Transcription

Presented by: Dr.Asma

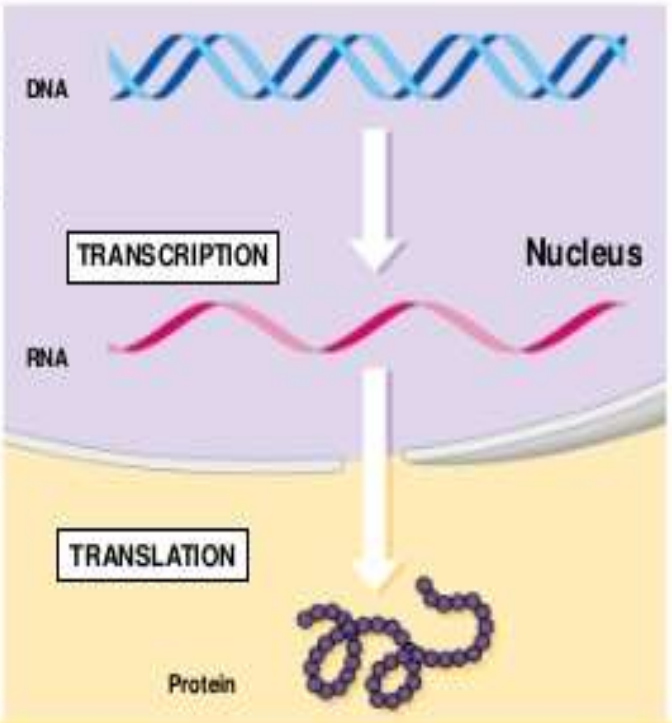
Definition

Synthesis of RNA using ssDNA as a template by DNA dependent RNA polymerase enzyme.

Similar to replication in terms of chemical mechanism, polarity, and use of template but differs in

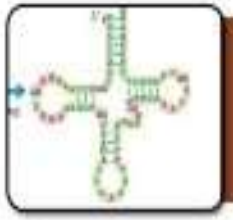
-does not require primers

-only a short segment of DNA is transcribed

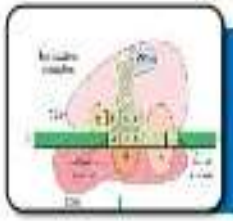


All 3 types of cellular RNA's are copied during transcription

Messenger RNAs (mRNAs) encode the amino acid sequence of one or more polypeptides specified by a gene.



Transfer RNAs (tRNAs) read the information encoded in the mRNA and transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis.



Ribosomal RNAs (rRNAs) are constituents of ribosomes, the intricate cellular machines that synthesize proteins.

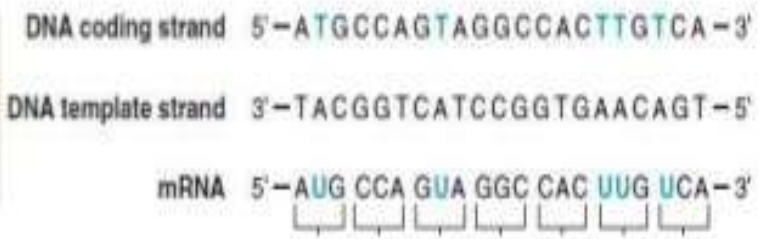
Salient features of transcription

- Synthesis of ALL types of RNA in Nucleus
- Only ONE STRAND of DNA participates
- Ribonucleotides are used in RNA synthesis.
- RNA Synthesis occurs in 5'-3' direction , DNA template is read from 3'-5' direction
- Synthesis follows Waston-Crick base pairing rules – A to U, G to C
- DNA dependent RNA polymerase or RNA polymerase

Basic Requirements of Transcription

Template

- ss DNA



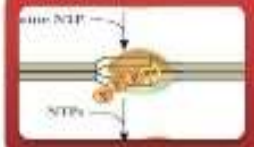
Enzyme

- DNA dependent RNA polymerase

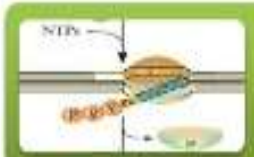
Ribonucleoside triphosphates

- ATP, GTP, CTP, UTP

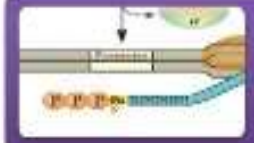
Steps involved in Transcription



INITIATION

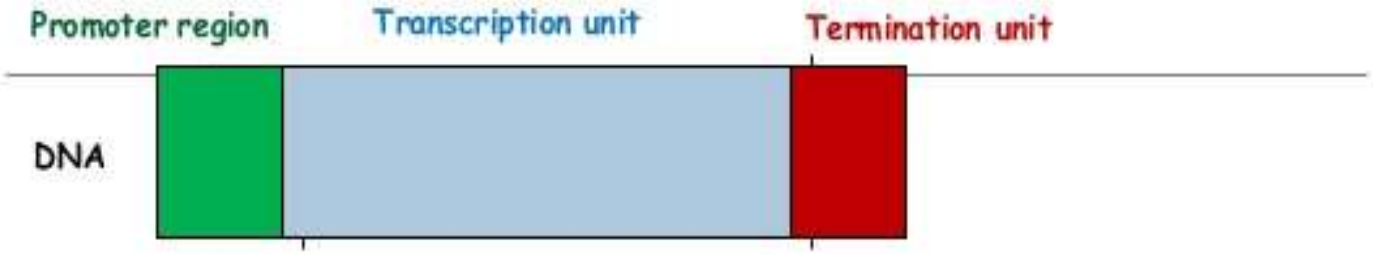


ELONGATION



TERMINATION & Release

Template DNA



1. **Promoter region:** It is the specific region in DNA ,where transcription is initiated.

2. **Transcription unit:** It is the region where DNA template is transcribed. Present in b/w promoter and terminating units.

3. **Termination unit:** It is the region where transcription terminates.

Initiation

- Starts with the recognition of promoter sequence on the DNA coding (anti-template) strand by RNA polymerase

- **Promoter sequence**

  Are specific areas on the DNA that act as starting signals for initiation process recognized by RNAP.

- Two common sequences are present on the upstream side of the start site of transcription .

