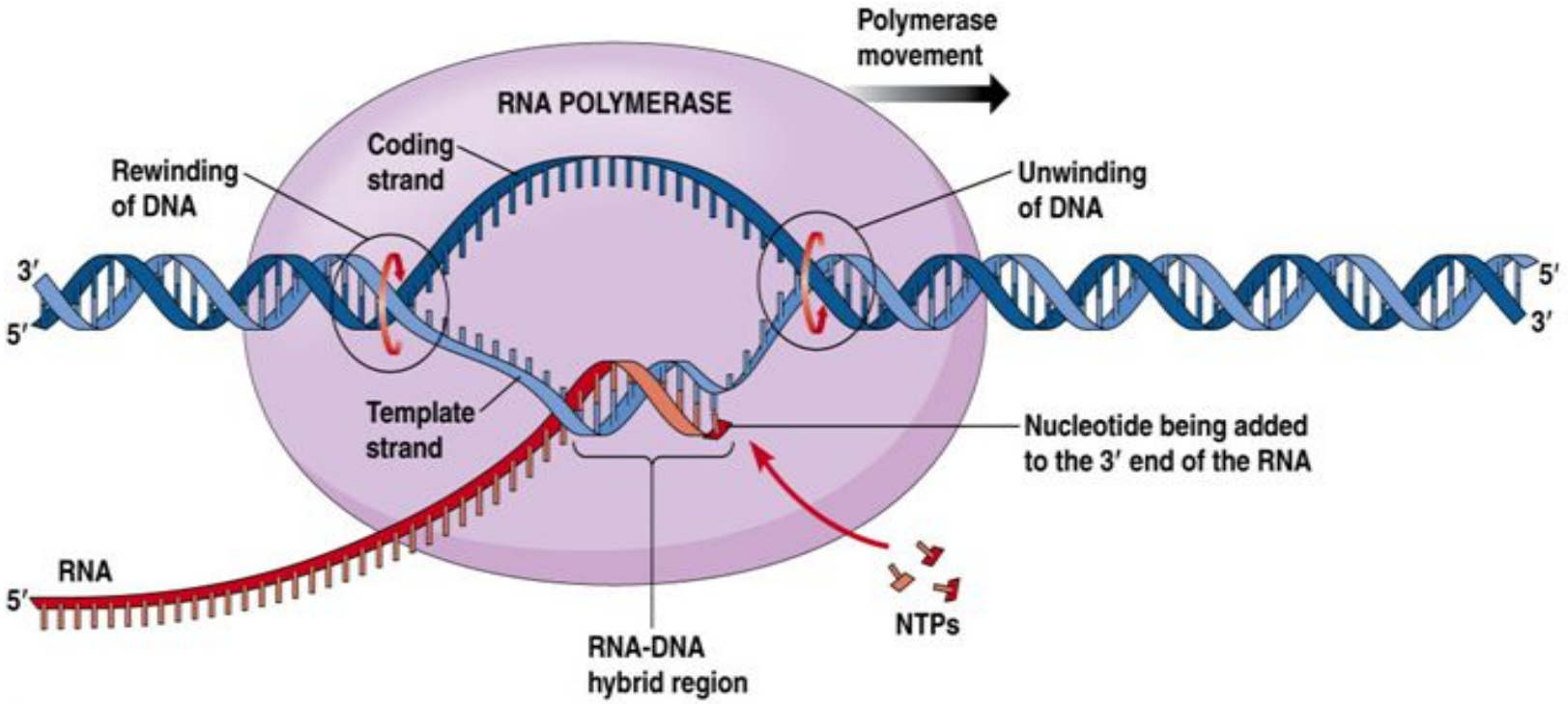
Bhagalpur National College,

(A Constituent unit of Tilka Manjhi Bhagalpur University, Bhagalpur)

PPT Presentation for B.Sc. III- Transcription



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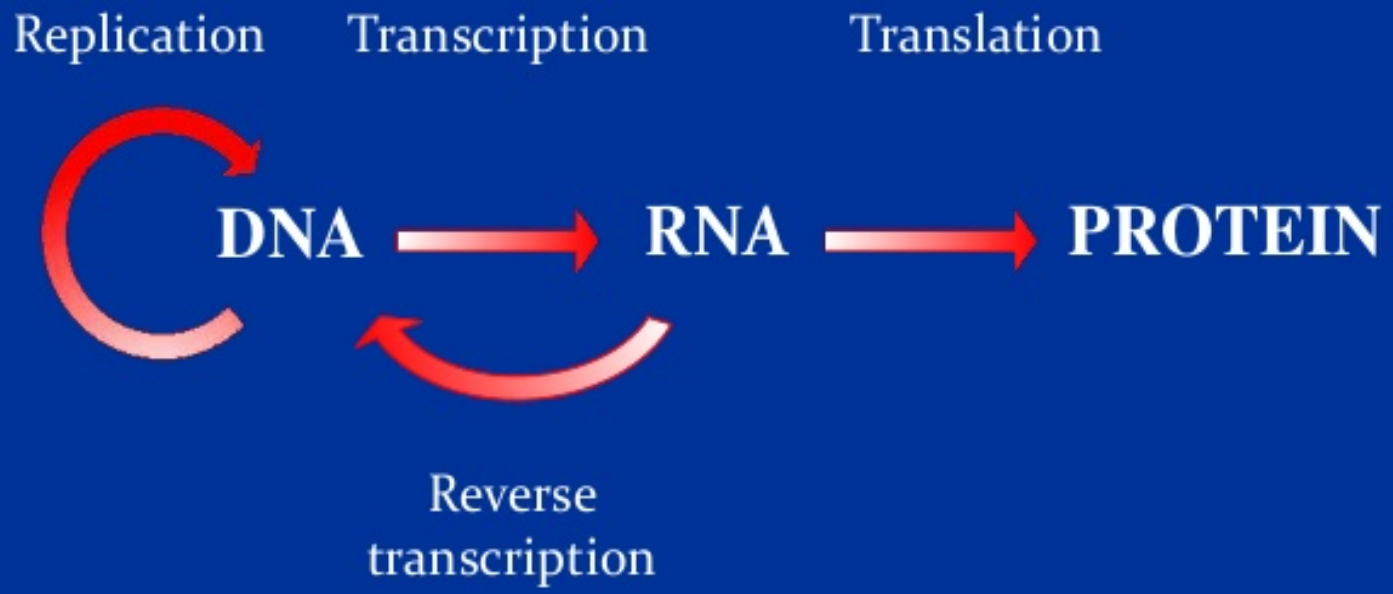
Central dogma



Transcription

The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.

Similarity between replication and transcription

- Both processes use DNA as the template.
- Phosphodiester bonds are formed in both cases.
- Both synthesis directions are from 5´ to 3´.

Differences between replication and transcription

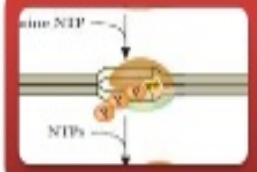
	replication	transcription
template	double strands	single strand
substrate	dNTP	NTP
primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
product	dsDNA	ssRNA
base pair	A-T, G-C	A-U, T-A, G-C

Features of transcription

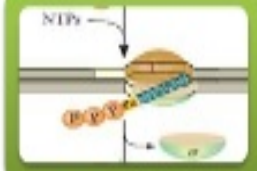
- **1)** It is highly selective.
- This selectivity is due to signals embedded in the nucleotide sequence of DNA.
- Specific sequences mark the beginning and end of the DNA segment which is to be transcribed.
- This signals instruct the enzyme

where to start & stop the transcription
when to start,
how often to start .

