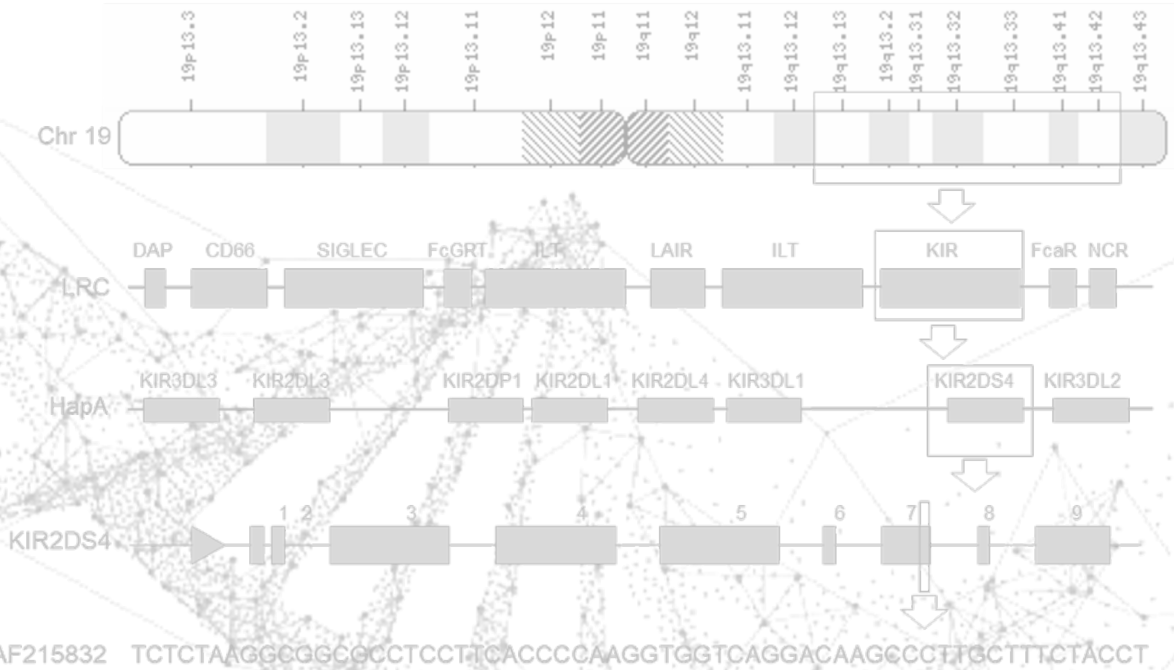

Transcription in prokaryotes and eukaryotes

San Luis Potosi State University (UASLP) Mexico
Molecular Biology Course, Faculty of Medicine post-graduate program

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Viral & Human Genomics BSL-3 Laboratory

Last updated October 07, 2025 v2

Introduction

Definition: The process by which RNA is synthesized from a DNA template.

- Catalyzed by RNA polymerase by reading the DNA template strand.
- RNA synthesized in 5′ to 3′ direction, while DNA is read in 3′ to 5′ direction.
- Promoter Region: Specific DNA sequences recruit RNA polymerase.
- Transcription Factors: Proteins that bind promtoers and regulate transcription.

Three Phases:

- Initiation: RNA polymerase binds to the promoter, and the DNA unwinds.
- Elongation: RNA polymerase synthesizes complementary RNA strand.
- Termination: Transcription stops at termination signal, releasing RNA transcript.

Types of RNA Polymerase (Eukaryotes):

- RNA Pol I – Synthesizes rRNA (except 5S rRNA).
- RNA Pol II – Synthesizes mRNA and some snRNA.
- RNA Pol III – Synthesizes tRNA and 5S rRNA.

Overview of prokaryote & eukaryote differences

Transcriptional unit.

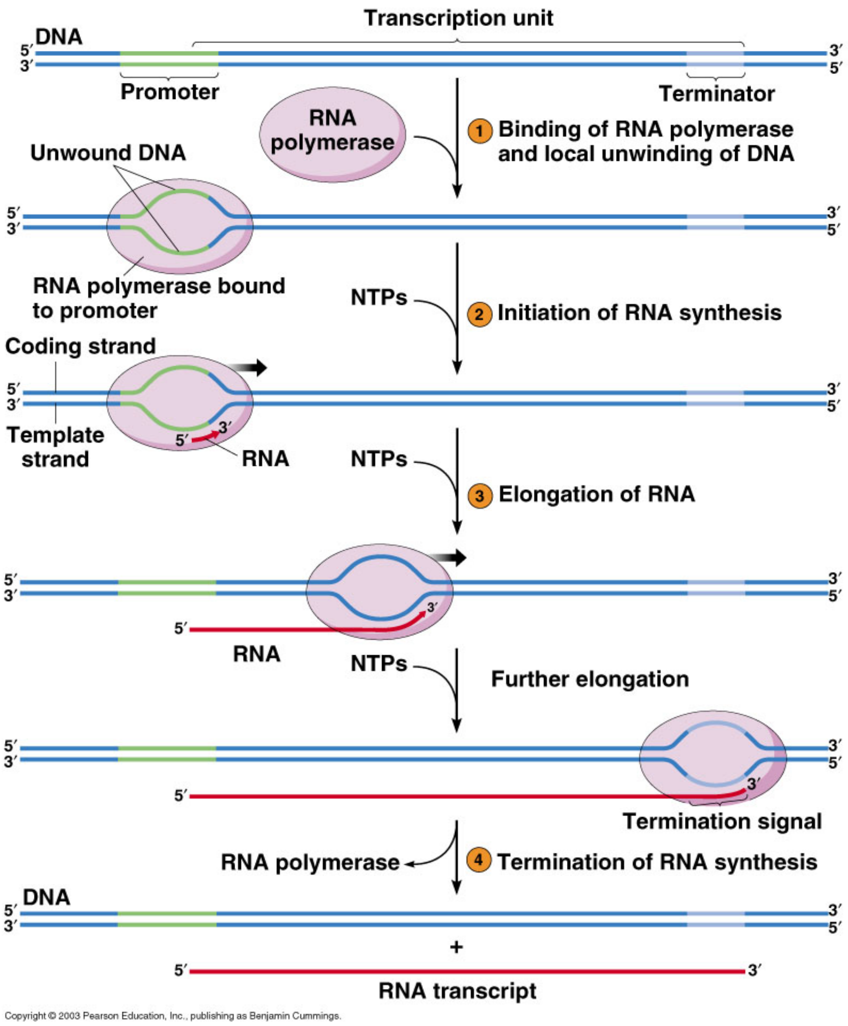
Promoter recognition.

Reference strand, coding strand, & RNA.

Phases:
- Initiation
- Elongation.
- Termination.

Prokaryotes:
- Transcript

Eukaryotes:
- Primary transcript (pre-mRNA)
- Mature transcript (mRNA)



Prokaryote promoters

Transcriptional unit.

Promoter recognition.

- Reference strand
- coding strand
- RNA.

Phases:

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Prokaryotes:

- Transcript

Eukaryotes:

- Primary transcript (pre-mRNA)
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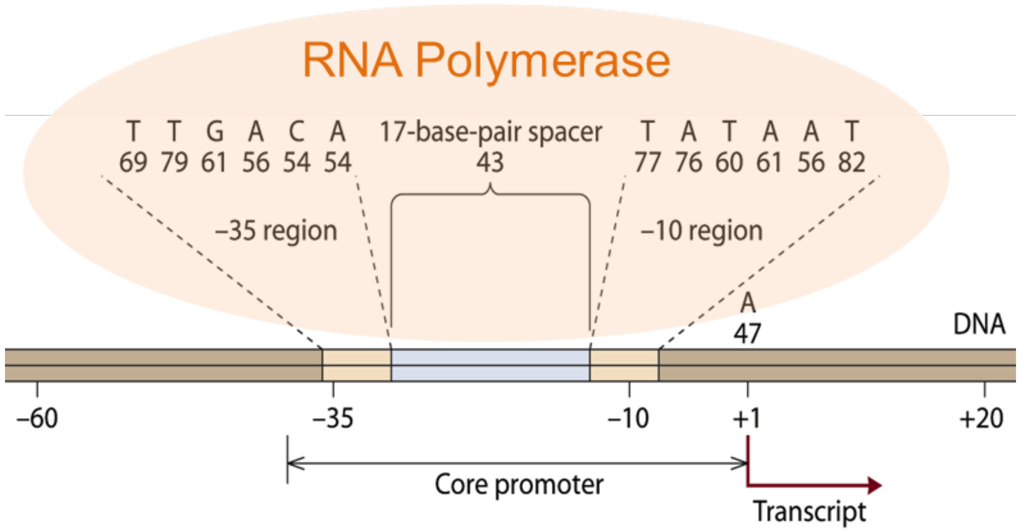


Prokaryote transcription phases

Transcription is regulated during initiation.

Binding of RNAP (σ subunit) to DNA at the promoter (regions -35 and -10).

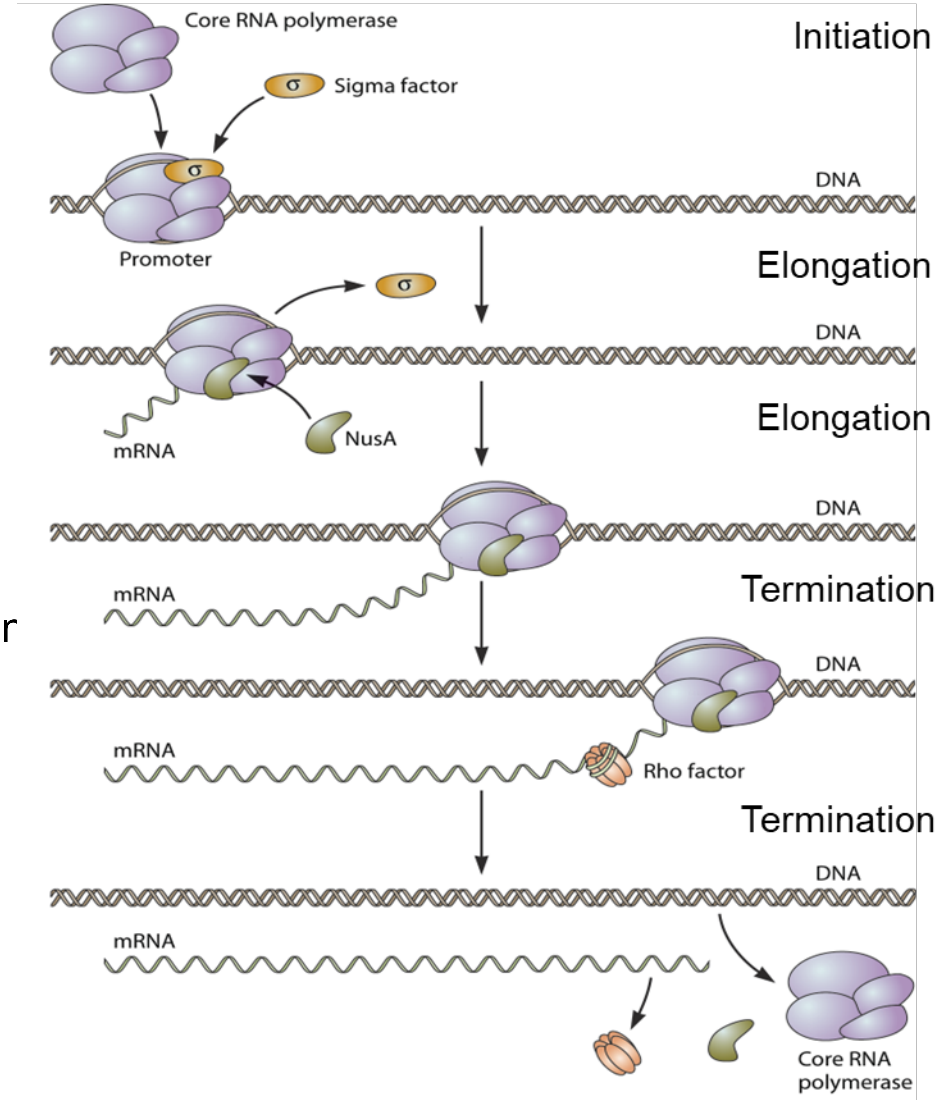
Approximately 12 bp are denatured to give access to RNA Pol.

The transcriptional bubble extends 18 bp to initiate transcription.

The σ subunit is released, and other elongatior factors are recruited to the site.

NusA elongation factor recruited during elongation.

Rho dependent and independent termination.



Prokaryote RNA polymerase

A single RNA polymerase synthesizes all types of RNA, including mRNA, rRNA, and tRNA.

The RNA polymerase consists of a core enzyme ($\alpha_2\beta\beta'\omega$) and a sigma ($\sigma$) factor, which is required for promoter recognition.

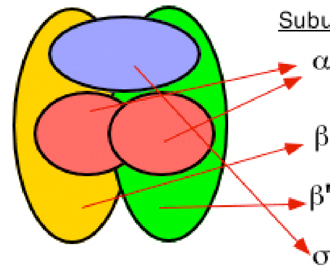
Promoters contain conserved -35 region sequences (TTGACA) and -10 region (Pribnow box, TATAAT), where the sigma factor binds to initiate transcription.

In 1959, the Nobel Prize went to Arthur Kornberg for what was then believed to be RNAP (actually another enzyme).

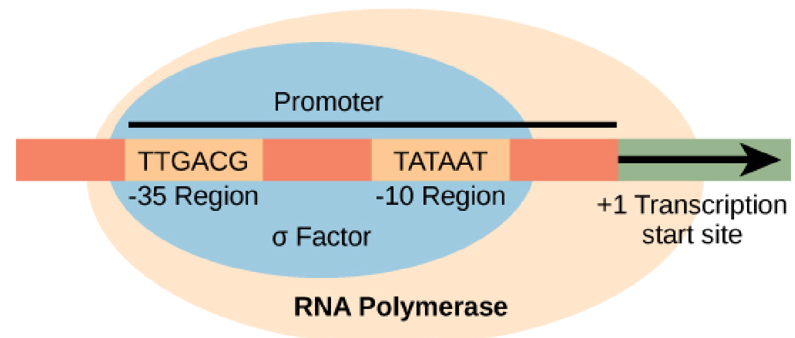
Finally discovered by Sam Weiss, Audrey Stevens, and Jerard Hurwitz in 1960.

Roger Kornberg Jr. photographed RNAP in action with EM.

Prokaryotic RNA Polymerase: Holoenzyme Enzyme



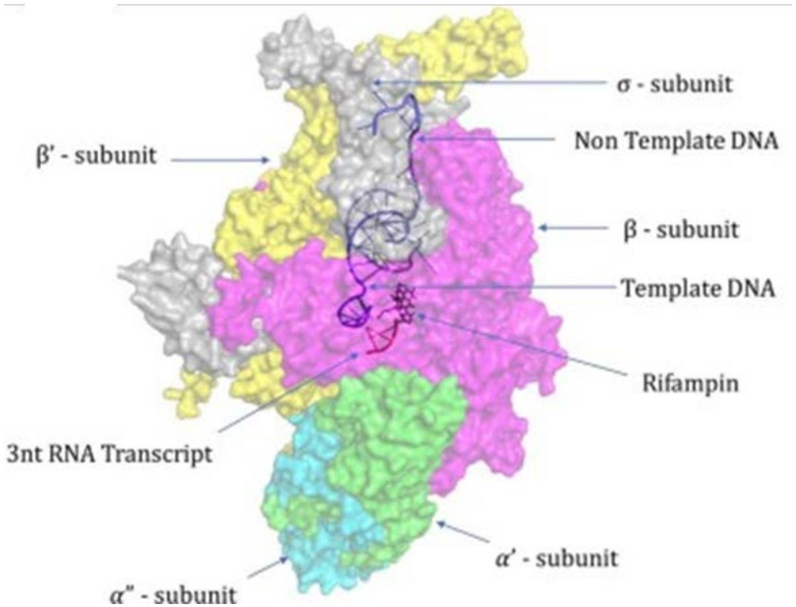
Subunit	Size	#/Molecule	Function
α	36.5 kD	2	chain initiation and interaction with regulatory proteins
β	151 kD	1	chain initiation and elongation
β'	155 kD	1	DNA binding
σ	70 kD	1	promoter recognition

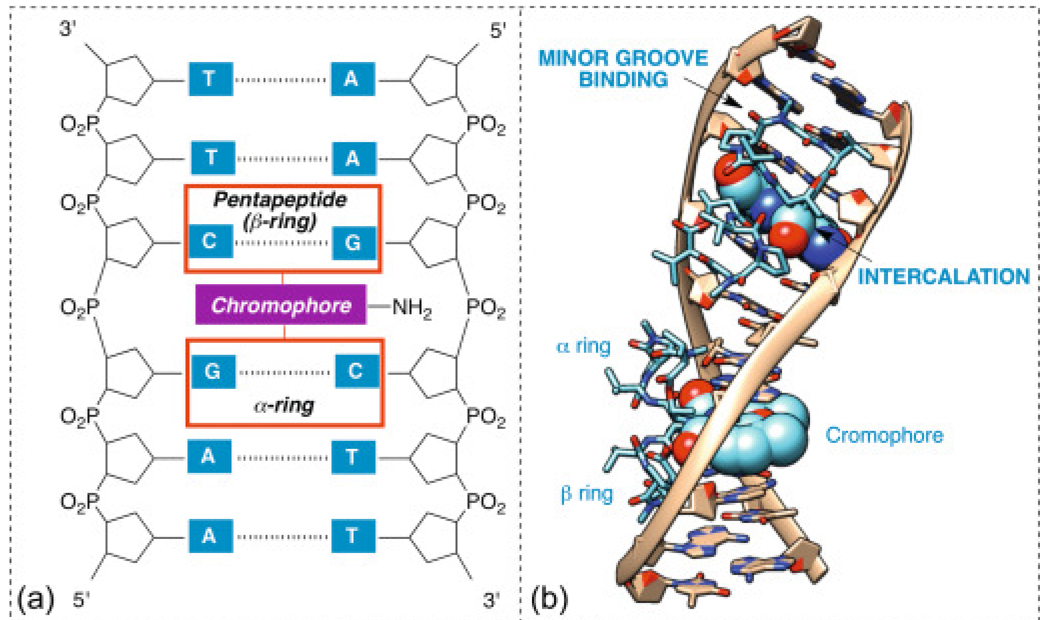


Prokaryote transcription and antibiotics

Rifampin (ansamycin antibiotic) blocks transcription initiation binding RNA polymerase,.

