Transcription

RNA (ribonucleic acid) is a key intermediary between a DNA sequence and a polypeptide. RNA is an informational polynucleotide similar to DNA, but it differs from DNA in three ways:

• RNA generally consists of only one polynucleotide strand.

• The sugar molecule found in RNA is ribose, rather than the deoxyribose found in DNA.

• Although three of the nitrogenous bases (adenine, guanine, and cytosine) in RNA are identical to those in DNA, the fourth base in RNA is uracil (U), which is similar to thymine but lacks the methyl (—CH_3) group

In the process of RNA synthesis, the information contained in DNA is transcribed into RNA. During transcription, the information in a DNA sequence (a gene) is copied into a complementary RNA sequence. The process occurs in the nucleus and the resulting RNA is carried to the cytoplasm, where the protein synthesis occurs.

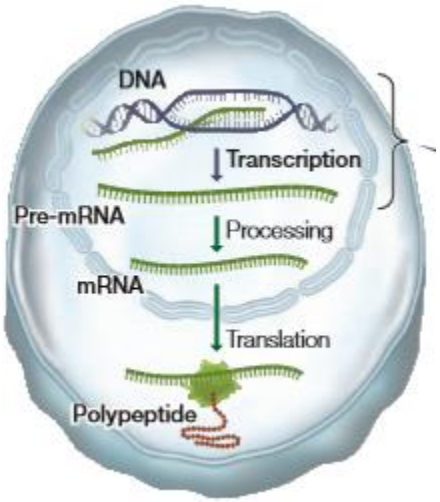


Figure 1- Sites of transcription and translation (protein synthesis) in a eukaryotic cell. Image courtesy- Sadava et al, Life: The science of Biology, 9th edition.

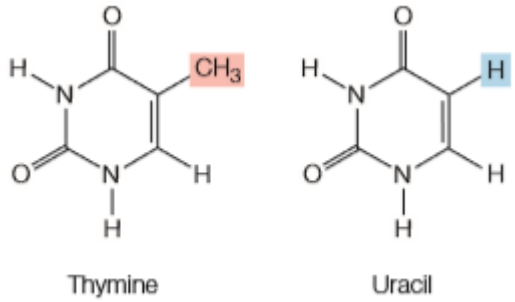


Figure 2- The nucleotide Thymine (present only in DNA) is replaced by Uracil (present only in RNA). The pairing of the ribonucleotides obeys the same complementary base-pairing rules as in DNA, except that adenine pairs with uracil instead of thymine. Image courtesy- Sadava et al, Life: The science of Biology, 9th edition.

Single-stranded RNA can fold into complex shapes by internal base pairing. Three types of RNA participate in protein synthesis:

• Messenger RNA (mRNA) carries a copy of a gene sequence in DNA to the site of protein synthesis at the ribosome.

• Transfer RNA (tRNA) carries amino acids to the ribosome for assembly into polypeptides.

• Ribosomal RNA (rRNA) catalyzes peptide bond formation and provides a structural framework for the ribosome.

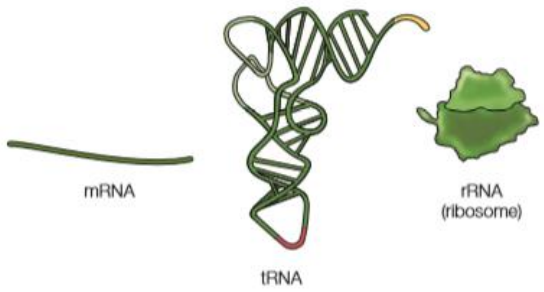


Figure 3- Types of RNA. Transcription is responsible for the synthesis of mRNA, tRNA and ribosomal RNA (rRNA), who play important roles in protein synthesis. Image courtesy- Sadava et al, Life: The science of Biology, 9th edition.

Transcription requires several components:

• A DNA template for complementary base pairing; one of the two strands of DNA

• The appropriate nucleoside triphosphates (ATP, GTP, CTP, and UTP) to act as substrates

• An RNA polymerase enzyme- Like DNA polymerases, RNA polymerases are *processive*; that is, a single enzyme–template binding event results in the polymerization of hundreds of RNA bases. But unlike DNA polymerases, RNA polymerases do not require a primer and do not have a proofreading function.

