Molecular biology and bacterial genetics

البايولوجي الجزيئي ووراثةالاحياء المجهرية

Lec 8, 9

د.مروة عباس كبة

2020-2019

RNA transcription

The directional flow of genetic information:

- The flow of genetic information in cells generally proceeds from DNA to RNA to protein

- DNA (more precisely, a segment of one DNA strand) first serves as a template for the synthesis of an RNA molecule, which in most cases then directs the synthesis of a particular protein.
- Francis Crick (1956) named the flow of information from DNA to RNAto protein the Central Dogma of molecular biology.

- RNA that is translated into protein is called messenger RNA (mRNA) because it carries a genetic message from DNA to the ribosome, where protein synthesis takes place

- In addition to mRNA two other types of RNA are involoved in protein synthesis, ribosomal RNA (rRNA) molecules, which are integral components of the ribosome, and transfer RNA (tRNA) molecules, which serves as intermediaries that translate the coded base sequence of messenger RNA and bring the appropriate amino acids to the ribosome.

- 2. Synthesis of an RNA molecule using a DNA template is called transcription. Only one of the DNA strands is transcribed. The enzyme used is RNA polymerase.
-
- There are four major types of RNA molecules:

a. Messenger RNA (mRNA) encodes the amino acid sequence of a polypeptide.

b. Transfer RNA (tRNA) brings amino acids to ribosomes during translation.

c. Ribosomal RNA (rRNA) combines with proteins to form a ribosome, the catalyst for translation.

d. Small nuclear RNA (snRNA) combines with proteins to form complexes used in eukaryotic RNA processing.

The Transcription (Process RNA Synthesis)

- Transcription, or gene expression, is regulated by gene regulatory elements associated with each gene.
- DNA unwinds in the region next to the gene, due to RNA polymerase in prokaryotes and other proteins in eukaryotes. In both, RNA polymerase catalyzes transcription

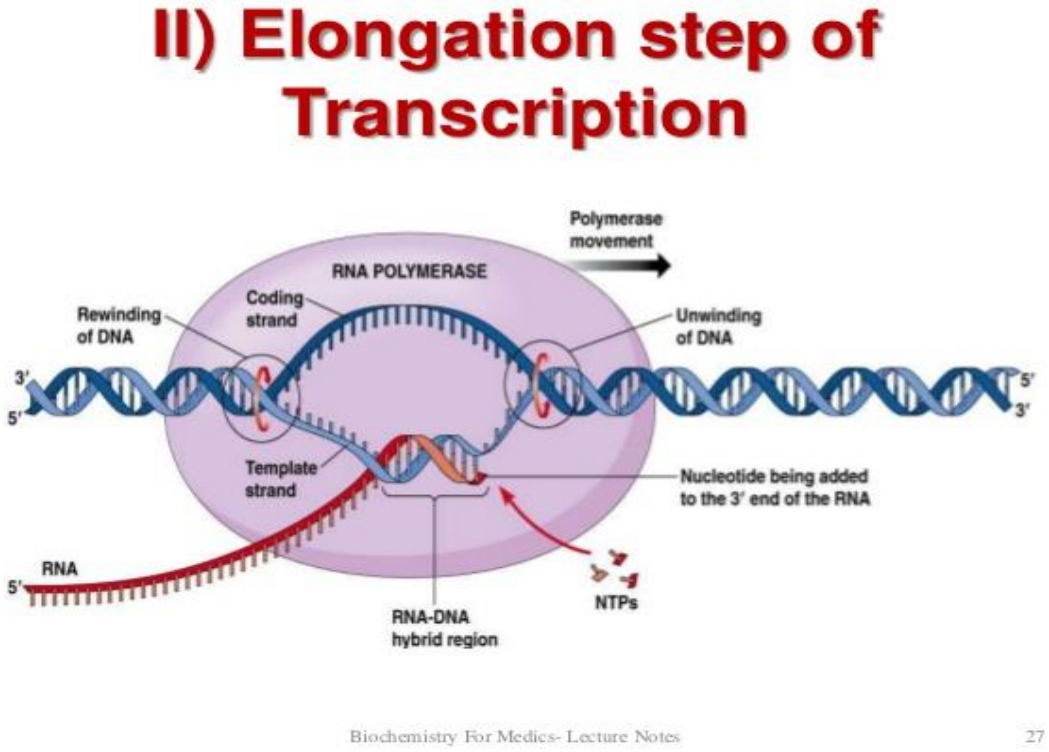


Figure 1: Transcription process.(Hardin, J.; Bertoni And L.J.Kleinsmith, World of the cell, 8th edition,2012, Beakers)

