SHRIMATI INDIRA GANDHI COLLEGE

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TRANSCRIPTION

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Class: I M.Sc Biochemistry

Code: P16BC21

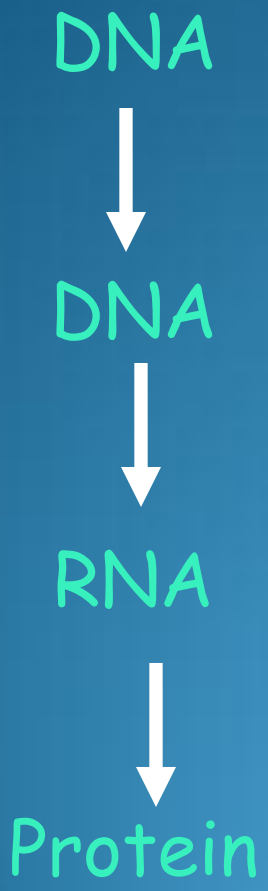
Subject: Molecular Biology

Semester: II

Synopsis

- Introduction
- Prokaryotic transcription
- Eukaryotic transcription
- mRNA splicing
- Capping
- Poly A tailing
- Processing of rRNA in prokaryotes
- Processing of rRNA in Eukaryotes
- Processing of tRNA in prokaryotes
- Inhibitors of transcription

DNA TRANSCRIPTION



Replication

Transcription
- Initiation
- Elongation
- Termination

Translation
- Initiation
- Elongation
- Processing
- Protein Targeting

Transcription

Transcription is the process through which a DNA sequence is enzymatically copied by an RNA polymerase to produce a complementary DNA

Types: Prokaryotic and Eukaryotic Transcription

Prokaryotic Transcription

Requirements: Template, Ribonucleotide triphosphates, RNA polymerase, metal ions and Rho protein.

Steps in Prokaryotic Transcription

I. Initiation II. Elongation III. Termination

Initiation: DNA polymerase recognize promoter region in DNA.

DNA is unwound and becomes single stranded called open complex.

The first nucleotide is added to the initiation complex.

Elongation: Proceeds by the addition of 2^{nd} nucleotide in 5'-3' direction.

After addition of 10 bases the sigma factor dissociates and it becomes available for another initiation of RNA polymerase.

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Termination: i. Rho dependent ii. Rho independent

Rho dependent: A specific protein, named p factor, binds to the growing RNA or weakly to DNA and in the bound state it acts as ATPase and terminates transcription and releases RNA. The p factor is also responsible for the dissociation of RNA polymerase from DNA.

Rho independent: In involves termination sequences within RNA to stop by forming stem- loop hairpin structure. This occurs due to the presence of palindromes. A palindrome is a word that reads alike forward and backward e.g. madam, rotor. The presence of palindromes in the base sequence of DNA template in the termination region is known.