Transcription occurs in three stages:

a) initiation,
b) elongation, and
c) termination

a) **INITIATION**- Transcription begins with initiation, which requires a promoter, a special sequence of DNA to which the RNA polymerase recognizes as a start site and binds very tightly. Eukaryotic genes generally have one promoter each, while in prokaryotes and viruses, several genes often share one promoter. Promoters are important control sequences that "tell" the RNA polymerase two things:

• Where to start transcription

• Which strand of DNA to transcribe (which of the two strands of DNA will act as template for producing a single RNA strand)

Part of each promoter is the initiation site, where transcription begins. Groups of nucleotides lying "upstream" from the initiation site (5′ on the non-template strand, and 3′ on the template strand) help the RNA polymerase bind.

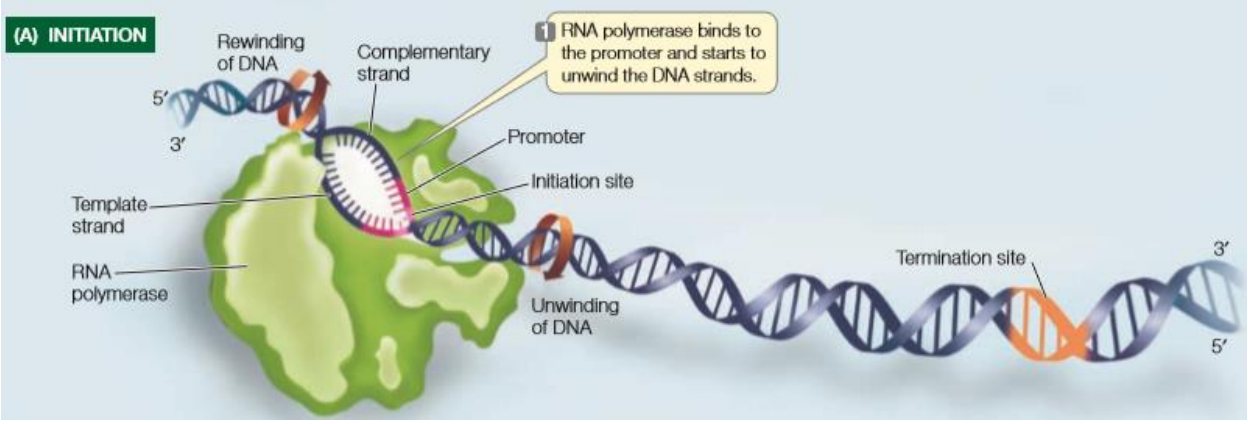


Figure 4- Initiation of RNA synthesis by RNA polymerase. Image courtesy- Sadava et al, Life: The science of Biology, 9th edition.

b) **ELONGATION**- Once RNA polymerase has bound to the promoter, it begins the process of elongation. RNA polymerase unwinds the DNA about 10 base pairs at a time and reads the template strand in the 3′-to-5′direction. Like DNA polymerase, RNA polymerase adds new nucleotides to the 3′ end of the growing strand, but does not require a primer to get this process started. The RNA transcript produced is antiparallel to the DNA template strand.

Because RNA polymerases do not proofread, transcription errors occur at a rate of one for every 10^4 to 10^5 bases. Because many copies of RNA are made, however, and because they often have only a relatively short life span, these errors are not as potentially harmful as mutations in DNA.

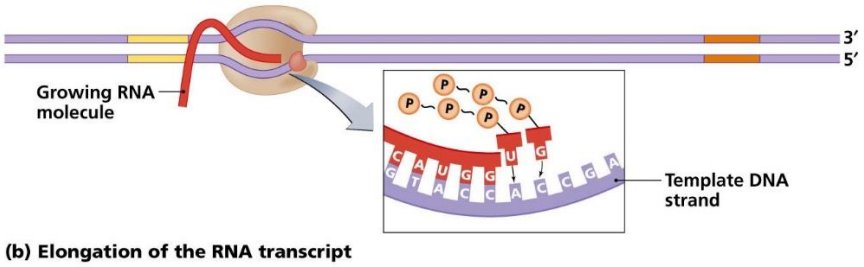


Figure 5- Successive addition of ribonucleotides to the 3' growing end of the newly synthesized RNA transcript.

c) **TERMINATION-** Just as initiation sites in the DNA template strand specify the starting point for transcription, particular base sequences specify its termination. For some genes, the newly formed transcript falls away from the DNA template and the RNA polymerase. For others, a helper protein pulls the transcript away.

