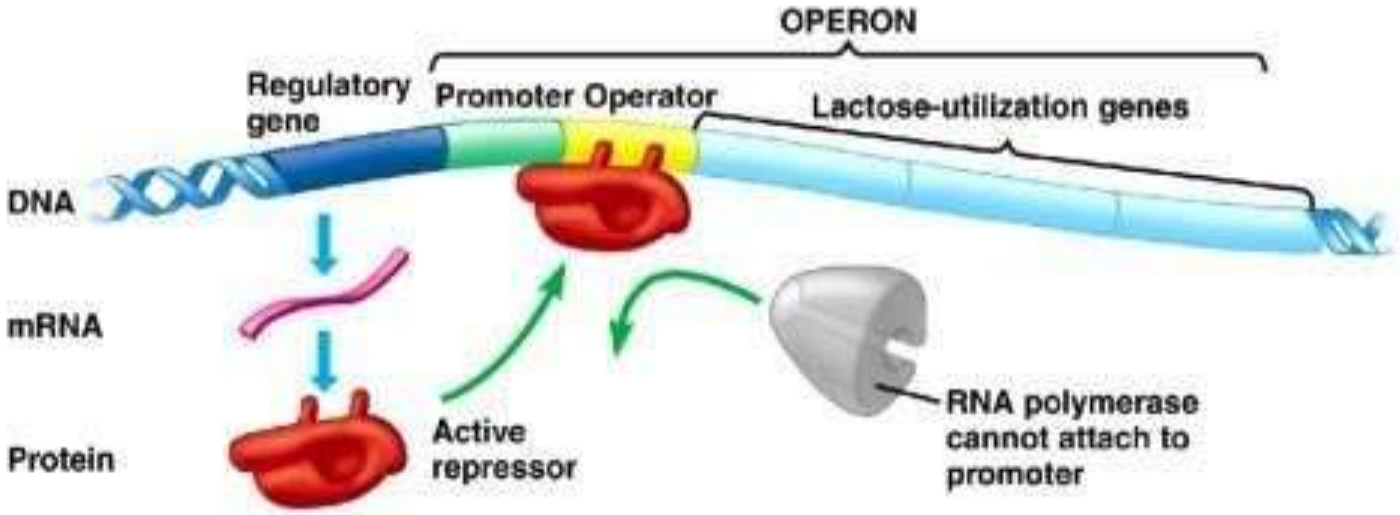
THE LAC OPERON



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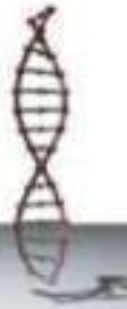
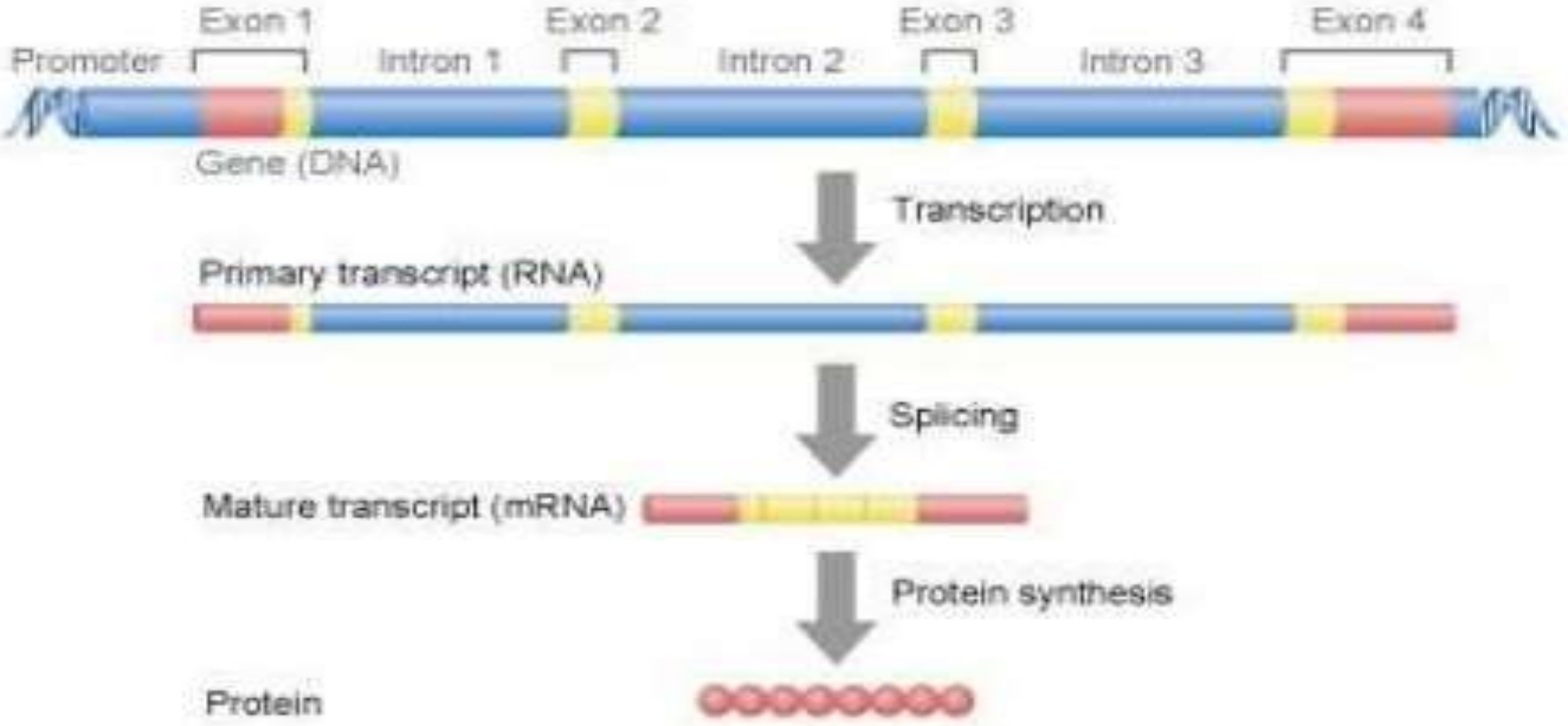
GENE EXPRESSION



- Gene expression is the process by which the information encoded in a gene is used to direct the assembly of a protein molecule.
- Gene expression is explored through a study of protein structure and function, transcription and translation, differentiation and stem cells.
- It is the process by which information from a gene is used in the synthesis of a functional gene product.
- These products are often proteins, but in non-protein coding genes such as ribosomal RNA (rRNA), transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA.
- The process of gene expression is used by all known life - eukaryotes (including multicellular organisms), prokaryotes (bacteria and archaea)

Gene Expression



TWO TYPES OF GENE EXPRESSION

1. When the expression of a gene is increased due to the presence of specific regulatory element – POSITIVE REGULATION.

2. When the expression of genetic information is decreased due to the presence of specific regulatory element – negative regulator is called NEGATIVE REGULATION.

Levels of regulation in bacterial gene expression

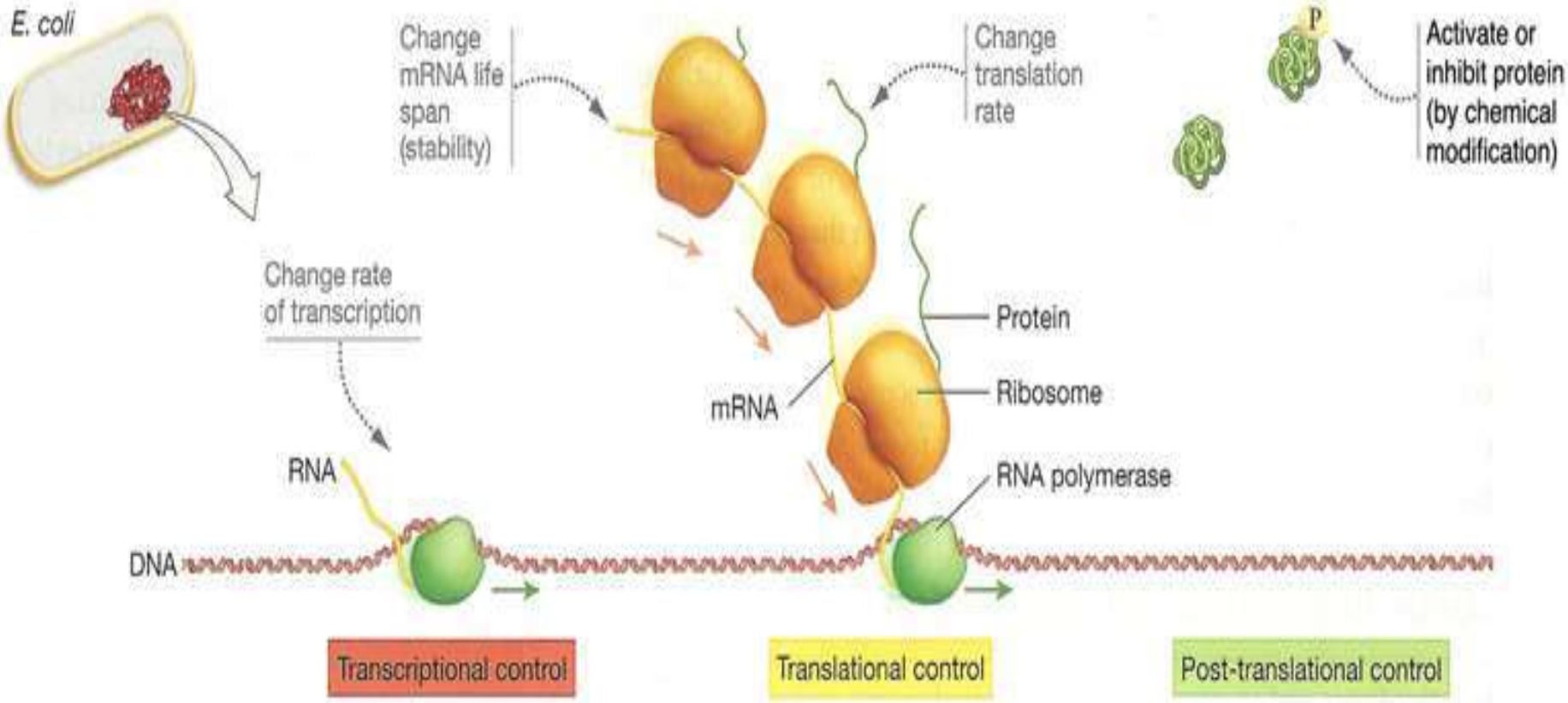


FIGURE 17.1 Gene Expression in Bacteria Can Be Regulated at Three Levels.

TRANSCRIPTION REGULATION

Depending on how RNAP interact with promoter 3 types of proteins involve: –

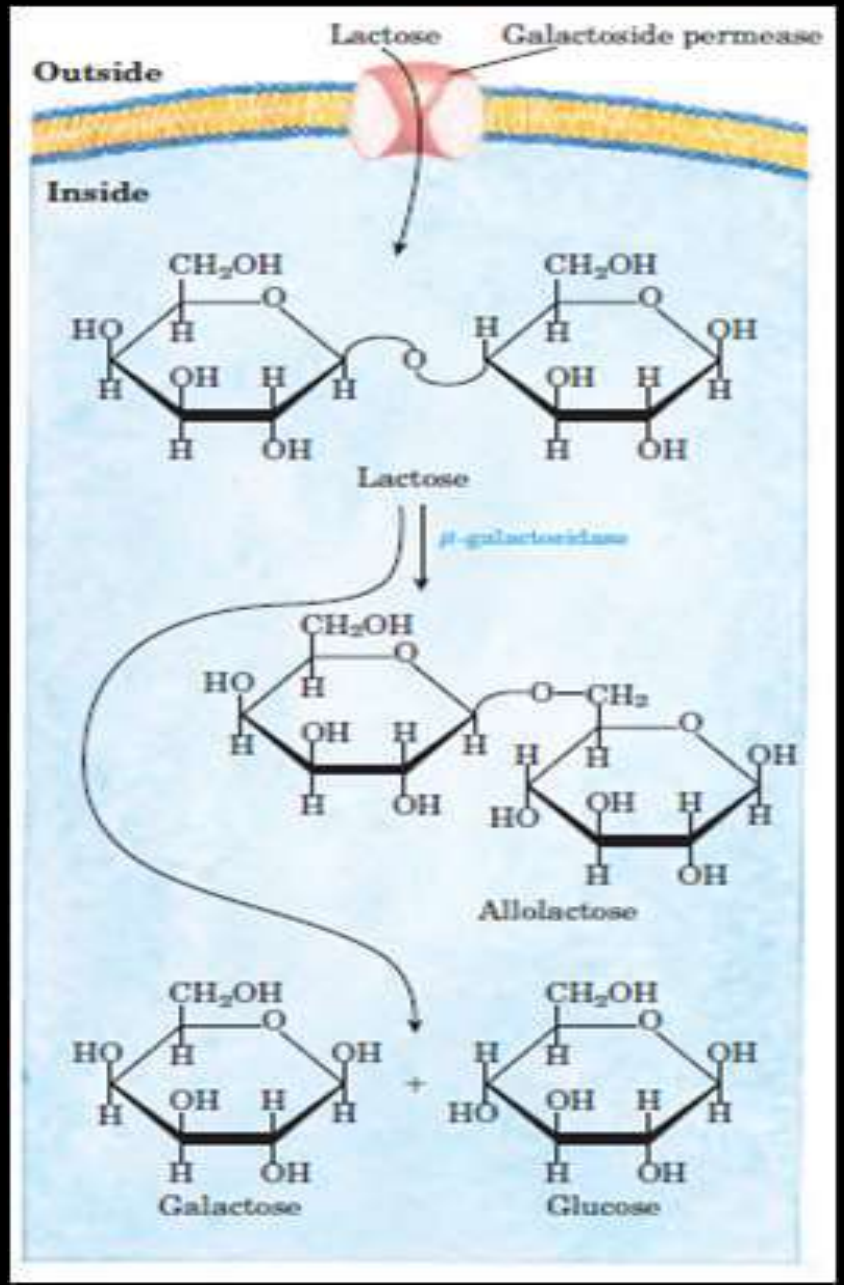
- SPECIFICITY FACTORS – alter the specificity of RNAP for a give promoters.

