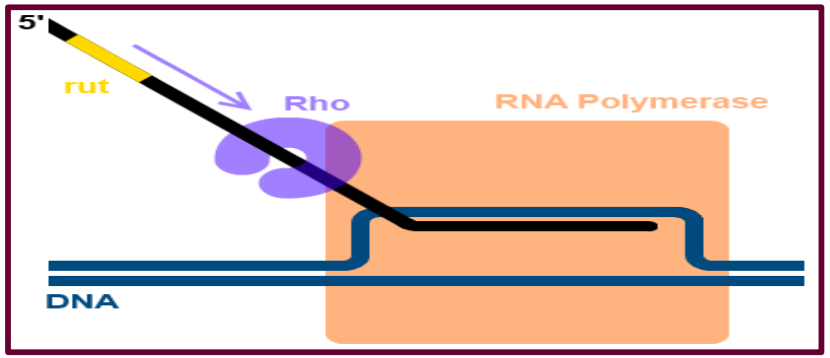
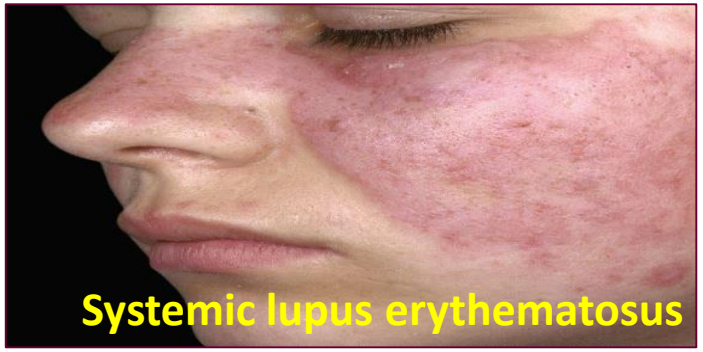
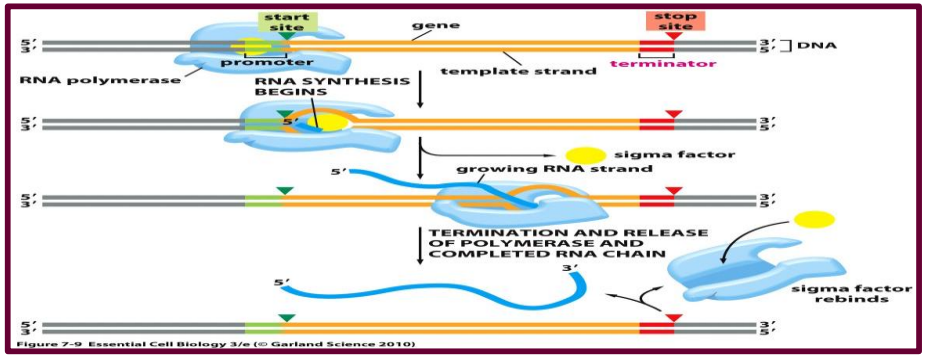
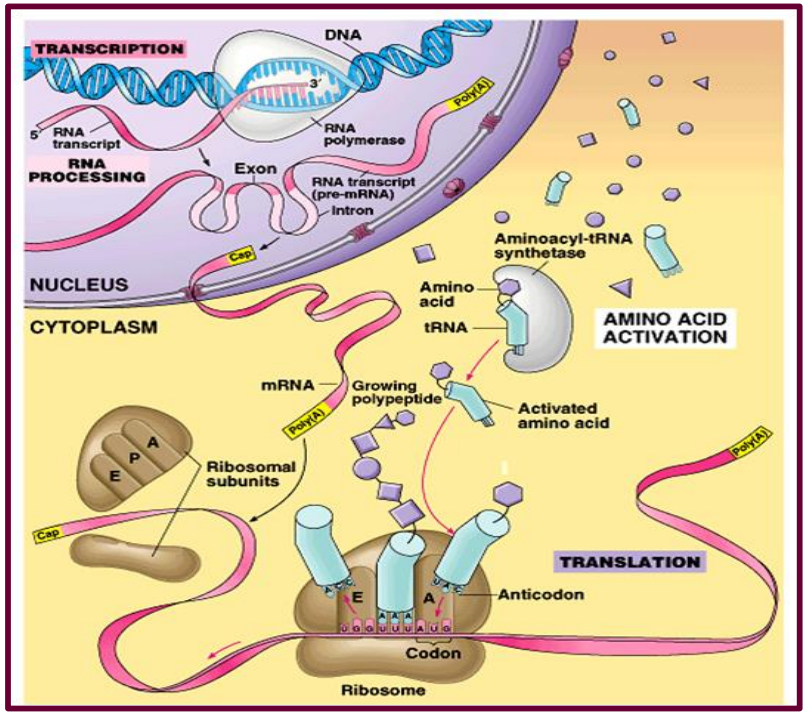


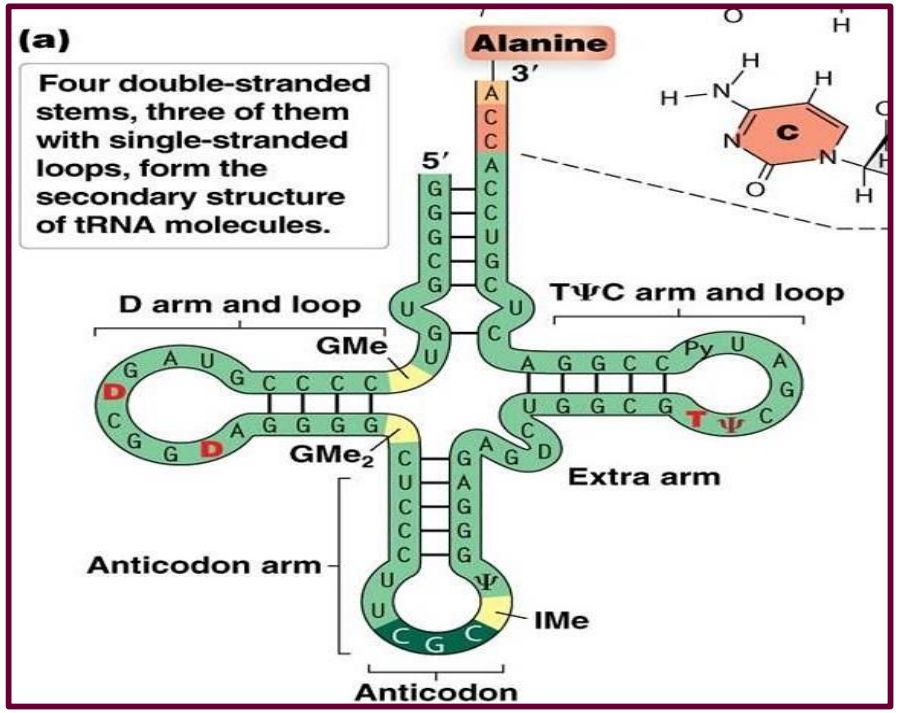




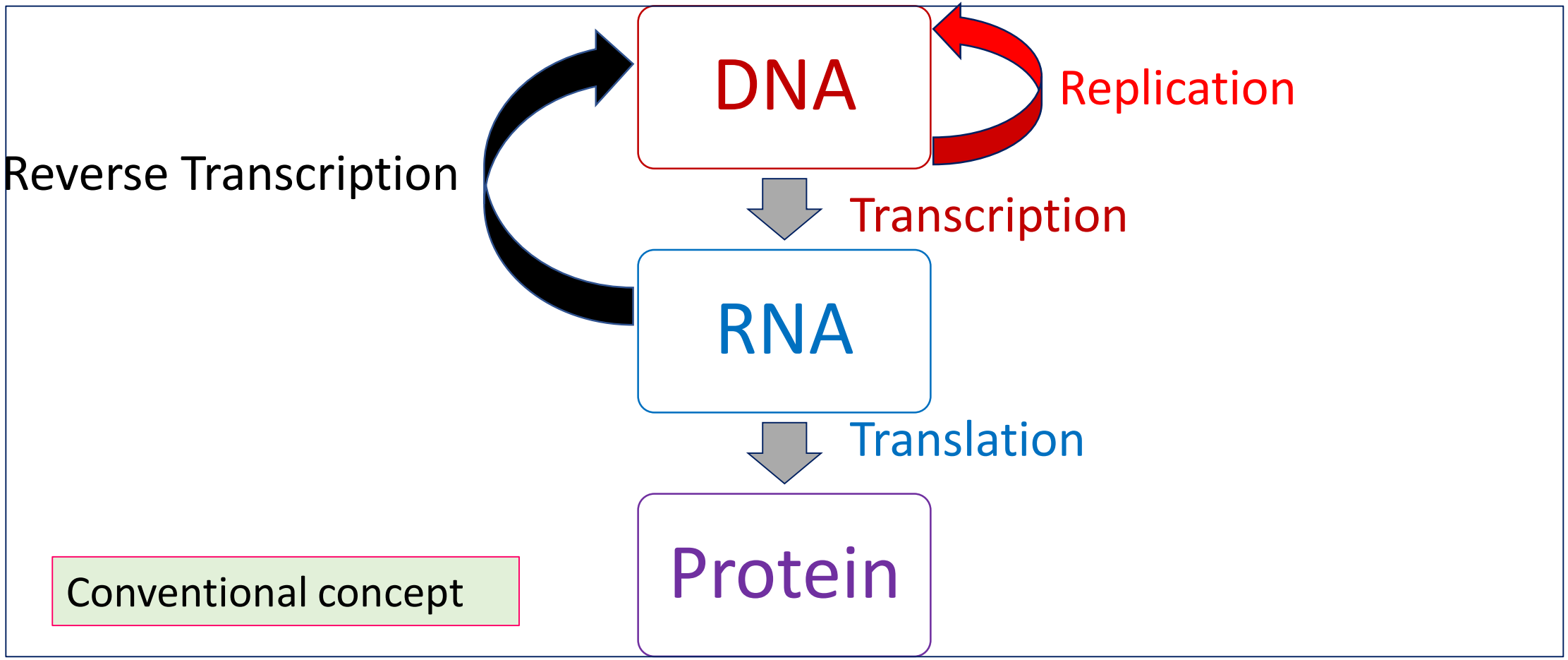
Prokaryotic and Eukaryotic Transcription with their clinical applications



Dr. Rohini C Sane



The central dogma of molecular biology

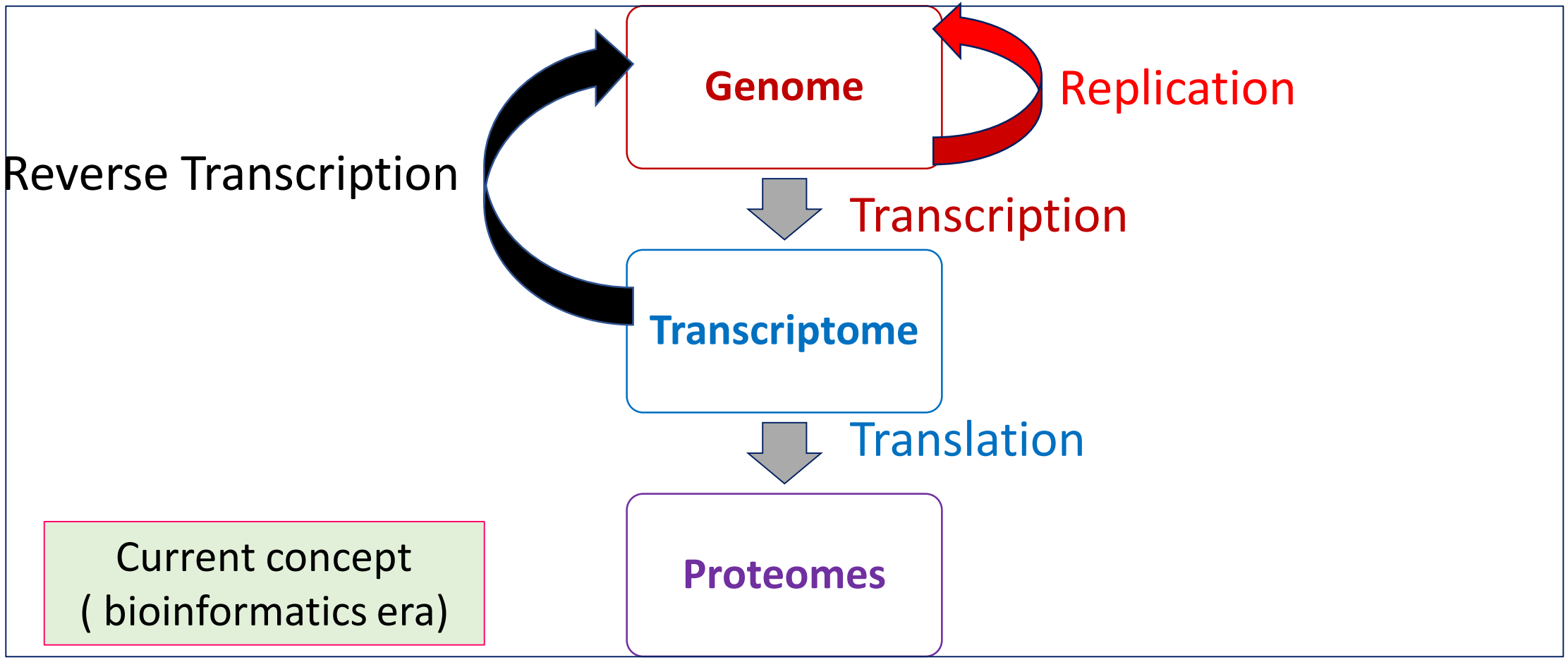


The flow of genetic information

The central dogma of molecular biology in bioinformatic era

- **Genome:** the total DNA (genetic information) contained in an organism or a cell . It is storehouse of biological information. It includes the chromosomes in the nucleus and the DNA in mitochondria.
- **Transcriptome:** the RNA copies of the active protein coding genes. It is the initial product of gene expression which directs synthesis of the protein.
- **Proteomes:** repository /storehouse / repertoire of cell's proteins. It represents the entire range of proteins and their biological functions in a cell.

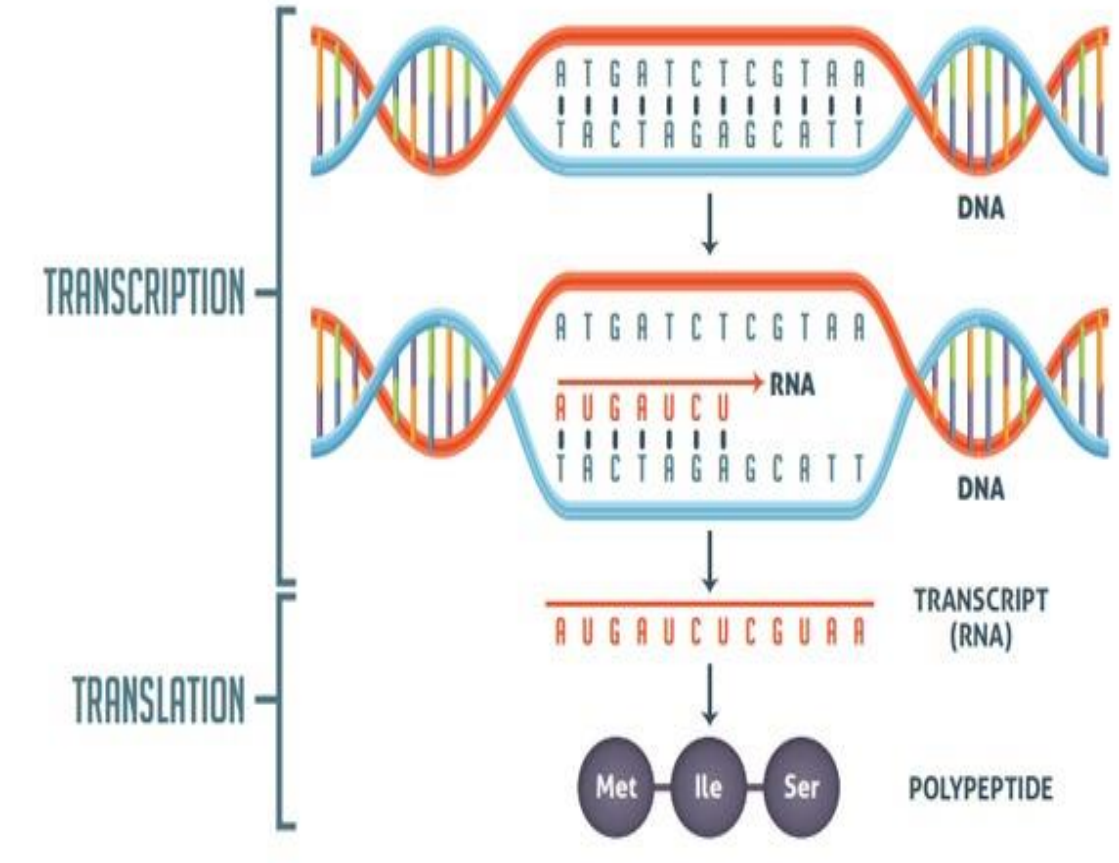
The central dogma of molecular biology

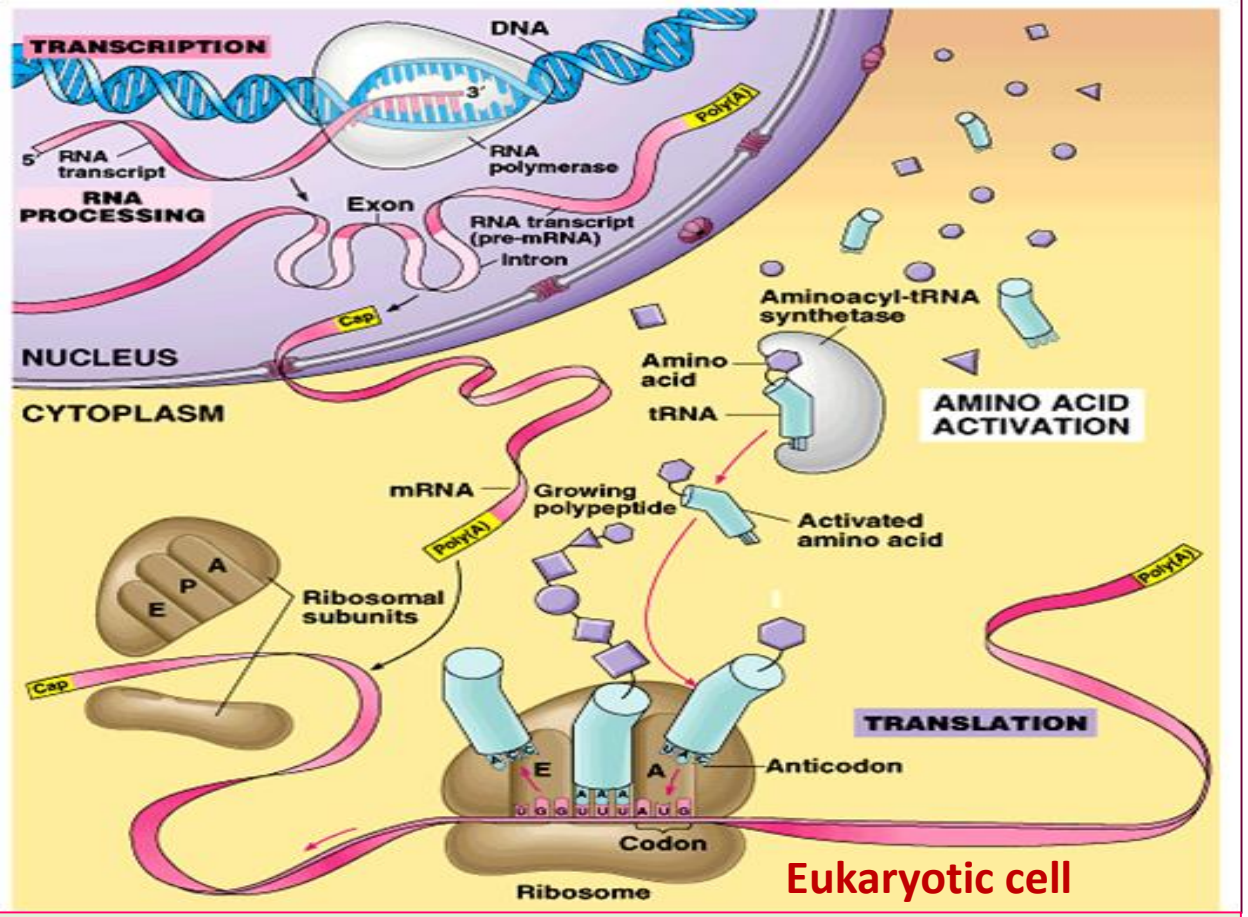


The flow of genetic information

Transcription(RNA synthesis)

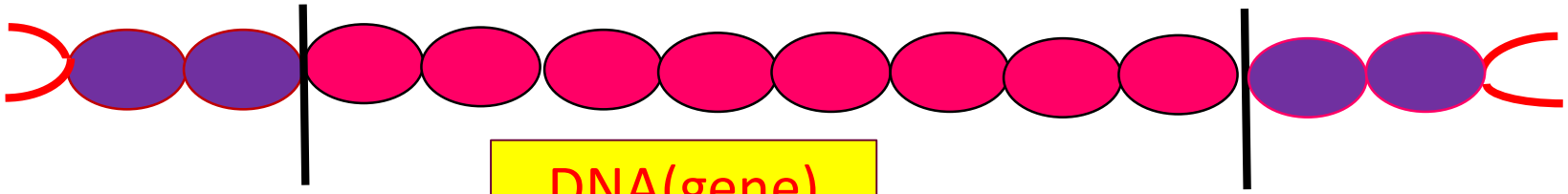
Transcription : the synthesis of RNA molecule using DNA as a template ,that results in the transfer of information stored in double stranded DNA into a single stranded RNA , which is used by the cell to direct its protein synthesis.





During transcription ,the message from DNA is copied in the language of nucleotides.(4 letter language).

Expression of genetic information by transcription

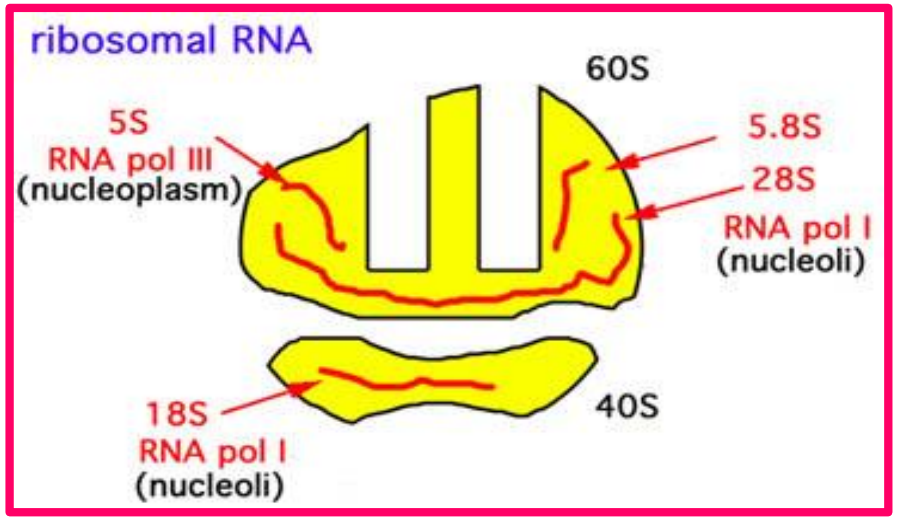


DNA(gene)

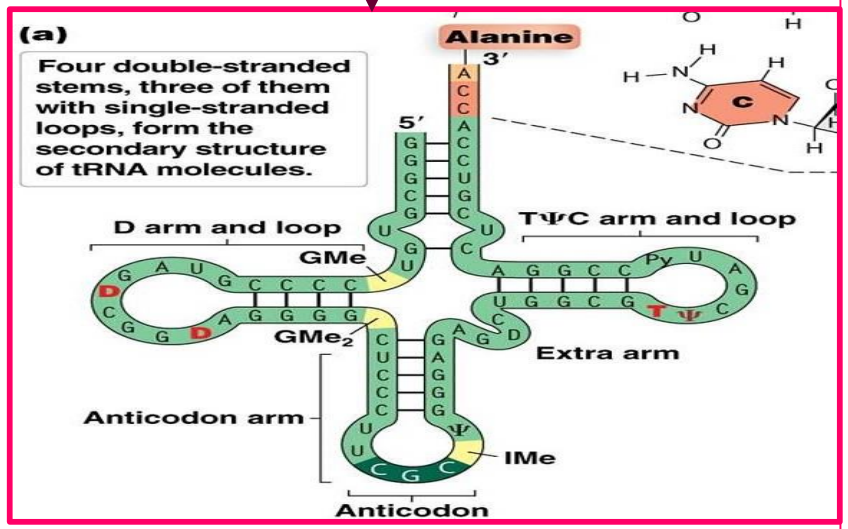
Transcription by RNA Polymerase

Meth7-Gppp^^^^^^ pApApA

Eukaryotic mRNA with cap(7-methylguanosine triphosphate) and Poly A tail(AAA)

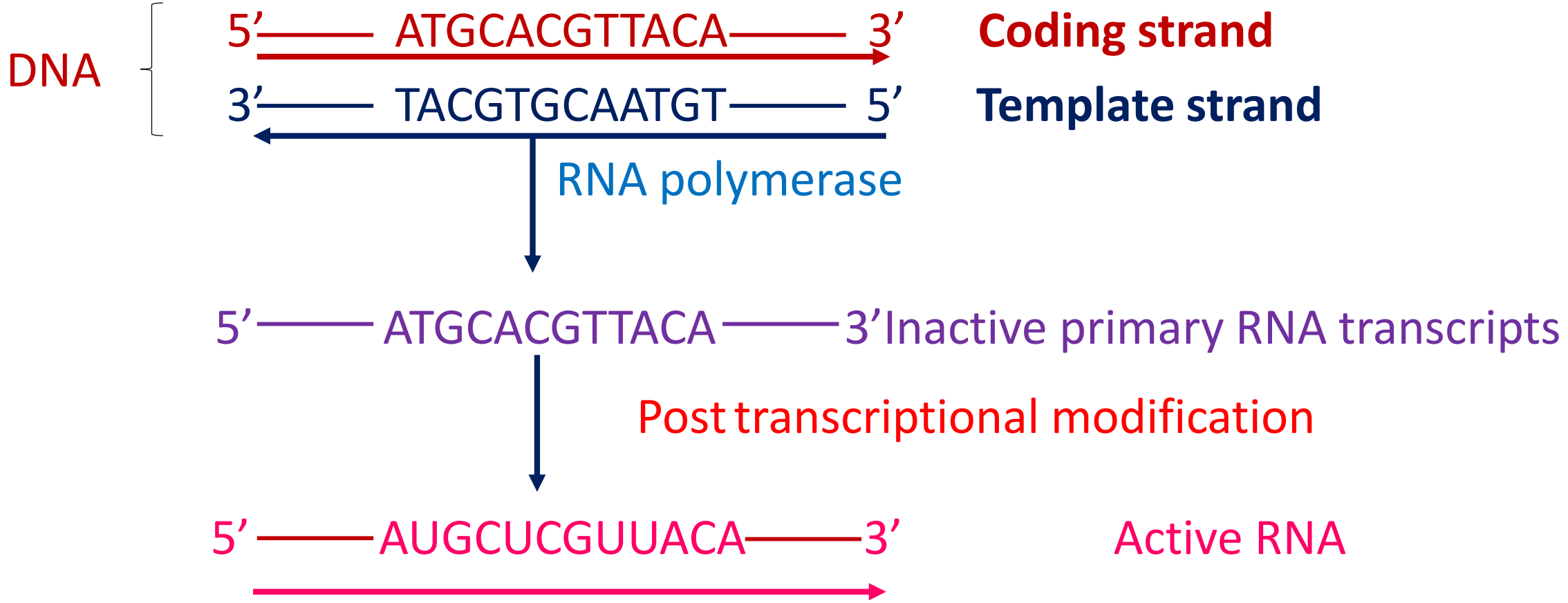


Eukaryotic rRNA



tRNA

Synthesis of RNA(transcription) from DNA



Transcription : the mRNA base sequence complementary to that of the template strand and identical to that of coding strand. In mRNA ,U replaces T.

Basic requirements of Transcription

- **Definition of Transcription:** is a process in which Ribonucleic acid(RNA) is synthesized from DNA template catalyzed by RNA polymerase .

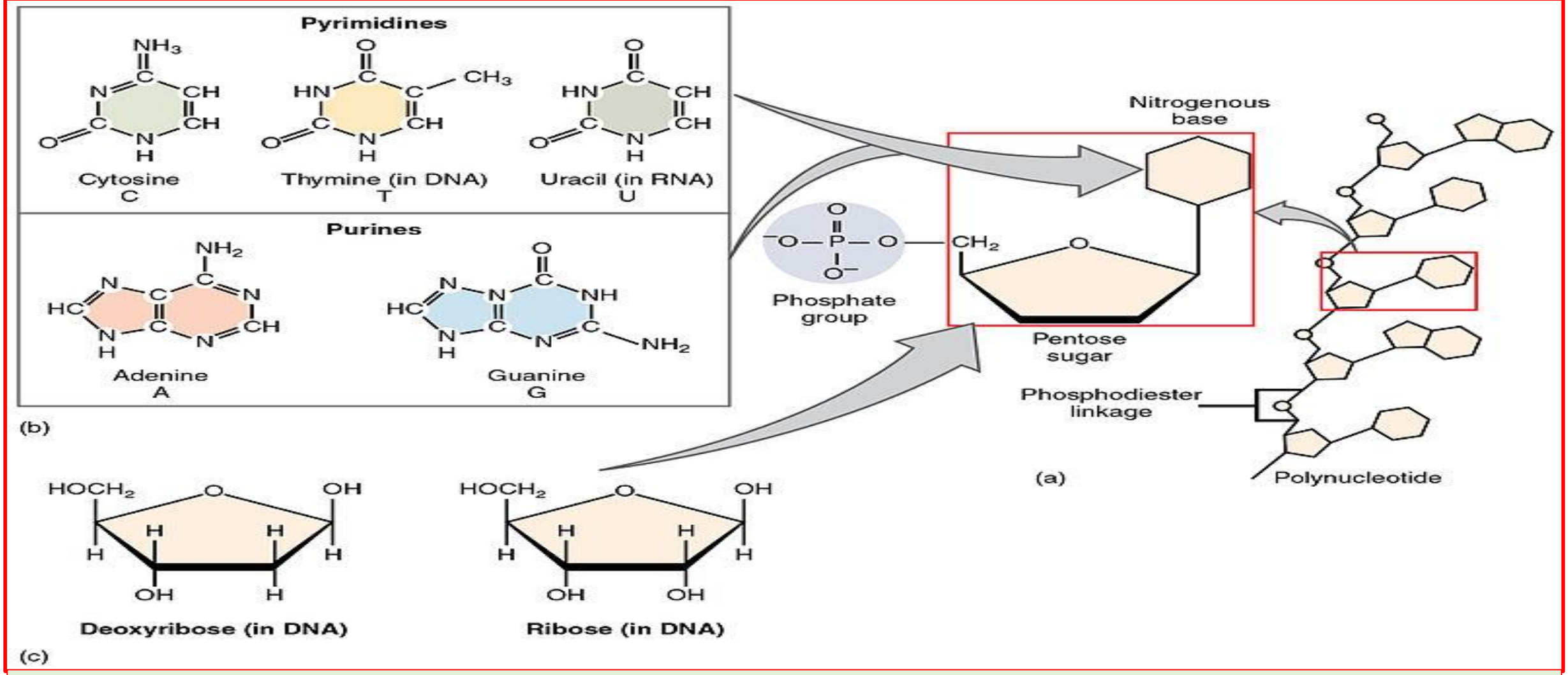
  $(NMP)_n + NTP \rightarrow (NMP)_{n+1} + PPi$

- **Gene:** a functional unit of DNA that can be transcribed.
- **Template strand of DNA :** One of two DNA strands(**non coding strand** or anti-sense strand)→to produces working copies of RNA molecules(formation of complementary copies of RNA molecule /transcript) .
- Other strand of DNA does not participate in transcription →referred as Coding strand of the gene or Sense Strand= non-template Strand
- **Coding strand** :commonly used with exception of T for U
- **Primary mRNA** :contains codons for same base sequence as coding strand
- **Substrates:** **4** ribonucleoside triphosphates (ATP,GTP, CTP,UTP)
- **Enzymes** : DNA dependent RNA polymerase (RNA polymerase → RNAP) responsible for the synthesis of RNA in 5'→3' direction using DNA template.

Characteristics of Transcription

- **Transcription** is **selective** i.e. entire molecule **is not expressed** in transcription.
- RNA are synthesized for selected regions of DNA, other regions of DNA, there may not be any transcription at all. (exact reason?→**Inbuilt signals** in the DNA molecule)
- **Three Stages of transcription** : initiation , elongation and termination
- The RNA synthesized is complimentary to one of the strand and identical to the other coding strand.
- Product of transcription : inactive primary RNA transcripts
- **inactive primary RNA transcripts** undergo post transcriptional modifications to produce functionally an active RNA molecules.
- Post transcriptional modifications : splicing, terminal base additions, base modifications etc.

Ribonucleotides (ATP,GTP, CTP,UTP) required for transcription



Substrates for transcription

Types of Cellular RNA

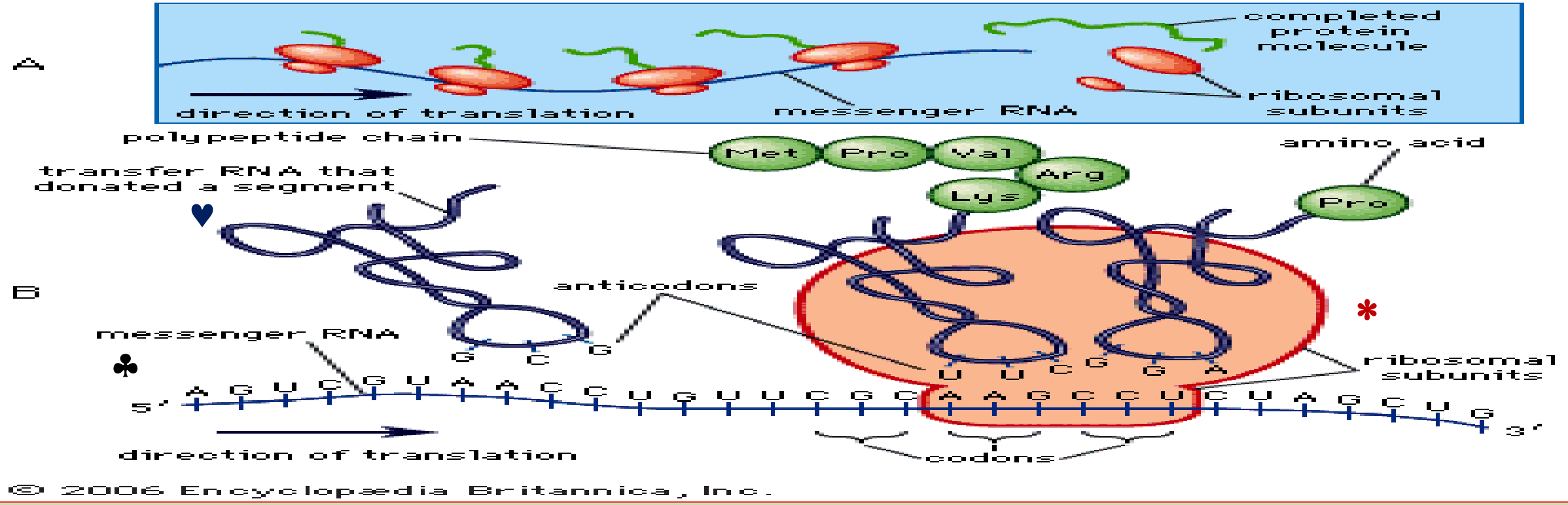
- Types of Cellular RNA include:

1. Messenger RNA(mRNA)
2. Ribosomal RNA(rRNA)
3. Transfer RNA(tRNA)

} involved in protein synthesis

4. Several small nuclear RNA (snRNAs)→ involved in mRNA splicing

Functions of RNAs in protein biosynthesis



♣**mRNA** : serve as a template for protein biosynthesis and transfer genetic information from DNA to protein synthesizing machinery.

♥**tRNA** : carries amino acids in an activated form to the ribosome for translations (protein synthesis) of information in the sequence of nucleotides of the m-RNA.

***r RNA**: maintains ribosomal structure and also participates in protein biosynthesis by binding of m-RNA to ribosome . It functions as an enzyme **Ribozyme** having catalytic activities.

Prokaryotic RNA polymerase

- Prokaryotes have **single RNA polymerase** that **transcribes** all **three RNAs** :

1. Messenger RNA(mRNA)
2. Ribosomal RNA(rRNA)
3. Transfer RNA(tRNA)

} involved in protein synthesis

RNPA contains four subunits (2 α,β' ,β) which form the core enzyme .

The **active enzyme** = **core enzyme** + fifth subunit (**sigma σ factor**)+ **omega subunit**

Function of sigma σ factor: binding of the polymerase to specific promoter regions of DNA template .

RNAP is **metalloenzyme** containing **Zinc** molecule .

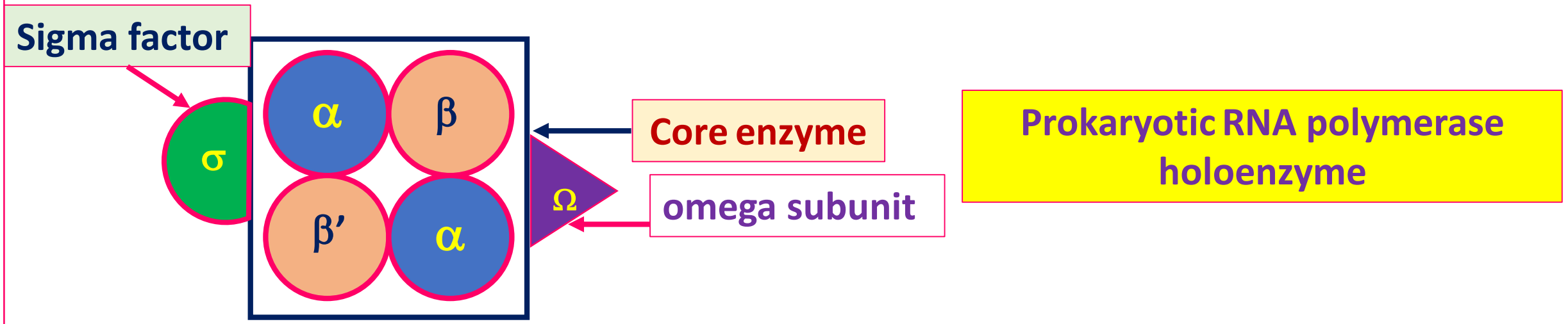
Subunits of Prokaryotic DNA dependent RNA polymerase

Prokaryotes have single RNA polymerase that transcribes all three RNAs i.e.

1. Messenger RNA(mRNA)
2. Ribosomal RNA(rRNA)
3. Transfer RNA(tRNA)

➢Subunits of DNA dependent RNA polymerase/RNA polymerase (**RNAP**):

2 alpha (α), beta (β), beta (β') , omega subunit and sigma (σ)



Eukaryotic RNA polymerases

- **In contrast to prokaryotes , eukaryotic cells have three types of RNA polymerases:**

1. RNA polymerase I
2. RNA polymerase II
3. RNA polymerase III

➢ **Eukaryotic RNA polymerases are :**

a. complex than prokaryotic RNA polymerase.
b. Differ in template specificity.
c. Differ in location in the nucleus.
d. Are responsible for the transcription of different sets of genes.

Types of Eukaryotic RNA polymerases and RNAs formed

Type of DNA dependent RNA polymerase(RNAP)	Location	Type precursors of RNA formed	Sensitivity towards Amanitin
RNA polymerases I or A	Nucleolus	18S ,5.8S, and 28S rRNA	Sensitive and inhibited
RNA polymerases II or B	Nucleoplasm	mRNA precursor, snRNA, snoRNA, miRNA	Not inhibited
RNA polymerases III or C	Nucleoplasm	tRNA, 5S rRNA, some snRNA, snoRNA	Moderately sensitive

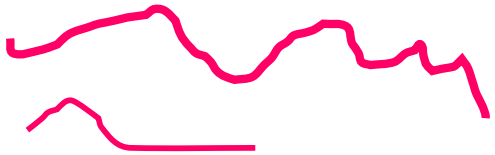
Eukaryotic RNA polymerases of are more complex than prokaryotic RNA polymerase and differ in their template specificity.

An overview of transcription in eukaryotes

RNA polymerases I

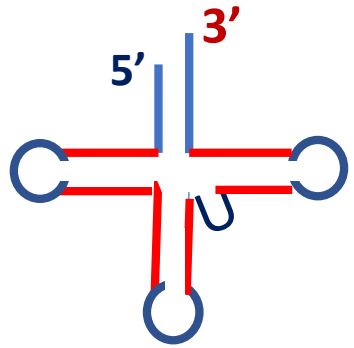
RNA polymerases II

RNA polymerases III



Ribosomal RNAs

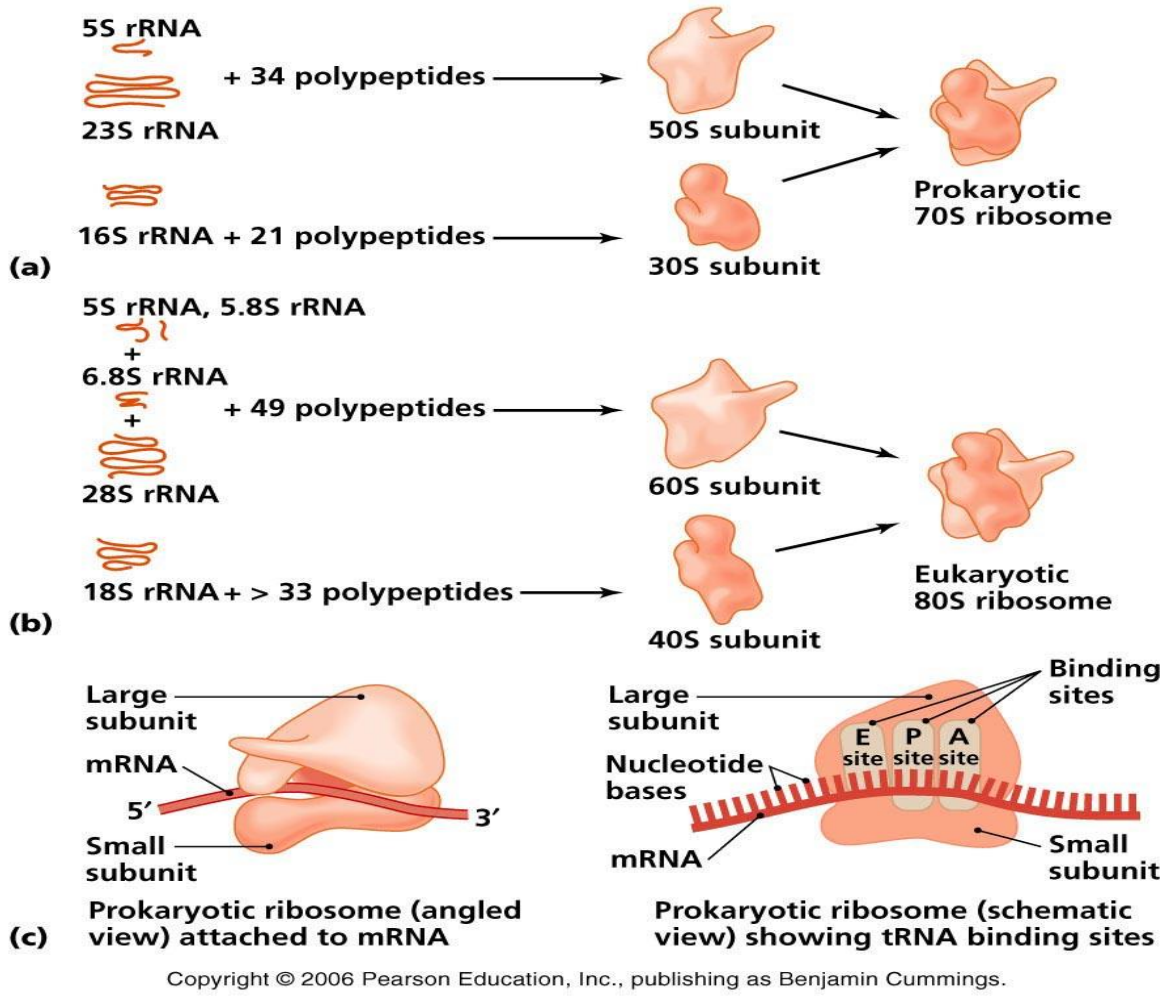
Messenger RNA,
snRNA,miRNA



Transfer RNA

Schematic diagram

Prokaryotic Ribosomal RNA(rRNA)



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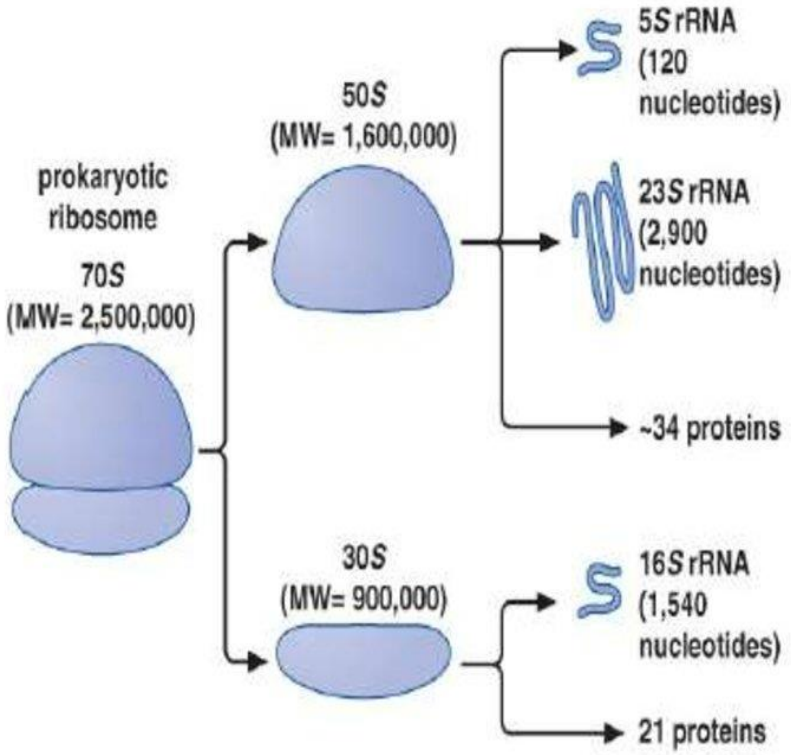
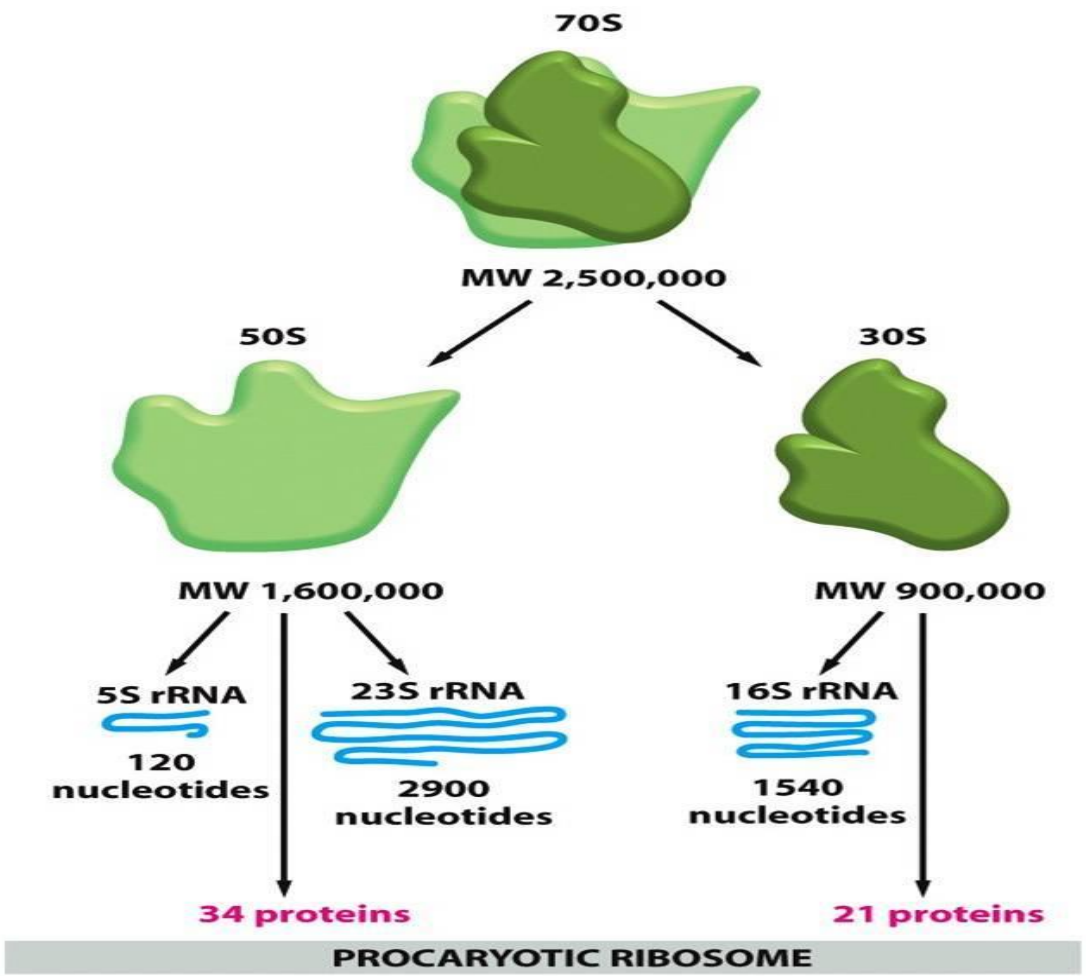
Ribosome: Structure

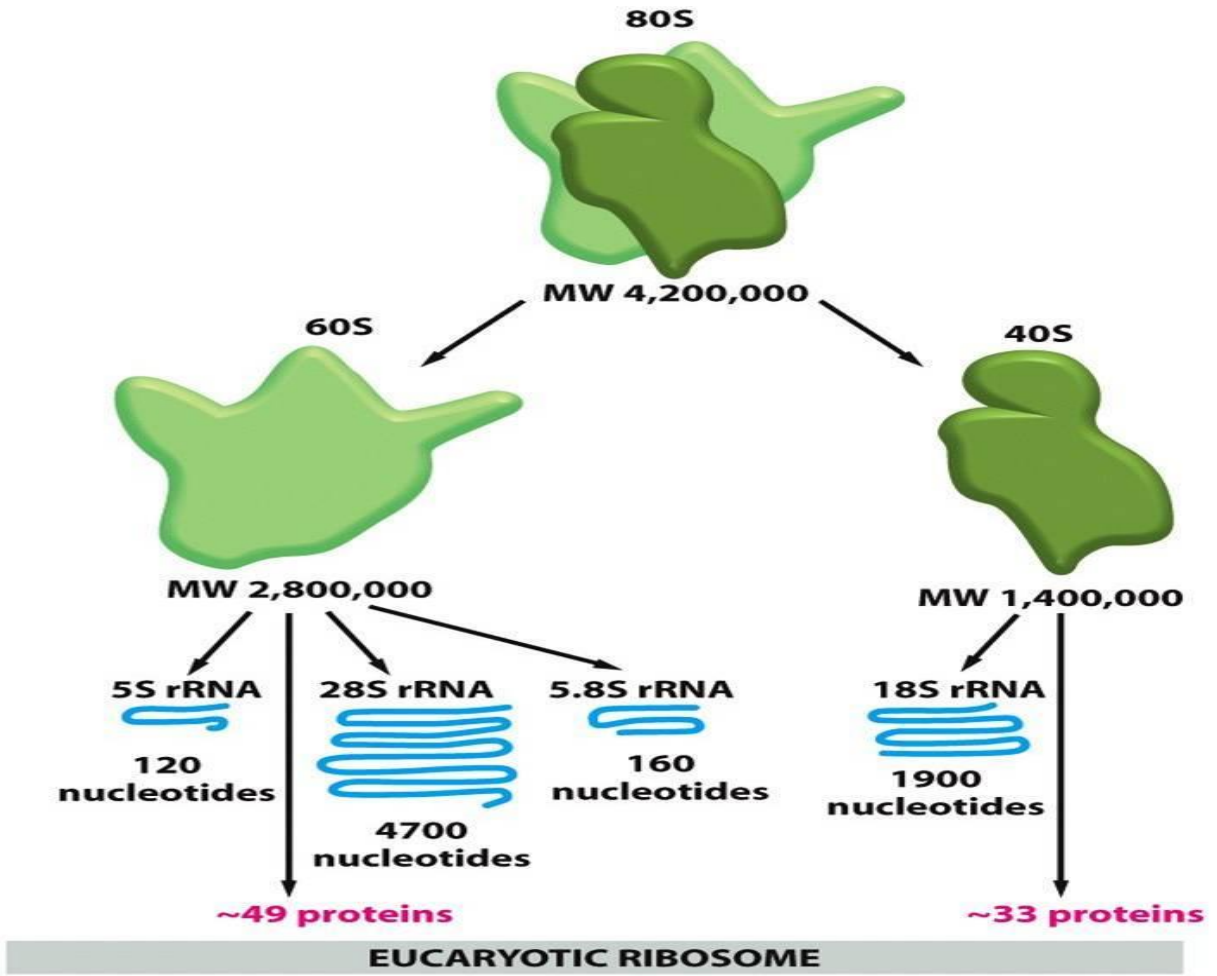


* Each ribosome contains one copy of the rRNAs and one copy of each of the ribosomal proteins, with one exception: One protein of the 50S subunit is present in four copies.