- Start site is denoted by +1

Promoters of prokaryotes

✓Pribnow box or TATAAT box:

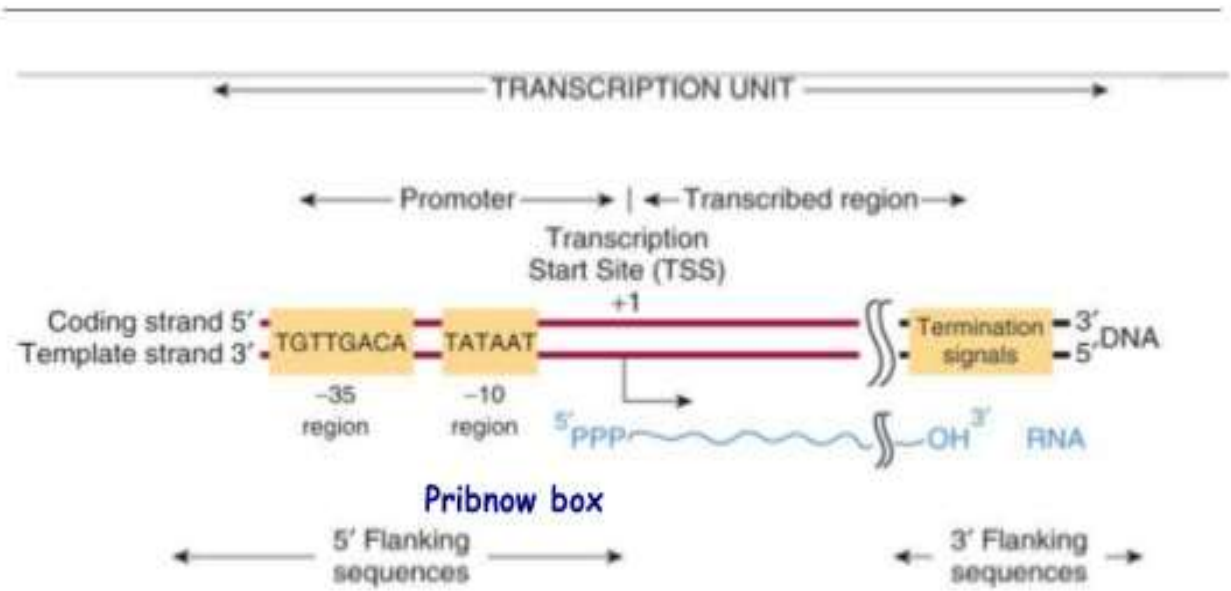
It contains 6 nucleotide bases TATAAT located -10 bases away on the left of origin of transcription.

✓ -35 sequence / 5`-TTGACA-3`seqence:

it is 35 bp upstream of the transcription unit

5` TTGACA 3`

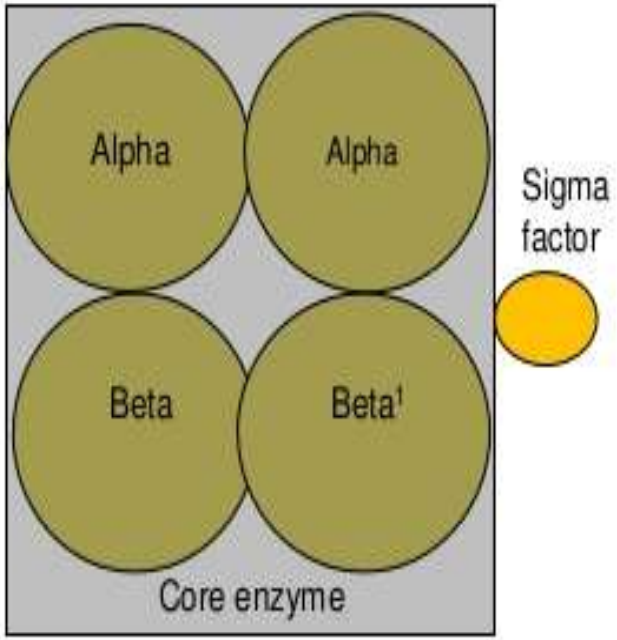
PROMOTER FOR PROKARYOTES



RNAP- prokaryotes

RNAP of prokaryotes has 5 sub units-2 alpha units , beta, beta^1 and sigma unit (2α,β,β',δ).

- RNA polymerase without σ subunit is called core enzyme
- Core enzyme contains the catalytic activity.
- Core enzyme with sigma factor is holo-enzyme



Functions of RNAP

Subunit	Role
α	Binds regulatory proteins
β	Forms phosphodiester bond
β′	Fixes RNAP to DNA template
σ	Recognizes and binds to promoter region of DNA, Initiates transcription.

In bacteria, one species of RNAP can synthesize all the RNA molecules (mRNA, tRNA, rRNA)

RNA polymerase differs from DNA Polymerase in two aspects

1. No primer is required for RNAP.
2. RNAP lacks a separate proofreading 3' to 5' exonuclease activity.

The error rate for transcription is higher.

Error rate is 10^4-10^5 times more than replication

Errors cause little damage – as they are not transmitted to daughter cell/ next generation.

Identification of promoter region:

Sigma unit of RNA Polymerase identifies the promoter region on template DNA .

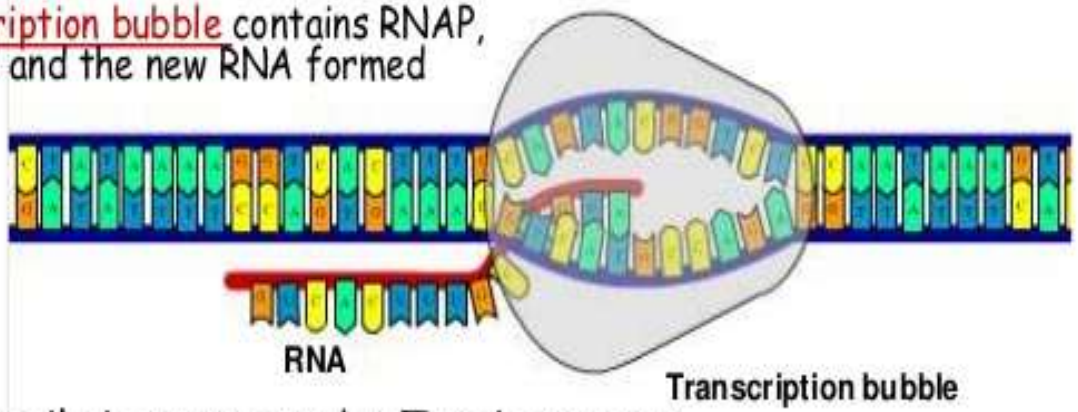
RNA polymerase binds to the promoter region of the template DNA

Beta unit fixes to the promoter region of the template strand and initiates transcription.