- REPRESSOR – bind to operators near the promoter- prevent access of RNAP to the promoter or movement along DNA after binding (negative regulation)

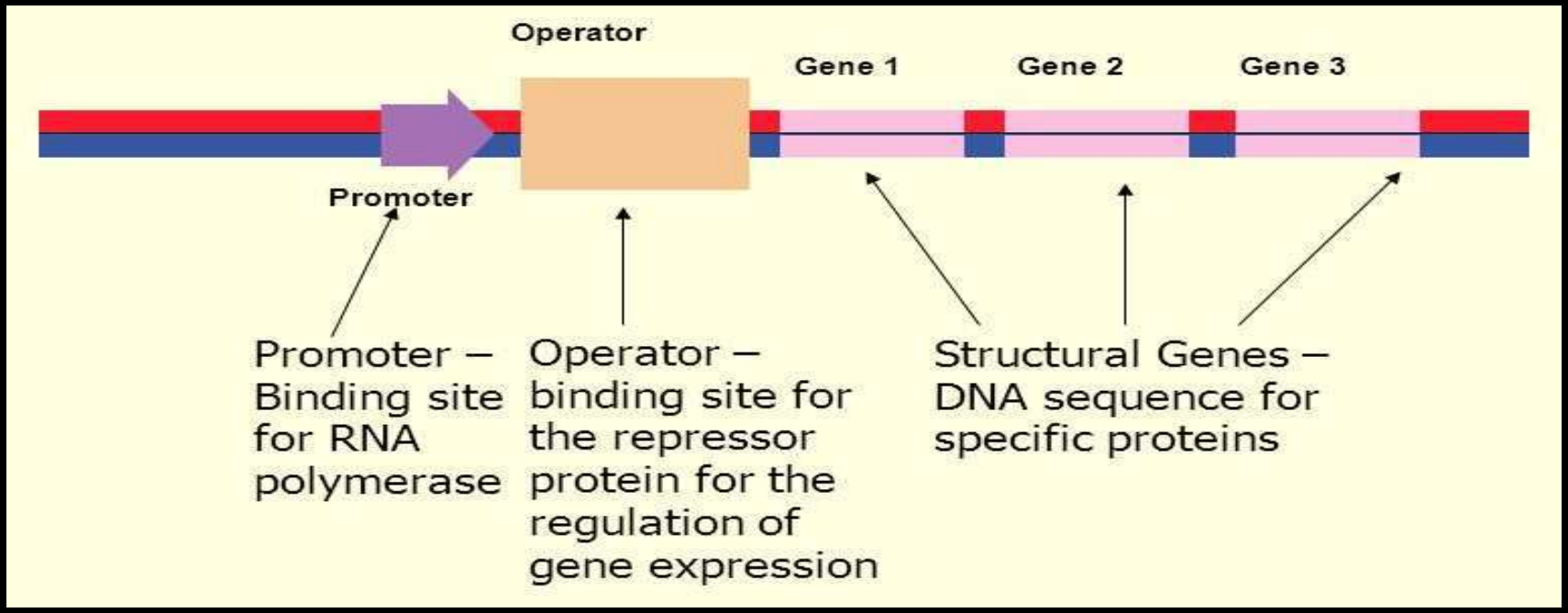
- ACTIVATOR – bind to enhancer DNA sites (near/distant from the promoter) & enhance the RNAP-promoter interaction – little transcription occurs in the absence of the activator.

Lactose metabolism:

- The first well defined studies – lactose metabolism in *E.coli* Lactose metabolism involve formation of:
  - β-GALACTOSIDASE (cleaves lactose to galactose and glucose)
  - GALACTOSIDE PERMEASE (transports lactose into the cell)
  - THIOGALACTOSIDE TRANSACETYLASE (modify toxic galactosides to facilitate removal from cell)
- When *E.coli* is presented with lactose or lactose analogs – the expression of β-galactosidase, galactoside permease and thiogalactoside transacetylase increased 100 fold – 1000 fold .



OPERON CONCEPT :



- An Operon is defined as several distinct genes situated in tandem, all controlled by a common regulatory region.
- Commonly consists of repressor, operator & structural genes.
- The message produced by an operon is polycistronic.
- Operons may be inducible or repressible.

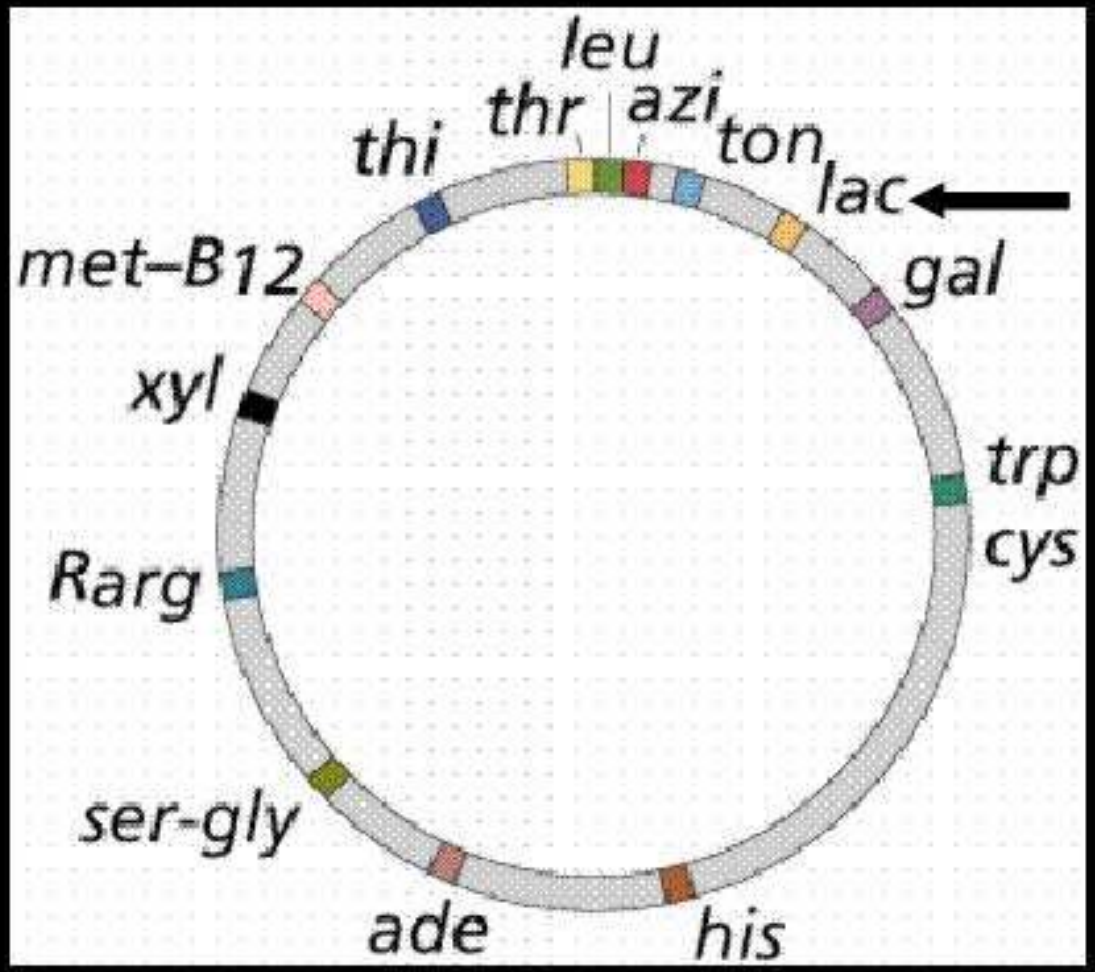
HISTORICAL

- Jacob and Monod in 1961 described their Operon model in a classic paper.
- Their hypothesis was to a large extent based on observations on the regulation of lactose metabolism by the intestinal bacterium E coli.
- Awarded the Nobel Prize in Physiology and Medicine 1965.

Francois Jacob

Jacques Monod

Escherichia coli



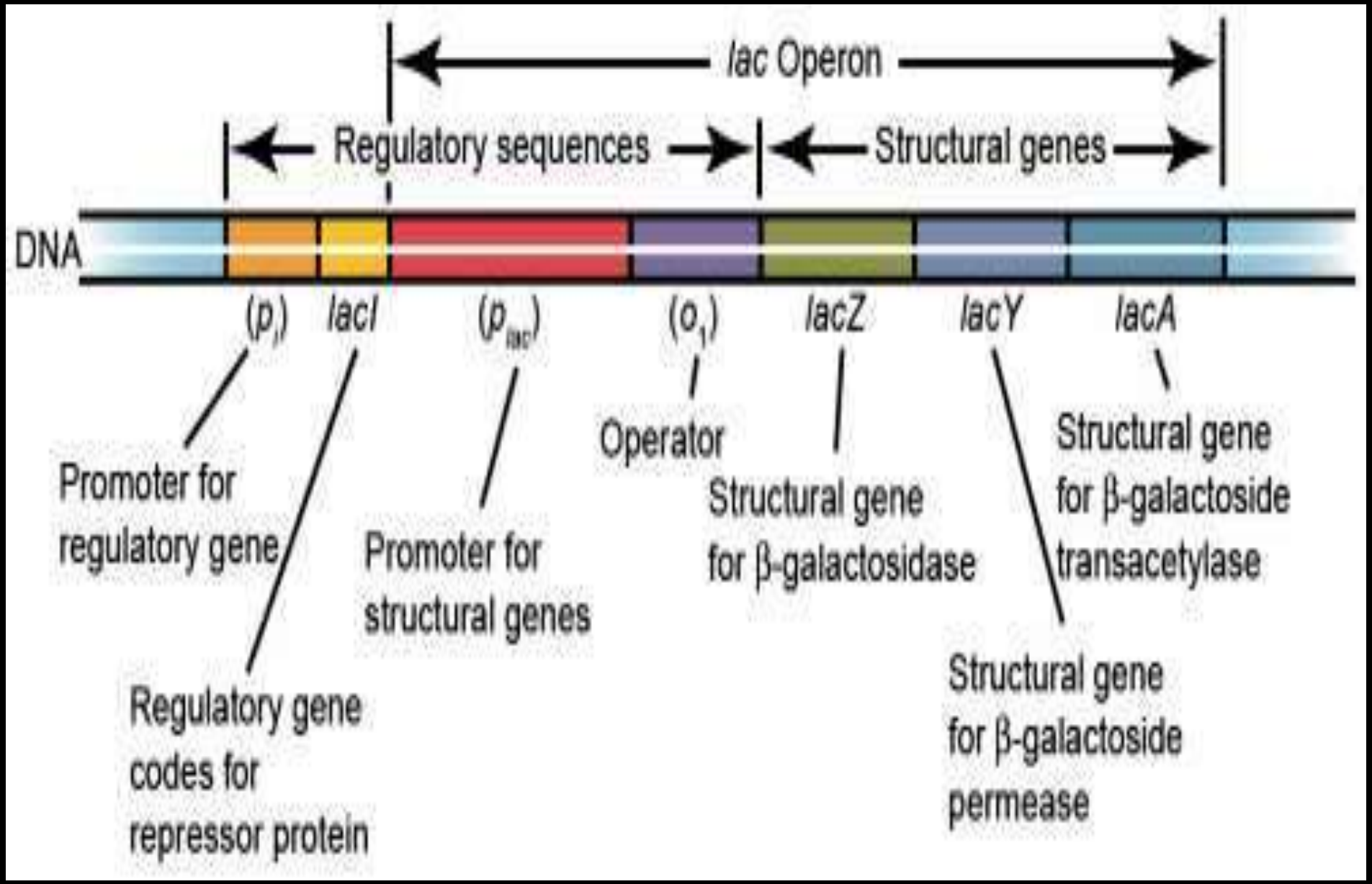
This is the mapped genome of the E. coli bacteria.

Note the lac operon.

Lac Operon – Basic concept

- Bacteria such as *E. coli* usually rely on glucose as their source of carbon and energy.
- However, when glucose is scarce, *E. coli* can use lactose as their carbon source even though this disaccharide does not lie on any major metabolic pathways.
- An essential enzyme in the metabolism of lactose is β-*galactosidase*, which hydrolyzes lactose into galactose and glucose

STRUCTURE OF LAC OPERON:



STRUCTURAL GENES

- The structural genes form long polycistronic mRNA molecule.
- The lactose operon contains following genes that are associated with lactose utilization:
- **lacZ gene :**
  - » encodes for the enzyme **β-galactosidases.**
  - » This enzyme cleaves the β,1 → 4 linkages of lactose & releases free monosaccharides.
  - » It is a tetramer of four identical subunits each with molecular weight of~500 KD.
- **lacY gene:**
  - » Codes for the enzyme **permeases.**
  - » it is a 30 KD membrane-bound transport protein.
  - » Helps in transportation of the lactose molecule from the medium which is then to be acted upon by the lac Z gene Product.
- **lacA gene:**
  - » Encodes for **transacetylase** enzyme.

