DNA->RNA: Transcription

The flow of information from genotype, the nucleotide sequence in DNA encoding a functional product, begins and often ends with the process of transcribing a copy of this information from DNA into RNA. The basics of this process are the same in prokaryotes and eukaryotes, but there are a lot of differences in details. You do need to know the basics and the differences for the USMLE exam. Start with the prokaryotes and then move to the eukaryotes. Notice the differences and think about the relationship between these and the differing organism complexities.

Readings
MBoC(6th) Ch6. FROM DNA TO RNA

There is more material covered in the chapters than you need to know. Use the text and illustrations below to help focus.

Need to know and understand:

Transcription
- 5', 3' ends of gene vs. 5', 3' ends of DNA
- Template strand
- Antisense strand
- Coding strand
- Sense strand
- Promoter
- DNA dependent RNA polymerases
- Transcription bubble

Bacterial
- -10 (Pribnow), -35 region
- consensus sequences
- directional activity
- E. coli RNA polymerase = core enzyme (α,α,β,β',ω) + non-catalytic accessory subunit sigma (σ).
- Regulation of promoter activity by sigma (σ) factors, and binding strength
- closed complex -> open complex (isomerization)

Termination
- Terminators
- rho (ρ) factor dependent and rho (ρ) factor independent

Eukaryotic
- TATA box
- 3 Different RNA polymerases in eukaryotes
  - RNA pol I - transcribes rRNAs
  - RNA pol II - transcribes mRNAs
  - RNA pol III - transcribes some snRNAs + tRNAs +5s rRNA
  - The eukaryotic preinitiation complex is huge, contains ~40 different proteins, almost as big as a ribosome. Associated non-RNApolII proteins are called transcription factors

transcription factors
- TFIID: Complex of TBP (TATA binding protein) and TBP-associated factors (TAFs)
- TFIIA: Contacts TBP and stabilizes its interaction with the TATA box
- The TFIIDA complex together is like the *E.coli* sigma factor. It helps mediate the

initial interaction between the holoenzyme and DNA and is then released when transcription starts.

Many others not described here.

primary transcript

RNA processing (post transciptional modification)

5' cap of 7-methylguanosine in 5'-5' linkage.

3' cleavage 10-35 nt 3' of sequence AAUAAA

3' poly adenylation (polyA tail added by polyadenylate polymerase)

split genes - RNA splicing

Introns/exons 5' GU/3'AG concensus sequence at intron boundaries

Spliceosome

Small nuclear ribonucleoproteins (snRPs , aka snurps)

Export of mRNAs from the nucleus

Classes of RNA and their basic functions

Ribosomal RNA (rRNA)

part of ribosomes, involved in structure of ribosomes and the binding of ribosomes at the start of translation

Transfer RNA (tRNA)

tRNAs contain many unusual bases. tRNAs specific for each amino acid. Involved in translation.

small nuclear RNA (snRNA)

involved in splicing in eukaryotes. Complexed with proteins to form small nuclear ribonucleoproteins (snurps)

Messenger RNAs (mRNA)

encoded protein, are translated.

miRNAs and siRNAs

regulate gene expression

lncRNAs

regulation of genome states - probably other undescribed functions as well.

heterologous nuclear RNA (hnRNA)

nuclear RNAs during processing. The RNA protein complexs during this stage are sometimes called heterologous nuclear ribonucleoprotein particles (hnRNPs).

RNAs can sometimes catalyze the breakage and formation of specific covalent bonds. ie. Some RNAs think they are enzymes. Called ribozymes.

Differences between DNA and RNA polymerases

RNA polymerases do not require a primer

RNA polymerases do not proof-read

RNA polymerases begin transcription at specific sequences

Differences in transcription and mRNAs between Prokaryotes and Eukaryotes

Medical Perspectives

Inhibition of PolII by Amanitas mushroom poisoning.

The Rifamycin family of antibiotics.

