4.3 TRANSCRIPTION IN EUKARYOTES 2

Transcription is the process of making RNA strand from the DNA template. Both prokaryotes and eukaryotes perform fundamentally the same process of transcription with few differences. The prokaryotes require only RNA polymerase while in eukaryotes three RNA polymerases are needed. The other difference is that the prokaryotic polymerase can bind to a DNA template on its own whereas eukaryotes require several other proteins called **transcription factors** which first bind to the promoter region and then help in the recruitment of the appropriate RNA polymerase.

Enzymes Involved in Eukaryotic Transcription

In eukaryotes there are three types of RNA polymerase which are responsible for transcribing different types of RNA.

- **RNA polymerase I:** RNA polymerase I is found in the **nucleolus** and transcribes r-RNA. r-RNAs is considered as functional RNA and has role in assembly of ribosomes. RNA pol I transcribes all r-RNA like 28 S, 18 S, and 5.8 S rRNA genes except for the 5S r-RNA molecules.
- **RNA Polymerase II:** It is located in the **nucleus** and synthesizes all protein-coding nuclear **pre -mRNAs** (heterogenous or hn RNA), small nuclear RNA s (sno RNAs) and small-nuclear RNAs (sn RNAs). It is a multiprotein complex which is 550 KDa having 12 subunits and the largest subunit contains a carboxyl terminal domain (CTD).
- **RNA polymerase III:** It is also located in the **nucleoplasm.** It transcribes genes for t RNA and 5 S rRNA. The t RNAs plays an important role in translation. They serve as adaptor molecules between the m RNA and growing polypeptide chain.

TABLE 4.2 *Location and products of the eukaryotic RNA polymerase.*

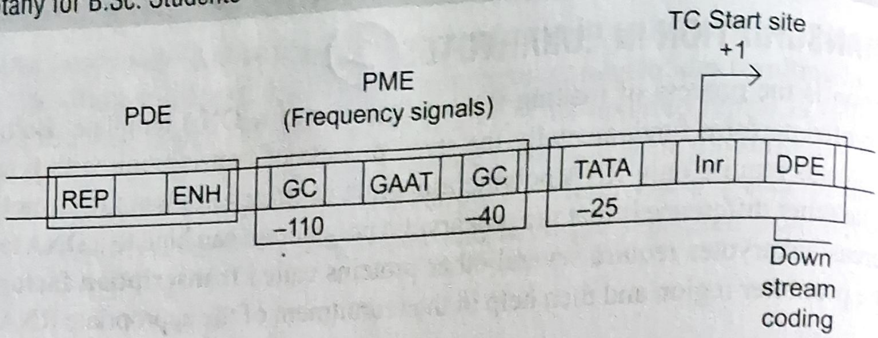
S. No	RNA pol	Location	Product
1.	RNA pol I	Nucleolus	All rRNAs except 5S rRNA
2.	II	Nucleus	All protein coding nuclear pre-m-RNAs
3.	III	Nucleus	5S rRNA,tRNAs and small nuclear RNAs

Process of Eukaryotic transcription can be categorized into three steps:

- Initiation
- Elongation
- Termination

***(i)* Initiation:** During initiation enzyme RNA polymerase II recognizes a specific site on the DNA called as **promoter site** where RNA pol comes and binds. So, **promoter** is a DNA sequence which is present on the non-template or coding strand of DNA. It has three types of initiation signals:

- TATA (Hogness box) which has a consensus sequence of TATA AA and is situated –25 to –30 bp upstream to the transcription start site.
- **Initiator sequences:** Initiator signals are also located somewhere around –3 to +5 bp.
- **Downstream promoter element (DPE):** DPE are also an initiator signals and located +25 bp region. Their initiation signals help to recognize and play an important role in binding of TFIID to the promoter site.



where

REP = Repressor

ENH = Enhance rate of Transcription regulation.

GC = Guanine, Cytosine rich region at –110 & –40 site

CAAT = CAAT box

TATA = TATA box (Hogness box)

Inr = Initiator sequences –3 to +5 bp.

DPE = Downstream promoter element.

PME = Promoter +25 bp proximal elements.

PDE = Promoter distal element.