OPERATOR GENE:

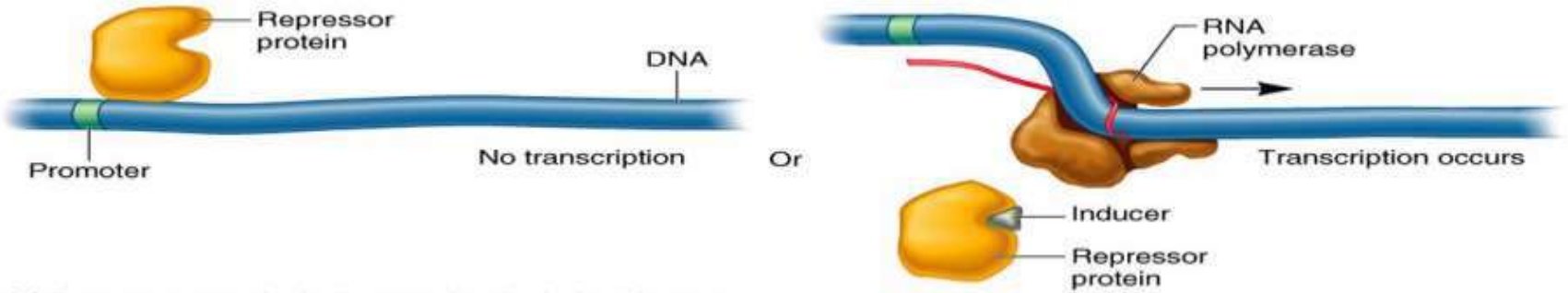
- About 28bp long ; adjacent to lacZ gene.
- Base pairs are palindromes.
- discovered by GILBERT & MAXAM (1973).
- overlaps the promoter region.
- repressor protein binds to the operator & forms an operator-repressor complex.

PROMOTER GENE:

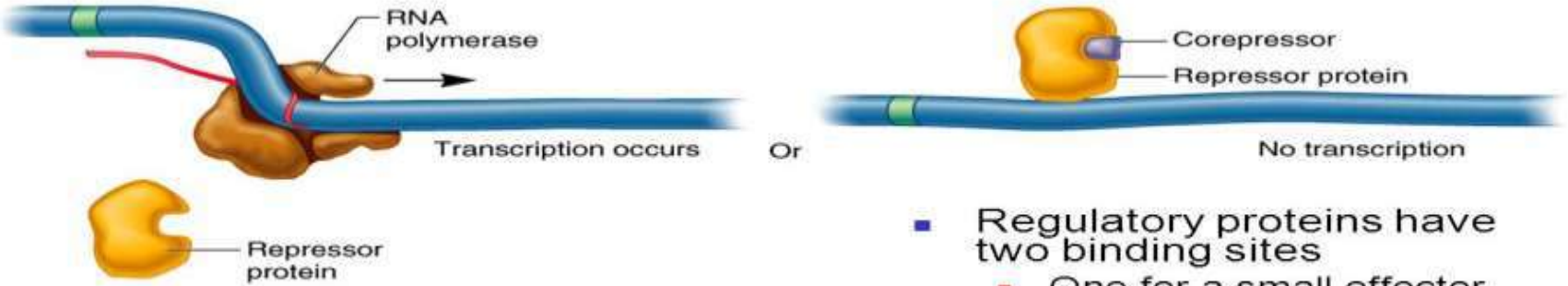
- About 100bp long & continuous with the operator
- Palindromic sequences present at CRP (cyclic receptor protein) site.

THE REPRESSOR GENE

- The repressor gene is the **lacI** i.e., inhibitor gene.
- Codes for the repressor protein that blocks the transcription of structural genes.
- Has its own **promoter (placI)** gene.
- **Repressor protein(inactive)** can be activated by binding with the **co-repressor.**
- This results in the formation of **repressor-co repressor complex.**



(a) Repressor protein, inducer molecule, inducible gene



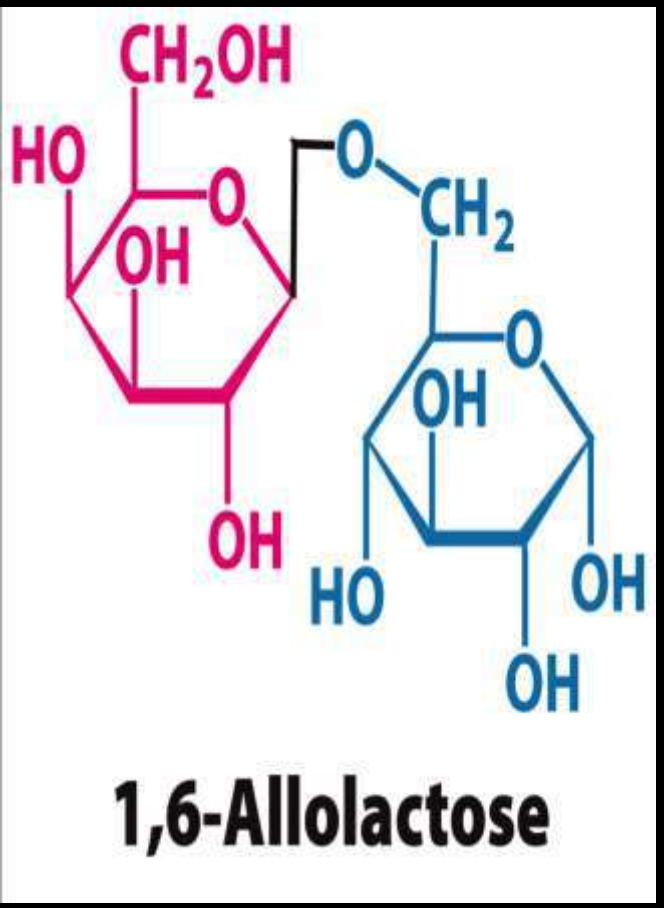
(b) Repressor protein, corepressor molecule, repressible gene

- Regulatory proteins have two binding sites
  - One for a small effector molecule
  - The other for DNA

How does the lac repressor inhibit the expression of the lac Operon?

- The *lac* repressor can exist as a dimer of 37-kd subunits, and two dimers often come together to form a tetramer.
- In the absence of lactose, the repressor binds very tightly and rapidly to the operator.

ALLOLACTOSE (the inducer molecule)



INDUCIBLE SYSTEM

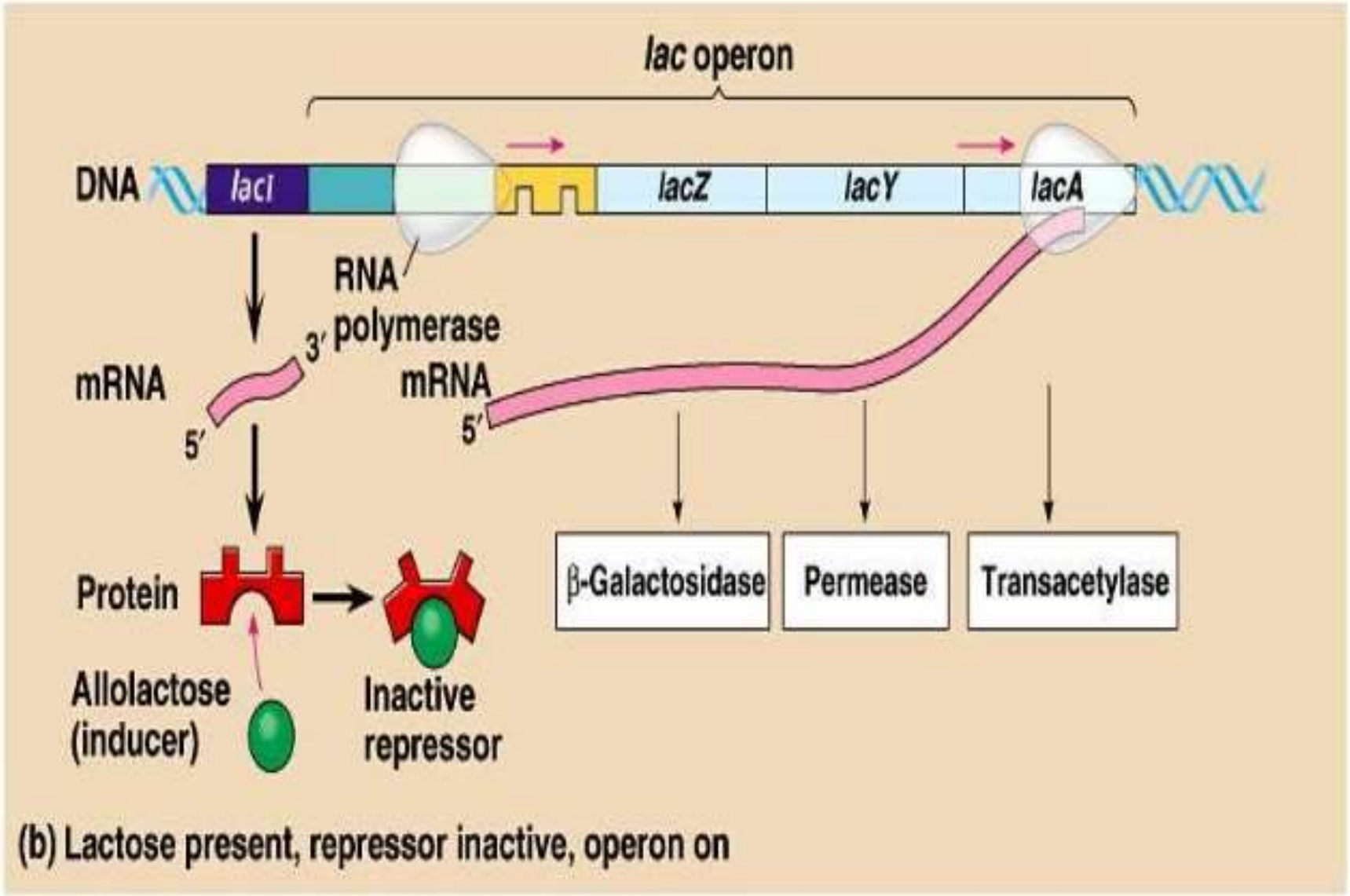
- Enzyme whose full level of transcription does not occurs unless their effectors molecules are present, called inducible enzyme, such effectors is called inducer and this regulation is called inducible system.
- Inducible products are made when the substrates (lactose) present in the environment and needs to be metabolized.
- E.g.-synthesis of beta galactosidase enzyme induced by lactose.
- Here lactose is inducer.

(it is actually an alternative form of lactose called allolactose.
When beta galactosidase breaks down into lactose to galactose and glucose, it rearranges small fraction of the lactose to allolactose (1, 6 galactosidic linkages).

POSITIVE REGULATION OF lac OPERON.

PRESENCE OF LACTOSE

- Lactose- acts as inducer
- Small amounts of lactose are able to enter the cell even in the absence of permease
- LacI repressor have a high affinity for the inducer. Binding of inducer to a repressor molecule attached to the operator locus-
- induces conformational change in the structure of the repressor- cause it to dissociate from the DNA (now its affinity to DNA is 10^3lower) .
- RNAP that attached to the promoter will begin transcription process
- Thus, an inducer derepresses the lac Operon.
- THIS IS CALLED **SWITCHING ON.**

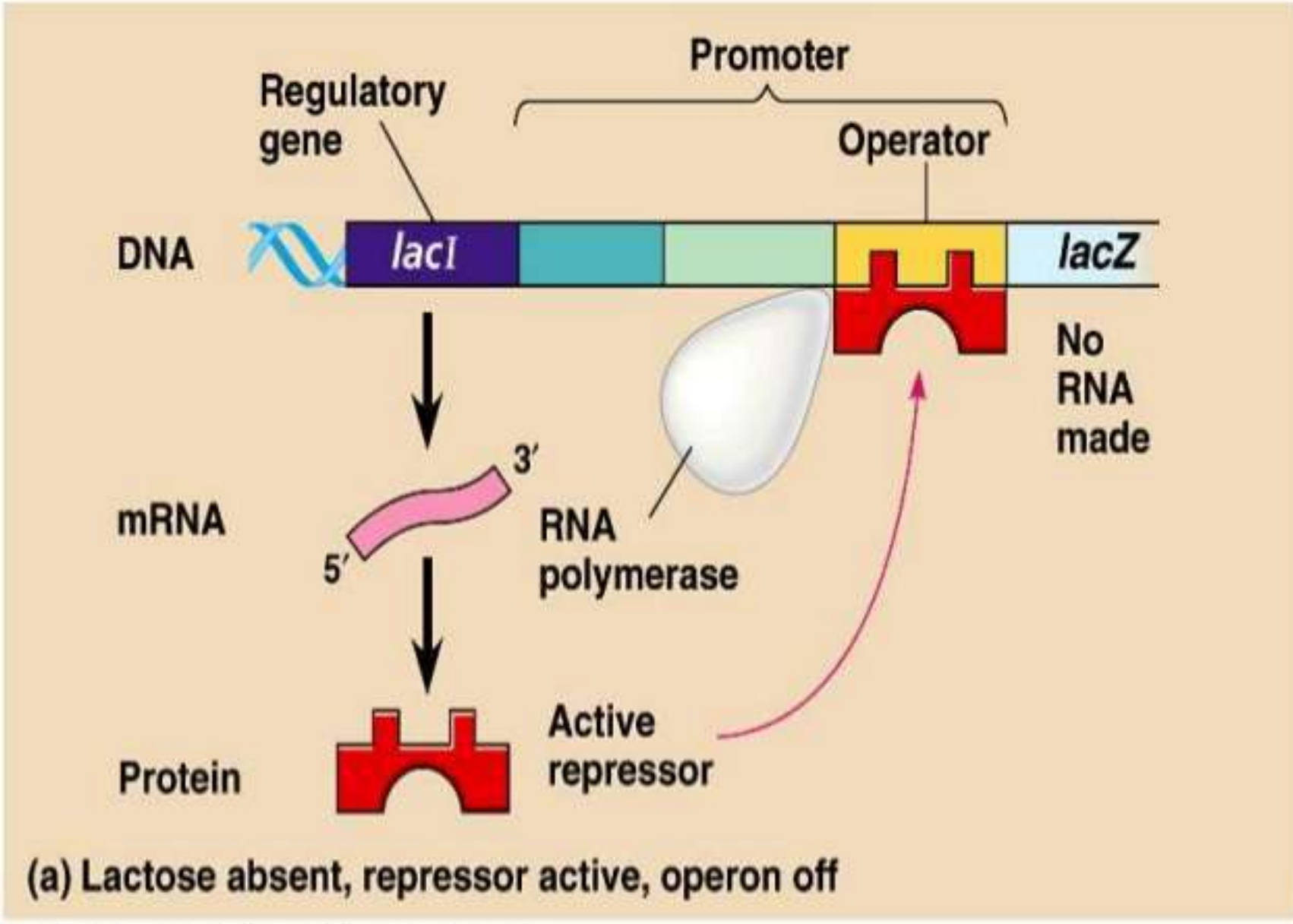


(b) Lactose present, repressor inactive, operon on

NEGATIVE REGULATION OF lac OPERON.