Focus illustrations: Source: Molecular Biology of the Cell: Sixth Edition (2015) Alberts et al., Garland Science, NY

text

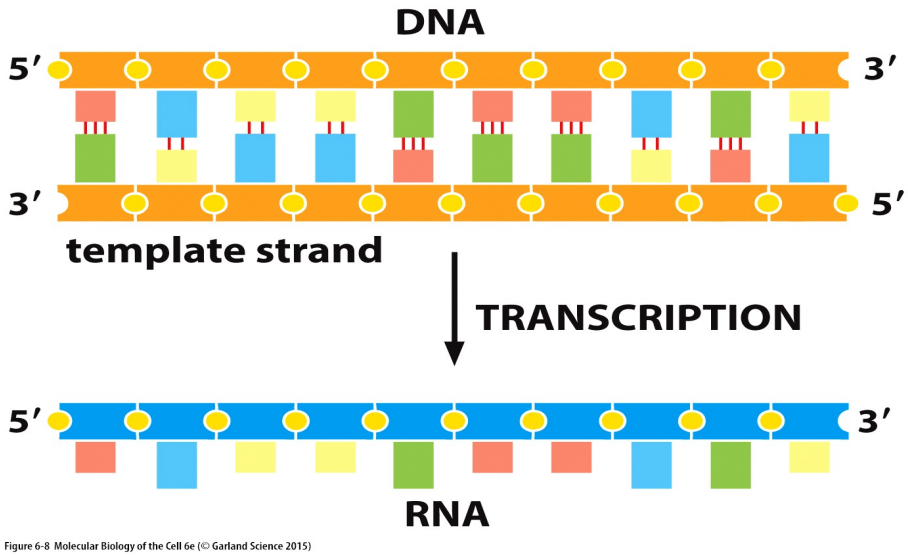

Figure 6–8 DNA transcription produces a single-stranded RNA molecule that is complementary to one strand of the DNA double helix. Note that the sequence of bases in the RNA molecule produced is the same as the sequence of bases in the non-template DNA strand, except that a U replaces every T base in the DNA.

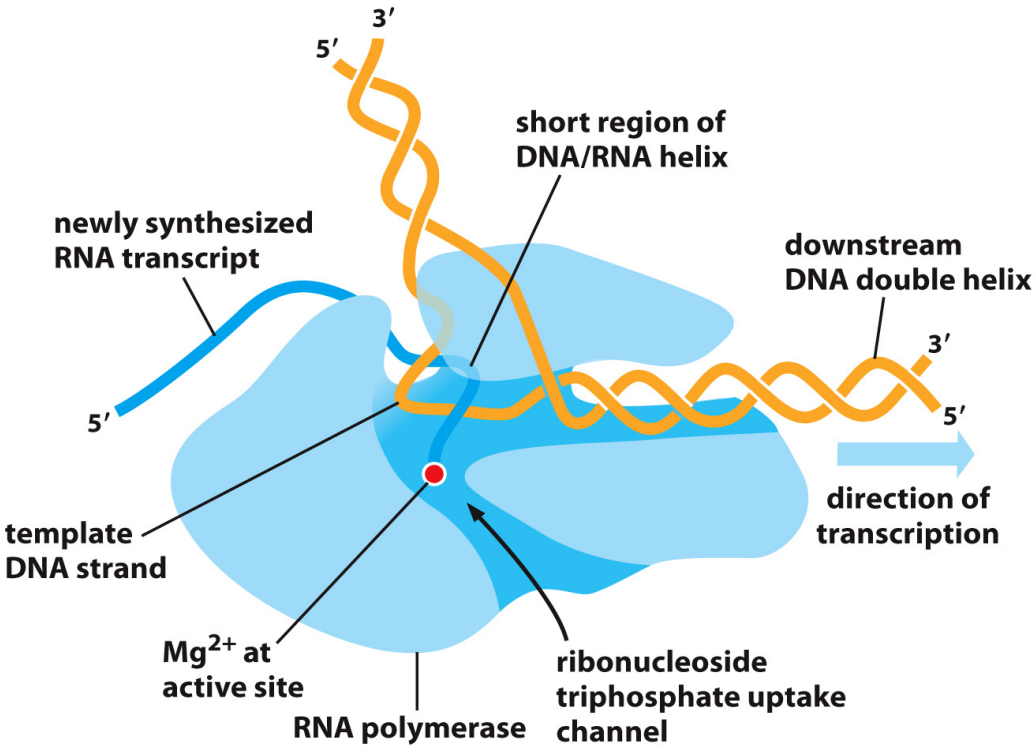

Figure 6-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–9 DNA is transcribed by the enzyme RNA polymerase. The RNA polymerase (pale blue) moves stepwise along the DNA, unwinding the DNA helix at its active site indicated by the Mg 2+ (red), which is required for catalysis. As it progresses, the polymerase adds nucleotides one by one to the RNA chain at the polymerization site, using an exposed DNA strand as a template. The RNA transcript is thus a complementary copy of one of the two DNA strands. A short region of DNA/RNA helix (approximately nine nucleotide pairs in length) is formed only transiently, and a "window" of DNA/RNA helix therefore moves along the DNA with the polymerase as the DNA double helix reforms behind it. The incoming nucleotides are in the form of ribonucleoside triphosphates (ATP, UTP, CTP, and GTP), and the energy stored in their phosphate– phosphate bonds provides the driving force for the polymerization reaction (see Figure 5–4). The figure, based on an x-ray crystallographic structure, shows a cutaway view of the polymerase: the part facing the viewer has been sliced away to reveal the interior (Movie 6.3). (Adapted from P. Cramer et al., Science 288:640–649, 2000; PDB code: 1HQM.)