Three types of **rRNA** molecules in E.Coli : 5S , 23S , 16 S .

Prokaryotic and Eukaryotic Ribosomal RNA(rRNA)





Three types of rRNA molecules in E.Coli : 5S , 23S , 16S .

Three types of Eukaryotic rRNA synthesized from 60 S preribosomal RNA (long precursor) : 5S, 28S, 5.8S and fourth 18s rRNA from 40s preribosomal RNA

Comparison of Prokaryotic and Eukaryotic Ribosomal RNA(rRNA)

Prokaryotic rRNAs

23 S rRNA

↑23S rRNA

←16S rRNA

←5S rRNA

S = Svedberg unit related to molecular weight and shape of molecule

Eukaryotic rRNAs (located in cytosol)

↑28S rRNA

←18S rRNA

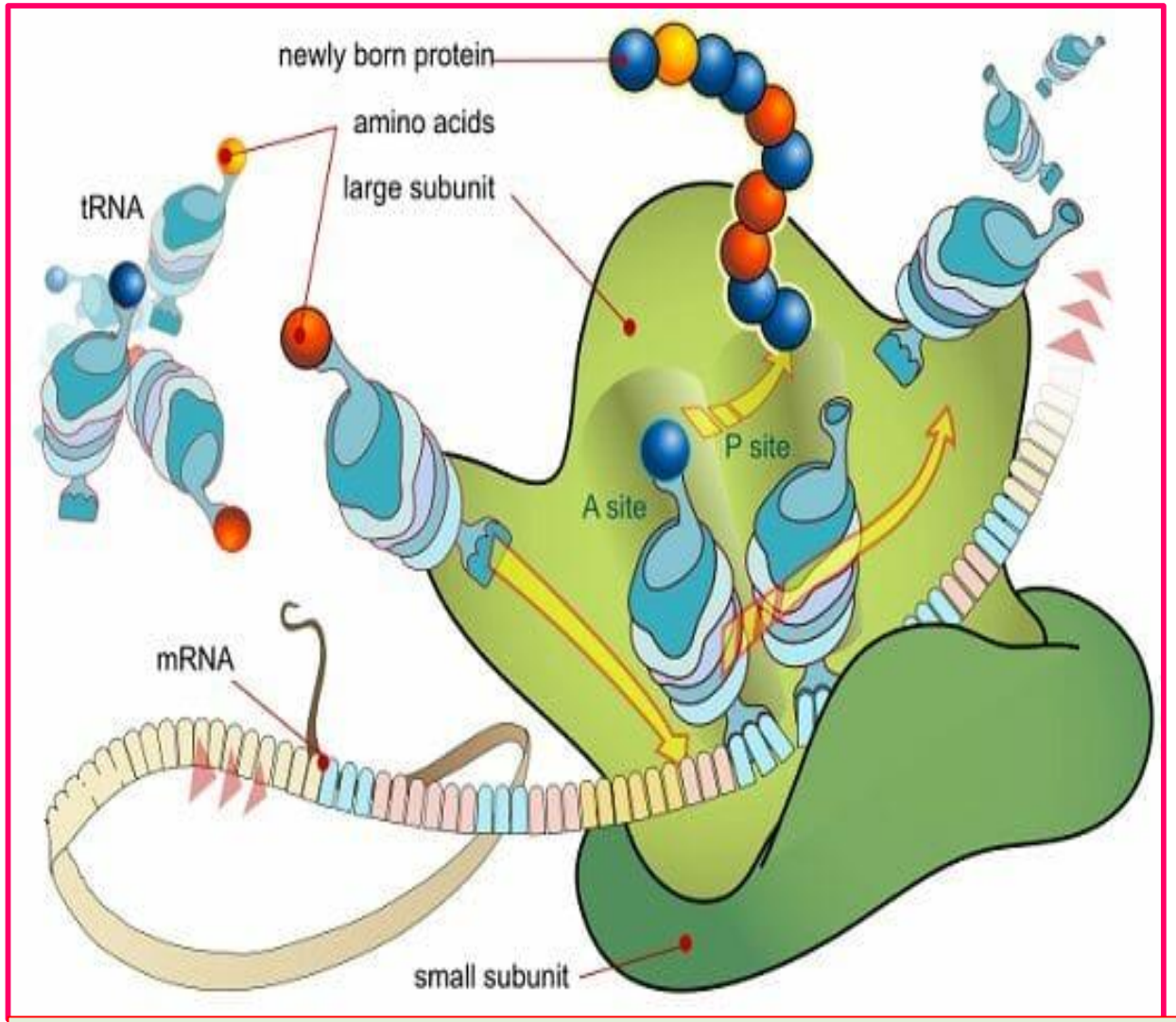
←5.8S rRNA

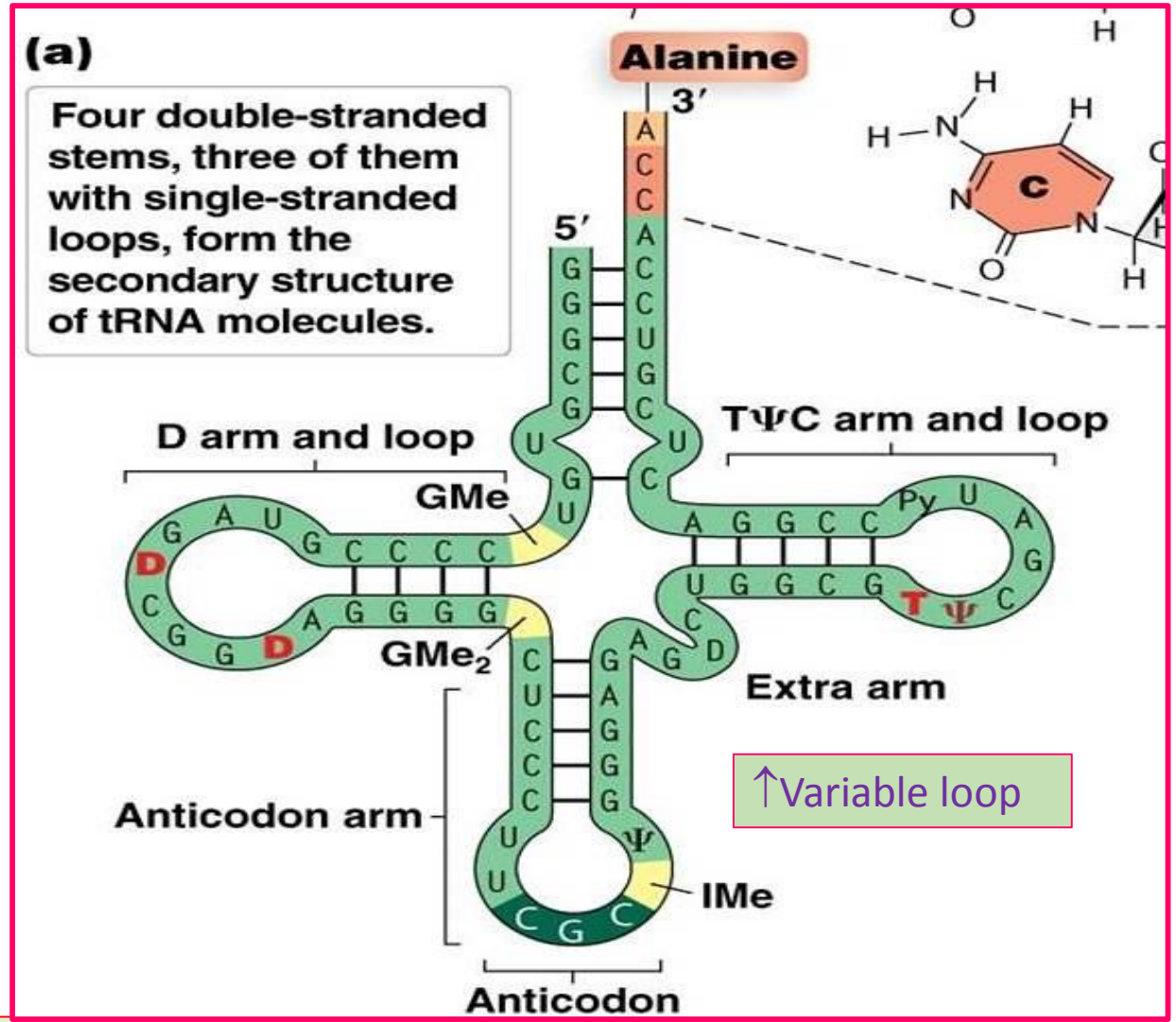
←5S rRNA

Schematic diagram

rRNA in association with several proteins as components of ribosomes serve as sites for protein synthesis . Some rRNA function as catalysts (termed as a **ribozyme**).

Structure and function of Transfer RNA(tRNA)





Function of Transfer RNA(tRNA): carrier of amino acids during protein synthesis. One specific type of tRNA molecule for each of 20 amino acids commonly found in proteins .

Small RNA

- Small RNA :

1. constitute ~ 1-2% of total RNA in the cell
2. 30 different varieties
3. Stable in nature
4. Subgroup : Small nuclear RNAs (SnRNAs) involved in mRNA splicing .

Mitochondrial RNA polymerase

- Mitochondria contain a single RNA polymerase that more closely resemble bacterial RNA polymerase than eukaryotic polymerases.

Micro-RNA(miRNA)

❑**Micro-RNA (miRNA):**

1. tiny RNAs produced by some genes
2. stable in nature
3. with 21-25 ribonucleotide bases
4. More than 200 varieties in human beings
5. Derived from **large primary transcript** inside the nucleus which is cleaved by certain **exonucleases** to reduce their length.
6. Have hairpin structure showing internal hybridization to make two strands(called **short hairpin RNA (shRNA).**
7. transported through **nuclear pore** into cytoplasm where one of the two strands is broken by **dicer nuclease**. The selected strand is called the **guide strand** ,which is incorporated into **RNA induced silencing complex (RISC) to form functional silencer of mRNA .**
8. The **guide strand** provides the **specificity** to **RISC** , which binds and then degrades complementary target mRNA in cytoplasm.
9. **Binds to matching pieces of messenger RNA , turn it into double stand and prevent it from doing job (alters function of mRNA). The process effectively blocks the production of the corresponding protein causing translational arrest.**

siRNA or interfering RNA or RNAi

❑**siRNA or interfering RNA or RNAi** :

1. double-stranded RNA which would silence gene corresponding to that RNA. It is the faster way to turn off genes.
2. with 21-25 ribonucleotide bases
3. degrade the mRNA through specific cytoplasmic organelle called **P bodies**.
4. decrease level of functional proteins in the cells(function similar to micro -RNA).
5. RNA interference is protective mechanism against virus . They can used to treat diseases (**silencing disease causing genes**)especially **HIV** infection.
6. used in preclinical trials in animal models for treating **incurable neurogenerative disorders.**
7. Andrew Fire and Craig Mello(Noble 2006) demonstrated that when double stranded RNA was given to round worms ,it would silence the gene corresponding to RNA.

Primary transcript of RNA

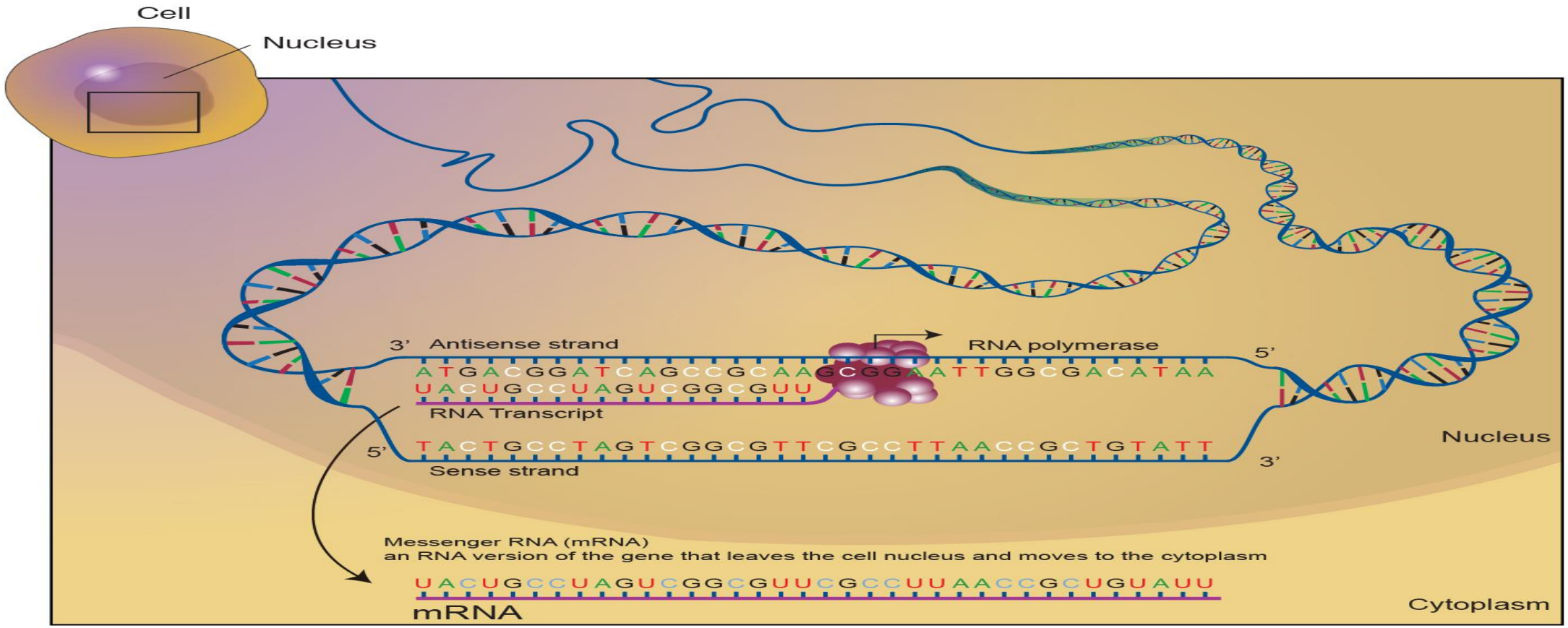
- **Transcription unit :** is the region of DNA that includes not only genes for mRNA synthesis but also the initiator, promoter regions ,introns and terminator regions.
- **Primary transcript of RNA:** Initial , linear RNA copy of transcription unit(the segment between specific initiation and termination sequences).
- The Primary transcript of both prokaryotic and eukaryotic tRNA and rRNA are post transcriptionally modified by cleavage of the original transcripts by ribonucleases.
- tRNA are then further modified to help each species to retain its unique identity.
- Prokaryotic mRNA is generally identical to its primary transcript.
- Eukaryotic mRNA is extensively modified both co and post transcriptionally.

Post transcriptional modifications of inactive primary RNA transcripts for synthesis of functional RNA

- **Post transcriptional modifications involve enzymatic alterations of inactive primary RNA transcripts (made by RNA polymerase) to functional RNA to be located in cytoplasm . These modifications are extensive in eukaryotes but not in prokaryotes (minor processing) .**
- **Post transcriptional modifications may involve either:**

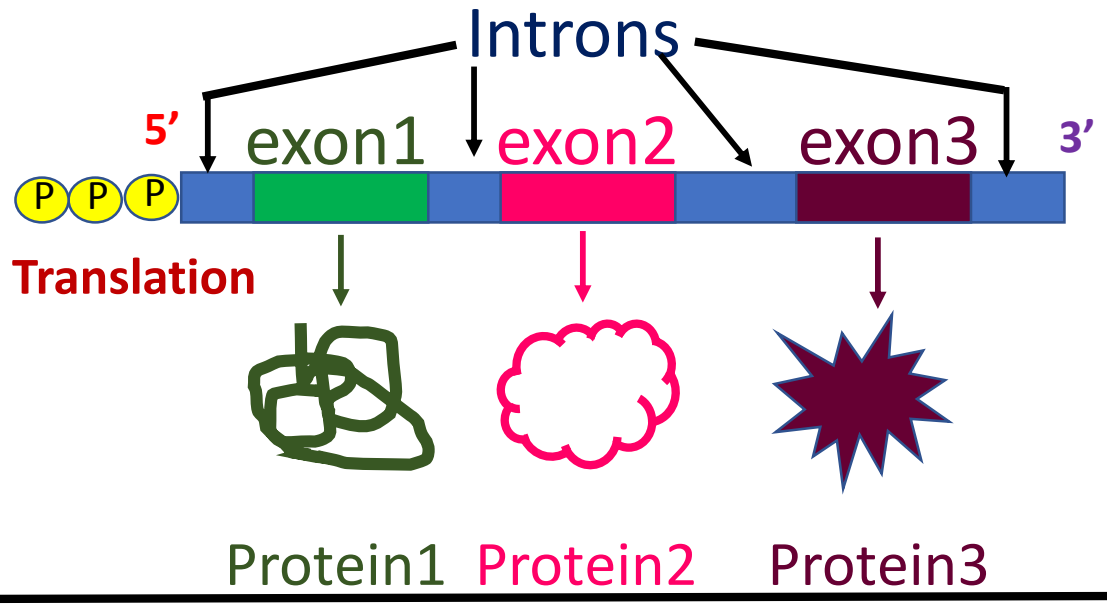
- **Cleavage** :of large precursor RNA for removal of excess sequences from the primary transcript by the action of **endonuclease** or **exonucleases** to a smaller molecule .
- **Splicing** : involves the removal of sequences called **introns** (sequences that do not code for proteins) from the primary transcript and joining of other sequences called exons (coding sequences) to each other to form functional RNA.
- **Terminal addition** of **nucleotides**
- **Nucleoside modification**

Template strand of DNA for synthesis of Messenger RNA(mRNA)



Template strand of DNA : One of two DNA strands(**non coding strand** or **anti-sense strand**)→to produces working copies of RNA molecules(formation of complementary copies of RNA transcript) . Other strand of DNA does not participate in transcription →referred as Coding Strand of gene or Sense Strand= non-template Strand

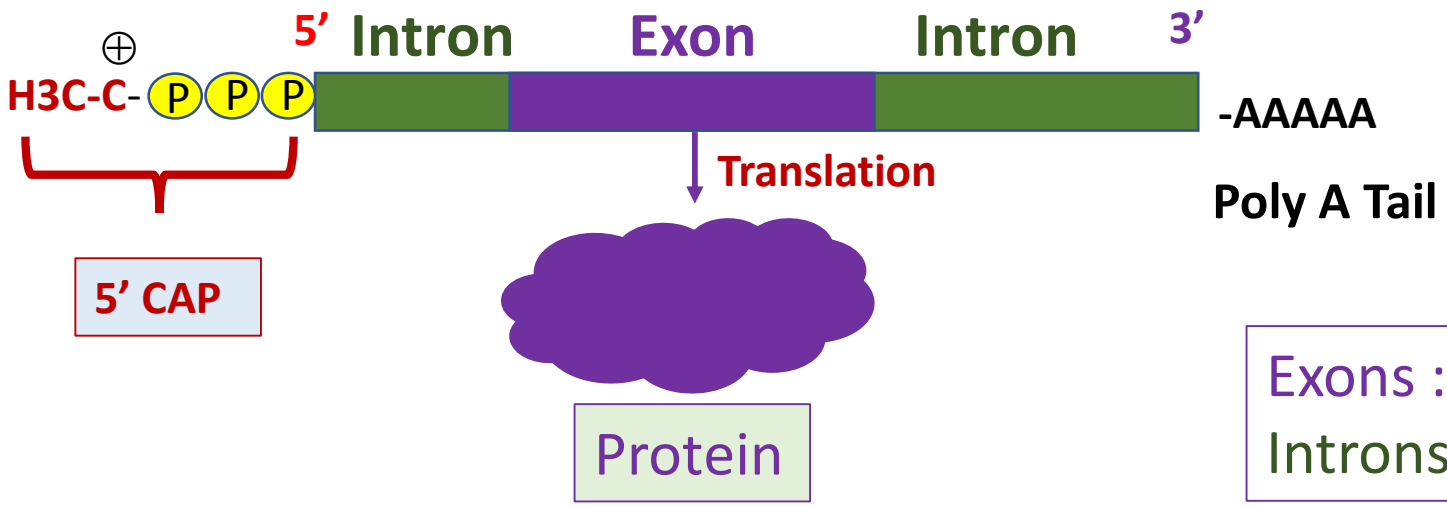
Comparison of Prokaryotic and Eukaryotic mRNA



Prokaryotic mRNA

Polycistronic

Schematic diagram



Eukaryotic mRNA

Monocistronic

Exons : coding sequences
Introns : non-coding sequences

Comparison of between RNA and DNA

Criteria	RNA	DNA
location	Mainly seen in cytoplasm	Mostly inside nucleus
Number of nucleotides	Usually 100-5000 bases	Million of base pairs
Number of strand/s	Generally single stranded	Double stranded
Constituent sugar	ribose	deoxyribose
Purines	Adenine ,Guanine	Adenine ,Guanine
Pyrimidine	Cytosine ,Uracil	Cytosine ,Thymine
Chargaff's rule	Guanine content not equal to cytosine and Adenine is not equal to Uracil	Guanine content equal to cytosine and Adenine is equal to Thymine.
Reaction with alkali	Destroyed by alkali	Alkali resistant

Comparison of Replication and Transcription

Criteria	Replication (DNA synthesis)	Transcription(RNA synthesis)
steps	Initiation, elongation, and termination	Initiation, elongation, and termination
Direction of synthesis	5'→3'	5'→3'
Watson's Crick base pairing	Followed	followed
Substrate	Deoxyribonucleotides (ATP, TTP, GTP, CTP)	Ribonucleotides (ATP, UTP, GTP,CTP)
Complementary base pair of Adenine	Thymine	Uracil
RNA primer	Required	Not required
Proof reading activity to excise mismatched nucleotides	Present and used by DNA polymerase	RNA polymerase lacks activity therefore no proof reading during transcription
Utilization of Genome in the process	The entire genome must be copied during DNA replication	A small portion of the genome is transcribed into RNA

Comparison of RNA and DNA polymerase

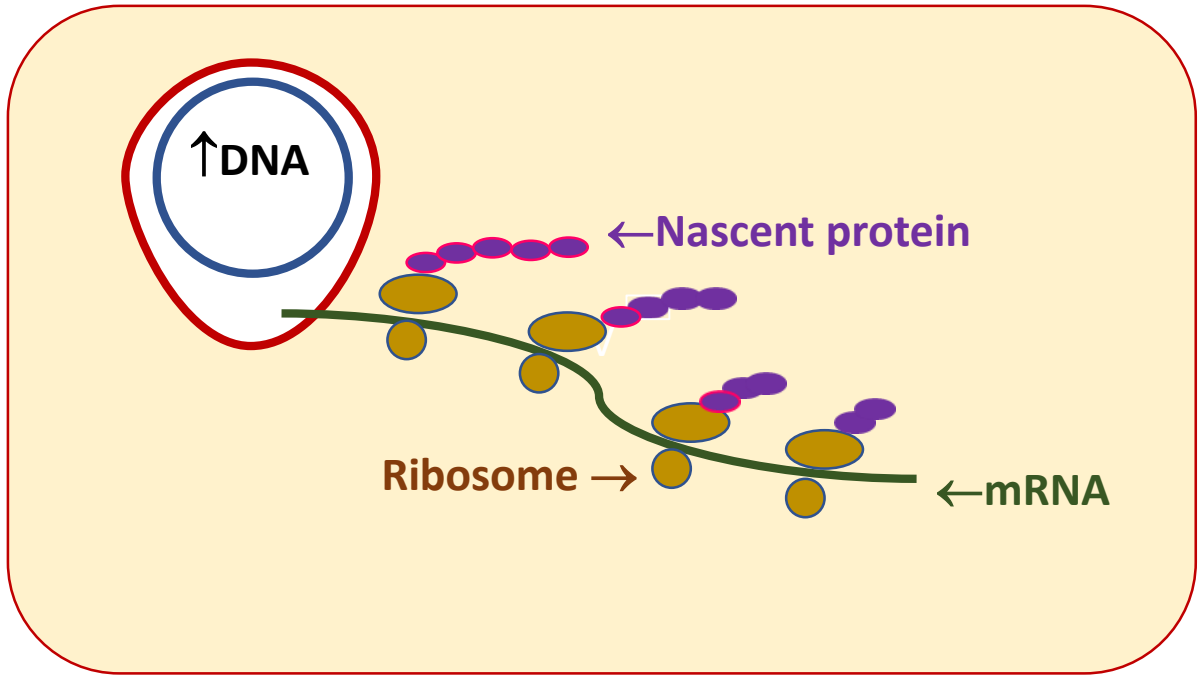
RNA polymerase	DNA polymerase
no primer required	primer required
no proof reading activity-less fidelity	proof reading activity—high fidelity
no exonuclease /endonuclease activity	exonuclease /endonuclease activity
no ability to repair mistakes (proofreading)	ability to repair mistakes (proofreading)
Mistakes made are less dangerous –RNA and are not transmitted to daughter cells.	Mistakes made are dangerous –DNA and are not transmitted to daughter cells.

Difference between prokaryotic and eukaryotic transcription

Criteria	Prokaryotic transcription	Eukaryotic transcription
Cellular compartment	transcription and translation occur in same cellular compartment.	transcription and translation occur in different cellular compartments. Transcription within the nucleus and translation outside nucleus .
Time factor for the course of translation	Bacterial mRNA translation begins during transcription.	Translation can occur only after transcription has finished.
Translation of primary transcript of mRNA	Occurs without undergoing processing of primary transcript of mRNA .	Occurs only after extensive processing of primary transcript of mRNA .
Base sequence of promoter site	**TATAAT** (**Pribnow box**) located at base -10 region and **TGTTGACA** at base -35 region	**TATAAA** located at base -25 region and **CAAT** located at base -75 region
Number of RNA polymerase to synthesize RNA	One/single RNA polymerase	Three types of RNA polymerases in nucleus

Schematic representation of prokaryotic transcription

DNA directed RNA synthesis

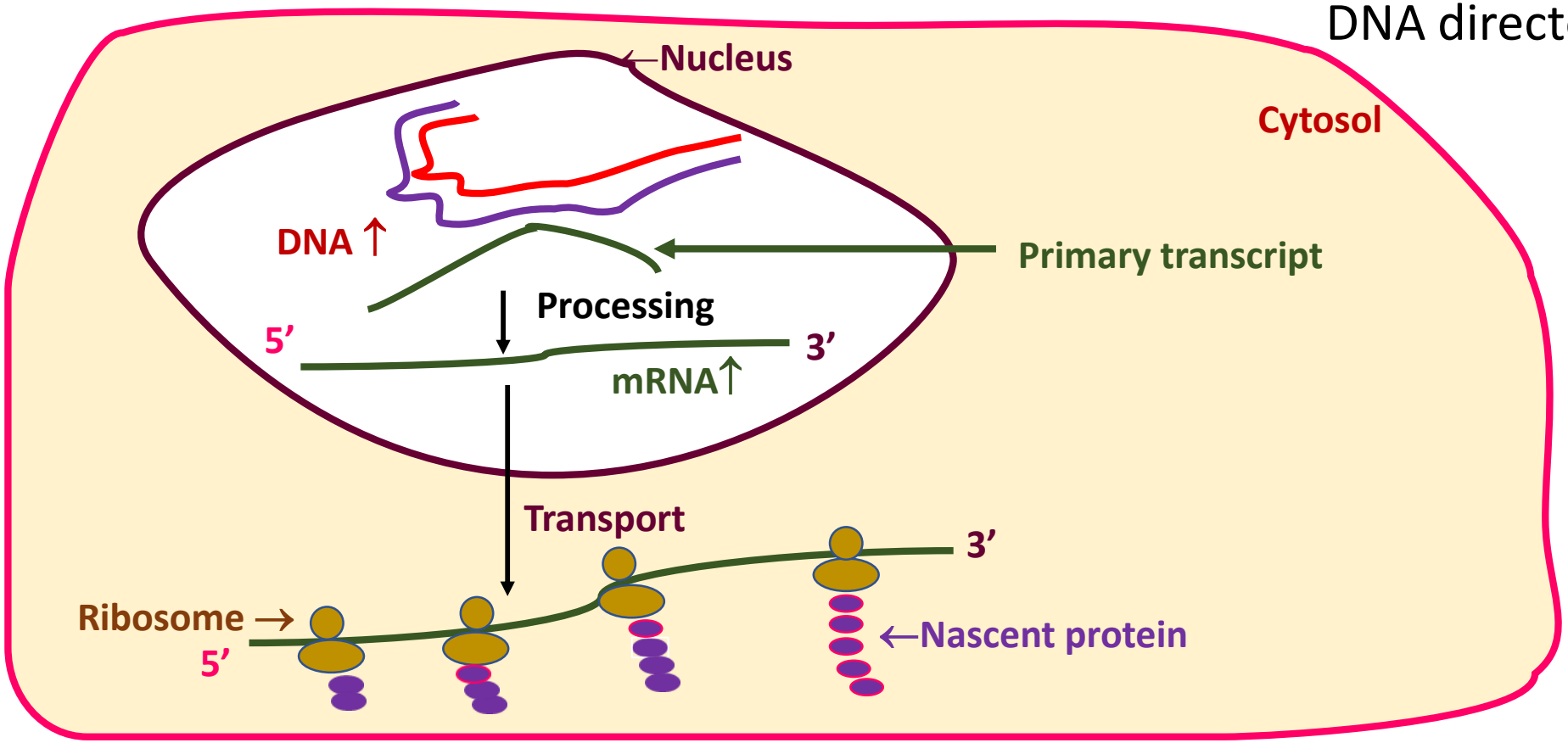


Prokaryotic cell

Steps of transcription Prokaryotic cell : Initiation , elongation and termination

Schematic representation of eukaryotic transcription

DNA directed RNA synthesis



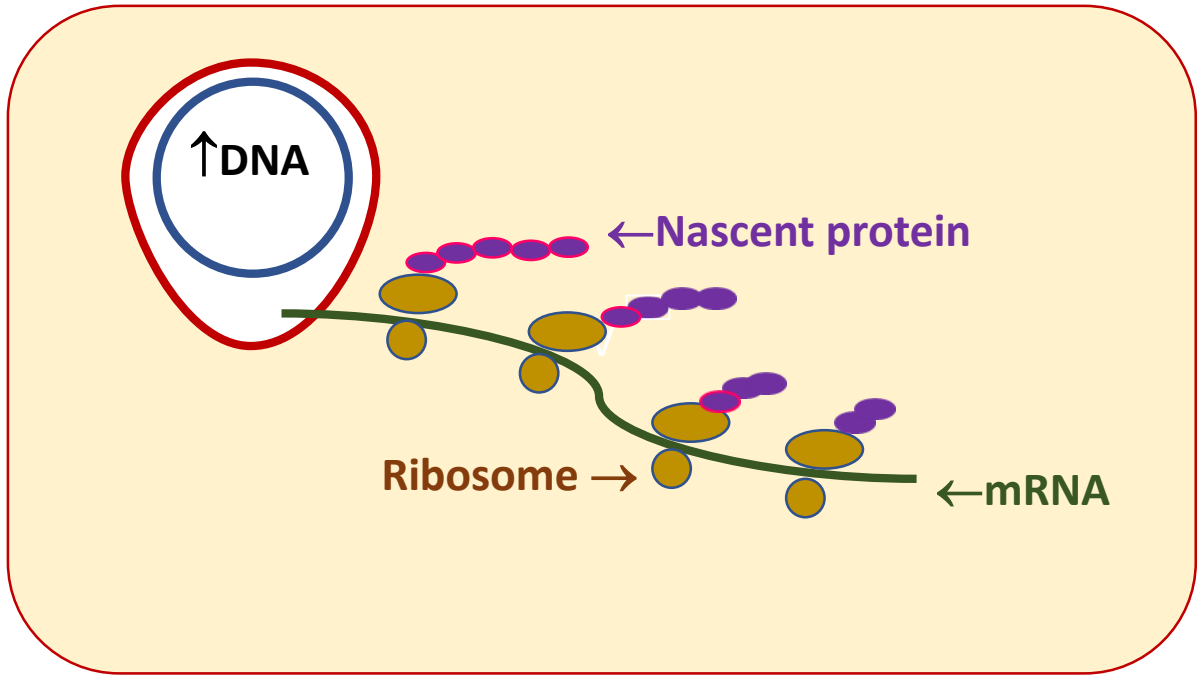
Steps of transcription Eukaryotic cell : Initiation , elongation and termination

❖Transcription (process of RNA synthesis) is best understood in prokaryotes . It is applicable to eukaryotes even though the enzyme involved and regulatory signals are different.

Transcription in prokaryotes

Schematic representation of prokaryotic transcription

DNA directed RNA synthesis



Roger Kornberg (Noble 2006): elucidated molecular basis of transcription.

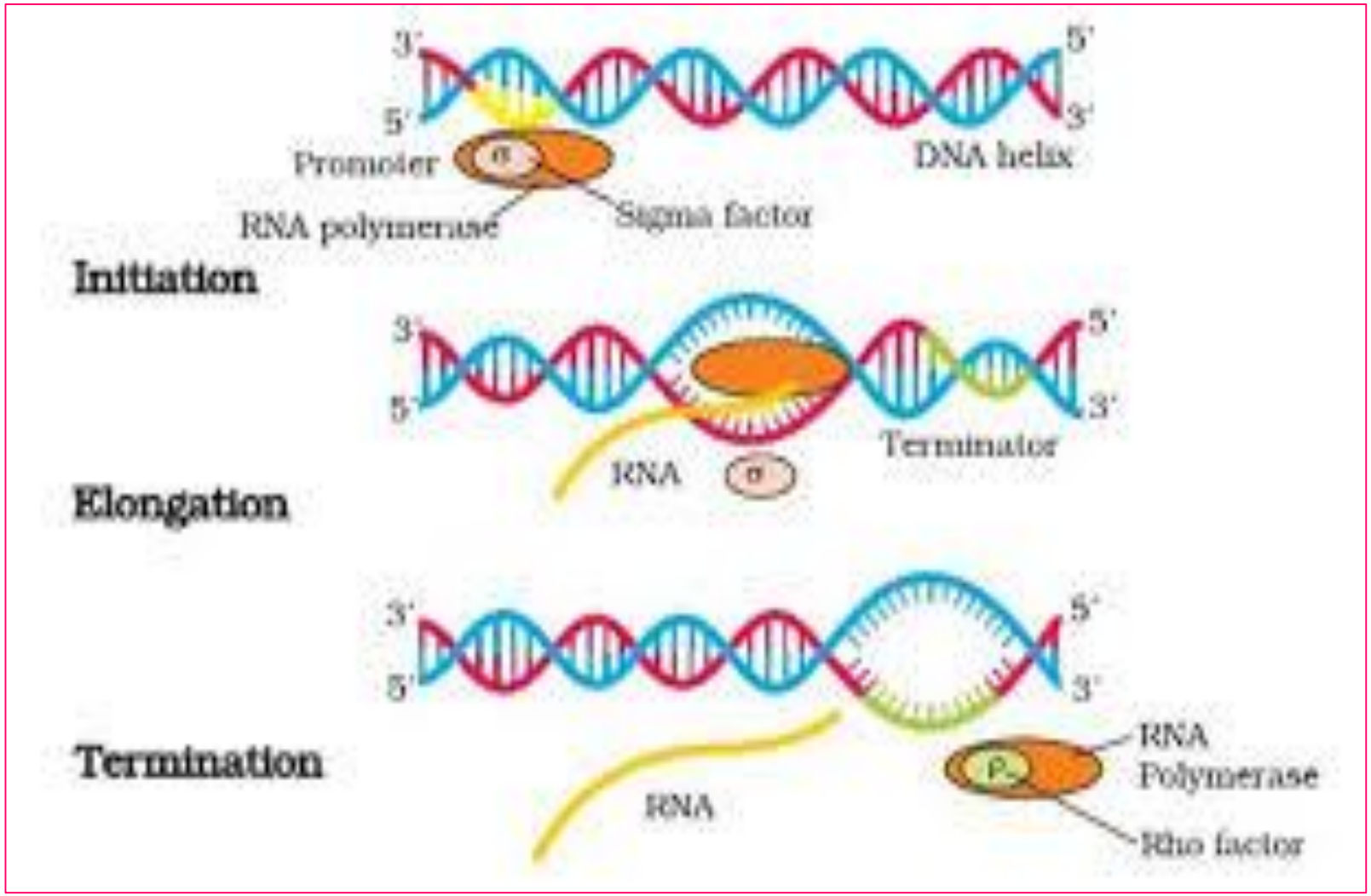
Prokaryotic cell

Steps of transcription Prokaryotic cell : Initiation , elongation and termination

Steps of Transcription in prokaryotes

❖**Four steps of Transcription in prokaryotes :**

1. Template recognition
2. Initiation
3. Elongation
4. Termination



RNA polymerase for Transcription in prokaryotes

- Enzyme required for **Transcription in prokaryotes : A single enzyme DNA dependent RNA polymerase** or **simply RNA polymerase**
- **Function of RNA polymerase :** synthesis of all three types of RNA
- **RNA polymerase** : mw 465000(465kDa) and holoenzyme has 5 polypeptide units viz 2Alpha,1 Beta,1 Beta', 1 ω(core enzymeα_2 β β'ω)+ Sigma factor (σ)
- **Cofactors of RNA polymerase: Mg^{+2} , Zn^{+2}**
- **Substrates of RNA polymerase :** four **5'** triphosphates **(**UTP, ATP ,CTP**,**GTP **) and** DNA template
- **Steps of Transcription in prokaryotes : Initiation ,Elongation, Termination**
- **Products of RNA polymerase in transcription :** primary transcript

Except prokaryotic m- RNA, all other primary transcript undergo **post-transcriptional changes.**

Subunits of RNA polymerase responsible for Transcription in prokaryotes

❖**subunits of RNA polymerase :** 2 Alpha,1 Beta, 1 Beta', 1 ω(core enzyme α_2 β β'ω) + Sigma factor (σ)

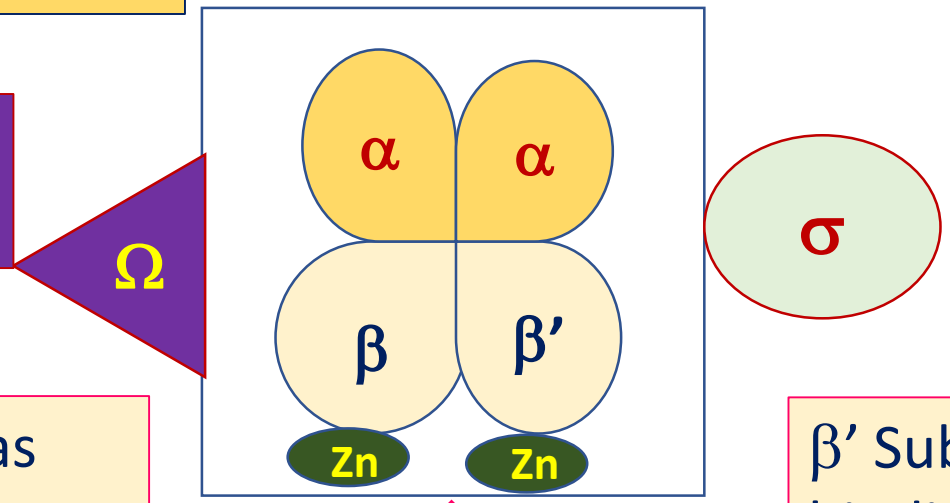
- **2 Alpha subunits (α_2) :** these are identical subunits ,each of which is encoded by the *rpo* A gene . Alpha subunit is essential for core protein assembly .
- **Beta subunit (β) :**This is encoded by the *rpo* B gene . The anti tuberculosis drug **rifampicin** binds to Beta subunit and inhibits transcription initiation, whereas **streptomycin** blocks transcription elongation.
- **Beta' subunit(β') :** this subunit is encoded by the *rpo* C gene ,binds to two Zn^{2+} that may be required as a cofactor for catalytic activity . Hairpin inhibits Transcription in vitro by binding to the Beta' subunit(β').
- **Sigma factor (σ factor):** binding of the polymerase to specific promoter regions of DNA template and increase the affinity of the holoenzyme to the promoter site.

Functions subunits of RNA polymerase of prokaryotes

α Subunits required for enzyme assembly

Schematic diagram

Omega subunit (function unclear)



Sigma factor recognizes the promoter site and increase affinity of holoenzyme to the promoter site.

β Subunit has 5'→3'polymerse activity

β' Subunit fixes at initiation site→ template binding.

Core enzyme

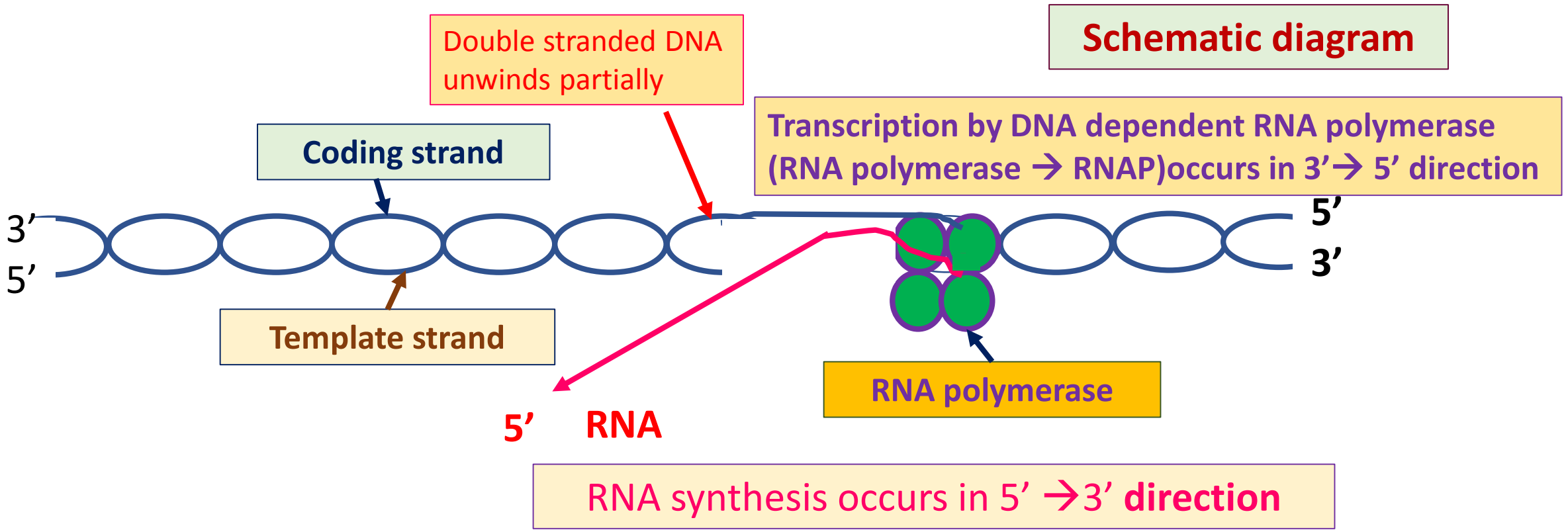
Zinc molecules serve as cofactor

Holoenzyme

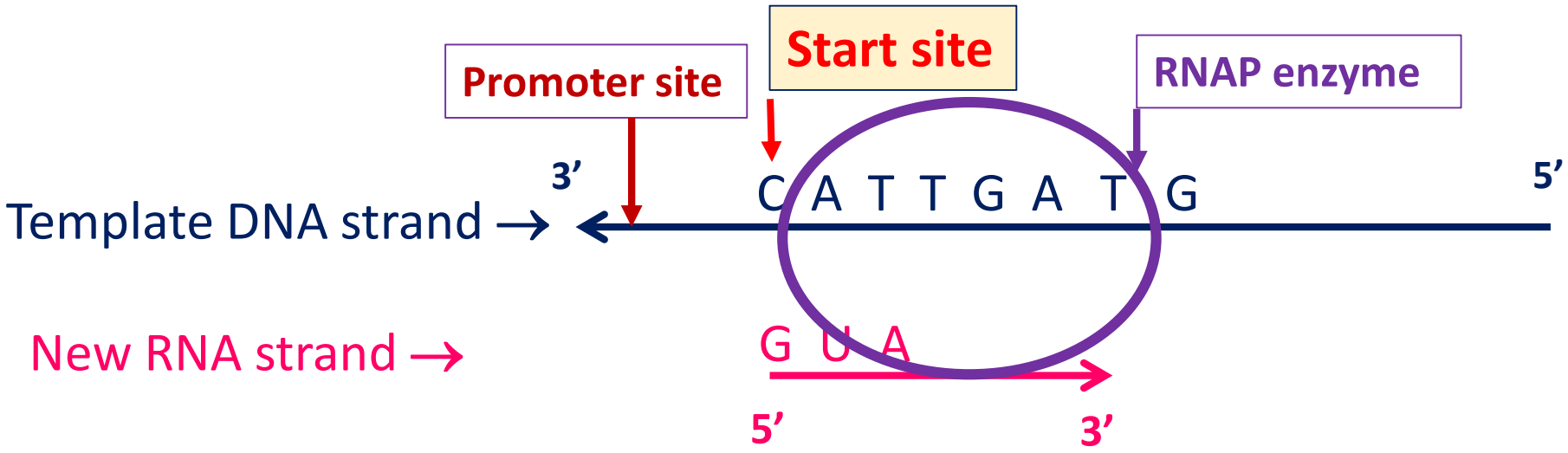
Role of Sigma factor (σ factor) of RNA polymerase in prokaryotes

- **Sigma factor^{70} (σ^{70}factor): is the most common Sigma factor in E.coli** that is responsible for transcription initiation by promoter . It enables the RNA polymerase holoenzyme to recognize and bind tightly to promoter sequences.
- Other **Sigma factors are expressed** under altered **environmental** conditions such as σ^{32} during heat shock and σ^{N} during nitrogen starvation have been recognized .