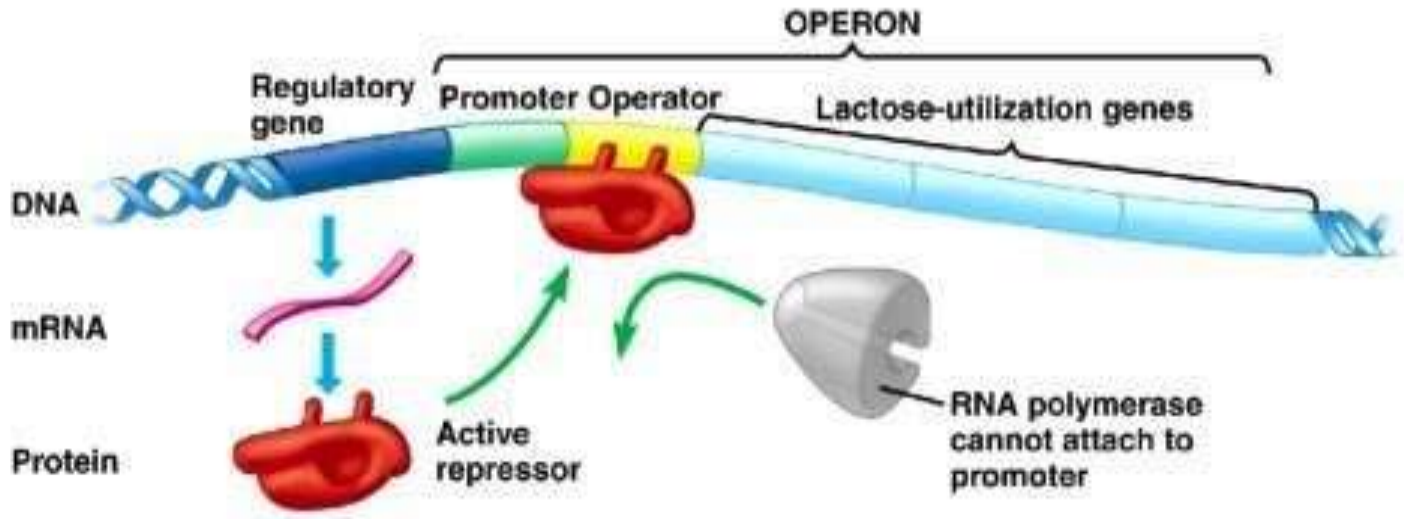
ABSENCE OF LACTOSE

- LacI gene is expressed at a constant rate – producing LacI repressor.
- Has high affinity to operator LacI repressor bind to operator locus – prevents transcription of the operator locus and lac Z, lac Y and lacA genes.
- Thus, LacI repressor- negative regulator; in its presence, expression of lac Z, lac Y and lacA is prevented.
- THIS IS CALLED **SWITCHING OFF.**



REGULATION OF

THE LAC OPERON



Glucose repression

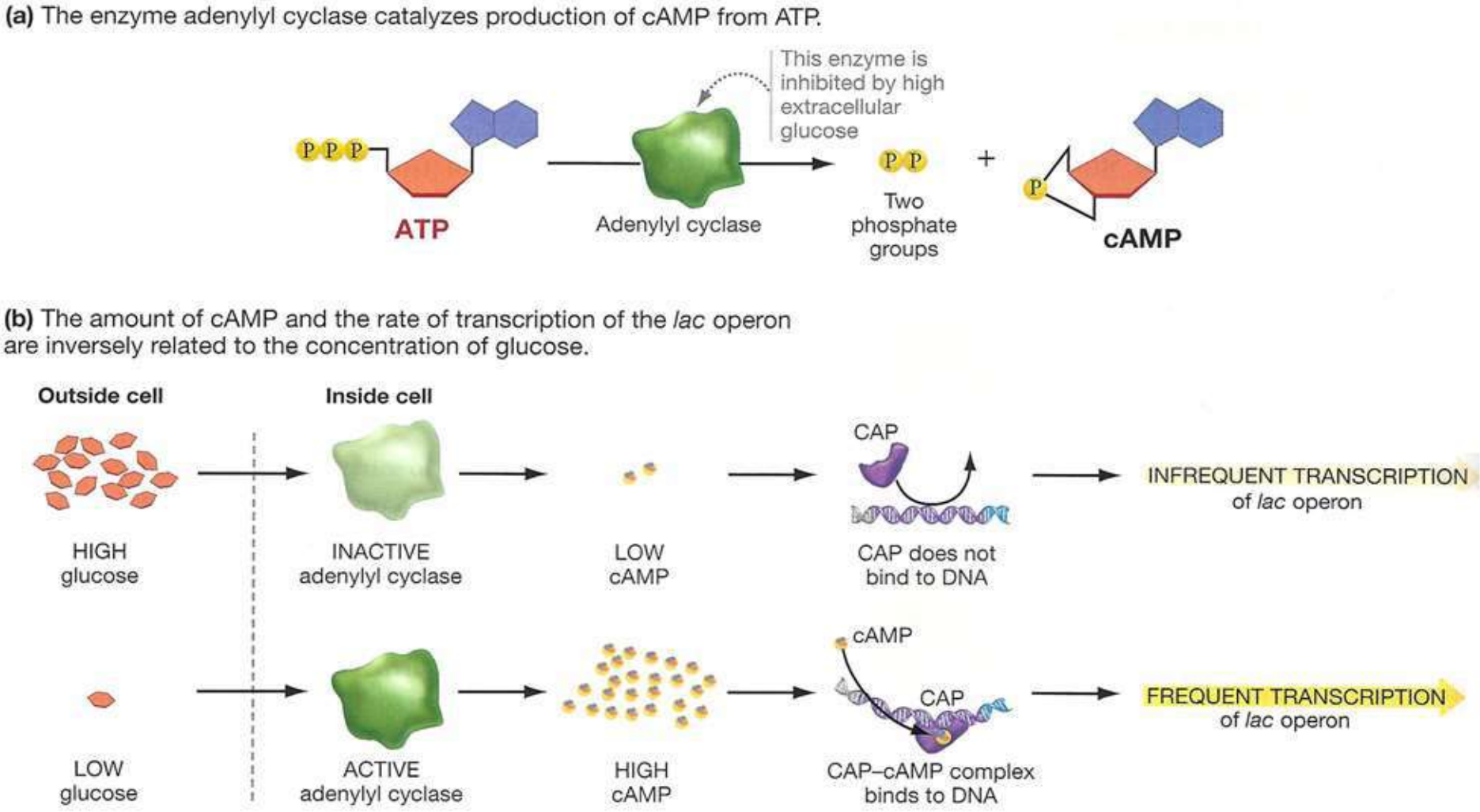
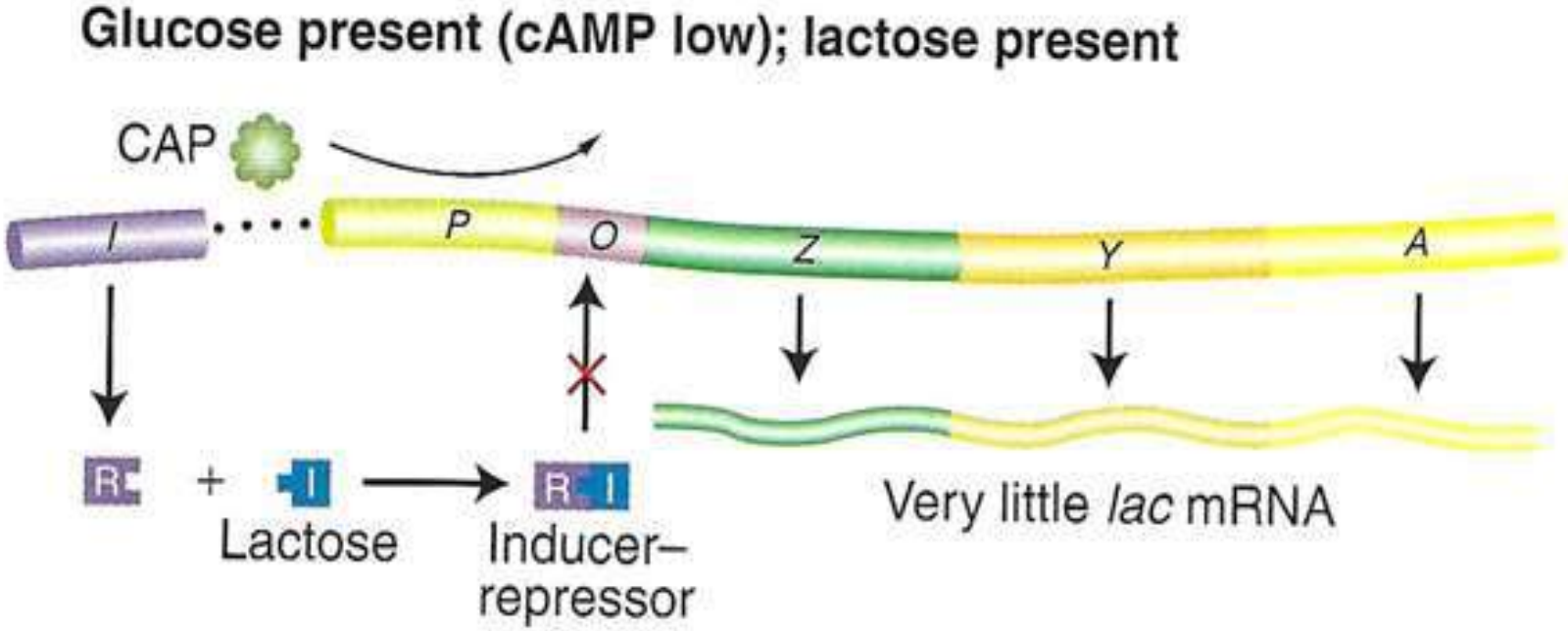


FIGURE 17.11 Cyclic AMP (cAMP) Is Synthesized When Glucose Levels Are Low.

WHEN BOTH GLUCOSE AND LACTOSE ARE PRESENT.

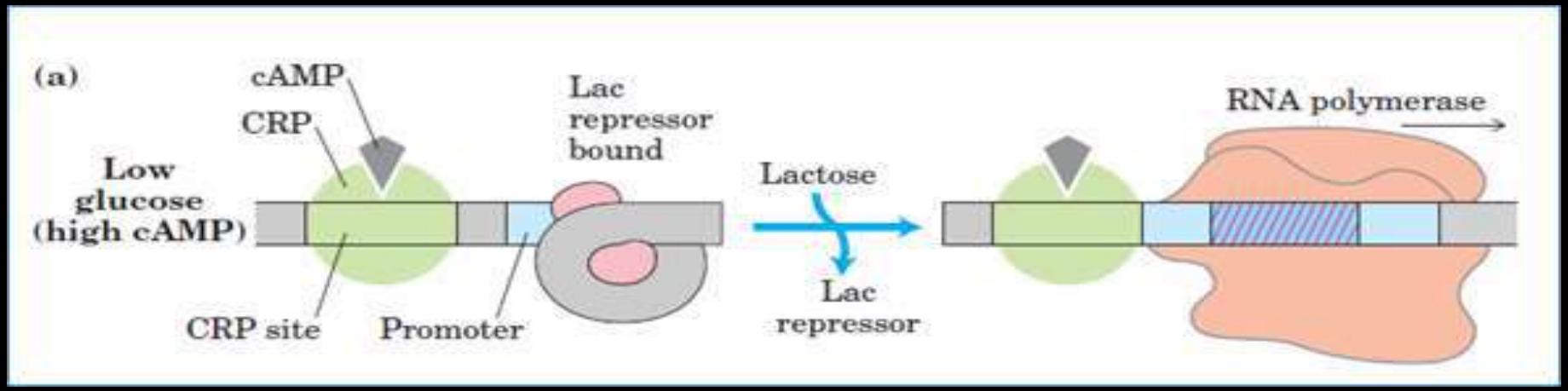
- When E.coli is exposed to both lactose and glucose as sources of carbon – it will first metabolize the glucose,
- and then temporarily stop growing until the genes of the lac Operon become induced to metabolize lactose .
- Glucose are more preferred energy source, can be metabolized directly in glycolysis .
- Other sugars can also be used for energy, but need extra steps and enzymes .
- Expressing the genes for proteins that metabolize sugars such as lactose is wasteful when glucose is abundant.