- RNA is transcribed 5'-to-3'. The template DNA strand is read 3'-to-5'. Its complementary DNA, the nontemplate strand, has the same polarity as the RNA.
- 4. RNA polymerization is similar to DNA synthesis (Figure 5.2), except:

a. The precursors are NTPs (not dNTPs).

b. No primer is needed to initiate synthesis.

c. No proofreading occurs.

d. Uracil is inserted instead of thymine.

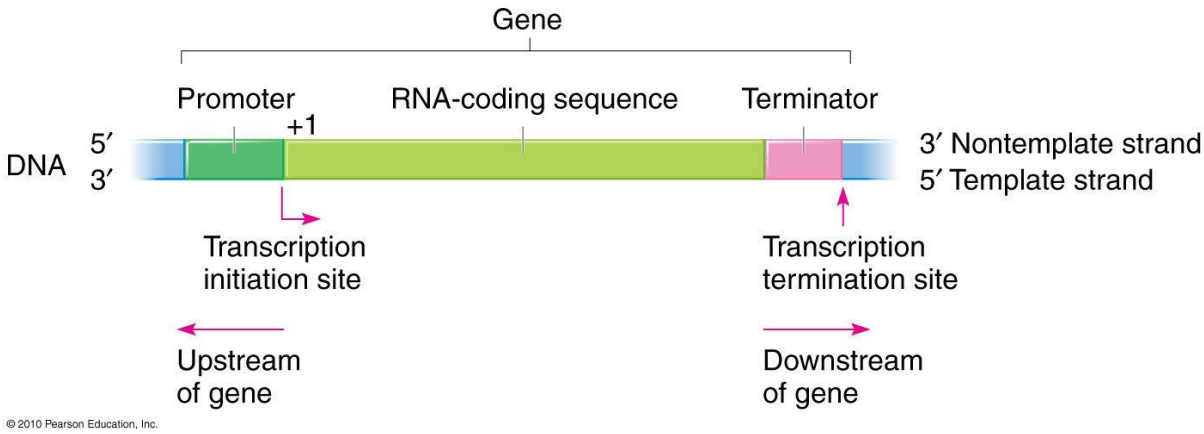
The Transcription Process Initiation of Transcription at Promoters

- Transcription is divided into three steps for both prokaryotes and eukaryotes. They are initiation, elongation and termination. The process of elongation is highly conserved between prokaryotes and eukaryotes, but initiation and termination are somewhat different.
- This section is about initiation of transcription in prokaryotes. E. coli is the model organism.
- A prokaryotic gene is a DNA sequence in the chromosome. The gene has three regions, each with a function in transcription:

a. A promoter sequence that attracts RNA polymerase to begin transcription at a site specified by the promoter.

b. The transcribed sequence, called the RNA-coding sequence. The sequence of this DNA corresponds with the RNA sequence of the transcript.

c. A terminator region downstream of the RNA-coding sequence that specifies where transcription will stop.



- Transcription initiation requires the RNA polymerase holoenzyme to bind to the promoter DNA sequence. Holoenzyme consists of:

a. Core enzyme of RNA polymerase, containing four polypeptides (two α, one β and one β').