TABLE 6–1 Principal Types of RNAs Produced in Cells	
Type of RNA	Function
mRNAs	Messenger RNAs, code for proteins
rRNAs	Ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	Transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	Small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	Small nucleolar RNAs, help to process and chemically modify rRNAs
miRNAs	MicroRNAs, regulate gene expression by blocking translation of specific mRNAs and cause their degradation
siRNAs	Small interfering RNAs, turn off gene expression by directing the degradation of selective mRNAs and the establishment of compact chromatin structures
piRNAs	Piwi-interacting RNAs, bind to piwi proteins and protect the germ line from transposable elements
lncRNAs	Long noncoding RNAs, many of which serve as scaffolds; they regulate diverse cell processes, including X-chromosome inactivation

TABLE 6–2 The Three RNA Polymerases in Eukaryotic Cells	
Type of polymerase	Genes transcribed
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	All protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, lncRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes, and genes for other small RNAs
The rRNAs were named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.	

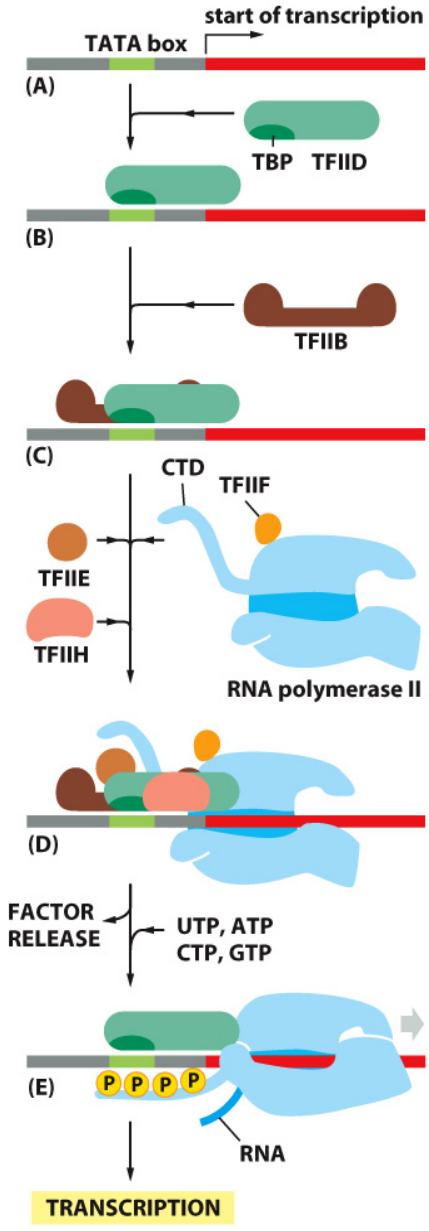

Figure 6-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–15 Initiation of transcription of a eukaryotic gene by RNA polymerase II. To begin transcription, RNA polymerase requires several general transcription factors. (A) The promoter contains a DNA sequence called the TATA box, which is located 25 nucleotides away from the site at which transcription is initiated. (B) Through its subunit TBP, TFIID recognizes and binds the TATA box, which then enables the adjacent binding of TFIIB (C). For simplicity the DNA distortion produced by the binding of TFIID (see Figure 6–17) is not shown. (D) The rest of the general transcription factors, as well as the RNA polymerase itself, assemble at the promoter. (E) TFIIH then uses energy from ATP hydrolysis to pry apart the DNA double helix at the transcription start point, locally exposing the template strand. TFIIH also phosphorylates RNA polymerase II, changing its conformation so that the polymerase is released from the general factors and can begin the elongation phase of transcription. As shown, the site of phosphorylation is a long C-terminal polypeptide tail, also called the C-terminal domain (CTD), that extends from the polymerase molecule. The assembly scheme shown in the figure was deduced from experiments performed in vitro, and the exact order in which the general transcription factors assemble on promoters probably varies from gene to gene in vivo. The general transcription factors are highly conserved; some of those from human cells can be replaced in biochemical experiments by the corresponding factors from simple yeasts.