Actinomycin D (anti-cancer phenoxazone antibiotic) intercalates DNA prevents transcription.



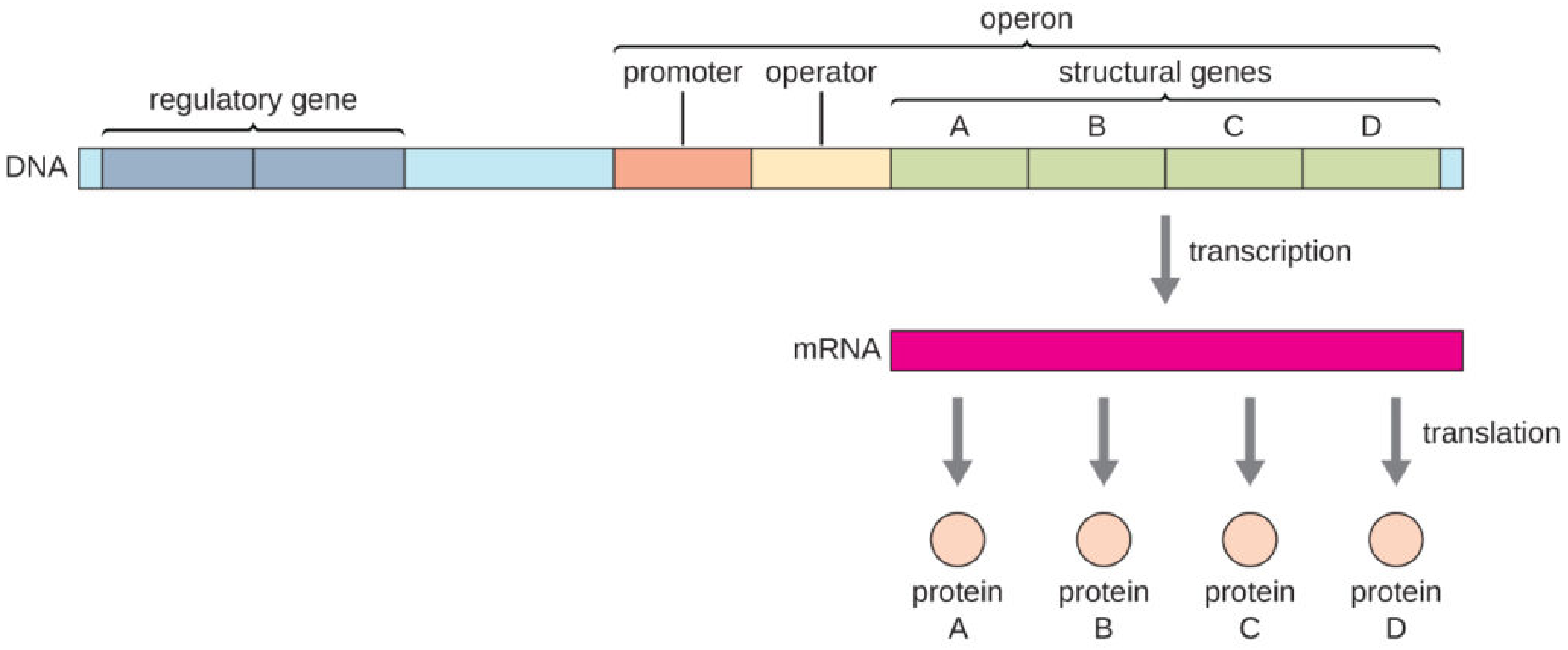


Prokaryote operons

Prokaryotic mRNA is often polycistronic (encodes multiple proteins).

Gene regulation in bacteria is mediated by operons.

Operons can be either **Inducible** or **Repressible**

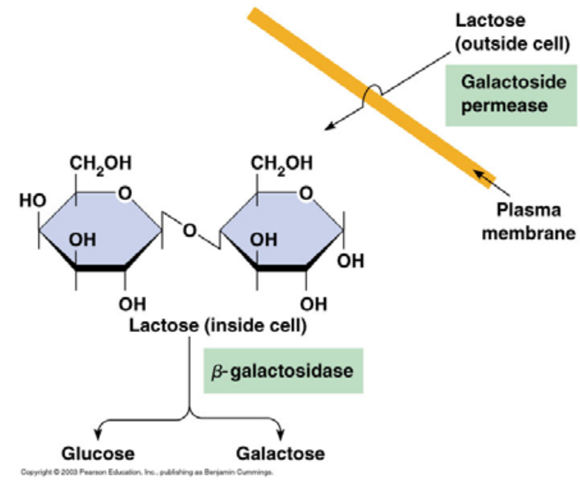


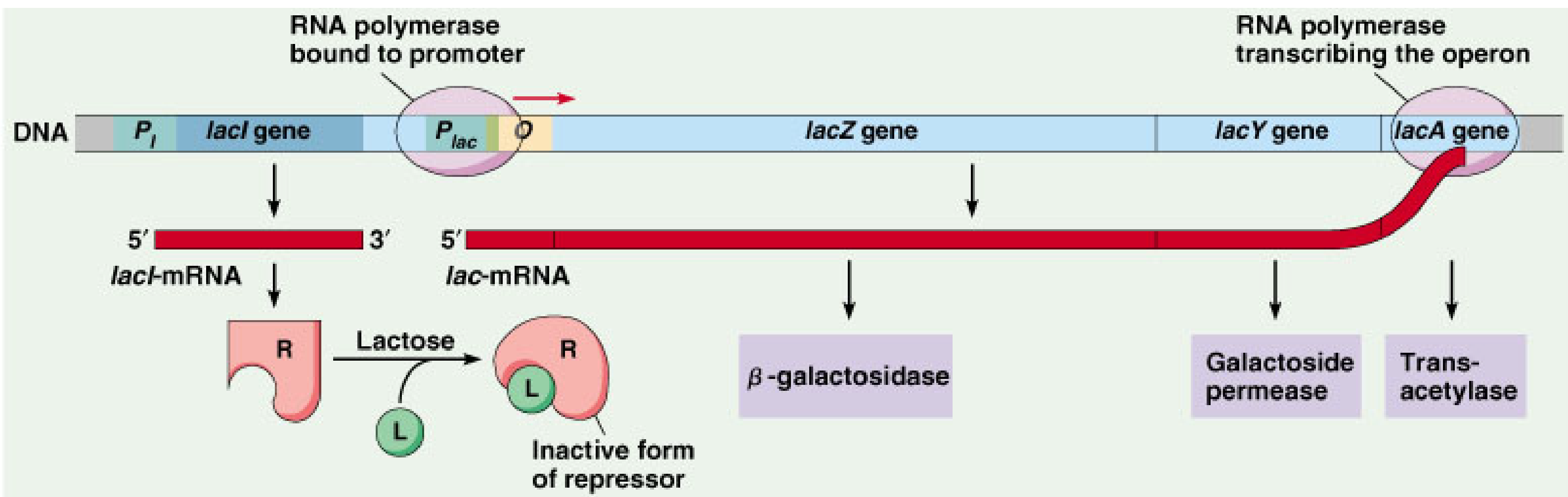
Prokaryote Lac operon (inducible operon)

In the presence of glucose, it is not necessary to expend energy to use lactose!

In the absence of glucose but presence of lactose, it would be beneficial to use lactose as energy source.

Requires:
- A transporter that allows lactose to enter
- An enzyme that digests lactose
- An enzyme that helps digest lactose (by transferring acetyl)





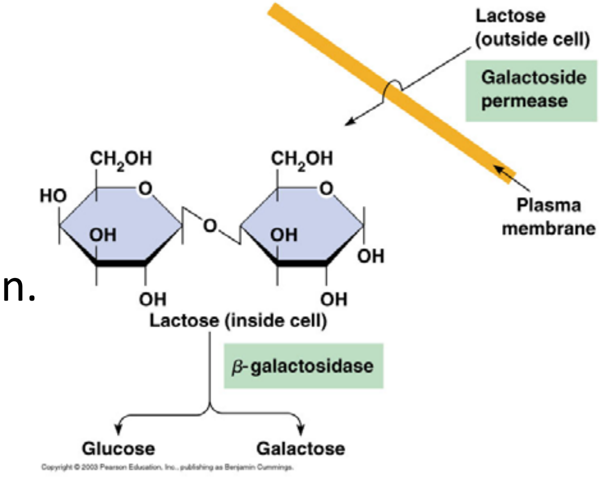
Prokaryote Lac operon (inducible operon)

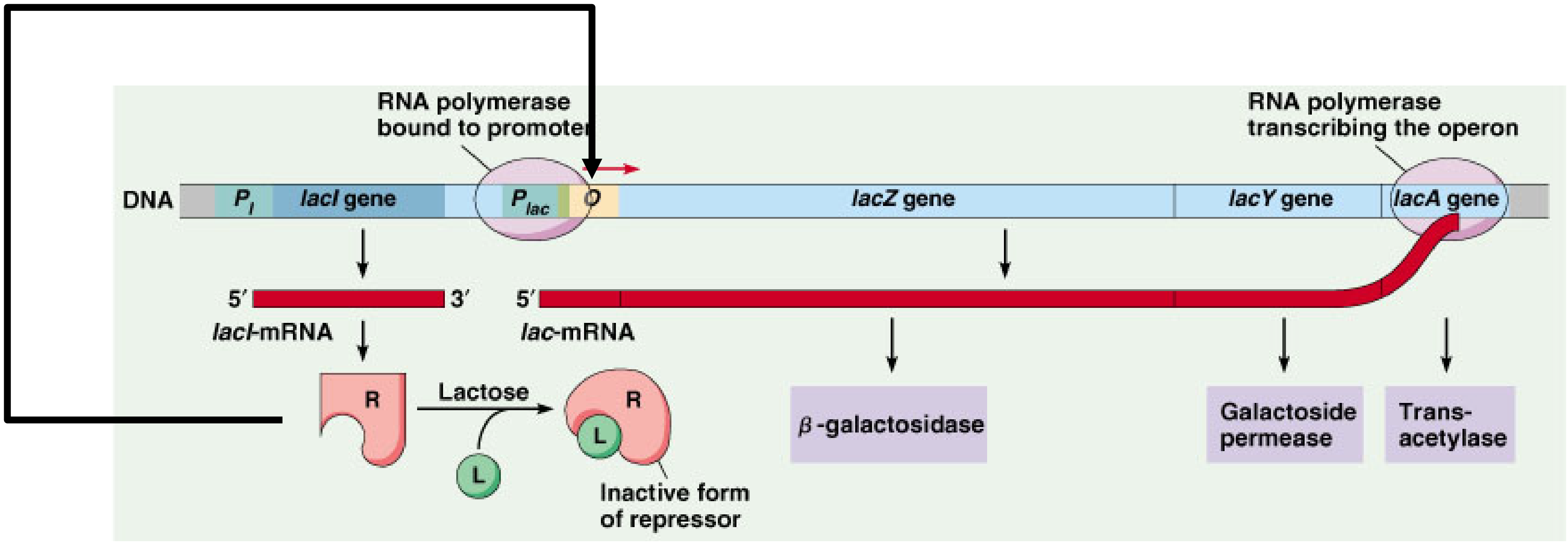
In the absence of lactose, there's no reason to keep this energetically expensive system running... It won't do any good.

Lactose doesn't bind to the repressor

Repressor recognizes and binds to operator, repressing the operon.

When either glucose or lactose are NOT available !





Prokaryote Lac operon (inducible operon)

Before the Lac operon is activated the bacterial cell has to acknowledge the absence of glucose…

No use in turning the Lac operon on if glucose is available.

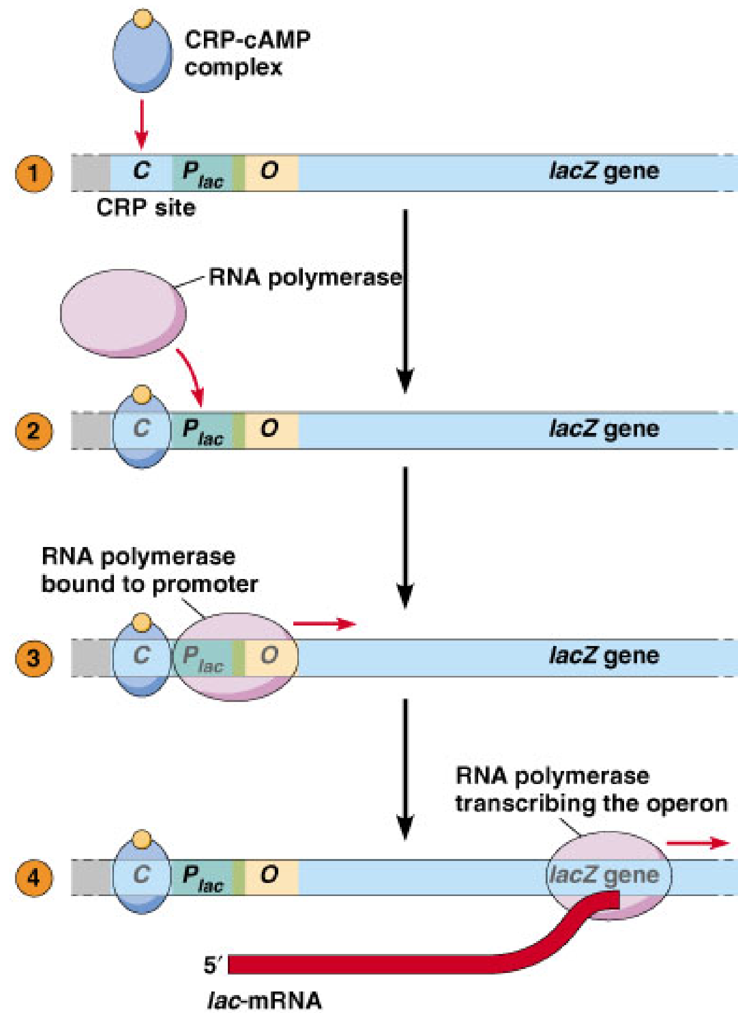
Sensing the amount of cAMP present.

Bacterial starvation leads to accumulation of cAMP.

cAMP is bound by CRP (cAMP receptor protein), a transcription activator protein.

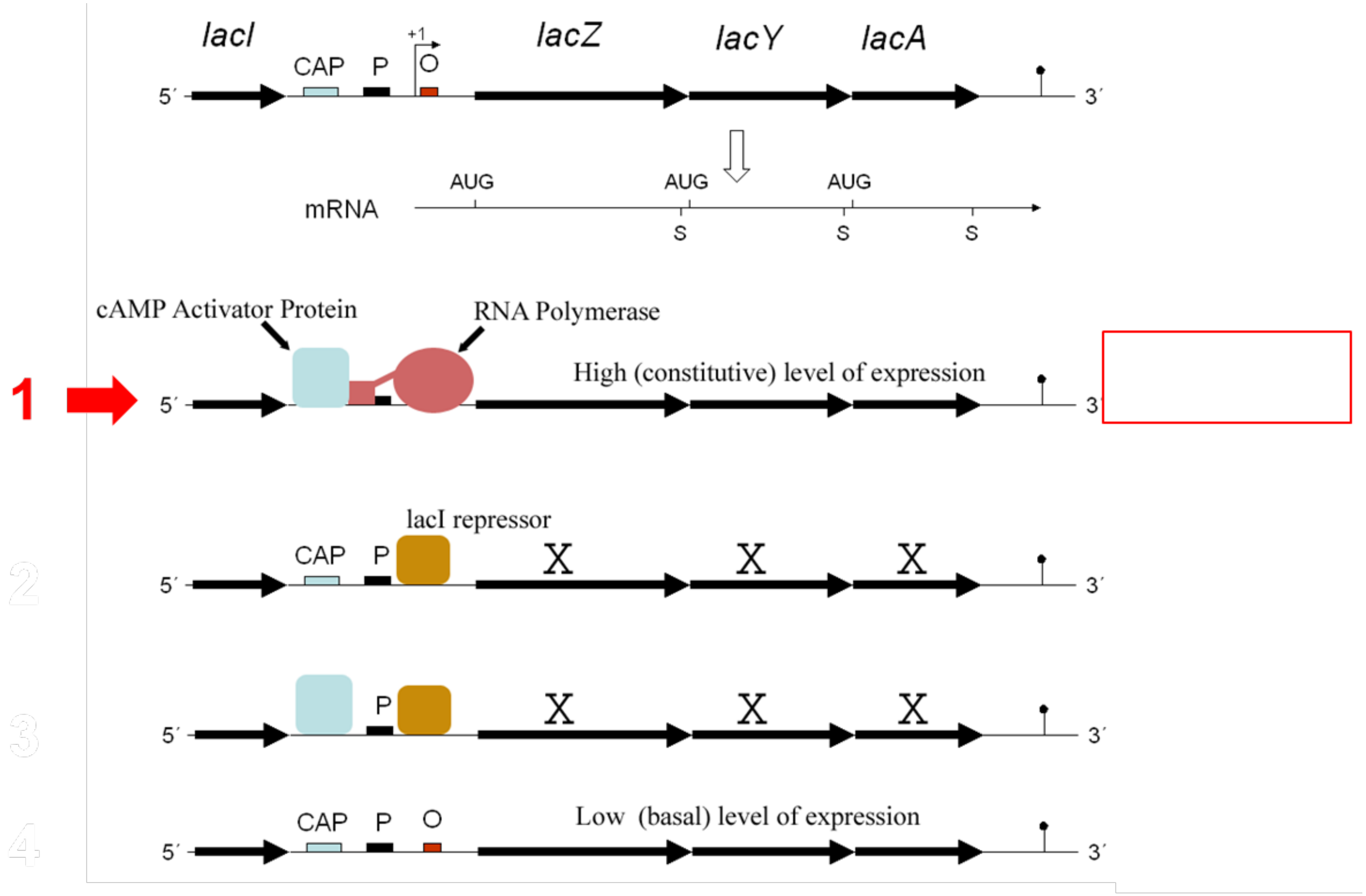
cAMP-CRP in CRP site recruit RNA Polymerase.