Glucose + lactose

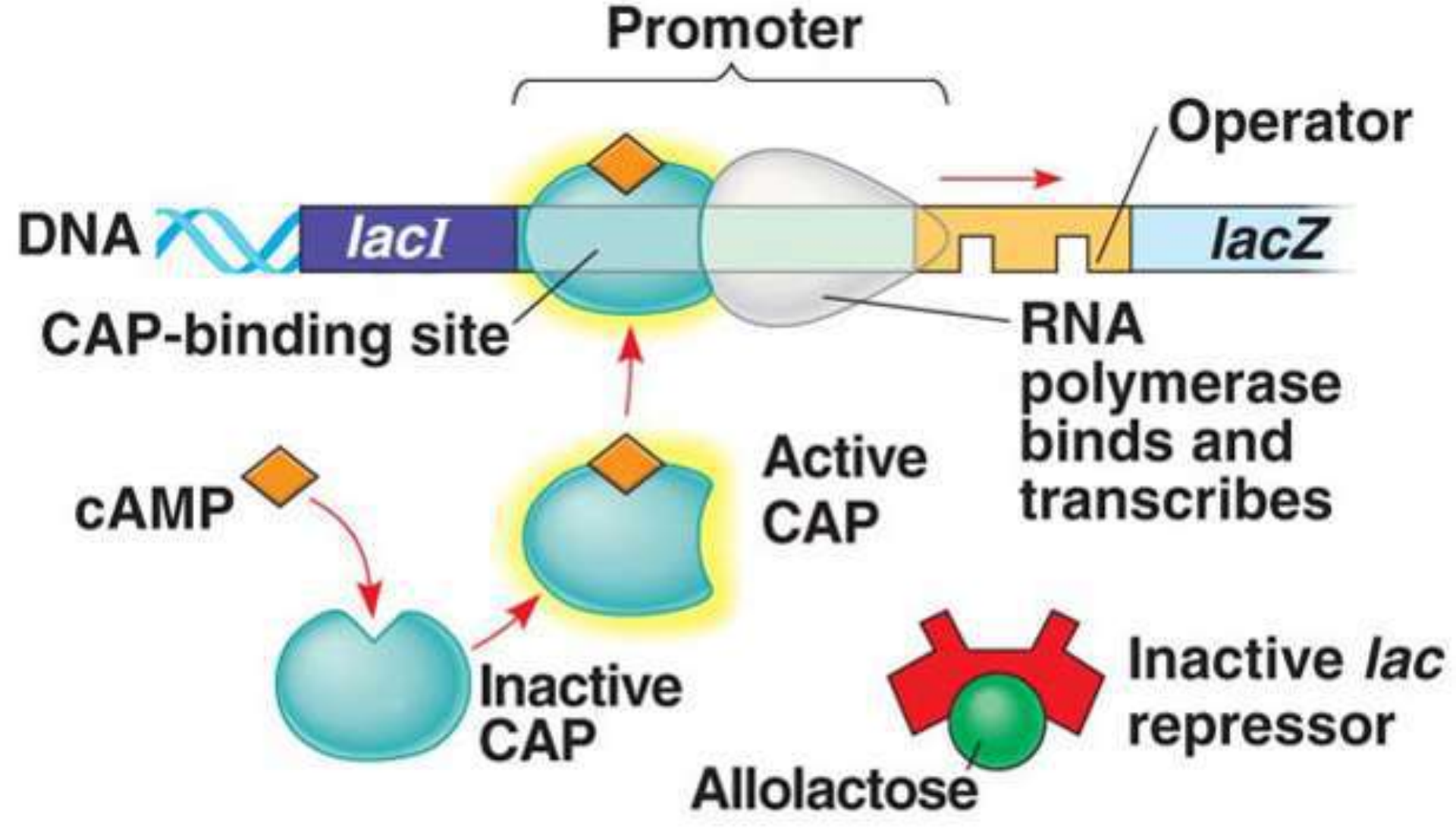


ROLE OF CATABOLITE GENE ACTIVATOR PROTEIN (CAP)

- Catabolite activator protein is a dimer and acts as a positive regulator of many catabolic Operons like the Lac Operon.
- Attachment of RNAP to the promoter site requires the presence of CAP bound to cAMP .
- Absence of glucose in the cell activates adenylate cyclase which catalyzes the synthesis of cAMP from ATP.
- cAMP will form CAP-cAMP complex .
- This complex binds to promoter site immediately. This stimulates the initiation of transcription of the lac Operon structural genes in the absence of repressor.

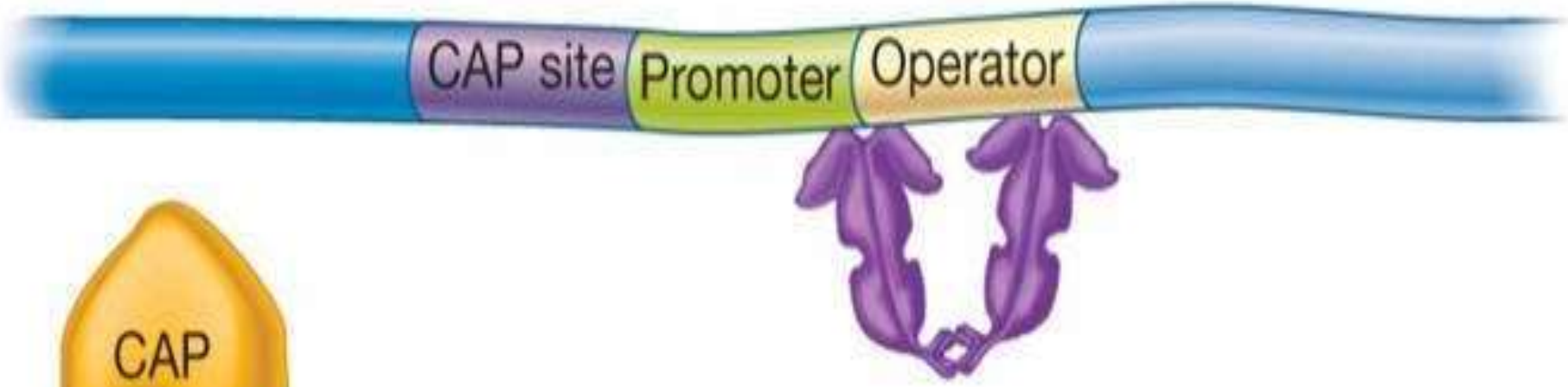


WHEN GLUCOSE IS ABSENT AND LACTOSE IS PRESNT



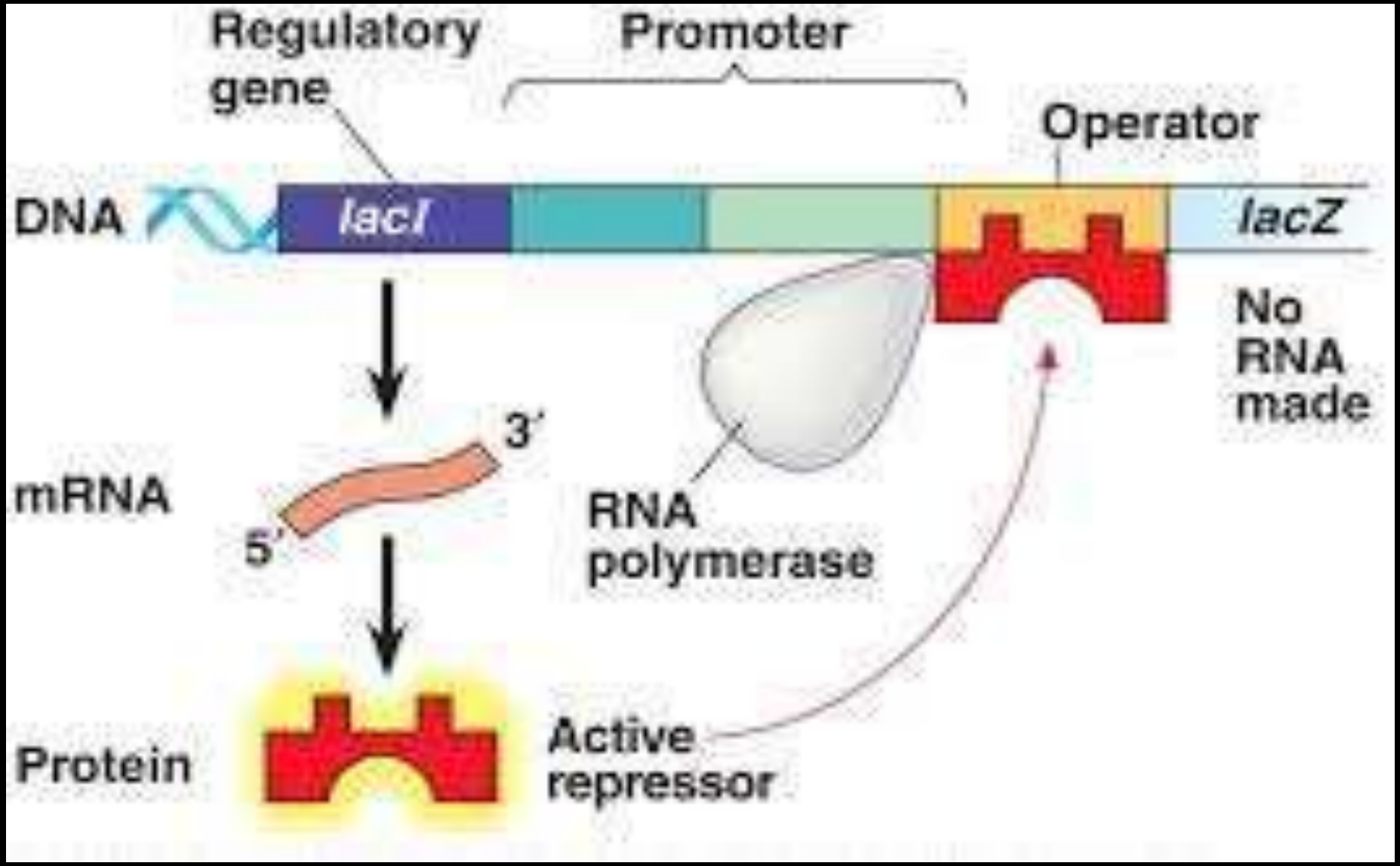
WHEN GLUCOSE IS PRESENT AND LACTOSE IS ABSENT.

- Catabolite repression occur – restrict expression of genes required to metabolized lactose or other sugars when glucose is present.
- In the presence of glucose, the synthesis of cAMP is inhibited .
- The effect of glucose on CAP is mediated by the cAMP interaction.
- CAP binds to the promoter only when it is complexed with cAMP.
- As (cAMP is declined) CAP binding to DNA declines, thereby decreasing the expression of the lac Operon



Glucose but no lactose

WHEN BOTH GLUCOSE AND LACTOSE ARE ABSENT.



GRATUITOUS INDUCERS

- Isopropyl thiogalactoside (IPTG), a structural analogue of lactose
- can induce the lac Operon.
- by inactivating repressor molecules
- IPTG cannot be hydrolyzes by β-galactosidase.