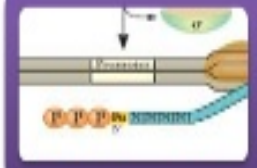
Steps involved in Transcription



INITIATION



ELONGATION

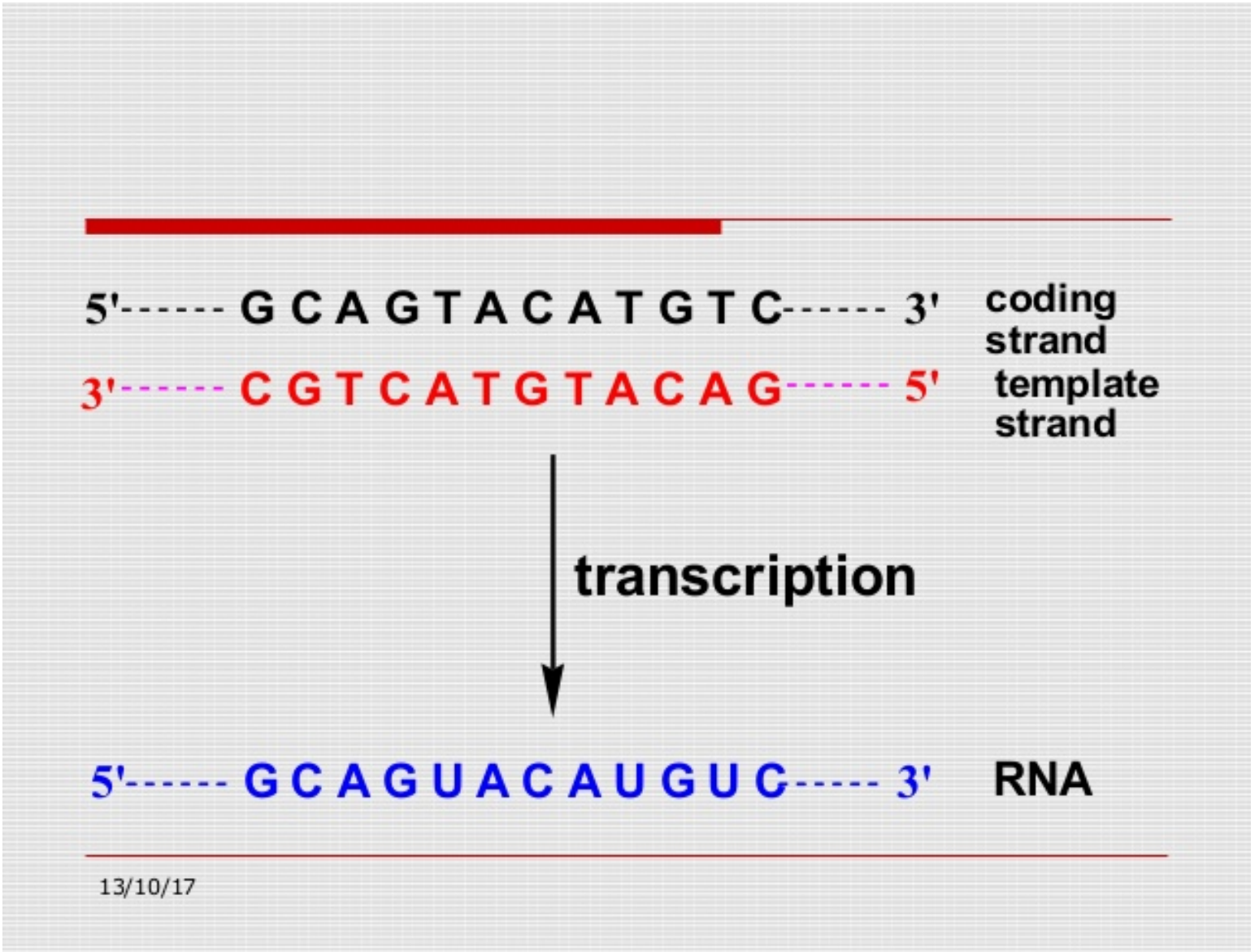


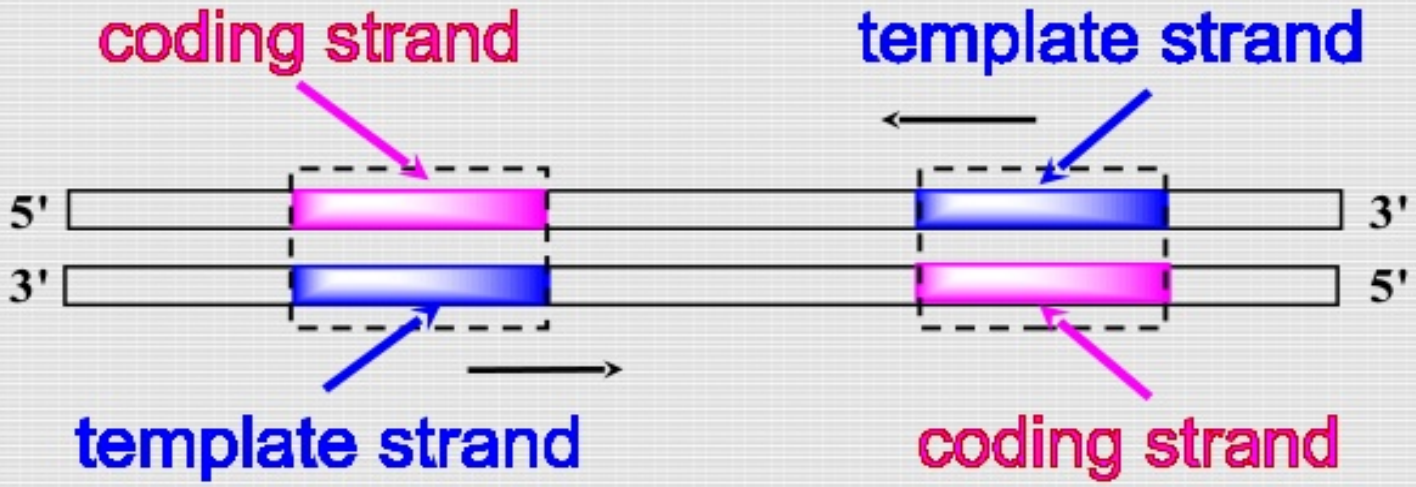
TERMINATION & Release

Template

The template strand is the strand from which the RNA is actually transcribed. It is also termed as **antisense strand**.

The coding strand is the strand whose base sequence specifies the amino acid sequence of the encoded protein. Therefore, it is also called as **sense strand**.


5'------ G C A G T A C A T G T C------ 3'
coding strand
3'------ C G T C A T G T A C A G------ 5'
template strand
transcription
5'------ G C A G U A C A U G U C----- 3'
RNA

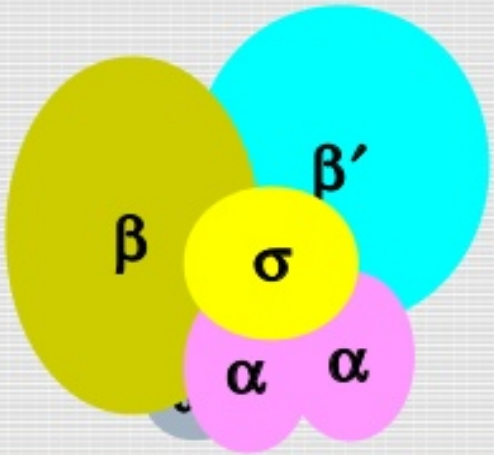

coding strand
template strand
5'
3'
3'
5'
template strand
coding strand

RNA Polymerase

- The enzyme responsible for the RNA synthesis is DNA-dependent RNA polymerase.
  - The prokaryotic RNA polymerase is a multiple-subunit protein of ~480kD.
  - Eukaryotic systems have three kinds of RNA polymerases, each of which is a multiple-subunit protein and responsible for transcription of different RNAs.

Holoenzyme

The holoenzyme of RNA-pol in *E.coli* consists of 5 different subunits: α_2 β β′ ωσ.