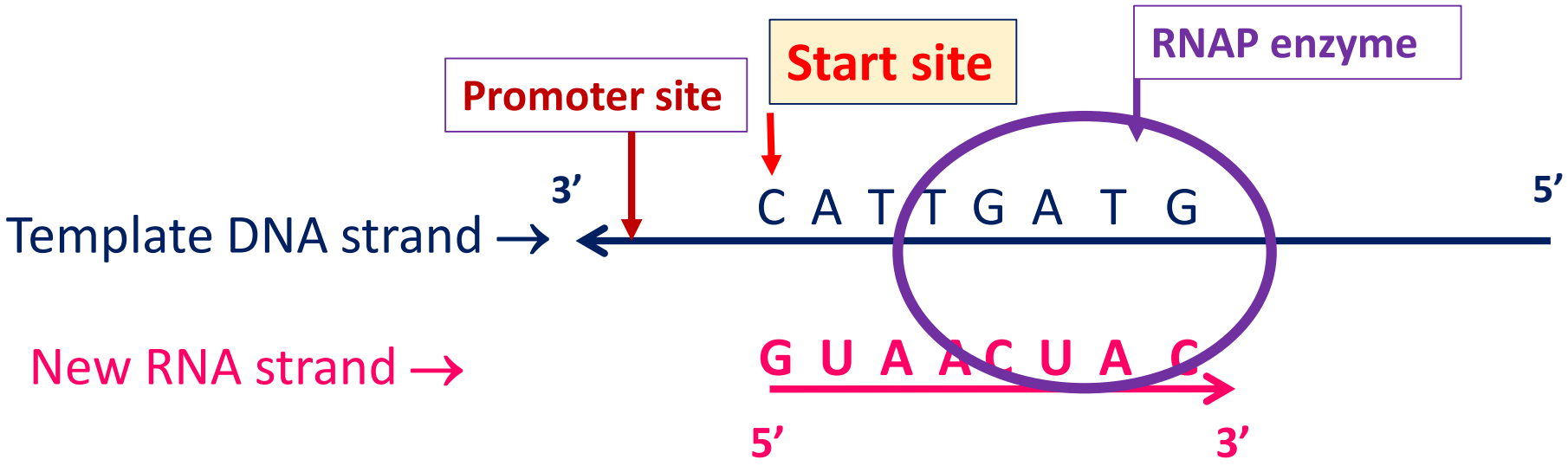
An overview of transcription in prokaryotes



Initiation of transcription



Elongation of transcription



Initiation of Transcription in prokaryotes

- **Initiation :** Transcription in prokaryotes is initiated by binding of RNA polymerase (holoenzyme=core enzyme +sigma factor)to a specific region of DNA strand called the Promoter region /site. (this binding is prerequisite for the transcription to start).
- **Promoter site** : are characteristic sequences of DNA .They are different in prokaryotes and eukaryotes.
- Transcription in prokaryotes begins at a specific site in DNA called the **start site /point** and stops at a terminator sequence.
- A **transcription unit** :The sequence extending between a Promoter region and a terminator sequence.
- **Upstream sequences** (denoted by a negative sign) : bases in DNA sequences before /prior to start point of gene to be transcribed.
- **Downstream sequences** (indicated by a positive sign) : bases in DNA sequences after/following start point of gene to be transcribed.

➢ Position +1 indicates the first nucleotide that will be transcribed into RNA.

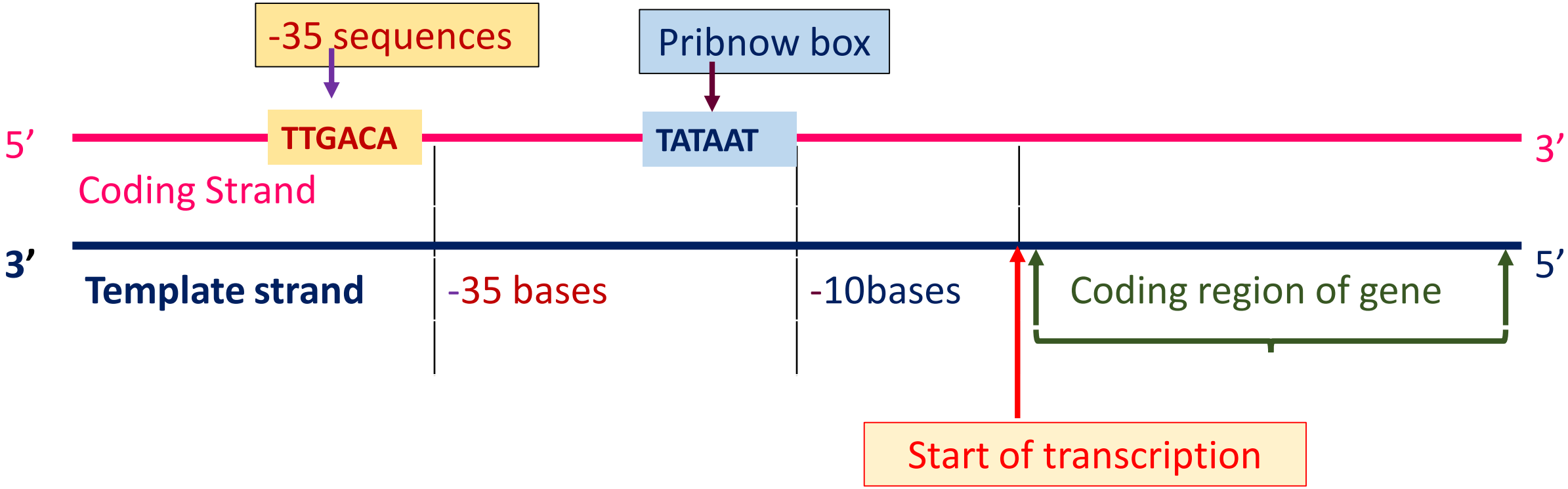
Promoter regions for Transcription in prokaryotes

- **Promoter region** : a specific region on DNA where RNA polymerase binds.
- Two base sequences on coding DNA strand which the sigma factor of RNA polymerase recognize for initiation of transcription in prokaryotes .
- **Prokaryotic genes have two promoter sequences :**

1. Pribnow box (TATA BOX) has 6 nucleotide bases of T A T A A T(consensus sequence**) and** usually located on the left side 10 base pairs away(upstream/ front) from the start point of transcription.

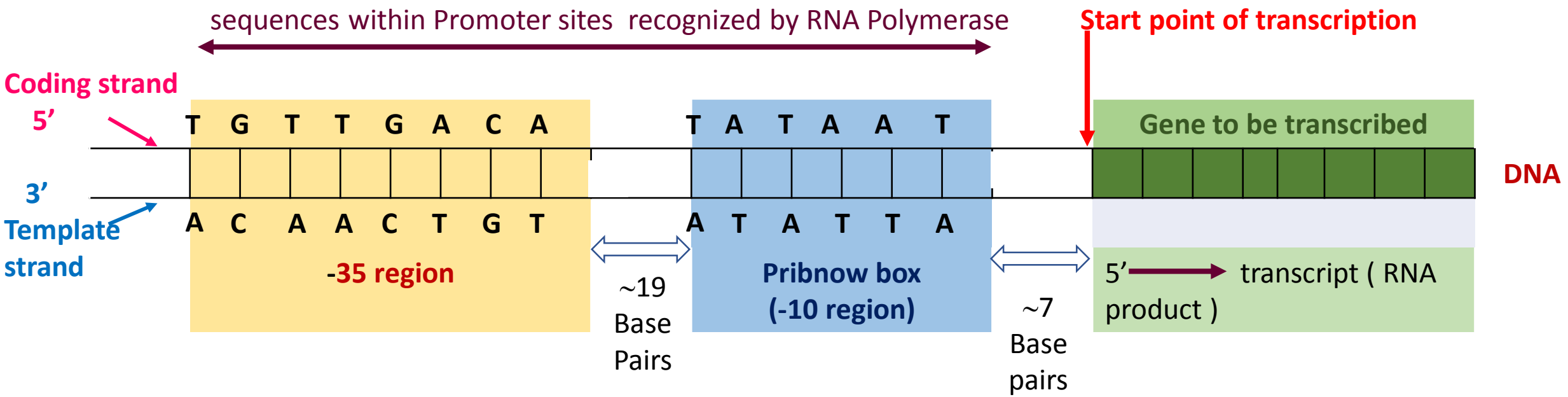
2. The **'-35 'sequence/region**: A second recognition side in the promoter region of DNA, with base(consensus) sequence **T T G A C A** and is located 35 base pairs (upstream hence**-35**) left side from the site of transcription start.

Promoter regions of DNA in prokaryotes



Schematic diagram

Promoter sites of prokaryotic transcription

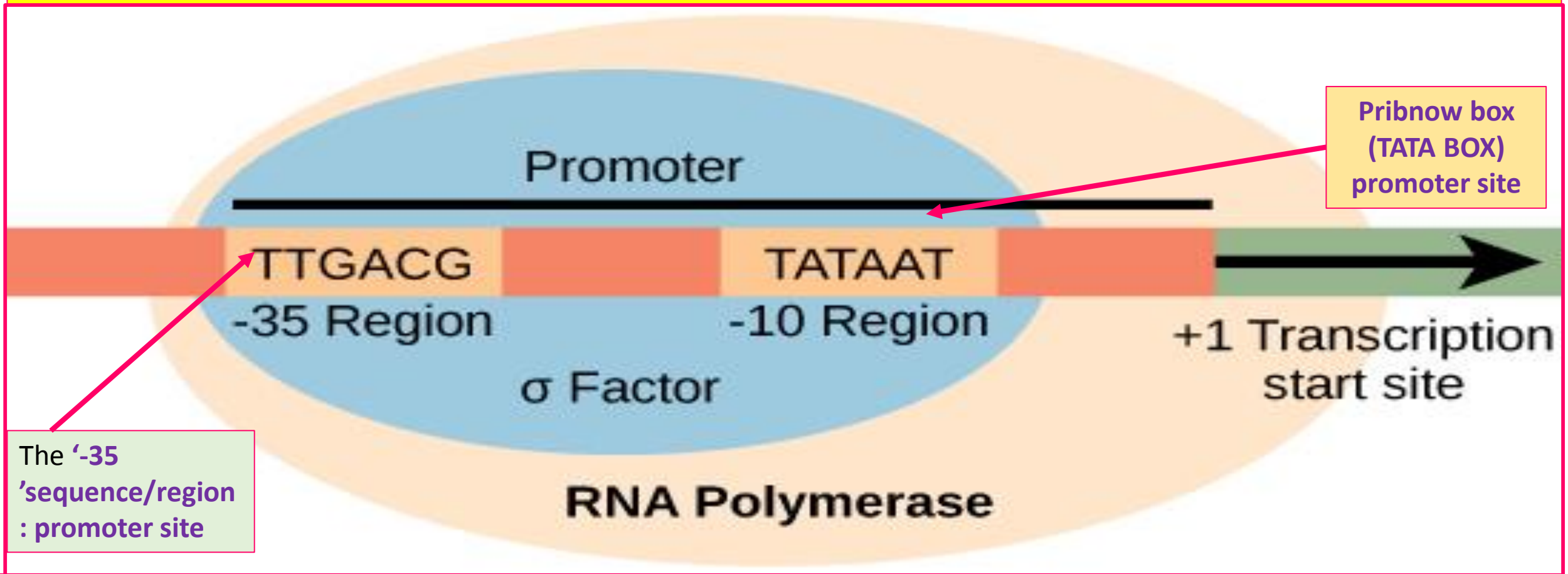


Prokaryotic genes have two promoter sequences:

1. Pribnow box of prokaryotic gene has the consensus sequence **T A T A A T** and is centered at -10 (usually found 10 base pairs upstream start point).

2. The other promoter sequence **(-35 region)** has the consensus sequence **T T G A C A(35 base pairs** upstream start point).

Promoter sequences and Sigma factor in E.coli



RNA polymerase recognizes and binds to -35 and -10 sequences on the promotor region.

Sigma factor^{70} (σ^{70}factor): is the most common Sigma factor in E.coli that is responsible for transcription initiation by promoter initiation. σ^{32} **are expressed** during heat shock and σ^{N} during nitrogen starvation have been recognized .

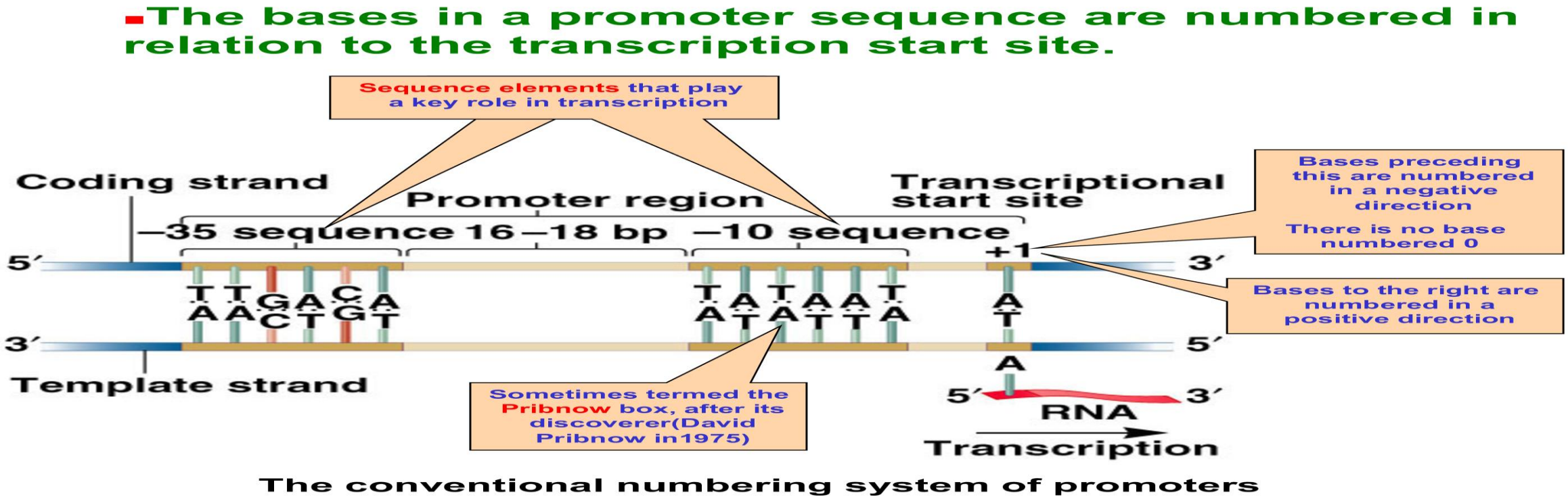
Silent features of Bacterial Promoter region

- E.Coli promoter sequence σ^{70} ranges between 40 -60 base pairs.
- RNA polymerase binds to the promoter around – 55 to +20.
- 4 features are conserved in bacterial promoters:

TTGACA …….16-18 bp …TATAAT …..5-8 bp….start point

1. Start point : is usually a purine (A or G) and is the central base in the sequence CG(A) T.
2. The -10 sequence : is present - 10 nucleotides upstream of the Start point contains 6 bp AT rich consensus sequence TATAAT with the following percentage of conservation: T_{80} A_{95} T_{45} A_{60} A_{50} T_{95}
3. The -35 sequence : is present - 35 nucleotides upstream of the Start point has consensus sequence TTGACA and functions as a recognition site for RNA polymerase and enhances interaction with **Sigma factor(σ)** .
4. The distance between the -10 sequence and the -35 sequence is 6-18bp.

The -35 sequence and The -10 sequence of bacterial promoter



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12-9

The -10 sequence : is present - 10 nucleotides upstream of the Start point contains 6 bp AT rich consensus sequence TATAAT with the following percentage of conservation: T_{80} A_{95} T_{45} A_{60} A_{50} T_{95}

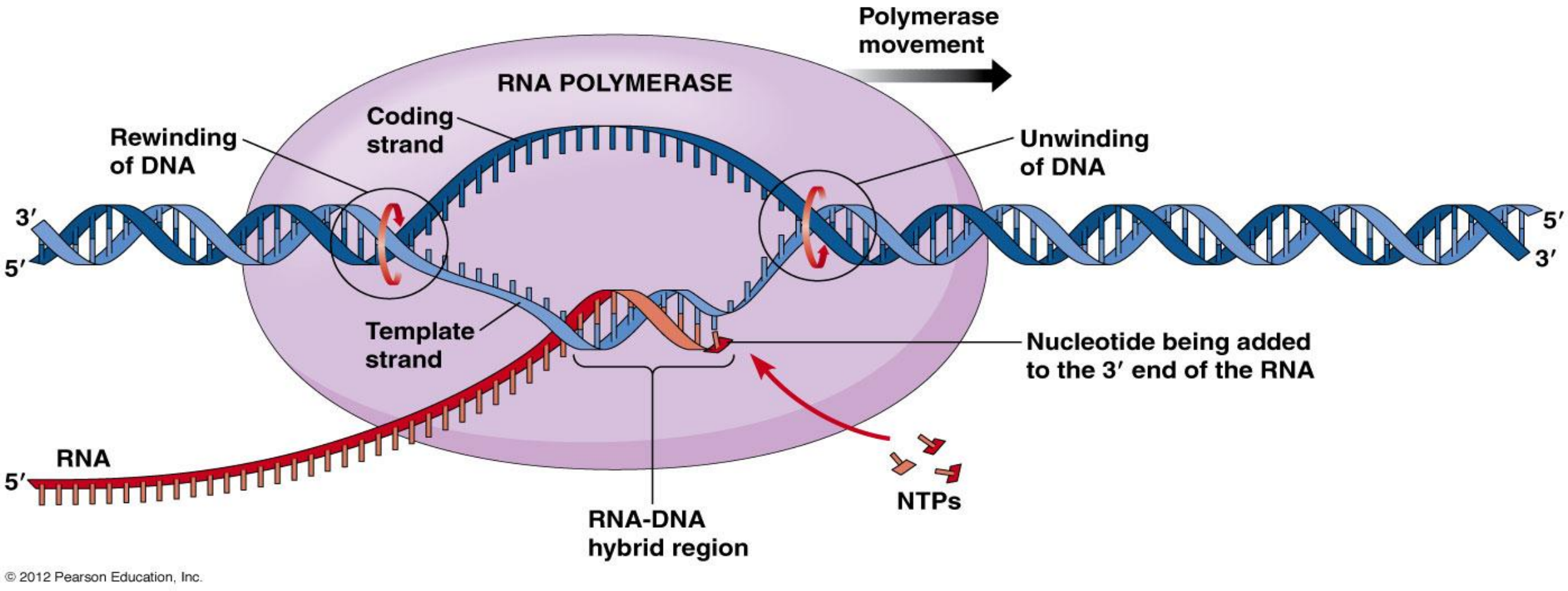
The -35 sequence : is present - 35 nucleotides upstream of the Start point has consensus sequence TTGACA and functions as a recognition site for RNA polymerase and enhances interaction with **Sigma factor(σ)** .

The distance between the -10 sequence and the -35 sequence is 6-18bp.

Process of Template recognition of Transcription in prokaryotes

1. RNA polymerase recognizes and binds to -35 and -10 sequences on the **promotor region.**
2. The **sigma factor** (σ) increases the affinity for promoter.
3. The initial binary complex of the enzyme and the promoter DNA is termed as the **closed complex.**
4. RNA polymerase unwinds the DNA double helix over a short distance of about 17bp converting **closed complex** to **open complex.**
5. DNA unwinding forms the **Transcription bubble and** makes the template stand available for Transcription.

Transcription bubble formed by unwinding of DNA by RNA polymerase

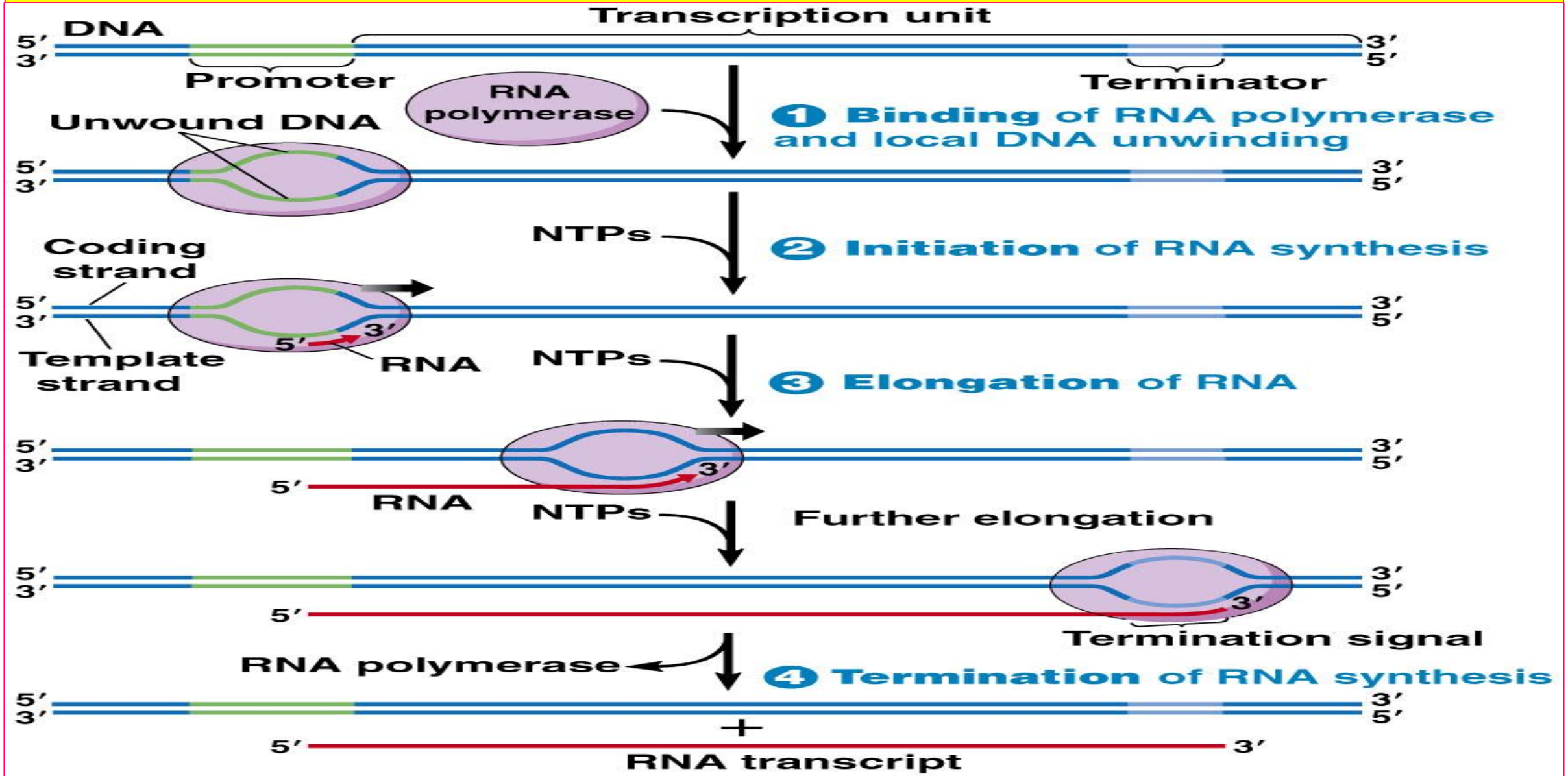


RNA polymerase unwinds the DNA over a short distance of about 17bp converting **closed complex** to **open complex** . DNA unwinding forms the **Transcription bubble and** makes the template stand available for Transcription.

Process of Initiation of Transcription in prokaryotes

1. Involves the formation of a phosphodiester bond between the first nucleotide (usually a purine nucleotide i.e. GTP or ATP) and second nucleotide (usually UTP or CTP), thereby creating a **ternary complex** of RNA polymerase-DNA –nascent RNA.
2. The 5’ triphosphate group on the first nucleotide is not hydrolyzed but remains intact throughout Transcription.
3. RNA polymerase makes short transcript of up to 9 bp releases them and reinitiates RNA synthesis by a process termed as **abortive initiation** .
4. The mean time for promoter Clearance by RNA polymerase is 1- 2 seconds.
5. Following initiation ,the sigma facto (σ) is released from the holoenzyme and the core enzyme undertakes elongation.

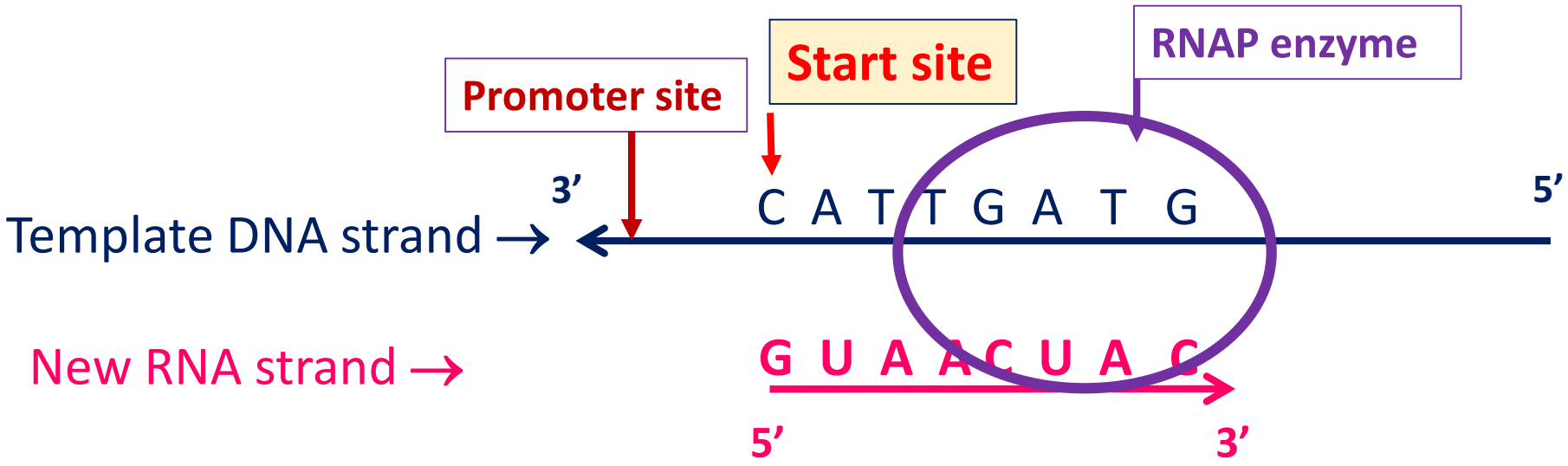
Process of Template recognition of Transcription in prokaryotes



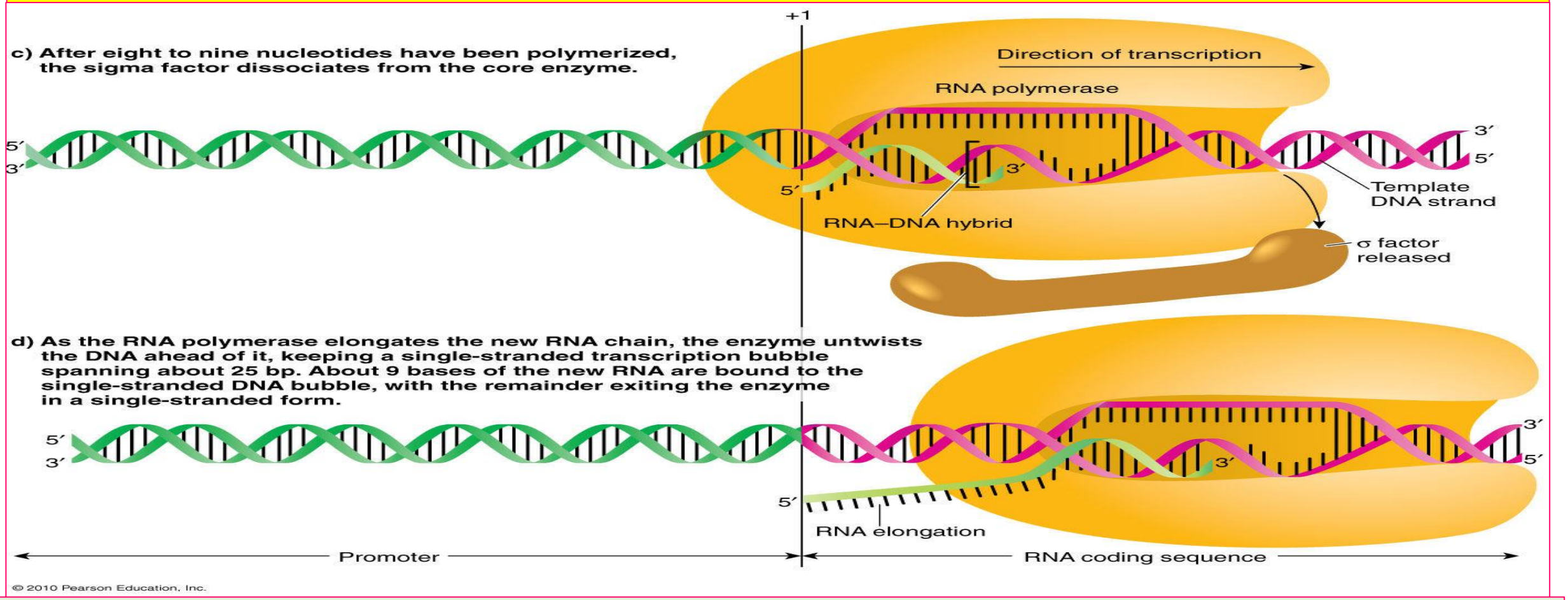
Elongation of transcription in prokaryotes

- As the holoenzyme RNA polymerase recognizes the **Promoter region,** the sigma factor is released and transcription proceeds .
- Double helical structure of DNA unwinds and as transcription goes on →results in supercoils(overcome by topoisomerase)

Elongation of transcription



Elongation of Transcription in prokaryotes



Double helical structure of DNA unwinds and as transcription goes on →results in supercoils(overcome by topoisomerase). As the holoenzyme RNA polymerase recognizes the **Promoter region,** the sigma factor is released and transcription proceeds .

Characteristics of Elongation of transcription in prokaryotes

- Site for binding of the binding of RNA polymerase to DNA : Promoter region
- **Direction of RNA synthesis**: from 5’ end to 3’(5’→3’anti-parallel to the DNA template).
- **Substrate for RNA polymerase for elongation of transcription:** Ribonucleotides ATP,GTP,CTP,UTP for the formation of RNA
- For the addition of each nucleotide to the growing chain ,a pyrophosphate (PPi) moiety is released.
- The sequence of nucleotide bases in mRNA complementary to the template DNA strand & identical to that of coding strand (except RNA contains U in place of T in DNA).

➢ Mistake in RNA synthesis are less dangerous as not transmitted to daughter cells.

➢ The double helical structure of DNA unwinds as the transcription goes on , resulting in supercoils.

➢ The problem of supercoils is overcome by topoisomerase.

Steps of Elongation of Transcription in prokaryotes

1. Elongation proceeds after the formation of the first phosphodiester bond.
2. RNA polymerase moves along DNA unwinding short region for elongation in front of transcription bubble and rewinds DNA behind it.
3. Ribonucleotides are successively added to the growing RNA chain.
4. The 3’ end of RNA forms a transient hybrid of 8-1 base pair with the template DNA.
5. The core enzyme then continues the elongation of the transcript.
6. By the 10 nucleotides have been added , the sigma factor dissociates.
7. The released sigma factor can combine with free core enzymes to form another holoenzymes that can initiate transcription.
8. The process of elongation of the RNA chain continues until a termination signal is reached.
9. RNA polymerase is released from elongation arrest by GreA and Gre B .

Process of termination of transcription in prokaryotes

❖**Transcription** continues until RNA polymerase encounters the terminator sequence/signals .

❖**Termination involves the following events :**

1. Release of RNA transcript from DNA
2. Dissociation of the RNA-DNA hybrid
3. Reformation of the DNA duplex

Two mechanisms of Termination of Transcription in prokaryotes

- The process of **Termination of Transcription in prokaryotes stops by Termination signals .**
- In prokaryotes ,termination of transcription occurs by one of the two well characterized mechanisms.
- **Two mechanisms of Termination of Transcription in prokaryotes:**

1. **Rho (ρ) factor dependent termination**
2. **Rho (ρ) factor independent termination**

Events of Termination in transcription in prokaryotes

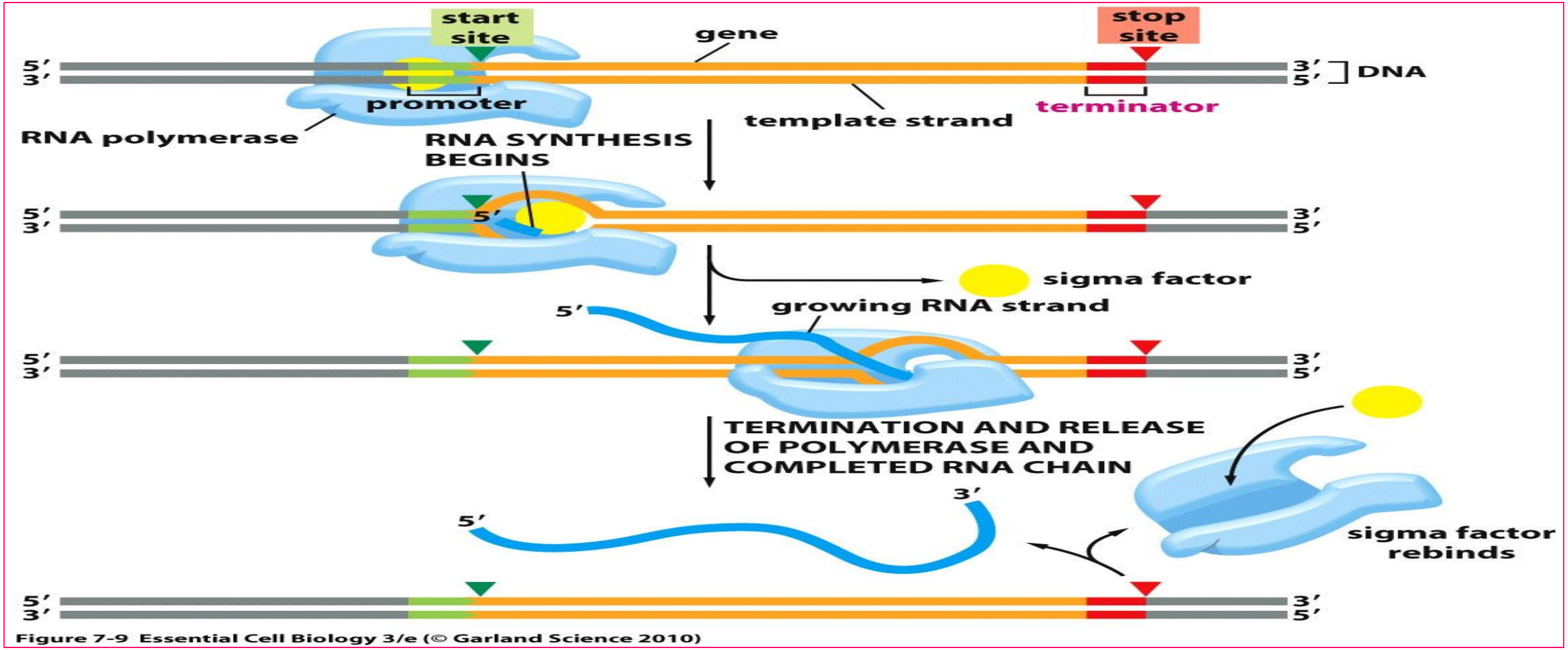


Figure 7-9 Essential Cell Biology 3/e (© Garland Science 2010)

Events of Termination : Release of RNA transcript from DNA ,Dissociation of the RNA-DNA hybrid ,Reformation of the DNA duplex

Rho(ρ) factor dependent Termination of Transcription in prokaryotes

❖**Rho-dependent termination , requires a protein factor called rho(ρ) factor which recognizes termination signal/sequence.**

❖**Rho (ρ) factor :**

1. is a specific protein(a hexamer with 5'→3' helicase activity and RNA –dependent ATPase activity)
2. binds to growing / nascent RNA upstream of the terminator(ATPase activity in bound state).
3. binds weakly to DNA.
4. doesn't bind to RNA polymerase .

❑**Functions** of **Rho (ρ) factor** at the termination sequence :

➢unwinds RNA-DNA duplex.

➢ dissociates/displaces RNA polymerase from DNA template as in a bound state(it acts as **ATPase),** resulting in termination of transcription(RNA synthesis)**.**

➢ dissociates RNA primary transcript from DNA template.

Rho (ρ) independent Termination of Transcription in prokaryotes

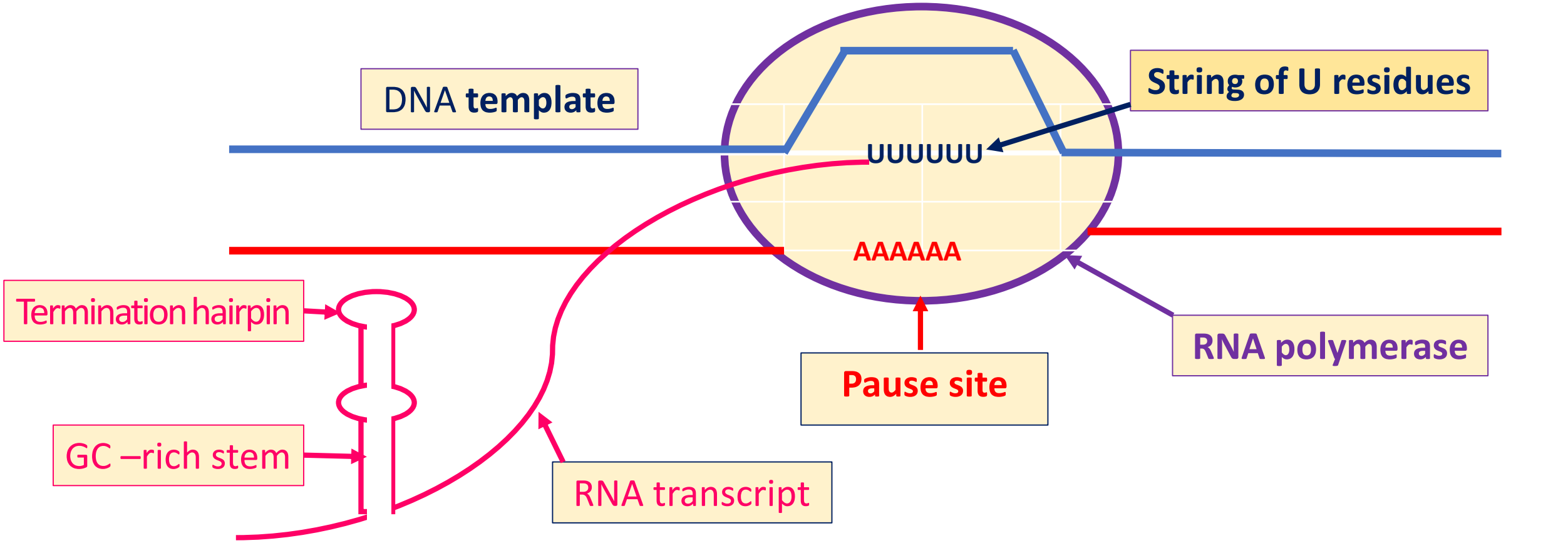
- **Rho (ρ) factor independent termination** : The termination is brought about by the formation of hairpin loop(secondary structure) of newly synthesized RNA. This occurs due to **Palindromes .**
- **Palindrome is a word that** reads alike forward & backward (MADAM ,ROTOR)
- The presence of palindromes in the base sequence of DNA template in the termination region is known .
- As a result of this, newly synthesized RNA folds to form hairpins (due to complementary base pairing) that cause the termination of Transcription. This dislodges the RNA polymerase from DNA template and release of the newly synthesized transcript.
- This hairpin loop structure is followed by a sequence of four or more uracil residues which are essential for termination. The RNA transcript ends within or just after then.

Mechanism of Rho (ρ) independent Termination of Transcription in prokaryotes

- **Rho (ρ) independent (intrinsic) termination** :is based on two structral features at the end of the RNA transcript**:**

1. A self complementary hairpin structure 15-20 nucleotide before the end of the RNA . The base pair of the hairpin which is GC rich, prevents RNA binding and disturbs the RNA-DNA hybrid .
2. A string of U residues that base pair weakly with corresponding A residues in the template DNA strand . As a result , RNA dissociates from the RNA-DNA hybrid.

Mechanism of intrinsic termination of transcription



A A T C C A C A G G C G C C A G X X X X X C T G G C G C A

T T A G G T G T C C G C G G T C X X X X X G A C C G C G T

A A U C C A C A G G C G C C A G U U C C G C U G G C G C A U U U U

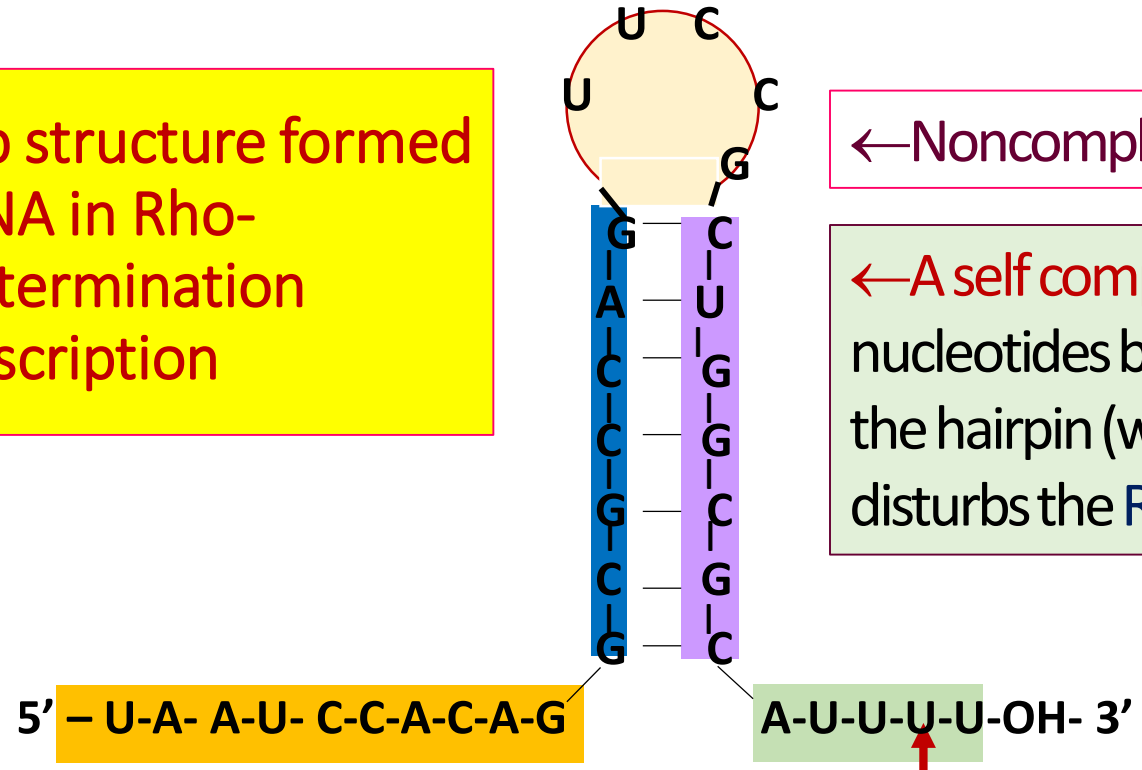
←Coding stand of DNA

←Template stand of DNA

Transcription

←Primary transcript of RNA

A hairpin loop structure formed by nascent RNA in Rho-independent termination phase of transcription



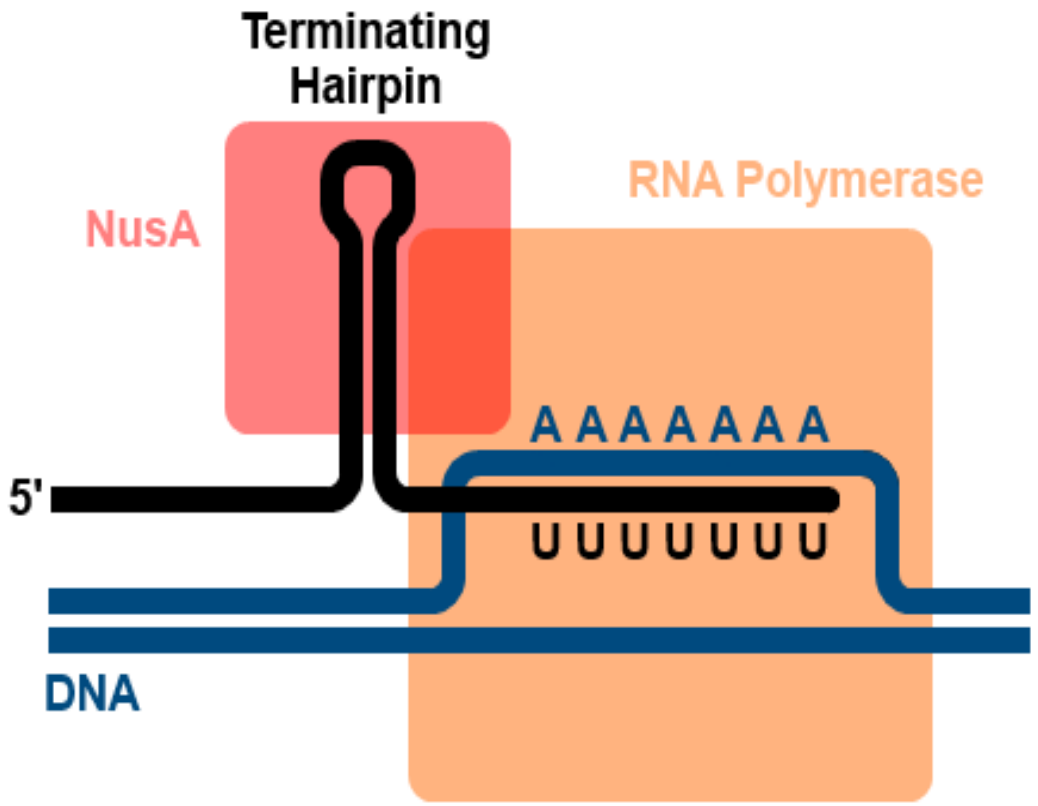
←Noncomplementary sequences in a primary transcript

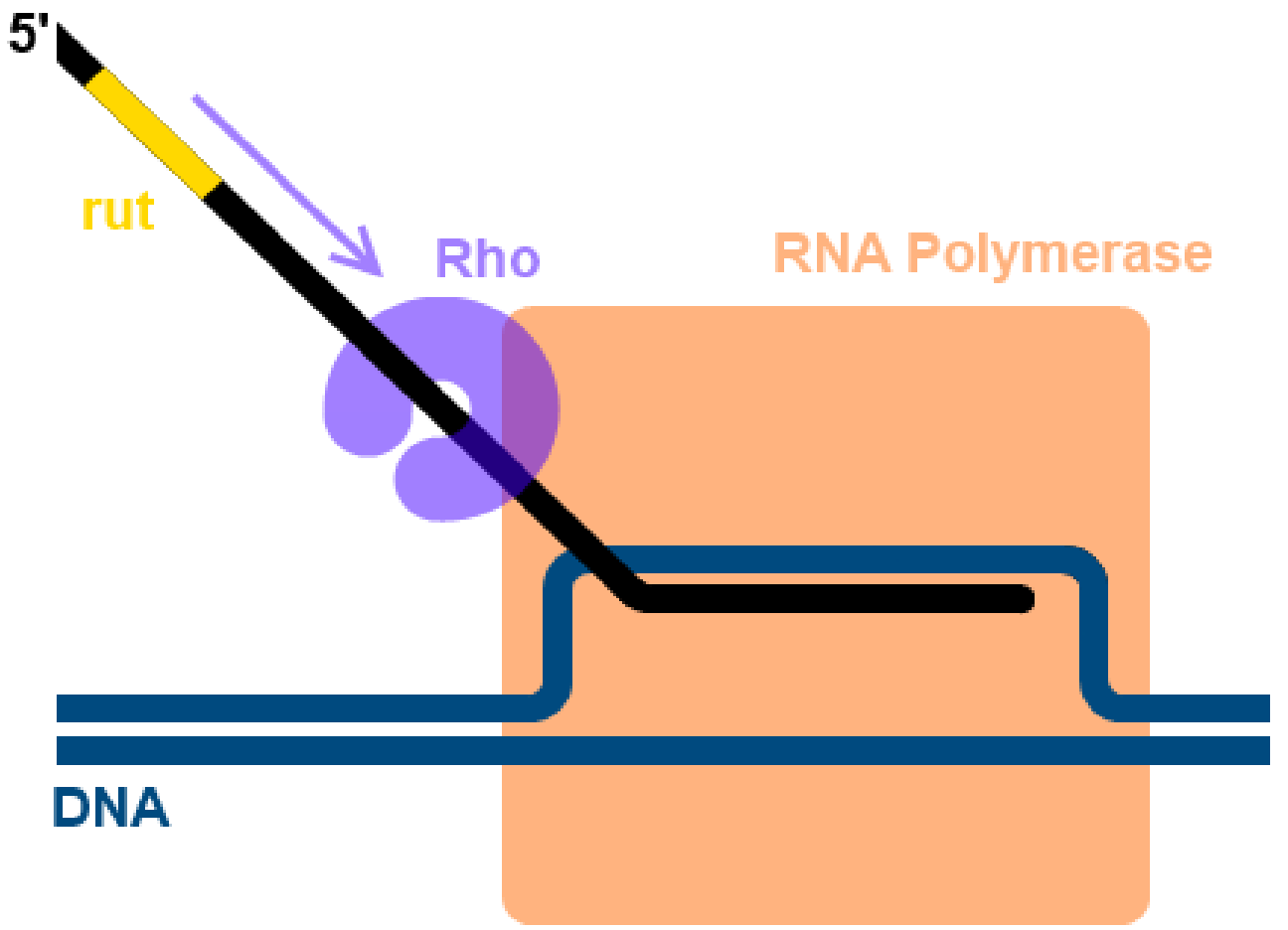
←A self complementary hairpin structure of 15-20 nucleotides before the end of the RNA . The base pairs of the hairpin (which is GC rich) prevents RNA binding and disturbs the RNA-DNA hybrid .