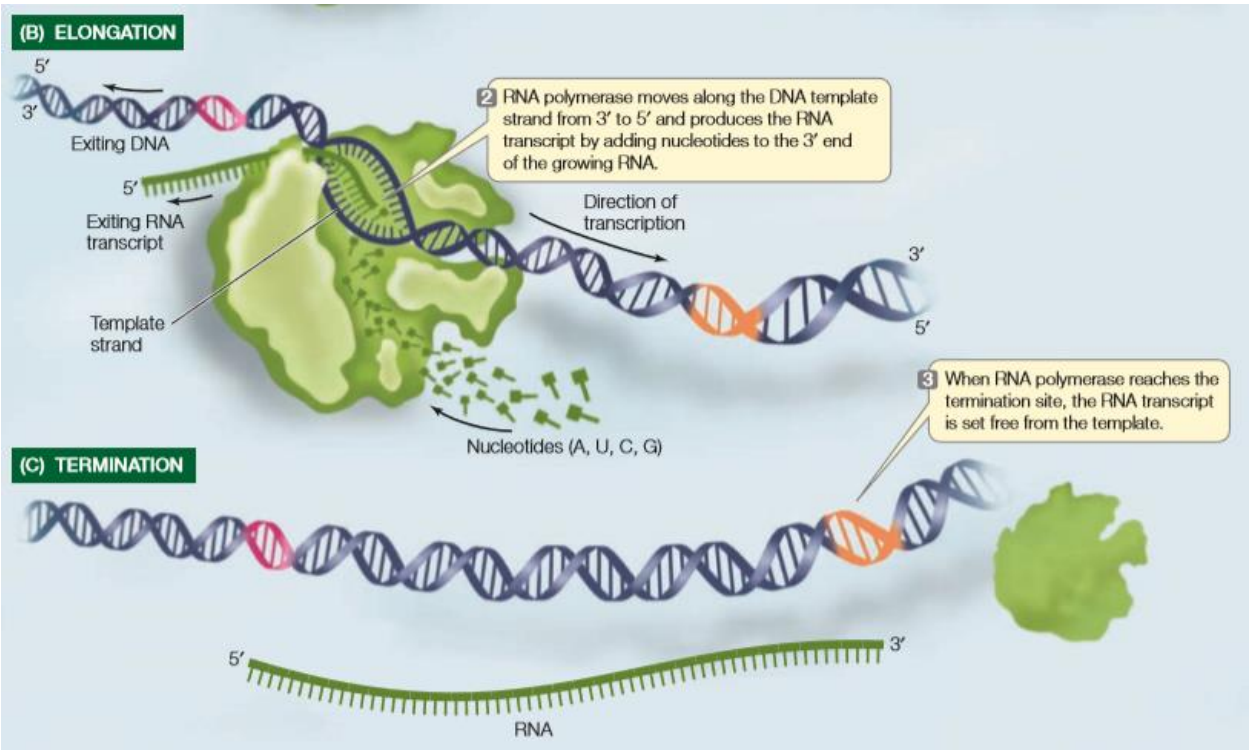


Figure 6- Process of elongation and termination in transcription of RNA. Image courtesy- Sadava et al, Life: The science of Biology, 9th edition.

Difference in transcription between Prokaryotes and Eukaryotes

- **Initiation**:

In bacterial cells, the holoenzyme (RNA polymerase plus sigma) recognizes and binds directly to sequences in the promoter. In eukaryotic cells, promoter recognition is carried out by accessory proteins (transcription factors) that bind to the promoter and then recruit a specific RNA polymerase (I, II or III) to the promoter.