RNA-pol of *E. Coli*

subunit	MW	function
α	36512	Determine the DNA to be transcribed
β	150618	Catalyze polymerization
β′	155613	Bind & open DNA template
σ	70263	Recognize the promoter for synthesis initiation

RNA-pol of other prokaryotic systems is similar to that of *E. coli* in structure and functions.

RNA-pol of eukaryotes

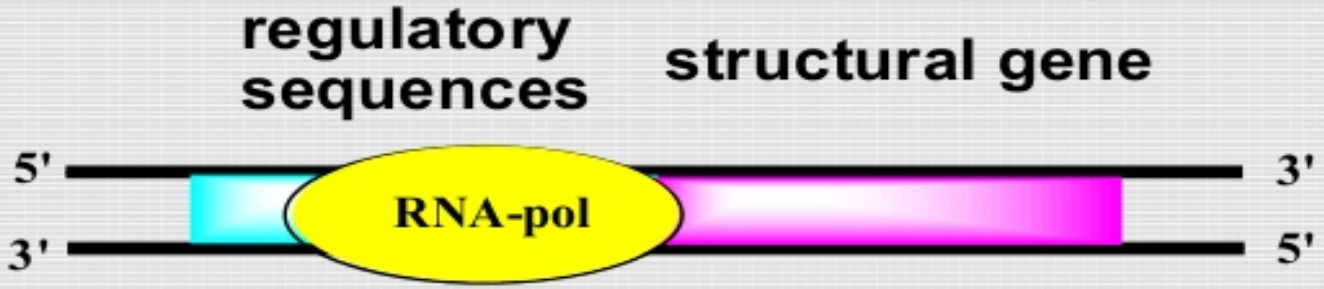
RNA-pol	I	II	III
products	45S rRNA	hnRNA	5S rRNA tRNA snRNA
Sensitivity to Amanitin	No	high	moderate

Amanitin is a specific inhibitor of RNA-pol.

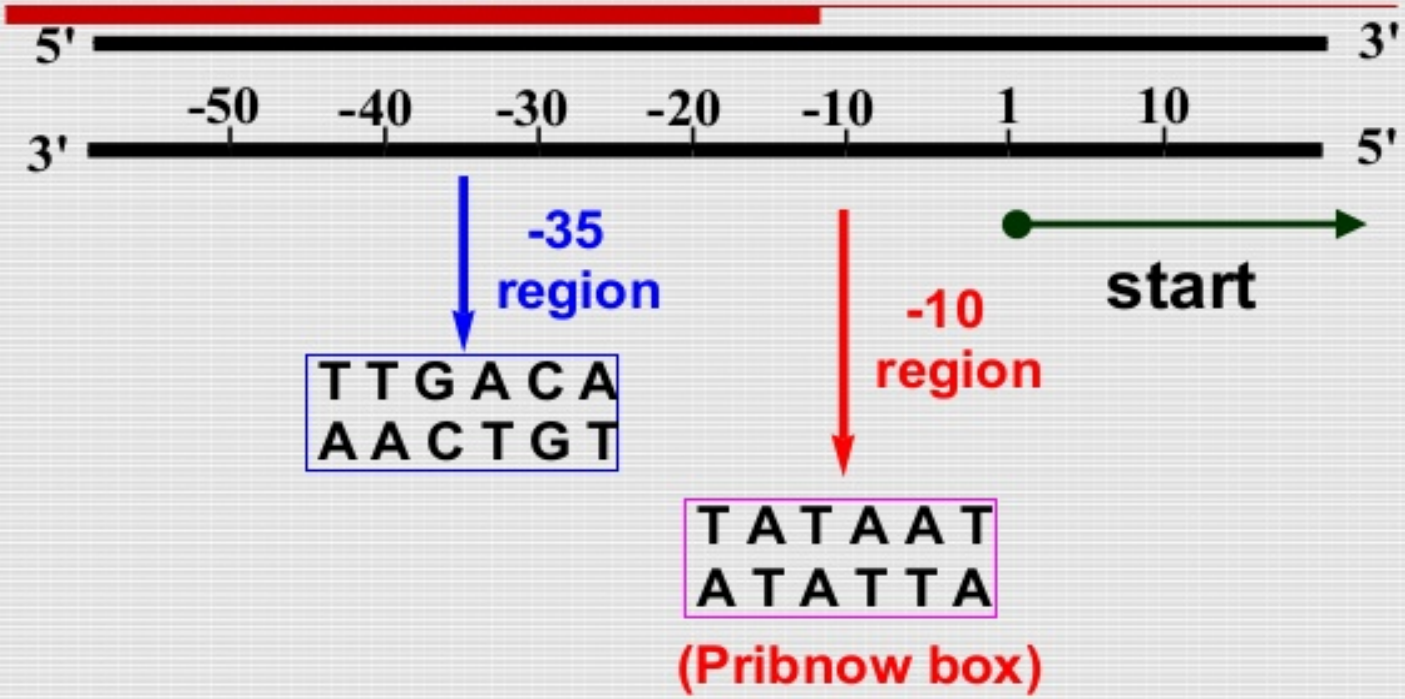
Recognition of Origins

- Each transcriptable region is called operon.
- One operon includes several structural genes and upstream regulatory sequences (or regulatory regions).
- The promoter is the DNA sequence that RNA-pol can bind. It is the key point for the transcription control.

Promoter

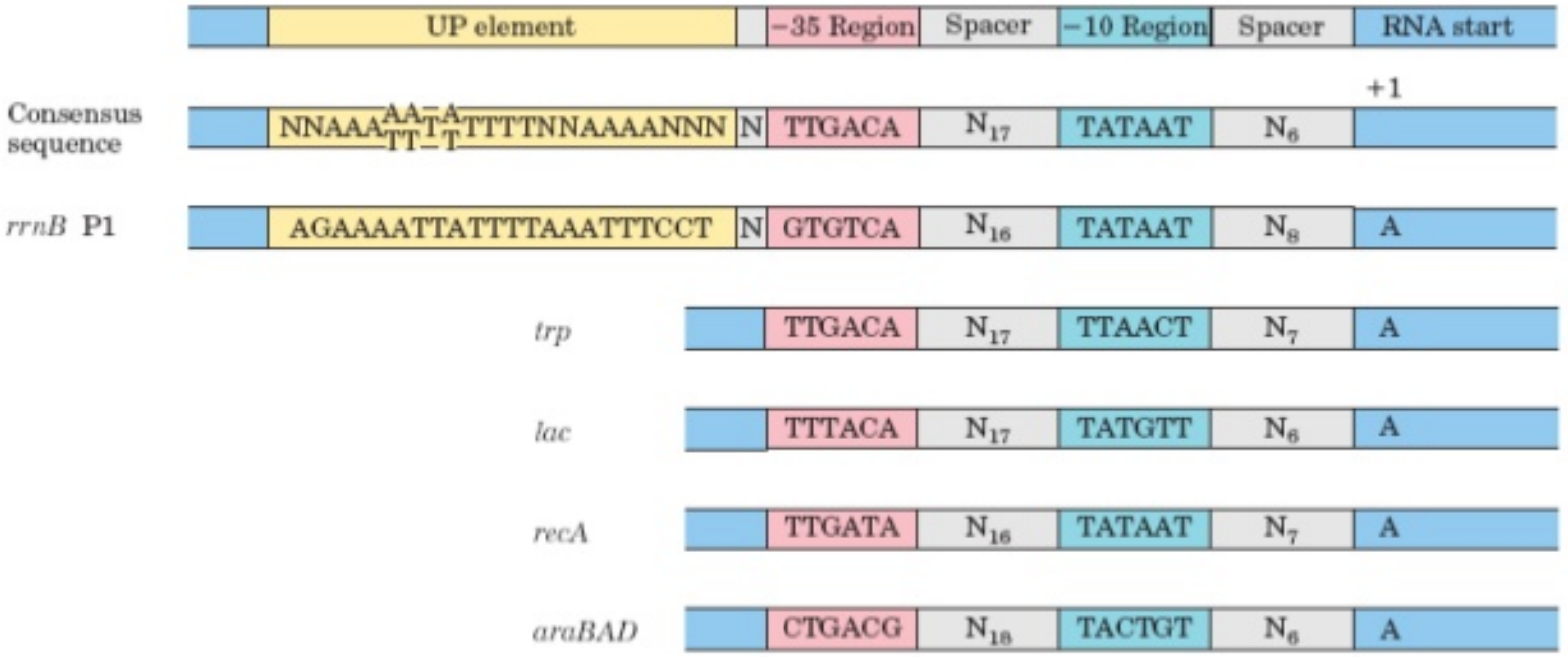


Prokaryotic promoter



Consensus sequence

Consensus Sequence



- The -35 region of TTGACA sequence is the recognition site and the binding site of RNA-pol.
- The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.

