- **a. Repressor Proteins** are involved in negative regulation, inhibiting gene expression.
- When a repressor protein binds to the operator, it physically blocks RNA polymerase from accessing the promoter, preventing transcription of the structural genes.
- Inducible operons and repressible operons both use repressor proteins to control gene expression.
- **b. Activator Proteins** are involved in positive regulation, enhancing gene expression.
- When an activator protein binds to a specific DNA sequence (enhancer), it facilitates the binding of RNA polymerase to the promoter, leading to increased transcription of the structural genes.

# Effector Molecules

- It includes inducers and corepressors.
- **Inducers** are small molecules interacting with repressor proteins, causing a conformational change that renders the repressor inactive.
- It allows RNA polymerase to bind to the promoter, initiating transcription.
- Inducible operons rely on the presence of inducers to activate gene expression.

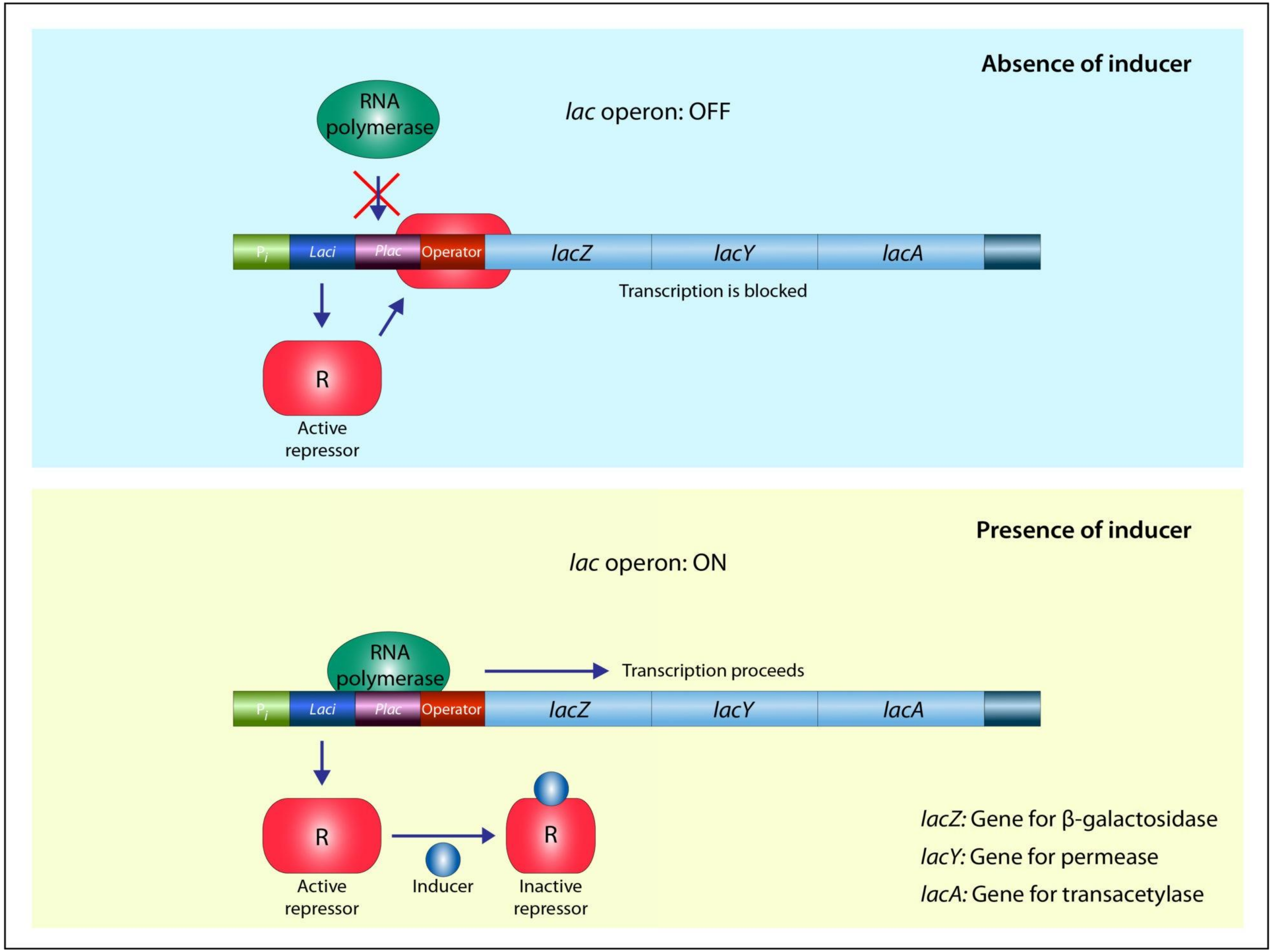
- **Corepressors** are small molecules interacting with repressor proteins, promoting their binding to the operator.
- It blocks RNA polymerase from initiating transcription, effectively inhibiting gene expression.
- Repressible operons require the presence of corepressors to turn off gene expression when the pathway’s end product is in excess.

# Types of Operons

- Operons can be classified based on their regulatory mechanisms, primarily into:
  - inducible operons
  - repressible operons
- These classifications reflect how operons respond to environmental cues, allowing prokaryotic cells to manage gene expression in response to changing conditions.

# Inducible Operons

- Inducible operons are typically inactive and require the presence of a specific inducer molecule to initiate transcription.
- A classic example of an inducible operon is the lac operon in *E. coli*, which is responsible for the metabolism of lactose.
- In the absence of lactose, a repressor protein binds to the operator, preventing transcription.
- When lactose is present, it is converted into allolactose, which acts as an inducer by binding to the repressor.
- This binding alters the repressor’s conformation, reducing its affinity for the operator and allowing RNA polymerase to access the promoter and initiate transcription.
- Inducible operons are advantageous in environments where the substrate is not always available, as they ensure that the metabolic machinery is only produced when needed, conserving cellular resources.