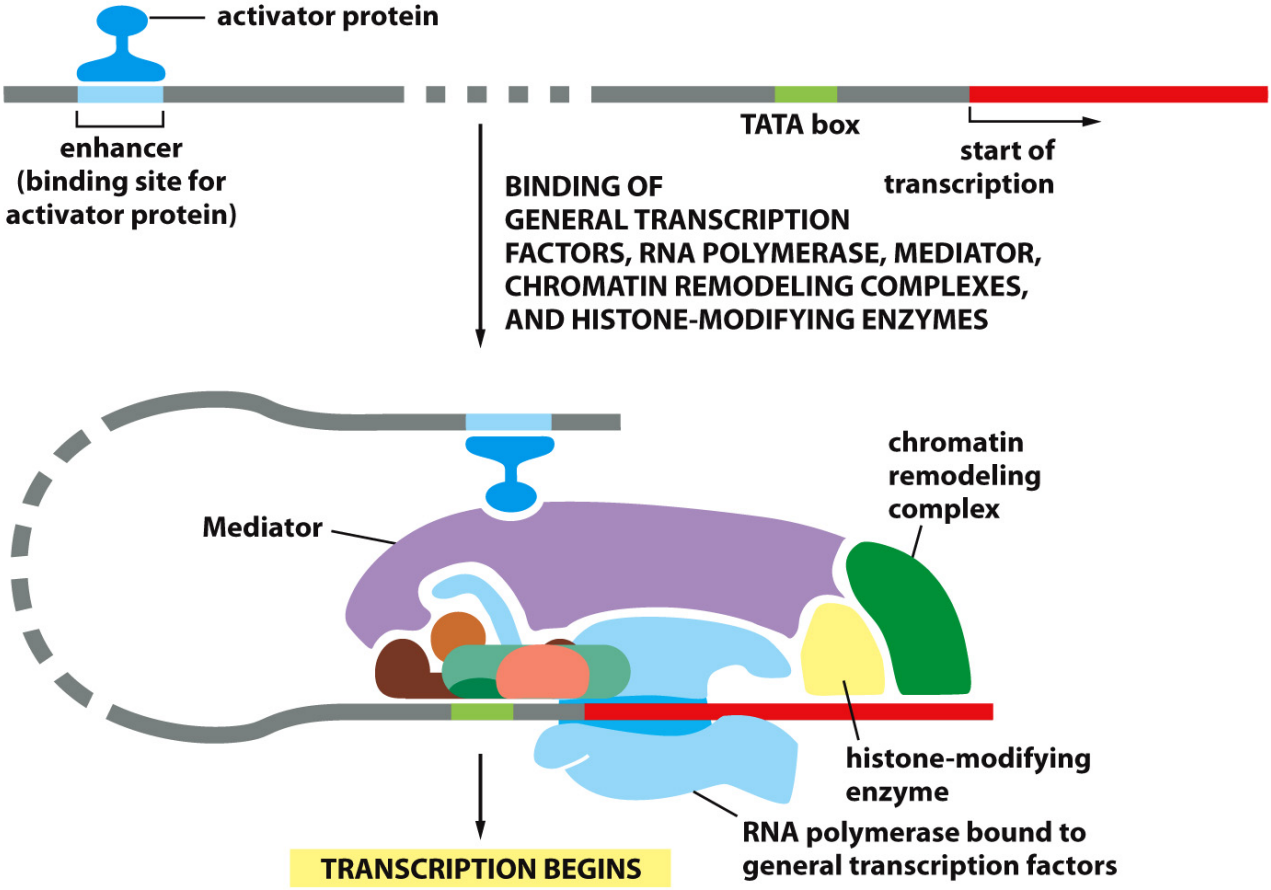

Figure 6-18 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–18 Transcription initiation by RNA polymerase II in a eukaryotic cell. Transcription initiation in vivo requires the presence of transcription activator proteins. As described in Chapter 7, these proteins bind to specific short sequences in DNA. Although only one is shown here, a typical eukaryotic gene utilizes many transcription activator proteins, which in combination determine its rate and pattern of transcription. Sometimes acting from a distance of several thousand nucleotide pairs (indicated by the dashed DNA molecule), these proteins help RNA polymerase, the general transcription factors, and Mediator all to assemble at the promoter. In addition, activators attract ATP-dependent chromatin remodeling complexes and histone-modifying enzymes. One of the main roles of Mediator is to coordinate the assembly of all these proteins at the promoter so that transcription can begin. As discussed in Chapter 4, the "default" state of chromatin is a condensed fiber (see Figure 4–28), and this is likely to be the form of DNA upon which most transcription is initiated. For simplicity, the chromatin is not shown in this figure.

