15.3 Eukaryotic Transcription

By the end of this section, you will be able to do the following:

- List the steps in eukaryotic transcription
- Discuss the role of RNA polymerases in transcription
- Compare and contrast the three RNA polymerases
- Explain the significance of transcription factors

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few key differences. The most important difference between prokaryote and eukaryote transcription is due to the latter's membrane-bound nucleus and organelles. With the genes bound in a nucleus, the eukaryotic cell must be able to transport its mRNA to the cytoplasm and must protect its mRNA from degrading before it is translated. Eukaryotes also employ three different polymerases that each transcribe a different subset of genes. Eukaryotic mRNAs are usually *monogenic*, meaning that they specify a single protein.

Initiation of Transcription in Eukaryotes

Unlike the prokaryotic polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called transcription factors, to first bind to the promoter region and then to help recruit the appropriate polymerase.

The Three Eukaryotic RNA Polymerases

The features of eukaryotic mRNA synthesis are markedly more complex than those of prokaryotes. Instead of a single polymerase comprising five subunits, the eukaryotes have three polymerases that are each made up of 10 subunits or more. Each eukaryotic polymerase also requires a distinct set of transcription factors to bring it to the DNA template.

RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes (Table 15.1). The rRNA molecules are considered structural RNAs because they have a cellular role but are not translated into protein. The rRNAs are components of the ribosome and are essential to the process of translation. RNA polymerase I synthesizes all of the rRNAs from the tandemly duplicated set of 18S, 5.8S, and 28S ribosomal genes. (Note that the "S" designation applies to "Svedberg" units, a nonadditive value that characterizes the speed at which a particle sediments during centrifugation.)

Locations, Products, and Sensitivities of the Three Eukaryotic RNA Polymerases

RNA Polymerase	Cellular Compartment	Product of Transcription	α-Amanitin Sensitivity
I	Nucleolus	All rRNAs except 5S rRNA	Insensitive
II	Nucleus	All protein-coding nuclear pre-mRNAs	Extremely sensitive
III	Nucleus	5S rRNA, tRNAs, and small nuclear RNAs	Moderately sensitive

Table 15.1

RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs. Eukaryotic pre-mRNAs undergo extensive processing after transcription but before translation. For clarity, this module's discussion of transcription and translation in eukaryotes will use the term "mRNAs" to describe only the mature, processed molecules that are ready to be translated. RNA polymerase II is responsible for transcribing the overwhelming majority of eukaryotic genes.

RNA polymerase III is also located in the nucleus. This polymerase transcribes a variety of structural RNAs that includes the 5S pre-rRNA, transfer pre-RNAs (pre-tRNAs), and **small nuclear** pre-**RNAs**. The tRNAs have a critical role in translation; they serve as the "adaptor molecules" between the mRNA template and the growing polypeptide chain. Small nuclear RNAs have a variety of functions, including "splicing" pre-mRNAs and regulating transcription factors.

A scientist characterizing a new gene can determine which polymerase transcribes it by testing whether the gene is expressed in the presence of α-amanitin, an oligopeptide toxin produced by the fly agaric toadstool mushroom and other species of *Amanita*. Interestingly, the α-amanitin affects the three polymerases very differently (Table 15.1). RNA polymerase I is completely

insensitive to α-amanitin, meaning that the polymerase can transcribe DNA in vitro in the presence of this poison. RNA polymerase III is moderately sensitive to the toxin. In contrast, RNA polymerase II is extremely sensitive to α-amanitin. The toxin prevents the enzyme from progressing down the DNA, and thus inhibits transcription. Knowing the transcribing polymerase can provide clues as to the general function of the gene being studied. Because RNA polymerase II transcribes the vast majority of genes, we will focus on this polymerase in our subsequent discussions about eukaryotic transcription factors and promoters.

RNA Polymerase II Promoters and Transcription Factors

Eukaryotic promoters are much larger and more intricate than prokaryotic promoters. However, both have a sequence similar to the -10 sequence of prokaryotes. In eukaryotes, this sequence is called the TATA box, and has the consensus sequence TATAAA on the coding strand. It is located at -25 to -35 bases relative to the initiation (+1) site (Figure 15.10). This sequence is not identical to the *E. coli* -10 box, but it conserves the A–T rich element. The thermostability of A–T bonds is low and this helps the DNA template to locally unwind in preparation for transcription.

Instead of the simple σ factor that helps bind the prokaryotic RNA polymerase to its promoter, eukaryotes assemble a complex of transcription factors required to recruit RNA polymerase II to a protein coding gene. Transcription factors that bind to the promoter are called *basal transcription factors*. These basal factors are all called TFII (for Transcription Factor/polymerase II) plus an additional letter (A-J). The core complex is TFIID, which includes a TATA-binding protein (TBP). The other transcription factors systematically fall into place on the DNA template, with each one further stabilizing the pre-initiation complex and contributing to the recruitment of RNA polymerase II.