Schematic diagram

A hairpin loop structure followed by a sequence of Uridine residues. A string of U residues that base pair weakly with corresponding A residues in the template DNA strand . As a result , RNA dissociates from the RNA-DNA hybrid.

Rho (ρ) factor dependent and Rho (ρ) factor independent termination of Transcription in prokaryotes:1

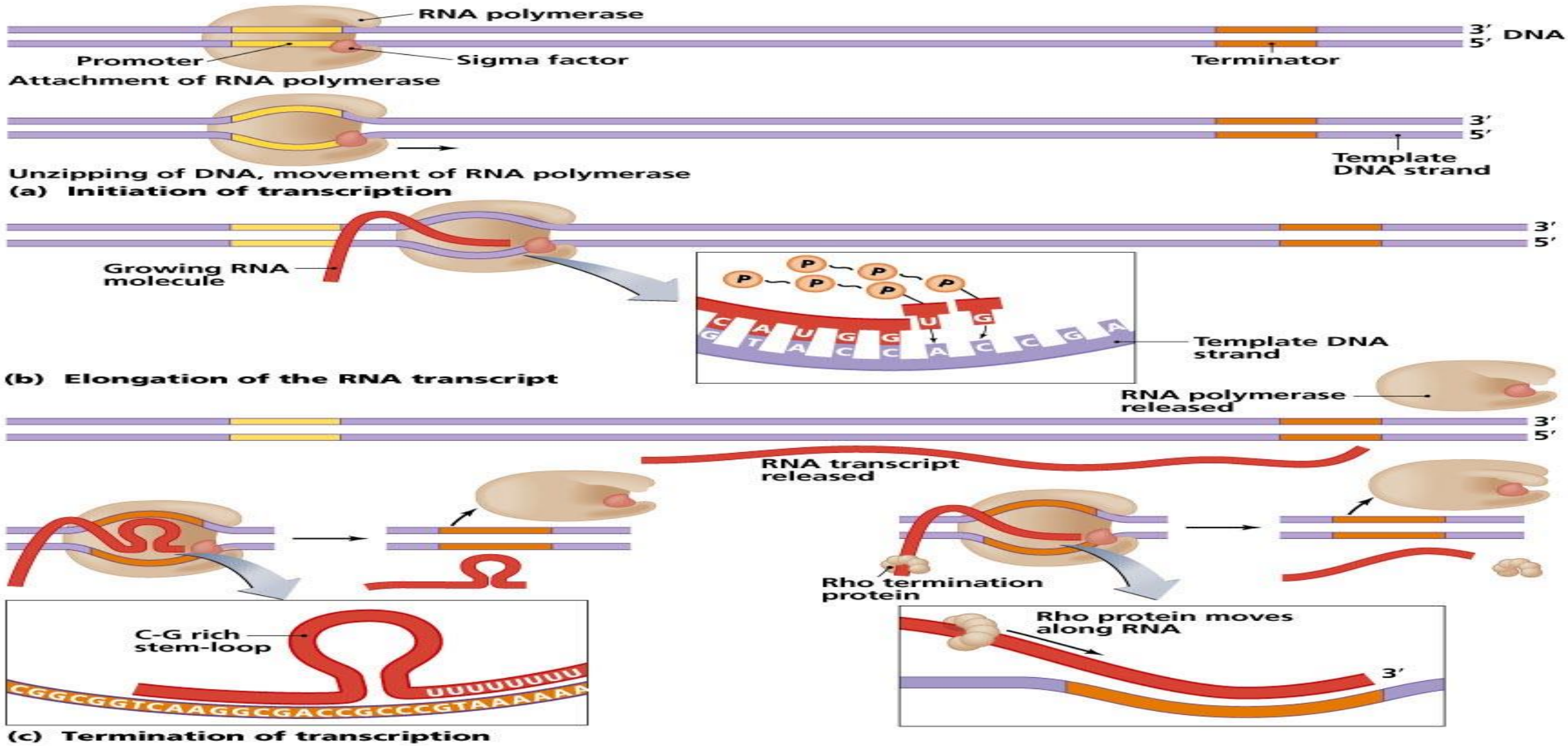




Rho (ρ) independent Termination of Transcription in prokaryotes : newly synthesized RNA folds to form hairpins (due to complementary base pairing) that cause the termination of Transcription.

Rho –dependent termination of Transcription in prokaryotes requires a protein factor called **rho(ρ)** factor which dissociates /displaces RNA polymerase from DNA template as In a bound state(it acts as **ATPase),** resulting in termination of transcription (RNA synthesis).

Rho (ρ) factor dependent and Rho (ρ) factor independent termination of Transcription in prokaryotes:2



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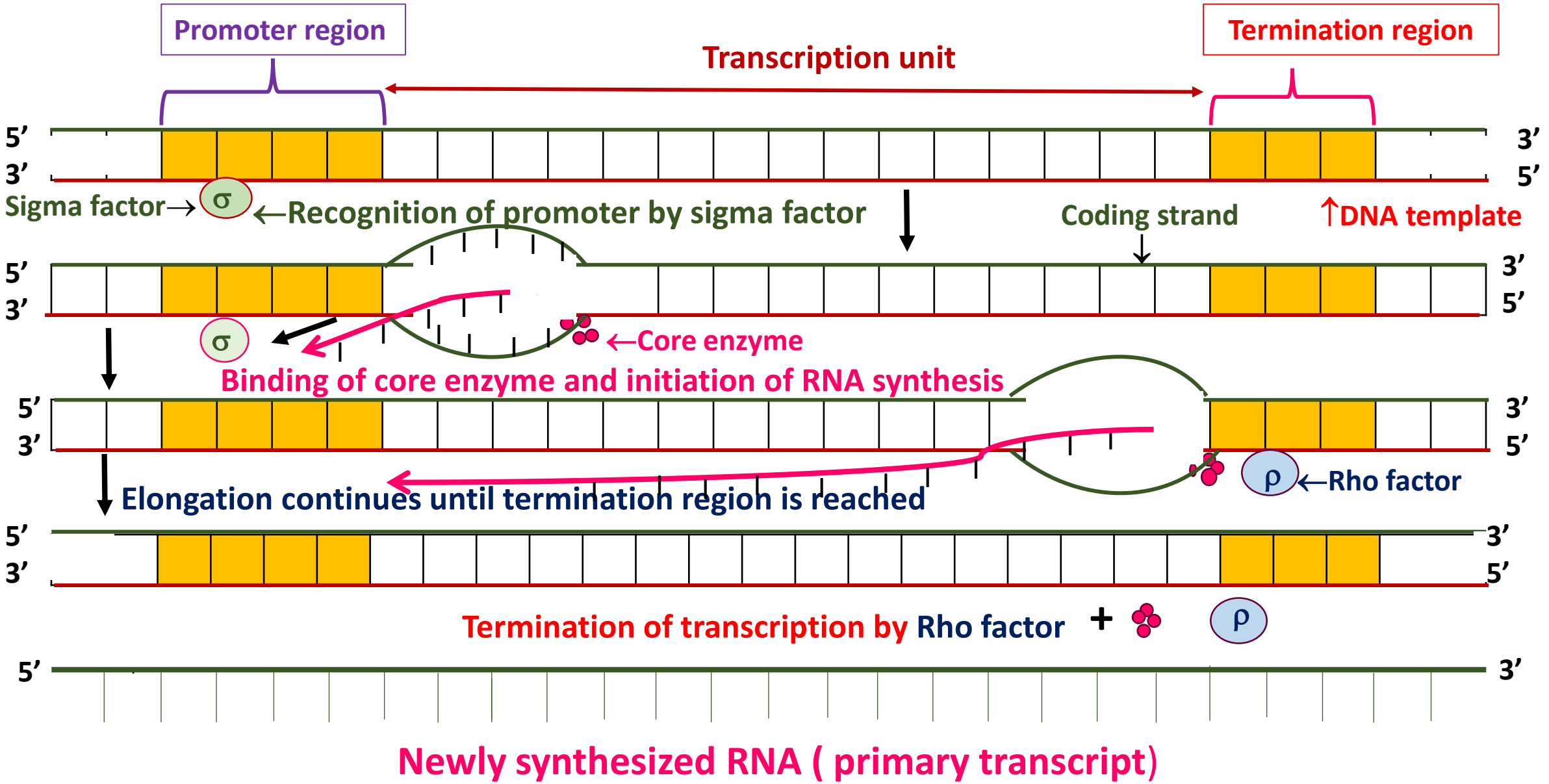
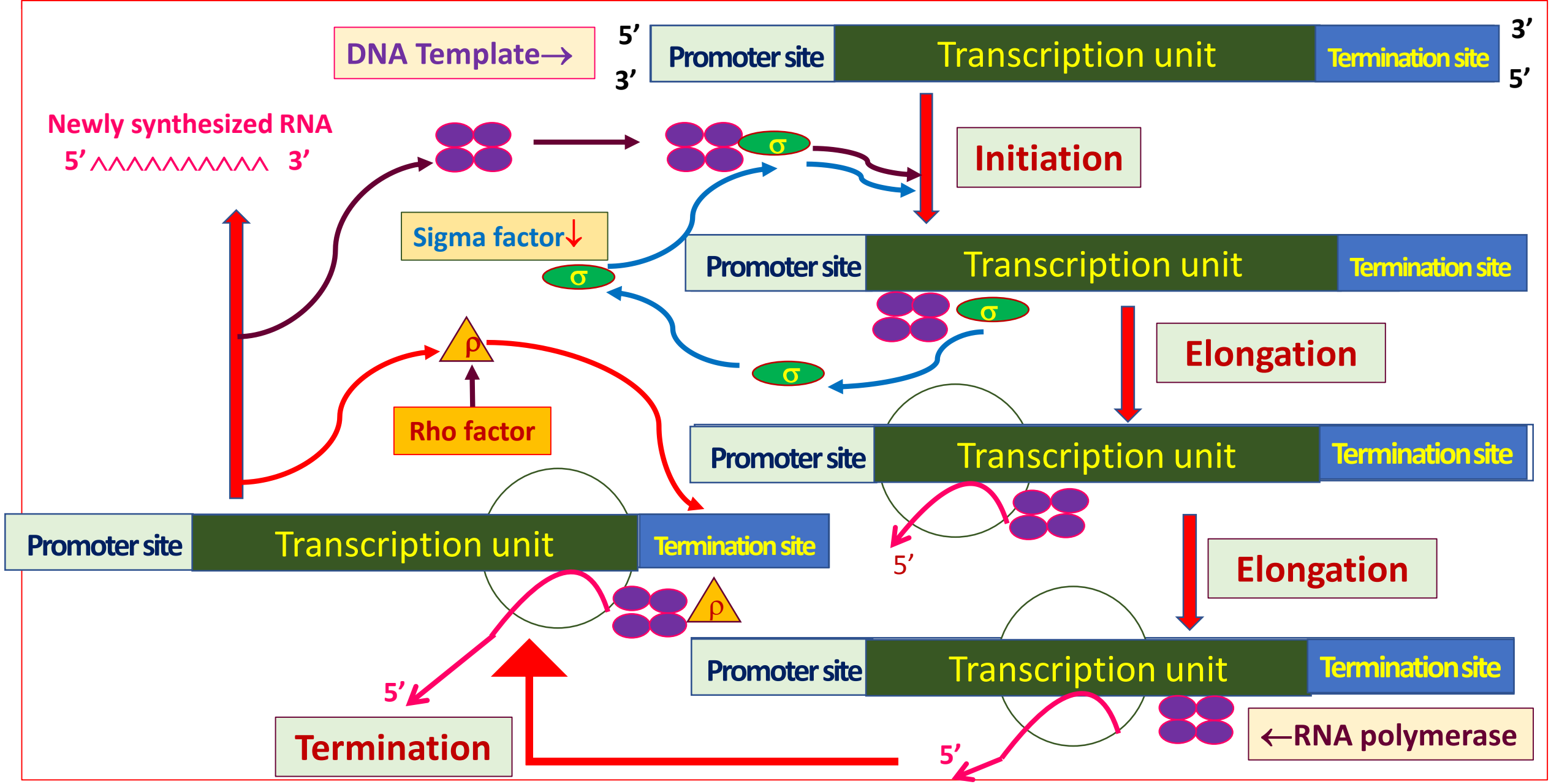
Process of transcription in prokaryotes



Summary of Synthesis of RNA from DNA template



Post transcriptional modifications of inactive primary RNA transcripts for synthesis of functional RNA

- **Post transcriptional modifications involve enzymatic alterations of inactive primary RNA transcripts (made by RNA polymerase) to functional RNA to be located in cytoplasm . These modifications are extensive in eukaryotes but not in prokaryotes (minor processing) .**
- **Post transcriptional modifications may involve :**

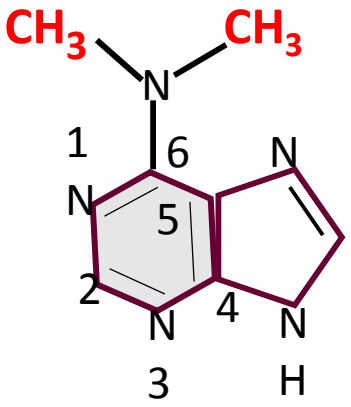
- **Cleavage** of large precursor RNA for removal of excess sequences from the primary transcript by the action of **endonuclease** or **exonucleases** to smaller molecules .
- **Splicing** : involves the removal of sequences called introns (sequences that do not code for proteins) from the primary transcript and joining of sequences called exons (coding sequences) to each other to form functional RNA.
- **Terminal** addition of nucleotides
- **Nucleoside modification**

Nucleoside (base) modifications as a Post transcriptional modification of inactive primary RNA transcripts for synthesis of functional RNA

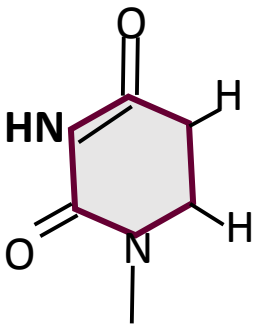
- **In prokaryotes , Nucleoside (base) modifications as a Post transcriptional modification of inactive primary RNA transcripts for synthesis of functional rRNA (in cytoplasm) is of two types:**

1. **involve modification of bases :** e.g. some bases of rRNA are methylated. Uridylate residues of **tRNA** are modified to form **ribothymidylate** and **pseudo uridylate**.
2. **involve modification of ribose unit of r RNA**

RNA processing by nucleoside modification

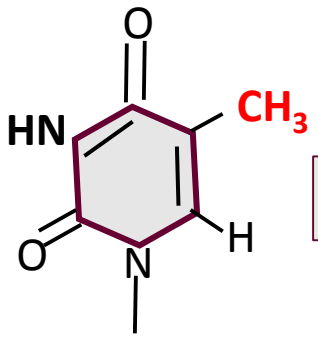


←Dimethyl adenosine (methylated base) in prokaryotes



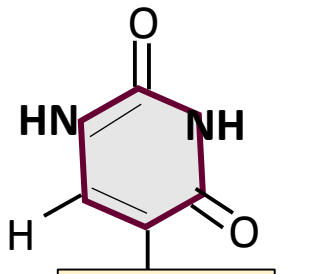
Ribose

Uridylate



Ribose

←Ribothymidylate



Ribose

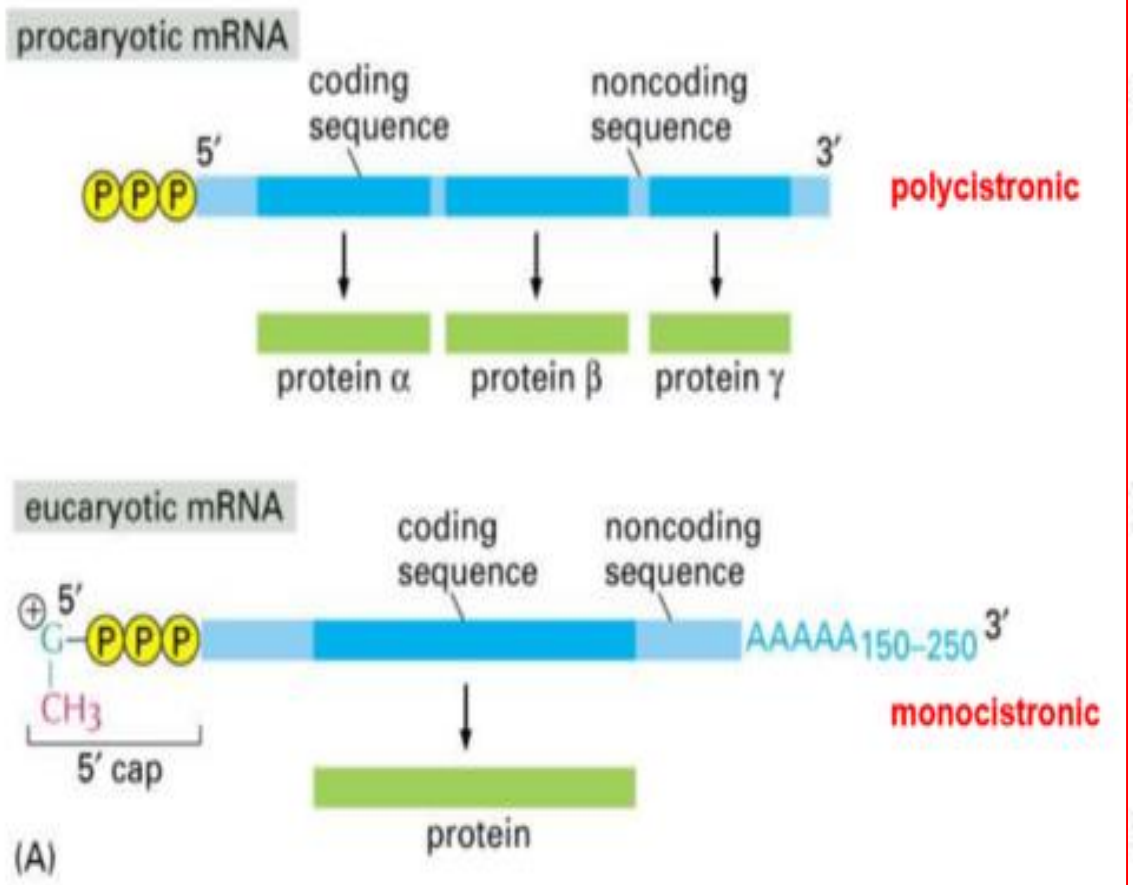
←Pseudo uridylate

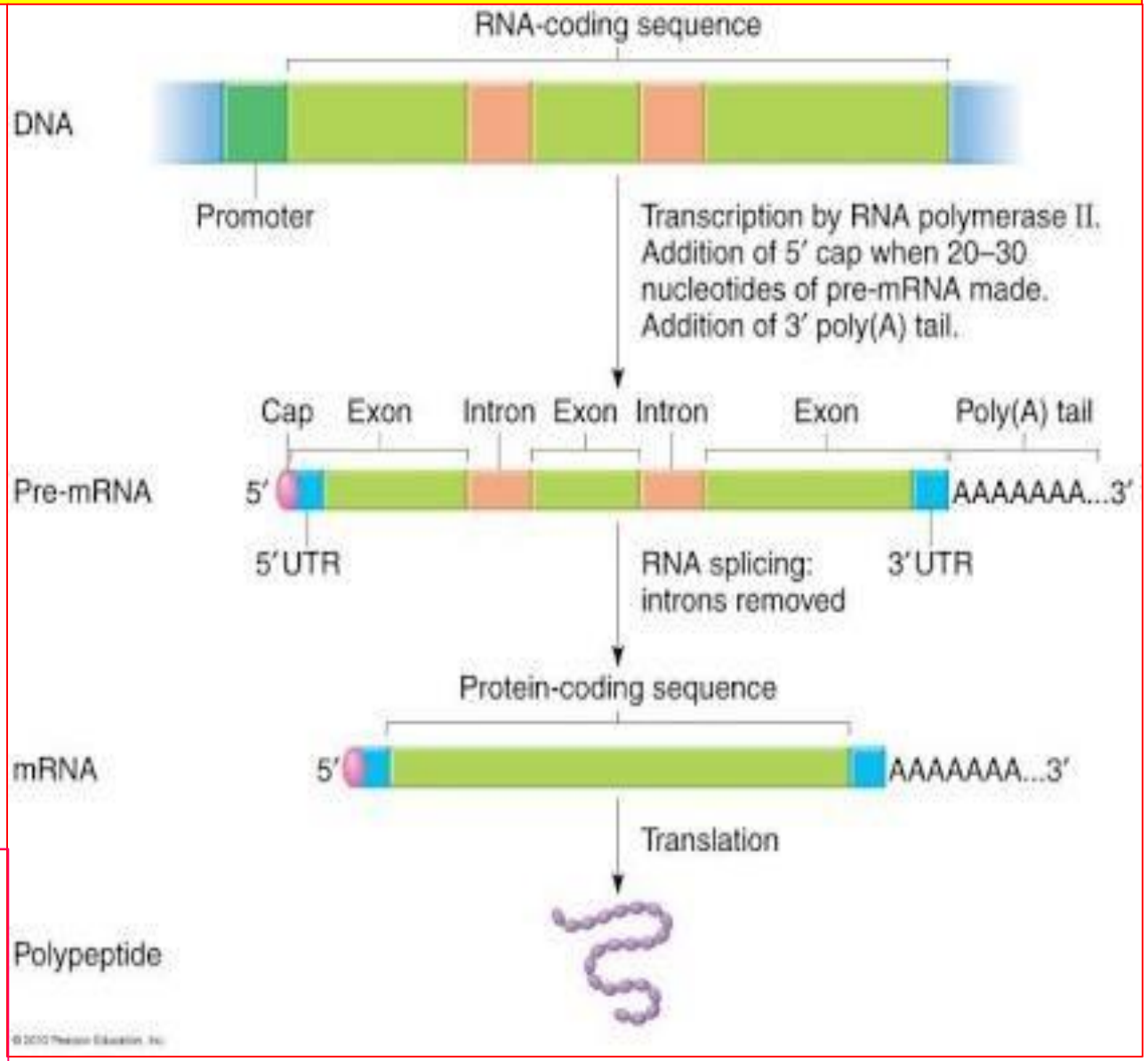
Cleavage as a Post transcriptional modification of inactive primary RNA transcripts for synthesis of functional RNA in Prokaryotes

❑**In prokaryotes:**

- **Synthesis of functional tRNA** and **rRNA** occurs by cleavage and modification of newly synthesized RNA chains (**Post transcriptional modification of inactive primary RNA transcripts/ nascent RNA)** .
- **mRNA** is **not Post transcriptionally modified** and is functional immediately after its synthesis . In fact ,its translation often begins before transcription is complete.
- **Three** types of **rRNA** molecules in E.Coli : 16 S, 23S , 5S .
- These three types rRNA molecules in E.Coli are cleaved (excised) from a single primary RNA transcript by highly precise **nucleases**(**ribonuclease P** and **ribonuclease III**). **Ribonuclease III cleaves** 16 S, 23S and 5S rRNA molecules from the primary transcript by cleaving double helical regions at specific sites. The primary RNA transcript contains spacer regions .
- All the **Transfer** RNA **(t- RNAs)** of prokaryotes and eukaryotes undergo post transcriptional modification. **tRNA molecules** in E.Coli is cleaved (excised) from a single primary RNA transcript by highly precise nuclease (ribonuclease P) which generates the correct 5’ terminus of all tRNA molecules. Ribonuclease P contains catalytically active RNA molecule.

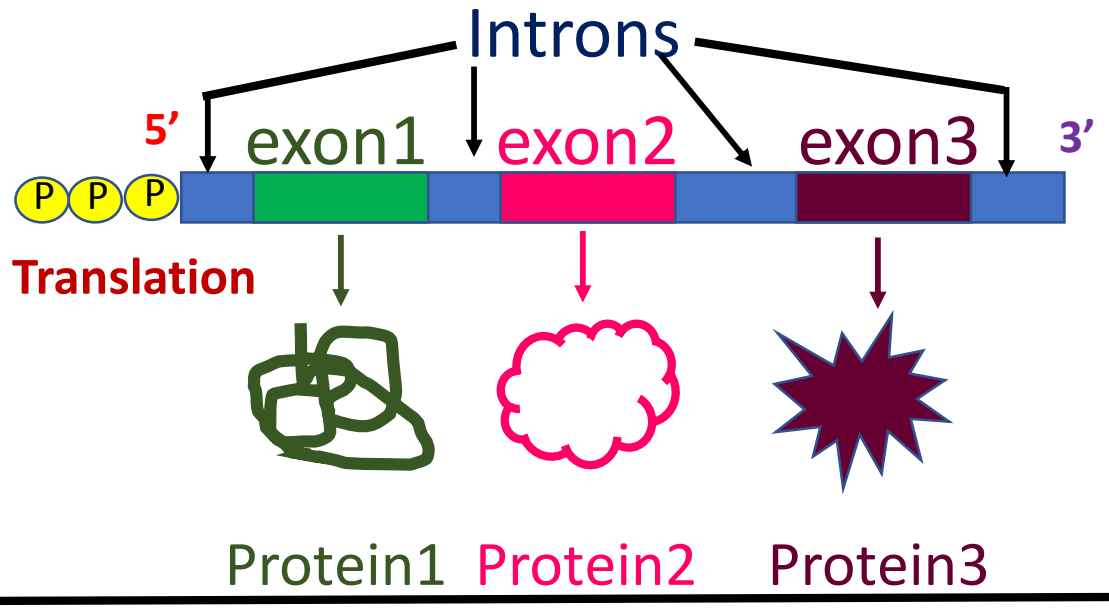
Post transcriptional modifications of inactive primary RNA transcripts for synthesis functional mRNA in Prokaryotic and Eukaryotic cells





In prokaryotes ,mRNA is **not Post transcriptionally modified** and is functional immediately after its synthesis . In fact, its translation often begins before transcription is complete.

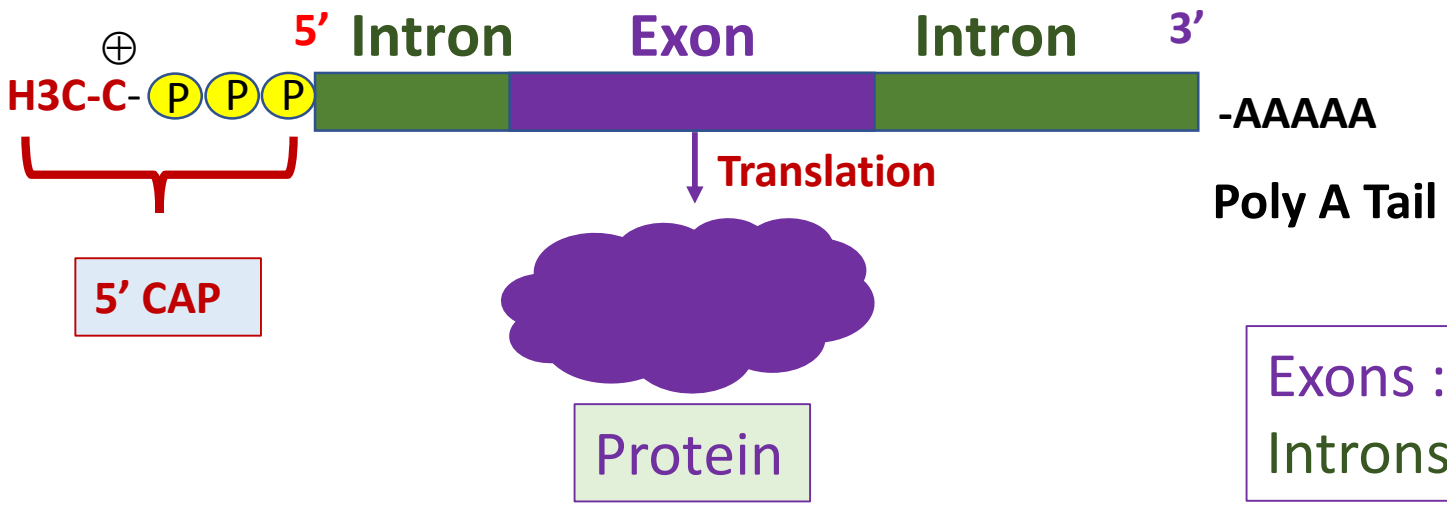
Comparison of Prokaryotic and Eukaryotic mRNA



Prokaryotic mRNA

Polycistronic

Schematic diagram

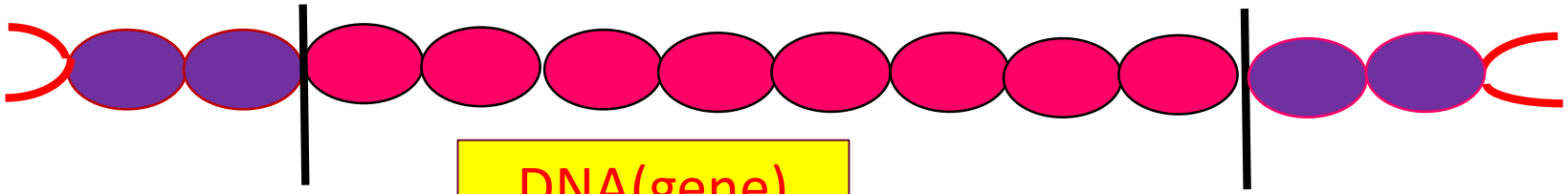


Eukaryotic mRNA

Monocistronic

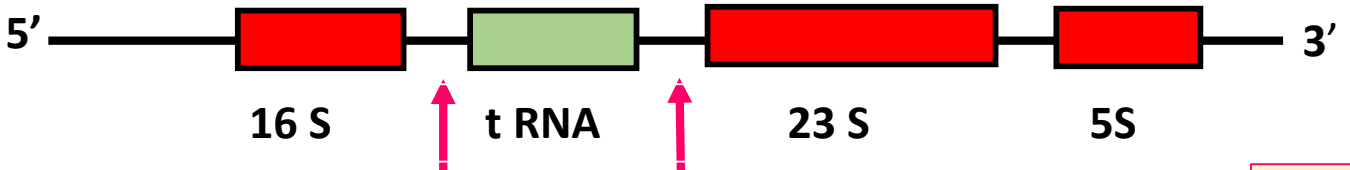
Exons : coding sequences
Introns : non-coding sequences

Post transcriptional modifications of inactive primary RNA transcripts for synthesis of rRNA in E.coli



DNA(gene)

Transcription by RNA Polymerase



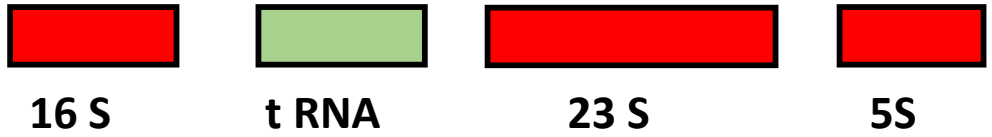
←Primary RNA transcripts

Spacer regions

Schematic diagram

Cleavage

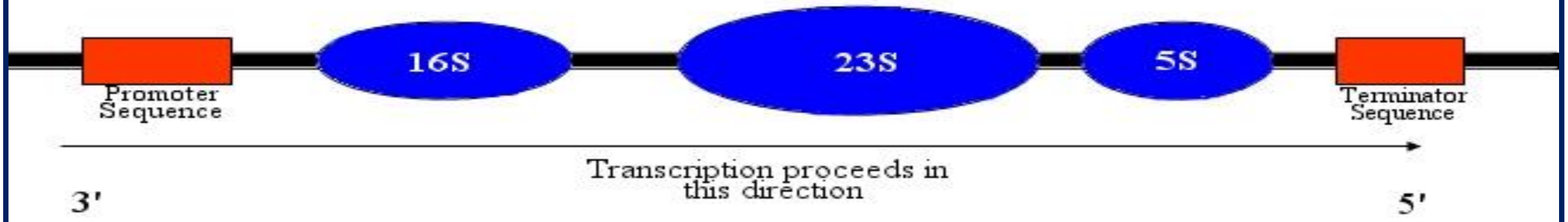
Ribonuclease P
Ribonuclease III



In E.coli chromosome, the genes for RNA are arranged in specified order specifying the 16S, 23S and 5S rRNA units . RNA polymerase transcribes the three genes in order.

Enzyme and Direction of rRNA synthesis in prokaryotes(E.Coli)

rRNA synthesis occurs in 5' →3' **direction**



Transcription by DNA dependent RNA polymerase (RNA polymerase → RNAP)occurs in 3'→ 5' direction

Enzymes and direction of transcription : DNA dependent RNA polymerase (RNA polymerase → RNAP) responsible for the synthesis of rRNA in 5'→3' direction

Terminal addition of nucleotides to inactive primary tRNA in Post transcriptional modifications

❖ Terminal addition of nucleotides to inactive primary tRNA in Post transcriptional modifications involve :

- Addition of CCA nucleotides to a 3’ terminal sequence of **inactive primary** tRNA to make it functional molecule in protein biosynthesis.*

Post transcriptional modifications of Prokaryotic Primary transcript of tRNA(prokaryotic transfer RNA processing)

- All the **Transfer** RNA **(tRNAs)** of prokaryotes and eukaryotes undergo post transcriptional modification of longer precursor molecule.
- Post translational modification of primary transcript into mature Eukaryotic tRNAs involve following alterations:

a. Cleavage of a 5' **leader sequence**(trimming**).** 16 nucleotide sequence at the 5' end is cleaved by RNAase P(ribozyme) .

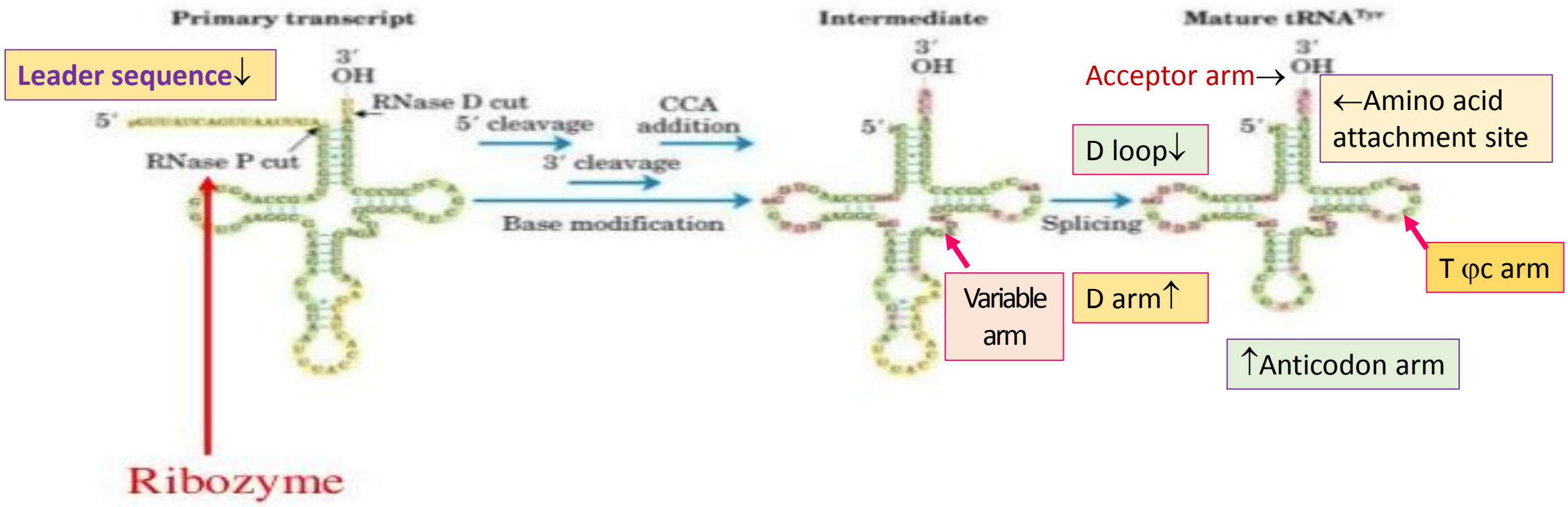
b. Splicing to remove introns i.e. 14 nucleotide introns in anticodon loop is removed by nucleases .

c. Replacement of the 3' terminal UU by CCA(addition of CCA nucleotides to 3' terminal end of tRNAs by nucleotide transferase) i.e. Uracil residues at the 3' end replaced by the CCA sequence found in all mature tRNAs.

d. Modification of several bases(converting the existing bases into unusual ones)i.e. many bases are converted to characteristic modified bases of tRNA .

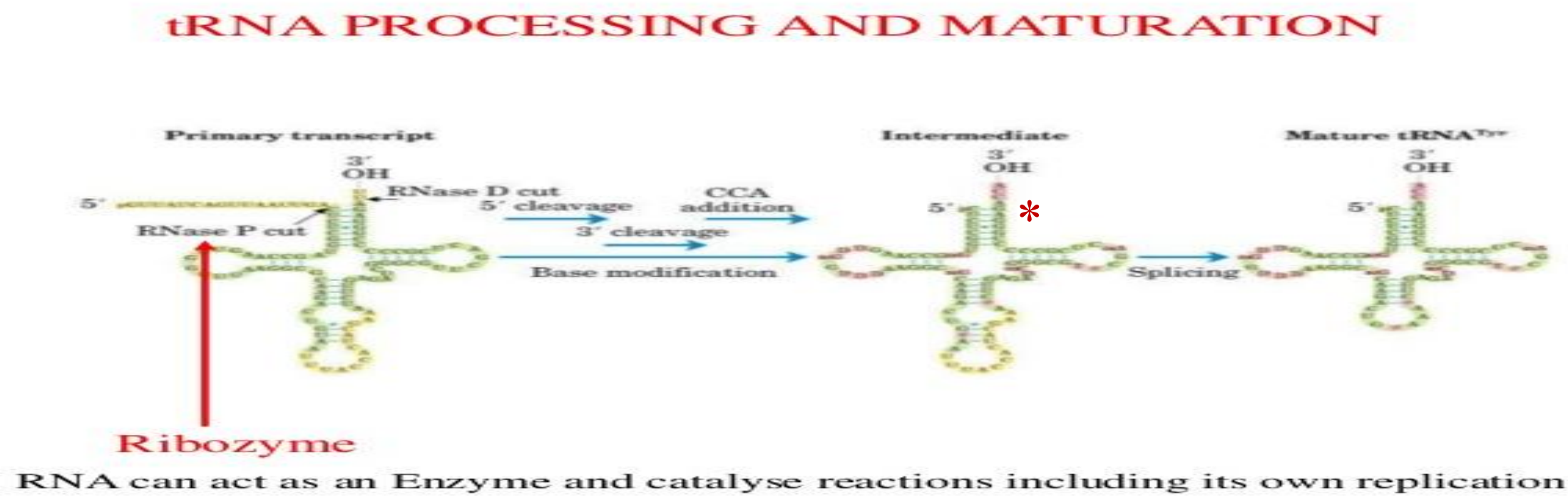
Processing of tRNA precursor to mature tRNA

tRNA PROCESSING AND MATURATION



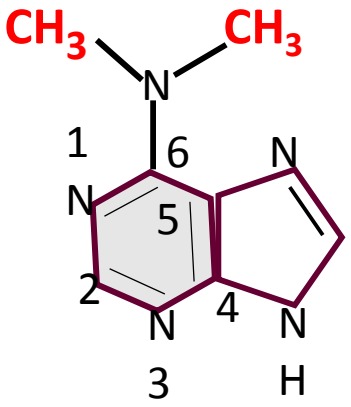
RNA can act as an Enzyme and catalyse reactions including its own replication

Post transcriptional modifications of inactive primary RNA transcripts for synthesis of t-RNA : splicing, terminal base additions, nucleoside (base) modifications

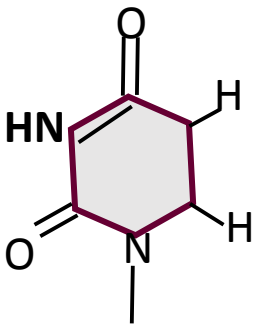


* Addition of CCA nucleotides to a 3' terminal sequence of **inactive primary** tRNA to make it functional molecule during protein biosynthesis . **tRNA molecules** in E.Coli is cleaved (excised) from a single primary RNA transcript by highly precise nuclease (ribonuclease P) which generates the correct 5' terminus of all tRNA molecules. Ribonuclease P contains catalytically active RNA molecule.

RNA processing by nucleotide modification

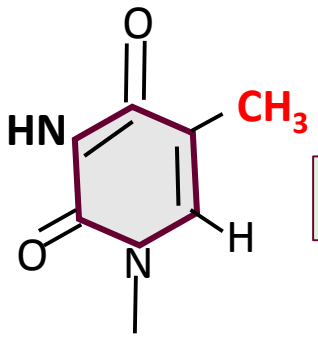


←Dimethyl adenosine (methylated base) in prokaryotes



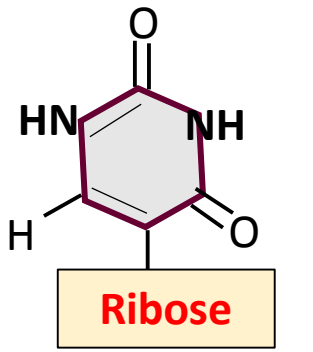
Ribose

Uridylate



Ribose

←Ribothymidylate



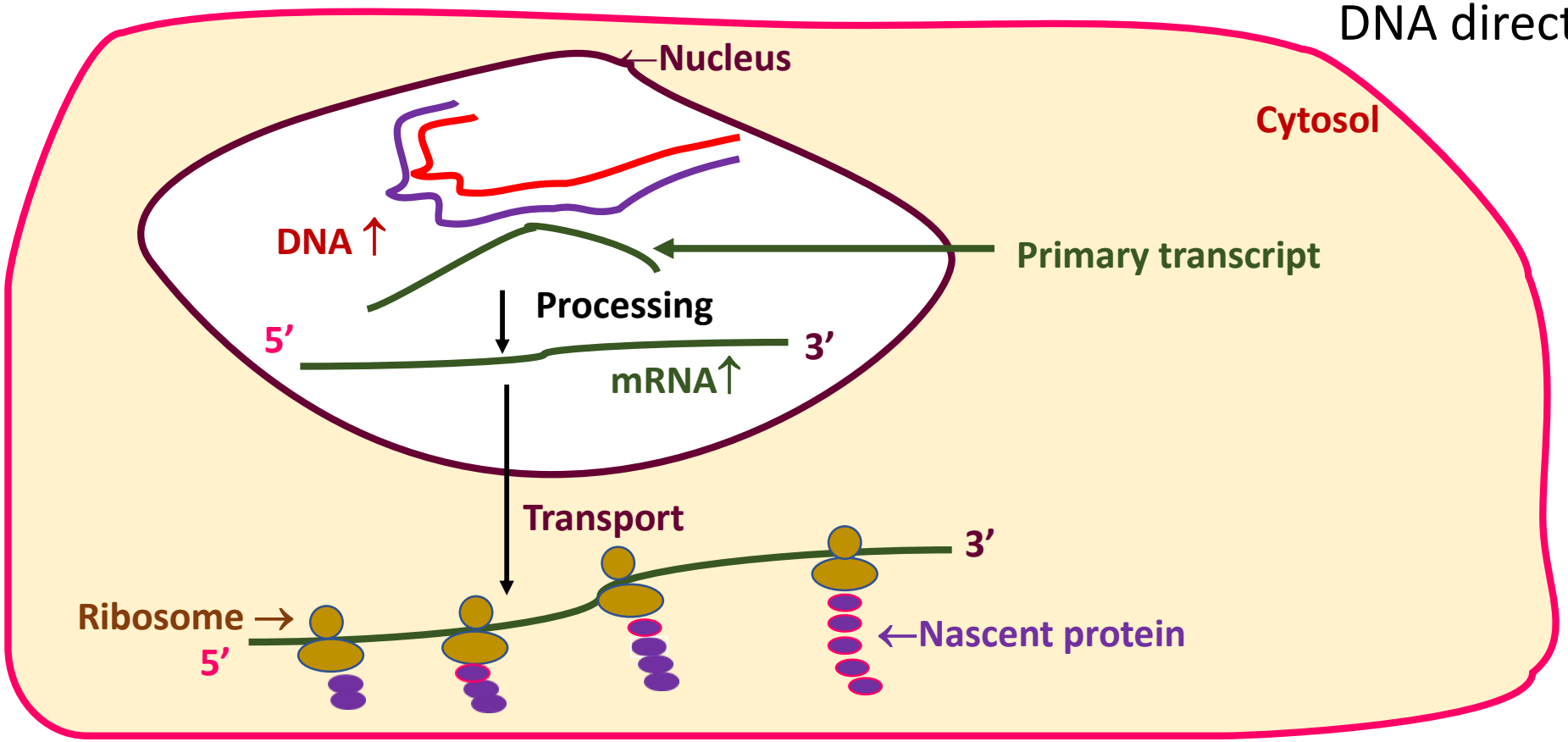
Ribose

←Pseudo uridylate

Transcription in Eukaryotes

Schematic representation of eukaryotic transcription

DNA directed RNA synthesis



Roger Kornberg (Noble 2006): elucidated molecular basis of transcription.

Steps of transcription Eukaryotic cell : Initiation , elongation and termination

Steps of transcription in Eukaryotes

Criteria	Initiation	Elongation	Termination
Requirements of step of transcription	Chromatin remodeling followed by binding of transcription factors and RNA polymerase to promoter regions located upstream or downstream the coding sequences/region (exon) to initiate transcription	Local unwinding of Duplex DNA helix	A termination signal sequences
Process involved in step of transcription	Enhanced by transcription factors bound to enhancer sequences.	The DNA template is read by RNA Polymerase in 3'→5' direction to synthesize RNA transcript in 5'→3' direction (elongation)	RNA polymerase and primary RNA transcript (newly synthesized RNA) released from DNA resulting in termination of transcription.

Silent features of Transcription in eukaryotes

1. Transcription in Eukaryotes is much more complicated than in prokaryotes.
2. Eukaryotic genes require promoters for transcription initiation .
3. Each of three types of polymerases has distinct promoters .
4. Promoters are always present on the same molecule as the gene they regulate. These promoters are referred as **cis-acting elements** .
5. Each type of RNA Polymerase use a different **Promoters** :

a. RNA **polymerase** I : have **single** type of promoter present in **rRNA gene**.

b. RNA polymerase II : contain a **TATA box** near the **transcription start site** .

c. RNA polymerase III : located in upstream position as well as downstream positions the initiation site of transcription .They are usually downstream of the start point.

- RNA **polymerase** I and RNA polymerase II are similar to the prokaryotic promoter in that they are upstream the start point.

Structural aspects of Eukaryotic RNA polymerases

❖**Structural aspects of Eukaryotic RNA polymerases are as follows :**

The three Eukaryotic RNA Polymerases are large complex proteins containing 2 large subunits of and about 10 smaller subunits . The subunits show homology with Alpha,1 Beta, 1 Beta' (α,β,β') subunits of E .coli RNA Polymerase (RNA Pol).

Eukaryotic RNA polymerases for Transcription

- The nuclei of **Eukaryotes contain t**hree distinct **RNA Polymerases .RNA Polymerases I,II and III** that differ in their sensitivity to the fungal toxin **α-amanitin . All t**hree **enzymes** require **transcription factor**s to initiate transcription .