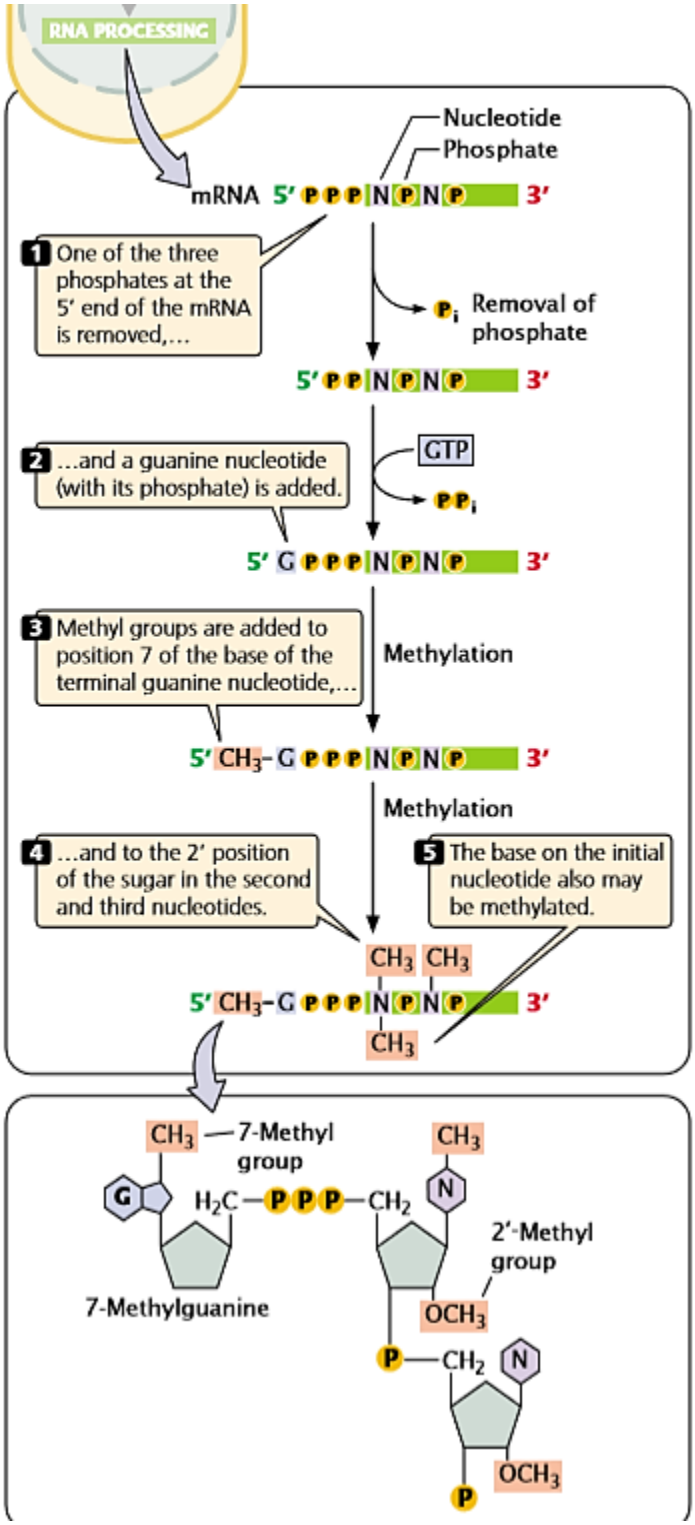
RNA processing:

In prokaryotes, several adjacent genes sometimes share one promoter; however, in eukaryotes, each gene has its own promoter, which usually precedes the coding region.

Eukaryotic genes undergo a systematic process called RNA processing to produce a mature mRNA from pre mRNA.

Eukaryotic genes may contain noncoding base sequences, called introns (intervening regions). One or more introns may be interspersed with the coding sequences, which are called exons (expressed regions). Both introns and exons appear in the primary mRNA transcript, called pre-mRNA, but the introns are removed by the time the mature mRNA—the mRNA that will be translated—leaves the nucleus (figure 1). Pre-mRNA processing involves cutting introns out of the pre-mRNA transcript and splicing together the remaining exon transcripts.

Eukaryotic gene transcripts are processed before translation: The primary transcript of a eukaryotic gene is modified in several ways before it leaves the nucleus: both ends of the pre mRNA are modified, and the introns are removed.



MODIFICATION AT BOTH ENDS Two steps in the processing of pre mRNA take place in the nucleus, one at each end of the molecule.

• A G cap is added to the 5′ end of the pre-mRNA as it is transcribed. The G cap is chemically modified (methylated) guanosine triphosphate (GTP). It facilitates the binding of mRNA to the ribosome for translation, and it protects the mRNA from being digested by ribonucleases that break down RNAs.