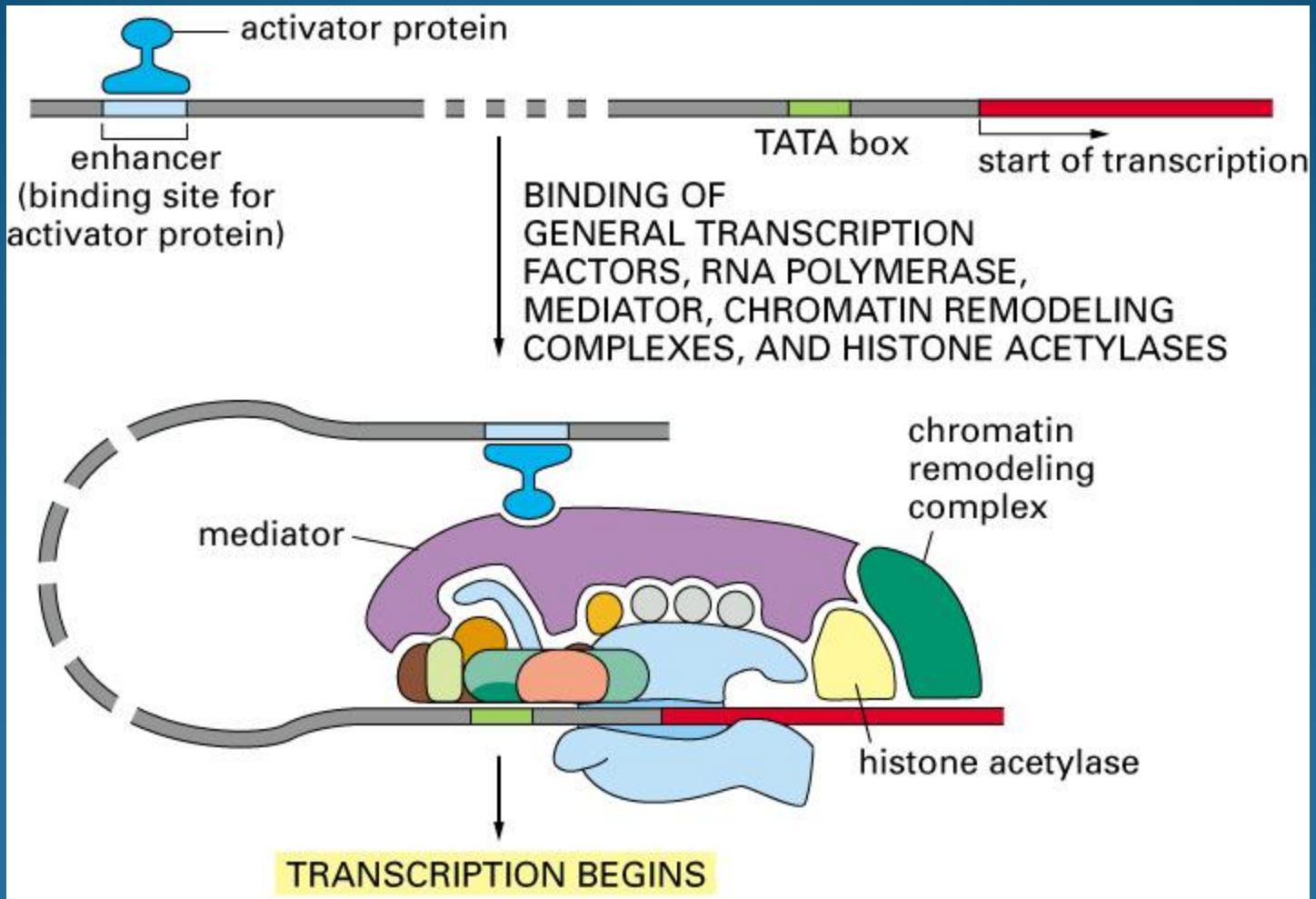


Figure 6–19. Molecular Biology of the Cell, 4th Edition.

Eukaryotic Transcription

Requirements: DNA template, Ribonucleotide triphosphates, RNA polymerase, metal ions, Transcription factors.

RNA Polymerase: Eukaryotes consist of three types of RNA polymerase namely I, II and III, each comprising of two large subunits and 12-15 smaller subunits. The two large subunits are homologous to the E. coli β and β' subunits. Two smaller subunits are similar to the E. coli α sub- unit. The eukaryotic RNA polymerase does not contain any subunit similar to the E. coli Sigma factor. Therefore, initiation should be mediated by other proteins

Most of the eukaryotic protein-coding genes contain segments called introns, which break up the amino acid coding sequence into segments called exons. The transcript of these genes is resulted as pre-mRNA (precursor-mRNA).

The pre-mRNA is then processed in the nucleus where the introns are removed and the exons are spliced together into a translatable mRNA.

The mRNA subsequently released from the nucleus and are translated in to proteins in the cytoplasm by ribosomes.

Mechanism of Transcription:

I. Preinitiation

II. Initiation

III. Promoter clearance

IV. Elongation

V. Termination

Preinitiation: In eukaryotes a sequence of DNA bases known as Hogness box is located on left from upstream and another site called CAAT box. These sites are identified by RNA polymerase.

Initiation: A collection of proteins called transcription factors mediate the binding of RNA polymerase and initiation of transcription.

Transcription factors: TFIIA, TFIIB, TFIID, TFIIF, TFIIE, TFIIH and TBP associated factors.

Promoter clearance: After the first bond is synthesized RNA polymerase must clear the promoter. Once the transcript reaches 23 nucleotides promoter clearance occur by phosphorylation.

Elongation: TFIIE and TFIIF remain associated throughout elongation. RNA polymerase II is dephosphorylated.

Inhibitors of Transcription

Actinomycin D – intercalates between G-C base pairs.