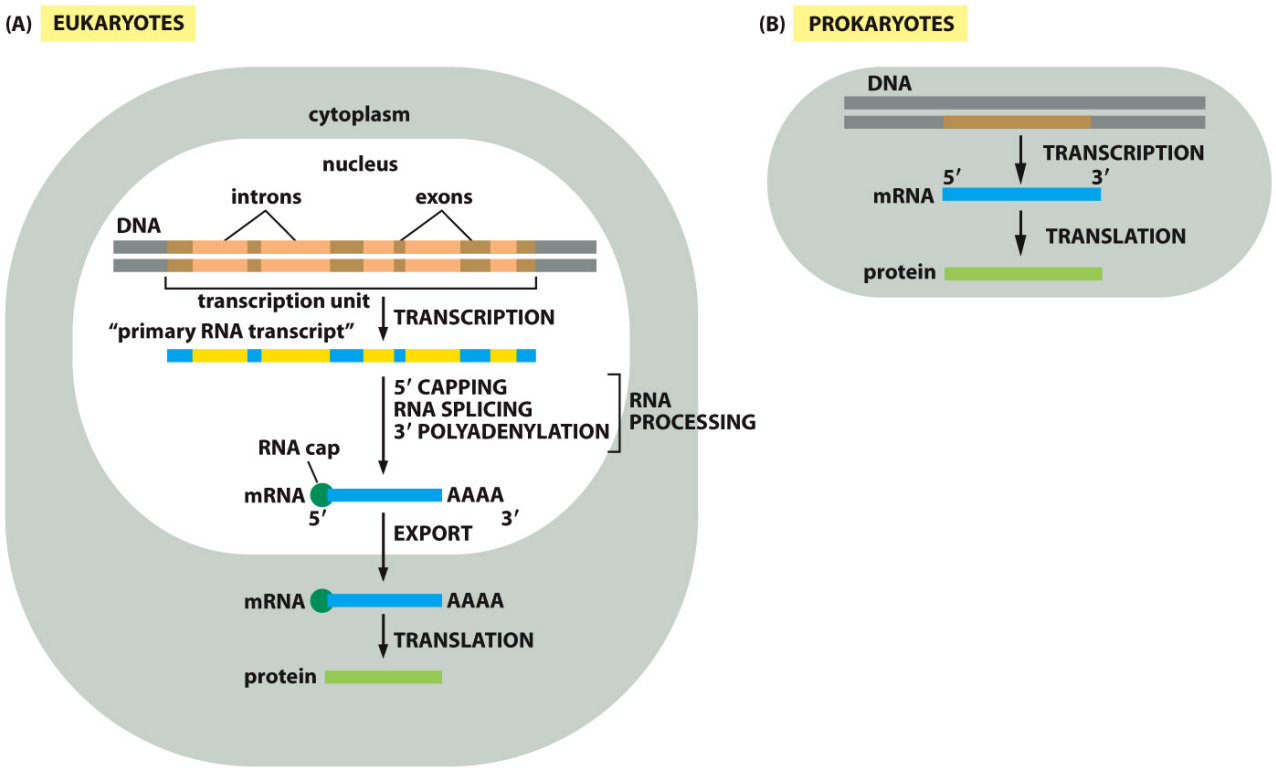

Figure 6-20 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–20 Comparison of the steps leading from gene to protein in eukaryotes and bacteria. The final level of a protein in the cell depends on the efficiency of each step and on the rates of degradation of the RNA and protein molecules. (A) In eukaryotic cells, the mRNA molecule resulting from transcription contains both coding (exon) and noncoding (intron) sequences. Before it can be translated into protein, the two ends of the RNA are modified, the introns are removed by an enzymatically catalyzed RNA splicing reaction, and the resulting mRNA is transported from the nucleus to the cytoplasm. For convenience, the steps in this figure are depicted as occurring one at a time; in reality, many occur concurrently. For example, the RNA cap is added and splicing begins before transcription has been completed. Because of the coupling between transcription and RNA processing, intact primary transcripts—the full-length RNAs that would, in theory, be produced if no processing had occurred—are found only rarely. (B) In prokaryotes, the production of mRNA is much simpler. The 5′ end of an mRNA molecule is produced by the initiation of transcription, and the 3′ end is produced by the termination of transcription. Since prokaryotic cells lack a nucleus, transcription and translation take place in a common compartment, and the translation of a bacterial mRNA often begins before its synthesis has been completed.