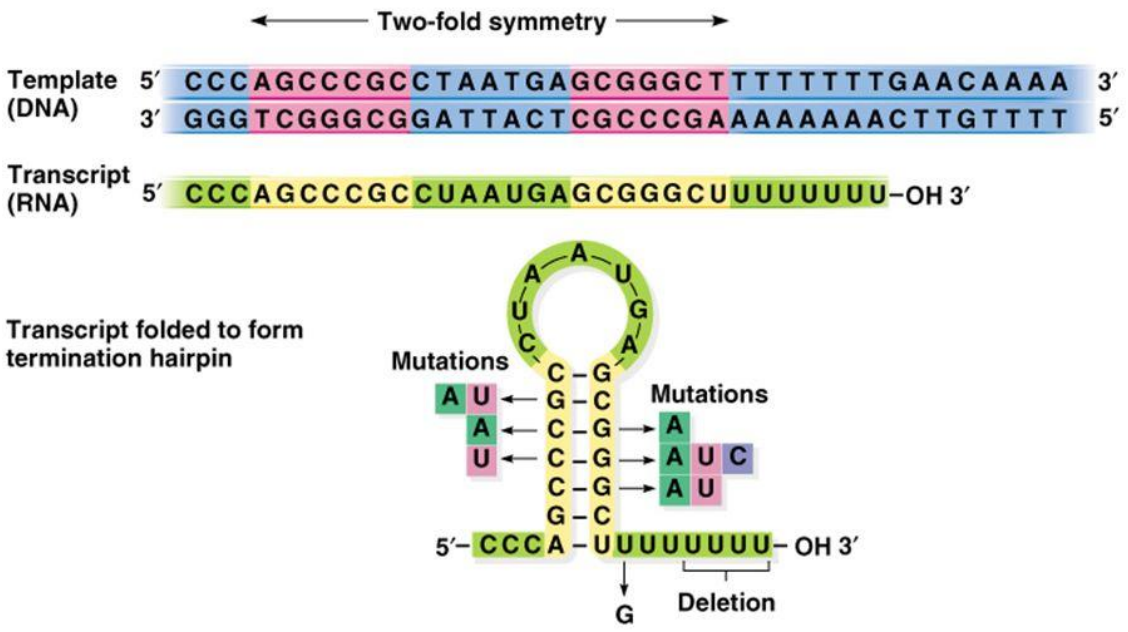
b. Sigma factor (σ).

7. Sigma factor binds the core enzyme, and confers ability to recognize promoters and initiate RNA synthesis. Without sigma, the core enzyme randomly binds DNA but does not transcribe it efficiently.

- Terminator sequences are used to end transcription. In prokaryotes there are two types:
  a. **Rho-independent (ρ-independent) or type I terminators** have two-fold symmetry that would allow a hairpin loop to form (Figure). The palindrome is followed by 4-8U residues in the trasncript, and together these sequences cause termination, possibly because rapid hairpin formation destabilizes the RNA-DNA hybrid.

b. Rho-dependent (ρ-dependent) or type II terminators lack the poly(U) region, and many also lack the palindrome. The protein ρ is required for termination. It has two domains, one binding RNA and the other binding ATP. ATP hydrolysis provides energy for ρ to move along the transcript and destablize the RNA-DNA hybrid at the termination region.

Fig. 5.5 Sequence of a ρ-independent terminator and structure of the terminated RNA



Transcription in Eukaryotes:

Eukaryotes have three different polymerases, each transcribing a different class of RNA. Processing of transcripts is also more complex in eukaryotes.

Eukaryotic RNA Polymerases

a. RNA polymerase I, located in the nucleolus, synthesizes three of the four rRNAs found in ribosomes: three of the RNAs (the 28S, 18S, and 5.8S rRNA molecules).

b. RNA polymerase II, located in the nucleoplasm, synthesizes messenger RNAs (mRNAs; translated to produce polypeptides) and some small nuclear RNAs (snRNAs), some of which are involved in RNA processing events.

c. RNA polymerase III, also located in the nucleoplasm, synthesizes the transfer RNAs (tRNAs), which bring amino acids to the ribosome; 5S rRNA, the fourth rRNA molecule found in each ribosome; and the small nuclear RNAs (snRNAs) not made by RNA polymerase II.

Transcription of Protein-Coding Genes by RNA Polymerase II

Prompters control the expression of protein coding genes. basal promoter elements are located near the transcription start site. Examples include:

i. The TATA box: its full sequence is TATAAAA. This element aids in local DNA denaturation, and sets the start point for transcription.

ii. The initiator element (Inr), a pyramiding-rich sequence near the transcription start site.

Characteristics of enhancers:

a. They are found in single or multiple copies.

b. They function in either orientation.

c. They function upstream, downstream or within the gene, although they are usually located upstream.

d. They may be several kb from the gene they control.

Eukaryotic mRNAs

a.Eukaryotes and prokaryotes produce mRNAs somewhat differently

Prokaryotes use the RNA transcript as mRNA without modification. Transcription and translation are coupled in the cytoplasm. Messages may be polycistronic.

b. Eukaryotes modify pre-RNA into mRNA by RNA processing. The processed mRNA migrates from nucleus to cytoplasm before translation. Messages are always monocistronic.