Transcription Process

General concepts

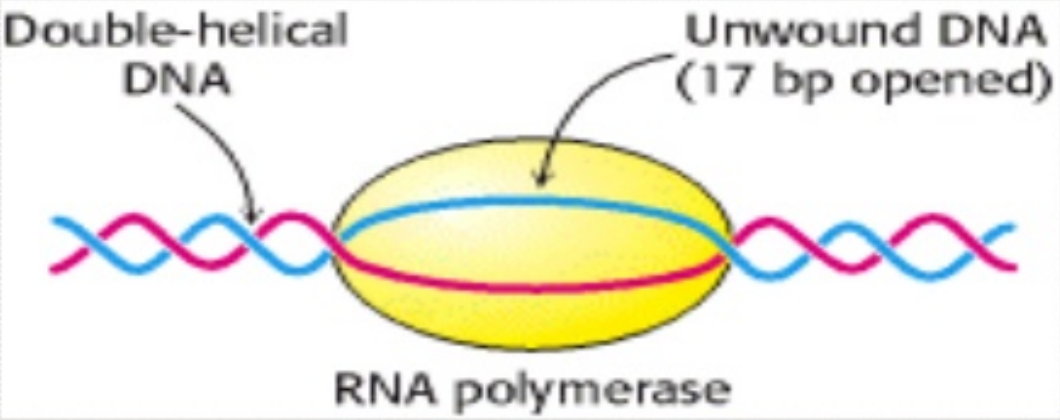
- Three phases: initiation, elongation, and termination.
- The prokaryotic RNA-pol can bind to the DNA template directly in the transcription process.
- The eukaryotic RNA-pol requires co-factors to bind to the DNA template together in the transcription process.

Transcription of Prokaryotes

- **Initiation phase**: RNA-pol recognizes the promoter and starts the transcription.
- **Elongation phase**: the RNA strand is continuously growing.
- **Termination phase**: the RNA-pol stops synthesis and the nascent RNA is separated from the DNA template.

a. Initiation

- RNA-pol recognizes the TTGACA region, and slides to the TATAAT region, then opens the DNA duplex.
- The unwound region is about 17±1 bp.



- The first nucleotide on RNA transcript is always purine triphosphate. GTP is more often than ATP.
- The pppGpN-OH structure remains on the RNA transcript until the RNA synthesis is completed.
- The three molecules form a transcription initiation complex.

RNA-pol ($\alpha_2\beta\beta'\sigma$) - DNA - pppGpN- OH 3′

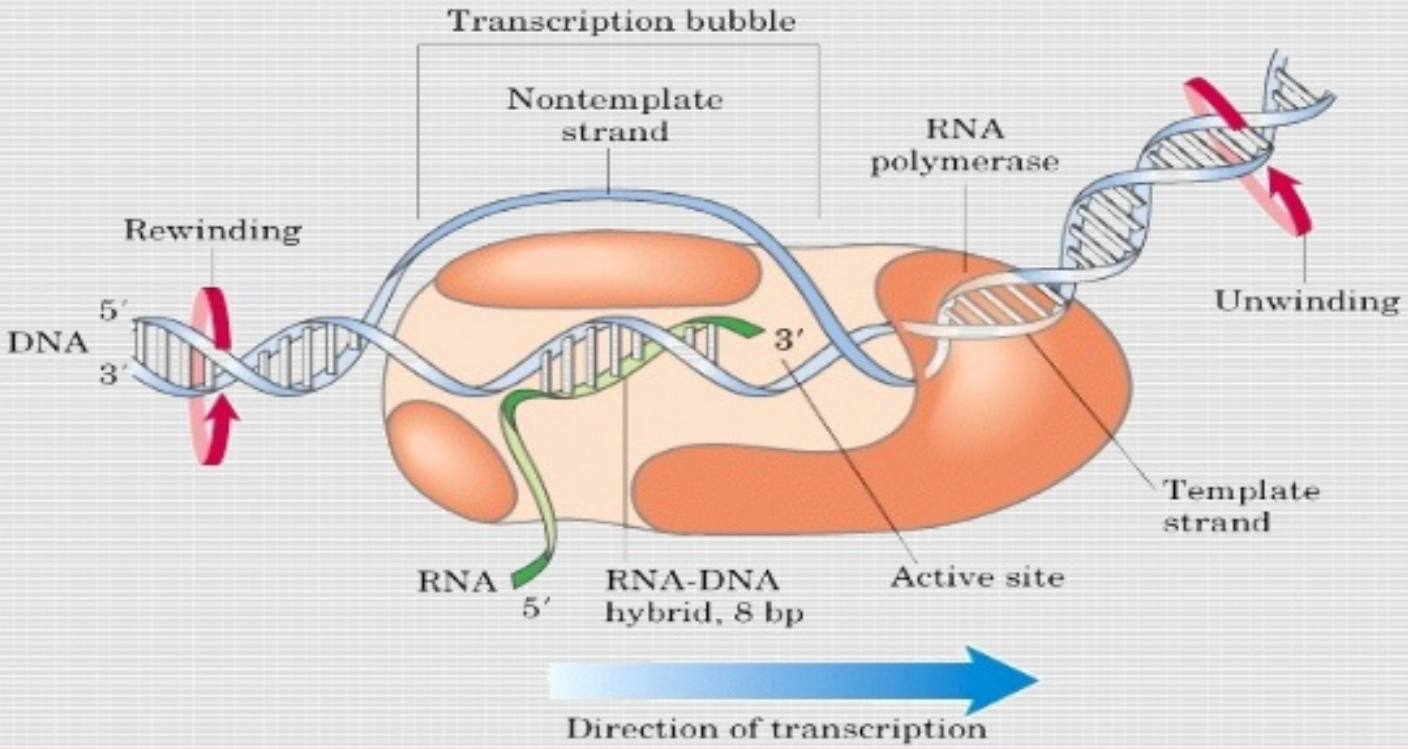
- No primer is needed for RNA synthesis.
- The σ subunit falls off from the RNA-pol once the first 3′,5′ phosphodiester bond is formed.
- The core enzyme moves along the DNA template to enter the elongation phase.