RNA polymerase can only transcribe if repressor is absent (linked to lactose).



(b) Action of CRP-cAMP complex (active CRP)

Lets exercise your knowledge



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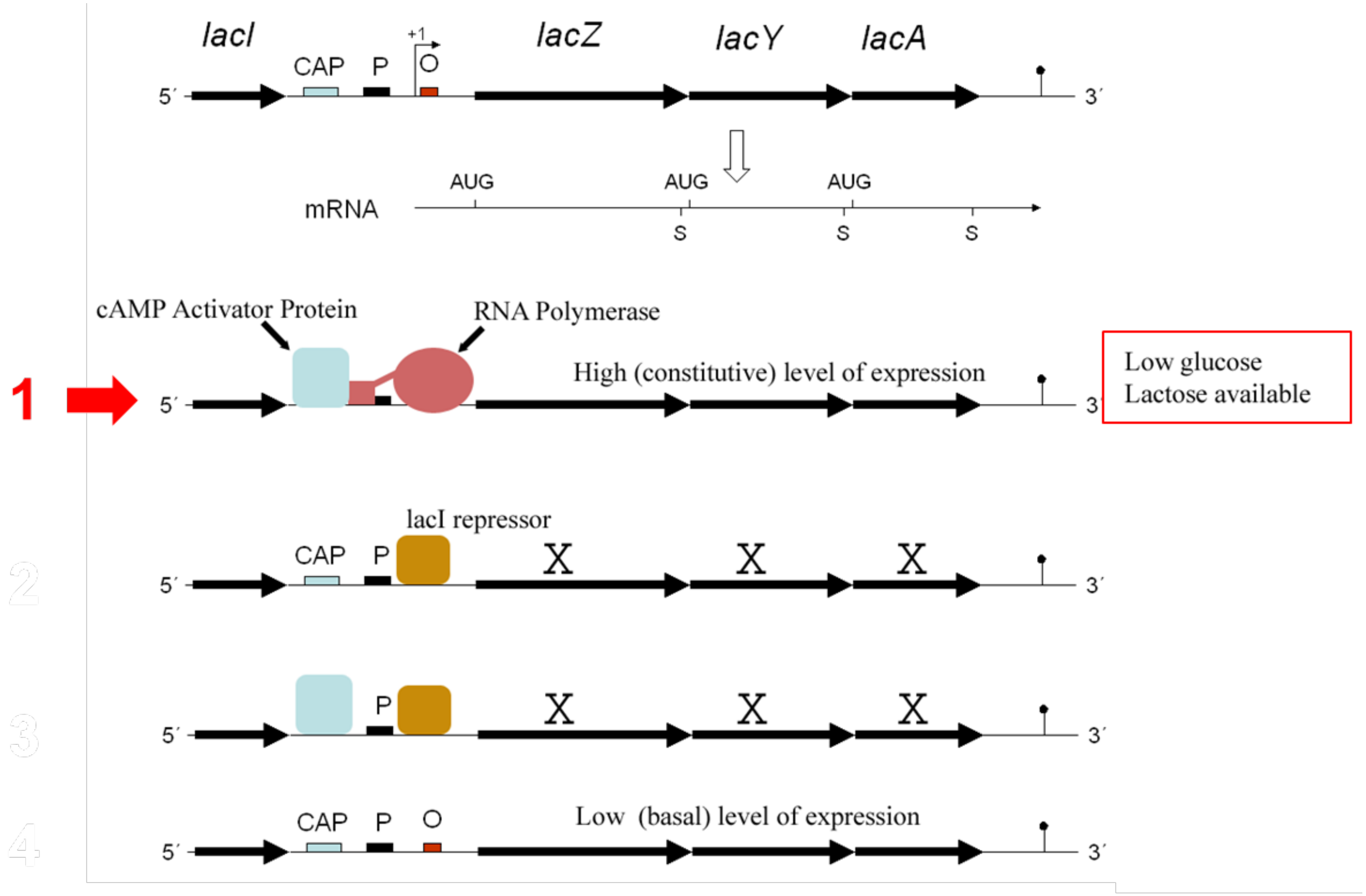

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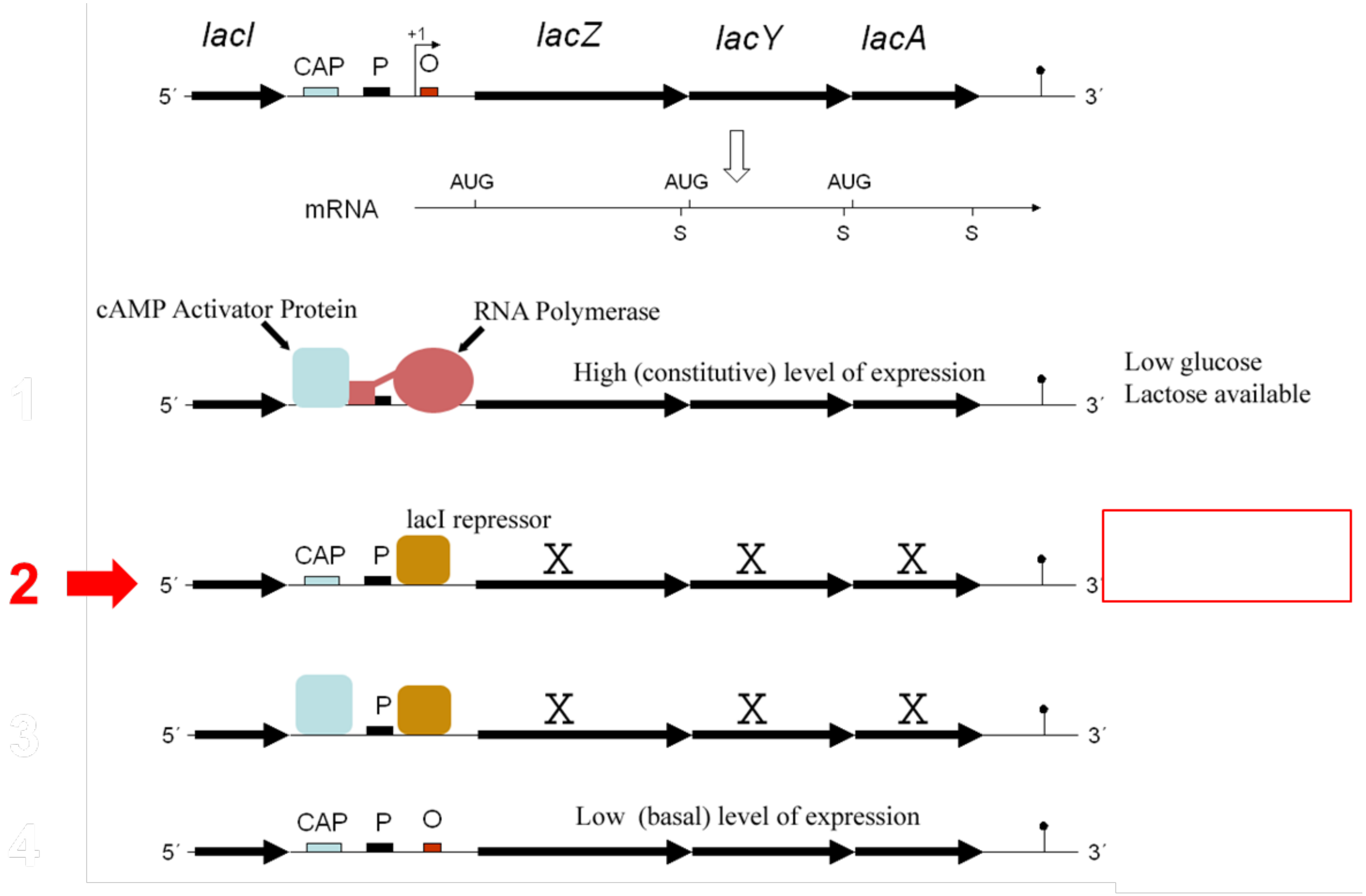

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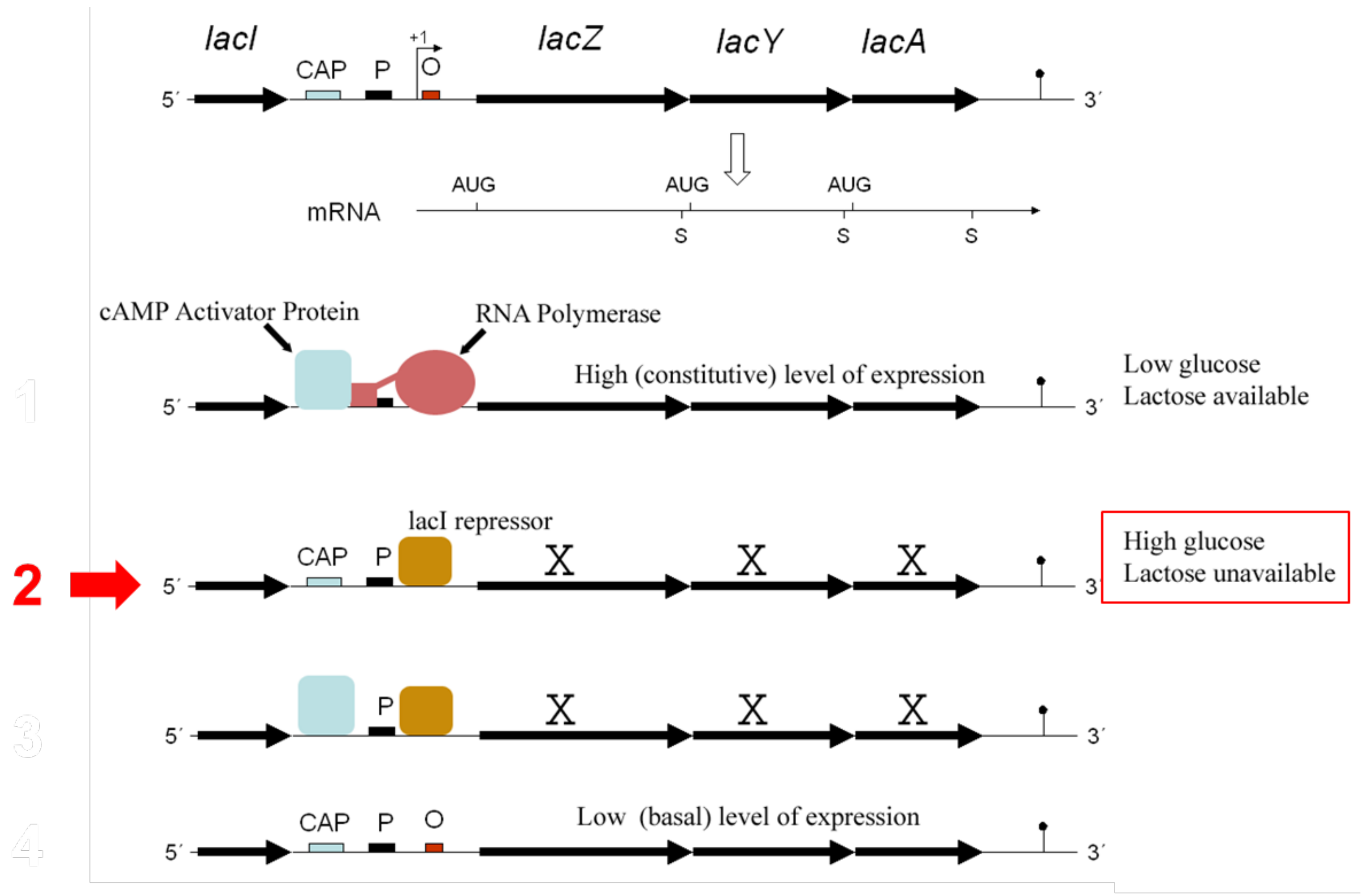

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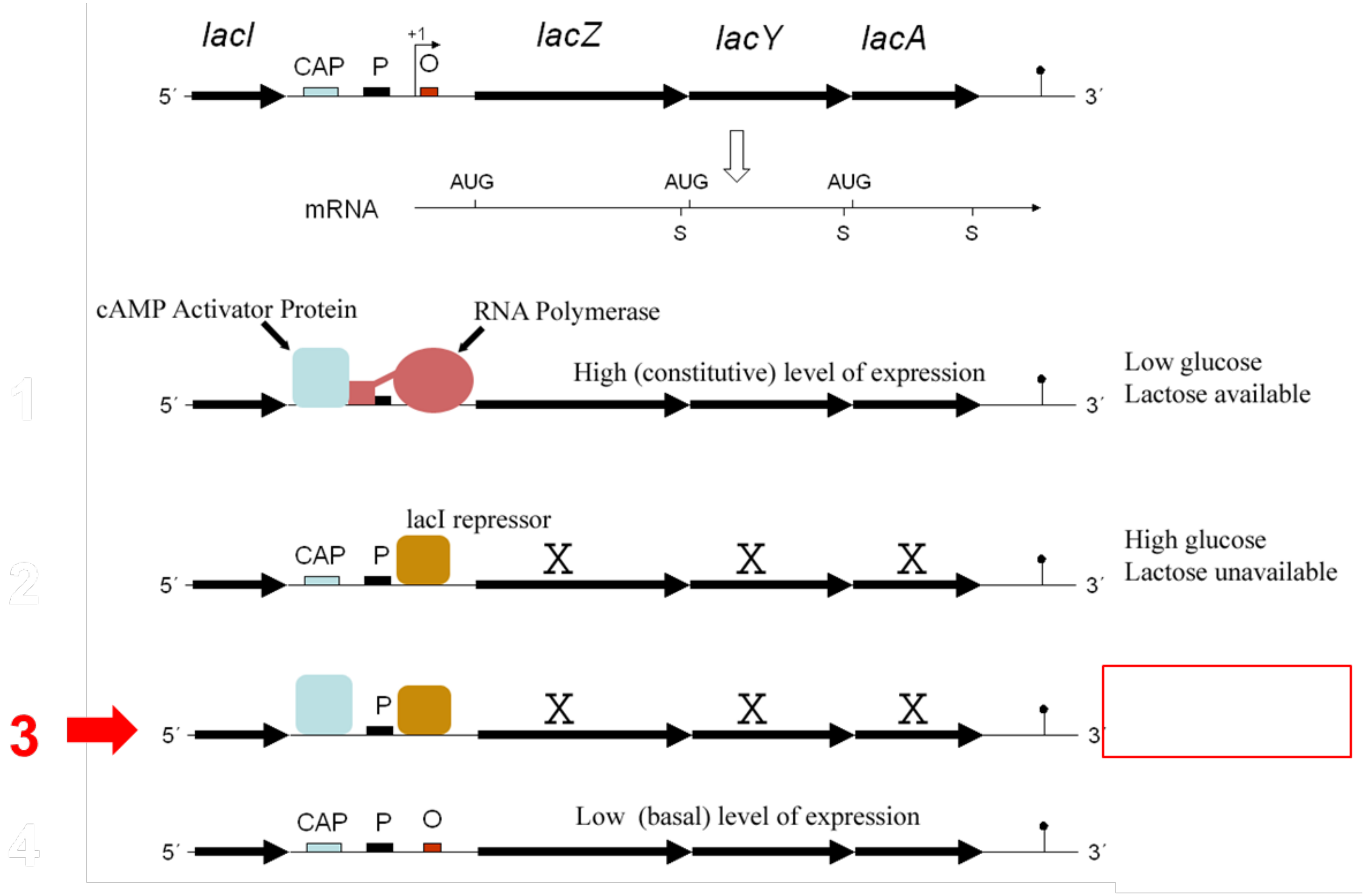