Sigma factor is released and RNAP in promoter region unwinds DNA helix.

A **Transcription bubble** is formed as the DNA unwinds down stream

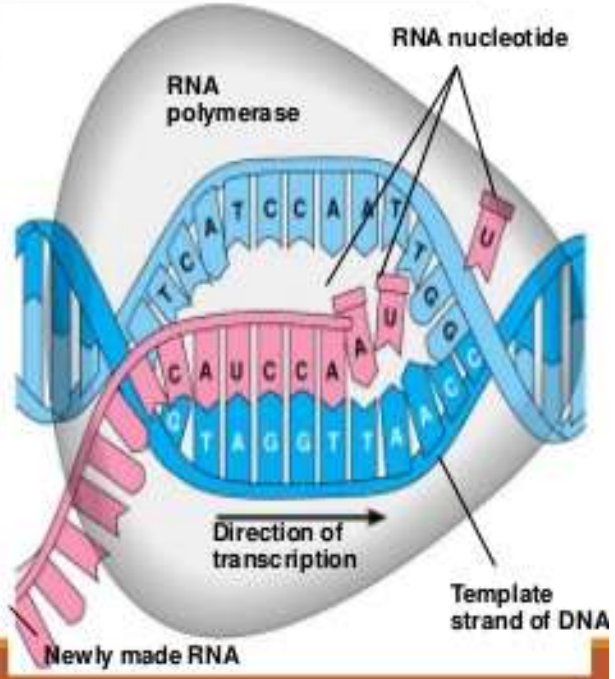
Transcription bubble contains RNAP, DNA and the new RNA formed



Supercoils is overcome by Topoisomerase

Elongation

- RNA polymerase elongates an RNA strand by adding ribonucleotide units to the 3'-hydroxyl end.
- RNAP uses ribonucleoside triphosphates, forms 3'5' PDE bond b/w adjacent ribonucleotide and releases ppi each time a nucleotide is added to the growing chain
- Each nucleotide in the newly formed RNA is selected by Watson-Crick base-pairing interaction. (G to C, A to U)



Termination

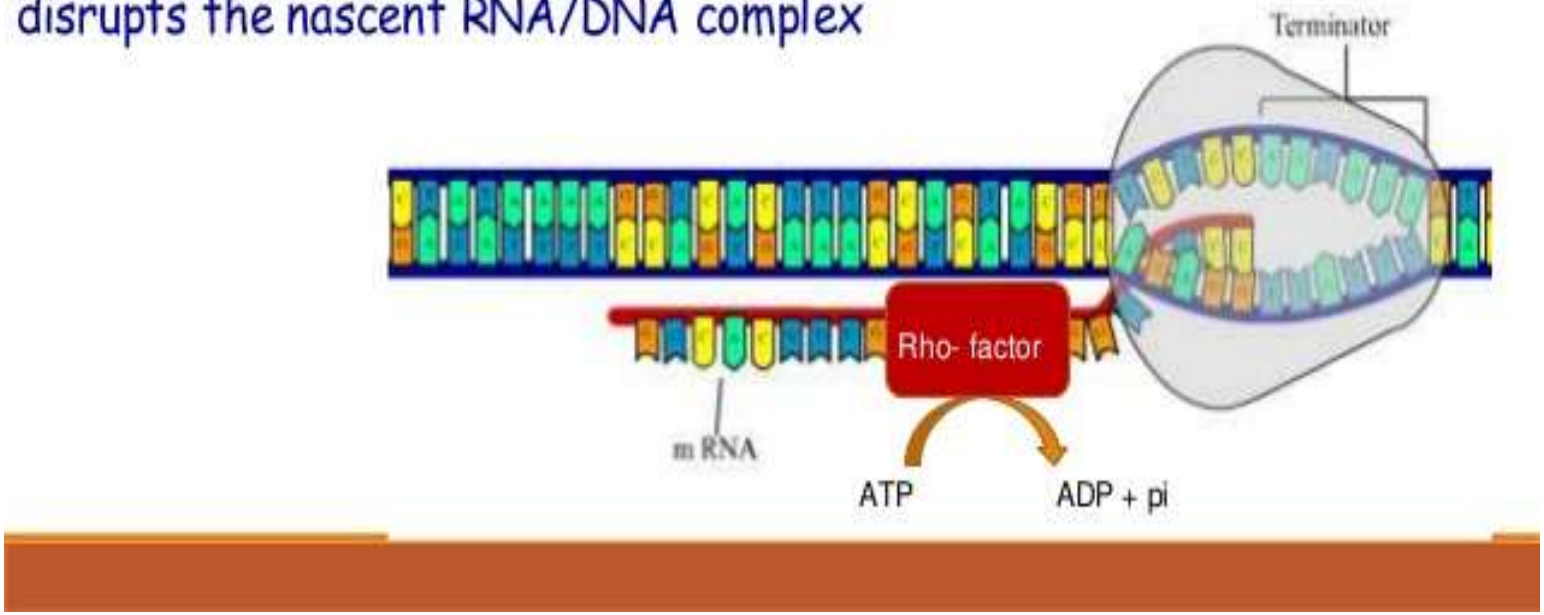
Rho dependent termination - A hexameric protein called **Rho factor** attaches to the DNA strand and RNAP can not move further and dissociates from DNA strand terminating the transcription

- ✓ **Rho independent termination** – palindrome like bases occurs at end sequence of DNA. Due to these sequences, the newly synthesized RNA folds on itself to form **hair pin loop** (due to complemenatry basepairing) which terminates the movement of RNAP.
- ✓ **palindrome** - word / phrases that read alike backwards and forwards - MADAM, MALAYALAM