b. Elongation

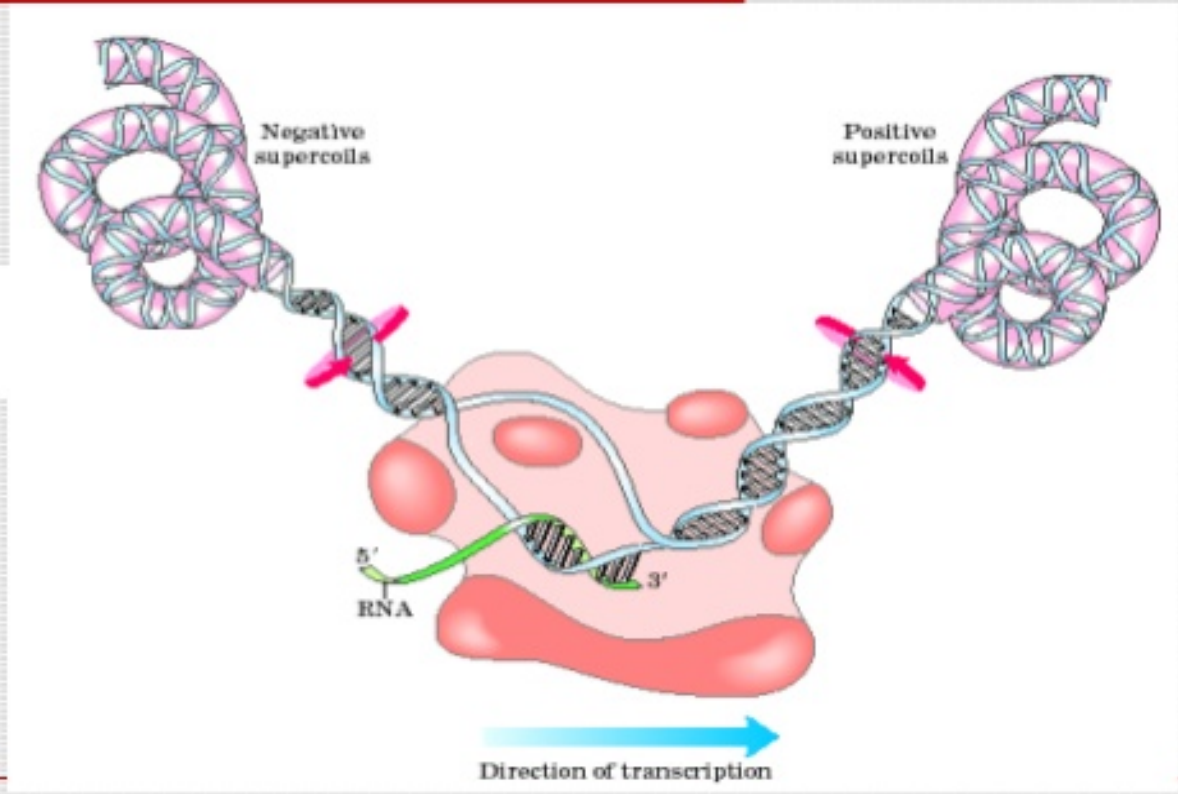
- The release of the σ subunit causes the conformational change of the core enzyme. The core enzyme slides on the DNA template toward the 3′ end.
- Free NTPs are added sequentially to the 3′ -OH of the nascent RNA strand.

- RNA-pol, DNA segment of ~40nt and the nascent RNA form a complex called the transcription bubble.
- The 3′ segment of the nascent RNA hybridizes with the DNA template, and its 5′ end extends out the transcription bubble as the synthesis is processing.

Transcription bubble



RNA-pol of *E. Coli*

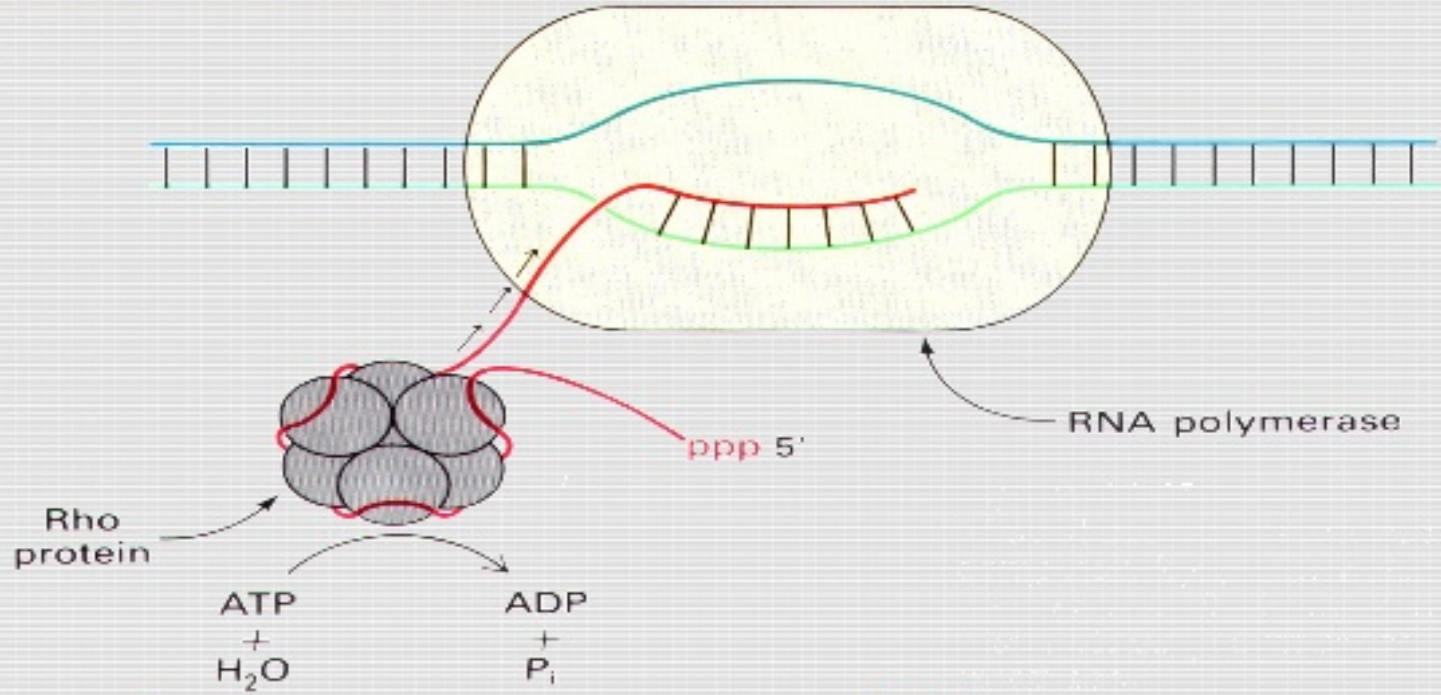


c. Termination

- The RNA-pol stops moving on the DNA template. The RNA transcript falls off from the transcription complex.
- The termination occurs in either ρ -dependent or ρ -independent manner.

http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animatic

The termination function of ρ factor



The ρ factor, a hexamer, is a ATPase and a helicase.

ρ-independent termination

- The termination signal is a stretch of 30-40 nucleotides on the RNA transcript, consisting of many GC followed by a series of U.
- The sequence specificity of this nascent RNA transcript will form particular stem-loop structures to terminate the transcription.

Prokaryotes	Eukaroytes
Simple	More complex
One RNAP	3 distinct RNAP
Promoter site - Pribnow box 35 sequence	Promoter site - TATA box - Hogness box , CAAT box
Initiation - Only requires sigma factor	Initiation - 6 Transcription factors interact with eukaryotic promoter region.
	POST TRANSCRIPTIONAL MODIFICATION

Promoters of eukaryotes

✓Goldberg -hogness box;