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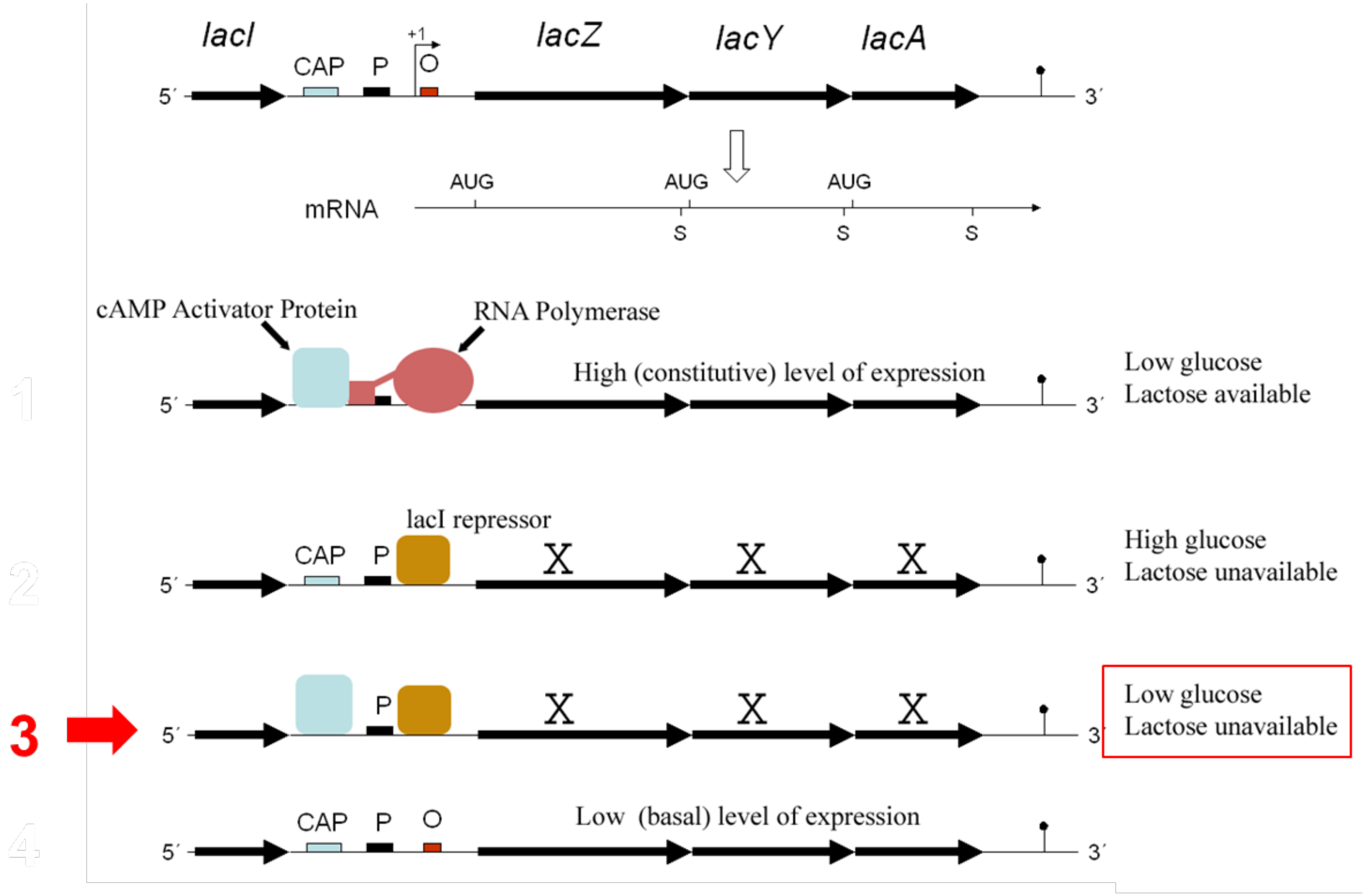

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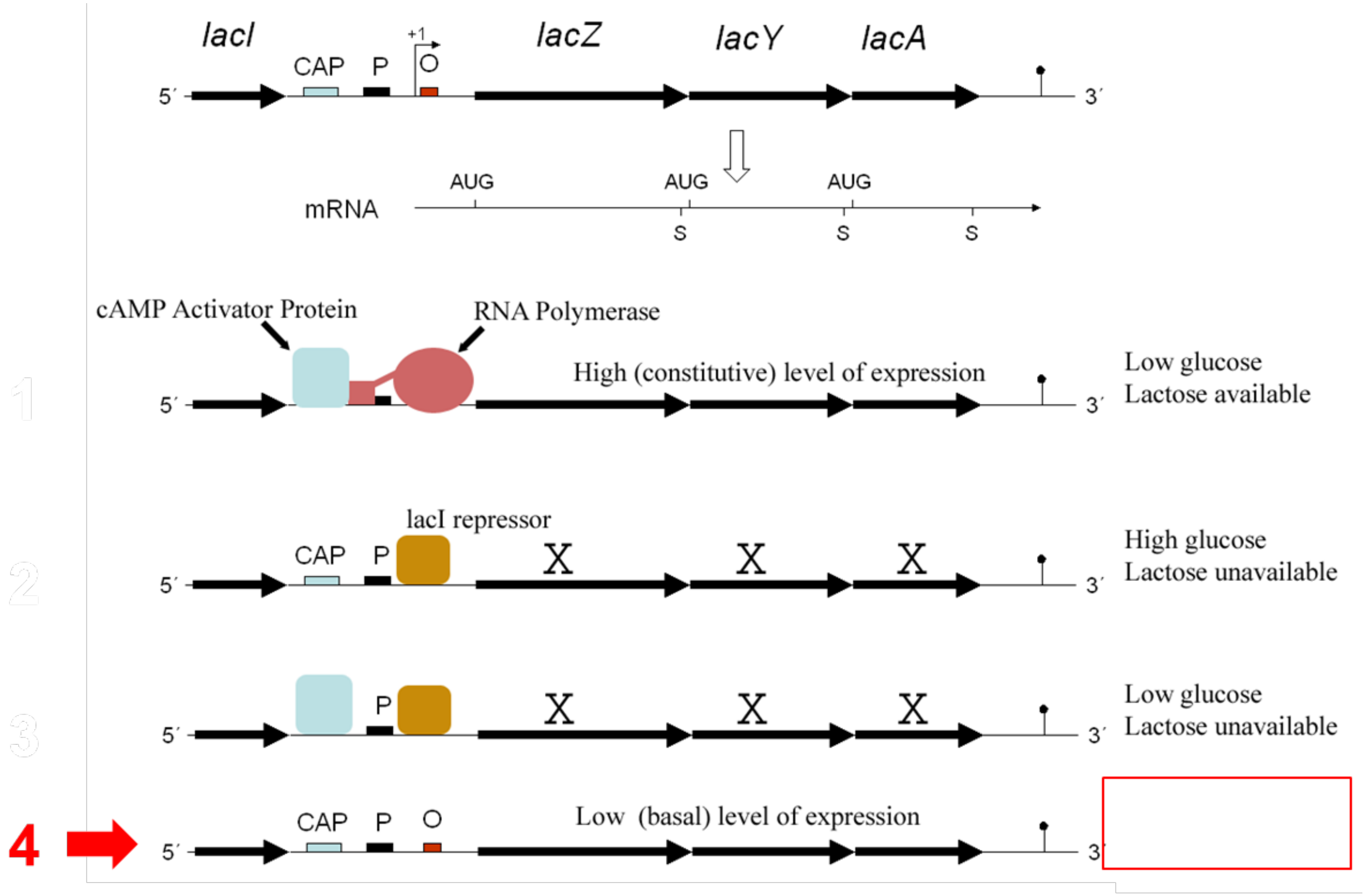

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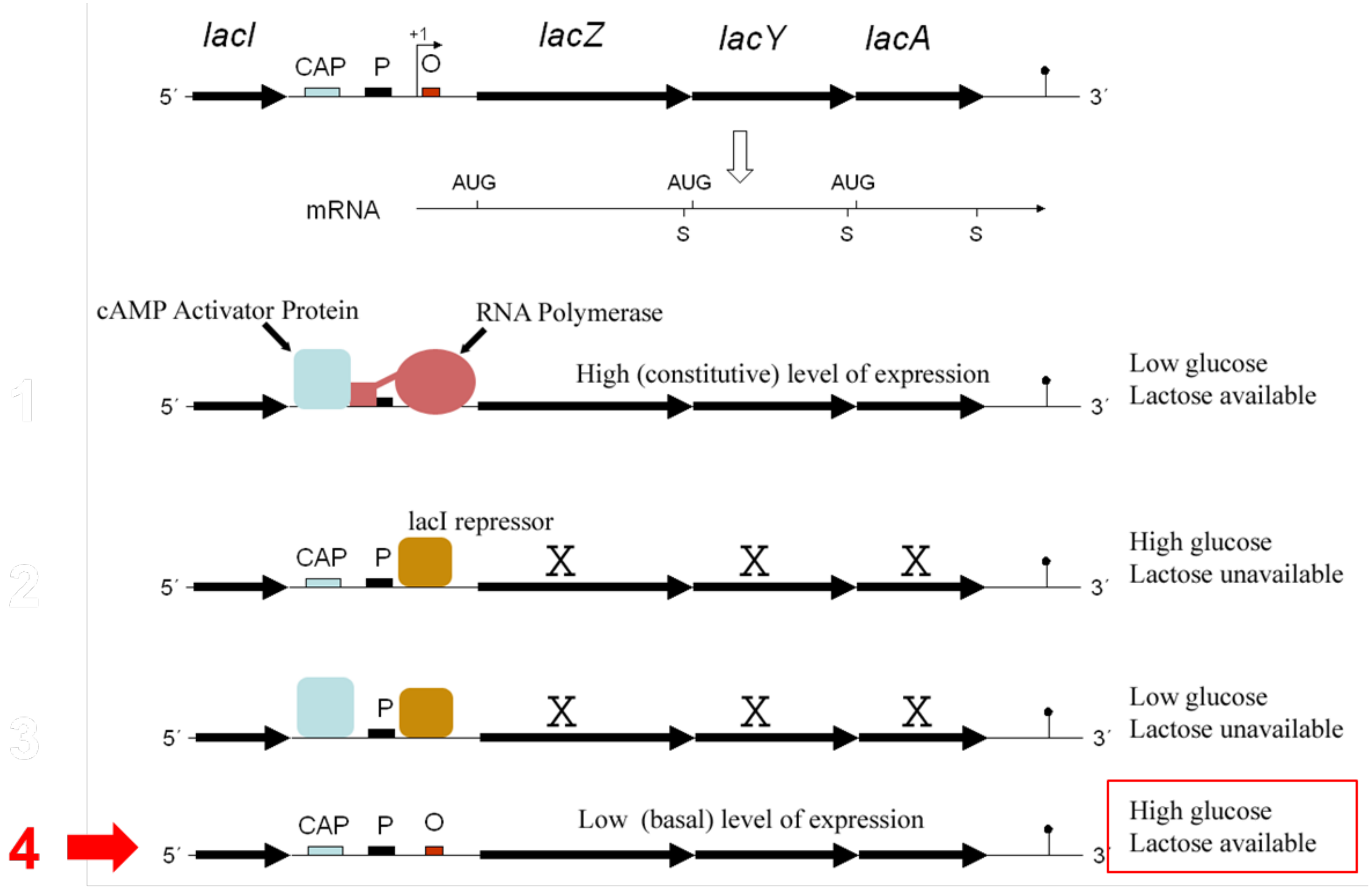


Prokaryote Trp operon (repressible operon)

Repressible operon – A repressor molecule only binds to an operator when an inducer is present.

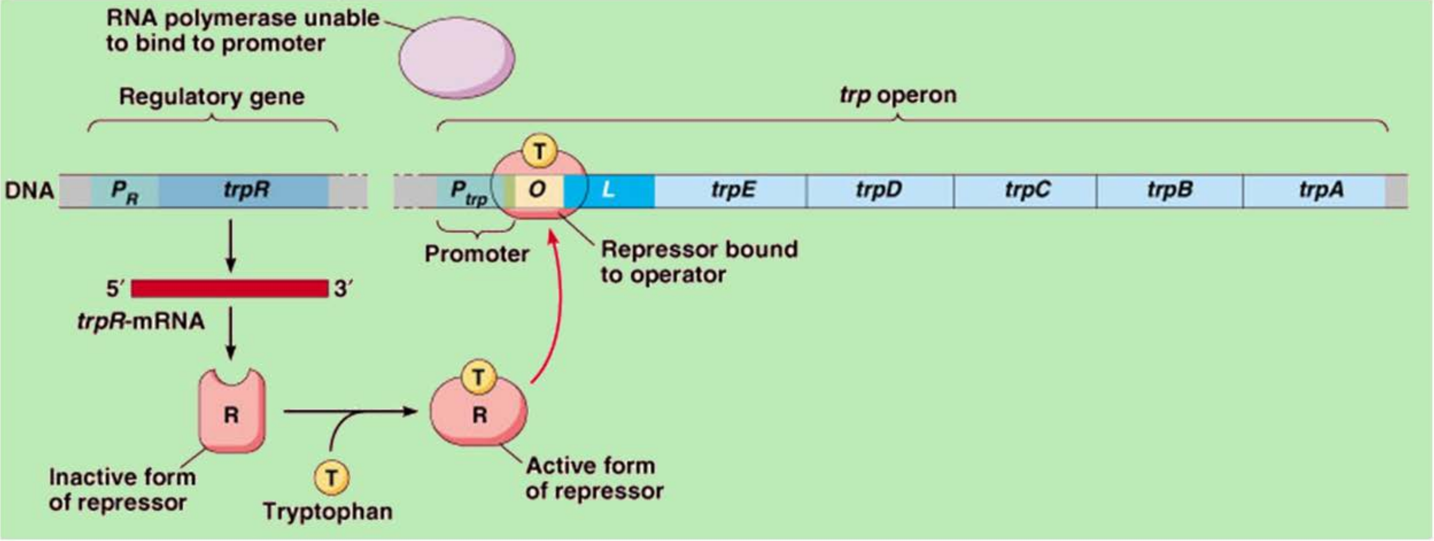
Bacteria require Trp for growth, whether or not it is provided by the environment.

When Trp is present in the environment, the bacteria shut down the operon that produces Trp.

This Trp itsel increases the repressor's affinity for the operator (DNA sequence).

This stops the Trp operon from being expressed.

Tryptophan present, repressor bound to operator, operon repressed



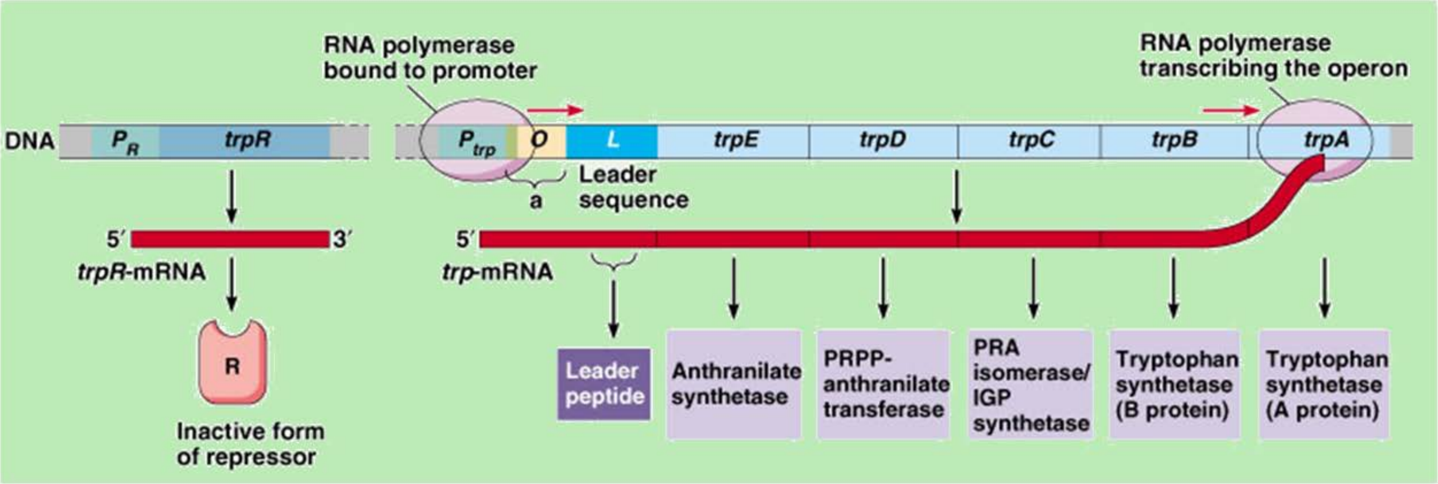
Prokaryote Trp operon (repressible operon)

In the absence of Trp, the repressor never binds to the operator nor can it block the passage of RNA pol.

RNA pol transcribes genes trpE through trpA.

These genes allow the bacterium to synthesize Trp itself without relying on environmental supply.