VISUAL CONNECTION

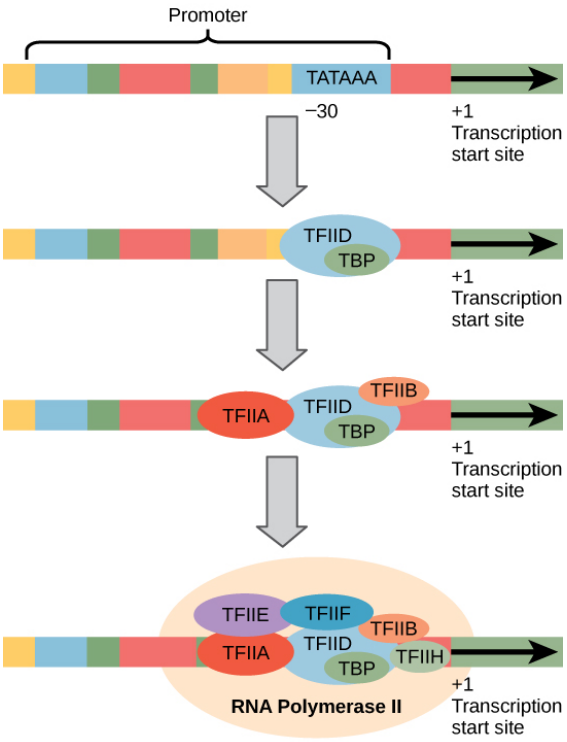

Figure 15.10 A generalized promoter of a gene transcribed by RNA polymerase II is shown. Transcription factors recognize the promoter. RNA polymerase II then binds and forms the transcription initiation complex.

A scientist splices a eukaryotic promoter in front of a bacterial gene and inserts the gene in a bacterial chromosome. Would you expect the bacteria to transcribe the gene?

Some eukaryotic promoters also have a conserved **CAAT box** (GGCCAATCT) at approximately -80. Further upstream of the TATA box, eukaryotic promoters may also contain one or more **GC-rich boxes** (GGCG) or **octamer boxes** (ATTTGCAT). These elements

bind cellular factors that increase the efficiency of transcription initiation and are often identified in more "active" genes that are constantly being expressed by the cell.

Basal transcription factors are crucial in the formation of a preinitiation complex on the DNA template that subsequently recruits RNA polymerase II for transcription initiation. The complexity of eukaryotic transcription does not end with the polymerases and promoters. An army of other transcription factors, which bind to upstream enhancers and silencers, also help to regulate the frequency with which pre-mRNA is synthesized from a gene. Enhancers and silencers affect the efficiency of transcription but are not necessary for transcription to proceed.

Promoter Structures for RNA Polymerases I and III

The processes of bringing RNA polymerases I and III to the DNA template involve slightly less complex collections of transcription factors, but the general theme is the same.

The conserved promoter elements for genes transcribed by polymerases I and III differ from those transcribed by RNA polymerase II. RNA polymerase I transcribes genes that have two GC-rich promoter sequences in the -45 to +20 region. These sequences alone are sufficient for transcription initiation to occur, but promoters with additional sequences in the region from -180 to -105 upstream of the initiation site will further enhance initiation. Genes that are transcribed by RNA polymerase III have upstream promoters or promoters that occur within the genes themselves.

Eukaryotic transcription is a tightly regulated process that requires a variety of proteins to interact with each other and with the DNA strand. Although the process of transcription in eukaryotes involves a greater metabolic investment than in prokaryotes, it ensures that the cell transcribes precisely the pre-mRNAs that it needs for protein synthesis.

EVOLUTION CONNECTION

The Evolution of Promoters

The evolution of genes may be a familiar concept. Mutations can occur in genes during DNA replication, and the result may or may not be beneficial to the cell. By altering an enzyme, structural protein, or some other factor, the process of mutation can transform functions or physical features. However, eukaryotic promoters and other gene regulatory sequences may evolve as well. For instance, consider a gene that, over many generations, becomes more valuable to the cell. Maybe the gene encodes a structural protein that the cell needs to synthesize in abundance for a certain function. If this is the case, it would be beneficial to the cell for that gene's promoter to recruit transcription factors more efficiently and increase gene expression.