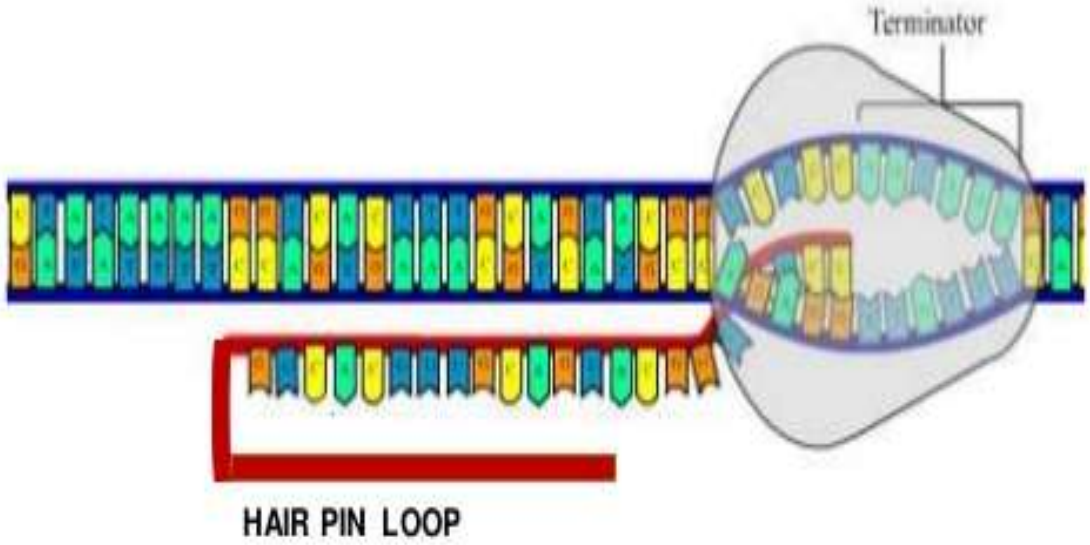
Termination

Rho factor is an ATP dependent RNA-DNA helicases

Recognizes and bind to the termination signals and disrupts the nascent RNA/DNA complex



Termination



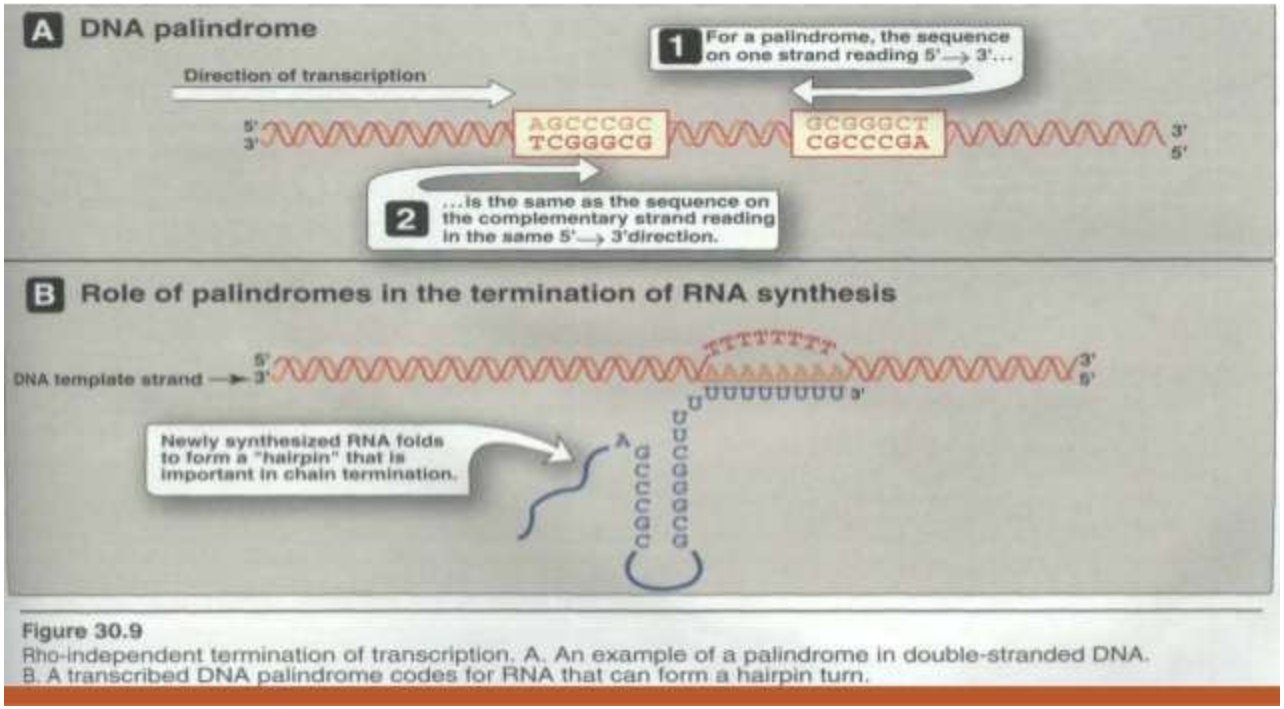
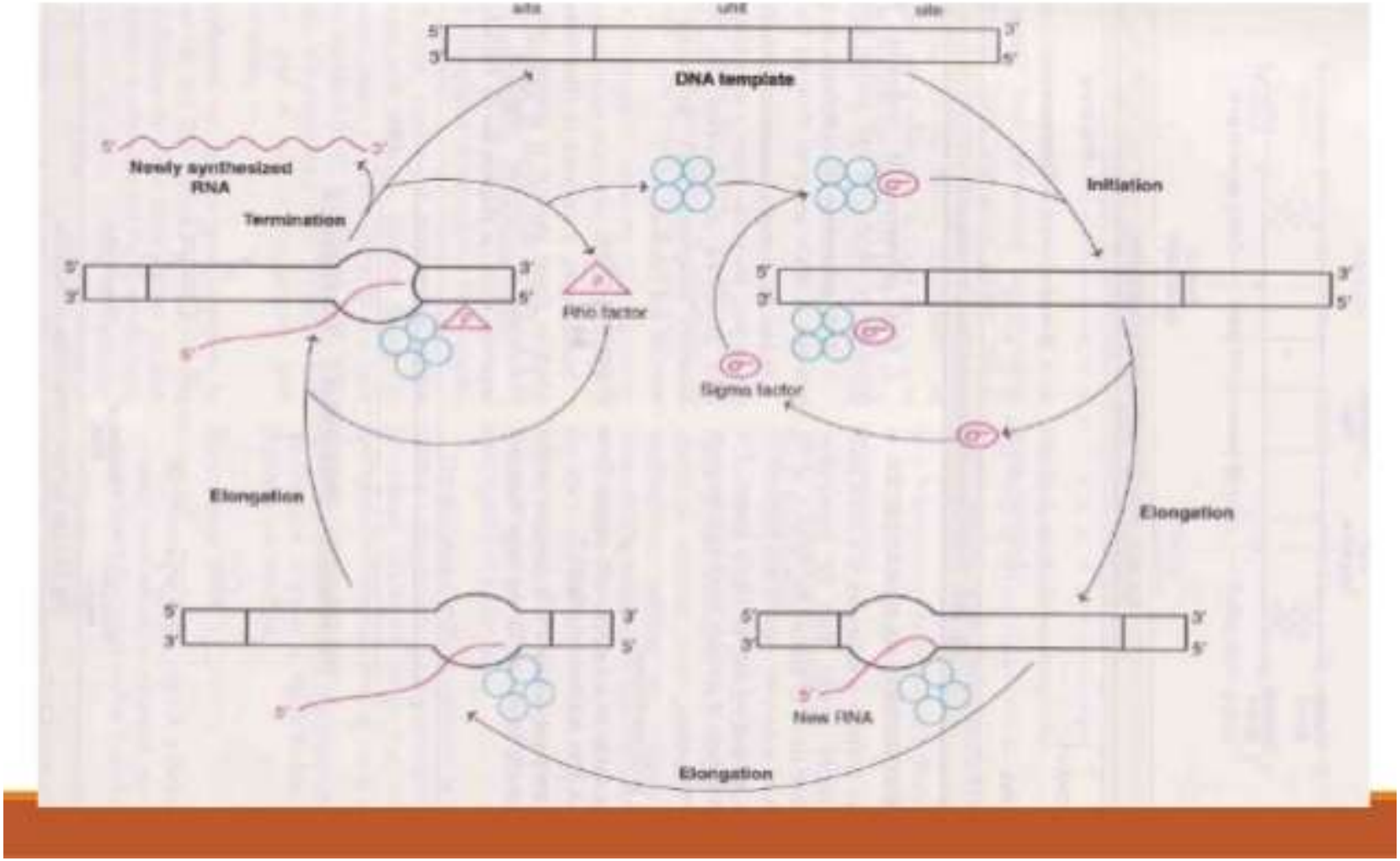