Absence of inducer
lac operon: OFF
RNA polymerase
Pi
Laci
Plac
Operator
lacZ
lacY
lacA
Transcription is blocked
R
Active repressor
Presence of inducer
lac operon: ON
RNA polymerase
Transcription proceeds
Pi
Laci
Plac
Operator
lacZ
lacY
lacA
R
Active repressor
Inducer
R
Inactive repressor
lacZ: Gene for β-galactosidase
lacY: Gene for permease
lacA: Gene for transacetylase

# Repressible Operons

- Repressible operons, in contrast, are generally active and are turned off in the presence of a specific corepressor molecule.
- The trp operon, which regulates tryptophan biosynthesis in E. coli, serves as a well-studied example.
- When tryptophan levels are low, the operon is active, allowing for the synthesis of enzymes required for tryptophan production.
- As tryptophan accumulates, it acts as a corepressor by binding to the repressor protein.
- This complex then binds to the operator, blocking transcription.
- Repressible operons are beneficial in maintaining homeostasis, as they prevent the overproduction of end products when they are already abundant.
- This feedback inhibition mechanism ensures that energy and resources are not wasted on synthesizing unnecessary proteins, allowing the cell to adapt efficiently to its nutritional environment.

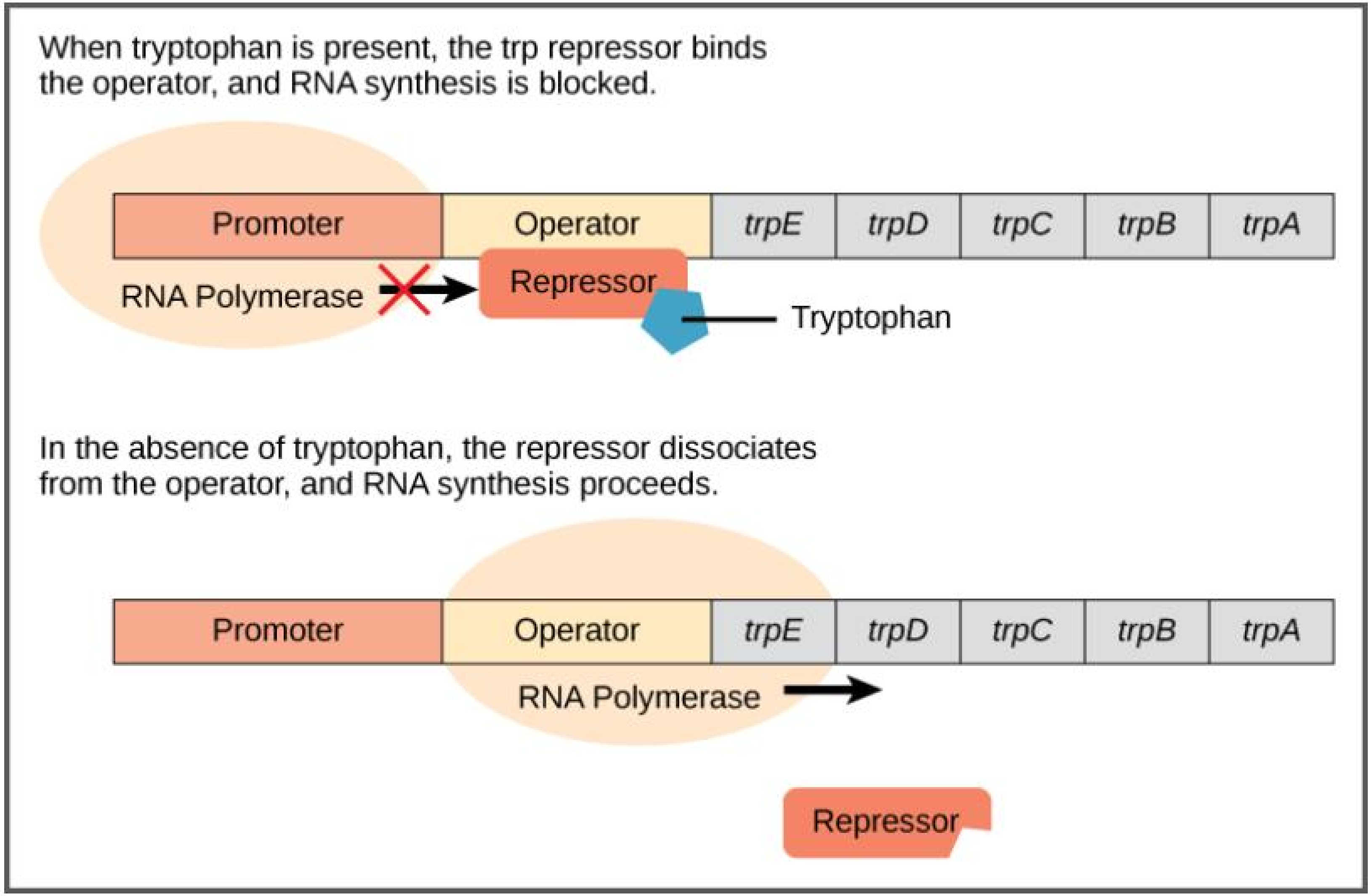



Figure 1. The five genes that are needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is plentiful, two tryptophan molecules bind the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan genes. When tryptophan is absent, the repressor protein does not bind to the operator and the genes are transcribed.