Fig. 5.9 Processes for synthesis of functional mRNA in prokaryotes and eukaryotes

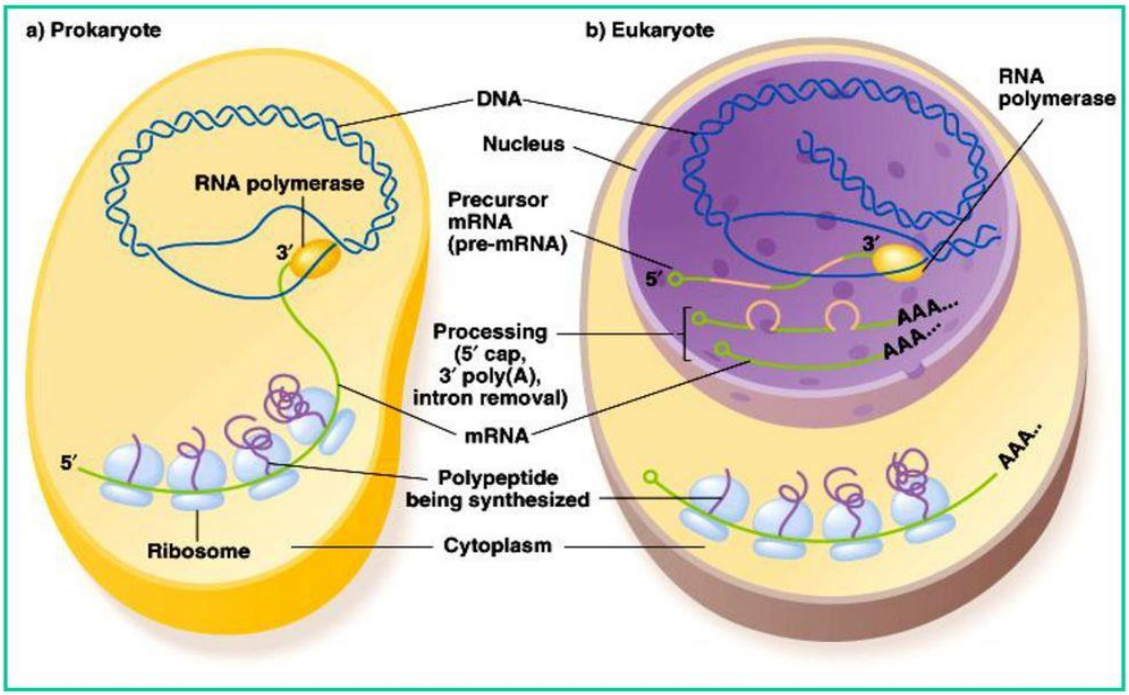


Figure: processes for synthesis of functional mRNA in prokaryotes and eukaryotes. (Russell, P., I Genetics, a molecular approach, 3rd edition, 2009, edited by Yue-Wen Wang. Pearsons, USA).

Production of Mature mRNA in Eukaryotes

Production of Mature mRNA in Eukaryotes

- Eukaryotic pre-RNAs often have introns (intervening sequences) between the exons (expressed sequences) that are removed during RNA processing
- 5’ and 3’ Modifications 1. The newly made 5’ end of the mRNA is modified by 5’ capping. A capping enzyme adds a guanine, usually 7-methyl guanosine (m7G), to the 5’ end using a 5’-to-5’ linkage (Figure 5.9). Sugars of the 2 adjacent nt are also methylated. The cap is used for ribosome binding to the mRNA during translation initiation.

- The 3’ end of the pre-RNA has 50–250 adenines added enzymatically to form a poly(A) tail. The poly(A) tail is important in mRNA stability, and also plays a role in transcription termination
- Removal of introns is necessary for mRNA maturation

Events in eukaryotic mRNA production are summarized in Figure They include:

a. Transcription of the gene by RNA polymerase II

.b. Addition of the 5’ cap.

c. Addition of the poly(A) tail.

d. Splicing to remove introns.

Events in splicing together two exons (designated 1 and 2):
a. cleavage occurs at the 5’ splice junction of exon 1 and the intron.

b. The G nucleotide at the free 5’ end of the intron joins with a specific A nucleotide (18-38 nt upstream of the 3’ spice junction) in the branch- point sequence of the intron, forming an RNA lariat structure.

c. The bond forming the lariat is a 2’-5’ phosphodiester linkage between the 5’ phosphate of the free guanine nt at the end of introns, and the 2’ OH of the adenine nt in the branch-point sequence

d. The introns lariat is excised, and the exons are joined to form a spliced mRNA. The introns RNA is degraded by the cell.

