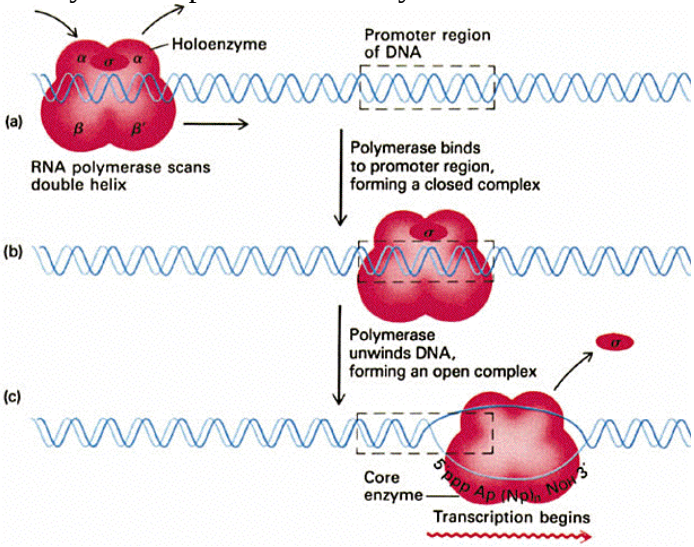
III. Prokaryotic transcription

A. Initiation

1. RNAP scans the DNA looking for promoters.
2. σ factor of RNAP binds the corresponding σ factor recognition sequence in the promoter.
3. Recent evidence suggests that at some promoters, the α subunit may bind to AT rich regions upstream of the sigma binding sites.
4. RNAP is bound covering approx. 60 basepairs. The DNA is still is a double helix (closed complex).
5. RNAP unwinds the DNA resulting in open complex formation.
6. First nucleotides are added to start RNA chain. Transcriptional initiation has occurred!
7. Accessory transcription factors may aid in all of the above listed steps.



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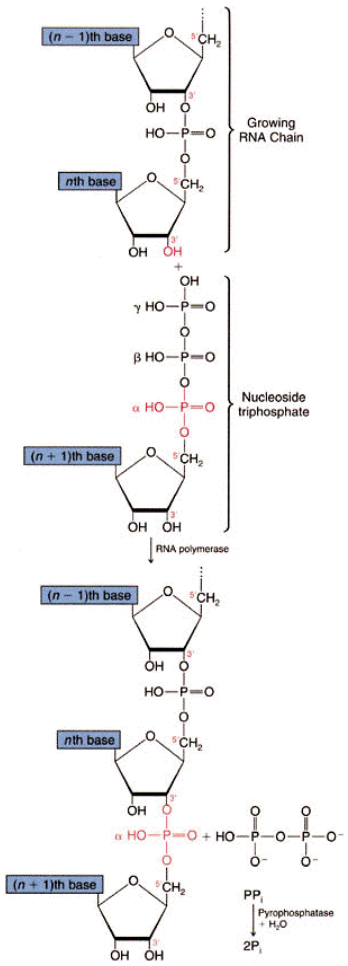
B. Elongation

1. Elongation is 5' → 3'
2. σ factor is ejected from RNAP after first 2-10 nucleotides are added.
3. Much less is known about this step for transcription than initiation. It was once believed that elongation occurred at a constant rate; however, recent work suggests that RNAP may pause during elongation. In fact, pausing is important in termination (see below).



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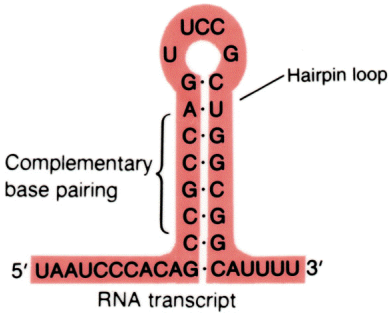
C. Termination (2 types)

1. Rho independent: A specific sequence at the end of the gene signals termination. The sequence is transcribed into RNA and it is the RNA sequence that is important. This sequence contains numerous Gs and Cs, which forms a hairpin structure, followed by a string of Us.