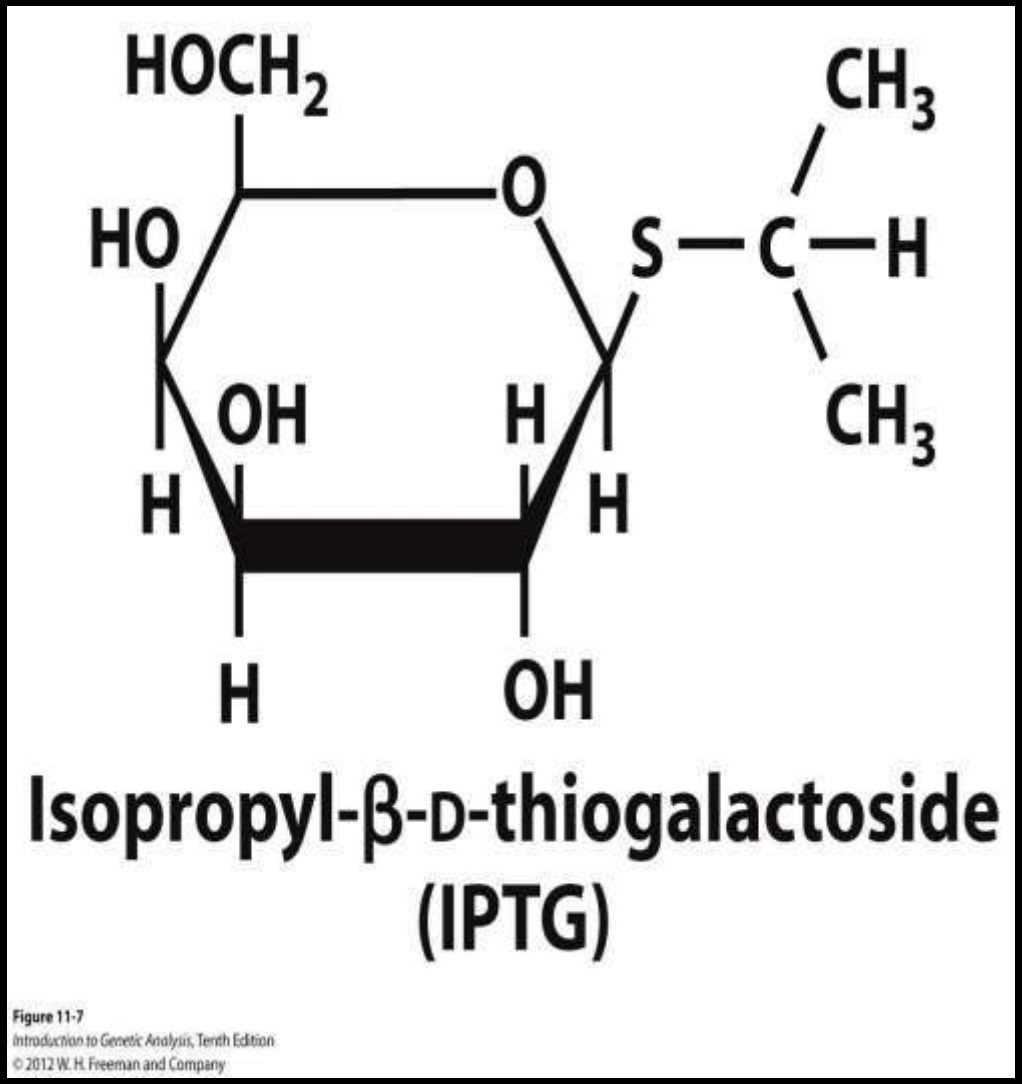


Figure 11-7
Introduction to Genetic Analysis, Tenth Edition

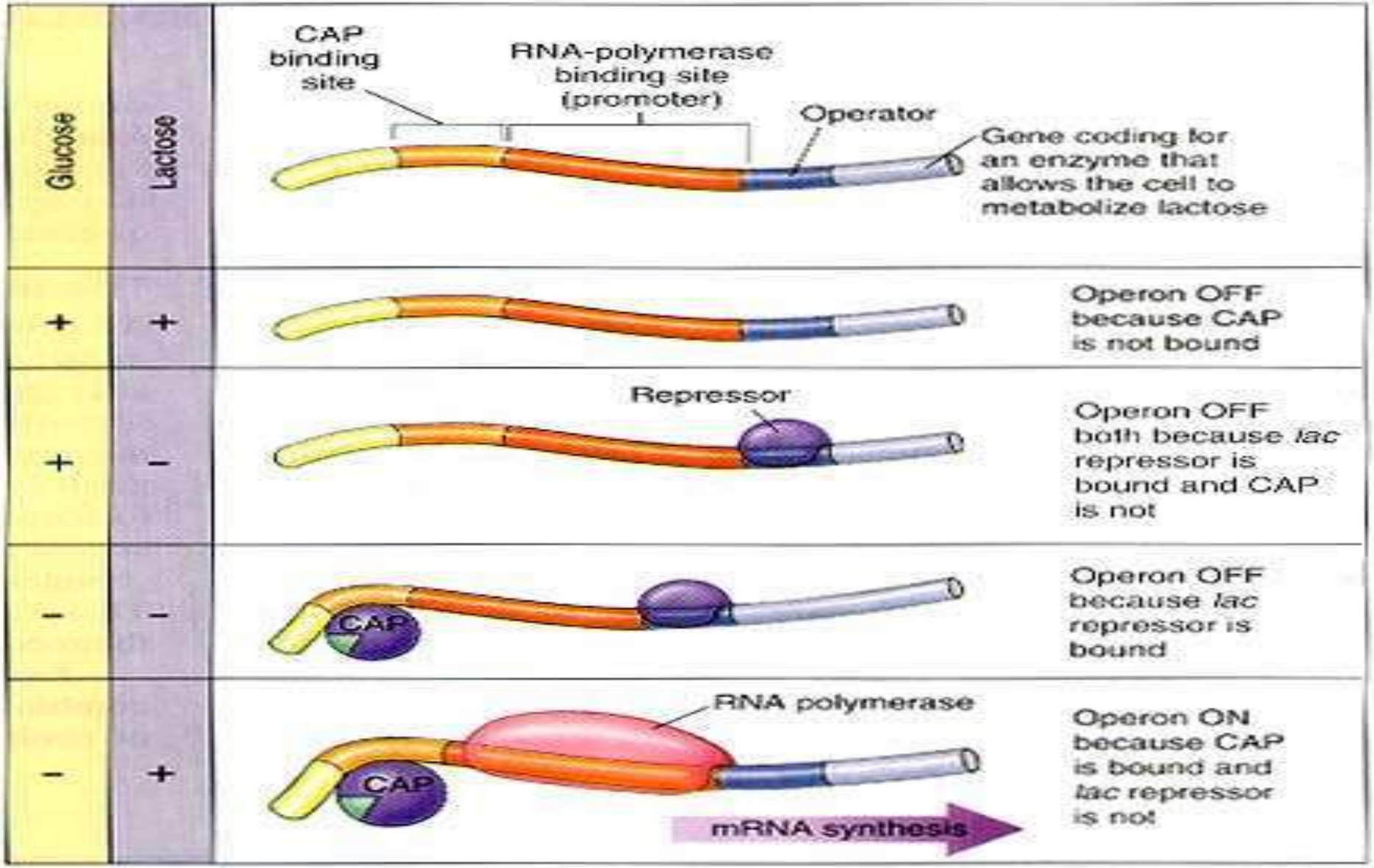


Figure **Activators and repressors at the *lac* operon.**

HOPE EVERYONE LIKED IT…..;0

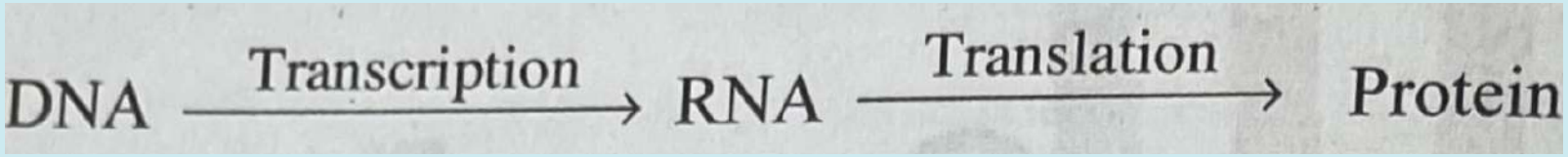
THANK YOU…..

Transcription

Synopsis

Introduction

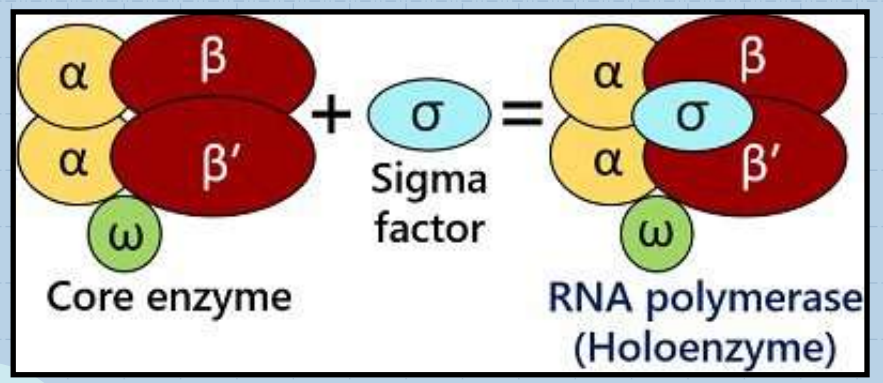
The sequencial transfer of information from DNA to protein via RNA is known as Central Dogma.

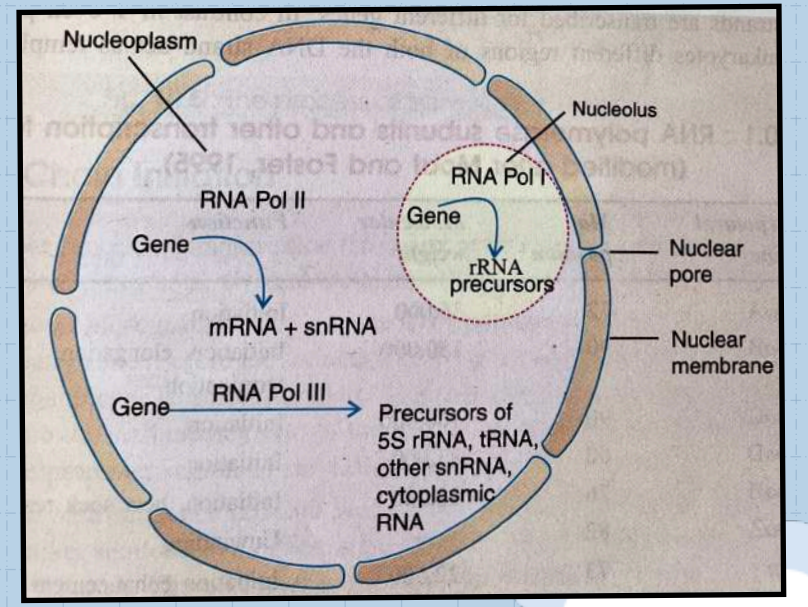


The tRNA, mRNA and rRNA are involved in the process of transcription. However, an enzyme transcriptase i.e. DNA-dependent RNA polymerase is required for the synthesis of RNA. Transcription is a process in which RNA is synthesised from the DNA sequence. The RNA transcript carries the information used to encode a protein.

RNA polymerase

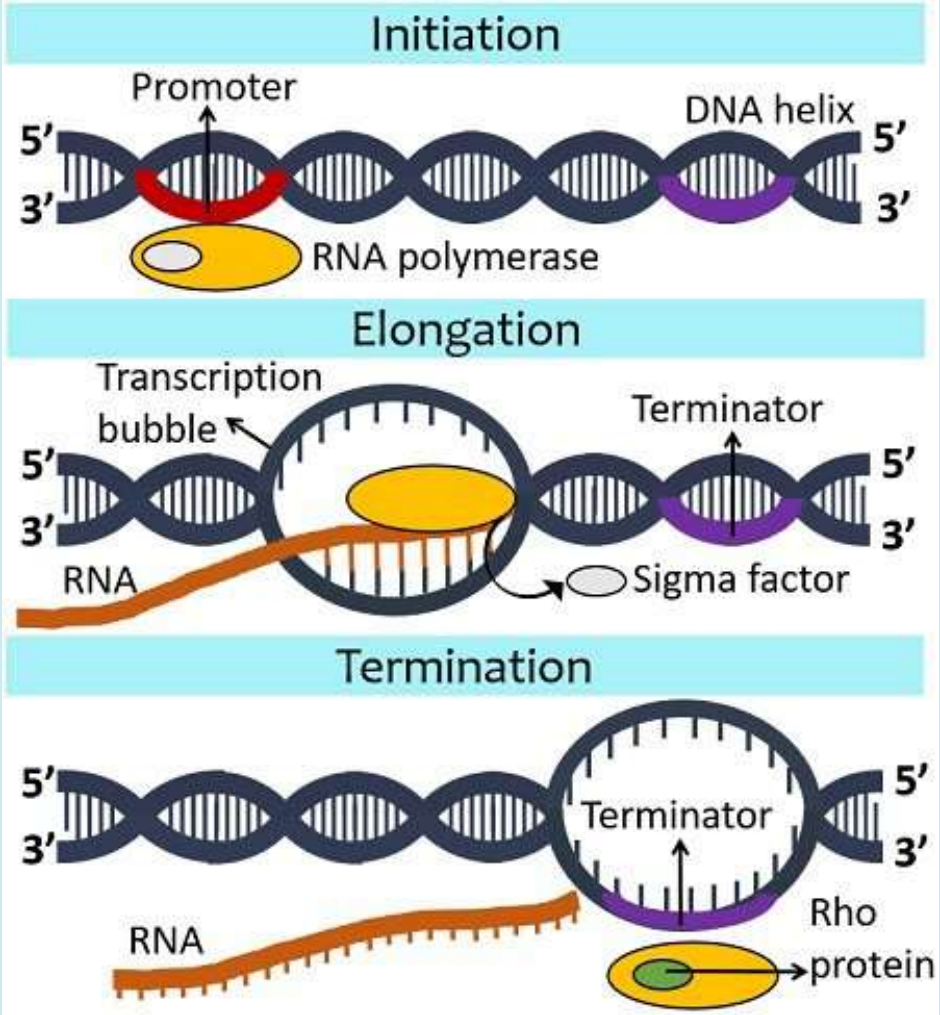
On a DNA template elongation of RNA chain at each step is catalysed by RNA polymerase. RNA polymerase is found both in prokaryotes and eukaryotes but the structure and function in these two groups of organisms differ. The holoenzyme is a complete RNA polymerase consisting of core, an enzyme and a sigma factor, hence it is a complex enzyme. The holoenzyme binds to DNA at specific sites called promoters and transcribes specific length of RNA. Thus sigma factor plays a significant role in promoter recognition by RNA polymerase.





Transcription Process

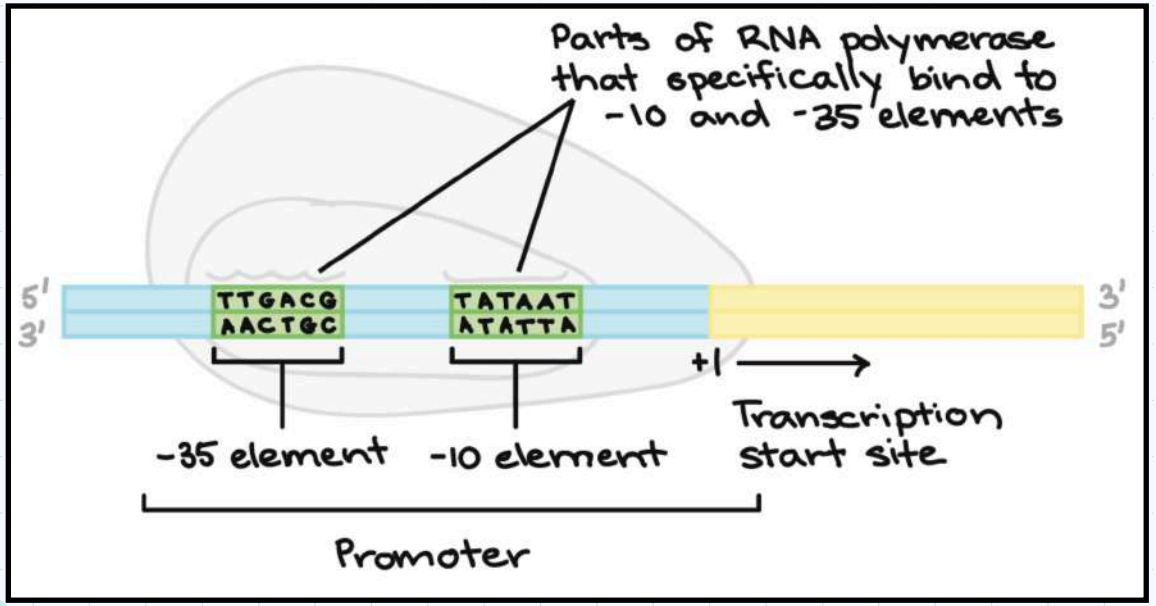
The process of transcription is accomplished in the following three main steps : chain initiation, chain elongation and chain termination.