Figure 30.9
Rho-independent termination of transcription. A. An example of a palindrome in double-stranded DNA. B. A transcribed DNA palindrome codes for RNA that can form a hairpin turn.


DNA template
Newly synthesized RNA
Termination
Rho factor
Initiation
Sigma factor
Elongation
Elongation
New RNA
Elongation

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;

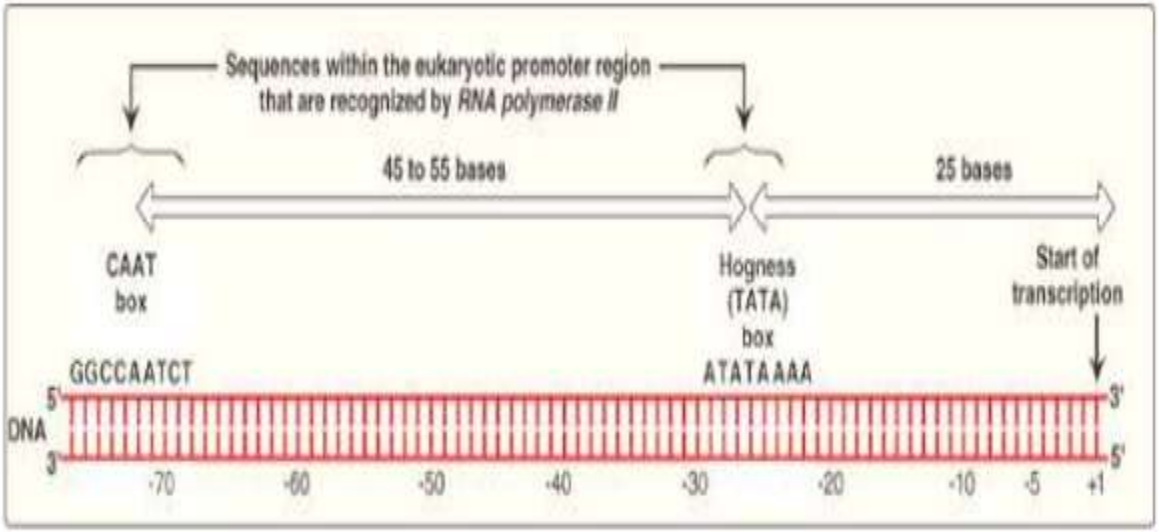
In eukaryotes a sequence TATAAA is located at 25-30 bp upstream to the start point it acts as signal to initiate the transcription.

✓CAAT box :

GGCAATCT Sequence is located 70 bp upstream to start point.