Tryptophan absent, repressor not bound to operator, operon activated



Prokaryote trasncription termination

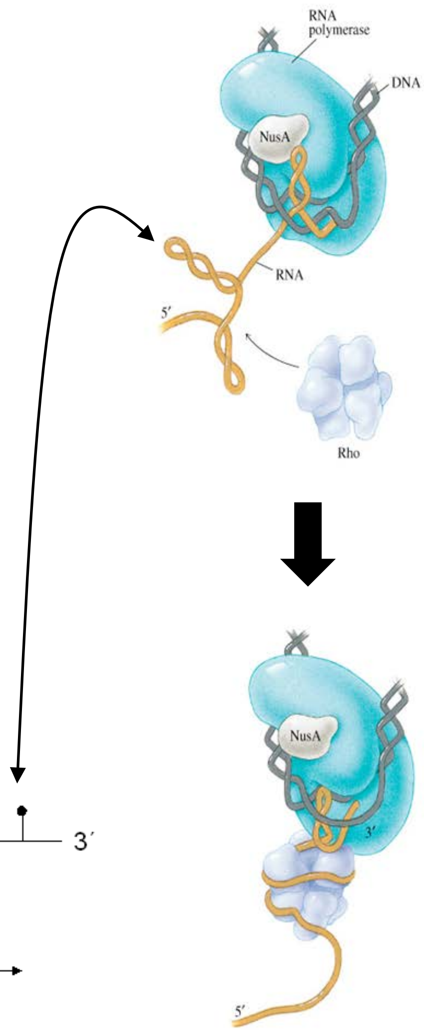
Rho-dependent

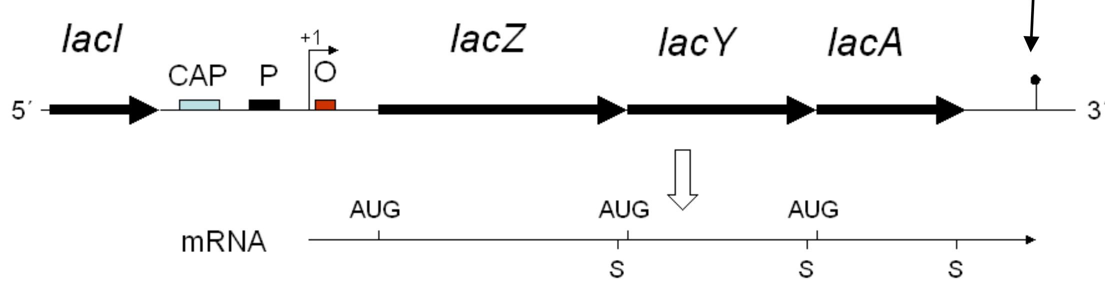
RNA pol passes the stop codon in DNA.

After the TER, it encounters a specific sequence: the Rho-Binding Site.

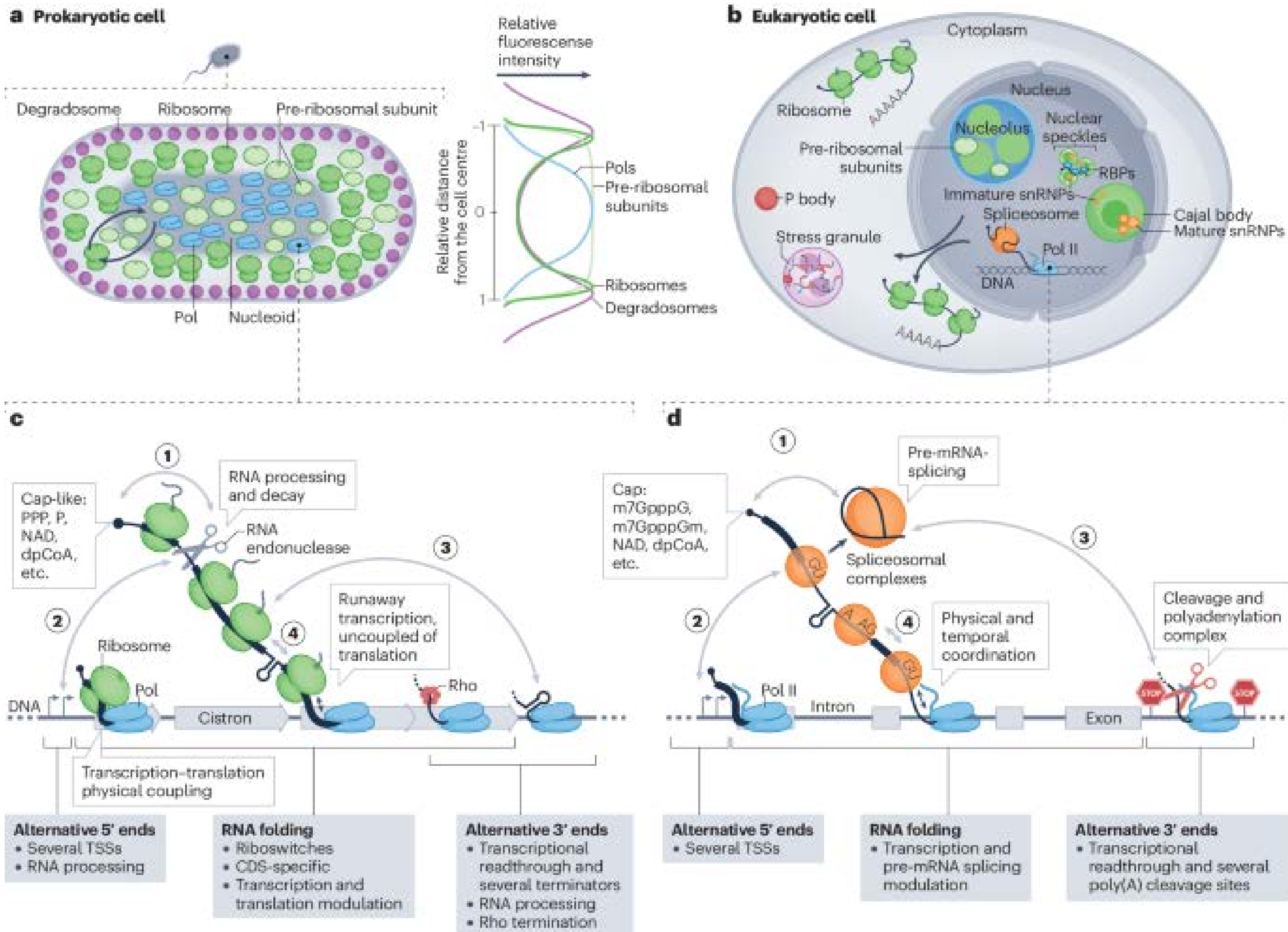
Rho protein recognizes this region and binds to it (a process that requires ATP).

Rho binding to mRNA "releases" the transcript and stops transcription.





Overview of prokaryote & eukaryote differences



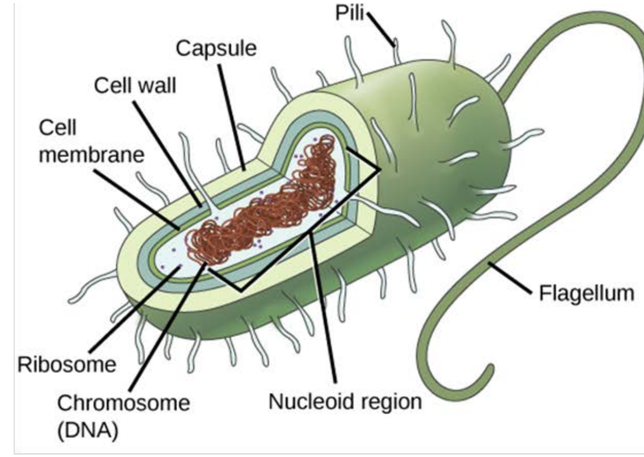
Prokaryote transcription

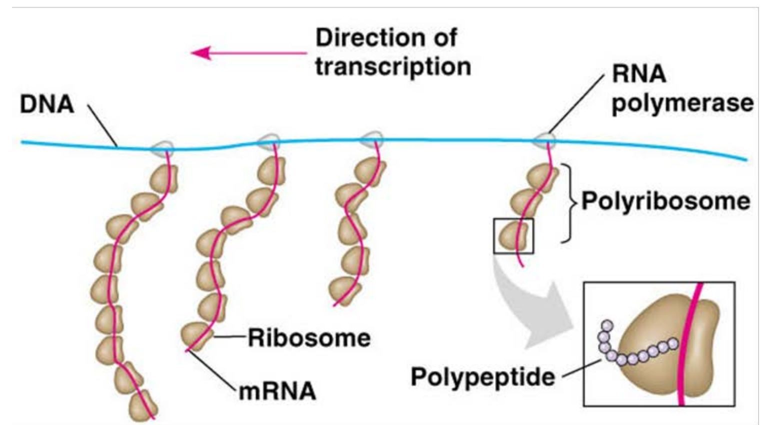
No physical separation between nucleoid & protoplasm.

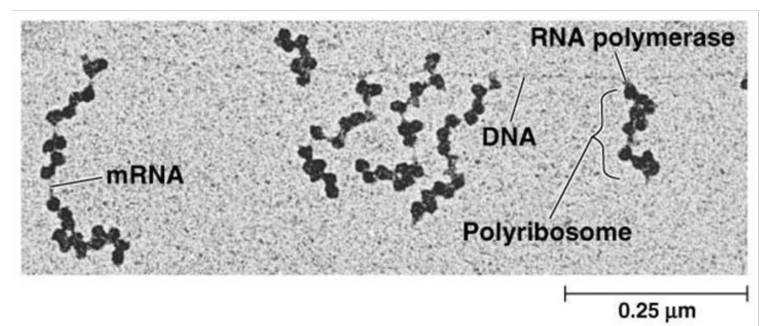
Transcription and translation are coupled: Ribosomes translate mRNA as it is being transcribed.

Termination

- rho-independent termination (GC-rich hairpin loop followed by a U-rich sequence), or
- rho-dependent termination, involving rho protein unwinding of RNA-DNA hybrid.







Tree of Life

Metaphor expresses idea that all life is related by common ancestor.

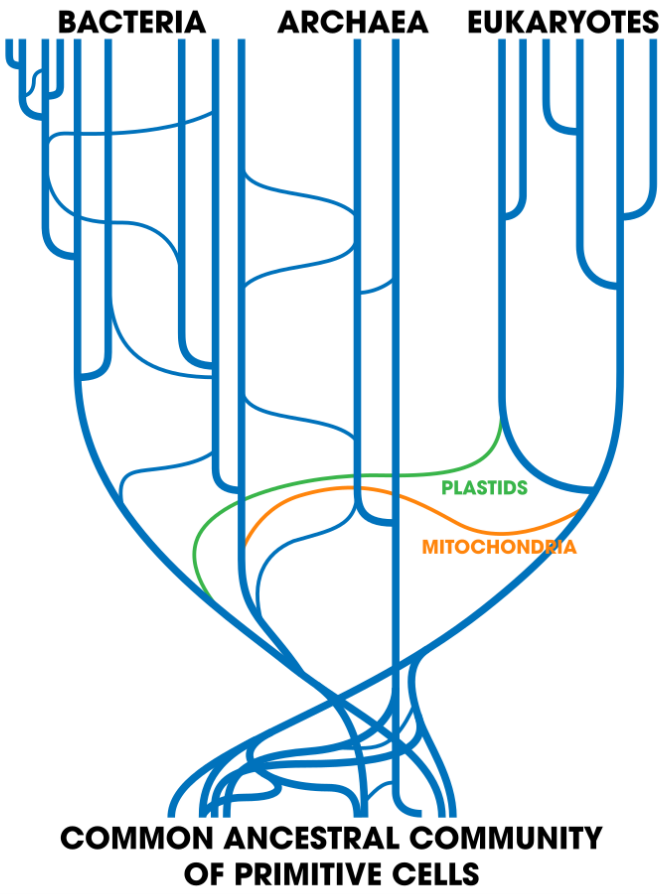
Product of traditional comparative anatomy, molecular evolution & molecular clock research.

It is now recognized that prokaryotes & archaea can transfer genetic information between them through horizontal gene transfer (HGT).

Archaea (αρχαία, "old ones") are single-celled organisms.

Originally discovered as extremophiles thriving at very high or very low temperatures, highly salty, acidic or alkaline water, geysers, black smokers, oil wells, and hot vents in the deep ocean.

In the past erroneously classed as prokaryotes (or Kingdom Monera) and named archaebacteria.



Eukaryote transcription

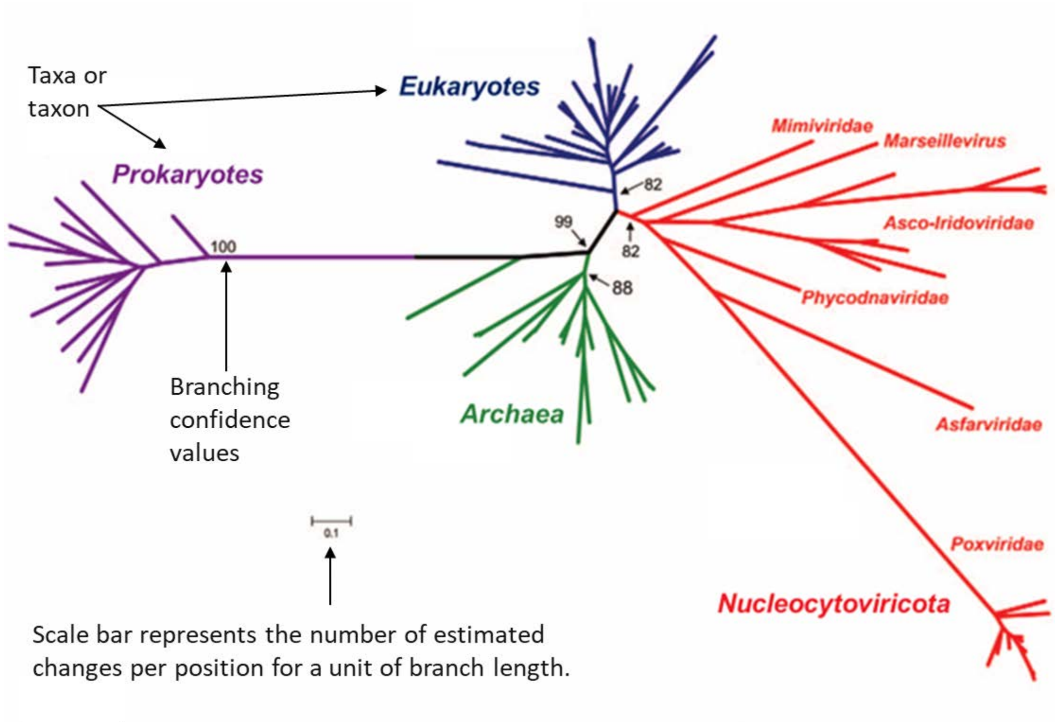
Eukaryotes use 3 RNA pols to synthesize the 3 different RNA.

Archaea possess a single RNA pol similar to eukaryotic RNA pol II.

Transcription occurs in the nucleus, translation in the cytoplasm.

Requires a greater number of TF.

The regulation of transcription in eukaryotes is more complex.



Eukaryotes have different promoters for each of the different RNA species.

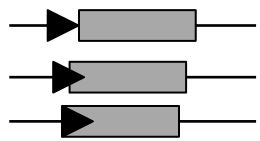
Phylogenetic tree of RNA polymerase II beta subunit.

Maximum likelihood unrooted tree inferred from 80 sequences.

RNA Polymerases

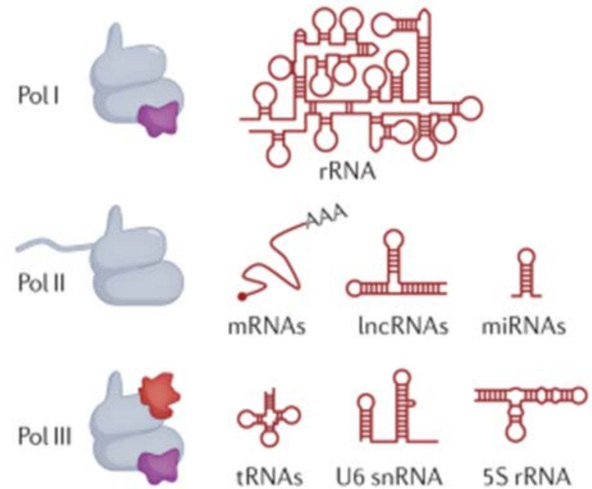
All are DNA-dependent RNA-polymerases (DdRp)

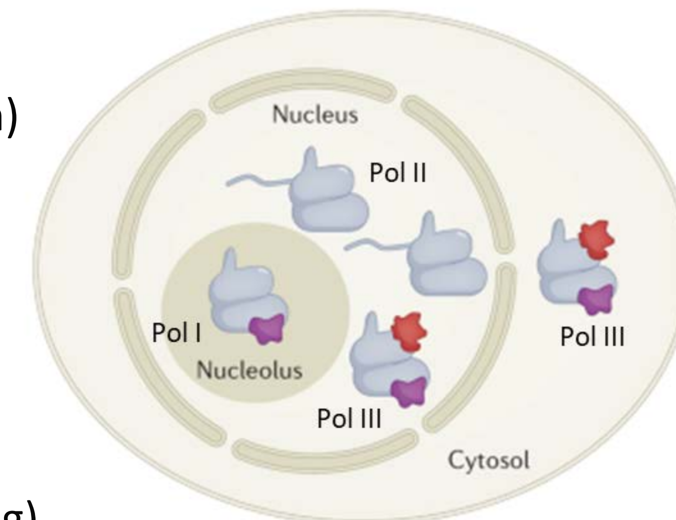
RNA Pol I transcribes 45S pre-rRNA:
•28S rRNA
•18S rRNA
•5.8S rRNA



RNA Pol II transcribes mRNA & non-coding RNAs.
- mRNA (codes proteins)
- snRNA (small nuclear RNA, splicing)
- miRNA (microRNA, post-transcriptional gene regulation)
- lncRNA (long non-coding RNA, regulatory roles)

RNA Pol III
- tRNA (RNA demodulation)
- 5S rRNA (the only rRNA not transcribed by Pol I)
- snRNA (U6, splicing factor)
- 7SL RNA (signal recognition particle for protein targeting)





Structural insights into nuclear transcription by eukaryotic DNA-dependent RNA polymerases. Girbig, M., *et al*. Nat Rev Mol Cell Biol 23, 603–622 (2022).