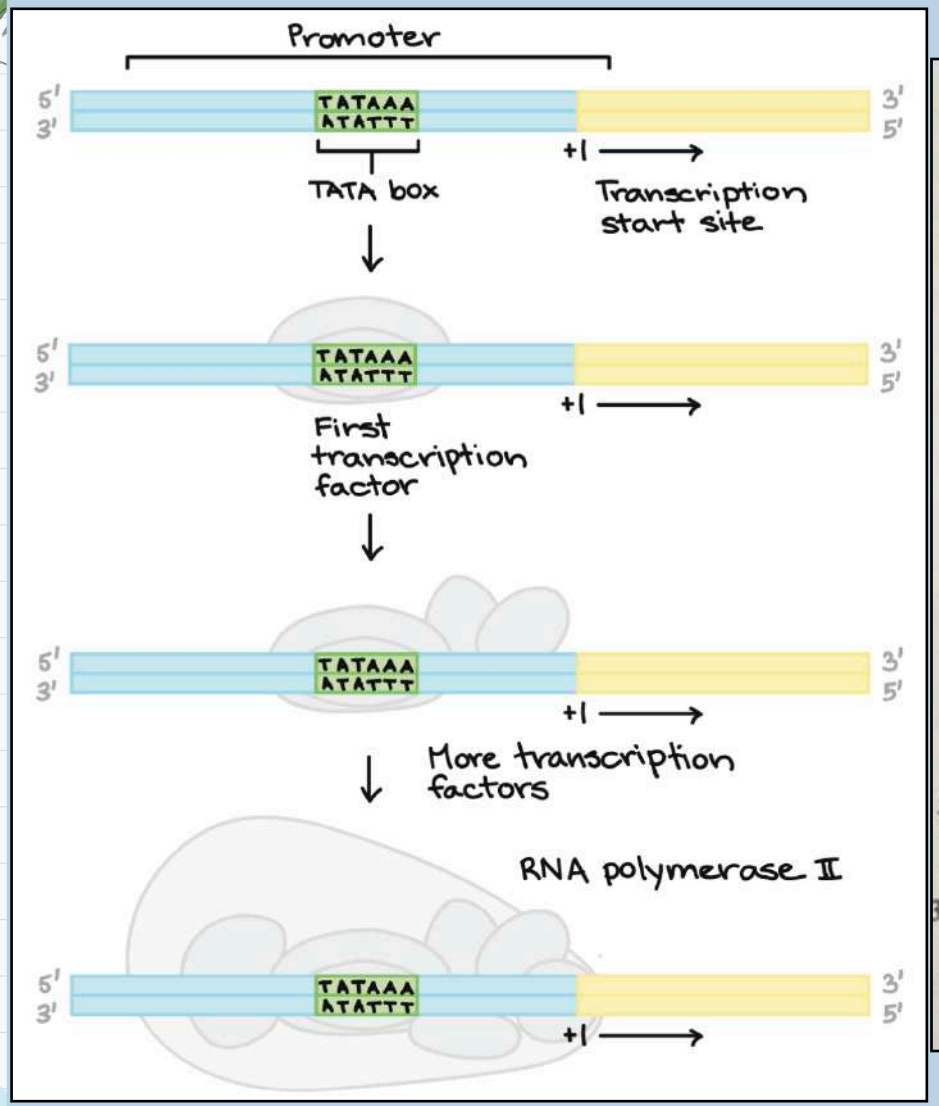


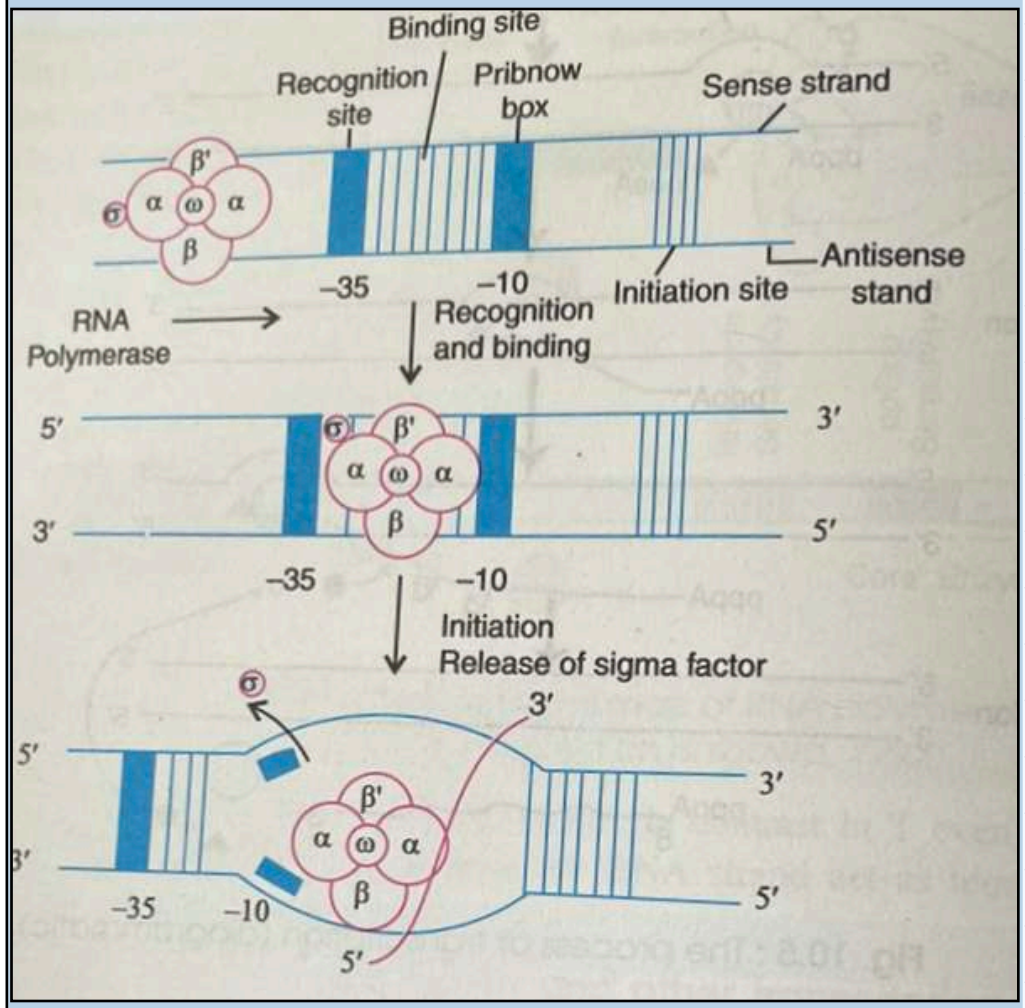
Chain Initiation

1 Promoter Recognition

The enzyme RNA polymerase plays a key role in recognition and binding of initiation site. Before the formation of complex enzyme, sigma factor interacts with core enzyme at B subunit site. This is required to check transcription of both the strands by core enzyme. The holoenzyme transcribes only one of the two DNA strands. The sigma factor of holoenzyme recognizes the promoter region of the DNA.




Promoter
5′
3′
TATAAA
ATATTT
3′
5′
+1
TATA box
Transcription start site
First transcription factor
More transcription factors
RNA polymerase II


Binding site
Recognition site
Pribnow box
Sense strand
Antisense stand
Initiation site
β′
α
ω
α
β
σ
–35
–10
RNA Polymerase
Recognition and binding
5′
3′
Initiation
Release of sigma factor

Chain Initiation

2 Unwinding of DNA double helix

Binding of omega factor of RNA polymerase results in unwinding of a DNA helix. Consequently, a short segment of DNA opens. The open complex then allows tight binding of the RNA polymerase with subsequent initiation of RNA synthesis.

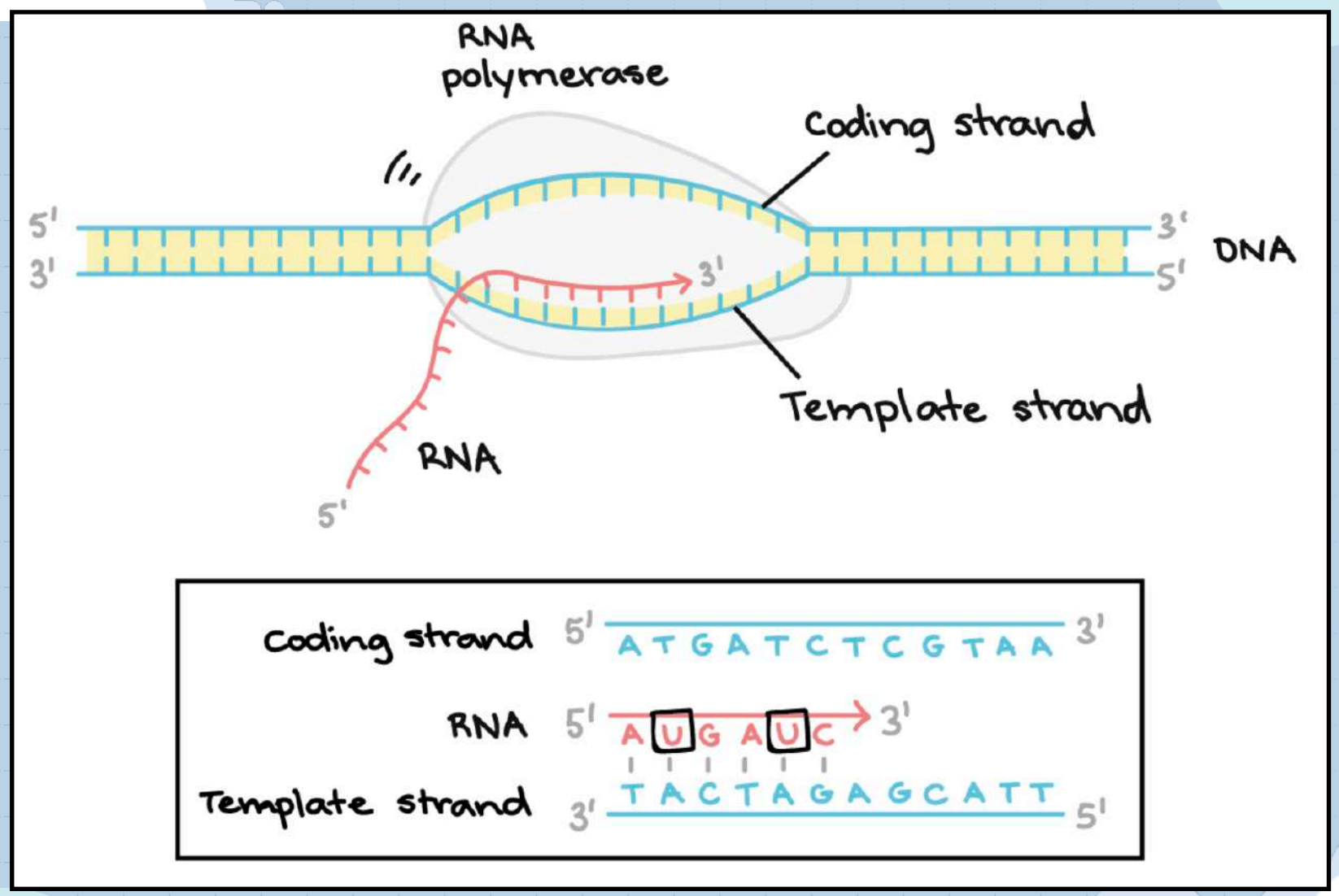
3 Synthesis of first base of RNA chain

The first base of RNA synthesised is always in the form of purine i.e. either triphosphate guanine (pppG) or adenine (pppA).

Chain Elongation

1. The sigma subunit falls off from the RNA-polymerase once the first 3’,5’-phosphodiester bond is formed. The core enzyme moves along the DNA template to enter the elongation phase. The release of the sigma subunit causes the conformational change of the core enzyme. The core enzyme slides on the DNA template toward the 3’ end.

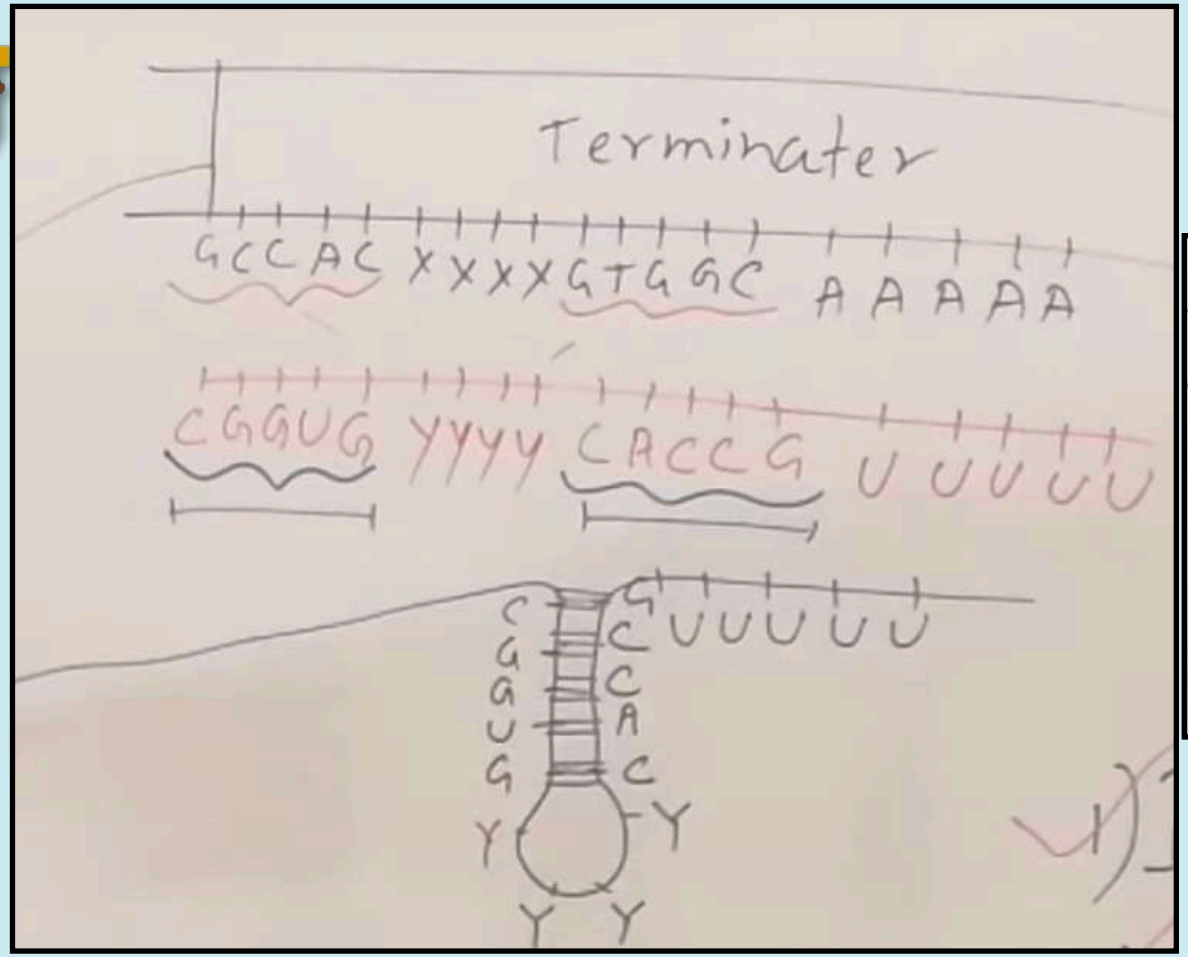
2. Pre-mRNA nucleotides are quickly paired with their complementary bases which correspond with the template strand of DNA. The pre-mRNA moves in the 5' to 3' direction while the template strand of DNA moves oppositely from the 3' to ;' direction. Pre-mRNA does not contain thymine, instead uracil is used as the complementarv base for adenine.