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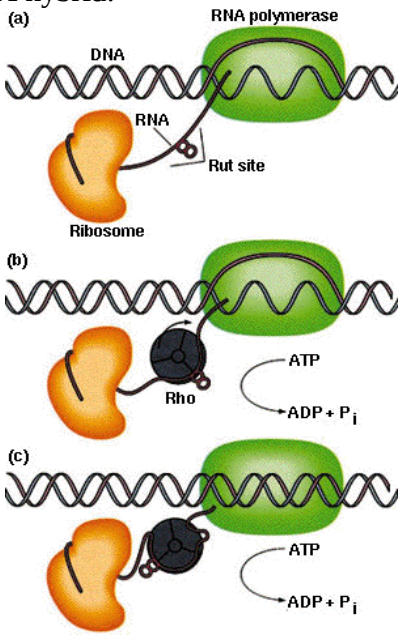
The hairpin destabilizes the DNA:RNA hybrid leading to dissociation of the RNA from the DNA.

2. Rho dependent: Rho protein binds to a sequence in the RNA (rut site – not well characterized). Rho moves along the RNA in the 3' direction until in eventually unwinds the DNA:RNA hybrid in the active site, thereby pulling the RNA away from the DNA and RNAP. Rut sites are located 5' to sites in the DNA that cause RNAP to pause. It is thought that this allows Rho to catch up to RNAP and the RNA-DNA hybrid.



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IV. Eukaryotic transcription

A. Initiation and elongation are similar to in prokaryotes; however, there are several important differences.

Table 13-8 Differences in Gene Expression between Prokaryotes and Eukaryotes

Prokaryotes	Eukaryotes
1. All RNA species are synthesized by a single RNA polymerase.	1. Three different RNA polymerases are responsible for the different classes of RNA molecules.
2. mRNA is translated during transcription.	2. mRNA is processed before transport to the cytoplasm, where it is translated. Caps and tails are added, and internal portions of the transcript are removed.
3. Genes are contiguous segments of DNA that are colinear with the mRNA that is translated into a protein.	3. Genes are often split. They are not contiguous segments of coding sequences; rather, the coding sequences are interrupted by intervening sequences (introns).
4. mRNAs are often polycistronic.	4. mRNAs are monocistronic.

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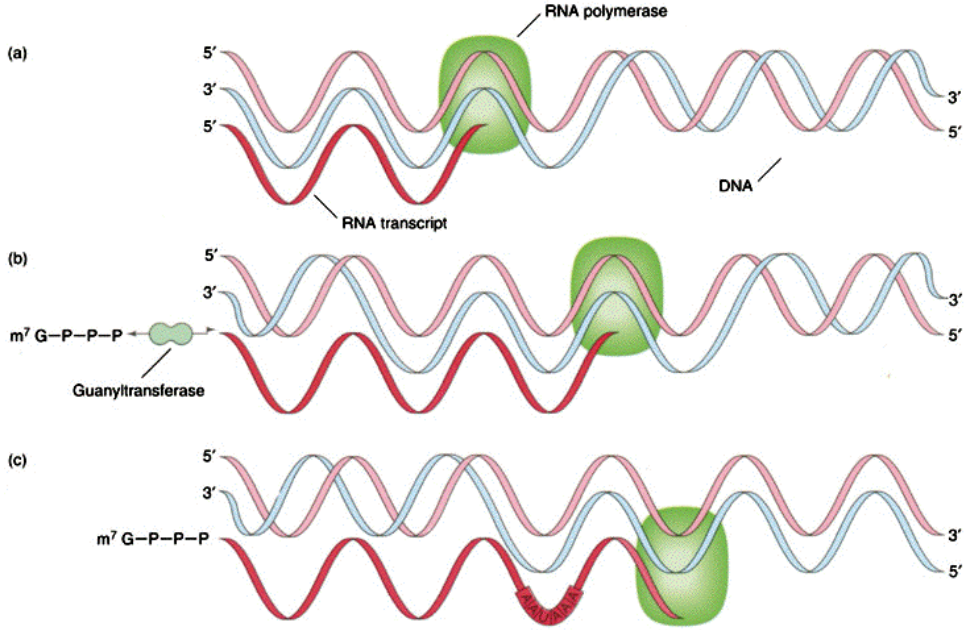
B. Termination of transcription in eukaryotes is poorly understood.