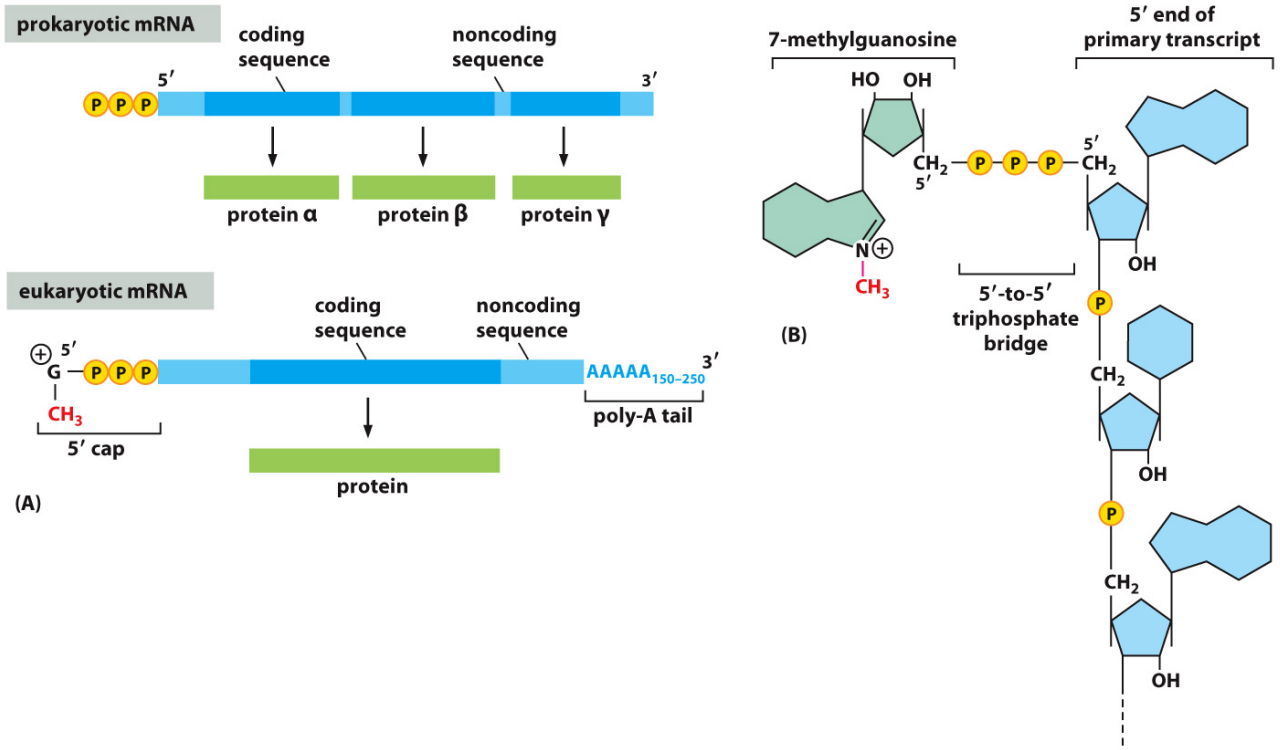

Figure 6-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–21 A comparison of the structures of prokaryotic and eukaryotic mRNA molecules. (A) The 5′ and 3′ ends of a bacterial mRNA are the unmodified ends of the chain synthesized by the RNA polymerase, which initiates and terminates transcription at those points, respectively. The corresponding ends of a eukaryotic mRNA are formed by adding a 5′ cap and by cleavage of the pre-mRNA transcript near the 3′ end and the addition of a poly-A tail, respectively. The figure also illustrates another difference between the prokaryotic and eukaryotic mRNAs: bacterial mRNAs can contain the instructions for several different proteins, whereas eukaryotic mRNAs nearly always contain the information for only a single protein. (B) The structure of the cap at the 5′ end of eukaryotic mRNA molecules. Note the unusual 5′-to-5′ linkage of the 7-methyl G to the remainder of the RNA. Many eukaryotic mRNAs carry an additional modification: methylation of the 2′-hydroxyl group of the ribose sugar at the 5′ end of the primary transcript (see Figure 6–23).