TATAAA

PROMOTER FOR EUKARYOTES



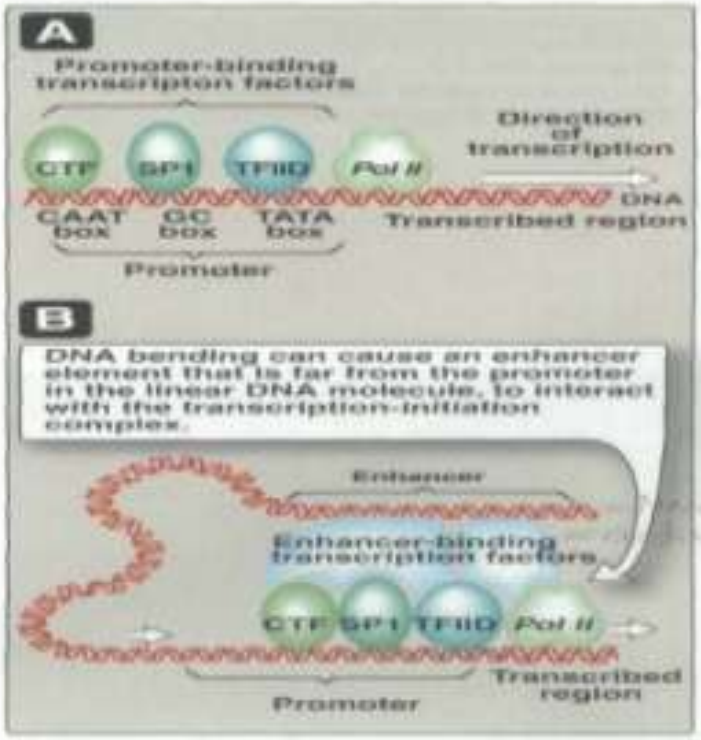
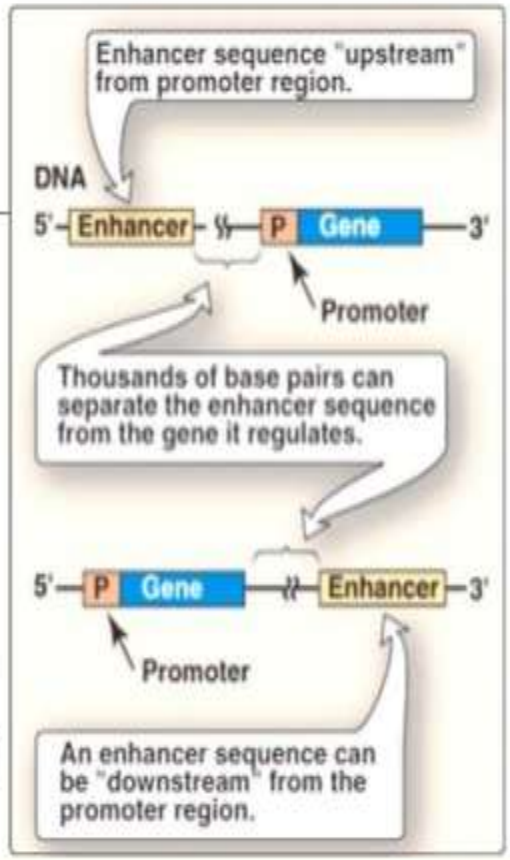


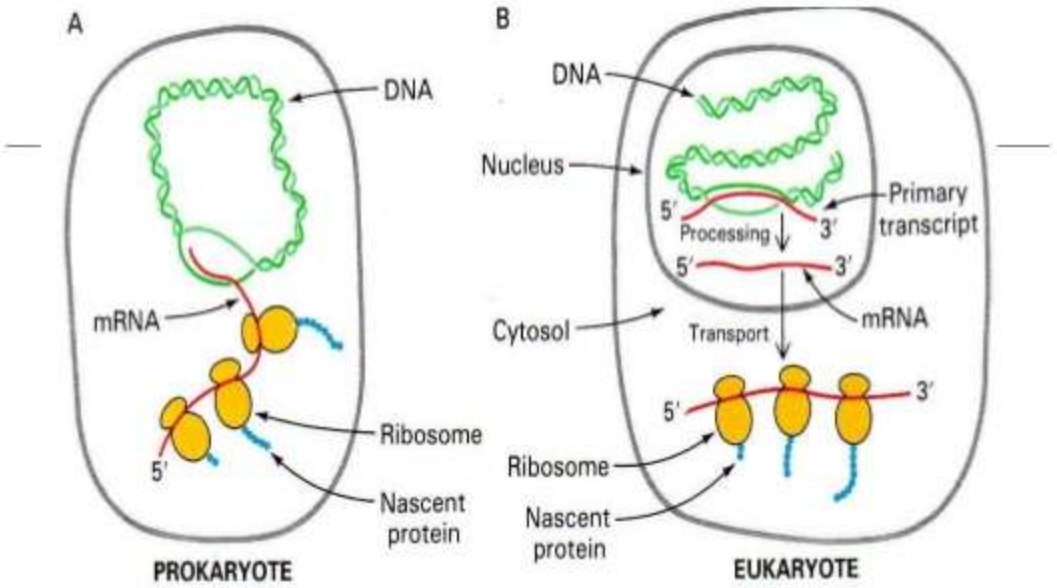
Figure 30.13
A. Eukaryotic general transcription factors bound to the promoter. CTF, SP1, and TFIID are general transcription factors. B. Enhancer stimulation of *RNA polymerase II*.

- Transcription factors binds to DNA sequences in promoter region
- Stimulated by enhancers
- **6 Transcription factors** - TFIID, TFIIA, TFIIB, TFIIF, TFIIE, TFIIH.
- Transcription factors binds to each other and in turn to enzyme RNAP - form preinitiation complex or basal transcription complex

- **Enhancer** - increases gene expression by 100 fold
- Enhancers bind to TFs to form Activators
- They have no Promoter activity of their own but stimulate the transcription gene.
- They can be present upstream, downstream or within a gene

- **Silencers** - DNA sequences which bind proteins that act to inhibit the rate of transcription.




A
DNA
mRNA
Ribosome
5′
Nascent protein
PROKARYOTE
B
DNA
Nucleus
5′
Processing
3′
Primary transcript
5′
3′
Cytosol
Transport
mRNA
5′
3′
Ribosome
Nascent protein
EUKARYOTE