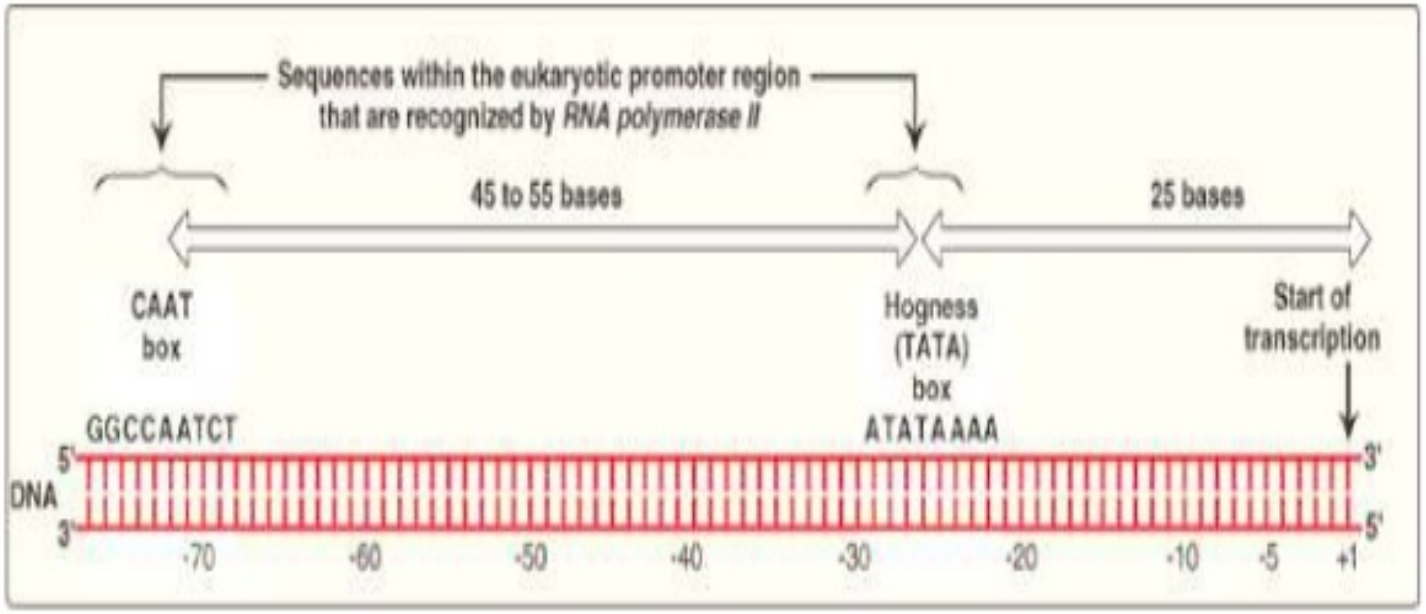
In eukaryotes a sequence TATAAA is located at 25-30 bp upstream to the start point it acts as signal to initiate the transcription.

✓CAAT box :

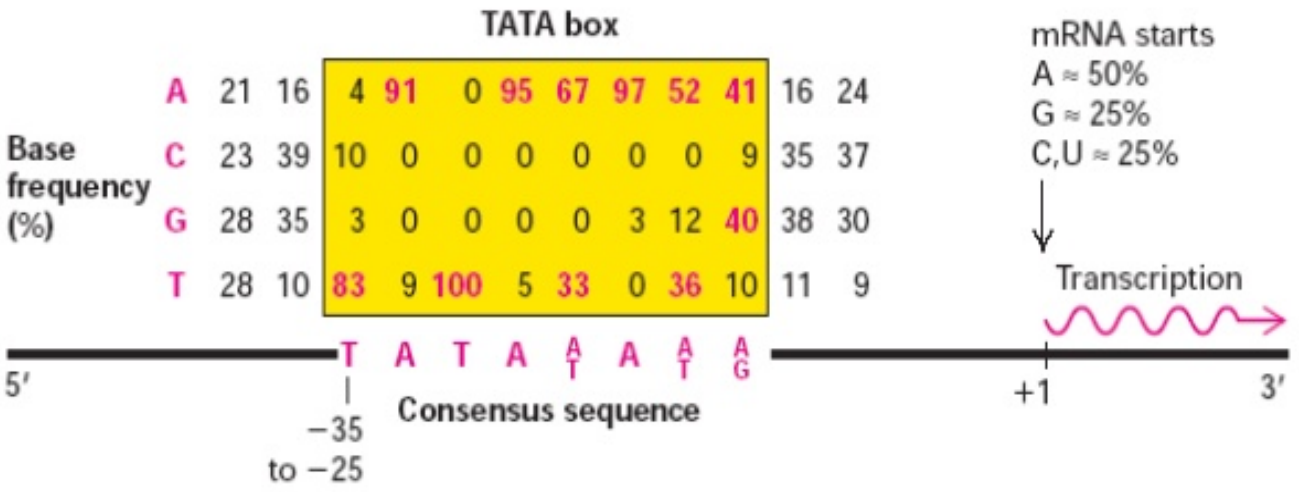
GGCAATCT Sequence is located 70 bp upstream to start point.

TATAAA

PROMOTER FOR EUKARYOTES



TATA box


TATA box
mRNA starts
A ≈ 50%
G ≈ 25%
C,U ≈ 25%
Base frequency (%)
A 21 16 4 91 0 95 67 97 52 41 16 24
C 23 39 10 0 0 0 0 0 0 9 35 37
G 28 35 3 0 0 0 0 3 12 40 38 30
T 28 10 83 9 100 5 33 0 36 10 11 9
Transcription
5′
T A T A A/T A A/T A/G
3′
+1
−35 to −25
Consensus sequence

RNAP of eukaryotes are of 3 types

RNAP-I synthesizes - rRNA

RNAP-II synthesizes - mRNA

RNAP-III synthesizes - tRNA

Transcription factors

- RNA-pol does not bind the promoter directly.
- RNA-pol II associates with six transcription factors, TFII A - TFII H.
- The trans-acting factors are the proteins that recognize and bind directly or indirectly cis-acting elements and regulate its activity.

b. Elongation

- The elongation is similar to that of prokaryotes.
- The transcription and translation do not take place simultaneously since they are separated by nuclear membrane.

c. Termination

- The termination sequence is AATAAA followed by GT repeats.
- The termination is closely related to the post-transcriptional modification.

Post transcriptional modifications

The mRNA formed from DNA is called the primary transcript or hnRNA.

It undergoes extensive modifications to become active and mature mRNA.

These modifications are called as post transcriptional modifications.

Eukaryotic RNA is processed before leaving the nucleus

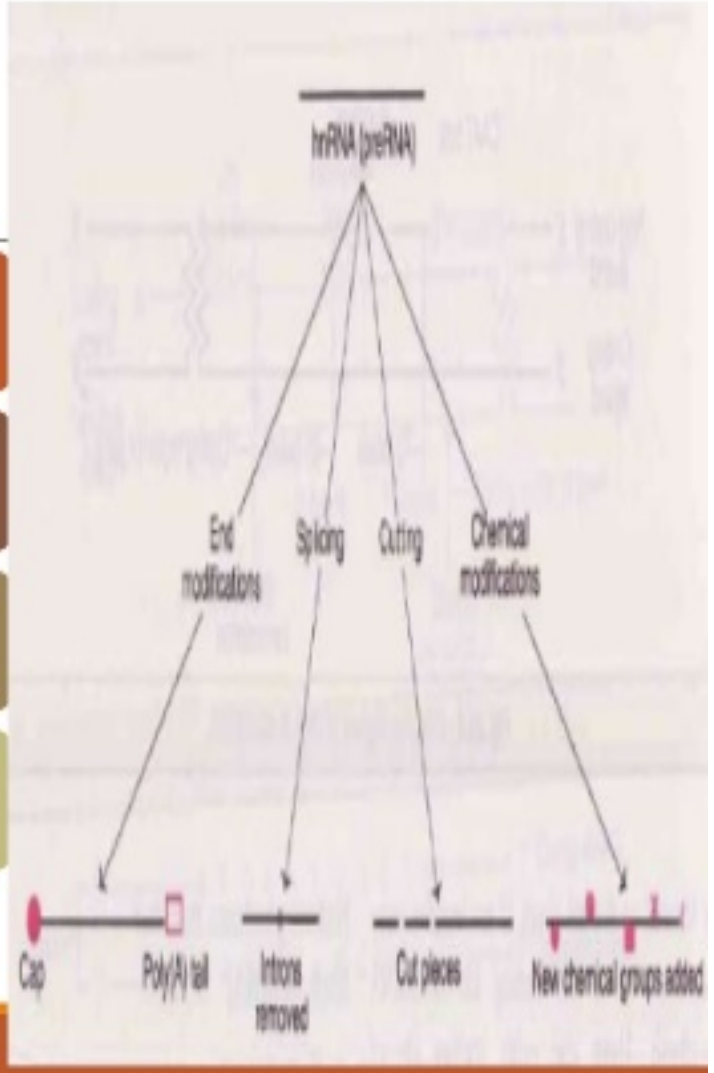
hnRNA to mRNA

1.capping at 5` end

2. Poly A tailing at 3'

3.Splicing - removal of introns

4. mRNA EDITING



Post transcriptional modifications

5' capping

- 7-methylguanylate attached by a unusual 5'-5' triphosphate linkage to the ribose at the 5'-end.
- Addition of GTP part of the cap is catalyzed by nuclear enzyme guanylyltransferase.
- Methylation of terminal guanine occurs in the cytosol- **SAM is the source of the methyl group**
- Catalysed by guanine-7-methyl transferase.