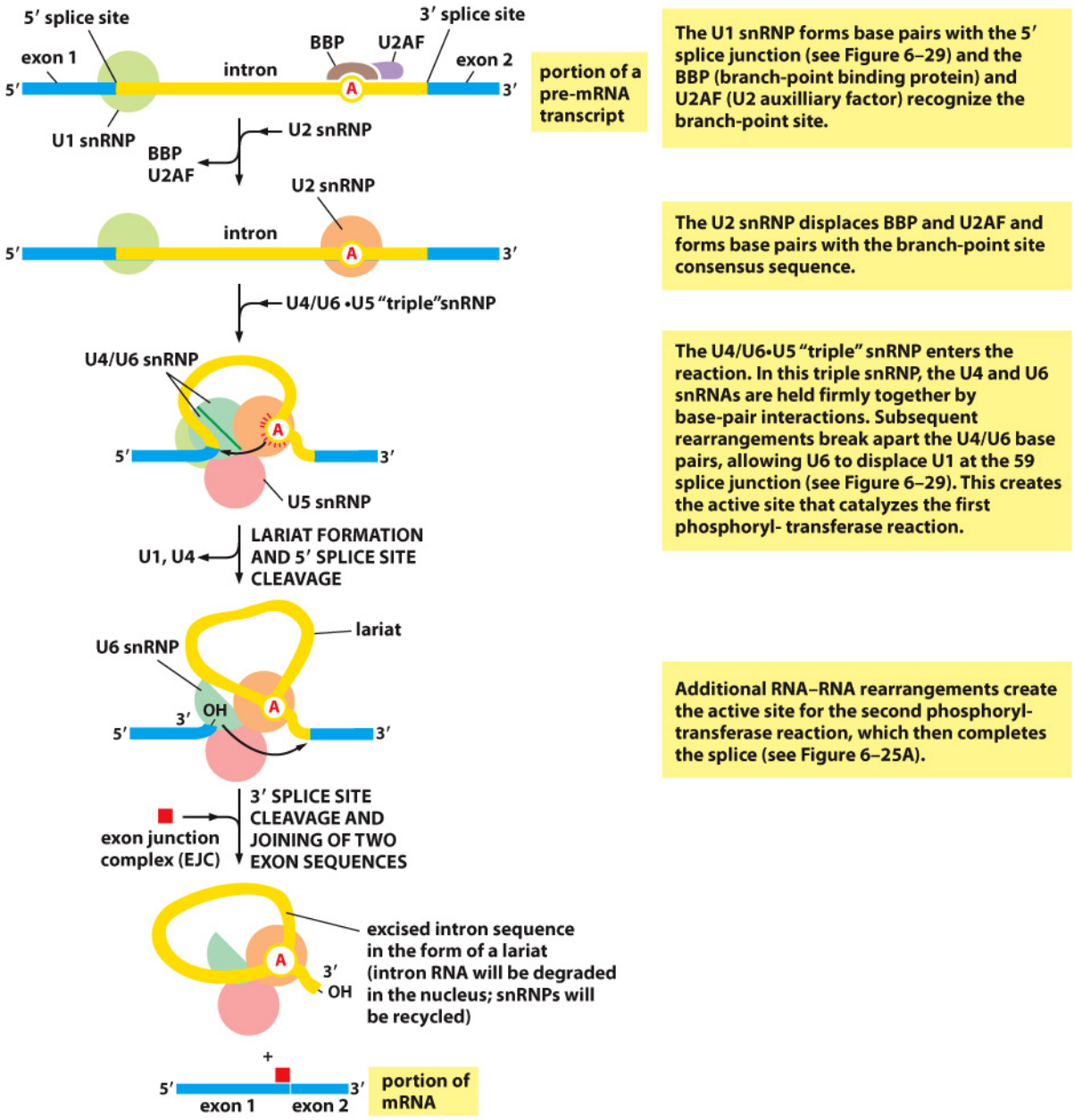

Figure 6-28 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–28 The pre-mRNA splicing mechanism. RNA splicing is catalyzed by an assembly of snRNPs (shown as colored circles) plus other proteins (most of which are not shown), which together constitute the spliceosome. The spliceosome recognizes the splicing signals on a pre-mRNA molecule, brings the two ends of the intron together, and provides the enzymatic activity for the two reaction steps required (see Figure 6–25A and Movie 6.5). As indicated, a set of proteins called the exon junction complex (EJC) remains on the spliced mRNA molecule.