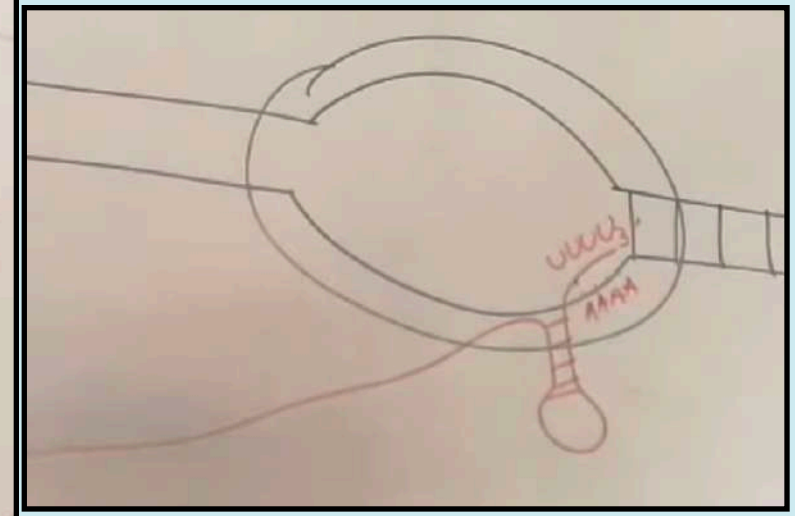

RNA polymerase
Coding strand
5'
3'
3'
5'
DNA
3'
RNA
5'
Template strand
Coding strand 5' ATGATCTCGTAA 3'
RNA 5' AUGAUC 3'
Template strand 3' TACTAGAGCATT 5'

Chain Termination

The process of termination of RNA chain ends with the events: (1) cessation of elongation, (2) release of transcript from the tertiary complex, and (3) dissociation of polymerase from the DNA template. There are two types of mechanisms that brings about termination: rho-independent and rho-dependent terminations.

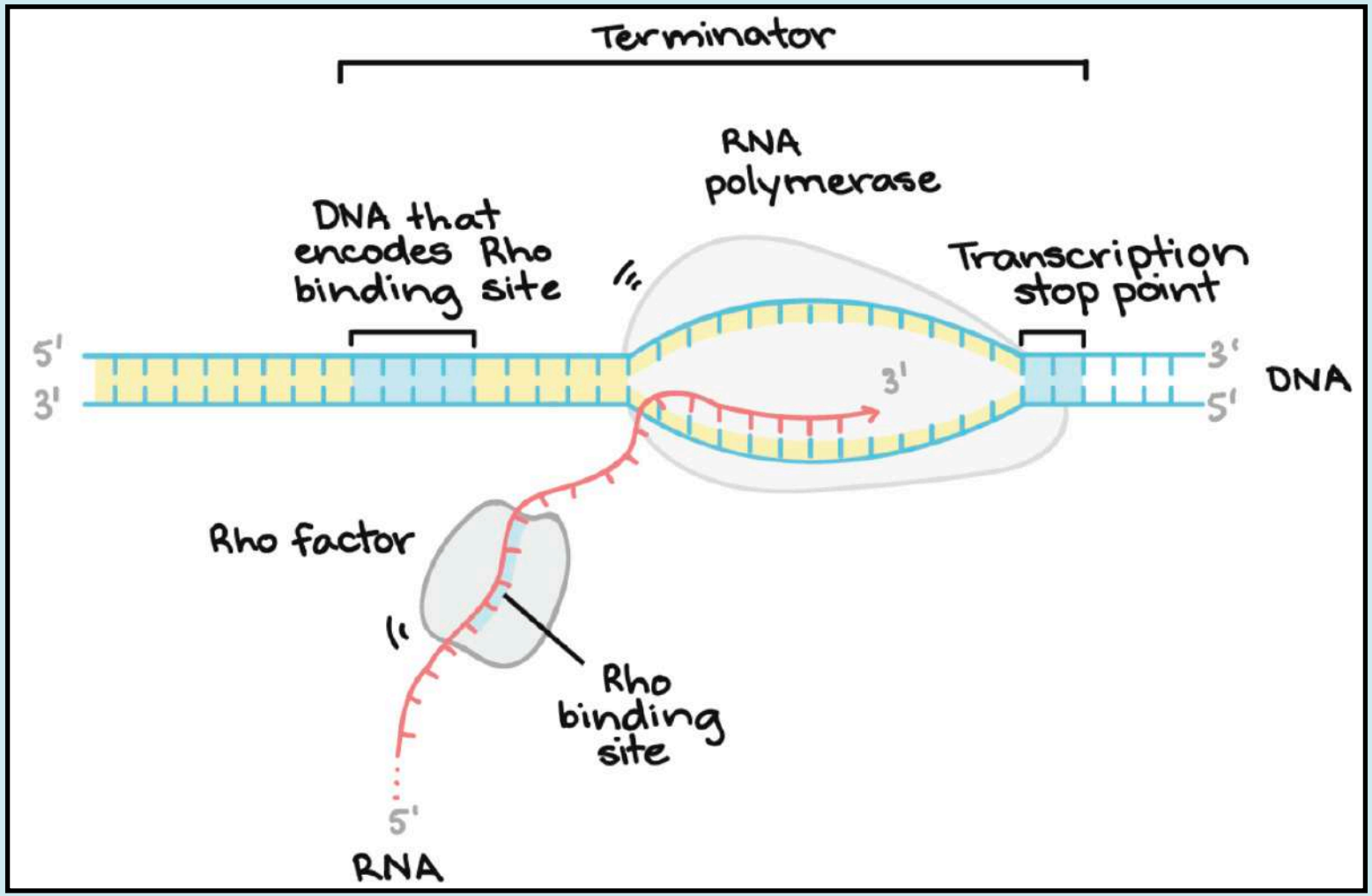
- Rho-independent Termination : The rho-independent signal for termination is recognised by DNA itself. It consists of GC rich region with dyad symmetry. RNA polymerase reads and extends polyA sequence on DNA template. It synthesises an RNA transcript that becomes folded to form a stem and loop structure of about 20 bases upstream from the 3'- OH terminus with a terminal stretch of 4-8 poly U residues.This RNA stem-loop structure causes RNA polymerase to pause and disrupt the RNA-DNA hybrid at 5' end. Poly U residues are present at 3' end of RNA-DNA hybrid molecule. The U-A hybrid base pairs are relatively unstable; therefore, it causes the 3' end of the hybrid to break and release the mRNA chain.


Terminater
GCCAC XXXXGTGGC AAAAA
CGGUG YYYY CACCG UUUUU
C
G
G
U
G
C
C
A
C
Y
Y
Y
Y
UUUUU



Chain Termination

- Rho-dependent Termination : One of the two transcription termination mechanisms prokaryotes use is Rho-dependent termination. The protein Rho factor exhibits helicase activity. The Rho protein binds to the RNA transcript and moves in a 5’-3’ direction with the RNA polymerase, which promotes the breaking of hydrogen bonds between the DNA template and RNA transcript. Rho factor separates the DNA/RNA hybrid as it approaches the transcription bubble, releasing the transcript from the bubble. When this takes place, it terminates the transcription process.


Terminator
RNA polymerase
DNA that encodes Rho binding site
Transcription stop point
5'
3'
3'
3'
5'
DNA
Rho factor
Rho binding site
5'
RNA

Post-Transcriptional process

1 Processing of rRNA

rRNAs of both prokaryotic and eukaryotic cells are generated from long precursor molecules called pre-rRNAs. The 23S, 16S, and 5S rRNA of prokaryotes are produced from a single pre-rRNA molecule, as are the 28S, 18S, and 5.8S rRNA of eukaryotes.
The pre-rRNAs are cleaved by ribonucleases to yield intermediate-sized pieces of rRNA, which are further processed (trimmed by exonucleases and modified at some bases and riboses) to produce the required RNA species. Post transcriptional processing of eukaryotic ribosomal RNA by ribonucleases (RNases).

2 Processing of tRNA

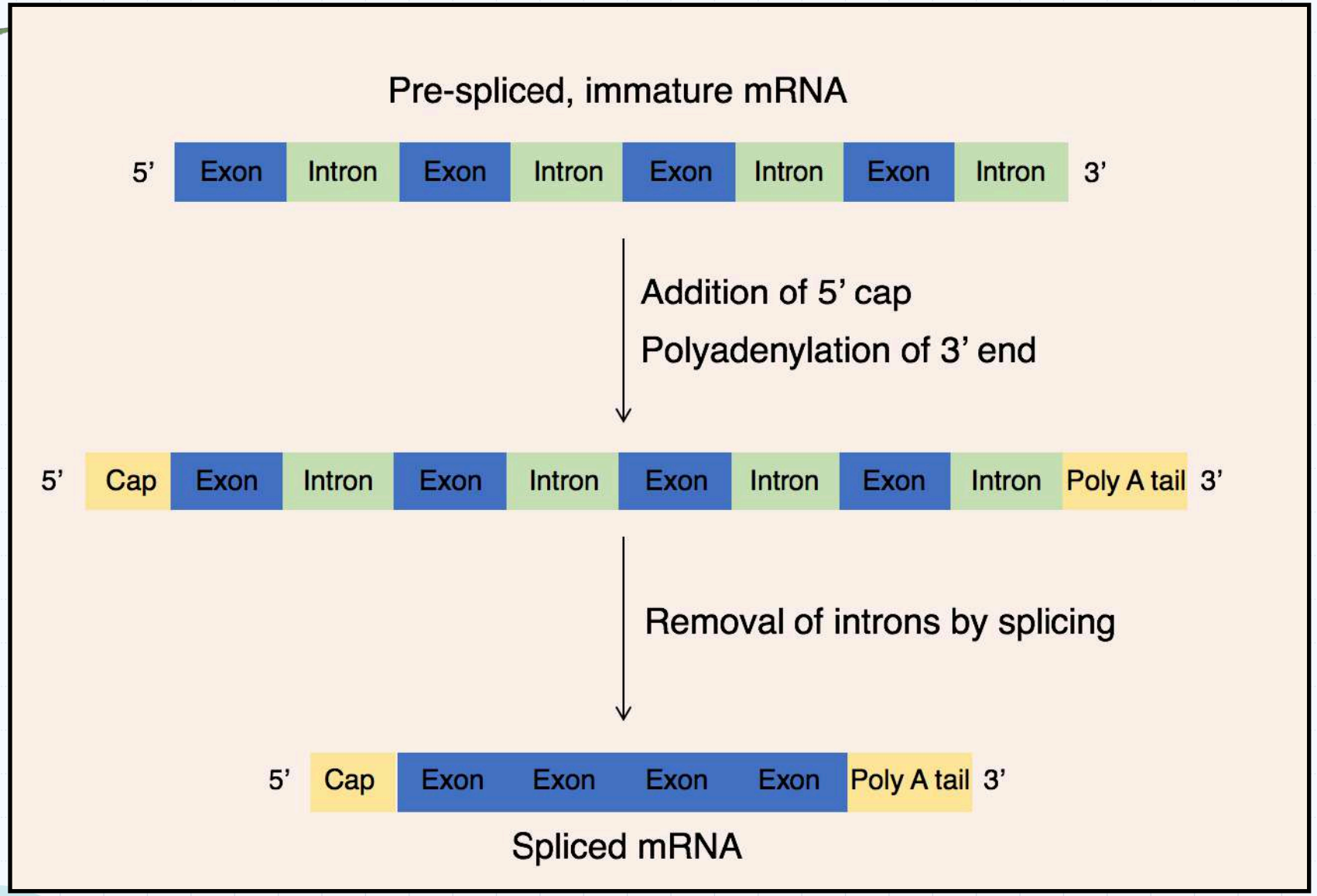
Mature tRNA molecules are generated by processing longer pre-tRNA transcripts.
Through a specific step, RNases D, E, F and P generate these mature tRNA molecules by exo-and endo-nucleolytic cleavage.

Post-Transcriptional process

3 Processing of mRNA

In prokaryotes, there is a little or no processing of mRNA transcripts. Prokaryotic mRNAs are degraded very rapidly. Therefore, to rescue from degradation it is translated before being finally transcribed. The collection of all the primary transcripts synthesized in the nucleus by RNA polymerase II is known as heterogeneous nuclear RNA (hnRNA). The pre-mRNA components of hnRNA undergo extensive co- and post transcriptional modification in the nucleus. These modifications usually include:

- 5’- Capping: 7-Methyl-guanosine
- 3’- Poly-A tail addition
- Removal of introns
- Alternative splicing of mRNA molecules


Pre-spliced, immature mRNA
5’
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
3’
Addition of 5’ cap
Polyadenylation of 3’ end
5’
Cap
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
Poly A tail
3’
Removal of introns by splicing
5’
Cap
Exon
Exon
Exon
Exon
Poly A tail
3’
Spliced mRNA

Thank You