Figure 7- Addition of 5' cap. Image courtesy- Genetics- : A Conceptual Approach; by Benjamin A. Pierce

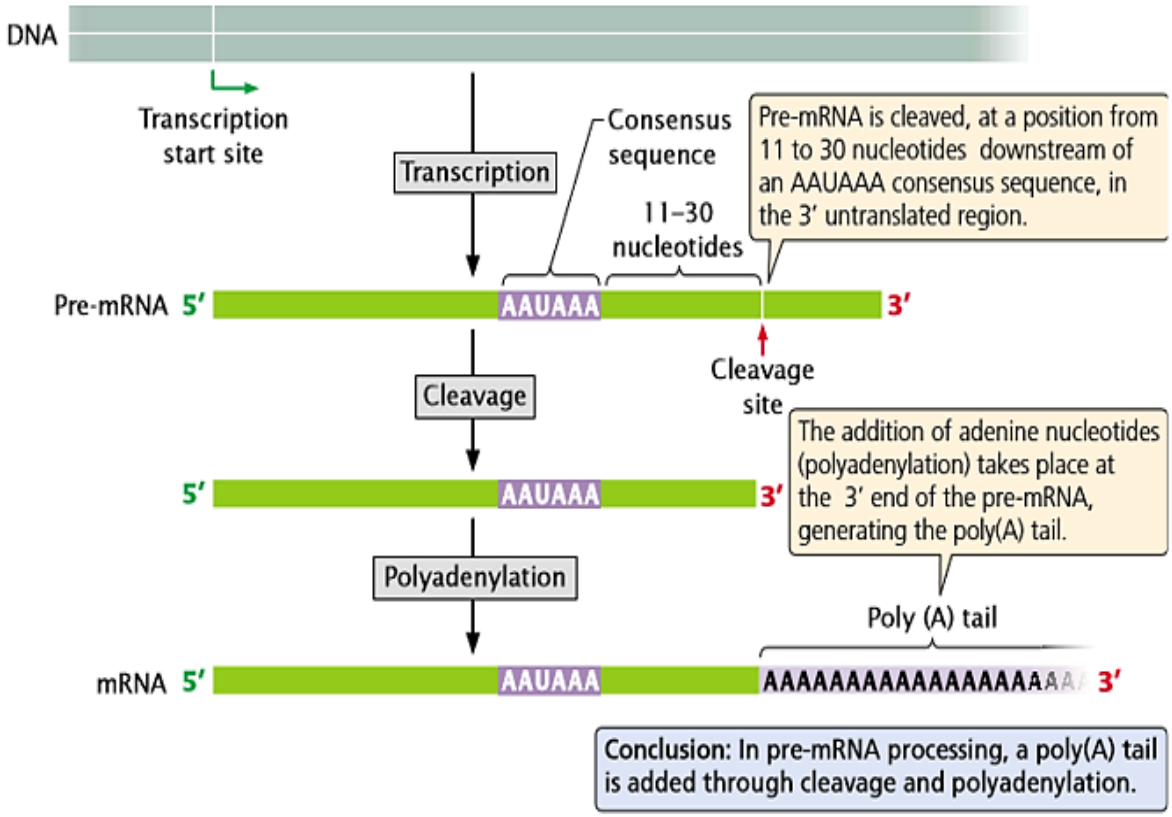
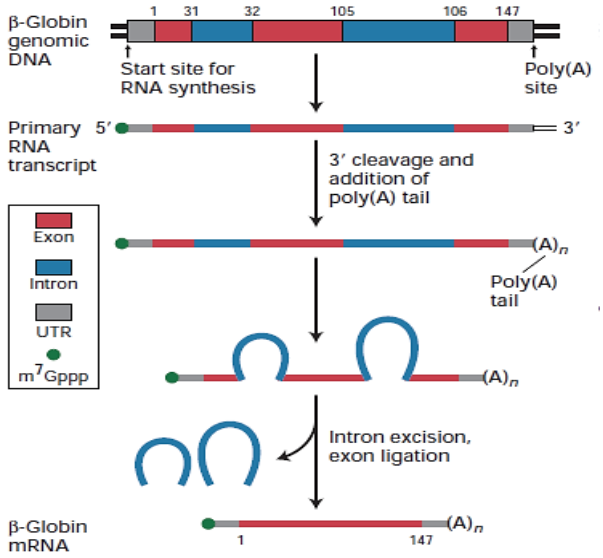