C. RNA processing

1. 5' capping: Occurs early in transcription. Guanosyltransferase adds 5' methyguanosine (Cap) to 5' end of mRNA. The Cap is important for translation initiation and for export from the nucleus.



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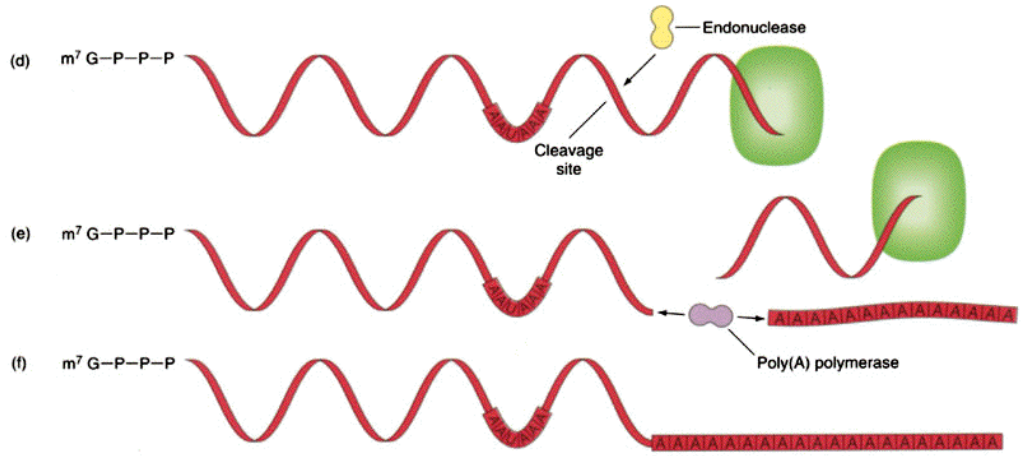
2. 3' poly(A) tail: AAUAAA sequence in the RNA signals a cleavage event in the RNA. Poly(A) polymerase then adds 150-200 A residues are added to the 3' end of the mRNA. The poly(A) tail increases the stability of the mRNA in eukaryotes.



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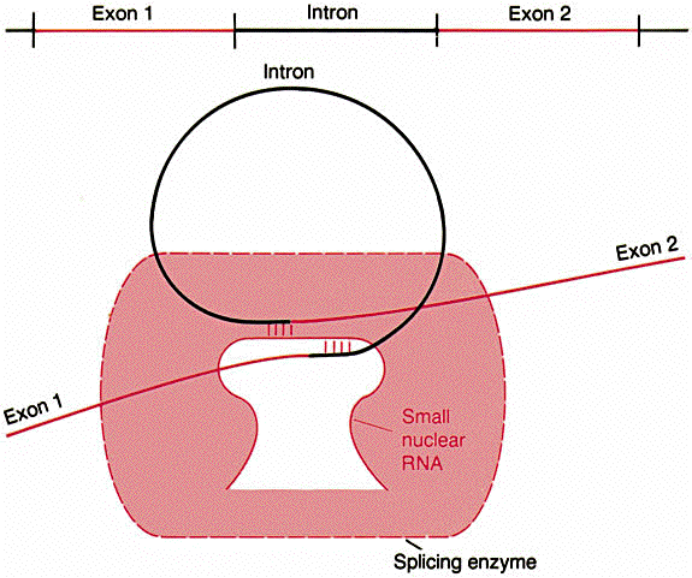
As a side note, recent evidence has demonstrated that there are poly(A) polymerases in prokaryotes and that some mRNAs have poly(A) tails. Interestingly though, the polyA tail destabilizes the mRNA in prokaryotes.