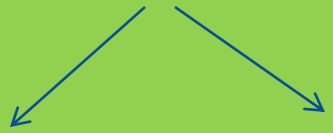
Acridine – inhibit RNA synthesis

Rifampicin – binds to beta subunit and prevent promoter clearance

Post transcriptional modification

Newly synthesized RNA

Primary Transcript



tRNA & rRNA mRNA

RNA Splicing

Eukaryotic mRNA contains non coding sequence called introns separated by Exons.

In a process called splicing introns are removed and exons are joined to form a continuous sequence.

Capping of mRNA

- 5’cap is formed by condensation of GTP at 5’ end.
- Guanine is methylated at N-7 and additional methyl groups are added at 2’OH end of first and second nucleotides adjacent to the cap.

Poly A tailing

- Poly A tailing in Eukaryotes protects mRNA
- Cleavage generates free hydroxyl group to which poly A tail are added by poly adenylate polymerase.

Processing of rRNA in prokaryotes

Pre ribosomal RNA

⬇

30s RNA precursor

⬇

Methylation of 2'OH of ribose

⬇

Cleavage

⬇

Mature RNA(16s, 23s, 5s)

Processing of rRNA in Eukaryotes

Pre ribosomal RNA

45s RNA precursor

Methylation of 2'OH of ribose

Cleavage

Mature RNA(18s, 5.8s, 28s)