Scientists examining the evolution of promoter sequences have reported varying results. In part, this is because it is difficult to infer exactly where a eukaryotic promoter begins and ends. Some promoters occur within genes; others are located very far upstream, or even downstream, of the genes they are regulating. However, when researchers limited their examination to human core promoter sequences that were defined experimentally as sequences that bind the preinitiation complex, they found that promoters evolve even faster than protein-coding genes.

It is still unclear how promoter evolution might correspond to the evolution of humans or other complex organisms. However, the evolution of a promoter to effectively make more or less of a given gene product is an intriguing alternative to the evolution of the genes themselves.[1]

Eukaryotic Elongation and Termination

Following the formation of the preinitiation complex, the polymerase is released from the other transcription factors, and elongation is allowed to proceed as it does in prokaryotes with the polymerase synthesizing pre-mRNA in the 5′ to 3′ direction. As discussed previously, RNA polymerase II transcribes the major share of eukaryotic genes, so in this section we will focus on how this polymerase accomplishes elongation and termination.

Although the enzymatic process of elongation is essentially the same in eukaryotes and prokaryotes, the DNA template is considerably more complex. When eukaryotic cells are not dividing, their genes exist as a diffuse mass of DNA and proteins called chromatin. The DNA is tightly packaged around charged histone proteins at repeated intervals. These *DNA–histone complexes*, collectively called nucleosomes, are regularly spaced and include 146 nucleotides of DNA wound around eight

1H Liang et al., "Fast evolution of core promoters in primate genomes," *Molecular Biology and Evolution* 25 (2008): 1239–44.

histones like thread around a spool.

For polynucleotide synthesis to occur, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome. This is accomplished by a special protein complex called **FACT**, which stands for "*facilitates chromatin transcription*." This complex pulls histones away from the DNA template as the polymerase moves along it. Once the pre-mRNA is synthesized, the FACT complex replaces the histones to recreate the nucleosomes.

The termination of transcription is different for the different polymerases. Unlike in prokaryotes, elongation by RNA polymerase II in eukaryotes takes place 1,000 to 2,000 nucleotides beyond the end of the gene being transcribed. This pre-mRNA tail is subsequently removed by cleavage during mRNA processing. On the other hand, RNA polymerases I and III require termination signals. Genes transcribed by RNA polymerase I contain a specific 18-nucleotide sequence that is recognized by a termination protein. The process of termination in RNA polymerase III involves an mRNA hairpin similar to rho-independent termination of transcription in prokaryotes.

15.4 RNA Processing in Eukaryotes

By the end of this section, you will be able to do the following:

- Describe the different steps in RNA processing
- Understand the significance of exons, introns, and splicing for mRNAs
- Explain how tRNAs and rRNAs are processed

After transcription, eukaryotic pre-mRNAs must undergo several processing steps before they can be translated. Eukaryotic (and prokaryotic) tRNAs and rRNAs also undergo processing before they can function as components in the protein-synthesis machinery.

mRNA Processing

The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated. Eukaryotic protein-coding sequences are not continuous, as they are in prokaryotes. The coding sequences (exons) are interrupted by noncoding introns, which must be removed to make a translatable mRNA. The additional steps involved in eukaryotic mRNA maturation also create a molecule with a much longer half-life than a prokaryotic mRNA. Eukaryotic mRNAs last for several hours, whereas the typical *E. coli* mRNA lasts no more than five seconds.

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus. The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5′ and 3′ ends of the molecule, and the removal of the introns (Figure 15.11). In rare cases, the mRNA transcript can be "edited" after it is transcribed.

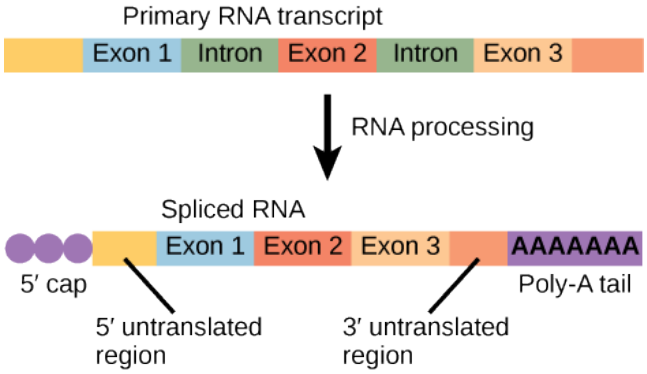

Figure 15.11 Eukaryotic mRNA contains introns that must be spliced out. A 5′ cap and 3′ poly-A tail are also added.

EVOLUTION CONNECTION

RNA Editing in Trypanosomes

The trypanosomes are a group of protozoa that include the pathogen *Trypanosoma brucei*, which causes nagana in cattle and sleeping sickness in humans throughout great areas of Africa (Figure 15.12). The trypanosome is carried by biting flies in the