Importance

- The cap binds mature mRNA to the ribosome during protein biosynthesis.
- Cap Stabilizes mRNAs against digestion by ribonucleases.
- Eukaryotic mRNAs lacking the cap are not translated efficiently.

Addition of poly A tail

- Poly A tail added at 3' end of mRNA
- 200-300 adenylate residues linked by PDE bonds
- ATP is the donor of adenylate group
- It is involved in stabilization of mRNA

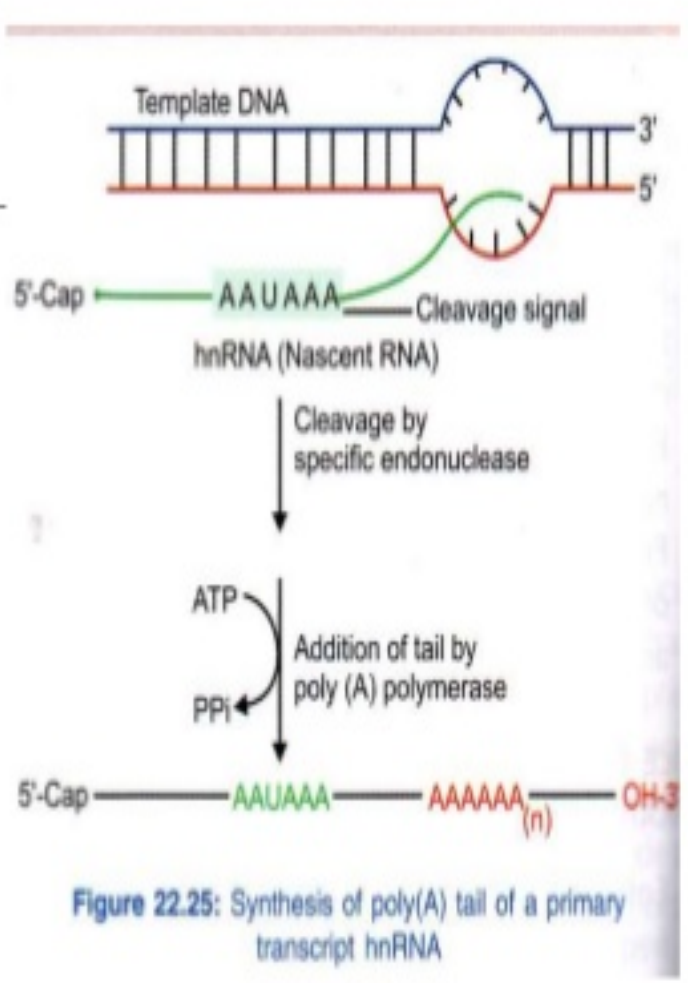
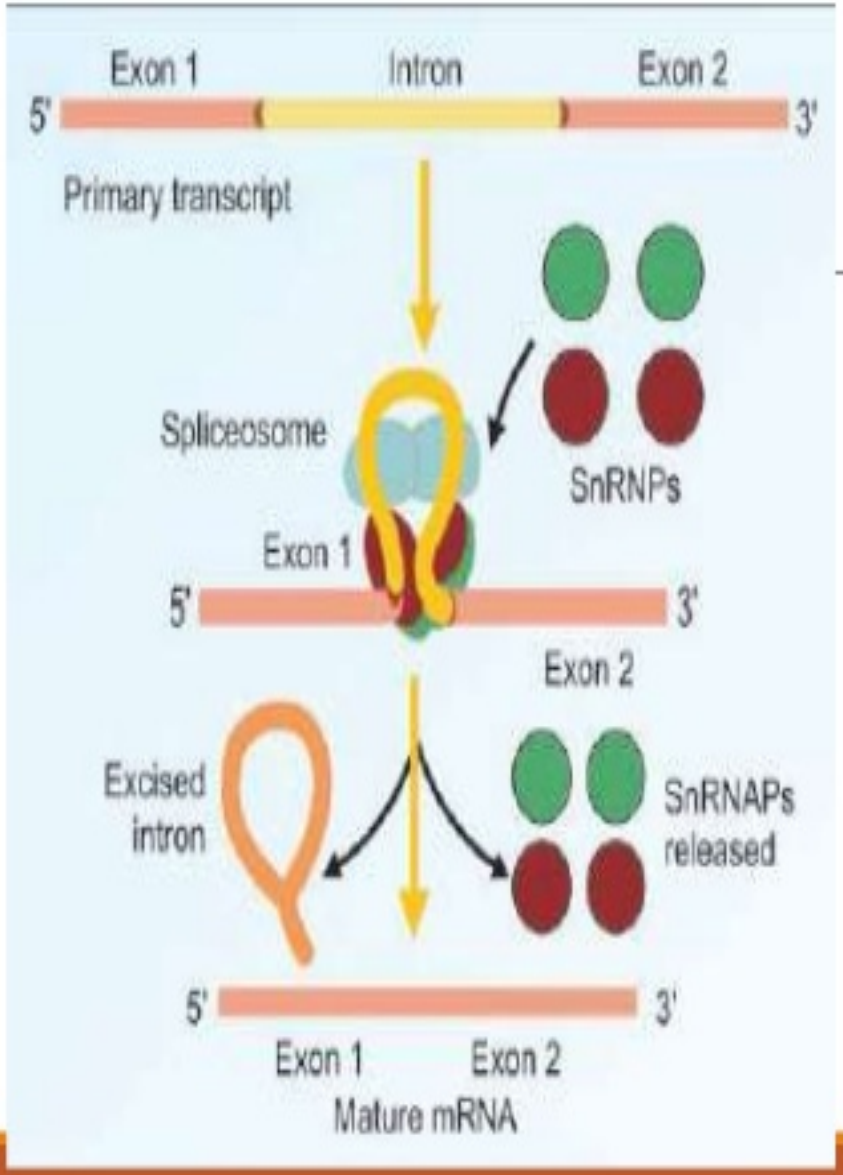
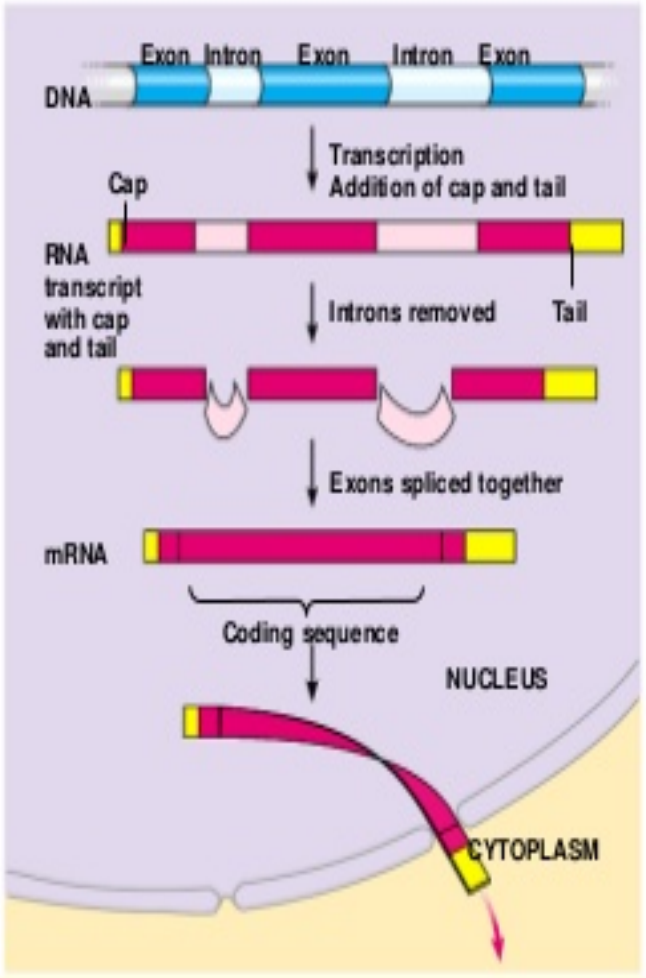