Eukaryote transcription factory

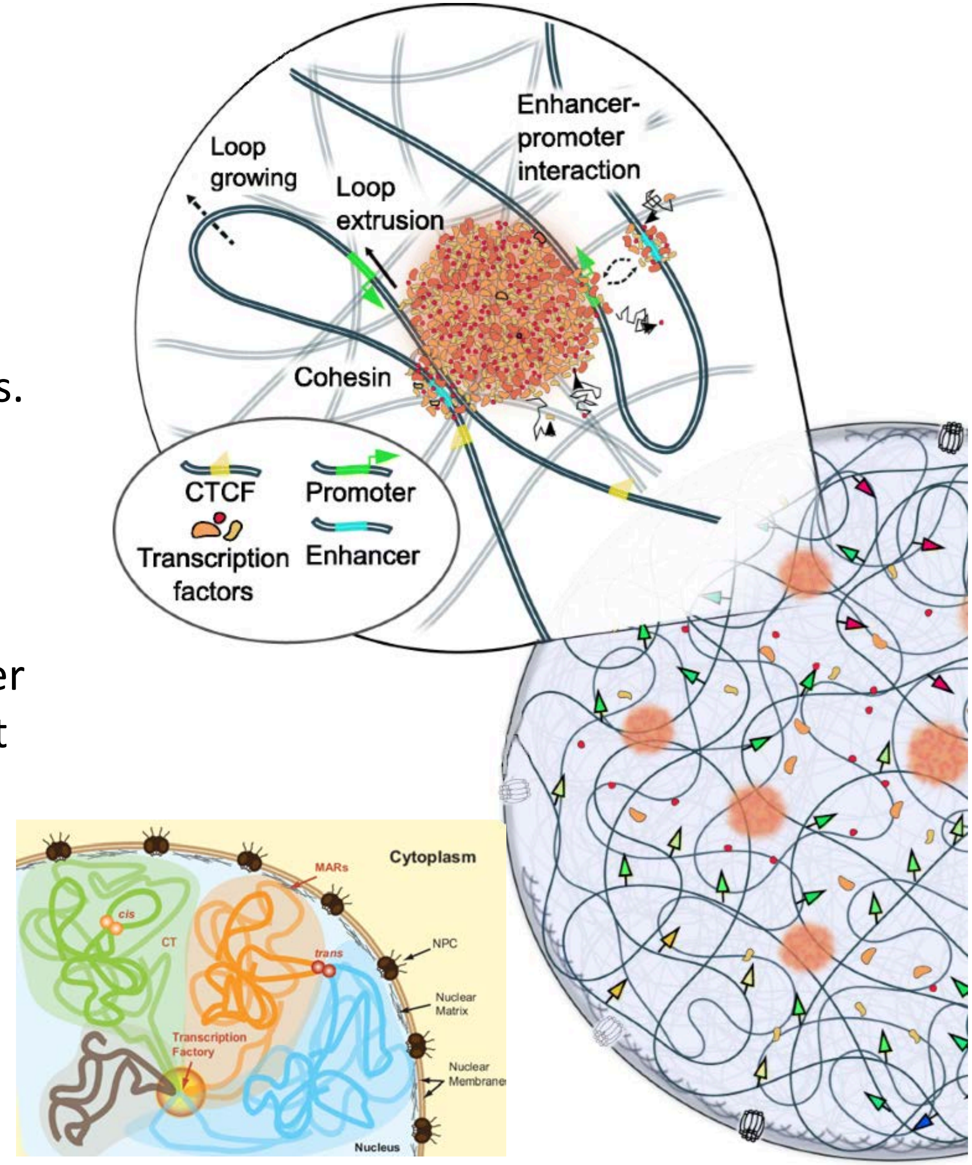
Specialized nuclear compartment where transcription of multiple genes occurs in a highly organized manner.

Clusters of RNA polymerase, transcription factors, and chromatin-associated proteins.

Facilitate efficient and coordinated gene expression.

Functional domains or loops bring together multiple active genes, often from different chromosomal locations, for simultaneous transcription.

Factories are dynamic and form or dissolve depending on cellular needs, stress, differentiation, or viral infections.



Wang, Xue Qing David & Crutchley, Jennifer & Dostie, Josée. (2011). Shaping the genome with Non-coding RNAs. Current genomics. 12. 307-21

Eukaryote RNA polymerases & amanitins

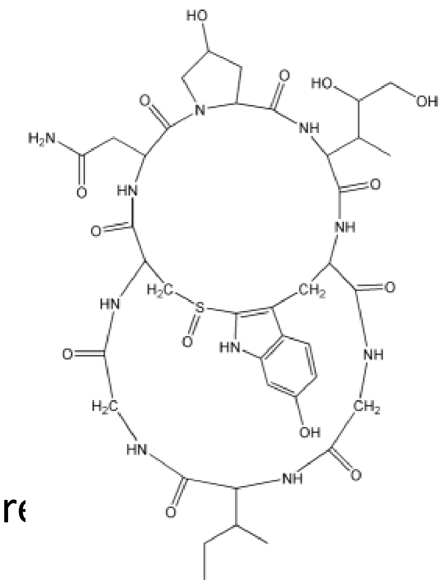
α-Amanitin is an octameric cyclic peptide.

Most deadly of all the amatoxins.

Toxins found in several species of Amanita.

The oral LD50 of amanitin is 100 µg/kg for rats.

Unlike most cyclic peptides, amatoxins (phallotoxins) are synthesized on ribosomes.



Eukaryotic RNA polymerases

RNA Polymerase	Location	Main Products	α-Amanitin Sensitivity
I	Nucleolus	Precursor for 28S rRNA, 18S rRNA, and 5.8S rRNA	Resistant
II	Nucleoplasm	Pre-mRNA and most snRNA	Very sensitive
III	Nucleoplasm	Pre-tRNA, 5S rRNA, and other small RNAs	Moderately sensitive*
Mitochondrial	Mitochondrion	Mitochondrial RNA	Resistant
Chloroplast	Chloroplast	Chloroplast RNA	Resistant

*In mammals.

Amanitin poisoning symptoms

Gastrointestinal Phase
(6–24 hours post-ingestion)

- Profuse watery diarrhea
- Nausea and intractable vomiting
- Severe abdominal cramping
- Hypovolemia, tachycardia & hypotension
- ⇩Na, ⇩ K, metabolic acidosis)

Hepatic Phase
(24–72 hours post-ingestion)

- Hepatocellular necrosis
- Jaundice
- AST, ALT > 1000 IU/L)
- Hyperbilirubinemia
- Coagulopathy
- Hepatic encephalopathy
- Lactic acidosis
- hHepatorenal syndrome

Multisystem Organ Failure Phase
(3–7 days post-ingestion)

- Fulminant hepatic failure
- Septic shock
- Metabolic acidosis
- Disseminated intravascular coagulation (DIC)
- Cardiovascular collapse
- Cerebral edema
- Death due to liver failure, renal failure, or sepsis

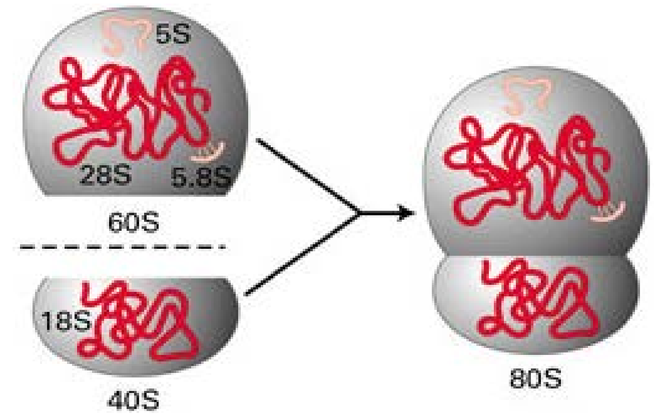
Key Laboratory Findings

- AST, ALT > 5000 IU/L
- Bilirubin > 5 mg/dL (severe cholestasis)
- Prolonged PT/INR > 2
- Metabolic acidosis w/high anion gap
- Elevated creatinine
- Hypoglycemia

Recovery Phase
(if liver transplant / supportive care successful)

Eukaryote ribosomal RNA (rRNA)

Eukaryotic ribosomes (80S ribosomes) consist of a large (60S) & small (40S) subunit.



18S rRNA (≈1,900 nucleotides)

- Core of 40S subunit.
- Essential for decoding mRNA and initiating translation.
- Binds to the mRNA and initiator tRNA.

28S rRNA (≈5,000 nucleotides)

- Largest rRNA
- Catalytic site of the ribosome (peptidyl transferase center).
- Responsible for transpeptidation reaction.

5.8S rRNA (≈160 nucleotides)

- Stabilizes ribosomal structure by interacting with 28S rRNA.

5S rRNA (≈120 nucleotides)

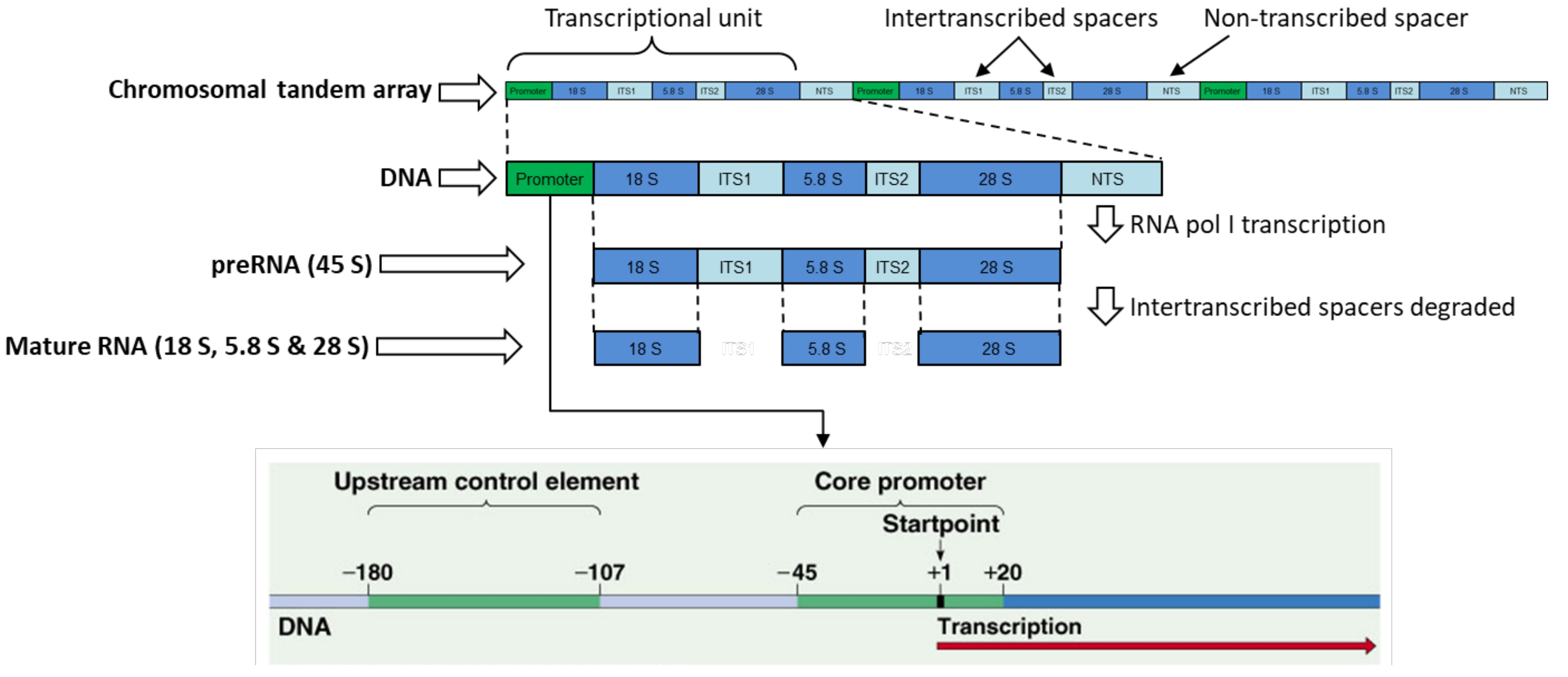
Transcribed by RNA pol III.

- Aids in ribosome assembly & structural integrity.

Eukaryote RNA Polymerase I promotor

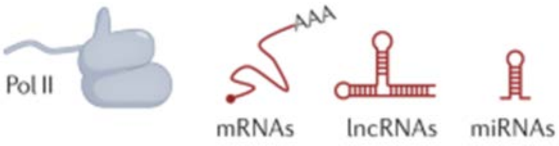
Eukaryotes have many copies of rRNA genes organized in tandem repeats.

Humans have approx 300–400 repeats present in 5 clusters, located on:
Chr 13 (RNR1), Chr 14 (RNR2), Chr 15 (RNR3), Chr 21 (RNR4) & Chr 22 (RNR5).



Eukaryote RNA Polymerase II

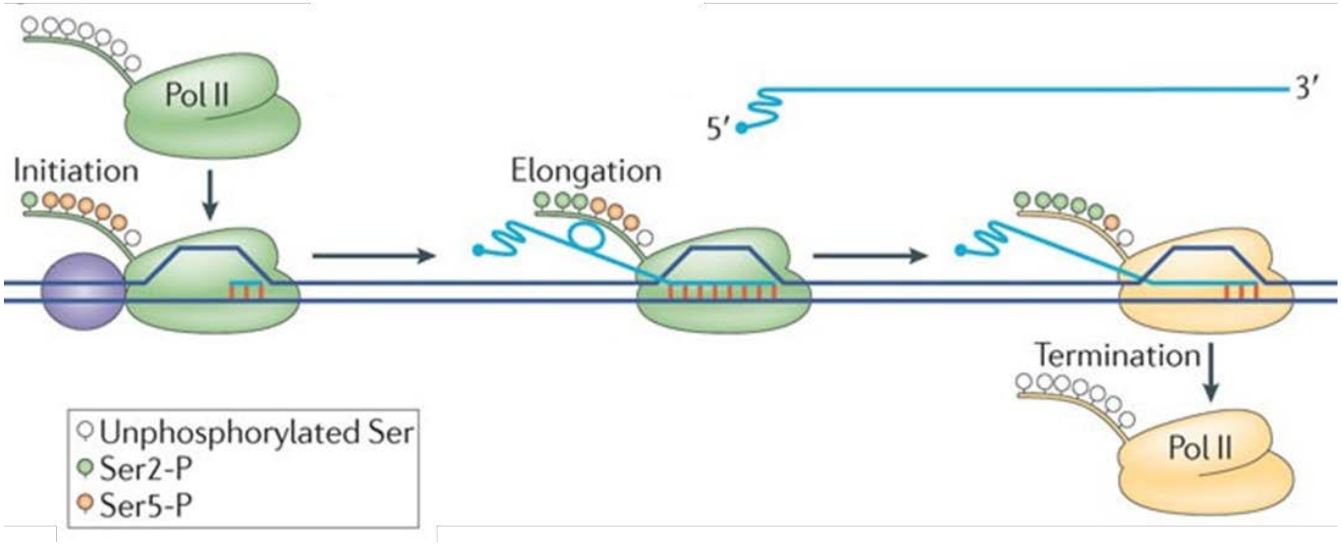
Transcribes mRNA and several ncRNAs.



12-subunit complex: catalytic core & C-terminal domain (CTD) that regulates transcription.

CTD Composed of heptapeptide repeats (YSPTSPS).

- Unphosphorylated: Promotes transcription initiation.
- Ser5 phosphorylation: Facilitates promoter escape & capping enzyme recruitment.
- Ser2 phosphorylation: Required for elongation, mRNA splicing & polyadenylation).



[Unravelling the means to an end: RNA polymerase II transcription termination. Kuehner, J., *et al*. Nat Rev Mol Cell Biol 12, 283–294 (2011).]

Eukaryote RNA Polymerase II promotor

Transcribes mRNA and several ncRNAs.