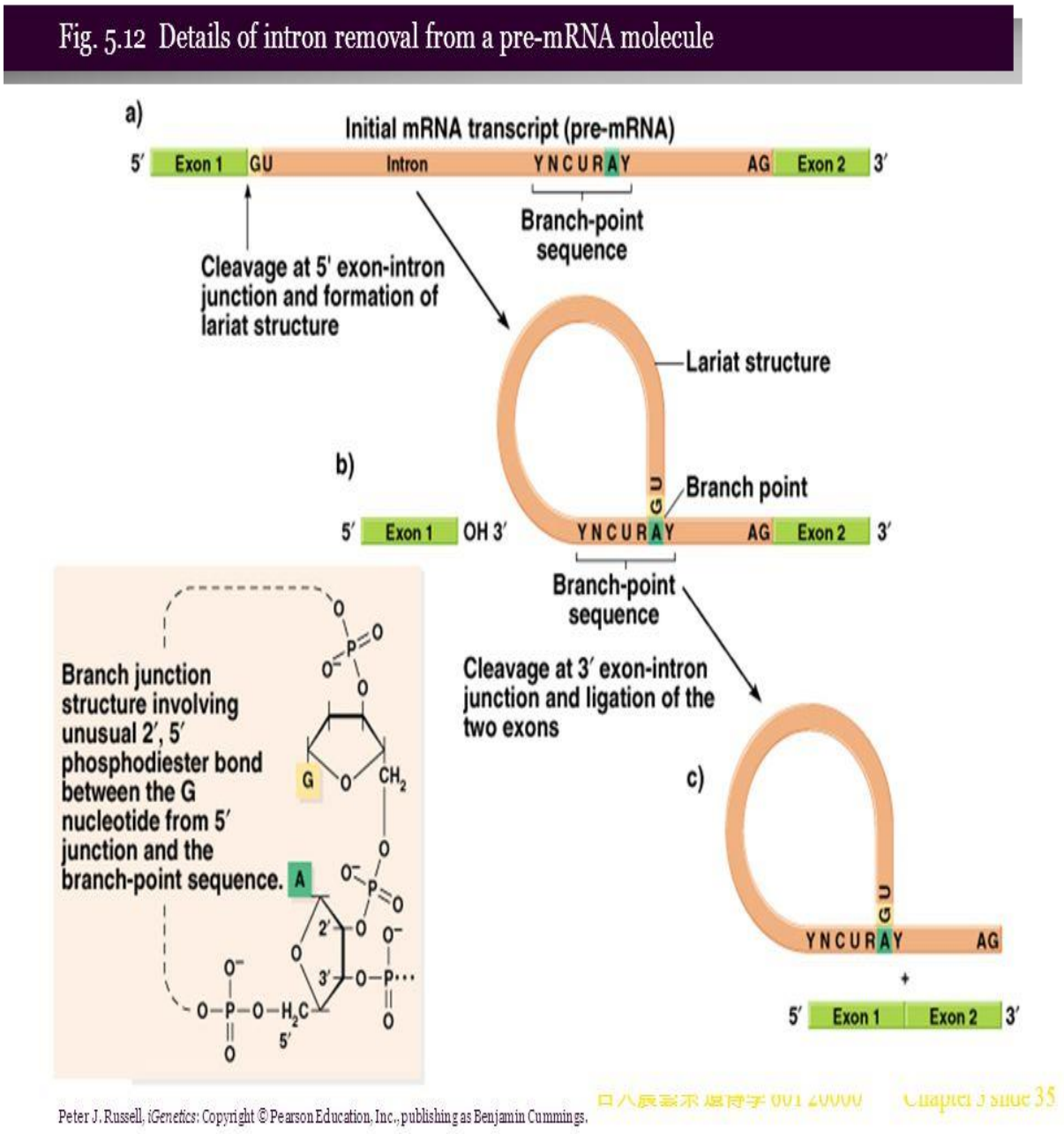


Figure: details of intron removal from a pre-mRNA molecule.(Russell,P. I Genetics, a molecular approach, 3th edition, 2009,edited by Yue-Wen Wang. Pearsons,USA).