Figure 22.25: Synthesis of poly(A) tail of a primary transcript hnRNA

Splicing

- Process by which **introns are removed** & **exons are joined** to form the functional mRNA .
- Requires **energy**
- Small nuclear RNAs associated with specific proteins to form complex -snRNPs (small nuclear ribonucleic protein particles) or snurps , involved in formation of spliceosomes.
- **Spliceosomes** –is a complex containing multiple snRNPs that contain snRNA that catalyze hnRNA to mRNA, by removing introns and joining exons.
- 15% genetic disease – due to splicing defects
- Faulty splicing – causes **βThalassaemia.**


Exon 1
Intron
Exon 2
5'
3'
Primary transcript
Spliceosome
SnRNPs
Exon 1
5'
3'
Exon 2
Excised intron
SnRNAPs released
5'
3'
Exon 1
Exon 2
Mature mRNA


Exon
Intron
Exon
Intron
Exon
DNA
Transcription
Addition of cap and tail
Cap
RNA transcript with cap and tail
Introns removed
Tail
Exons spliced together
mRNA
Coding sequence
NUCLEUS
CYTOPLASM

mRNA editing

- ✓0.01% of the mRNAs undergoes editing.
- ✓ enzyme mediated alteration of base sequence of RNA (not by splicing)
- ✓Ex:- conversion of CAA codon in mRNA (of apoprotein B gene) to UAA by the enzyme cytidine deaminase .
- ✓ Originating from the same gene, the liver synthesizes a 100-kDa protein (apoB 100) while the intestinal cells synthesize 48-kDa protein (apoB 48).
- ✓This happens due to formation of a termination codon UAA from CAA in RNA editing.

tRNA

- Cleavage of a 5'leader sequence.
- Splicing to remove intron.
- Replacement of the 3'terminal UU by CCA &
- Modification of several bases – dihydrouridine, pseudouridine, Thymine, methylated bases.

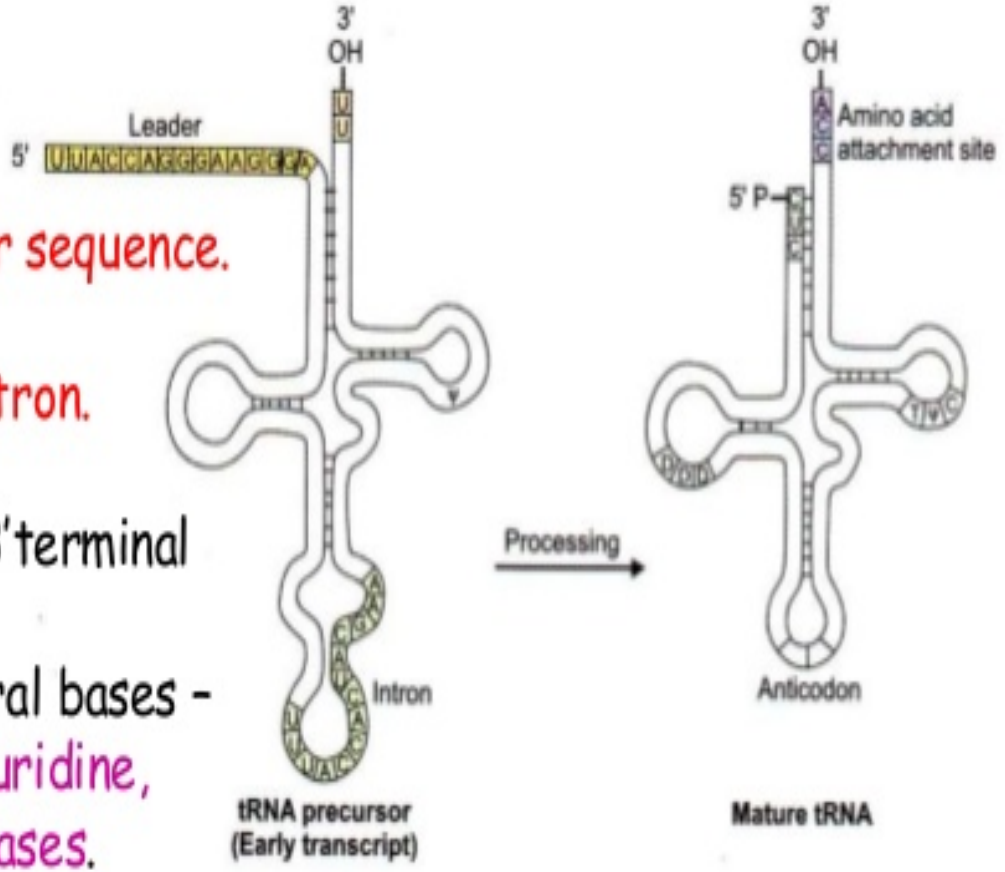


Figure 22.24: Processing of tRNA precursor to mature tRNA

rRNA

- 28 s, 18s, 5.8 s are synthesized as long precursor - Preribosomal 45S RNAs
- This is cleaved and trimmed to produce mature functional rRNA
- 5 S rRNA is produced by transcription of 5S gene by RNA polymerase III & modified separately.

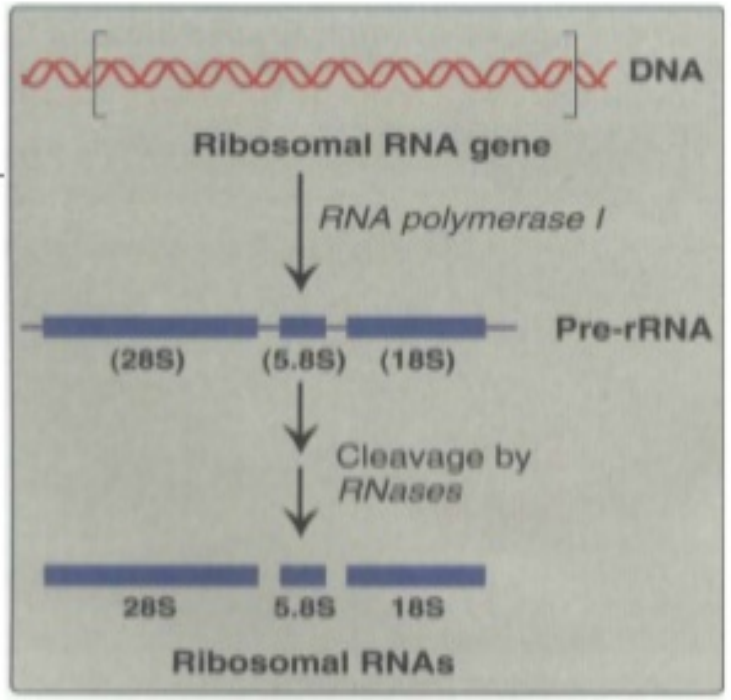


Figure 30.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases.


Thank
You