1. **RNA Polymerase I/A** : present in **nucleolus** and responsible for the synthesis of precursors for the large ribosomal RNA(rRNA**)** .It transcribes all the rRNA except 5S rRNA. It is insensitive to **α-amanitin.**
2. **RNA Polymerase II/B** : present in the nucleoplasm and responsible for the synthesis of the precursors for messenger RNA (mRNA**)** & small nuclear RNAs (Sn- RNA**)**. It is highly sensitive to **α-amanitin.**
3. **RNA Polymerase III/C :**located in the nucleoplasm and transcribes genes for **transfer** RNA **(tRNA) & small ribosomal RNAs (5SrRNA) , U6snRNA and other small RNA .** It is moderately sensitive to **α-amanitin.**

- In Eukaryotes ,besides the three distinct RNA Polymerases , there exist Mitochondrial RNA polymerase (resembles prokaryotic RNA polymerase in structure & functions).

Types of Eukaryotic RNA polymerases and RNAs formed

Type of DNA dependent RNA polymerase(RNAP)	Location	Type precursors of RNA formed	Sensitivity towards α-Amanitin
RNA polymerases I or A	Nucleolus	18S ,5.8S, and 28S rRNA	Sensitive and inhibited
RNA polymerases II or B	Nucleoplasm	mRNA precursor, snRNA, snoRNA, miRNA	Not inhibited
RNA polymerases III or C	Nucleoplasm	tRNA, 5S rRNA, some snRNA, snoRNA	Moderately sensitive

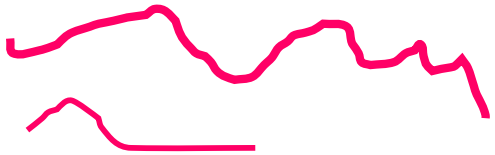
Eukaryotic RNA polymerases of are more complex than prokaryotic RNA polymerase and differ in their template specificity.

An overview of transcription in eukaryotes

RNA polymerases I / A

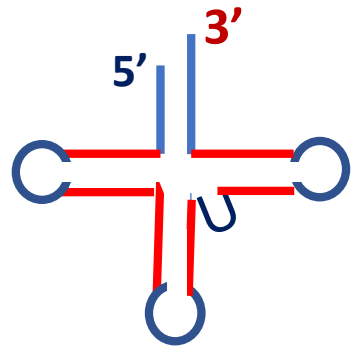
RNA polymerases II/B

RNA polymerases III/C



18S ,5.8S, and 28S **Ribosomal RNAs**

Messenger RNA, snRNA , snoRNA, miRNA



Transfer RNA

Schematic diagram

Comparison of Eukaryotic RNA polymerases used in Transcription

Criteria	RNA Polymerase I	RNA Polymerase II	RNA Polymerase III
Location in a cell	**nucleolus**	nucleoplasm	nucleoplasm
Transcribes genes for	large ribosomal RNA (rRNA**)** .It transcribes all the rRNA except 5S rRNA.	messenger RNA (mRNA**)** & small nuclear RNAs (SnRNA snoRNA, miRNA)	**transfer** RNA **(tRNA) & small ribosomal RNAs (5SrRNA) ,** U**6snRNA and other small RNA**
Promoters	have **single** type of promoter present in **rRNA gene**.	contain a **TATA box** near the **transcription start site** .	located in upstream position as well as downstream positions the initiation site of transcription .
sensitivity towards the fungal toxin **α - amanitin**	insensitive	highly sensitive	moderately sensitive to α-**amanitin**

Mitochondrial RNA polymerase

- Mitochondria contain a single RNA polymerase that more closely resemble bacterial RNA polymerase than eukaryotic polymerases.

Promoters of eukaryotic polymerases

- Eukaryotic genes ,like prokaryotes require promoters for transcription initiation.
- Each of three types of eukaryotic polymerases has distinct promoters.
- Promoters are always present on the same molecule of DNA as the gene they regulate. These promoters are referred as **cis-acting elements** . Such sequences serve as binding sites for **Transcription Factors**, which in turn interact with each other and with RNA polymerase II.

Silent features of Eukaryotic Promoter region

- Each type of eukaryotic RNA polymerase uses different promoters . The promoters used by **RNA polymerase I and II** are similar to **prokaryotic promoter** in that they are upstream of the start point gene to be transcribed.
- Promoters of RNA polymerase (RNA pol III) are downstream of the start point gene to be transcribed.
- RNA polymerase (RNA pol II) located in the nucleoplasm catalyzes the transcription of protein coding mRNA genes and some snRNA genes .
- **RNA polymerase (RNA pol II) Promoter region contains following sequence :**

1. Minimal core **Promoter elements include :TATA box located** 25-35 nucleotides upstream of the Start site /point with consensus sequence 5'- TATA(A/T)A (A/T)-3' which positions (RNA pol II) for correct transcription initiation .
2. Initiator (INR) element : present around the transcription **Start site** and has consensus sequence 5'-C/TC/TANT/AC/TC/T-3'.
3. Upstream regulatory element (**URE**) located several hundred base pairs Upstream of core **Promoter and determines the frequency of** transcription.

Upstream regulatory element (URE) in Eukaryotes

❑Upstream regulatory element (**URE**) include :

1. **GC boxes** that bind Sp1 and Sp2.
2. **CAAT box** that binds CAAT binding factor (**CTF**)
3. **Enhancers and silencers :**that increase or decrease the rate of transcription initiation respectively . These are found Upstream or downstream from transcription start site respectively and can exert their effects even when located thousand base pairs away from transcription unit.
4. **Insulators :** sequences between silencers and enhancers that prevent interference between these sequences.
5. **Response elements** : identify the particular groups of genes and include **heat shock response elements (HSE) ,glucocorticoid response elements (GRE) and metallothionein unit(MRE) .**
6. **Other regulatory sequences for transcription:** repressor, inducers , derepressors and hormone response element (**HRE**).

Promoter sites of Transcription in Eukaryotes

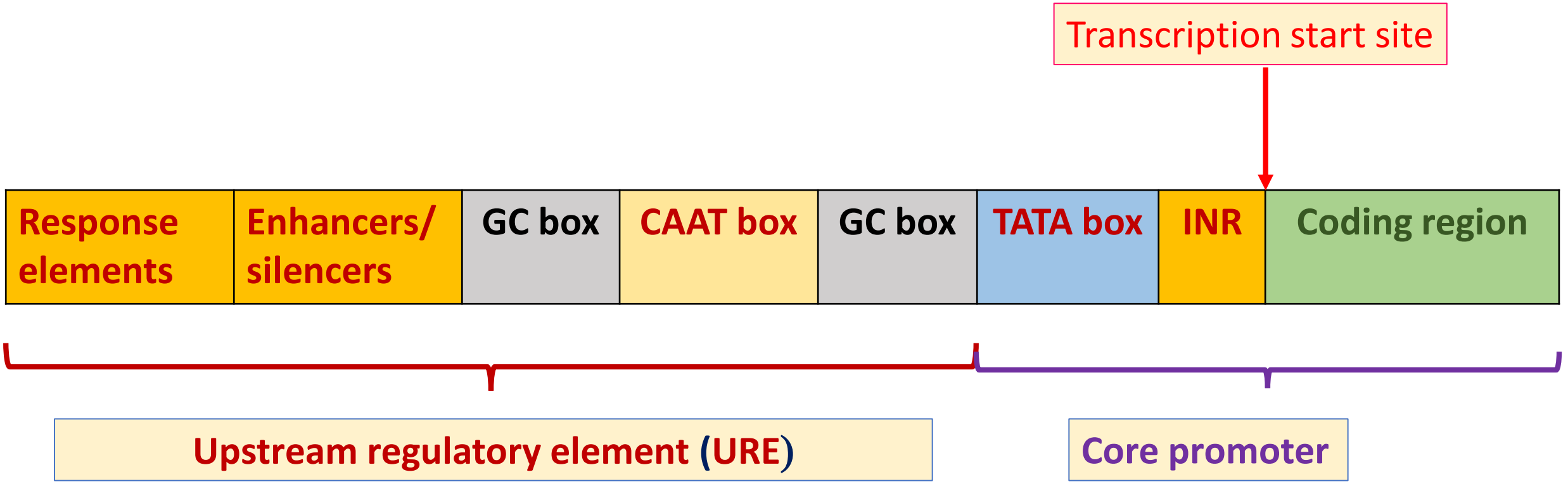
- Many eukaryotic genes encoding proteins have promoter sites with TATAA consensus sequence. Many eukaryotic promoters also have CAAT and GC box.
- **Promoter sites** (a sequence of DNA bases) **in Eukaryotes:**

- **is identical to Pribnow box in prokaryotes with a TATAA consensus sequence of DNA bases known as Goldberg-Hogness box or TATA box.**
- located on the left side about 25 nucleotides away(upstream, centered at -25) from the **start site** of m- RNA synthesis .
- Other recognition sites are located between 70 -80 nucleotides away (upstream, centered at -75)from the start of transcription. These include:

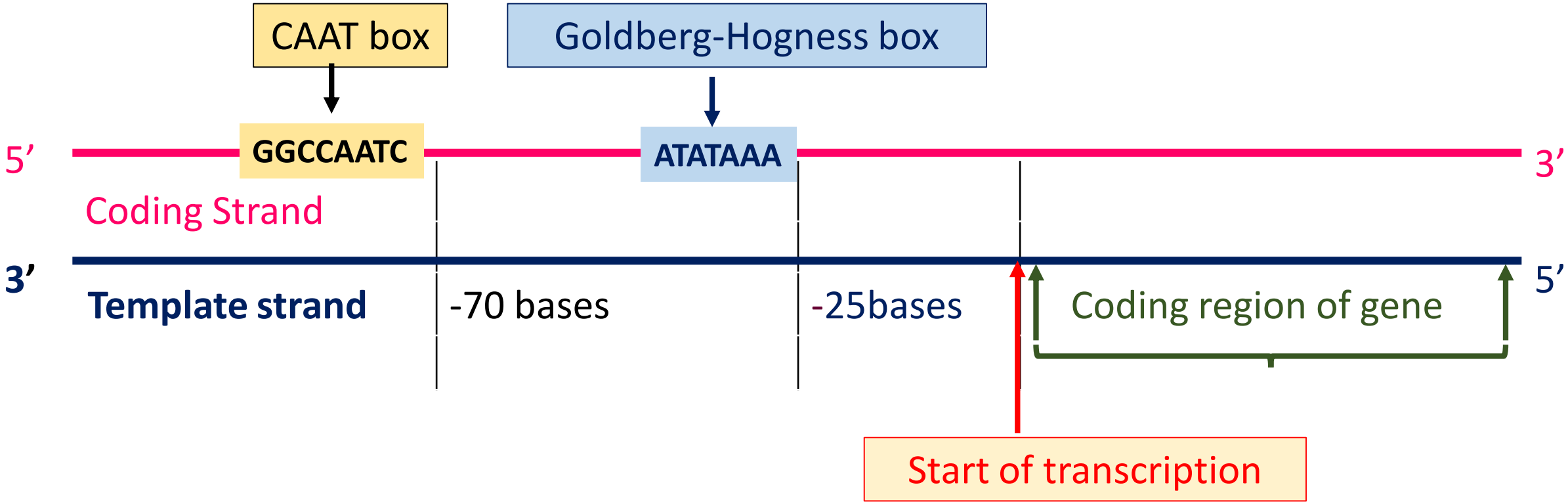
1. **CAAT box** with GGCAATCT consensus sequence located at -75 .
2. **CG box** with **GGGCG** consensus sequence.

- One of these two sites (or sometimes both)helps in RNA polymerase II to recognize the requisite sequence of DNA for Transcription.
- **Enhancer sequences** : stimulates transcription of eukaryotic genes(located quite distant from the start site either on the its 5' or its 3' sites .

Components of RNA polymerase II promoter

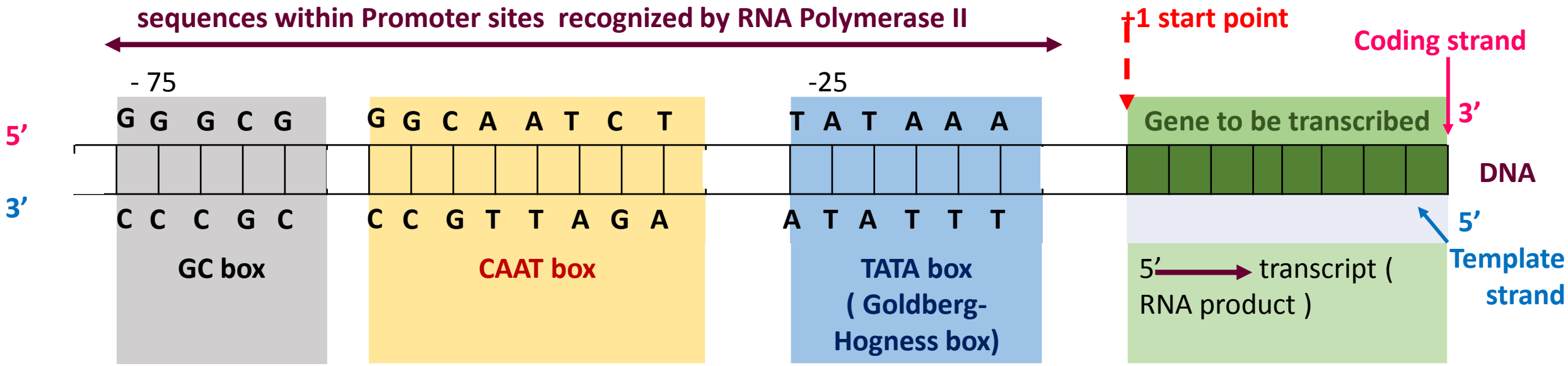


Promoter regions of DNA in Eukaryotes



Schematic diagram

Promoter sites of eukaryotic transcription



Eukaryotic genes encoding proteins have **promoter sites** a TATAA consensus sequences called **TATA box or Goldberg-Hogness box**, centered at about -25.

Many eukaryotic promoters have a **CAAT box** with GGCAATCT consensus sequences centered at -75 and **G C box with GGGCG** consensus sequence.

Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a specific site known as **start point** or **initiation site**.

Transcriptional initiation does require primer (like replication).

Promoter sites of Transcription in Eukaryotes

- Many eukaryotic genes encoding proteins have promoter sites with TATAA consensus sequence. Many eukaryotic promoters also have CAAT and GC box.
- **Promoter sites** (a sequence of DNA bases) **in Eukaryotes:**

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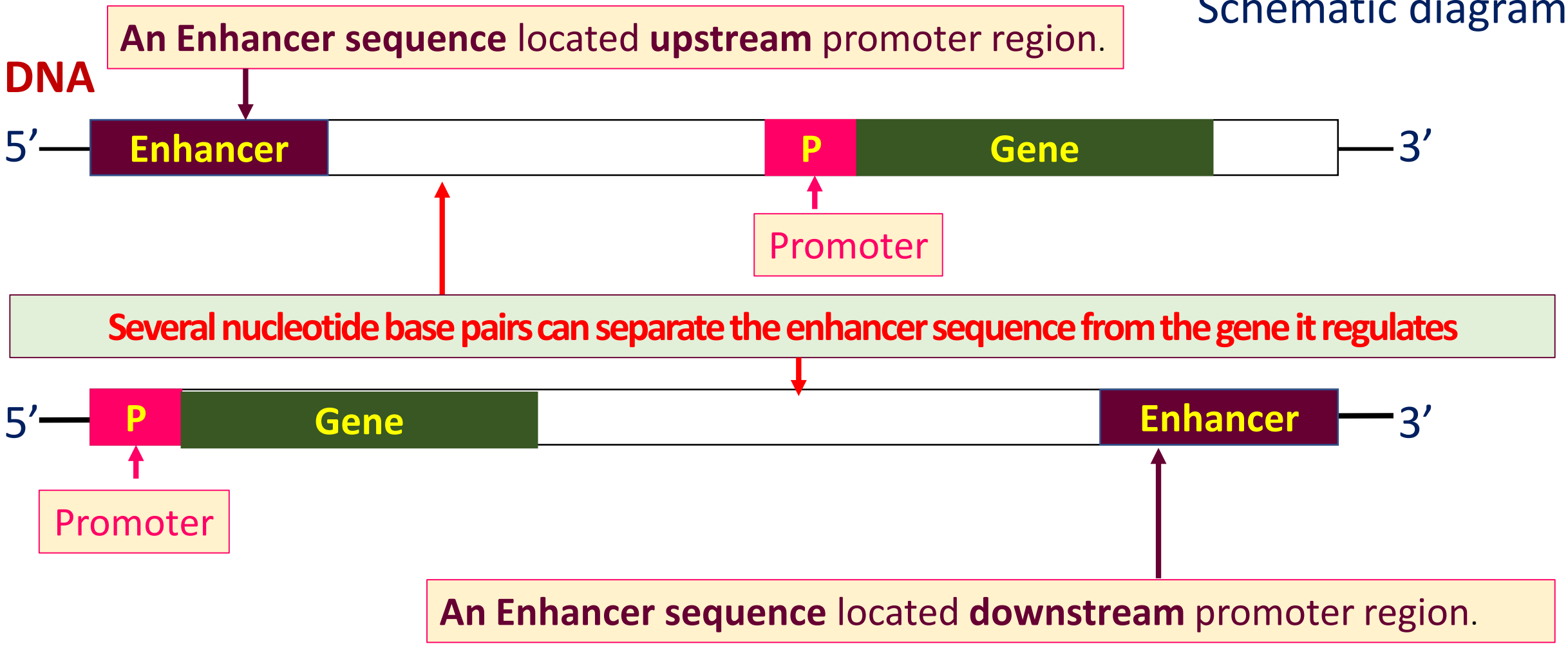
- **Enhancer sequences** : stimulates transcription of eukaryotic genes(located quite distant from the start site either on the its 5' or its 3' sites .

Locations of enhancer sequences

Schematic diagram



Silent features of Enhancers of Transcription in Eukaryotes

- Enhancers of Transcription in Eukaryotes are DNA sequences that **regulate** the **frequency** of **transcription** of genes of eukaryotes cells. They can increase gene expression by about 100 folds by increasing rate of initiation by RNA polymerase II.
- This is made possible by binding of Enhancers(contain DNA sequences called response elements) in close vicinity of specific Transcription factors to form **activators** . The chromatin forms a loop that allows the transcription factor bound to promoter and enhancer to be close together in space to facilitate Transcription .
- Thus they are another type of **cis-acting DNA sequences/ elements** . They can be upstream(to the 5' side), downstream(to the 3' side) or within genes. Moreover, they are effective when present on either DNA strand.
- They have **no promoter activity of their own** but can stimulate the transcription of genes .
- They **differ from promoters** in that their sequences are dissimilar and they may be located thousands of base pairs away from the start point of transcription .
- A particular enhancer is **effective only in certain cells** e.g. the immunoglobulin enhancers functions only in B- lymphocytes but not elsewhere.

Cancer and Enhancers of Transcription in Eukaryotes

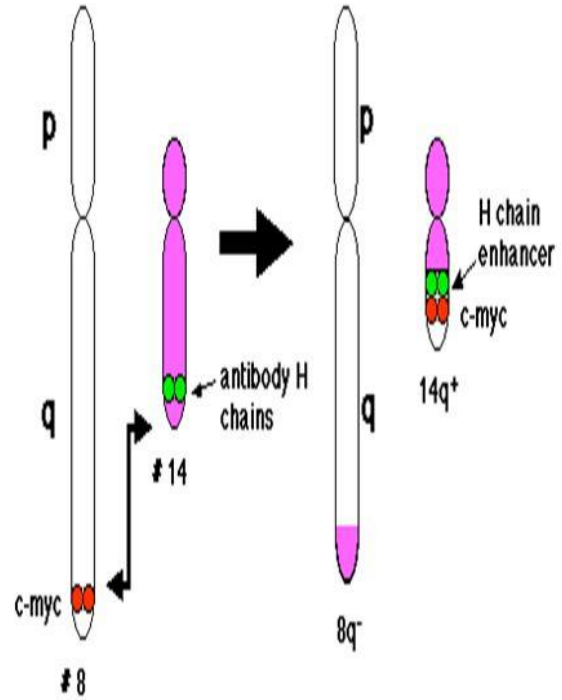
❖Cancer can result if **relation between genes** and **enhancers** is **disturbed**.

e.g. a **chromosomal translocation** brings the **protooncogenes myc under the control of immunoglobulin enhancer** which leads to dysregulation of **myc gene** and play role in development of **cancer** ,**Leukemia** and **Burkitt lymphoma**.

Dysregulation of myc gene and Burkitt lymphoma

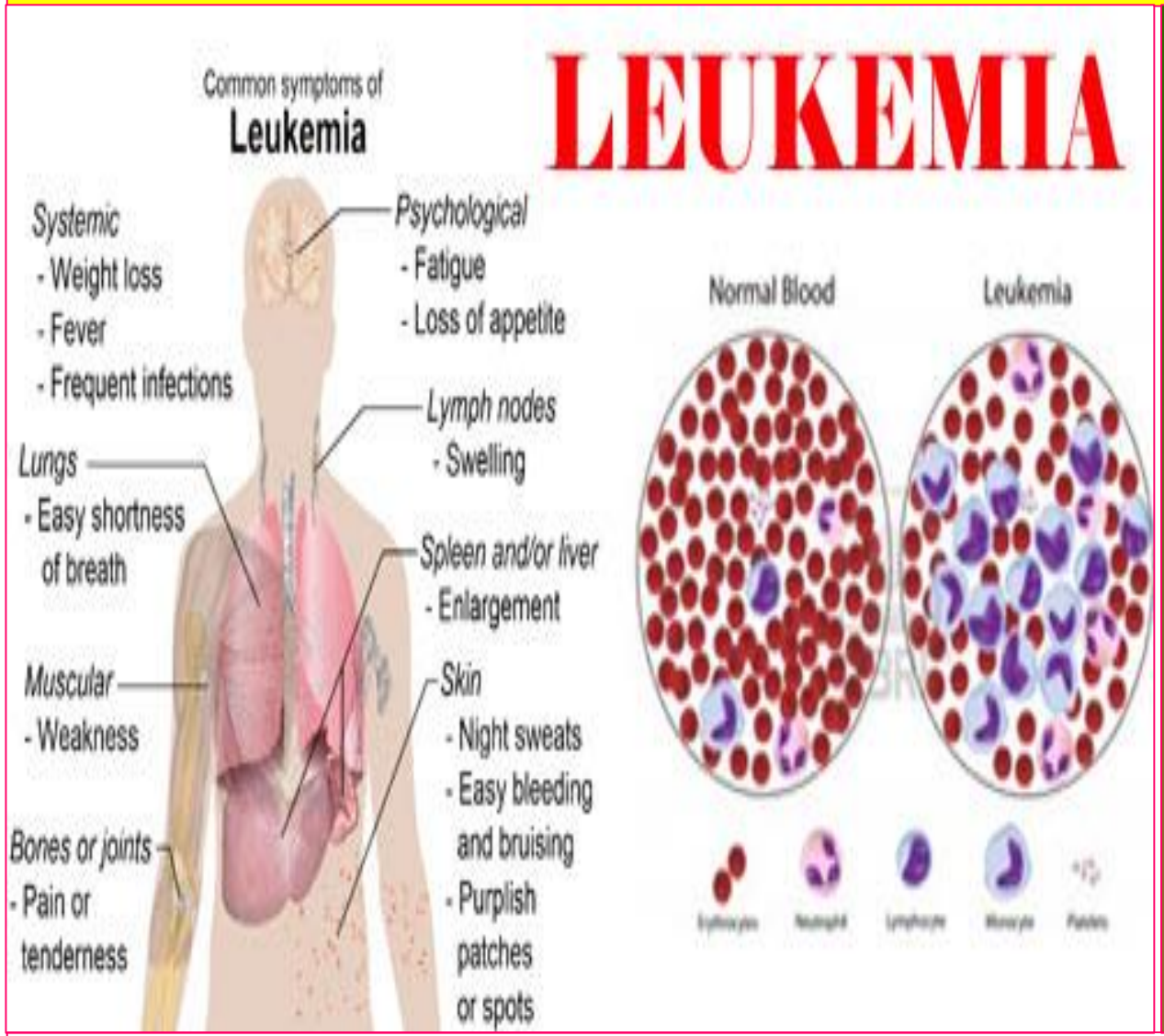
Burkitt's lymphoma

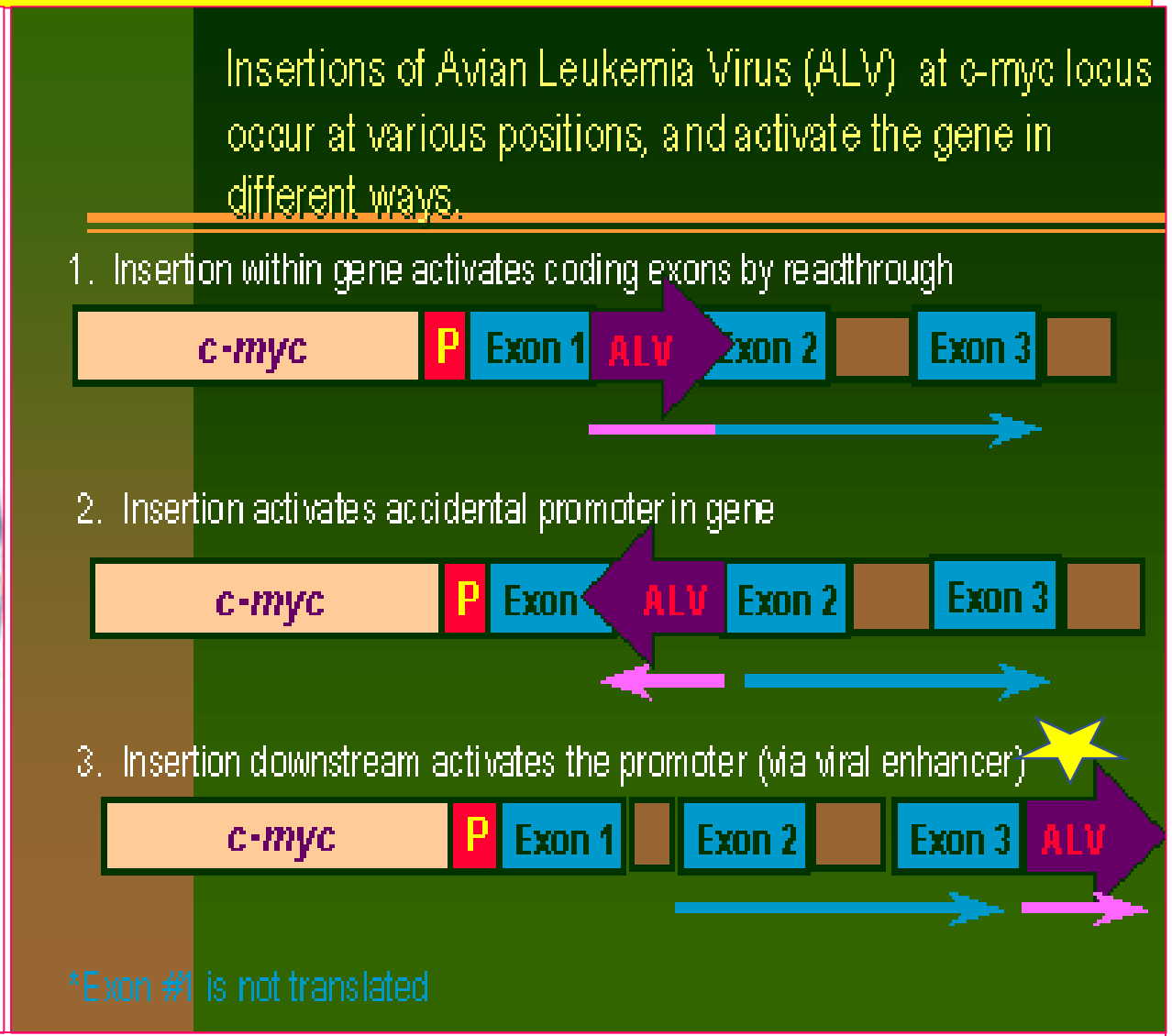
A reciprocal translocation has moved the proto-oncogene ***c-myc*** from its normal position on chromosome **8** to a location close to the enhancers of the antibody heavy chain genes on chromosome **14**.



A **chromosomal translocation** brings the **protooncogenes myc under the control of immunoglobulin enhancer** which leads to dysregulation of **myc gene** and play role in development of **cancer ,Leukemia** and **Burkitt lymphoma**.

Dysregulation of myc gene and Leukemia





A **chromosomal translocation** brings the **protooncogenes myc under the control of immunoglobulin enhancer** which leads to dysregulation of **myc gene** and play role in development of **cancer ,Leukemia** and **Burkitt lymphoma.**

Role of silencer of transcription in eukaryotes

❖**Silencer of transcription in eukaryotes :**

1. are DNA sequences which bind proteins that act to inhibit the rate of transcription(reduce gene expression).
2. Are similar to enhancers in that they act over long distances.

Initiation of Transcription in Eukaryotes:1

❖**The molecular events required for Initiation of Transcription in Eukaryotes are more complex** than in prokaryotes. Transcriptional initiation does not require a primer.

❖ **Function of promoter sequences :** responsible for directing RNA polymerase to initiate transcription at **start point** or **initiation site**

❖**Three stages of Initiation of Transcription in Eukaryotes:**

1. Chromatin containing the promoter sequence is made accessible to the Transcription machinery.
2. Binding of **Transcription factors**(**TFs**) to DNA sequences in the promoter region .
3. Stimulation of Transcription by **enhancers**.

Initiation of eukaryotic transcription:2

- Multiple Initiation factors are needed for eukaryotic transcription because complexity of their RNA polymerases and diversity of promoters .
- The binding of the RNA polymerase to DNA template results in the unwinding of the DNA double helix.
- The RNA polymerase catalyzes the formation of phospho-diester bond between the first two bases. The first base is usually a purine nucleoside triphosphate.

Transcription factors(TF) of in Eukaryotes

- A large number Transcription factors(TFs) interact with the Eukaryotic promoter regions .

❑**Transcription factors**:

a. bind to each other and in turn to the enzyme RNA polymerase II.

b. Recognize and bind to their specific DNA sequences through a variety of motifs , such as helix-loop-helix, zinc finger and leucine zipper. The chromatin structure in that region must be altered (remodeled) to allow assess to the DNA.

c. are encoded by different genes, synthesized in cytosol and must transit to their sites of action (called **trans-acting factors**).

d. are minimal requirements for recognition of the promoter , recruitment of RNA polymerase II to the promoter and initiation of transcription. (Eukaryotic RNA polymerase II does not itself recognize and bind the promoter).

e. Specific TFs (**transcriptional activators**) bind to sequences within and outside the core promoter. They are required to modulate the frequency of initiation, to mediate the response to signals such as hormones and to regulate genes expressed at a given point of time.

f. Specific TFs bind other proteins (**coactivators**) recruiting them to the transcription complex. Coactivators include the HAT enzymes involved in chromatin remodeling.

➢A typical protein coding eukaryotic gene has binding sites for many such TFs.

Types of Transcription factors(TF) in human

- A large number Transcription factors(TFs) interact with the Eukaryotic promoter regions . They are encoded by different genes, synthesized in cytosol and must transit to their sites of action (called trans-acting factors).
- Each eukaryotic RNA polymerase has its own promoters and TFs.
- In human ,about six Transcription factors have been identified:

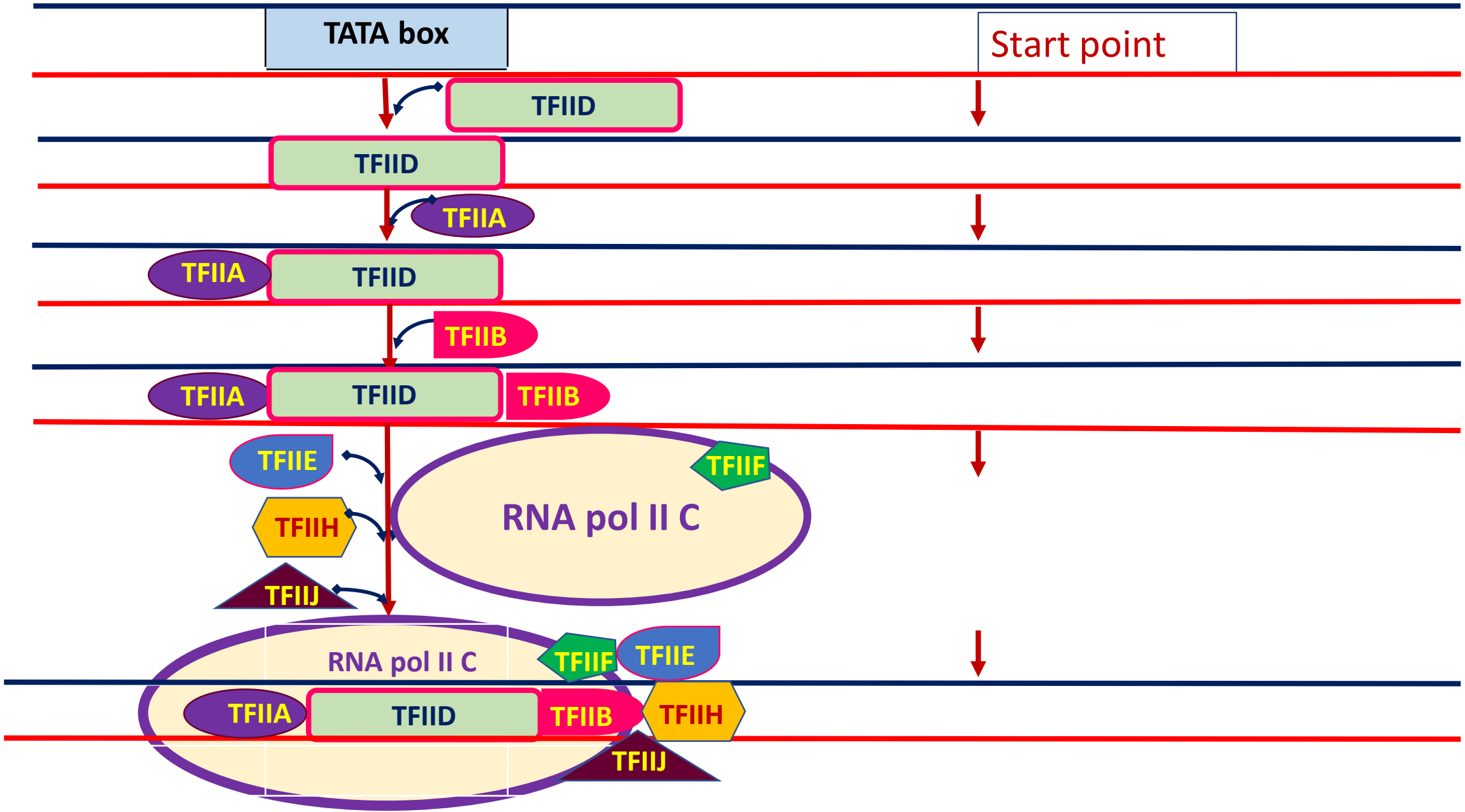
1. TFIID(recognizes and bind the TATA box through its TATA binding protein(TBP)component. In addition it has 11 TBP –associated factors)
2. TFIIA
3. TFIIB
4. TFIIF(targets RNA pol II by decreasing RNA pol binding to nonspecific sites and thus brings the RNA pol polymerase II to promoter).It is required for promoter clearance.
5. TFIIE
6. TFIIH(with helicase activity melts DNA and its kinase activity phosphorylates phosphorylase allowing it to clear promoter. Thus ,It is required for promoter clearance along with TFIIF). It helps RNA pol II to gain access to the template strand.

Formation of preinitiation complex(PIC):1

- Transcription by RNA pol II involves the formation of a **preinitiation complex(PIC)** followed by initiation , elongation and termination stages of transcription.
- **Requirements for Formation of preinitiation complex(PIC): RNA pol II and the general TFs** viz **TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH and TFIIJ.**
- **Steps involved in Formation of preinitiation complex(PIC):**

1. Binding of **TFIID** to promoter through its TATA binding protein (TBP) component.
2. Binding of **TFIIA** and **TFIIB.**
3. Binding of RNA pol II with **TFIIF** followed by binding of **TFIIE ,H and J. TFIIF** targets RNA pol II by decreasing RNA pol binding to nonspecific sites and thus brings the RNA pol polymerase II to promoter.
4. Binding of **TFIIH .** After synthesizing short lengths of RNA promoter at the promoter , **TFIIH** phosphorylates the C-terminal domain(CTD) of RNA pol II due to which enzyme undergoes a conformational change that facilitates promoter clearance.
5. The Transcription factors are cleared and RNA pol II continues transcription.

Formation of preinitiation complex(PIC):2



Process of Elongation of Transcription in Eukaryotes

1. Elongation proceeds after the formation of the first phosphodiester bond.
2. RNA polymerase moves along DNA unwinding short region for elongation in front of transcription bubble and rewinds DNA behind it.
3. Ribonucleotides are successively added to the growing RNA chain.
4. The 3’ end of RNA forms a transient hybrid of 8-1 base pair with the template DNA.
5. The core enzyme then continues the elongation of the transcript.
6. By the 10 nucleotides have been added , the sigma factor dissociates.
7. The released sigma factor can combine with free core enzymes to form another holoenzymes that can initiate transcription.
8. The process of elongation of the RNA chain continues until a termination signal is reached.
9. RNA polymerase is released from elongation arrest by GreA and Gre B .

Rho (ρ) independent Termination of Transcription in Eukaryotes

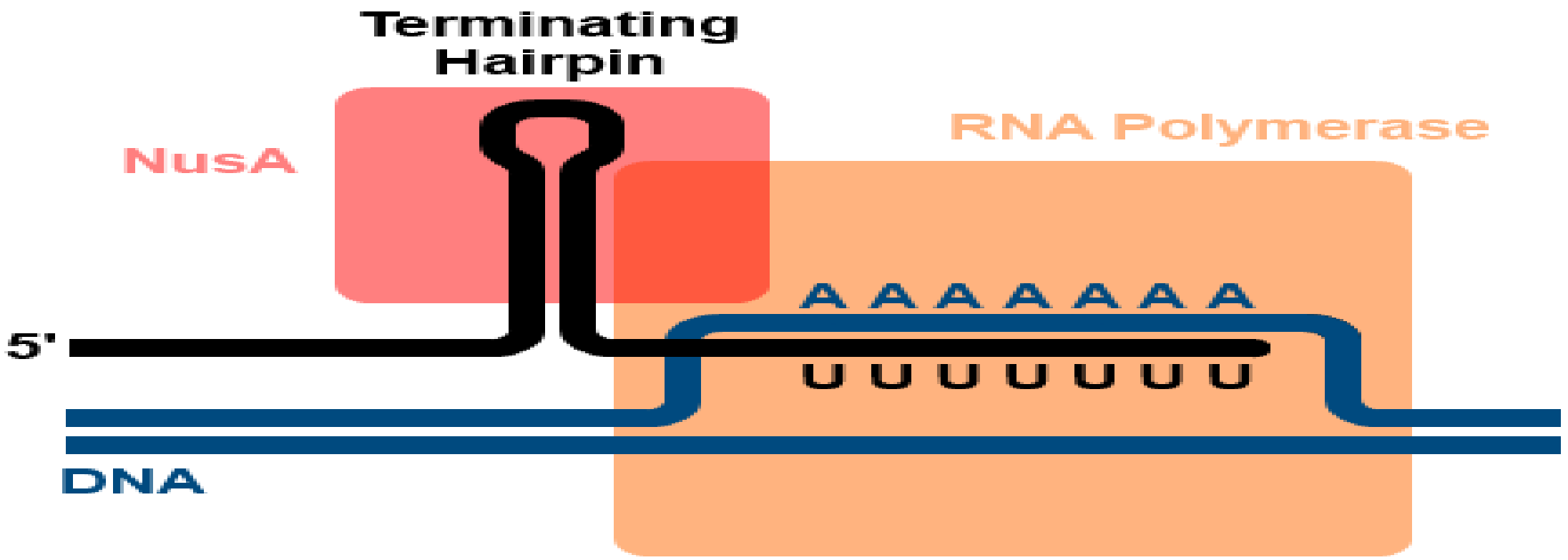
- **In eukaryotes cells termination is less well defined . It is believed that it is similar to rho independent prokaryotic termination.**
- **Rho (ρ) factor independent termination** : The termination is brought about by the formation of hairpin loop(secondary structure) of newly synthesized RNA. This occurs due to **Palindromes .**
- **Palindrome is a word that** reads alike forward & backward i.e. MADAM,ROTOR.
- The presence of palindromes in the base sequence of DNA template in the termination region is known .
- As a result of this, newly synthesized RNA folds to form hairpins (due to complementary base pairing) that cause the termination of Transcription. This dislodges the RNA polymerase from DNA template and release of the newly synthesized transcript.
- This hairpin loop structure is followed by a sequence of four or more uracil residues which are essential for termination. The RNA transcript ends within or just after then.

Mechanism of Rho (ρ) independent Termination of Transcription in Eukaryotes

- **Rho (ρ) independent (intrinsic) termination** :is based on two structral features at the end of the RNA transcript**:**

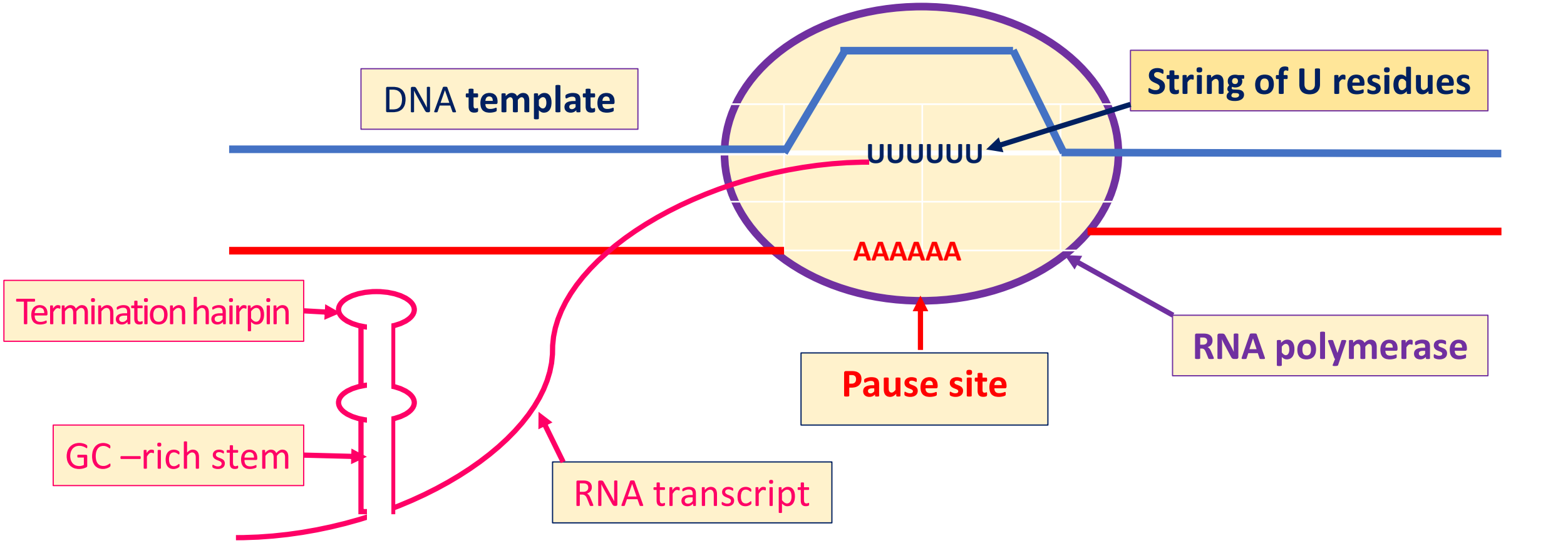
1. A self complementary hairpin structure 15-20 nucleotide before the end of the RNA . The base pair of the hairpin which is GC rich, prevents RNA binding and disturbs the RNA-DNA hybrid .
2. A string of U residues that base pair weakly with corresponding A residues in the template DNA strand . As a result , RNA dissociates from the RNA-DNA hybrid.

Rho (ρ) factor independent termination of Transcription in Eukaryotes



Rho (ρ) independent Termination of Transcription in Eukaryotes : newly synthesized RNA folds to form hairpins (due to complementary base pairing) that cause the termination of Transcription. A string of U residues that base pair weakly with corresponding A residues in the template DNA strand . As a result , RNA dissociates from the RNA-DNA hybrid.

Mechanism of intrinsic termination of transcription



A	A	T	C	C	A	C	A	G	G	C	G	C	C	A	G	X	X	X	X	X	C	T	G	G	C	G	C	A

←Coding stand of DNA

T	T	A	G	G	T	G	T	C	C	G	C	G	G	T	C	X	X	X	X	X	G	A	C	C	G	C	G	T

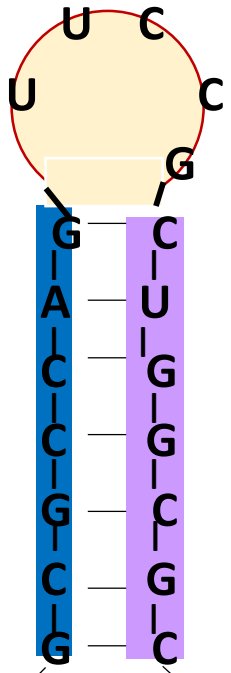
←Template stand of DNA

Transcription

A	A	U	C	C	A	C	A	G	G	C	G	C	C	A	G	U	U	C	C	G	C	U	G	G	C	G	C	A	U	U	U	U

←Primary transcript of RNA

A hairpin loop structure formed by nascent RNA in Rho-independent termination phase of transcription



←Noncomplementary sequences in a primary transcript

←A self complementary hairpin structure of 15-20 nucleotides before the end of the RNA . The base pairs of the hairpin (which is GC rich) prevents RNA binding and disturbs the RNA-DNA hybrid .