Figure 8- Addition of 50 to 250 adenine nucleotides at the 3 end is called Polyadenylation, which occurs after transcription is completed. Image courtesy- Genetics- : A Conceptual Approach; by Benjamin A. Pierce

• A poly A tail is added to the 3′ end of the pre-mRNA at the end of transcription. In both prokaryotic and eukaryotic genes, transcription begins at a DNA sequence that is upstream (to the "left" on the DNA) of the first codon (i.e., at the promoter), and ends downstream (to the "right" on the DNA) of the termination codon. In eukaryotes, there is usually a "polyadenylation" sequence (AAUAAA) near the 3′ end of the pre-mRNA, after the last codon. This sequence acts as a signal for an enzyme to cut the pre mRNA. Immediately after this cleavage, another enzyme adds 100 to 300 adenine nucleotides (a "poly A" sequence) to the 3′ end of the pre-mRNA. This "tail" may assist in the export of the mRNA from the nucleus and is important for mRNA stability.

Splicing-



The next step in the processing of eukaryotic pre-mRNA within the nucleus is removal of the introns. If these RNA sequences were not removed, a very different amino acid sequence, and possibly a nonfunctional protein, would result. A process called RNA splicing removes the introns and splices the exons together.

Figure 9- The process of splicing beta globin gene. (UTR- untranslated region).

Alternative splicing

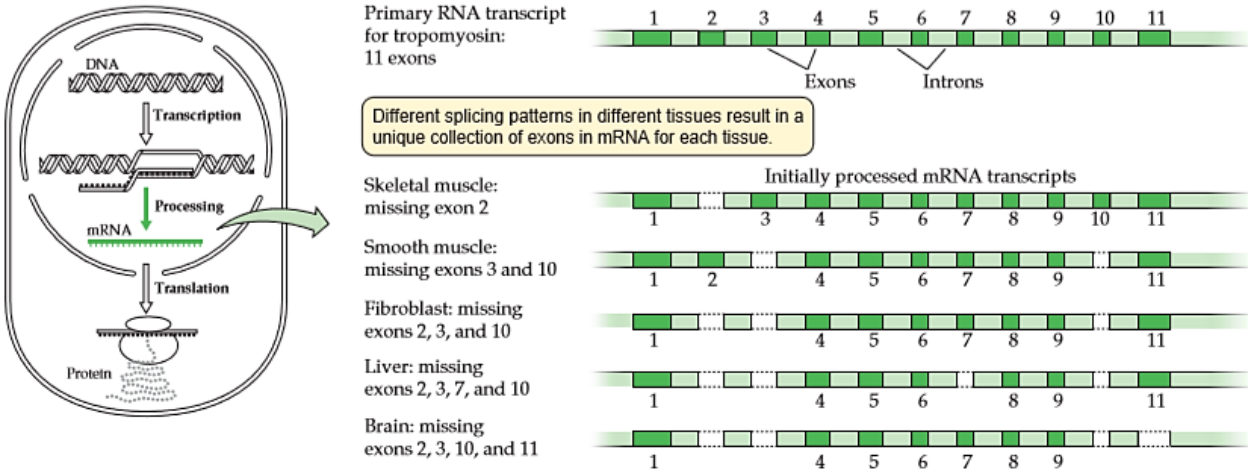


Figure 10- Alternative Splicing Results in Different mRNAs and Proteins In mammals, the protein tropomyosin is encoded by a gene that has 11 exons. Tropomyosin pre-mRNA is spliced differently in different tissues, resulting in five different forms of the protein.

Alternate splicing is a mechanism in which the differential splicing of the same pre- mRNA gives rise to different proteins, which may have different functions. It can be a deliberate mechanism for generating a family of different proteins from a single gene. For example, a single pre-mRNA for the structural protein tropomyosin is spliced differently in five different tissues to give five different mature mRNAs. These mRNAs are translated into the five different forms of tropomyosin found in these tissues: skeletal muscle, smooth muscle, fibroblast, liver, and brain.