There are several general transcription factors (GTFs) and mostly called as TF (transcription factor II) because they are transcription factors of RNA polymerase II whi binds to the promoters. It interacts with transcription factors to from pre-initiation complex.

First the TATA box binding protein (TBP) recognizes the TATA box and binds to it. Th TBP is associated with TATA box binding protein Associated factor (TAFs) which consist 13-14 TAFs and TBP in combination with TAFs constitutes TF II D. TF II D is the first GTF that bind to the promoter region then comes another factor i.e., TF II A which stabilizes bindin of TBP to promoter. After this TF II B comes into the picture. TFIIB plays an important role the recruitment of RNA polymerase II and forms a complex with TF II F.

The TF II F binds tightly to RNA pol II TF II B and it prevents binding of RNA pol II t any other non-specific DNA sequences. Then TF II H which helps in the stimulation of ATPas and helicase activity of TF II H. At the end TFIIH binds to the promoter site which posses helicase activity and unwinds DNA at the promoter region and leads to the formation of a open- complex. The phosphorylation of CTD of RNA pol II occurs because of kinase activity of TF II H.

***(ii)* Elongation:** Transcription elongation is aided by various elongation factors. The elongation process involves addition of 5′ phosphate of ribo-nucleotides to the 3′-OH group of elongating heterogenous m-RNA. This addition of nucleotides continued until specific termination signals are encountered.

***(iii)* Termination:** The termination begins by stopping the RNA polymerase. There is a Eukaryotic consensus sequences for termination which is 5′AAUAAA-3′ in the m-RNA and is called as polyadenylation signal or poly-A tail.

TABLE 4.3 *Name and function of general transcription factors.*

S. No.	*Factor*	*Function*
1.	TF II D	Recognition of the TATA box and regulation of TBP binding.
2.	TF II A	Stabilizes TBP and TAF binding.
3.	TF II B	Recruitment of pol II & TF II F; start site recognition for RNA pol II
4.	TF II F	Promoter targeting for pol II
5.	TF II F	TF II H recruitment, modulation of TF II H helicase and ATPase activity
6.	TF II H	Promoter melting; Promoter clearance via phosphorylation of CTD.

Post-Transcriptional Modification of m-RNA

The primary eukaryotic m-RNA transcript is much longer and localized into the nucleus and transported into the cytosol through nuclear-pore. The RNA- transcript is called as **heterogenous nuclear RNA** (hn-RNA) or pre-mRNA.

There are certain post-transcriptional modifications which occurs on both the ends of RNA-transcript. At 5′ end addition of 5′ Cap and addition of poly A tail at 3′ end. On the pre-mRNA strand removal of introns and splicing of exons also occurs simultaneously.

(*i*) 5′end modification of m-RNA (Capping): Capping involves a reaction between the 5′ triphosphate of the terminal nucleotide and the triphosphate of GTP nucleotide. The γ-phosphate of the terminal nucleotide is removed resulting in a 5′–5′ unusual bond. This reaction is carried out by the enzymes **guanylyl transferase**. The second step of capping reaction convert new terminal Guanosine into **7-methylguanosine** by attachment of a methyl group to Nitrogen (N-7) of the purine ring. This reaction is catalyzed by **Guanine methyl transferase** (GMT).

$$\overset{\alpha}{\text{G}}\ \overset{\beta}{\text{p}}\ \overset{\gamma}{\text{p}}\ \text{p} + \overset{\gamma}{\text{p}}\ \overset{\beta}{\text{p}}\ \overset{\alpha}{\text{p}}\ \text{N}$$
$$\downarrow \text{Guanylyl transferase}$$
$$\text{G p p p N - 5}'\text{ -}$$
$$\downarrow \text{Guanine methyl transferase}$$
$$\text{7 - Me - G p p p N - 5}'\text{ -}$$

Fig. 4.7. *Post-transcriptional modification at 5′ end.*

Function of Capping

- The capping of pre-m RNA transcript protects it from degradation.
- The cap also facilities the transport of mature RNA out of the nucleus.
- The cap in needed for proper splicing of a pre- m RNA.

(*ii*) 3′ end modification of eukaryotic mRNA (polyadenylation): The 2nd type of end modification is the addition of 50 to 250 adenine nucleotides at the 3’end forming a poly A tail. The process of adding poly A to m RNA is called polyadenylation.

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