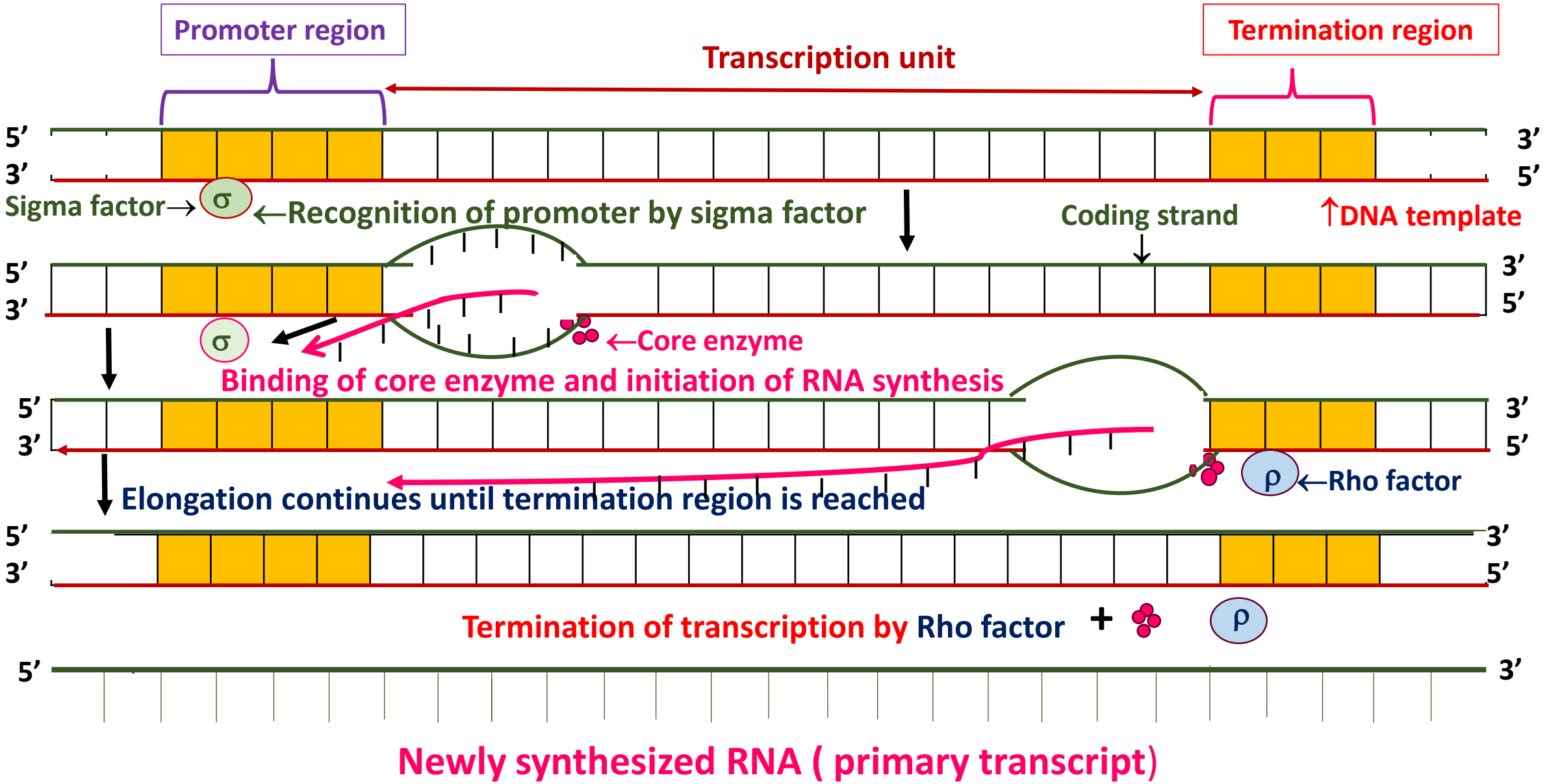
5’ – U-A- A-U- C-C-A-C-A-G

A-U-U-U-U-OH- 3’

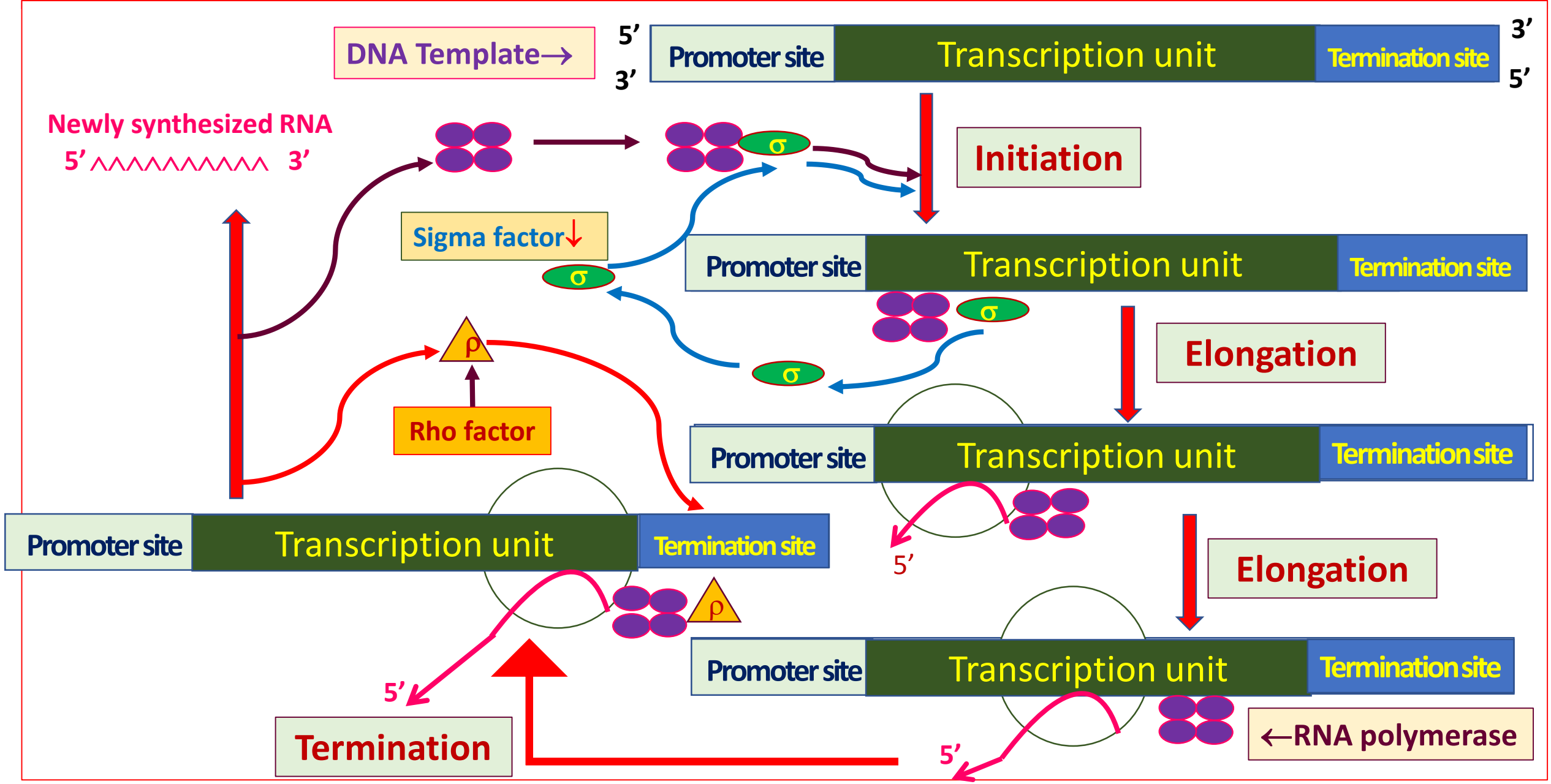
Schematic diagram

A hairpin loop structure followed by a sequence of Uridine residues. A string of U residues that base pair weakly with corresponding A residues in the template DNA strand . As a result , RNA dissociates from the RNA-DNA hybrid.

Process of transcription in Eukaryotes



Synthesis of RNA from DNA template



Ribozymes in Eukaryotes

- A **Ribozyme is a catalytic RNA molecule (RNA enzyme).**
- **Ribozyme is seen in both prokaryotes and Eukaryotes.** They exhibit Michaelis –Menten kinetics. The ribozymes are vestigial remnants of nucleic acids which were biological catalysts in precellular era.
- **Example of Ribozymes include the following :**

1. **RNase P :** a ribonucleoprotein that functions as an endonuclease and generates the 5' end of mature of tRNA. The RNA component is sufficient to function as an endonuclease and the protein required to stabilize the RNA or facilitate its functions.
2. **The self –splicing group I introns L-19 intervening sequence (IVS):** in **Tetrahymena thermophili** L-19 RNA behaves as a **nucleotidyl transferase** and catalyzes transesterification reactions during r**RNA** splicing **.**
3. **Peptidyl transferase :** present in the lager subunit of ribosome and hence used for protein biosynthesis.

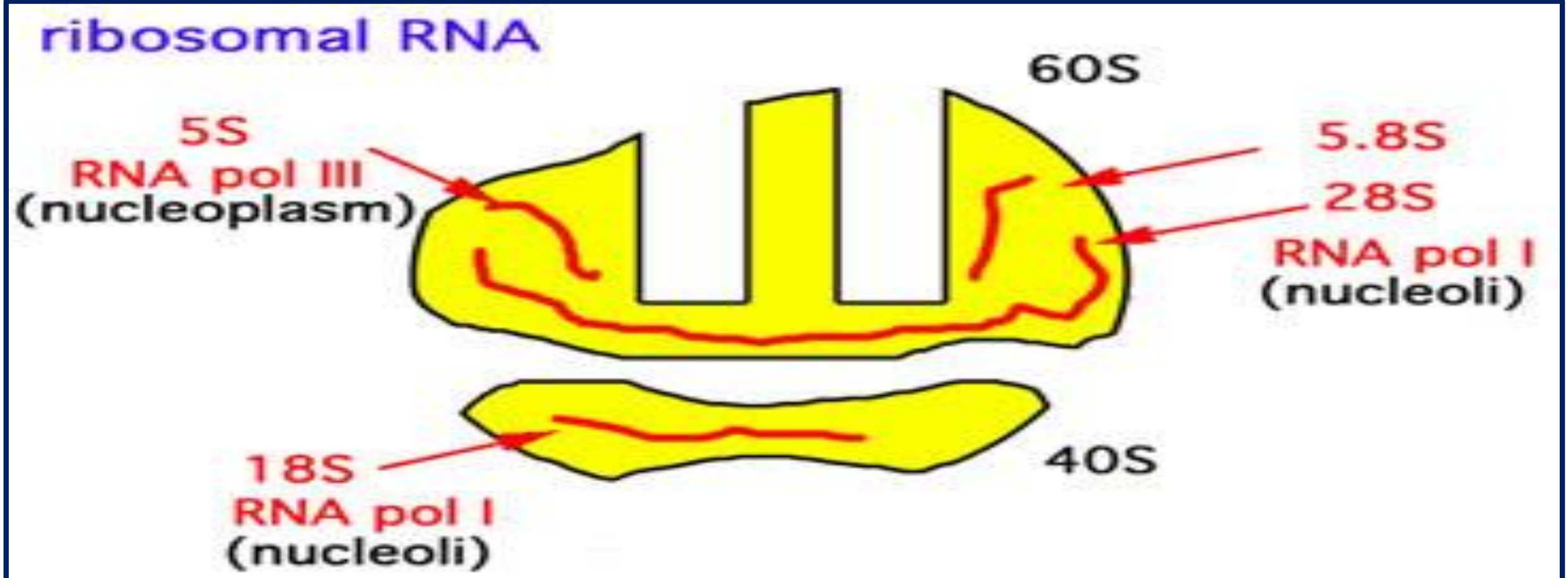
Post transcriptional modifications in Eukaryotic cells

- Eukaryotic post-transcriptional process is more **extensive** than in prokaryotes. This is partly due to presence of well distinct nucleus from which most RNAs must be transported . RNAs are processed during this transport .
- **Purpose** : processing gives them the characteristics they need to be functional in the cytoplasm.
- **Substrate** :The transcription products of all three eukaryotic polymerases are processed.

Comparison of Post transcriptional modifications of different types Primary transcript RNAs in Eukaryotic cells

Post transcriptional modifications of Primary transcript of rRNA	Post transcriptional modifications of Primary transcript of tRNA	Post transcriptional modifications of Primary transcript of mRNA
Cleavage		Splicing to remove introns (non-coding sequences)and join exons(coding sequences)
Ribose sugar modification	Addition of CCA at 3’ end	Addition of 7methylguanosine cap at 5’ end and poly A tail at 3’ end
Nucleotide Base modification	Nucleotide Base modification	
Trimming	Trimming	

Eukaryotic Ribosomal RNA(rRNA)



Three types of Eukaryotic rRNA synthesized from 45S preribosomal RNA (long precursor) : 28S, 18S, 5.8S.

The forth types of Eukaryotic rRNA synthesized by transcription of 5S gene by RNA polymerase III : 5S (it is modified separately).

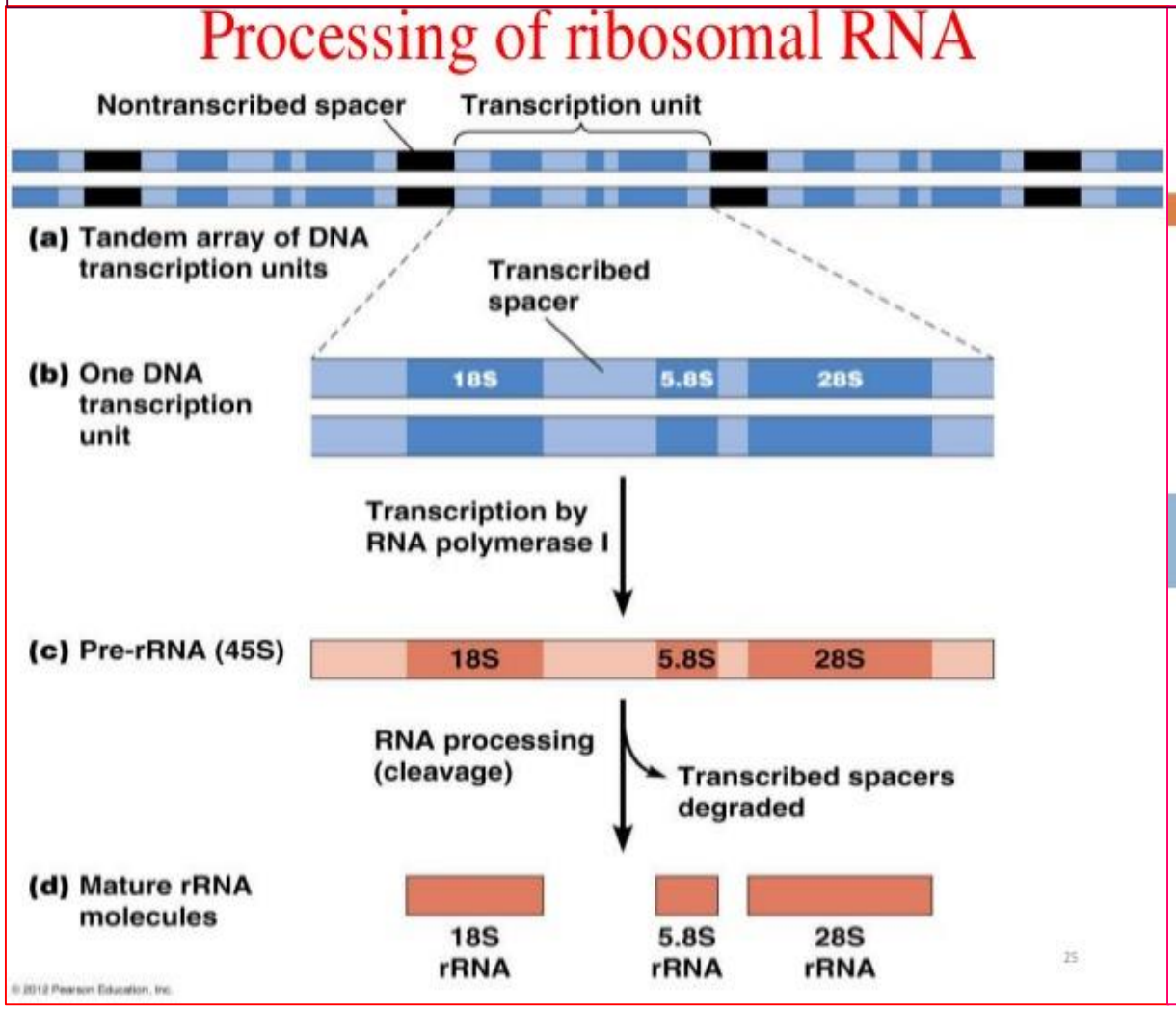
Post transcriptional modification of Eukaryotic rRNA

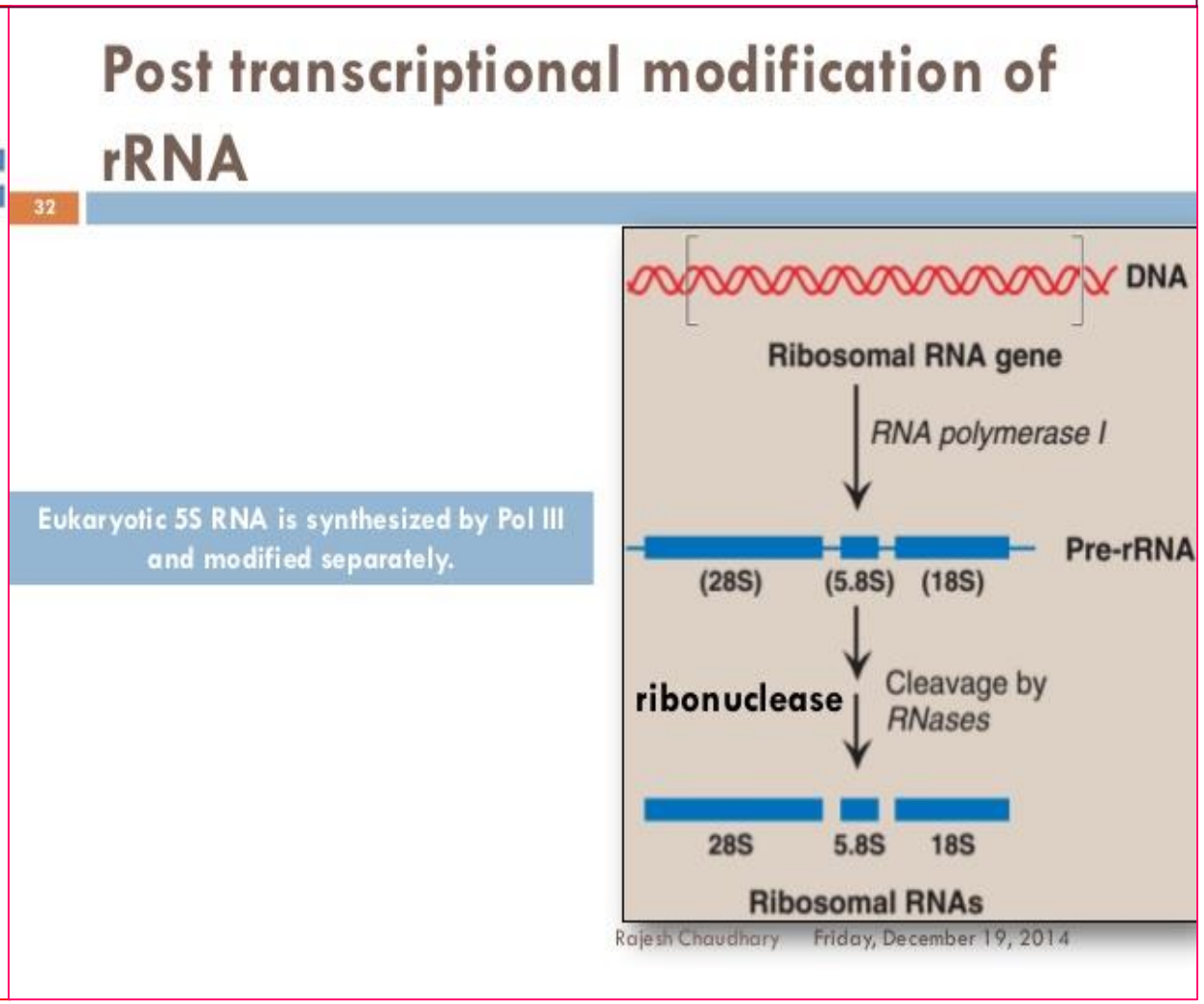
- **Eukaryotic rRNA processing** is **similar** to that of **prokaryotes**.
- **Three types of Eukaryotic rRNA synthesized from 45S preribosomal RNA (long precursor) : 28S, 18S, 5.8S**
- **Post transcriptional modification of 45S preribosomal RNA :** cleaved and trimmed by specific **endonucleases** to produce mature functional rRNA molecule (as in prokaryotes spacer sequences are removed).
- **The forth types of Eukaryotic rRNA by transcription of 5S gene by RNA polymerase III : 5S (** it is modified separately).

➢ The 5.8S rRNA base pairs with the 28S rRNA during **formation of ribosomal subunits** , which is completed before transport from nucleus to cytoplasm.

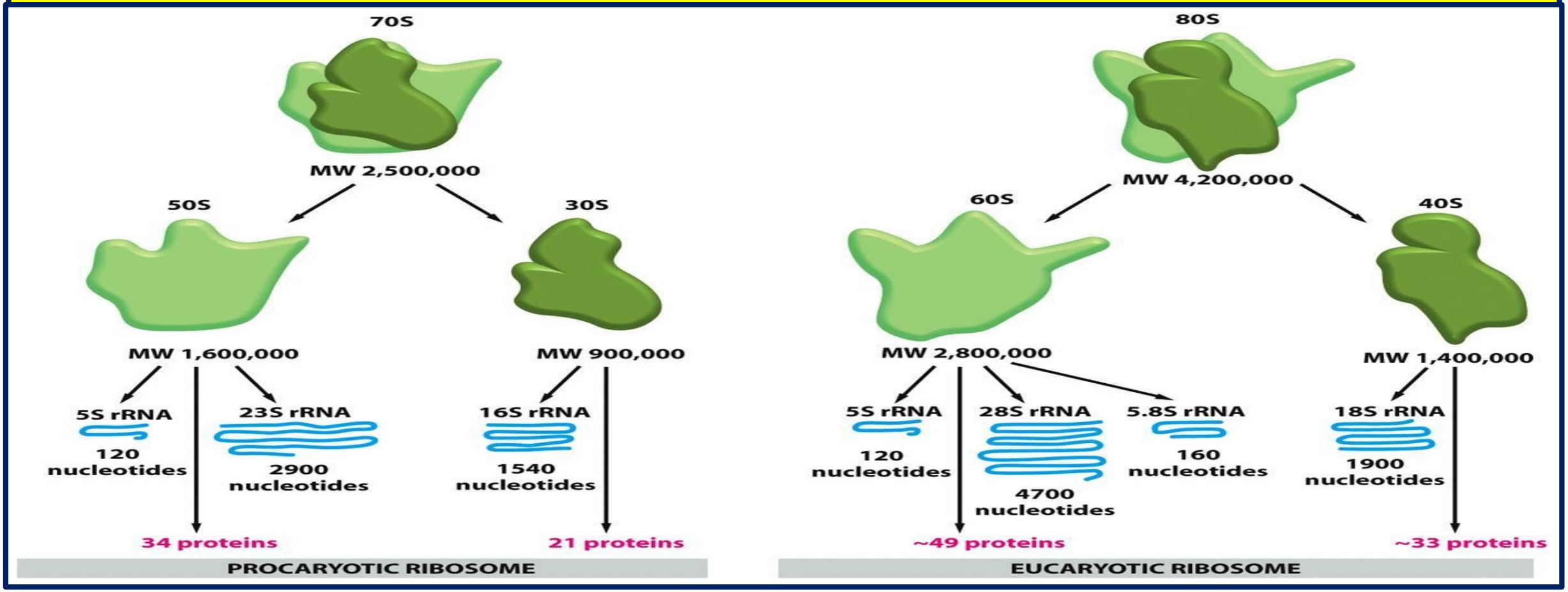
Post transcriptional modifications of inactive primary RNA transcripts for synthesis of Eukaryotic rRNA

The preribosomal RNAs originally synthesized are converted to ribosomal RNAs (r RNA) by a series of post transcriptional modifications.





Comparison of Ribosomal RNA(r- RNA) in prokaryotes and Eukaryotes



Three types of Eukaryotic rRNA synthesized from 45 S preribosomal RNA (long precursor) : 28S, 18S, 5.8S and the forth types of Eukaryotic rRNA by transcription of 5S gene by RNA polymerase III : 5S

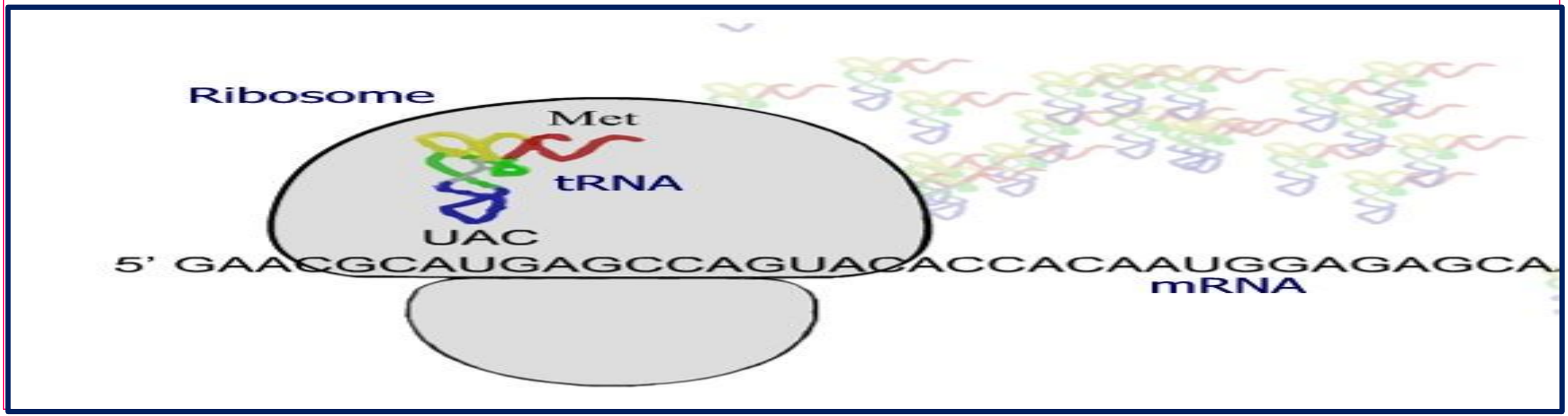
Eukaryotic mRNA processing

Eukaryotic mRNA processing

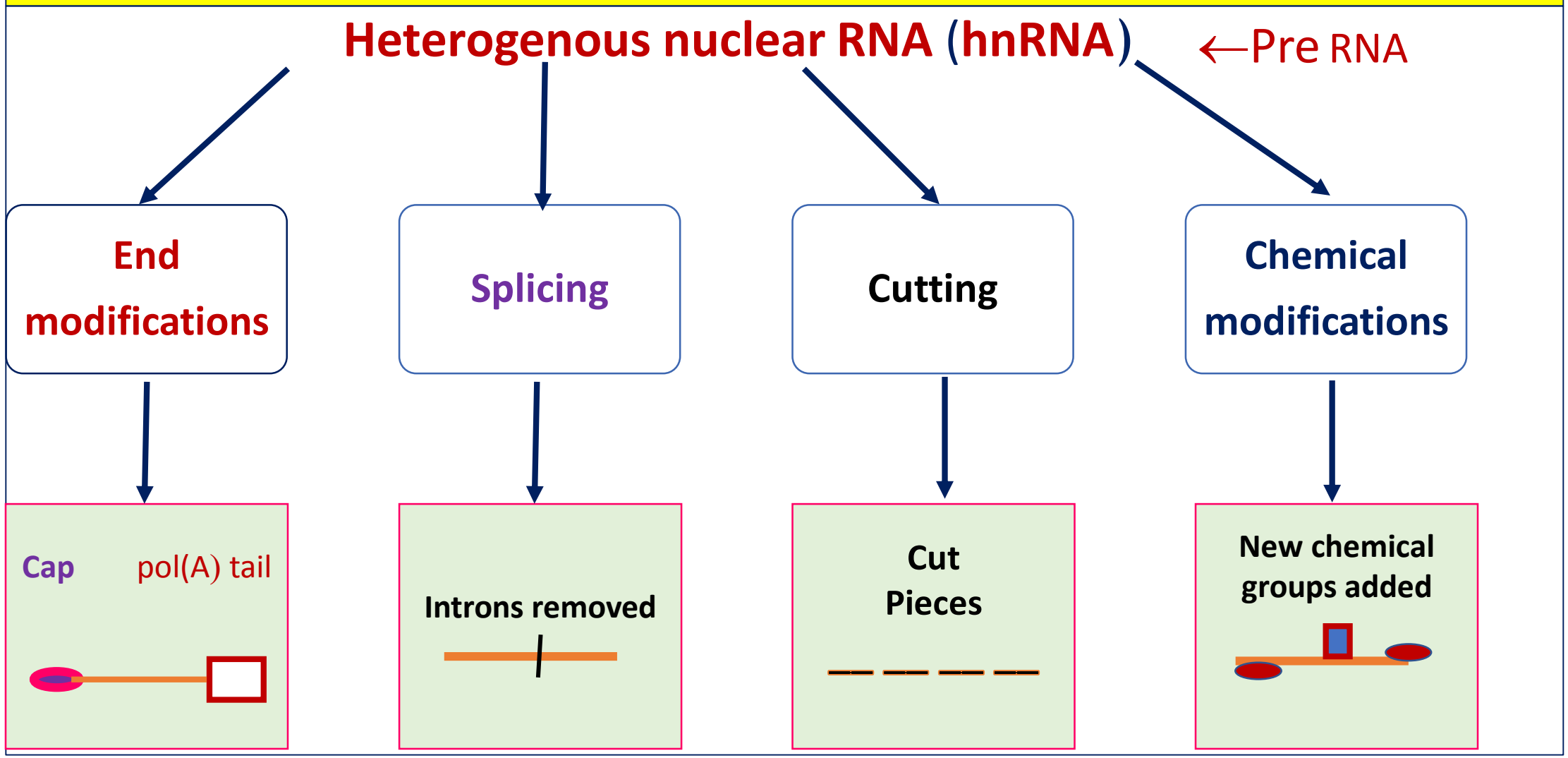
- Mature ,functional mRNA is formed from extensive processing of large precursor called **Heterogenous nuclear RNA** (hnRNA) primary transcript product of RNA polymerase II .
- (hnRNA) primary transcript is extensively modified after transcription.

Heterogenous nuclear RNA (hnRNA) in Eukaryotes

- The collection of all **inactive primary RNA transcripts** produced by RNA polymerase II in nucleus of eukaryotes is often referred as **Heterogenous nuclear RNA** (hnRNA)**.**
- Post transcriptional modifications of Heterogenous nuclear mRNA occurs in the nucleus to produce mRNA .The mature mRNA then enters the cytosol to perform its function of protein synthesis(translation).



Post transcriptional modifications of Heterogenous nuclear RNA in Eukaryotes



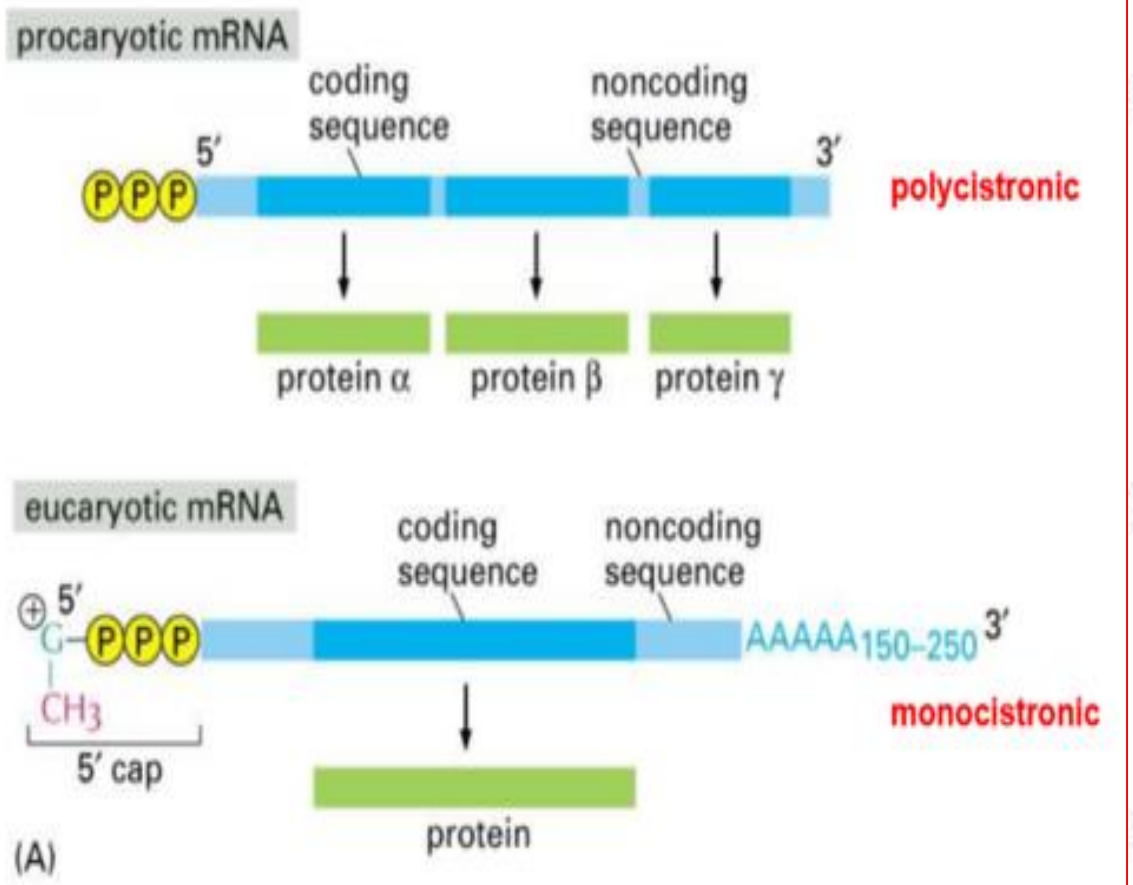
Post transcriptional modification of Eukaryotic Messenger RNA(mRNA)

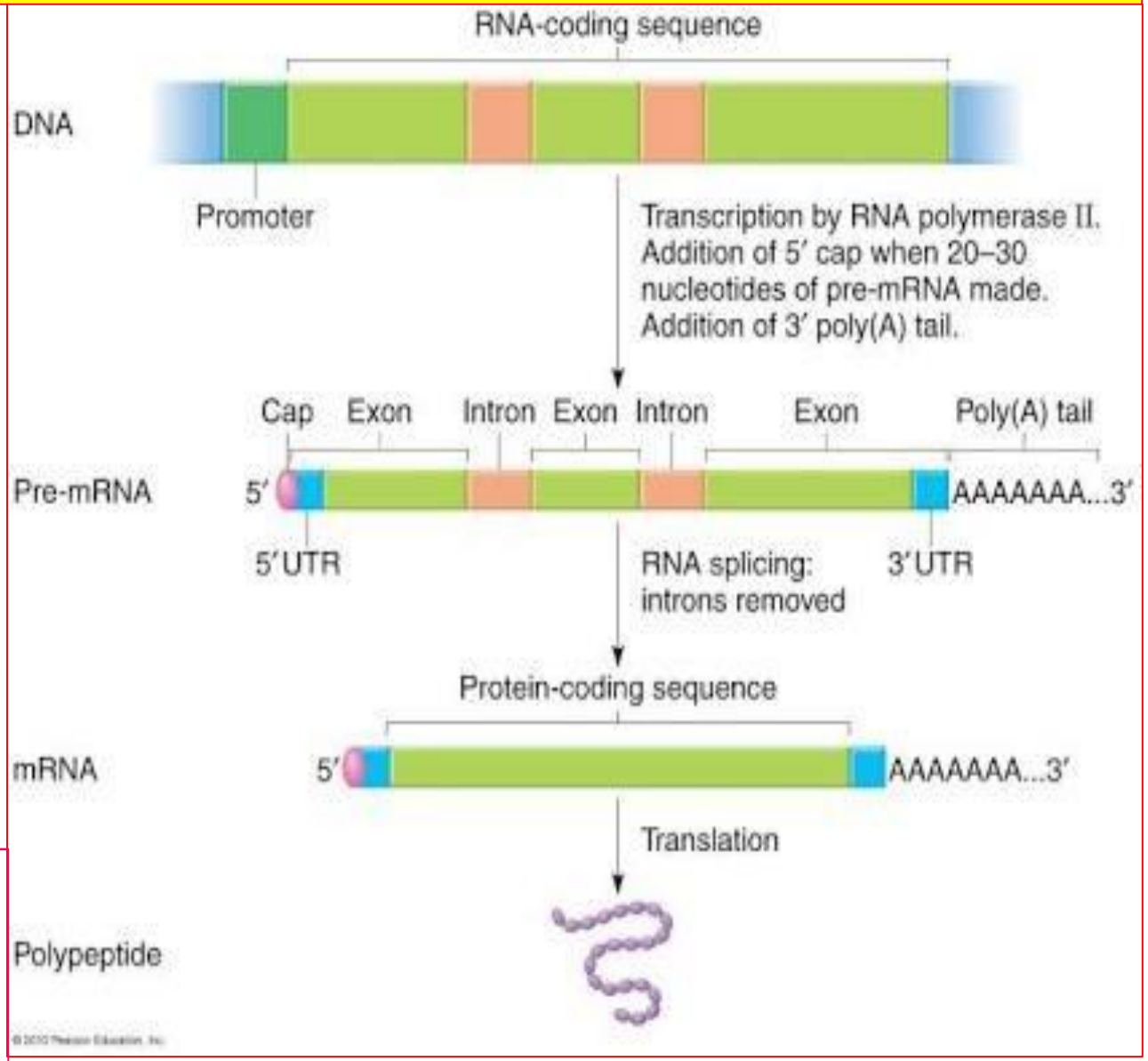
The inactive primary transcript of mRNA components of **Heterogenous nuclear RNA** (hnRNA) undergoes extensive co and post transcriptional modifications before functional mRNA is produced in the nucleus . This transcript is a product of RNA polymerase II.

Modifications of primary mRNA transcript include:

1. **The 5' capping** : The 5'end of m-RNA is capped with 7-methylguanosine attached by an unusual **5'** → **5'** triphosphate linkage to ribose at 5' –end. 5-Adenosylmethionine is donor of methyl group . This cap is required for translation and stabilizes the structure of mRNA.
2. **Poly -A tail :** A large number of Eukaryotic mRNA possess an adenine nucleotide chain at the 3'end. This **Poly -A tail** as such is not produced during transcription. It is later added to stabilize mRNA. **Poly -A tail** get reduced as the mRNA enters cytosol.

Post transcriptional modifications of inactive primary RNA transcripts for synthesis functional mRNA in Prokaryotic and Eukaryotic cells

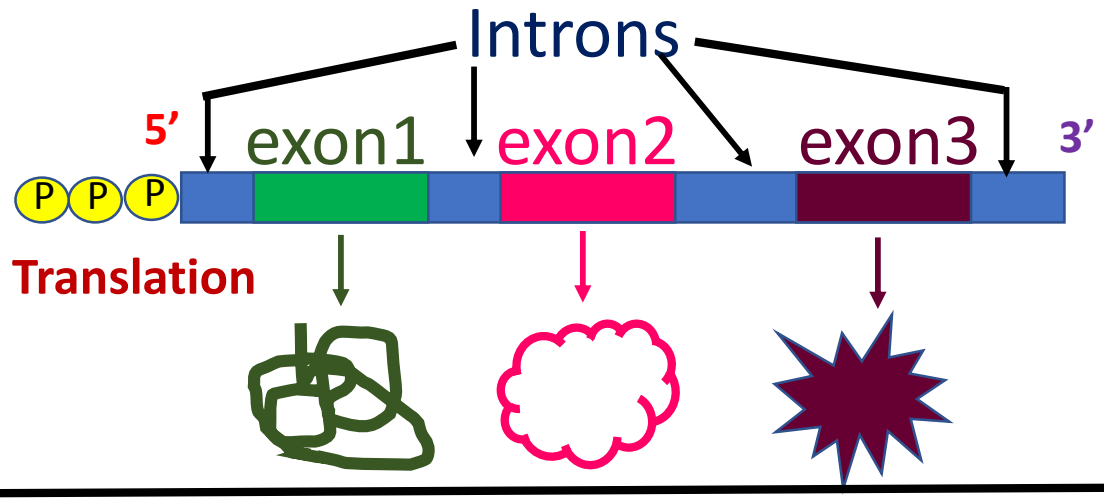




In prokaryotes ,mRNA is **not Post transcriptionally modified** and is functional immediately after its synthesis . In fact, its translation often begins before transcription is complete.

Comparison of Prokaryotic and Eukaryotic mRNA

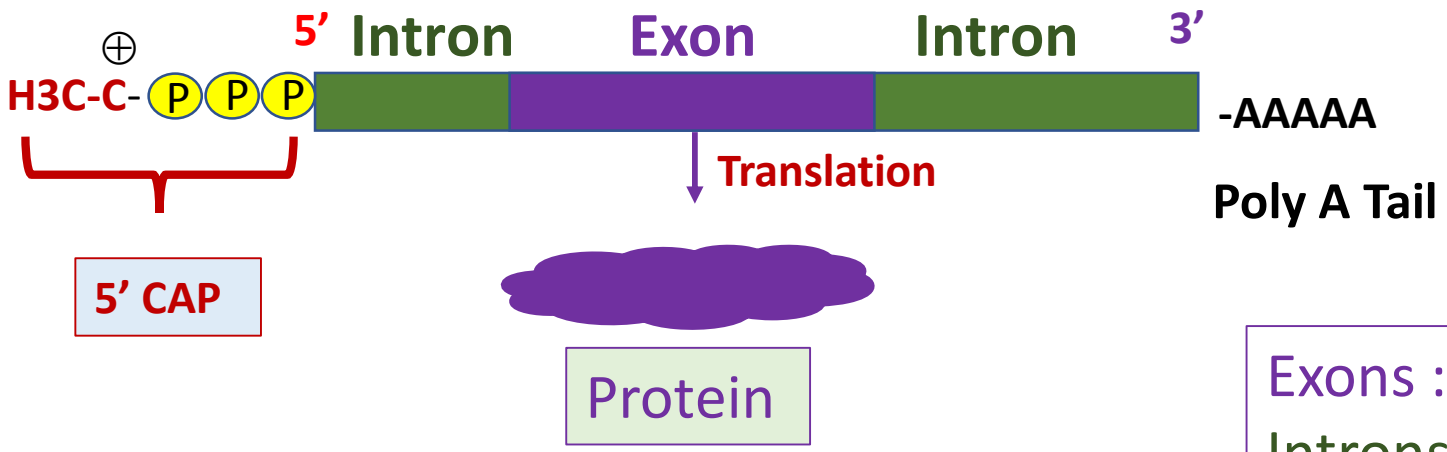
Prokaryotic mRNA



Polycistronic

Schematic diagram

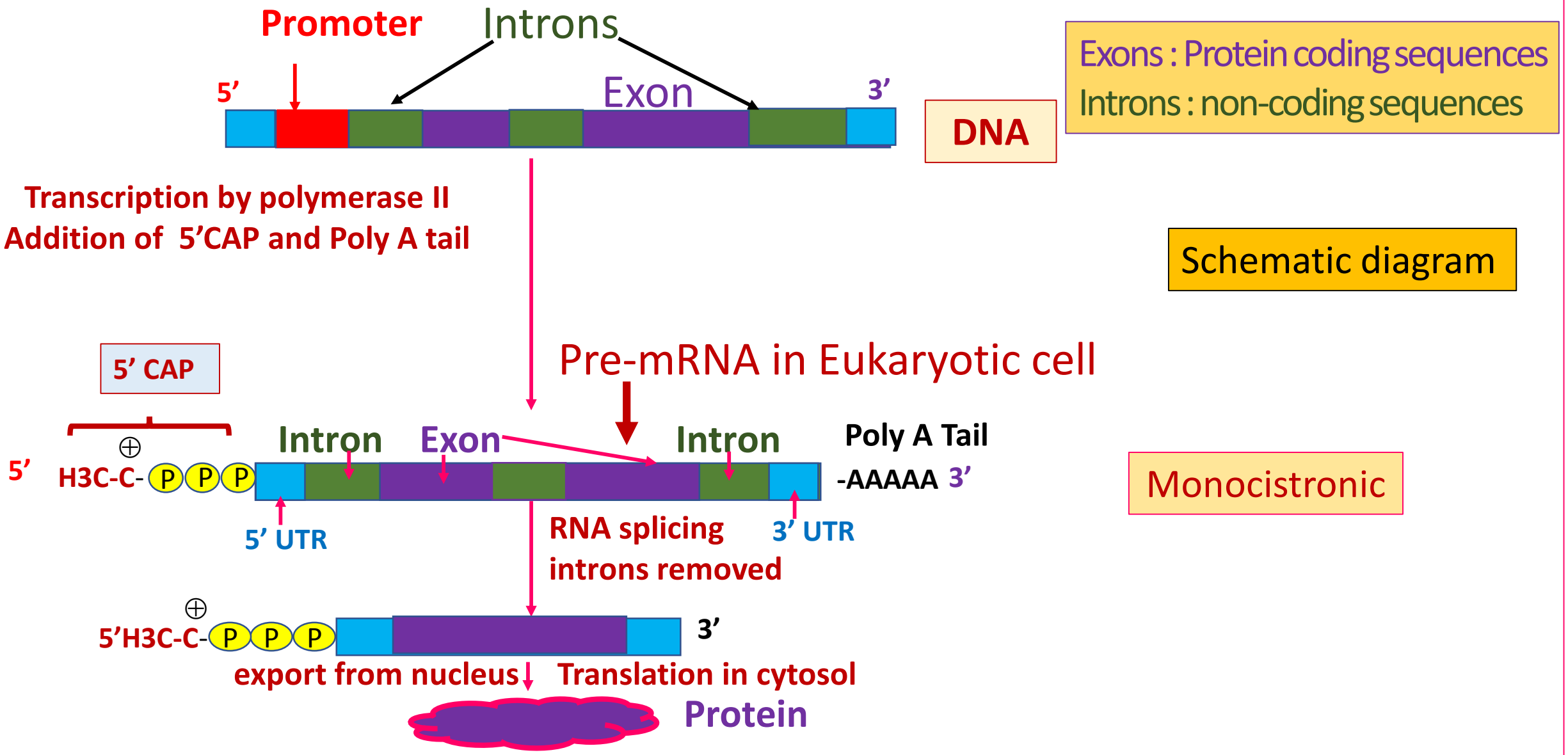
Eukaryotic mRNA



Monocistronic

Exons : coding sequences
Introns : non-coding sequences

Post transcriptional modifications of inactive primary RNA transcripts for synthesis functional mRNA in Eukaryotic cells



Capping at 5'end of primary transcript

- The 5' end of eukaryotic mRNA consist of cap of 7-methylguanylate attached to triphosphate linkage to the ribose at the 5'-end.

❑Processing reactions of 5' capping of hnRNA :

a. Is the **first** processing **reaction for pre mRNA.**

b. Creation of cap requires removal of the γ from 5' triphosphate of the pre mRNA followed by **addition** of GMP (from guanosine triphosphate GTP) catalyzed by nuclear enzyme **guanyl** transferase.

c. **Methylation** of this **terminal guanine** occurs in the cytosol and is catalyzed by guanine -7-methyl transferase using S-adenosyl-methionine(**SAM**) as a source of the methyl group .

❑Role/functions of 5'cap:

a. Helps in the binding of mature mRNA to the ribosome during initiation of protein biosynthesis.

b. Facilitates stabilization of mRNAs by protecting them from digestion by nucleases that degrade RNAs from their 5' end.

c. Eukaryotic mRNA lacking the cap are not translated efficiently.

Characteristics of Poly-A tail of hnRNA

- **Location of Poly A tail:** 3’ end of most eukaryotic mRNA (polynucleated→ Poly A)
- **Characteristics of Poly A tail of eukaryotic mRNA :**

a. A chain which have 20 to 300 adenylate residues linked by phosphodiester bonds (with several exceptions e.g. mRNA of Histones).

b. is not transcribed from DNA but added after transcription by polyadenylate polymerase using ATP as the substrate.

- **Synthesis of Poly A tail** :

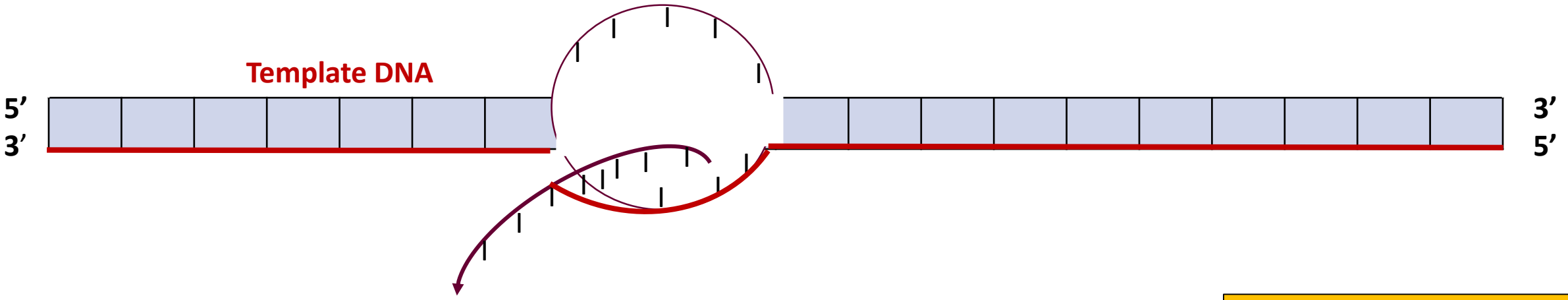
a. Eukaryotic primary transcripts are cleaved downstream of consensus sequence by a specific endonucleases that recognizes the **polyadenylation signal sequence (AAUAAAA) found near 3’ end of the RNA.**

b. cleavage does not occur if this sequence or a segment of some 20 nucleotides on its 3’ end is deleted.

c. After cleavage by endonuclease, A poly A polymerase adds about 200 -300 adenylate residues to the 3’ end of transcript.

d. ATP is the donor of adenylate.

Addition /synthesis of poly(A) tail of a primary transcript hnRNA



Schematic diagram

5′ Cap —— AAUAAA —— cleaving signal

hn RNA (Nascent RNA)

↓ Cleavage by specific endonuclease

ATP
Ppi

↓ Addition of tail by poly (A) polymerase

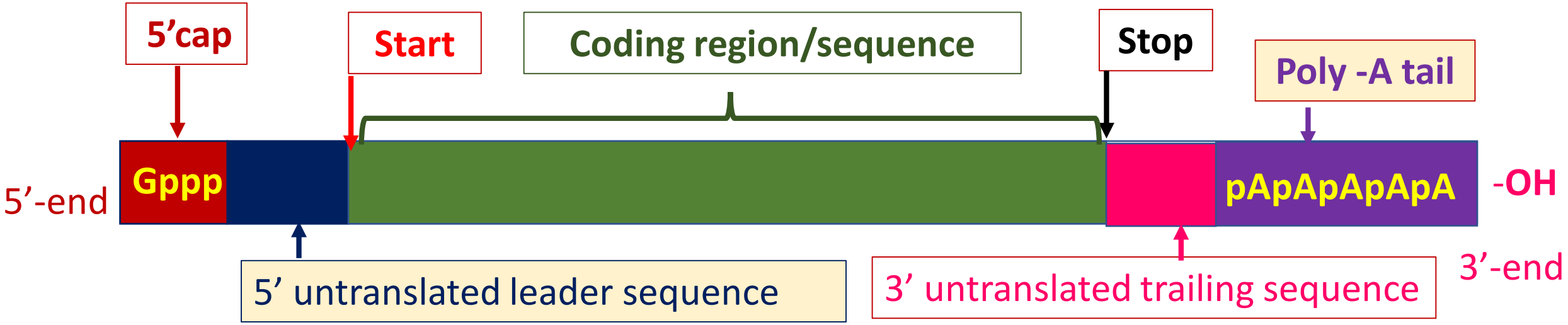
5′ Cap —— AAUAAA —— $AAAAAA_{(n)}$ —— OH 3′

Functions of Poly A tail

❑Functions of Poly A tail:

1. It is involved in stabilization of mRNA and its exit from nucleus.
2. It may enhance translation efficiency.
3. mRNA molecule devoid of poly A tail is less effective template for protein synthesis than is one with poly A tail.
4. Other functions?
5. After the mRNA enters the cytosol , the poly-A tail is gradually shortened.