Introducing: Transcription

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

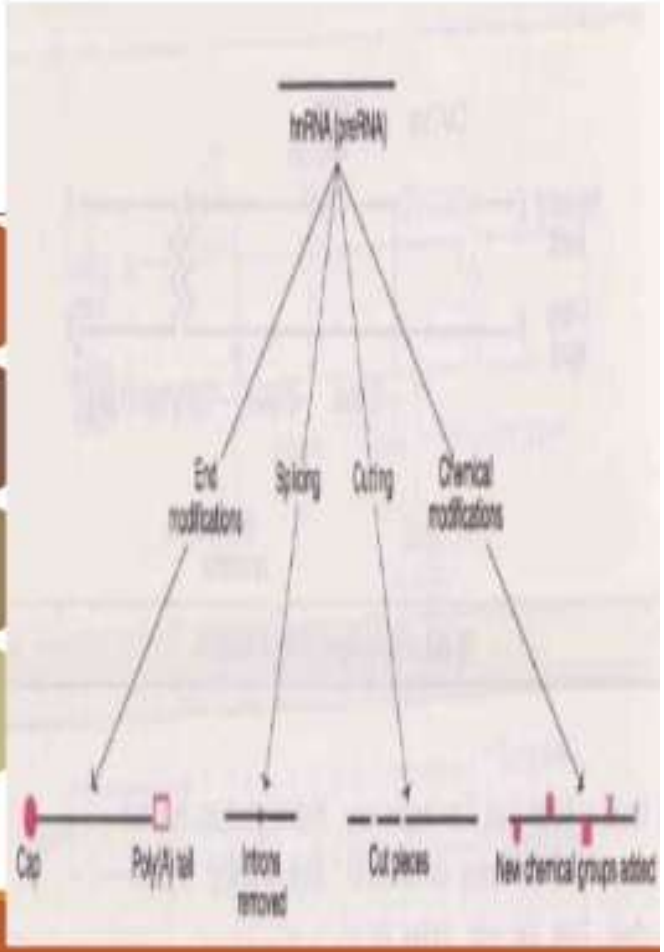
hnRNA to mRNA

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

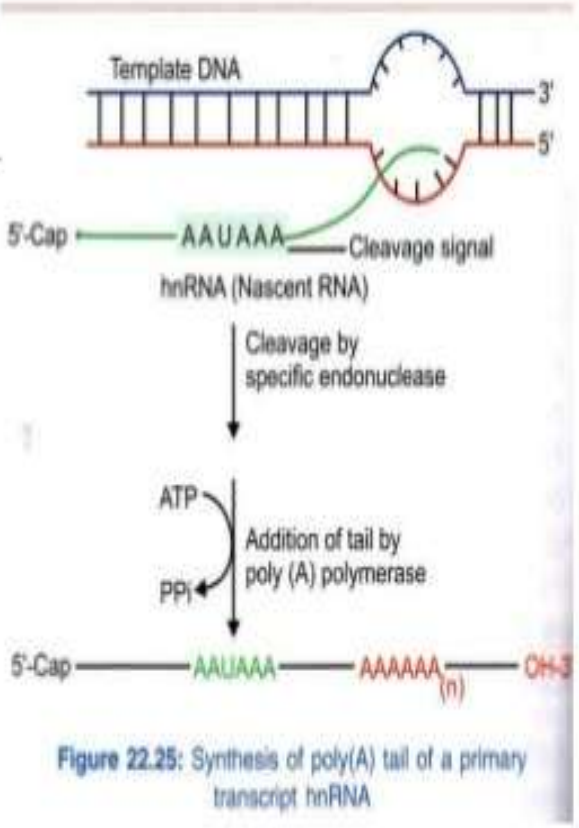
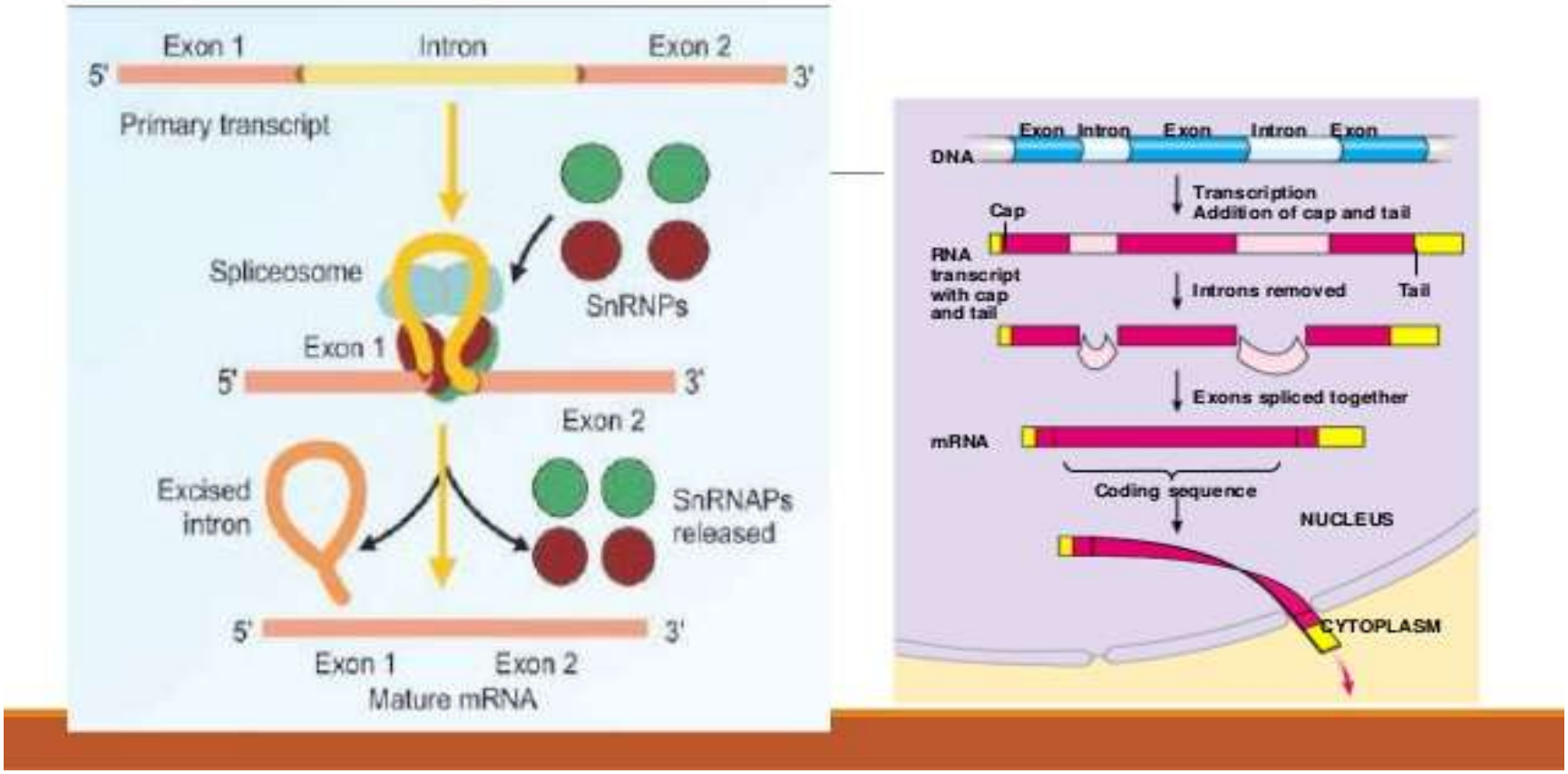


Figure 22.25: Synthesis of poly(A) tail of a primary transcript hnRNA

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
Splicing

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.