Processing of tRNA in prokaryotes and Eukaryotes

Removal of 5’ leader sequence by RNase P

Removal of 3’ trailer sequence by endo and exonuclease

Addition of CCA to 3’ end

Splicing of introns in eukaryotes

Modification of multiple residues

Separation of multiple tRNA

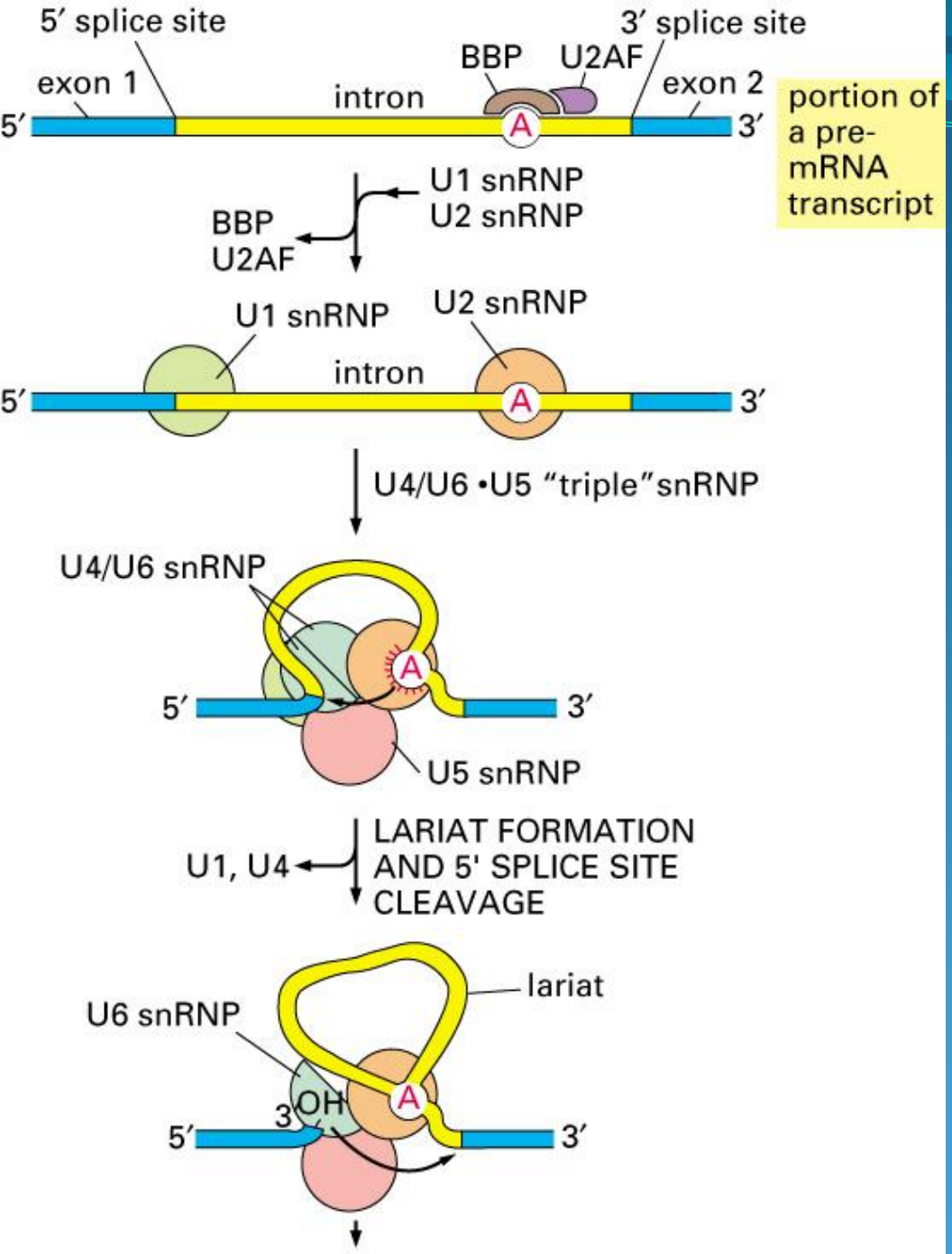


Figure 6–29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

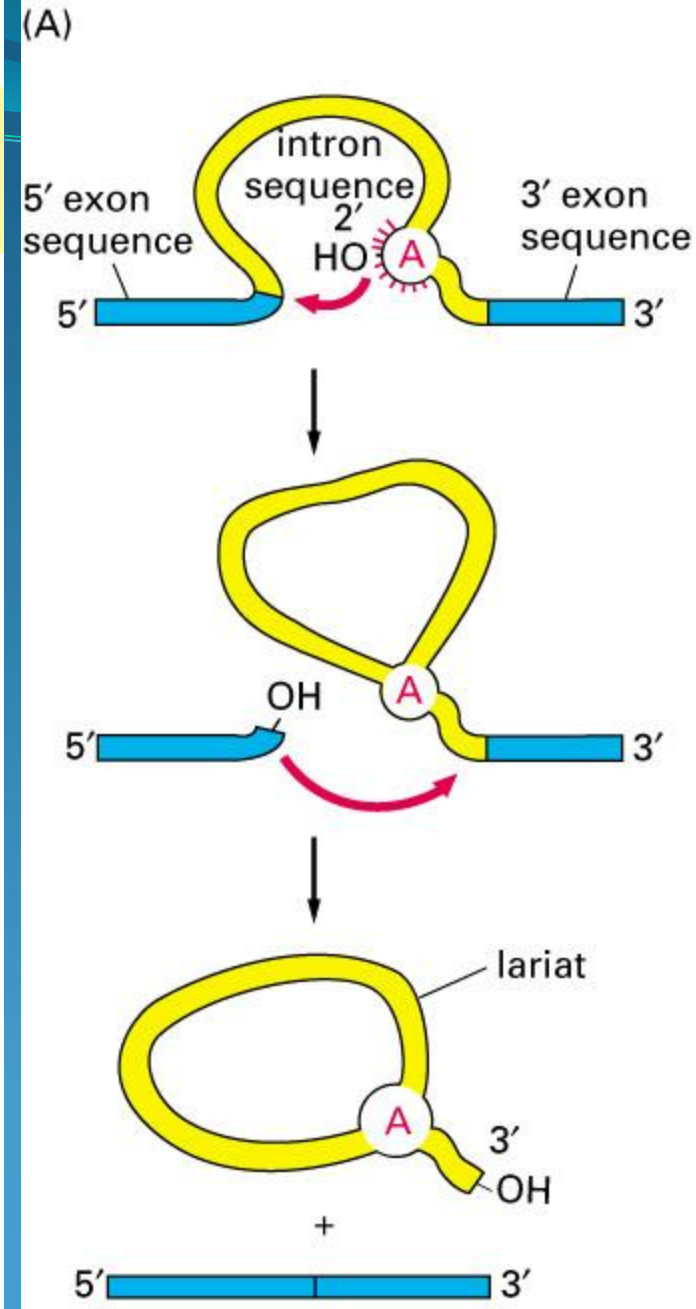


Figure 6–26 part 1 of 2. Molecular Biology of the Cell

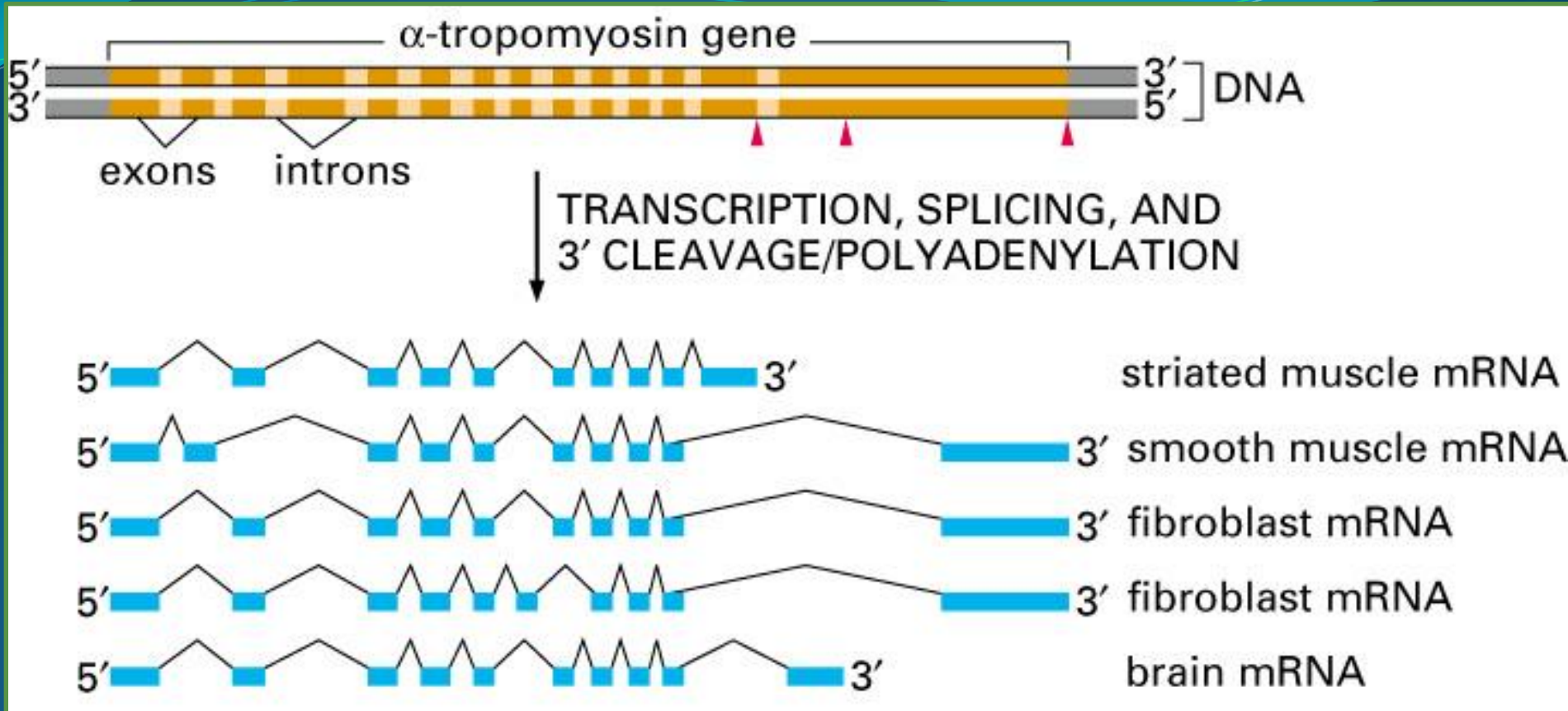


Figure 6–27. Molecular Biology of the Cell, 4th Edition.

Transcript export

- Proteins associated with mRNA mark it for export
- Only mature mRNA is exported from nucleus
- Exit via nuclear pore complexes

Possible Questions

1.Write in brief about Prokaryotic RNA polymerase

2.Explain about Prokaryotic and Eukaryotic Transcription

3. Describe Eukaryotic RNA polymerase

4. Define promoters and Enhancers

5.Explain Rho dependent and Rho independent termination

6. Write a note on Transcriptional inhibitors

7. Describe Poly “A” tailing and Capping

8. Enumerate on RNA splicing

9. Write an essay on post transcriptional modification of tRNA and rRNA in prokaryotes and Eukaryotes.