Some α2-thalassemias (anemia due to imbalance of α and β hemoglobin subunits) have been attributed to a defect in polyadenylation. Specifically, there is a mutation in the cleavage site from AAUAAA → AAUAAG.

3. Splicing: The primary transcripts often contain intervening sequences (introns) that are removed from the RNA prior to translation by a cleavage reaction catalyzed by snRNPs (small nuclear ribonuclear proteins which contain RNA and protein). Frequently, the splicing site in the intron has a GU at the 5' end and an AG at the 3' end. The snRNP aligns these ends in a lariat formation to allow precise splicing.



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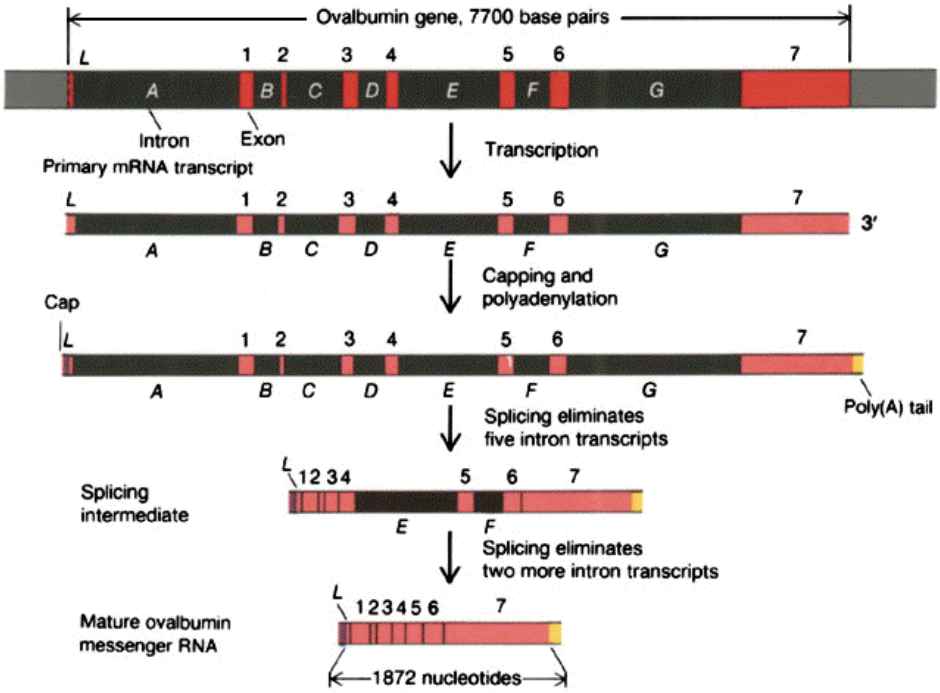
Complexes containing the snRNP, mRNA, and associated proteins are called spliceosomes.

Splicing is important (1) splicing allows variations of a gene and therefore gene product to be made (2) it has been suggested that exons correspond to functional motifs in proteins and thus the presence of genes that require slicing allows for evolutionary tinkering (3) many viruses have spliced mRNAs and so understanding the process may lead to new therapeutic approaches.

As an interesting aside, people with systemic lupus erythematosus have antibodies directed against snRNP protein subunits. The significance of this is unknown at this time.

Example of processing of eukaryotic mRNA:



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D. RNA export: RNA synthesis and processing occurs in the nucleus. The mature mRNA is then transported through the nuclear pores in the nuclear envelope to the cytoplasm. There is a nuclear complex that is involved in the transport. This complex recognizes the 5' CAP of the mRNA.