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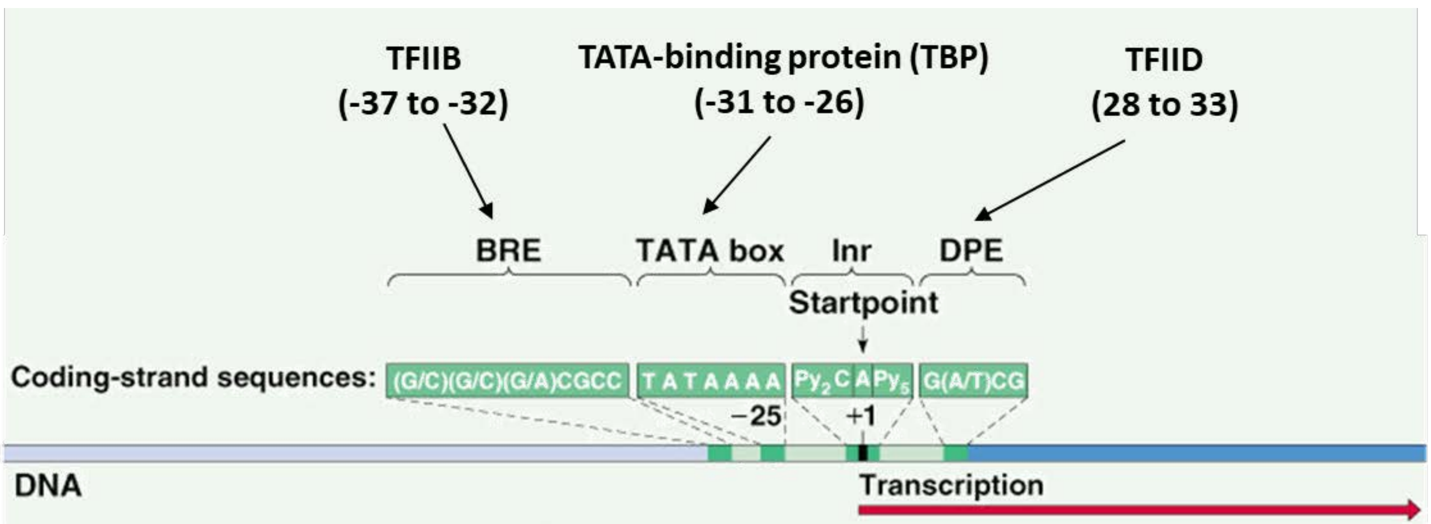
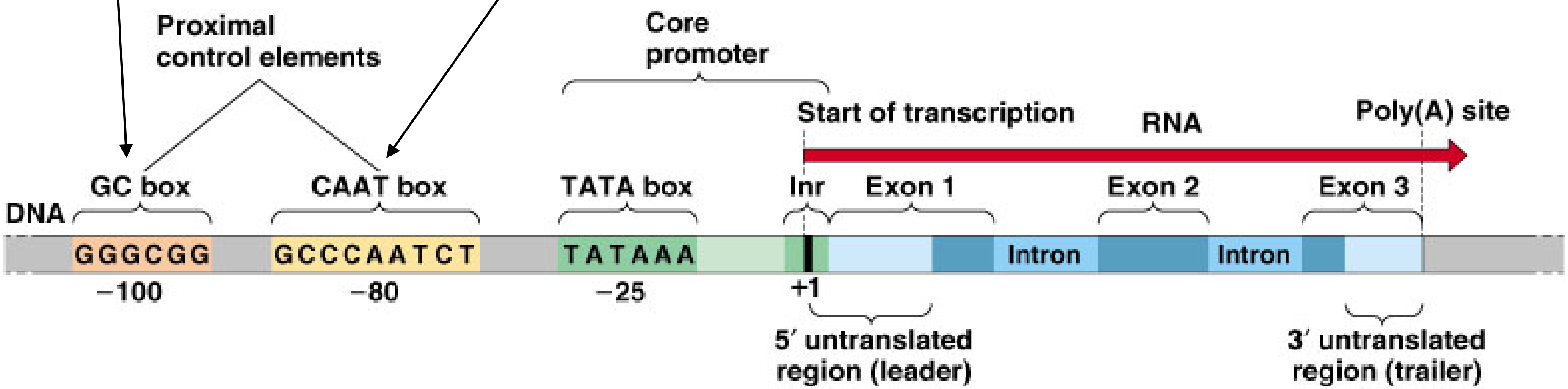


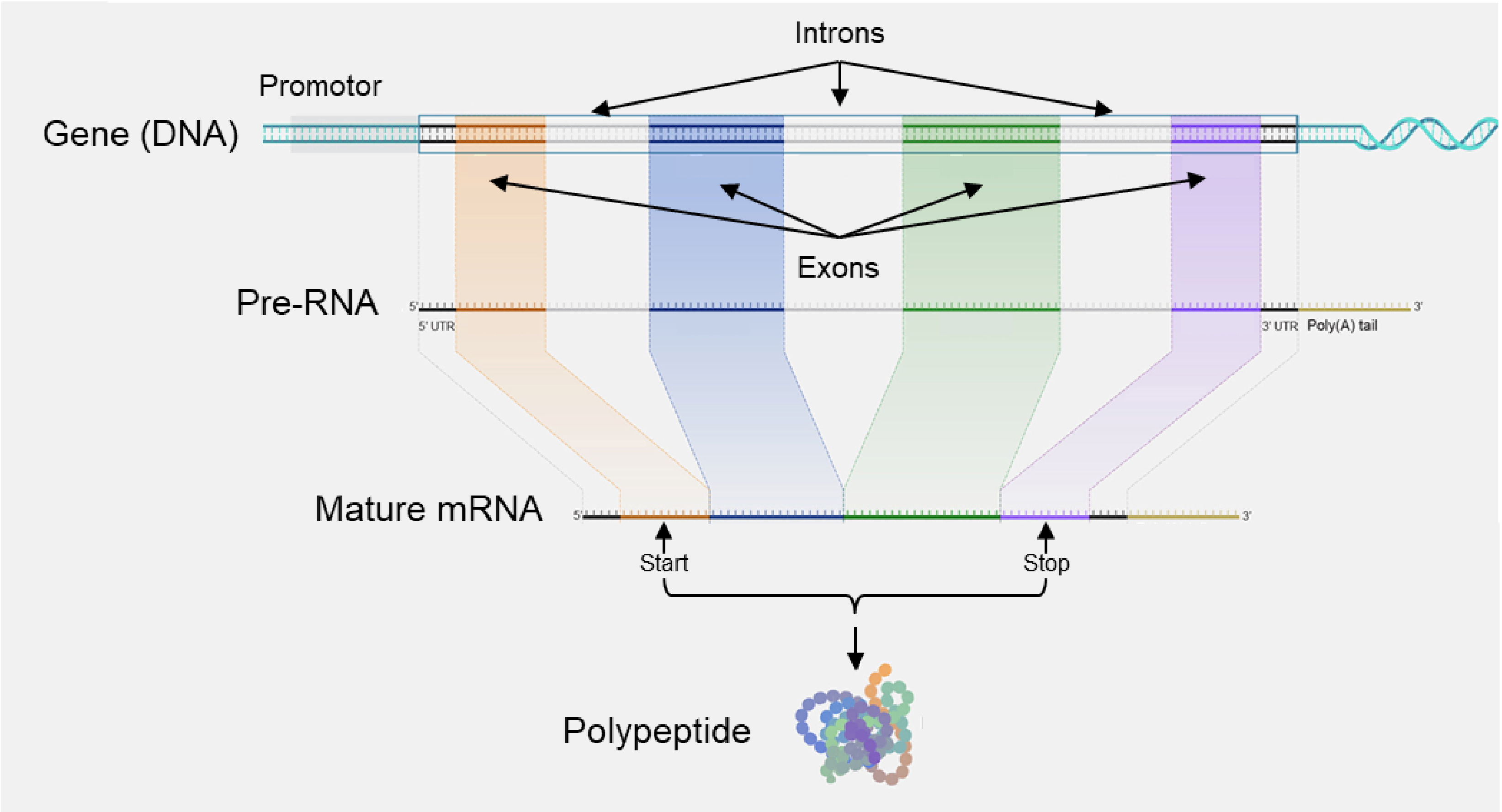
Eukaryote RNA Polymerase II promotor

GC box-binding proteins
(Sp1, Krox/Egr, Wilms' tumor, MIGI, CREA & zinc finger TF).

CCAAT/enhancer binding proteins (C/EBPs)



Eukaryote mRNA transcripts



Eukaryote RNA Polymerase III

Responsible for transcribing tRNA, 5S rRNA & small non-coding RNAs essential for protein synthesis and other cellular functions.

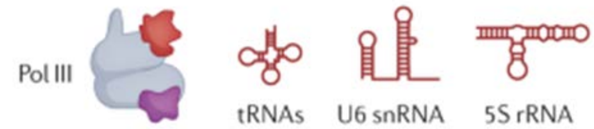
No helicase required (Pol III can open DNA on its own).

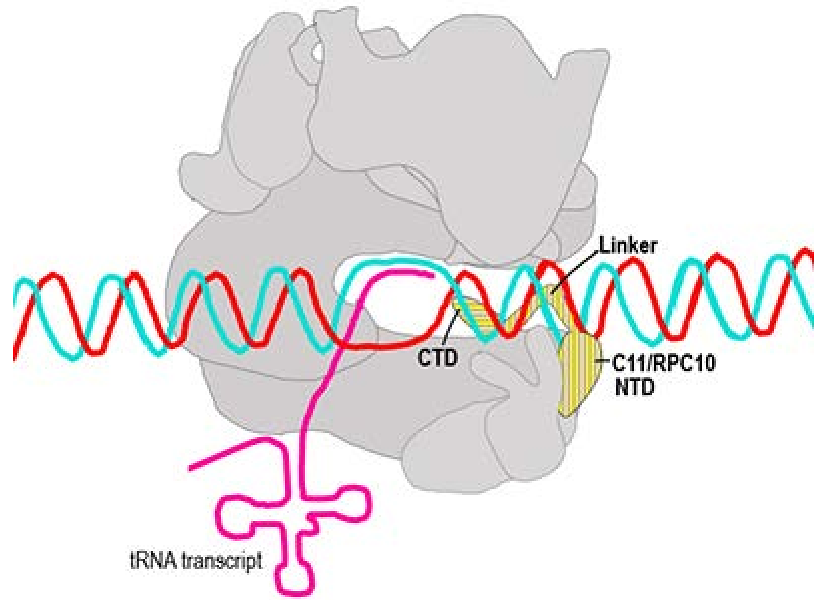
17-subunit complex, structurally similar to Pol II.

Minimal pausing during elongation compared to Pol II.

Intrinsic termination via poly(U) tract (similar to rho-independent termination in bacteria).

No cleavage/polyadenylation required.





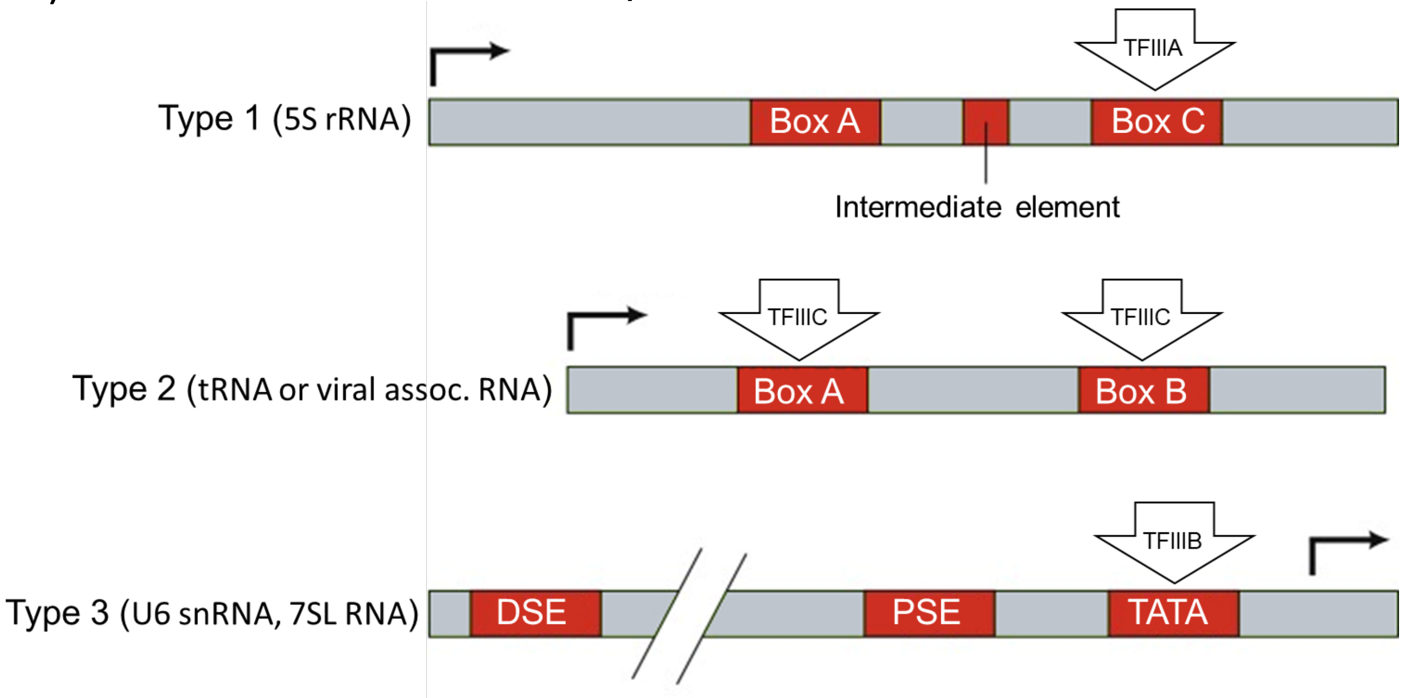
Type	Function
tRNA (transfer RNA)	Information demodulation
5S rRNA	Structural component of the 60S ribosomal subunit.
snRNA (U6)	Participates in RNA splicing (spliceosome function).
7SL RNA	Component of signal recognition particle (SRP) for protein targeting.
Y RNA	Involved in DNA replication and RNA quality control.
Vault RNA (vtRNA)	Possible role in drug resistance and cellular transport.

Eukaryote RNA Polymerase III promotor

Pol III promoters are unique because they can be internal (within the gene) or upstream.

Type 1 (Internal Promoter) - 5S rRNA - Contains Box A and Box C inside the gene.
Type 2 (Internal Promoter) - tRNA genes - Contains Box A and Box B inside the gene.
Type 3 (Upstream Promoter) - U6 snRNA, 7SL RNA- Has TATA box.

TFIIIC binds to internal promoter elements (Box A/B).
TFIIIB (with TBP) recruits Pol III to the transcription start site.



Eukaryote enhancers

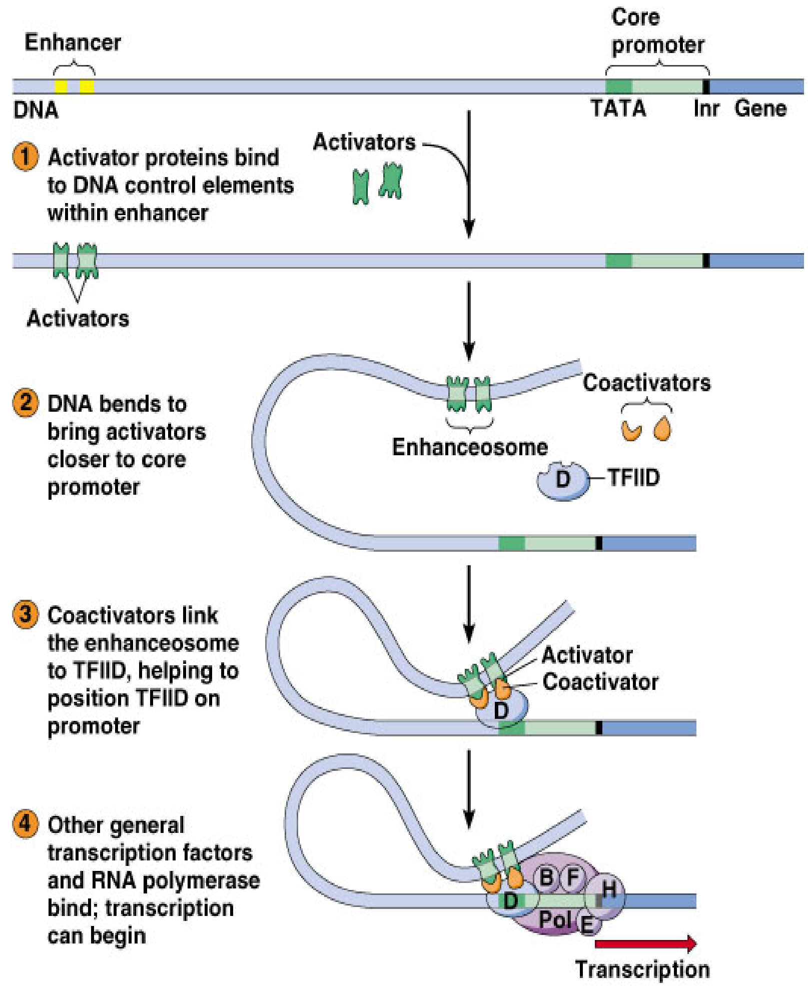
Regulatory TFs include ACTIVATORS that exert their effect on ENHANCERS "up-stream" to the promoters.

They are additional regulatory sequences.

The "enhanceosome" bends the DNA allowing it to approach the promoter.

Coactivators bind to activators helping to recruit TFIID to the promoter.

Other TFs are recruited later.

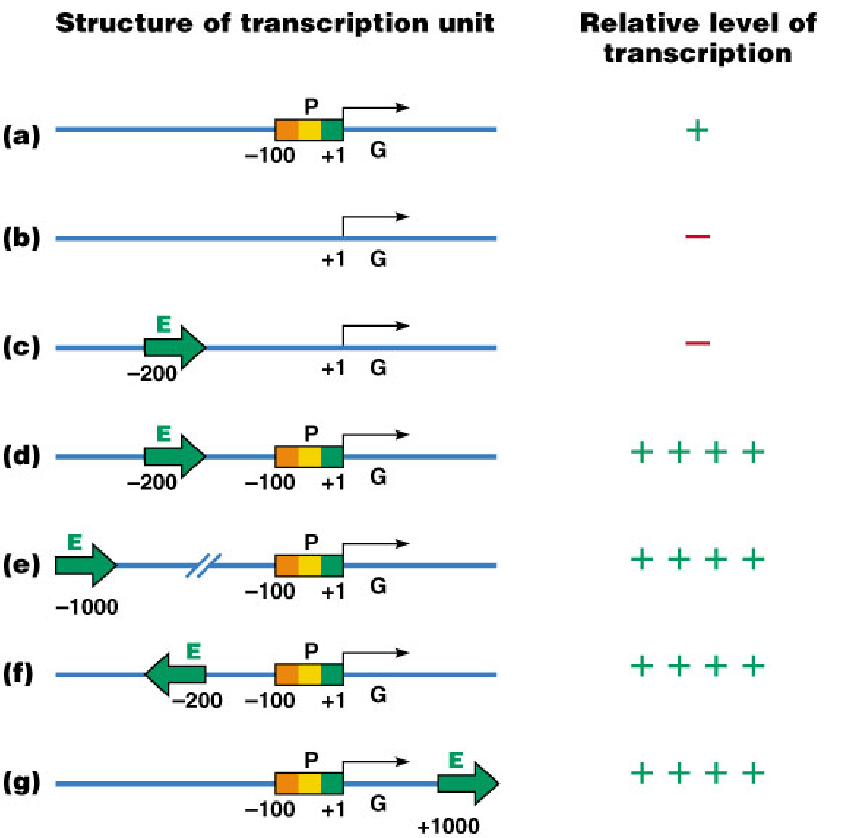


Eukaryote enhancers

Correlating presence and direction of enhancers with basal activity of the chorus promoter.