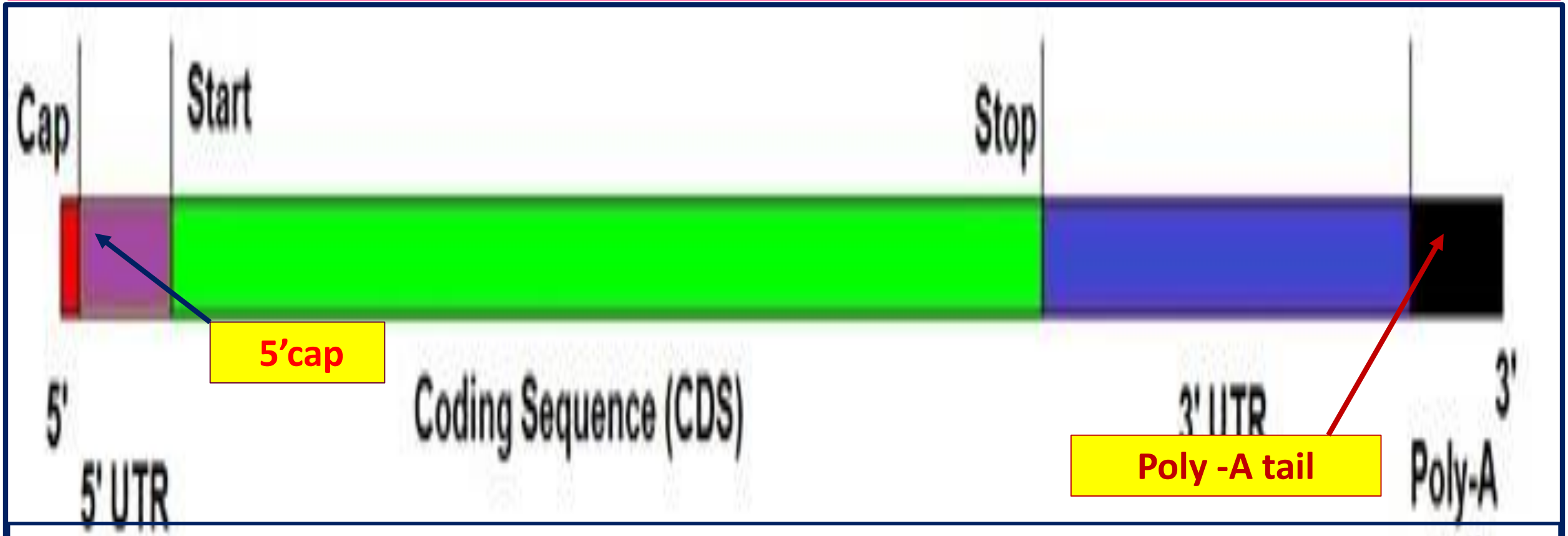
Structure of Eukaryotic messenger RNA



Structure of 5'cap: The 5'end of m-RNA is capped with 7-methylguanosine attached by an unusual **5'** → **5'** triphosphate linkage to ribose at 5' –end. 5-Adenosylmethionine is donor of methyl group . This cap is required for translation and stabilizes the structure of mRNA.

Structure of Poly A tail : A chain which have 20 to 300 adenylate residues linked by phosphodiester bonds. It is not transcribed but added after transcription

Mature and functional messenger RNA (mRNA)



Functions of 5'cap: helps in the binding of mature mRNA to the ribosome during protein biosynthesis .It facilitates stabilization of mRNAs by protecting them from digestion by nucleases that degrade RNAs from their 5' end.

Functions of Poly A tail : involved in stabilization of mRNA and its exit from nucleus. It enhances translation efficiency .

Untranslated regions of mRNA

- Protein synthesis is often regulated at the level of initiation of translation , is a critical step.
- The regulation occurs by cis-regulatory elements ,which are located in the 5’ and 3’ UTRs(untranslated regions) and trans-acting factors.
- A breakdown in the regulatory machinery can perturb cellular metabolism, leading to various physiological abnormalities.
- The highly structured UTRs ,along with features such as GC -richness , upstream open reading frame, internal ribosome entry site significantly influence the rate of translation of mRNAs.
- Changes in cis-regulatory sequences of the UTRs (point mutation and truncation) influence expression of specific genes at level of translation.

Clinical manifestations related to alterations in cis-regulatory sequences of the UTRs

- Changes in cis-regulatory sequences of the UTRs may alter physiological balance from healthy to disease state suggesting crucial role of UTRs as that of coding sequences in health and disease.
- Diseases associated with changes in cis-regulatory sequences of the UTRs:

a. Hereditary thrombocytopenia
b. Breast cancer
c. Alzheimer disease
d. Fragile X syndrome
e. Bipolar effective disorder

Half life of messenger (mRNA)

- Half life of an mRNA may be determined in part by rate of degradation of its poly A tail.
- **Location of degradation /shortening of mRNA** : cytoplasm
- **Initiation of Degradation of mRNA** : only after removal of poly A tail

➢ **Storage of some mRNA :** in an unadenylated form and receive the poly A tail only when translation is imminent .

Splicing in Messenger RNA primary transcript in Eukaryotes

3. Introns removal:

- **Introns** : are the intervening nucleotide sequences (genes)in mRNA which do not code for proteins(non-coding sequences between exons) . A few eukaryotic primary transcripts contain no introns . e.g. those from Histone genes lack introns. The primary transcript for α chain of collagen contain >50 introns.
- **Exons (expressed portion of the gene)of mRNA** : possess genetic code/coding sequences of genes and responsible for protein synthesis .
- **Process of splicing :** introns are excised from primary transcript and exons are linked to form functional mature mRNA . Spliceosome accomplishes this task.
- **Promotion of The splicing and excision of the Introns :** by small nuclear ribonucleic protein particles (snRNPs pronounced as **snurps**) .
- **Formation of snRNPs:** by the association of small nuclear RNA(snRNA) with proteins.
- **Spliceosome** : represent the snRNP association with hn-RNA at the exon-intron junction .
- **Location of Post transcriptional modifications of Heterogenous nuclear RNA** : nucleus

➢ The mature mRNA then enters the cytosol to perform its function (translation) .

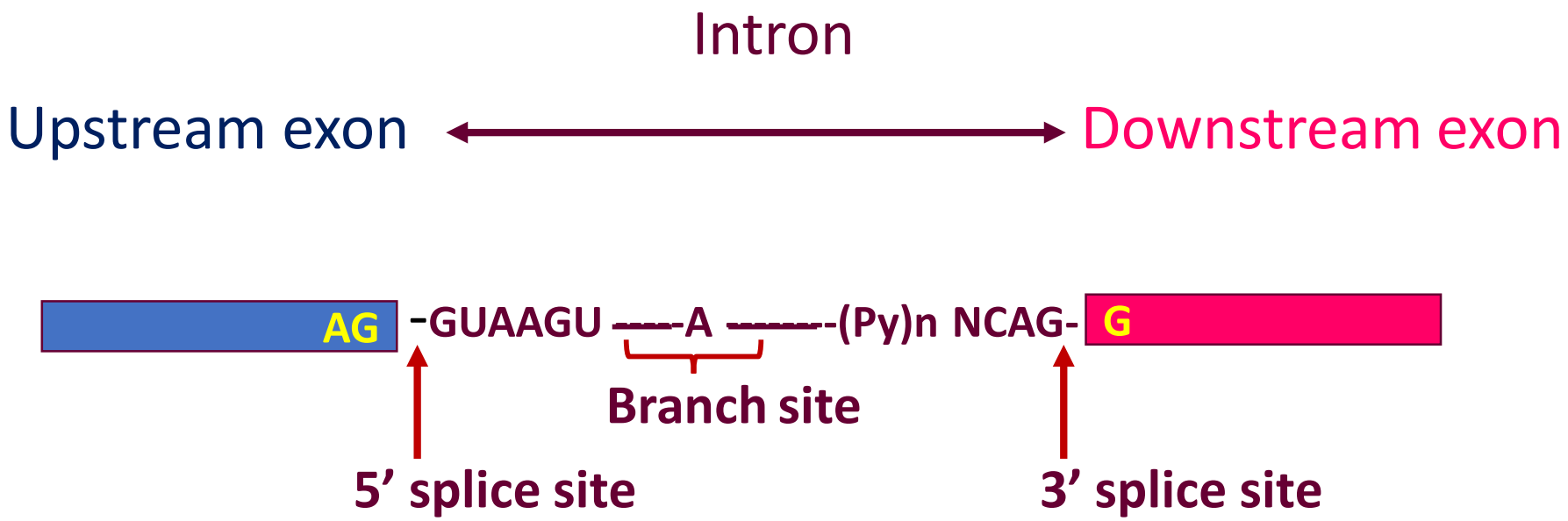
Splice site

The consensus sequences at the introns /exon boundaries of the hnRNA (primary transcript) :

a. are **AGGU.**
b. almost may vary to some extent on the exon side of boundary.
c. almost all introns begin with a 5’ GU and end with a 3’ AG.
d. at the 5’ splice in vertebrates is **AGGUAAGU.**
e. At the 3’ end of an intron, the consensus sequence is a stretch of 10 pyrimidine (U or C), followed by any base and then by C and ending with the invariant AG.

- Since every 5’ GU and 3’ AG combination does not result in a functional spice site , indicating other features within the exon and intron define appropriate splice sites . These indicate other features within the exon and intron define the approximate spice site.
- Introns also have an internal site located between 20 and 50 nucleotides upstream of 3’ spice site , it is called the **Branch site.**
- **In** mammals, variety of branch site sequences are found.

Schematic representation of Splice sites



Consensus sequences for 3’ and 5’ splice site where Py = Pyrimidine , N= any nucleotide

Characteristics of splicing in mRNA

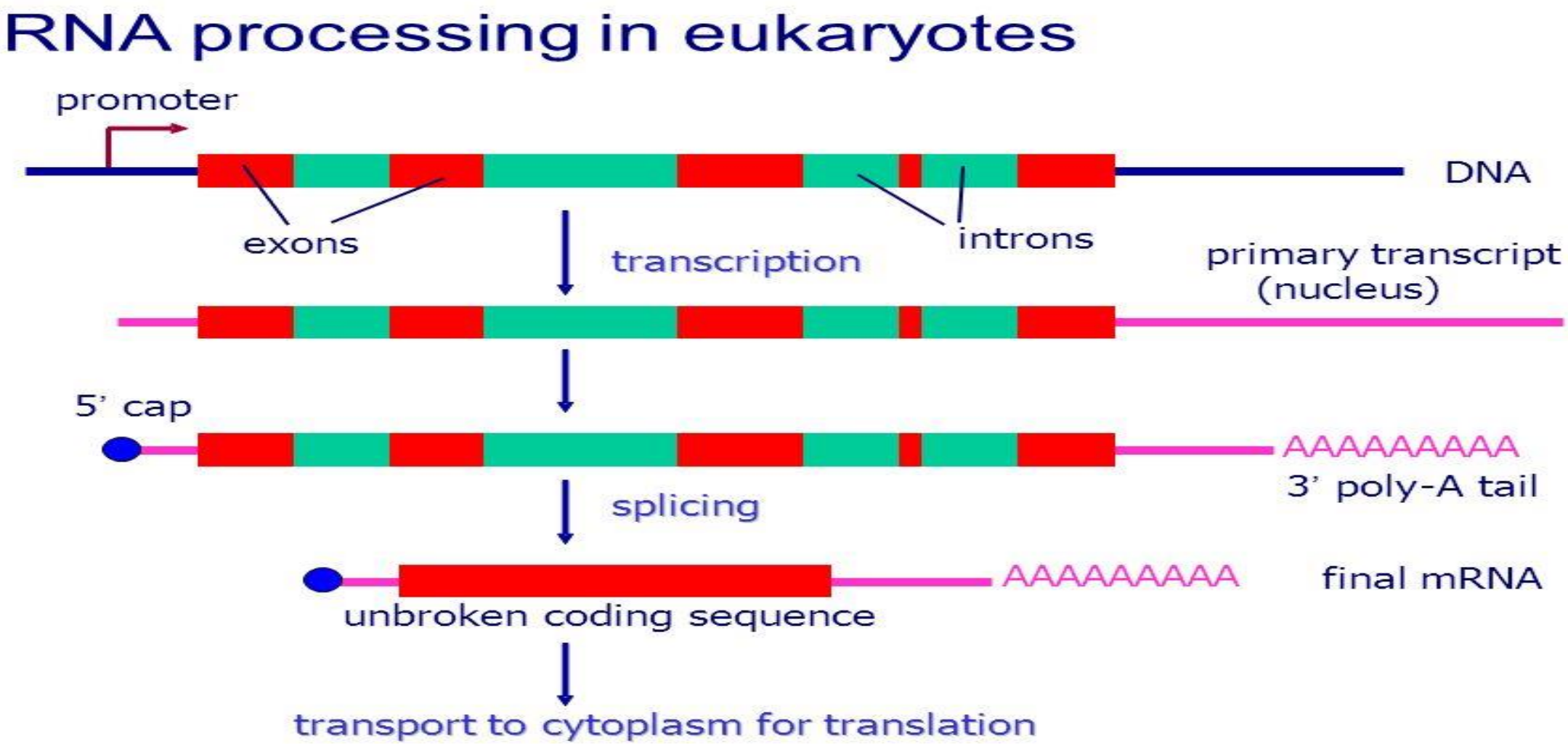
❑**Characteristics of splicing :**

1. Accurate process
2. Very sensitive
3. happens quite often

Splicing in mRNA eukaryotes:1

- Most genes in higher eukaryotes are composed of exons and introns.
- The process by which introns are excised and exons are linked to form functional mRNA is called **splicing**.
- Different genes have different numbers of introns of different sizes.
- The splicing must be very accurate and very sensitive .
- One nucleotide slippage in a splicing point would shift the reading frame on the 3’-side of splice to give entirely different amino acid sequence.

Splicing in mRNA eukaryotes:2

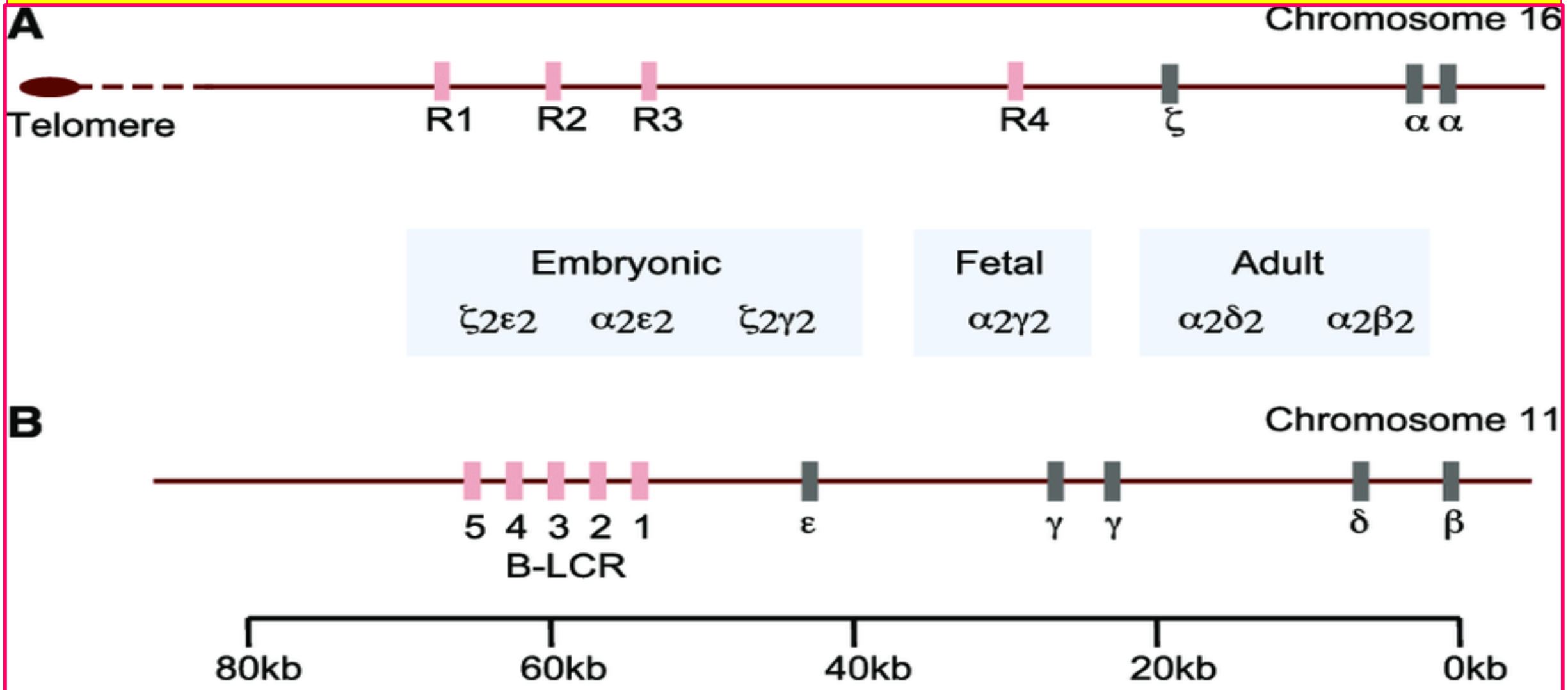


Process of splicing : introns are excised and exons are linked to form functional mRNA .

Faulty splicing can cause diseases

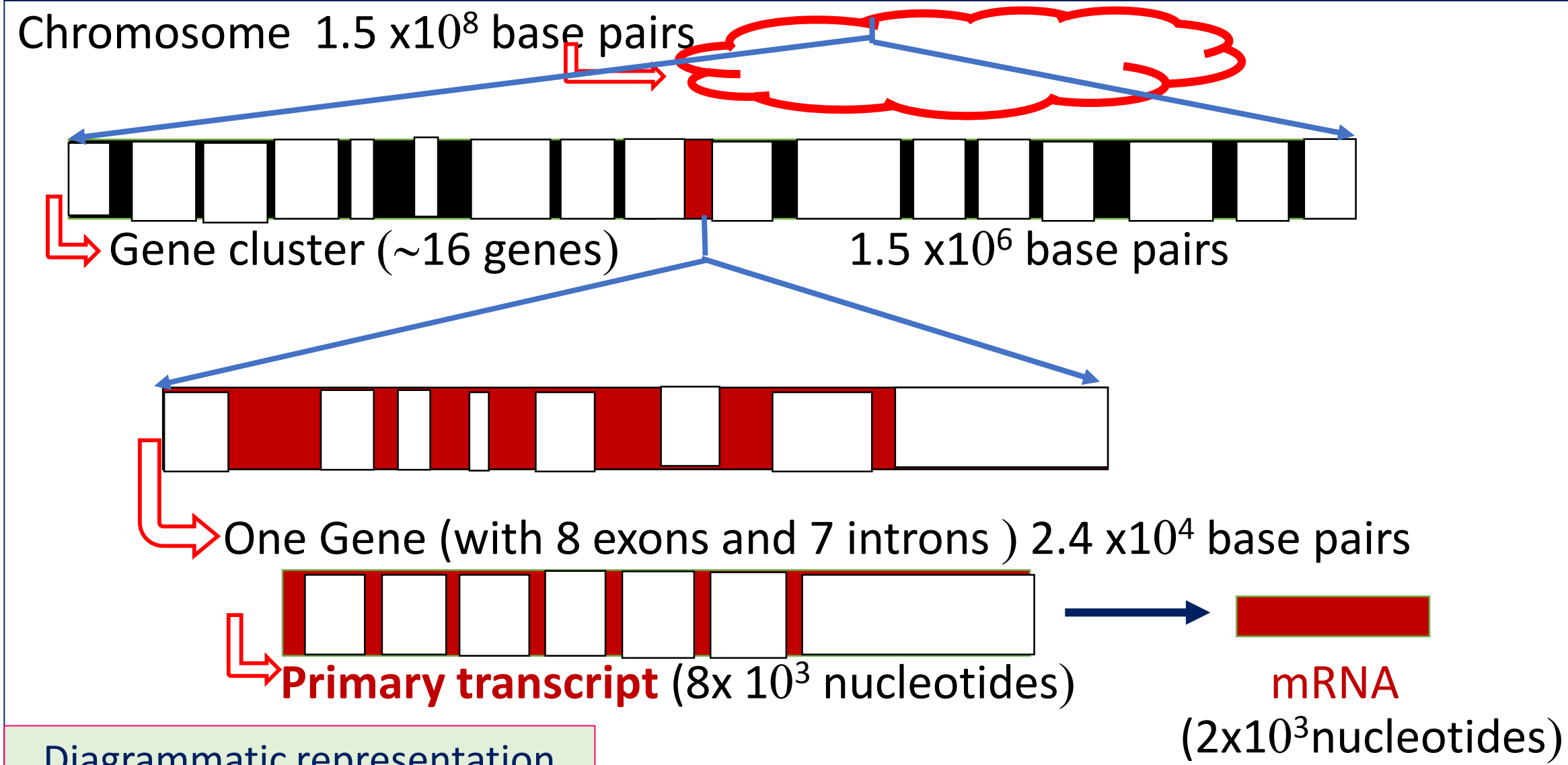
- Splicing of hn-RNA has to be performed with precision to produce functional mRNA.
- Faulty /aberrant splicing causes some forms of diseases e.g. β thalassemia in human
- β thalassemia is due to a mutation that results in a nucleotide change at an exon-intron junction .
- The result is a diminished or lack of synthesis of β chain of hemoglobin ,and consequently the disease β thalassemia.

Splicing of hnRNA has to be performed with precision to produce functional mRNA.



Aberrant splicing: causes some forms of thalassemia

Relationship between eukaryotic chromosomal DNA and mRNA



Diagrammatic representation

Splicing mechanism

❑**Splicing of primary mRNA transcript** :

a. is a complicated and multistep process.

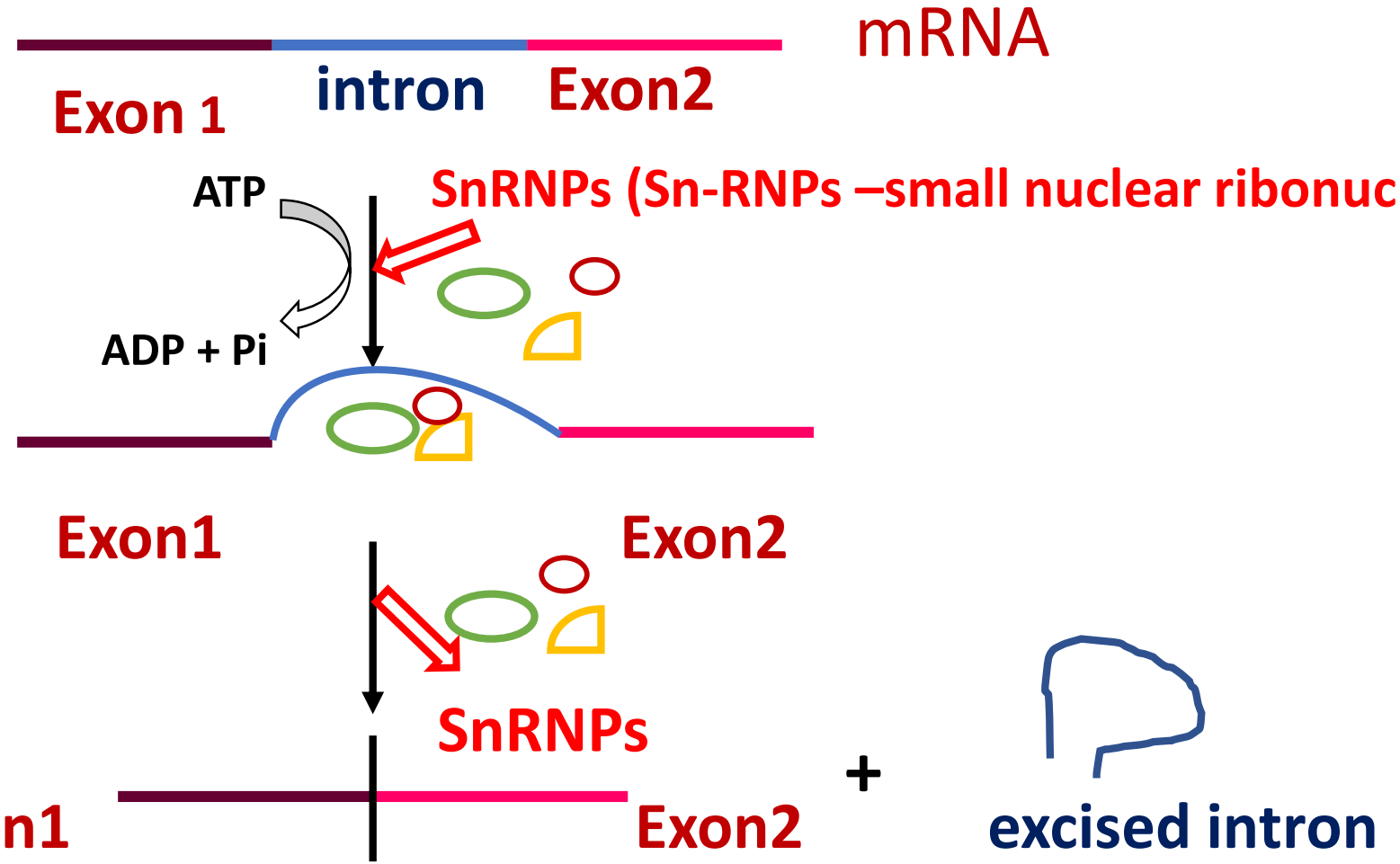
b. Requires several small RNAs (**small nuclear RNAs= snRNA**) and proteins that form a large complex called a **spliceosome** .

c. **small nuclear RNAs** molecules are associated with specific proteins to form complex termed as small nuclear riboproteins particles(**snRNPs**) and known as **snurps .** The binding of **snRNPs** brings the sequences of neighboring exon into the correct alignment for splicing.

❑**Snurps** :

1. rich in **uracil.**
2. identification by numbers preceded by a U(few designated as U1, U2, U4,U5, and U6).
3. involved in the formation of spliceosome.
4. are essential for splicing mRNA precursors.

Formation of mature RNA from eukaryotic mRNA



Small nuclear ribonucleoprotein particles(snRNPs) involved in the splicing of hnRNA

Small nuclear ribonucleoprotein: In association with protein ,Uracil-rich small nuclear RNAs form **small nuclear ribonucleoprotein** particles (**snurps**) designated as U1 U2 etc. that mediate splicing . They facilitate the removal of introns by formation of base pairs with the consensus sequences at each end of the intron and formation of spliceosomes. They all are located in the nucleus.

Small nuclear ribonucleoprotein particles(snRNPs)	Function
U_1	Binds the 5’ splice site and then 3’ splice site
U_2	Binds the branch site of the introns
U_4	Masks the catalytic activity of U6
U_5	Binds the 5’ splice site
U_6	Catalyzes splicing

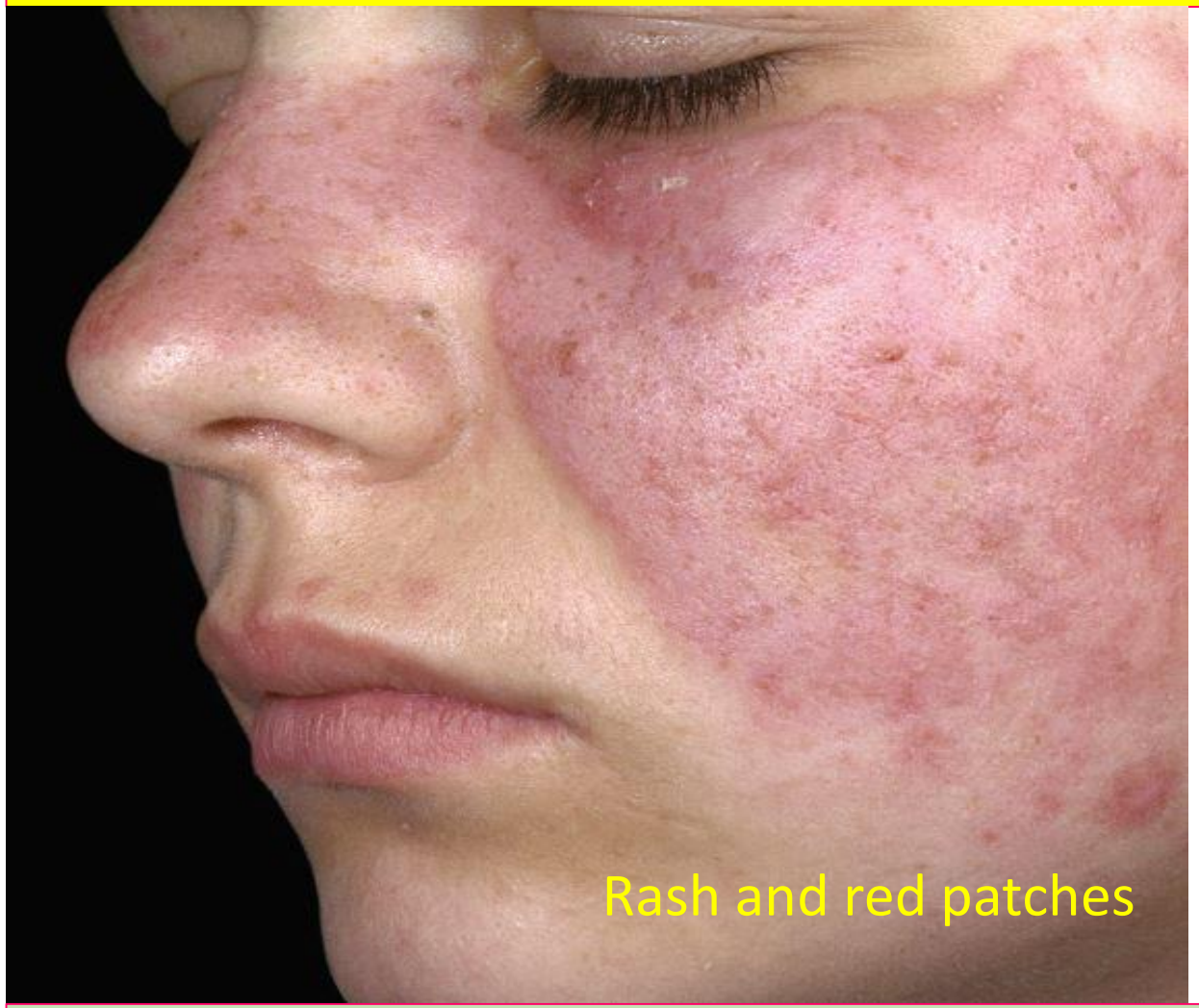
U= Uracil-rich

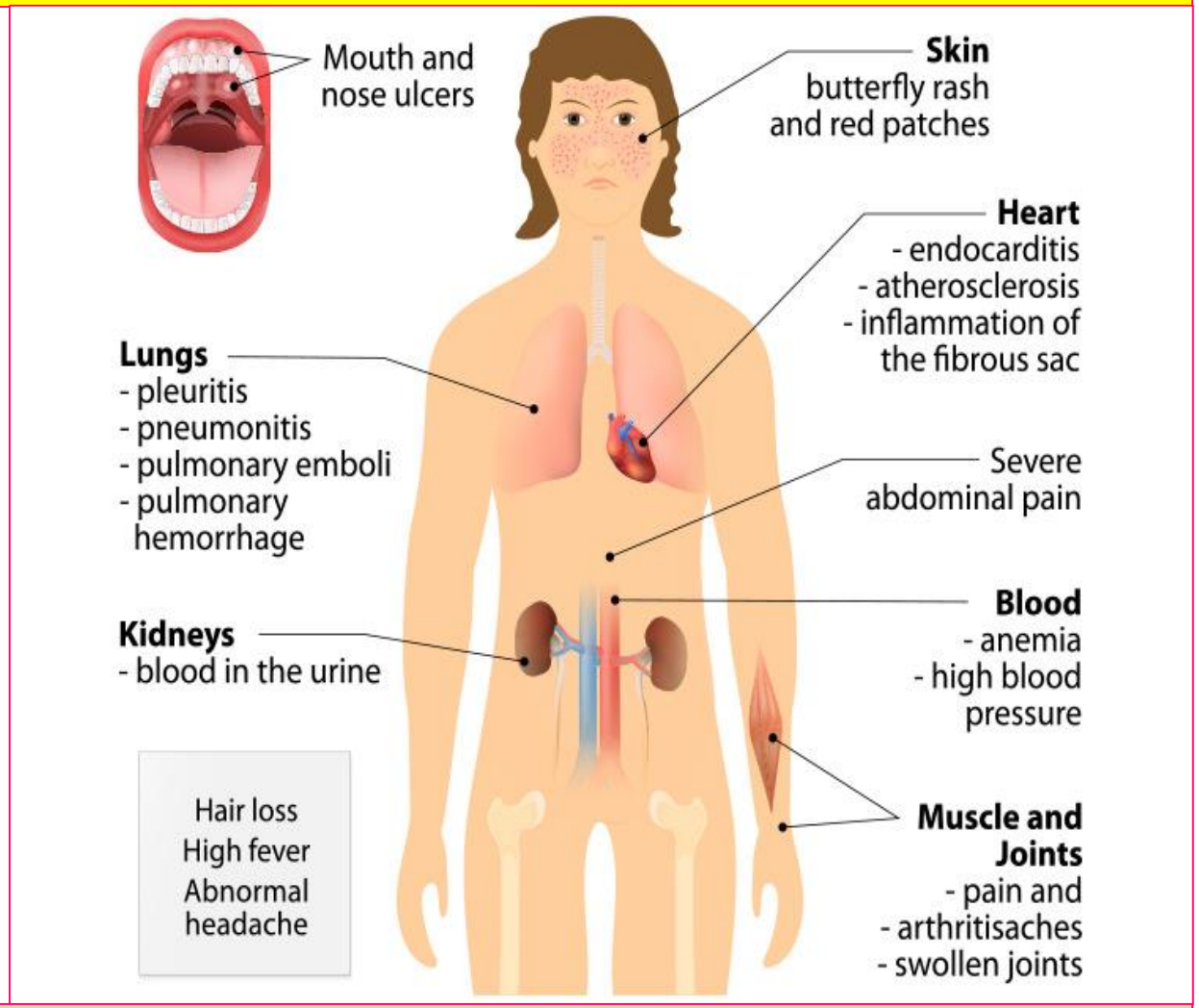
Small nuclear RNAs and Systemic lupus erythematosus:1

- **Systemic lupus erythematosus(SLE):**

1. Fatal inflammatory autoimmune disease
2. Results from an autoimmune response to produce antibodies against own nuclear proteins such as “snurps”(small nuclear RNAs= snRNPs) in patients.

snurps and Systemic lupus erythematosus:2





Systemic lupus erythematosus: Results from an autoimmune response to produce antibodies against own nuclear proteins such as “snurps”(small nuclear RNAs= snRNPs) in patients.

Biochemistry of splicing process in eukaryotes :1

- Splicing starts with the cleavage of the phosphodiester bond between the upstream exon (exon-1) and 5’ end of the intron.
- The phosphate attached to G at the 5’ end of the intron forms a 2’ 5’ phosphodiester bond between 2’ hydroxyl group of the adenine nucleotide residue at branch site of intron and the 5’ terminal phosphate of the intron and cleavage occur at the end of the first exon which continues to be held in place by the spliceosome. This reaction is called **transesterification** . This generates a new 3’ hydroxyl group at 3’ end of exon-1.
- The Adenylate residue is also joined to other nucleotides by normal 3’ , 5’ phosphodiester bonds . Hence , a branch is generated at this site .
- A second cleavage occurs at the 3’ end of the intron after the A G sequence.

The newly formed 3’ hydroxyl terminus of exon 1 attacks the phosphodiester bond between exon 2 and 3’ end of the intron (3’ splice site). This is a second transesterification reaction.

Splicing is thus accomplished by two transesterification reaction rather than by hydrolysis.

1. The first reaction generates a free 3’ –OH group at the end of exon 1 and
2. Second reaction links this group to the 5’ phosphate of exon 2.

Biochemistry of splicing process in eukaryotes :2

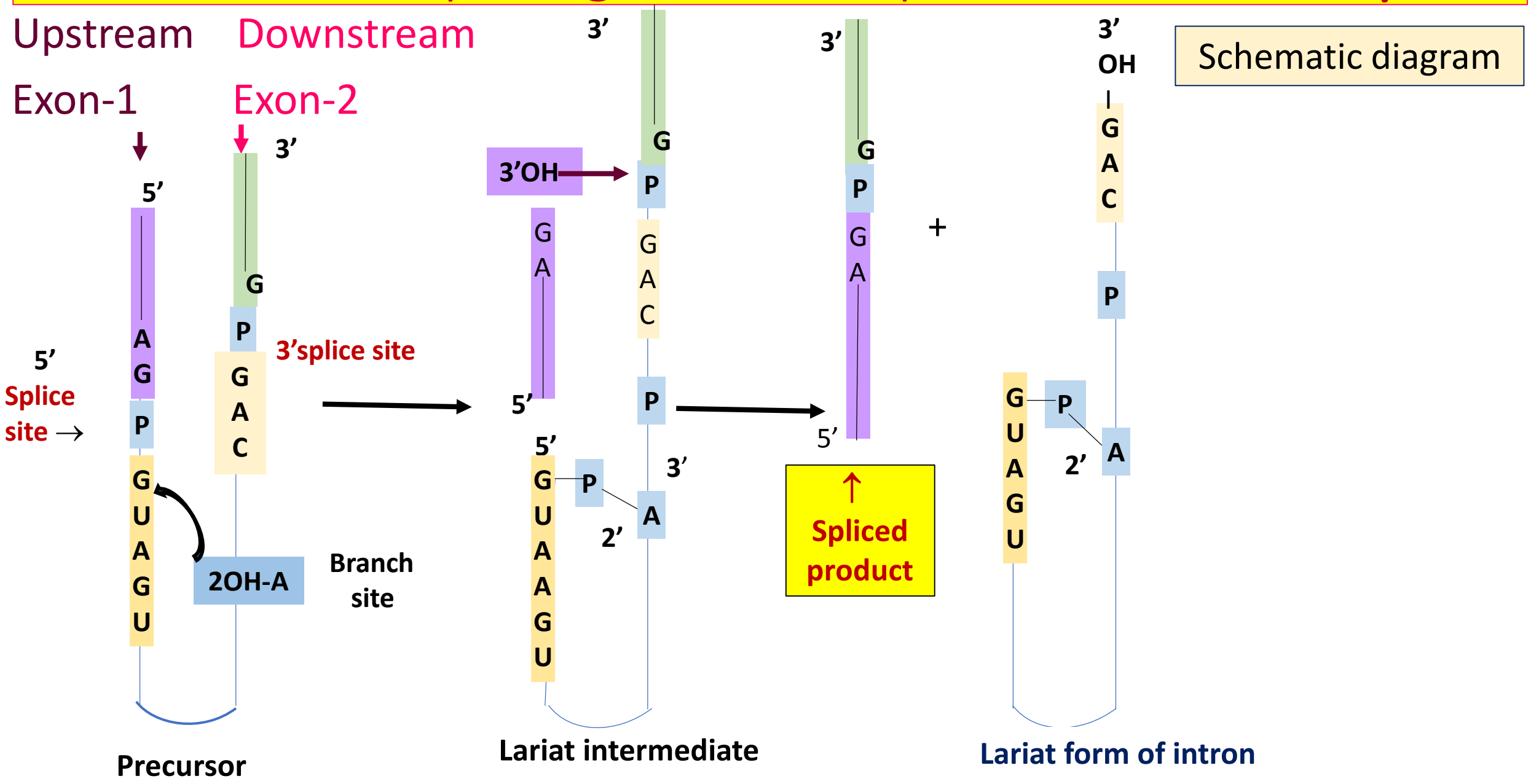
The exons 1 and 2 are joined and the intron is released in the form of a lariat (a rope with noose at one end used for catching cattle).

The number of phosphodiester bonds stays the same during these steps ,which is essential because it allows the splicing reactions itself to proceed without an energy source such as ATP and GTP.

The mature mRNA molecule leave the nucleus and pass into cytosol through pores in nuclear membrane.

The introns of primary transcript of tRNA are removed by different mechanism.

Mechanism of splicing for mRNA precursor in eukaryotes

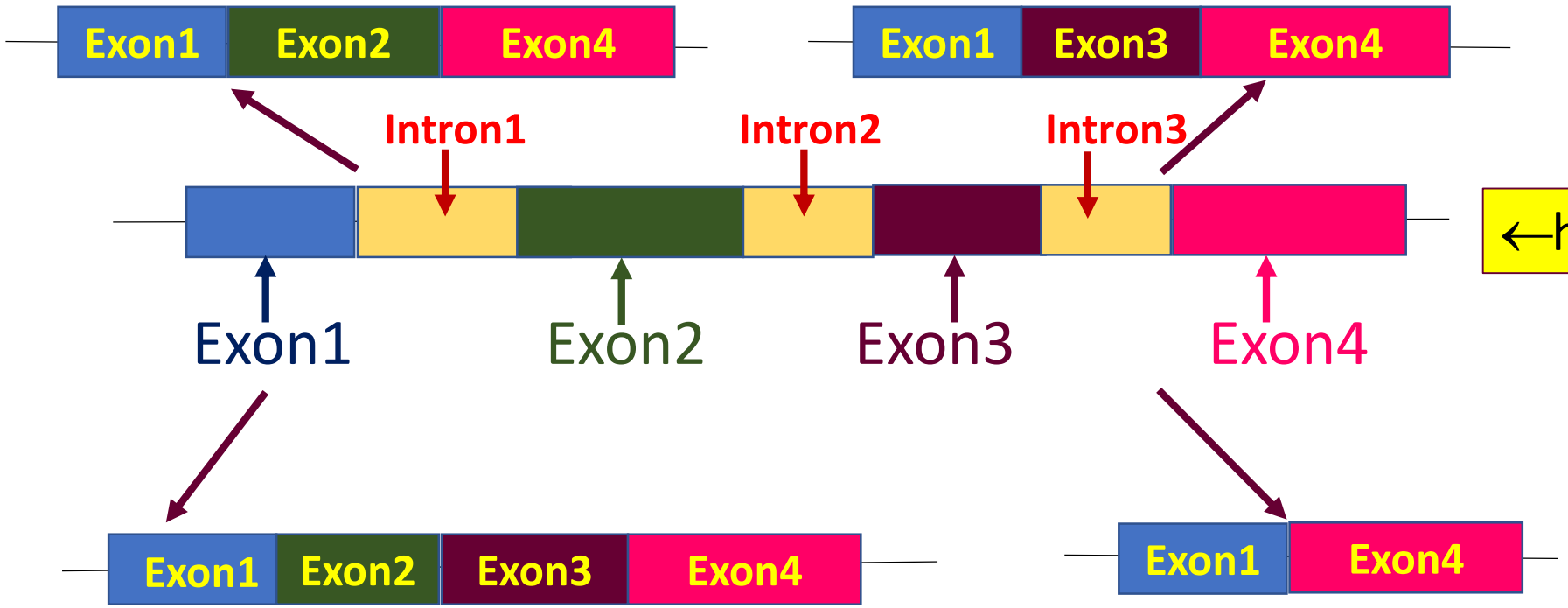


Different mRNAs produced by alternate splicing in eukaryotes

- A hnRNA with multiple exons is sometimes spliced in different ways in different tissues.
- Some hnRNA can be spliced in alternative ways to yield different mRNA molecules which can produce different proteins .
- Alternate splicing results in mRNA heterogenicity and a mechanism for producing divert set of proteins from limited set of genes. In fact, the processing of hnRNA molecules becomes a site for the regulation of gene expression .
- By selecting the exons in a given hnRNA (precursor mRNA) it should be possible to generate different mRNA from the same section of genomic DNA.
- E.g. in eukaryotic cells ,the mRNA for tropomyosin ,an actin filament-binding protein of cytoskeleton undergoes tissue specific alternative splicing with multiple isoforms of the tropomyosin protein.

Alternative splicing results in mRNA heterogenicity in eukaryotes

Schematic diagram



Alternate splicing of **Calcitonin** gene yields an RNA that synthesizes **calcitonin** in **thyroid** and **calcitonin –gene related peptide (CGRP) in brain .**

Alternate splicing in the alpha **Tropomyosin** transcript produces 8 different mRNAs .

Proteins undergo alternative RNA splicing

1. Actin
2. Troponin
3. Tropomyosin
4. Myosin
5. Fibrinogen
6. Calcitonin
7. Alcohol dehydrogenase
8. Aldolase
9. Fibronectin

Clinical importance of splicing in mRNA

❑**Importance of splicing in mRNA :**

1. Provides a mechanism for expanding the versality of genome sequence.
2. One nucleotide slippage in a splice point would **shift the reading frame** on the 3' side of the splice to give an entirely **different amino acid sequence .**
3. Sequence of glucokinase = 10 exons + 9 introns , expression of GK gene is regulated differently in liver and pancreas because of two different promoters in these tissues . Presence of 2 different promoters in these tissue → differential splicing of exons → differential expression of genes in liver and pancreas.
4. **Splicing defects** : are responsible for 15% of all genetic diseases.
5. **Aberrant splicing(splice site mutation)**: Mutation that cause the incorrect splicing of beta globin mRNA responsible for some forms of βthalassemia(defective synthesis of beta chain of hemoglobin).
6. Studies on the mechanism of splice site selection will be crucial to the field of proteomics. Alternate splicing identified in case of at least 40 different genes.

RNA editing

- The sequence in the DNA determines the coding sequence in mRNA and final the amino acid sequence in the protein.
- A change in the base sequence of RNA after transcription by process other than RNA splicing is called **RNA editing**.

❑**RNA editing:**

1. Involves the enzyme mediated alteration of base sequence of RNA in the cell nucleus before translation.it occurs in organelle as a co- or post transcriptional event.
2. Involve the insertion , deletion or substitution of nucleotides in the RNA molecule.
3. Results in the synthesis of a different polypeptide.

➢Substitution of one nucleotide for another has been observed in human and can result in tissue specific differences in transcript e.g. gene of Apoprotein B, Apo B gene.