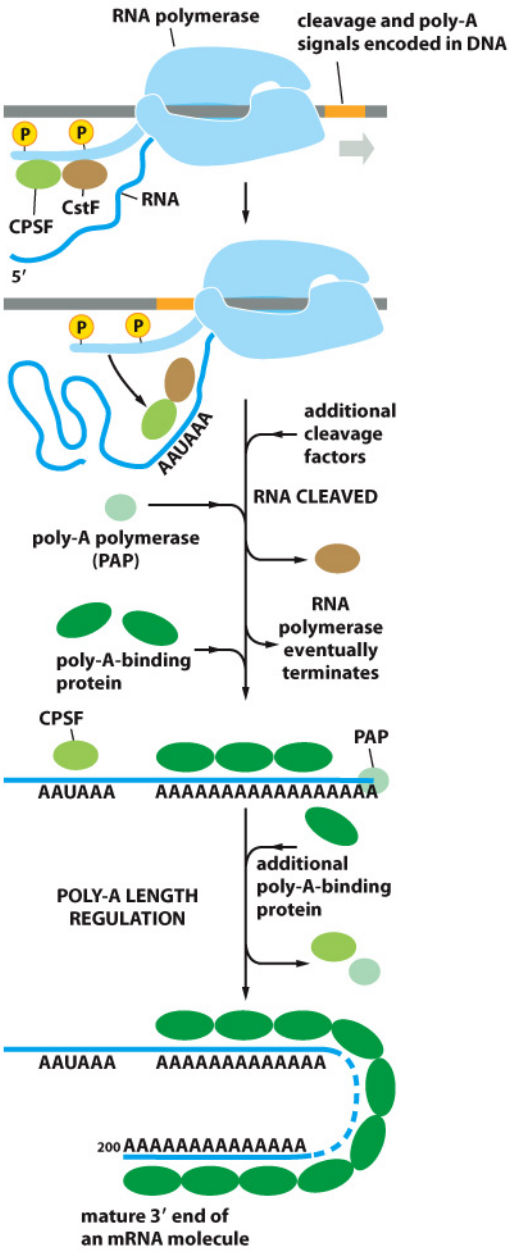

Figure 6-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–35 Some of the major steps in generating the 3′ end of a eukaryotic mRNA. This process is much more complicated than the analogous process in bacteria, where the RNA polymerase simply stops at a termination signal and releases both the 3′ end of its transcript and the DNA template (see Figure 6–11).

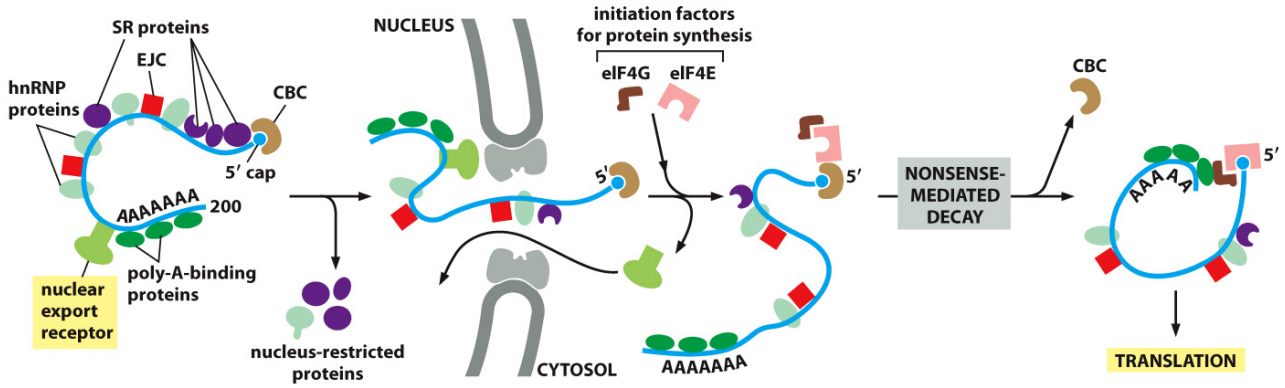

Figure 6-38 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 6–38 Schematic illustration of an export-ready mRNA molecule and its transport through the nuclear pore. As indicated, some proteins travel with the mRNA as it moves through the pore, whereas others remain in the nucleus. The nuclear export receptor for mRNAs is a complex of proteins that binds to an mRNA molecule once it has been correctly spliced and polyadenylated. After the mRNA has been exported to the cytosol, this export receptor dissociates from the mRNA and is re-imported into the nucleus, where it can be used again.