A- Isolated promoter
B- Without promoter
C- Without promoter with enhancer
D- Promoter with close enhancer
E- Promoter with distant enhancer
F- Promoter with anti-sense enhancer
G- Promoter with “down-stream” enhancer

Similar to the Lac Operon

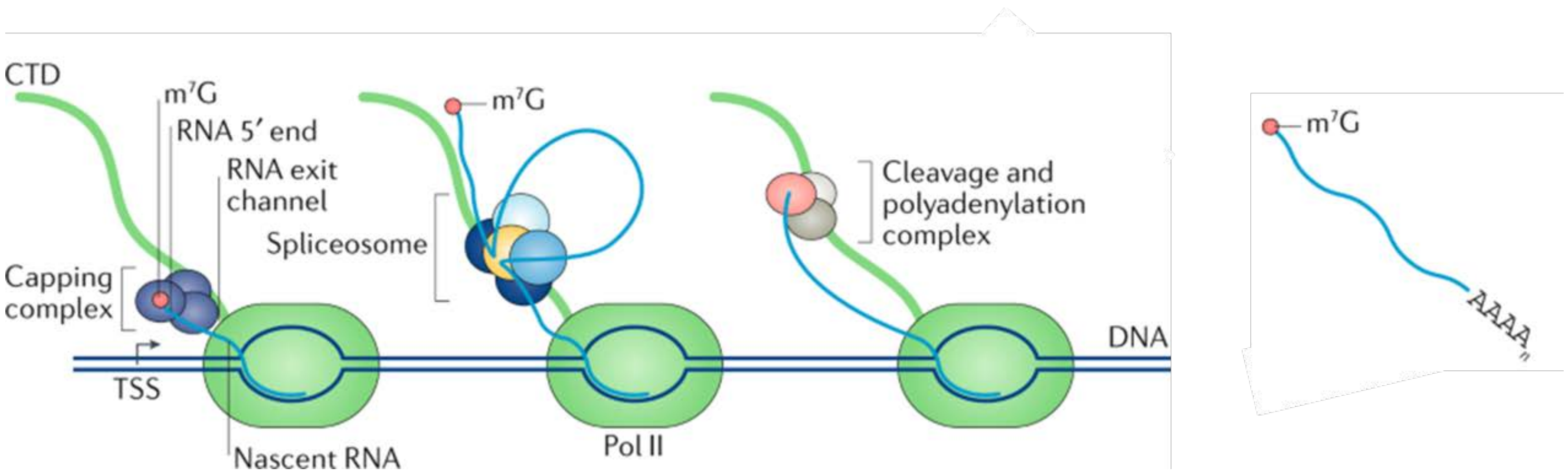


mRNA processing in eukaryotes

5′ Capping
Addition of a 7-methylguanosine cap for stability and translation initiation.

Splicing
Removal of introns and joining of exons by the spliceosome.

Polyadenylation
Addition of a poly(A) tail to the 3′ end for mRNA stability and transport.



[Targeting mRNA processing as an anticancer strategy. Desterro J, *et al*. Nat Rev Drug Discov. 2020 Feb;19(2):112-129.]

mRNA 5’ end capping

Addition of a 7-methylguanosine cap for stability and translation initiation.

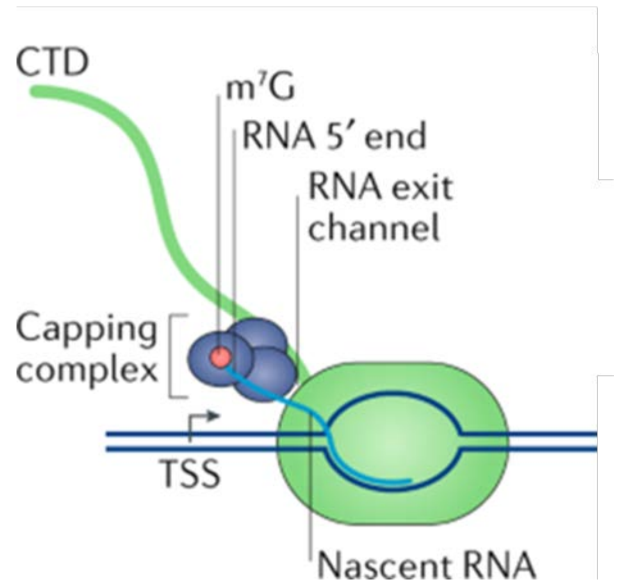
Guanine nucleotide connected to the mRNA via an unusual 5' to 5' triphosphate linkage.

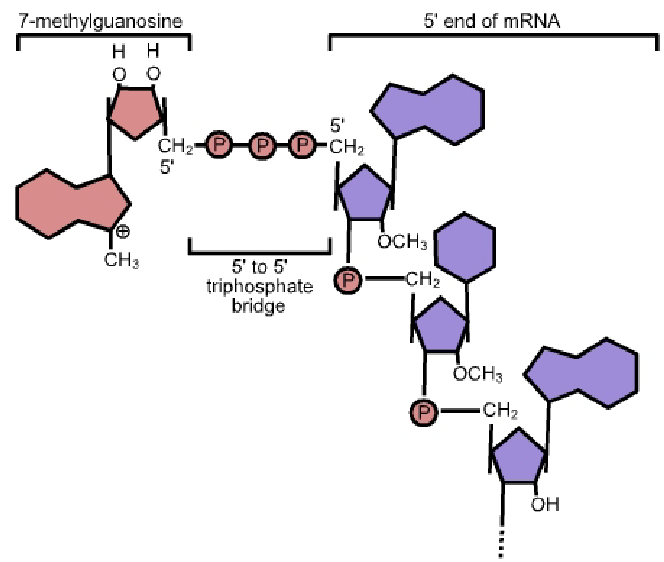
This guanosine is methylated on C7 (7-methylguanosine) or m7G.

Methylation of the 2’-OH of the first 3 ribose sugars.

Functionally the 5’-cap looks like the 3' end of an RNA molecule.

Offers resistance to 5' exonucleases.





mRNA splicing

Removal of introns and joining of exons by the spliceosome.

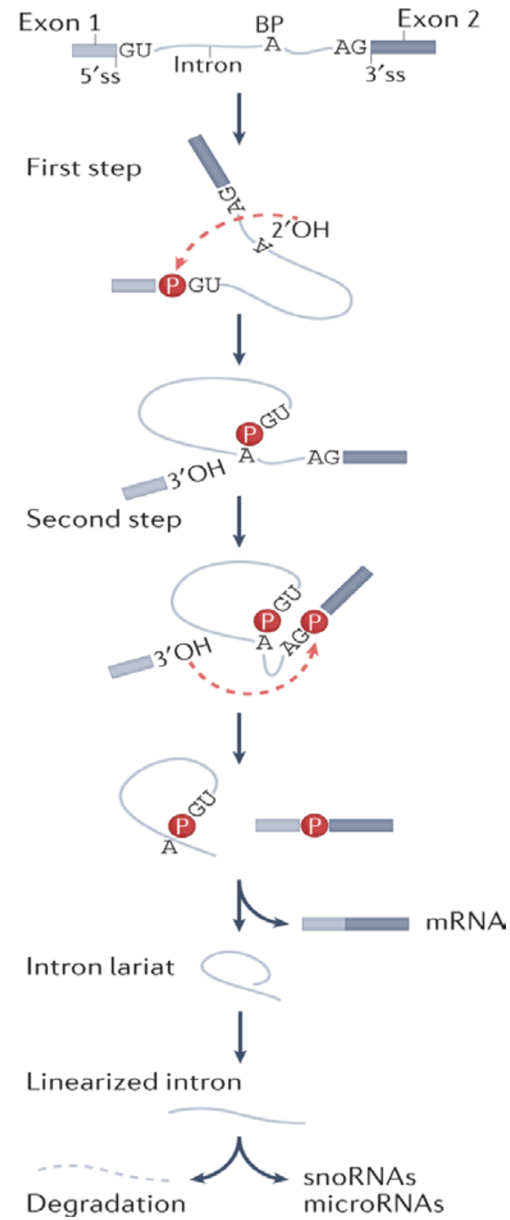
Occurs in the nucleus of eukaryotic cells.

Only properly spliced mRNAs are translated

Alternative splicing allows a single gene to produce multiple protein isoforms.

Errors in splicing can lead to diseases such as cancer, neurodegenerative disorders, and inherited syndromes.

snRNP	**Function**
U1	Binds to the 5' splice site (GU)
U2	Binds to the branch point (A residue)
U4/U6	Keeps U6 inactive until catalysis
U5	Aligns the exons for ligation
U6	Catalyzes spliceosome activity



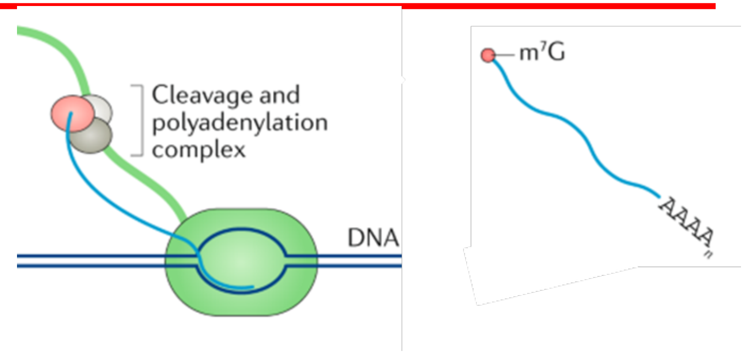
Splicing related diseases

Result from mutations in:

- Splice sites (5' or 3' splice sites)
- Branch point sequences
- Regulatory elements (ESE, ESS, ISE, ISS)
- Splicing factors (snRNPs, SR proteins, hnRNPs)

Disease	Splicing Defect	Consequence
Spinal Muscular Atrophy	SMN2 exon 7 skipping	Motor neuron loss
Myotonic Dystrophy	Sequestration of MBNL1	Muscle wasting, cardiac defects
Frontotemporal Dementia & Amyotrophic Lateral Sclerosis	Mutations in TDP-43, FUS, and C9orf72 genes.	Accumulation of RNA/protein aggregates, neuronal death.
Bcl-x Splicing in Cancer	Bcl-xL ↑ (anti-apoptotic)	Chemotherapy resistance
p53 Splicing in Tumors	Loss of functional p53	Uncontrolled cell growth
Cystic Fibrosis	CFTR exon skipping	Thick mucus, lung infections
Beta-Thalassemia	Incorrect HBB splicing	Severe anemia
Duchenne Muscular Dystrophy	Dystrophin exon skipping	Muscle degeneration
Diabetes	Altered INSR splicing	Insulin resistance
Lupus (SLE)	Autoantibodies target snRNPs (U1 snRNA).	Autoimmune inflammation
Cancer and Tumorigenesis	Increased Bcl-xL via splicing factor SRSF1)	Resistance to apoptosis
	Mutations in MDM2 and p53 mRNA splicing	Inhibit tumor suppression
	Aberrant tumor suppressors (BRD9, BAP1)	Incr. tumor growth, metastasis.
	Bcl-xL increase (anti-apoptotic)	Chemotherapy resistance

mRNA 3’ end polydenylation



Addition of 50 to 250 Adenosine residues to the 3′ end.

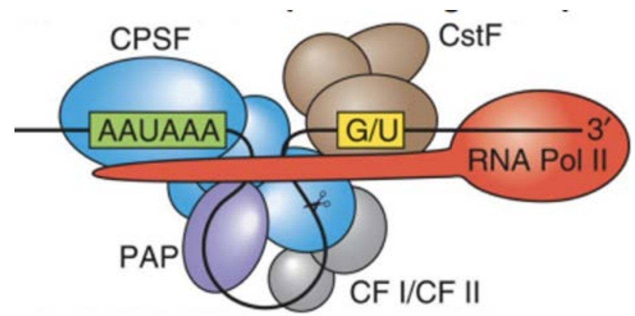
The mRNA needs to be modified to stabilize it (by labeling it or altering its conformation).

Places between 50–250 adenosine residues.

Enhances mRNA stability (prevents rapid degradation).

Facilitates nuclear export.

Regulates gene expression (affects mRNA half-life).



Protein Complex	Function
Cleavage & Polyadenylation Specificity Factor (CPSF)	Recognizes AAUAAA signal and cleaves pre-mRNA.
Cleavage Stimulation Factor (CstF)	Binds GU-rich region, promotes cleavage.
Cleavage Factors (CFI & CFII)	Aid in RNA cleavage.
Poly(A) Polymerase (PAP)	Adds adenosine residues after cleavage.
Poly(A) Binding Proteins (PABP)	Regulates poly(A) length and mRNA stability.
RNA Polymerase II CTD	Recruits CPSF and CstF

Transcription summary videos

https://www.youtube.com/watch?v=t5jroSCBBwk

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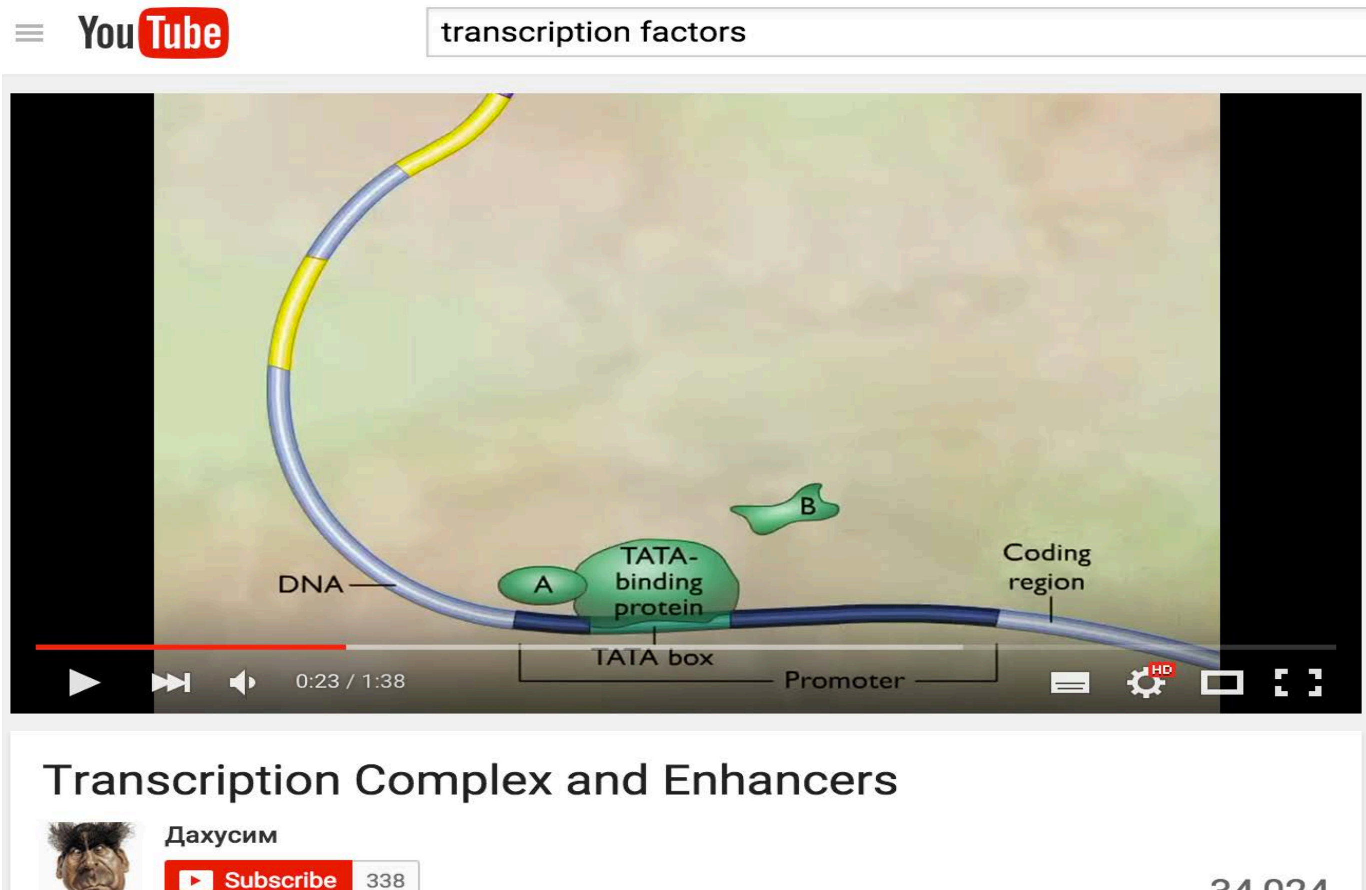

https://www.youtube.com/watch?v=ysxtZJUeTCE

Laboratorio de Genómica Viral y Humana

Instalaciones de Alta Contención Biológica Nivel de Bioseguridad 3 (BSL-3) CDC-certificadas

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