➢Two Forms of Apoprotein B:

a. 14.1 kbp apo B-100 (4536 amino acid residues)
b. 7.0 kbp apo-B -48 (2152 amino acid residues)

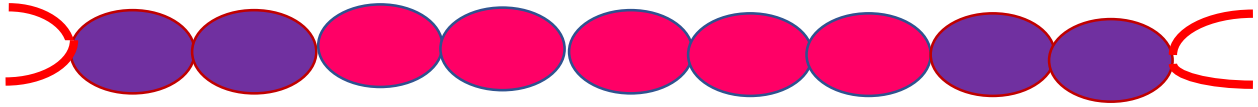
Types of RNA editing

Criteria	Simple editing	Insertional editing	Pan editing	Polyadenylation editing
mechanism	Involve single residue conversion.	Involve insertion of a single nucleotide or small runs of nucleotides such as G insertions during transcription .	Involve insertion/deletion of multiple uridine residues .	In which Pre mRNA lacking a stop codon is polyadenylated with the first one or two adenylate residues providing missing information.
example	Apoprotein mRNA gives rise to truncated protein in small intestine because the codon for **Glutamine CAA** is converted to **stop codon UAA** by C to T transition.	Transcription of the paramyxovirus P gene.		

mRNA editing of Apo B gene

- The sequence in the DNA determines the coding sequence in mRNA, and final the amino acid sequence in the protein.
- Changes in the coding information by editing mRNA have been reported in recent years.
- about $0.01\,\%$ of mRNA undergoes editing .
- e.g. the conversion of CAA codon in mRNA (of apoprotein B gene) to UAA by the enzyme cytidine deaminase . As a result of ,originating from the same gene , the liver synthesizes a 100 kDa protein(apoB 100)while the intestinal cells 48 kDa protein (apoB 48). Thus, codon for **Glutamine** is changed to a **termination codon**.
- This happens due to formation of termination codon UAA from CAA in m-RNA editing.

mRNA editing of Apo B gene



Schematic diagram

ApoB gene

Transcription

CAA (Glutamine codon)

5’ 3’

←Unedited mRNA

RNA editing by cytidine deaminase

NH_4

UAA(stop codon)

Translation in liver

Edited mRNA

ApoB 100 (4536 amino acid residues)

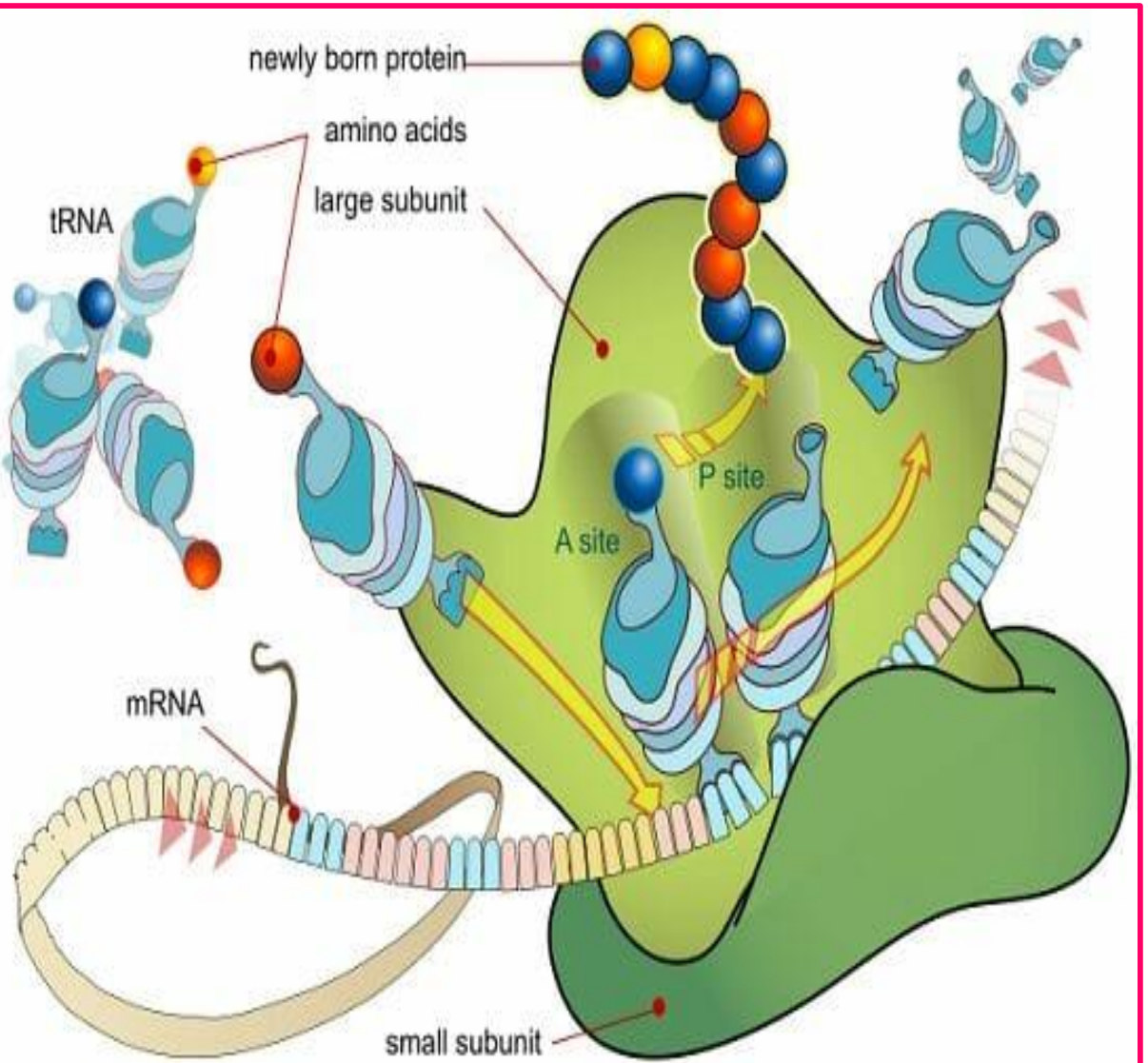
Translation in intestine

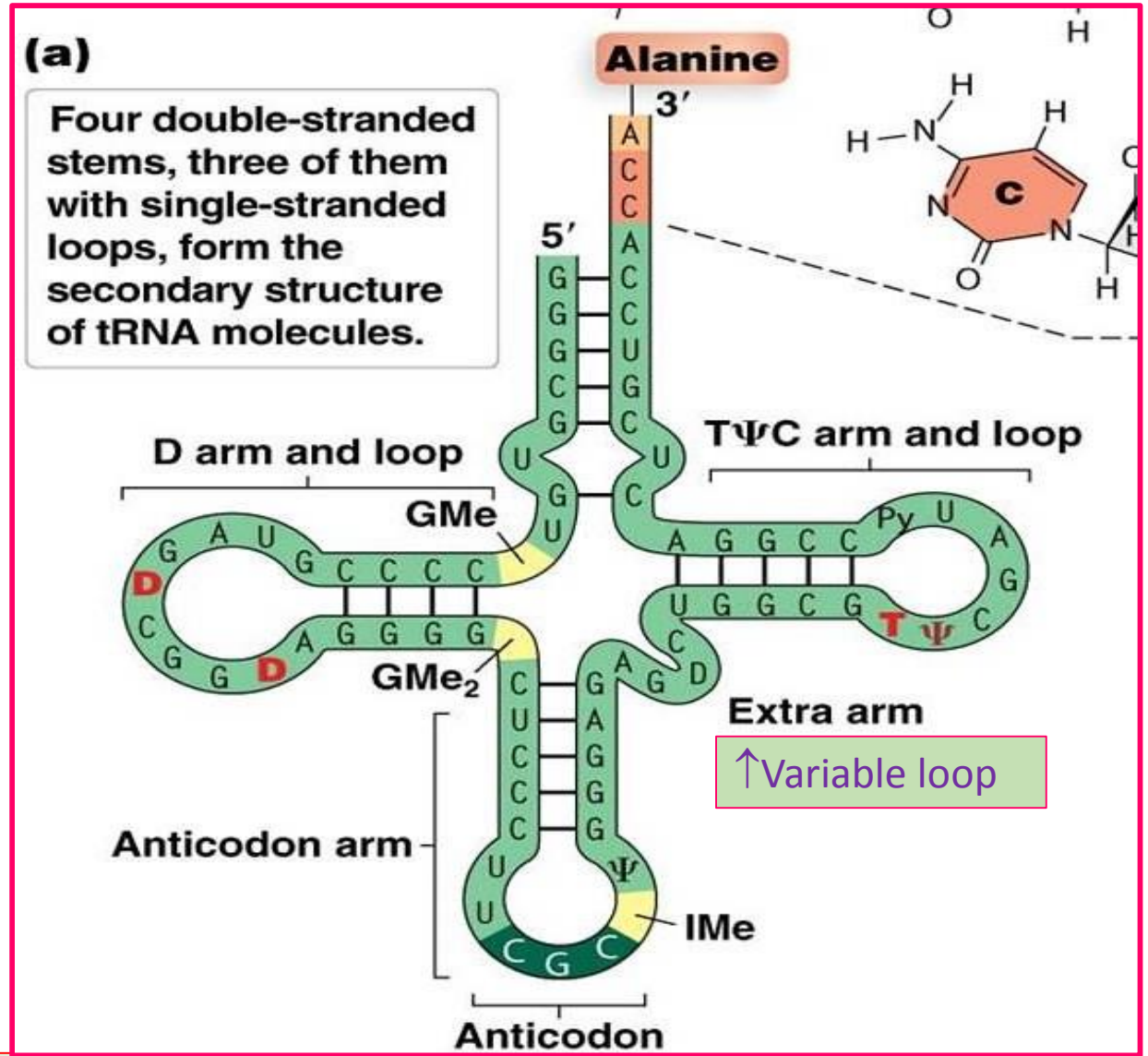
Apo B 48 (2152 amino acid residues)

Apoprotein B

Criteria	Apoprotein B 100	Apoprotein B 48
Organ involved synthesis	liver	Small intestine
Gene for apoprotein B Apo B encodes	14.1 kbp mRNA transcript encodes Apoprotein B- 100	7.0 kbp mRNA transcript encodes Apoprotein B -48
Number of Amino acid residues	4536 amino acid residues	2152 amino acid residues
		A cytidine residue of mRNA is deaminated to uridine which changes the codon at residue 2153 from **CAA** (Glutamine) to **UAA** (**stop codon**). Deamination is catalyzed by deaminase present in the small intestine but not the liver and is expressed only at certain developmental stages.

Structure and function of Transfer RNA(tRNA)





Function of Transfer RNA(tRNA): carrier of amino acids during protein synthesis

Post transcriptional modifications of Eukaryotic Primary transcript of tRNA(Eukaryotic transfer RNA processing)

- All the **Transfer** RNA **(tRNAs)** of prokaryotes and eukaryotes undergo post transcriptional modification of longer precursor molecule . The primary transcript folds into characteristics tRNA structure with stem and loops.
- Post translational modification of primary transcript into mature Eukaryotic tRNAs involve following alterations:

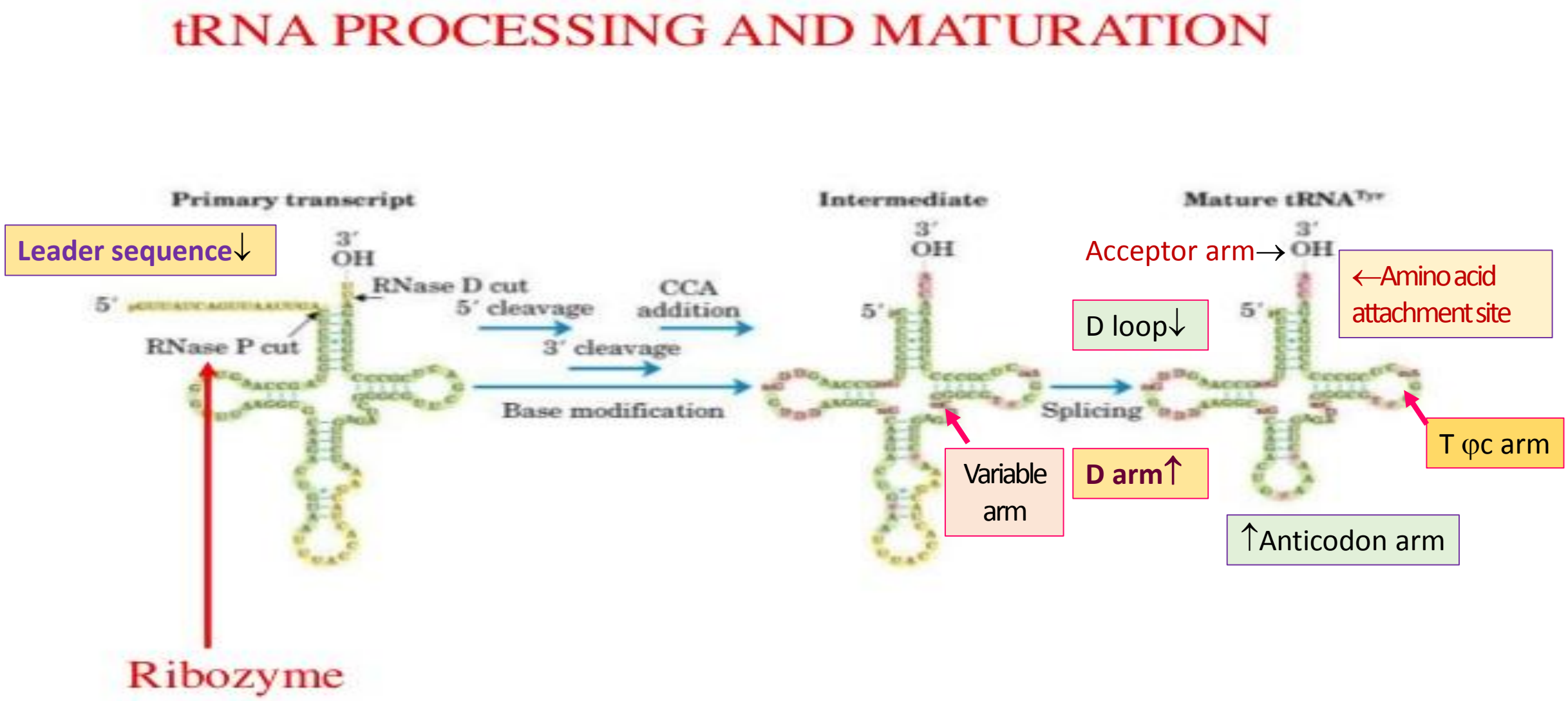
a. Cleavage of a 5' **leader sequence**(trimming**).** 16 nucleotide sequence at the 5' end is cleaved by RNAase P(ribozyme) .

b. Splicing to remove introns i.e. 14 nucleotide introns in anticodon loop is removed by nucleases .Splicing Exons.

c. Replacement of the 3' terminal UU by CCA(addition of CCA nucleotides to 3' terminal end of tRNAs by nucleotide transferse) i.e. Uracil residues at the 3' end replaced by the CCA sequence found in all mature tRNAs.

d. Modification of several bases(converting the existing bases into unusual ones)i.e. many bases are converted to characteristic modified bases of tRNA .

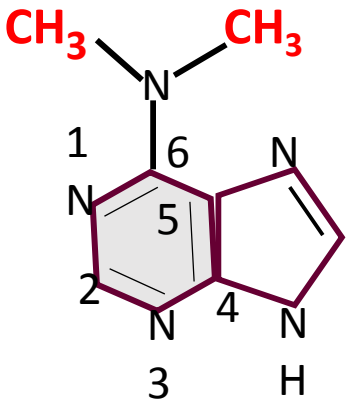
Processing of **Eukaryotic** tRNA precursor to mature tRNA



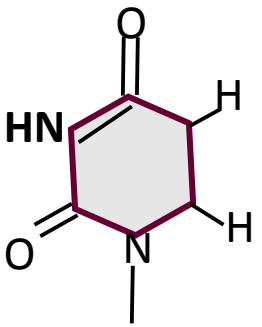
RNA can act as an Enzyme and catalyse reactions including its own replication

Alterations involved in Post translational modification of primary transcript into mature tRNA s : Cleavage of a 5’ leader sequence ,Splicing to remove introns , Replacement of the 3’ terminal UU by CCA and Modification of several bases

RNA processing by nucleotide modification

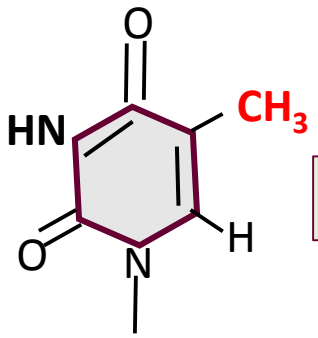


←Dimethyl adenosine (methylated base) in prokaryotes



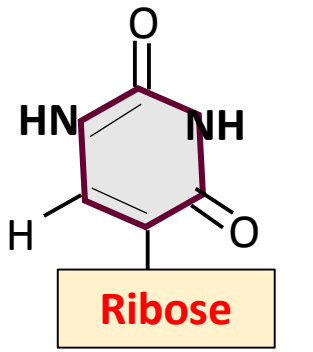
Ribose

Uridylate



Ribose

←Ribothymidylate



Ribose

←Pseudo uridylate

Genetic code

- The information needed to direct the synthesis of protein is contained in the mRNA in the form of a Genetic code.
- The Genetic code is the system of nucleotide sequences of mRNA that designates particular amino acid sequences in the process of translation .
- **Codons** : are a group of three adjacent bases that specify the amino acids of protein (the genetic code is the relation between the sequences of bases in DNA and the sequences of amino acids in protein).

Relation between the Genetic code(sequences of bases in DNA) and the sequences of amino acids in protein

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC		UCC		UAC		UGC	
UUA	Leu	UCA		UAA	Stop	UGA	Stop
UUG		UCG		UAG		UGG	Trp
CUU		CCU	Pro	CAU	His	CGU	Arg
CUC		CCC		CAC		CGC	
CUA		CCA		CAA	Gln	CGA	
CUG		CCG		CAG		CGG	
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUA		ACC		AAC		AGC	
AUC		ACA		AAA	Lys	AGA	Arg
AUG	Met/Start	ACG		AAG		AGG	
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUA		GCC		GAC		GGC	
GUC		GCA		GAA	Glu	GGA	
GUG		GCG		GAG		GGG	

The Genetic code is the system of nucleotide sequences of mRNA that designates particular amino acid sequences in the process of translation i.e. the genetic code is the relation between the sequences of bases in DNA and the sequences of amino acids in protein.

Genetic code

1st letter	Second Letter: U		Second Letter: C		Second Letter: A		Second Letter: G		3rd letter
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Characteristics of genetic code:1

1. Total Number of possible codons : 64(4 nucleotide bases A,G, C and U used to produce the three base codon therefore 4^3 or 64 different combinations of bases possible codon sequences.
2. **Stop** or **termination** or **nonsense codons** :three (UAA, UAG, and UGA) do not code for any amino acids and normally signal termination of polypeptide chains. These are arbitrarily named **Amber , Ochre** and **Opal.**
3. **Code** is **degenerate** but **unambiguous:** there are 61 codons for 20 amino acids , one amino acid has more than one codon and the code is referred to as degenerate indicating that there are redundancies , i.e. although an amino acid may have more than one codon , each codon specifies only one amino acid . Thus genetic code is unambiguous . Degeneracy minimizes the deleterious effects of mutations.

Characteristics of genetic code:2

4. Codons that designate the **same amino acids** are called **synonyms**. E.g. UUU and UUC code for Phenylalanine .
5. Two amino acids **Methionine (AUG) and Tryptophan(UGC)** have only one codon.
6. Remaining amino acids have multiple codons e.g. specific six different codons **of Arginine CGU, CGC,CGA,CGC ,AGA and AGG.**
7. **Universal nature of codon :** each codon is the same in almost all known organisms . Exception genetic code found in human mitochondria e.g. UGA codes for Tryptophan instead of serving as a stop codon . AUA codes for Methionine instead of Isoleucine and CUA codes for threonine instead of Leucine.
8. Code is **non-overlapping** and **without punctuation** : code is read sequentially without spacer bases , from a starting point as a continuous sequence of bases , taken 3 at a time. E.g. AUG/CUA/GAC/UUU read as AUGCUAGACUUU without punctuation between the codons.

Exceptional genetic codons found in human mitochondria		
Codon	**Codes for all organisms**	**Human mitochondria**
UGA	stop codon	Tryptophan
AUA	Isoleucine	Methionine
CUA	Leucine	Threonine

Wobble hypothesis

- The genetic code assumes that each codon base pairs in antiparallel fashion with the anticodon of the tRNAs that are specific for the amino acid corresponding to the code word.
- The first two bases of these codons are the same , whereas the third is different “ wobble ”.
- Wobble allows some tRNAs to recognizes more than one codon.
- The non-standard base pairing occurs in the third position of the codon, the position that has the least effect on specifying a particular amino acid.

Rules for Wobble hypothesis codon-anticodon interactions:1

❑Rules of **Wobble hypothesis proposed by Crick :**

1. The first two bases of a codon pair in the standard way i.e. always form strong Watson Crick base pairs with corresponding bases of the anticodon and confer most of the coding specificity .
2. For a given amino acid codons that differ in either of the first two bases must be recognized by different t-RNAs .e.g. different t-RNAs for codes for UUA and CUA , both coding for Leucine.
3. The first base of an anticodon (reading in the 5'→ 3' direction) determines whether a particular tRNA reads more than one codon for given amino acid:

Rules for Wobble hypothesis codon-anticodon interactions:2

❑Four rules of **Wobble hypothesis proposed by Crick :**

- When the first base of the anticodon is C or A it can read only one codon.
- When it is U or G , it can read two different codons.
- When the wobble base of an anticodon is I (Inosine) or certain other modified bases , it can read three different codons.
- Thus , part of the degeneracy of the genetic code arises from wobble (imprecision) in the pairing of third base of the codon . That is the reason , why there is frequent appearance of inosine , one of the unusual nucleotides in anticodons.

4. It is not necessary to have 61 different types of tRNA to read all 61 possible code words. A minimum of 32 tRNA is required to translate all 61 different codons for the amino acids .

Role of the bases of an anticodon(5'→3')in wobble hypothesis

Criteria	can read
First base of the anticodon is C or A	One codon
First base of the anticodon is U or G	two different codons
Wobble base of an anticodon is I (Inosine) or modified bases	Three different codons

Inhibitors of transcription

- The synthesis of RNA is inhibited by certain antibiotics and toxins . Some bind to DNA and other to RNA polymerase .Antibiotics (serve as therapeutic drug) inhibit RNA synthesis in prokaryotes but not in eukaryotes.
- **Actinomycin D (Dactinomycin):** It is synthesized by Streptomyces . It binds specifically and tightly with double stranded DNA and thereby prevents it from being an effective DNA template stand for transcription. Thus ,it blocks the movement of RNA polymerase needed for RNA synthesis . It is extensively used as an inhibitor of transcription in both prokaryotes and eukaryotes without affecting DNA replication or translation. It inhibits the growth of rapidly dividing cells makes it an effective therapeutic agents for cancer .It is the first antibiotic used in treatment of cancer.
- **Rifampin** : Rifampin binds to the beta subunit of prokaryotic RNA polymerase and inhibits its activity. Thus it inhibits the initiation of transcription. It has no effect on eukaryotic nuclear RNA polymerase . It is widely used in treatment of tuberculosis and leprosy.
- α -**Amanitin**: toxin produced by the mushroom (**Amanita phalloides** is **delicious** in taste but **poisonous= death cap**) . It binds with RNA polymerase II of eukaryotes and inhibits transcription (hence protein synthesis).

Comparison of Inhibitors of transcription

Criteria	Actinomycin D (Dactinomycin)	Rifampin	α- Amanitin
synthesized by(Source)	Streptomyces	synthetic	**Amanita phalloides** is **delicious** in taste but **poisonous mushroom.**
Mechanism of action	It binds specifically and tightly with double stranded DNA and thereby prevents it from being an effective DNA template stand. Thus ,it blocks the movement of RNA polymerase needed for RNA synthesis .	binds to the beta subunit of prokaryotic RNA polymerase and inhibits its activity. It has no effect on eukaryotic RNA polymerase .	It binds with RNA polymerase II of eukaryotes and inhibits transcription.
Therapeutic use	inhibits the growth of rapidly dividing cells makes it an effective therapeutic agent for cancer .It is the first antibiotic used in treatment of cancer.	widely used in treatment of tuberculosis and leprosy.	Antibiotic

Actinomycin D as an Inhibitor of transcription

❑**Actinomycin** D(**Dactinomycin**):

- **Source** : synthesized by Streptomyces .
- **Mechanism of action :** It binds specifically and tightly with double stranded DNA(intercalates DNA) and thereby prevents it from being an effective DNA template stand. Thus, it blocks the movement of RNA polymerase needed for RNA synthesis .
- **Therapeutic use**: It is extensively used as an inhibitor of transcription in both prokaryotes and eukaryotes without affecting DNA replication or translation.

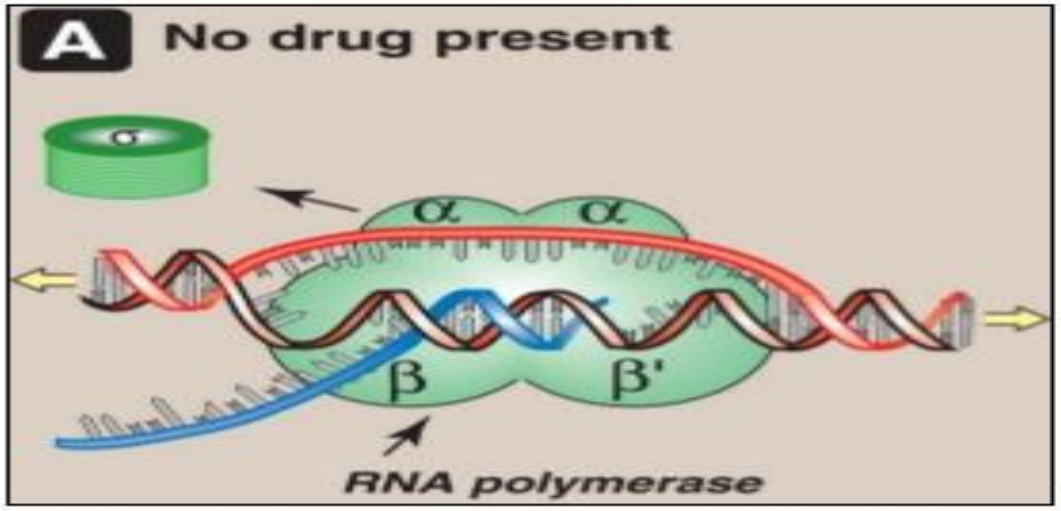
It inhibits the growth of rapidly dividing cells makes it an effective therapeutic agent . It is the first antibiotic used in treatment of cancer.

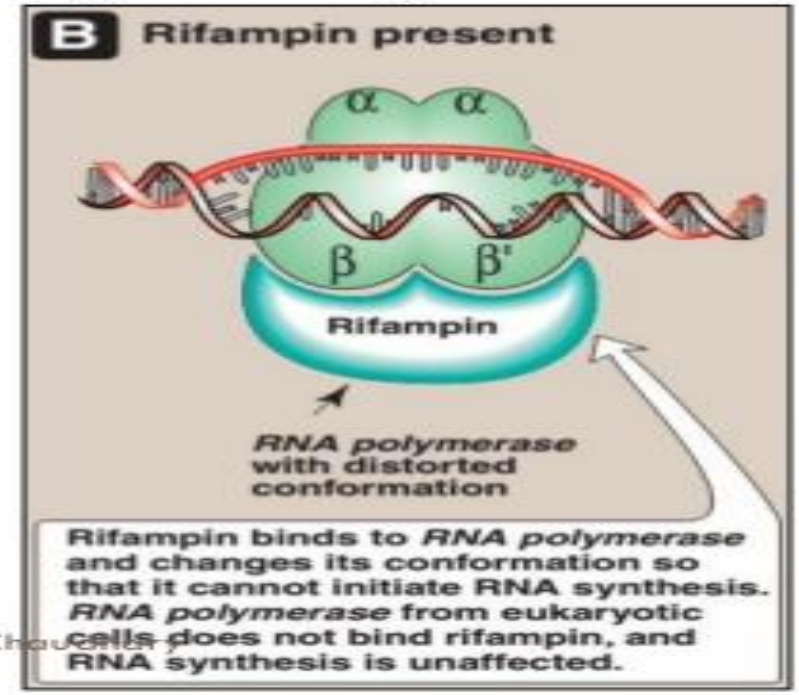
Rifampin as an Inhibitor of transcription

Action of antibiotics

21

- Antibiotics prevent bacterial growth by inhibiting RNA synthesis.
  - E.g.: rifampin, Actinomycin D





Rajesh Chaudhary

Rifampin : used in treatment of tuberculosis and leprosy. Rifampin binds to the beta subunit of prokaryotic RNA polymerase and inhibits its activity. It has no effect on eukaryotic RNA polymerase .

α-Amanitin as an Inhibitor of transcription

- **Source** : toxin produced by **Amanita phalloides** which is **delicious** in taste but **poisonous** mushroom .
- **Mechanism of action** : It binds with RNA polymerase II of eukaryotes and inhibits transcription.
- **Therapeutic use**: effective antibiotic

3’-detoxyadenosine as an Inhibitor of transcription

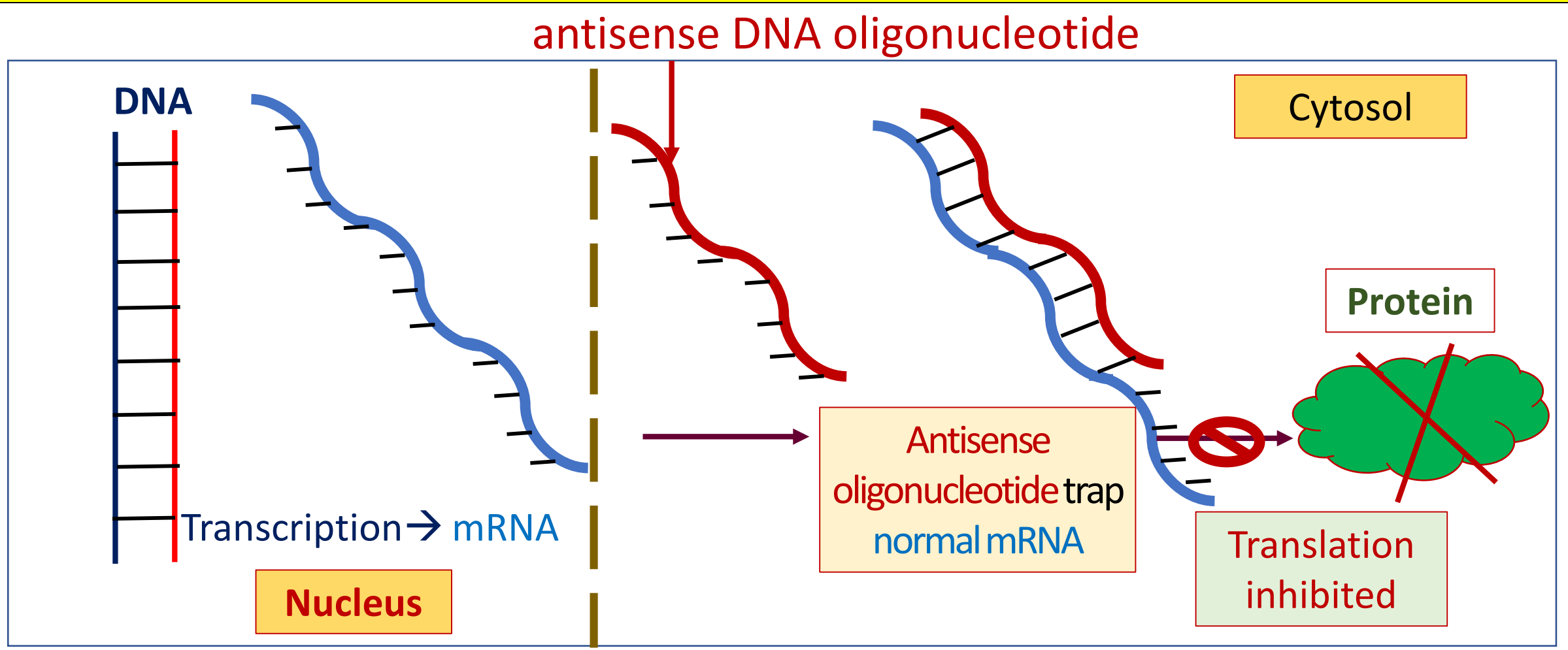
❑3’-detoxyadenosine as an Inhibitor of transcription :

- **Source : a** synthetic analog
- **Mechanism of action :** incorrect entry into chain causing chain termination

Principle of Antisense therapy:1

- The mRNA contains a message/information or sense to be translated into protein.
- If nucleotide having complementary sequence to mRNA is made , it is said to be antisense.
- When oligonucleotide (either RNA or DNA) is added ,it will trap the normal mRNA and so protein synthesis is inhibited . This is said **antisense strategy.**
- **Small oligonucleotides about 7 to 10 nucleotides in length can act as antisense molecules. The antisense nucleotides are delivered into cells by liposome encapsulation.**
- **Clinical are trials on cancer and HIV are being conducted using antisense molecule.**

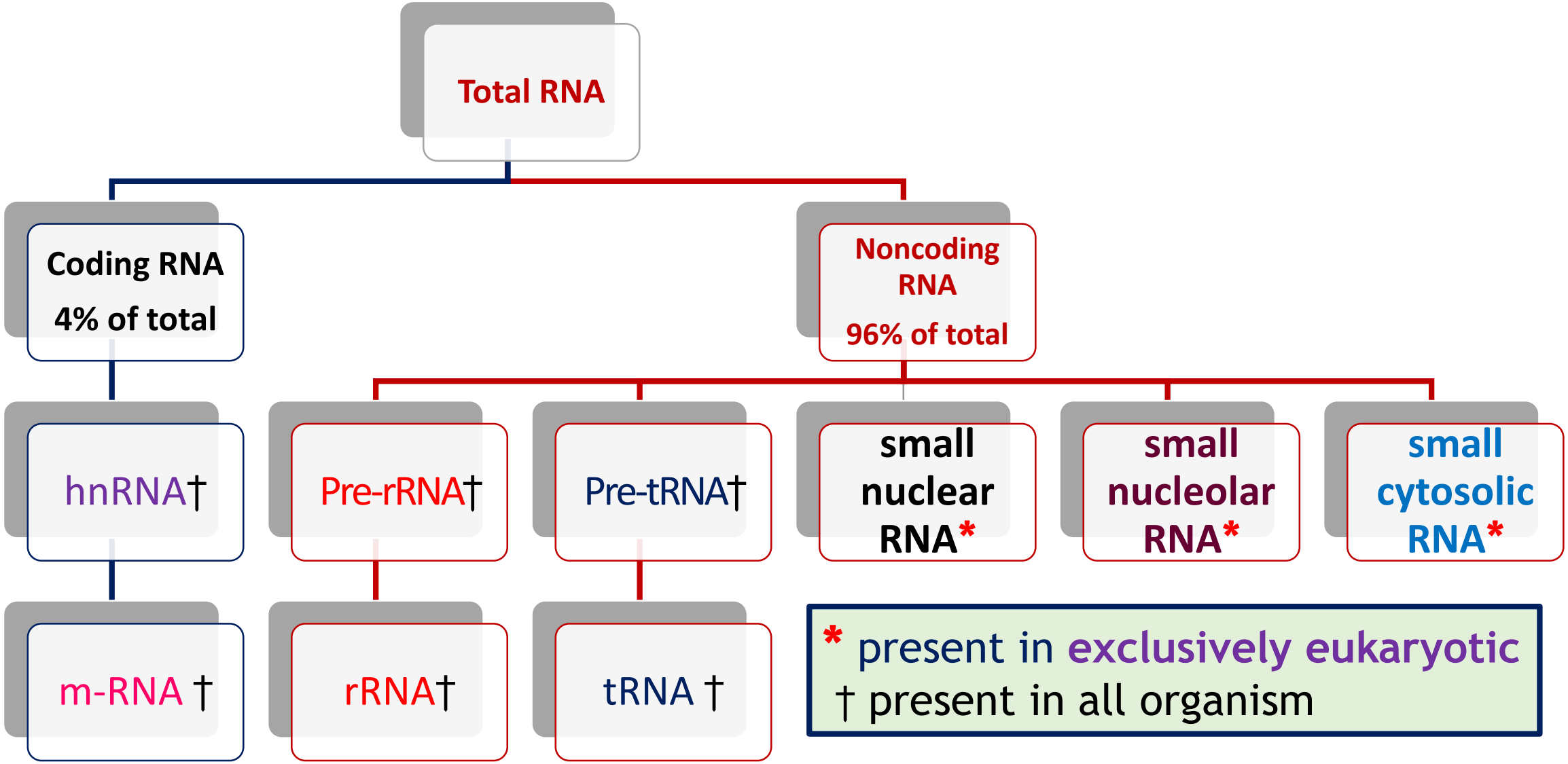
Principle of Antisense therapy:2



Cellular RNA contents:1

- A typical bacterium contains 0.05- 0.10pg of RNA which contributes to about 6% of the total cell weight .
- A mammalian cell ,being larger in size contains 20- 30pg of RNA which contributes to about 1% of the total cell weight .
- Transcriptome ,represents the RNA derived from protein coding genes actually constitutes only 4 %, while 96% is the non-coding RNA.
- **The non-coding RNAs:** ribosomal RNA, transfer RNA, small nuclear RNA ,small nucleolar RNA and small cytosolic RNA.

Cellular RNA contents:2



Cellular RNAs and their functions

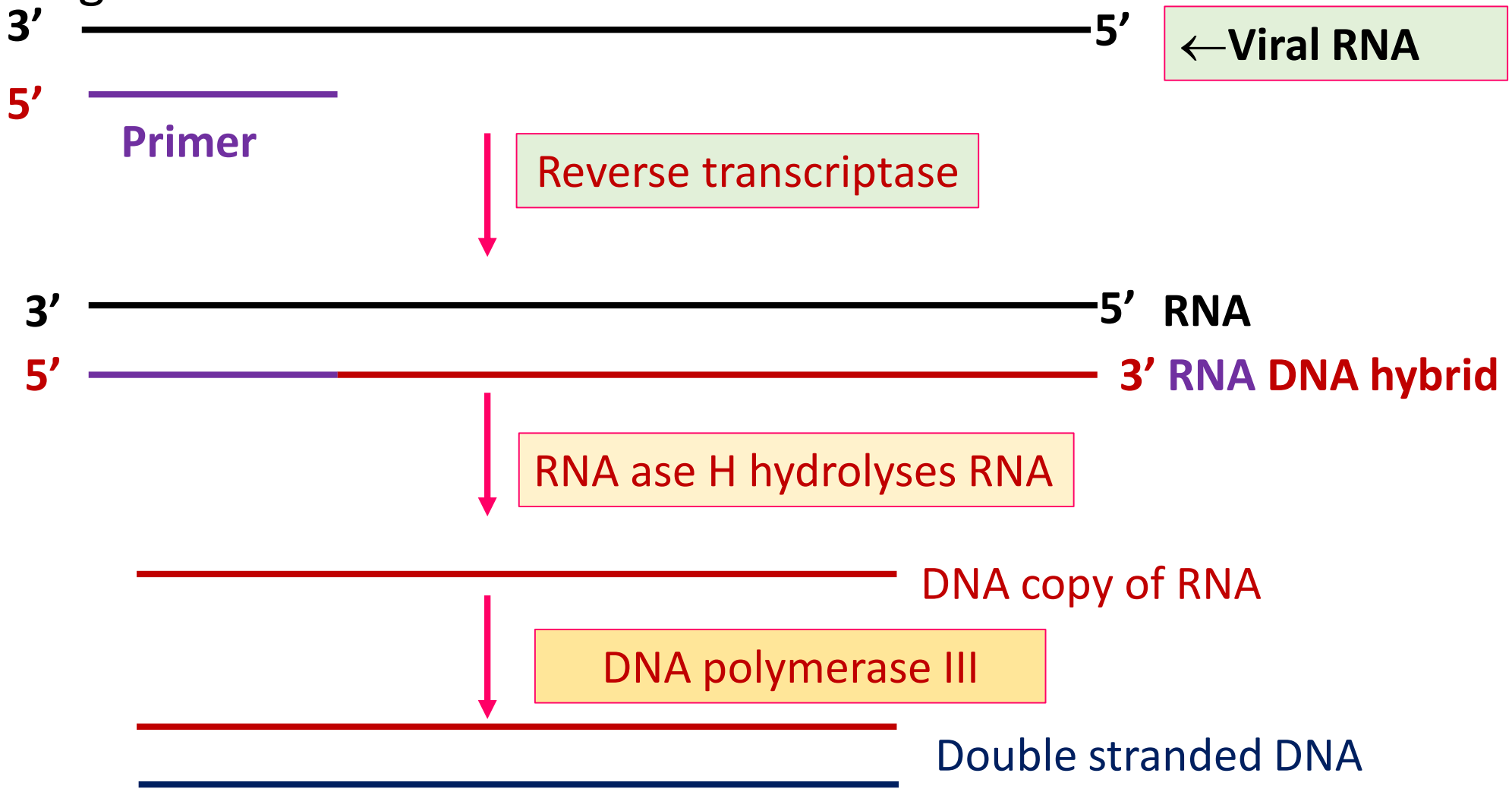
Type of RNA	Abbreviations	Functions
Heterogenous nuclear RNA	hnRNA	Serves as a precursor for mRNA and other RNAs.
Messenger RNA	mRNA	Transfer genetic information from genes to ribosomes to synthesize proteins.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes.
Transfer RNA	tRNA	Transfers amino acid to mRNA for protein biosynthesis.
Small nuclear RNA	snRNA	Involved in mRNA processing.
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export .
Transfer-messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

Reverse Transcription

- **Transcription** : transfer of genetic information from gene on DNA to mRNA by DNA dependent RNA polymerase .
- However ,genetic material of some plant and viruses is made of RNA.
- Some of the viruses known as **retroviruses** possess **RNA** as the genetic material and causative agents of **AIDS** .
- **Reverse Transcription:** transfer of genetic information from RNA to DNA by RNA dependent DNA polymerase (reverse transcriptase).
- **Tumor Retroviruses : oncogenic** cause cancer in animals and found in the transformed cells of the tumors .
- **RNA dependent DNA polymerase = reverse transcriptase:** responsible for the **formation of a new DNA from RNA**. This DNA is complimentary(cDNA) to viral RNA and can be transmitted into host DNA.
- Temin and Baltimore (Noble1970) isolated enzyme isolated **reverse transcriptase.**

Reverse transcription in RNA retrovirus

Schematic diagram



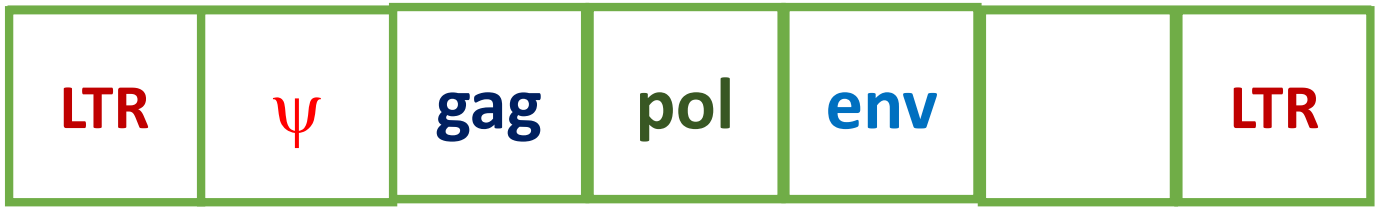
Howard Temin and David Baltimore (Noble Prize 1975) isolated enzyme **Reverse transcriptase.**

Three genes of Retroviruses

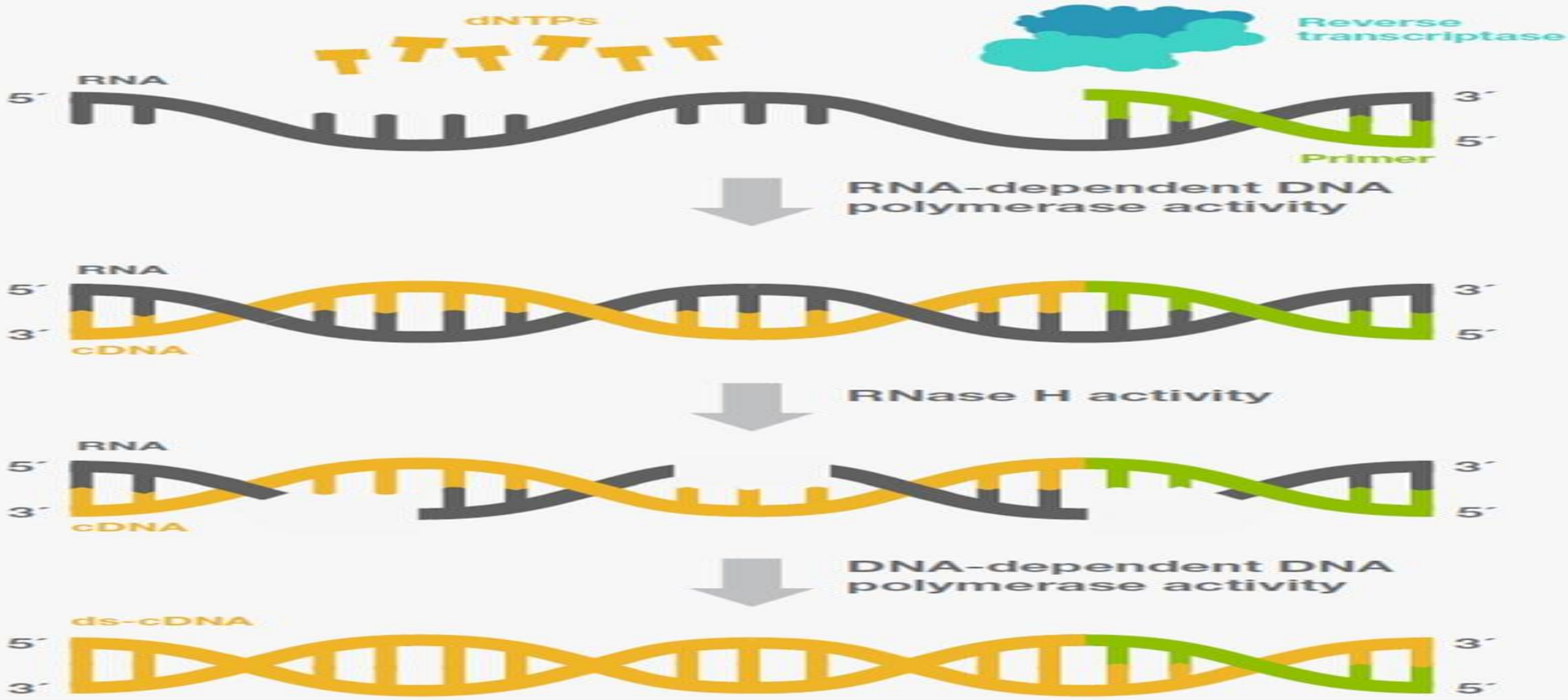
❖**Retroviruses contain three genes :**

1. **gag** : that encodes proteins that form the core of protein particles.
2. **pol** : that encodes **reverse transcriptase and integrase**
3. **env :** that encodes viral envelop proteins.
4. These genes are flanked by long terminal repeats (**LTR**) that help in viral integration into host genome.
5. The RNA genome in Retroviruses replicate via a double stranded DNA intermediate that gets integrated in host cell genome.

The Retroviral genome



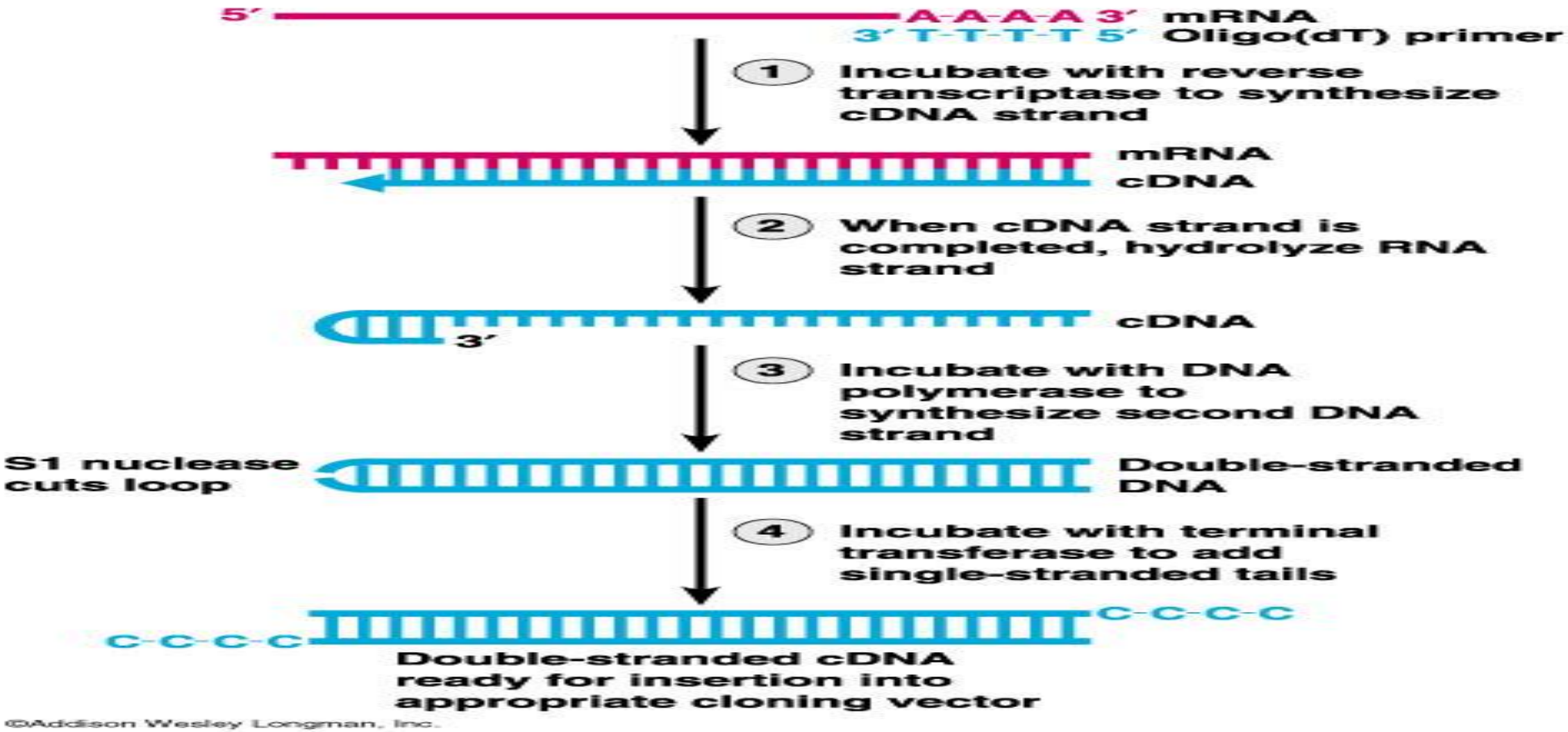
Reverse Transcription



Synthesis of complimentary(cDNA) from mRNA:1

- The DNA expresses the genetic information in the form of RNA.
- **The mRNA** serves as a template for synthesis of double-stranded **complimentary(cDNA)** by using **reverse transcriptase and** determines the amino acid sequence in a protein.
- **Use of Complimentary(cDNA):** used as a probe to identify the sequence of DNA in genes .

Synthesis of complimentary(cDNA) from mRNA:2



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