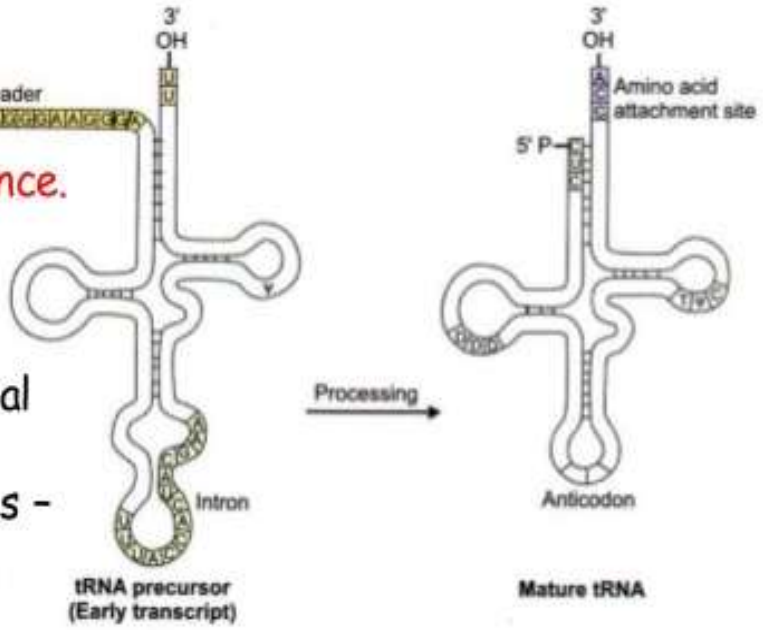


Figure 22.24: Processing of tRNA precursor to mature tRNA

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.

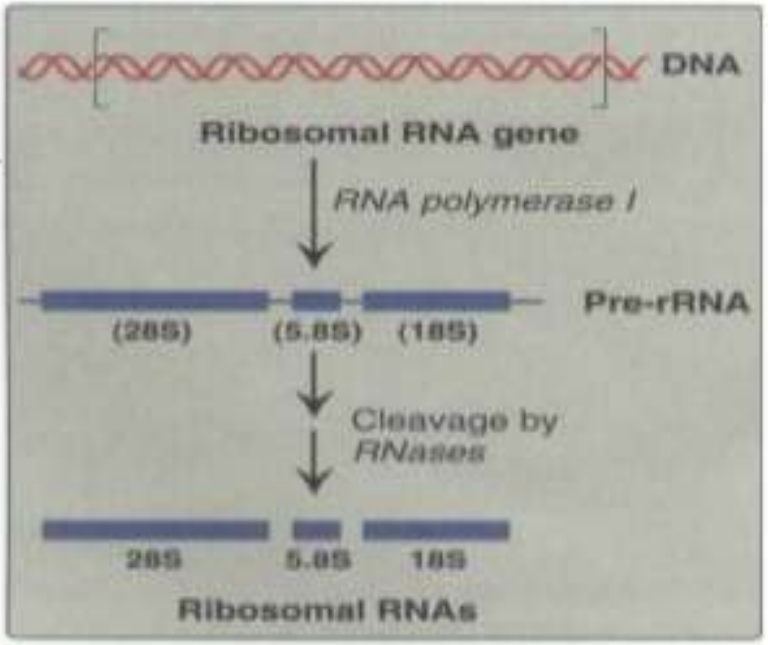


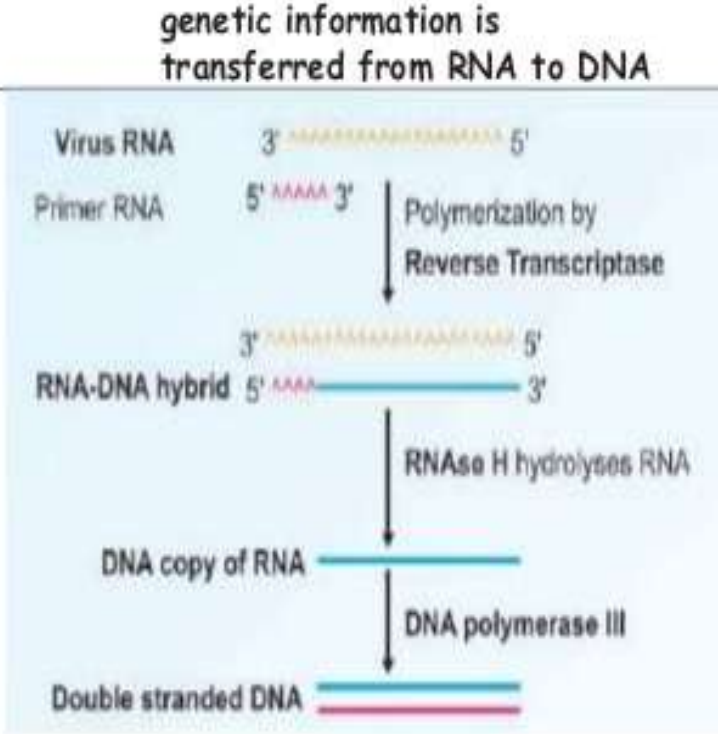
Figure 30.15
Posttranscriptional processing of eukaryotic ribosomal RNA by ribonucleases.

Inhibitors of transcription

Inhibitors			Mode of action
Rifampicin	antitubercular drug	Only inhibits prokaryotes	binds to beta subunit of RNA polymerase
Actinomycin D	synthesized by Streptomyces	Both	Binds with DNA strand & blocks movements of all forms of RNA.
a-Amanitin	toxin produced by Mushroom, Amanita phalloides	Eukaryotes	tightly binds with RNA polymerase II & inhibits transcription

Reverse transcription

- ✓Generation of DNA from RNA is reverse transcription
- ✓Enzyme catalyzing this is reverse transcriptase or RNA dependent DNA polymerase
- ✓Usually seen in RNA viruses of retrovirus group
- ✓ Some of the tumor viruses
- ✓Eg: HIV causing AIDS is a retrovirus.



Ribozyme

Enzymes made up of RNA are called ribozymes

Catalytic RNA molecules with sequence specific cleavage activity

exhibit Michaelis-Menten kinetics

Ex:-

- **Spliceosomes** - contains ribozymes as well as protein. Involved in Post transcriptional modification.
- **RNAse-P-** generates the ends of tRNAs.
- **Peptidyl transferase** -used for protein biosynthesis