Transcription in a Nutshell

Initiation

Prokaryotic

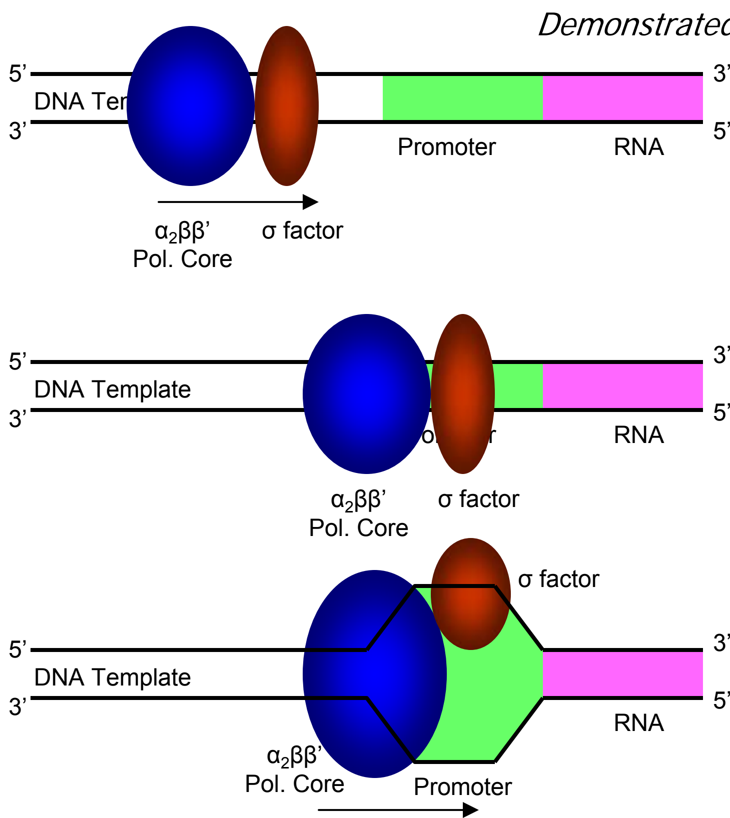
	-35	-10	+1
DNA Template	TTGACA	TATAAT	
	-35 Region	Pribnow Box	Start of RNA

Eukaryotic

	-75	-25	+1
DNA Template	GGNCAATCT	TATAAA	
	CAAT box (sometimes present)	TATA box	Start of RNA

The RNA Polymerase is going to start at the Promoter. In prokaryotes, the promoter consists of two consensus sequences: the -35 Region and the Pribnow box. In Eukaryotes, the TATA box is almost always found, while the CAAT box is found part of the time. It is important to note that these consensus sequences are always found upstream of the coding region of the gene. Also the sequence of the areas identified can control gene expression. The better the sequence match between the polymerase and the consensus sequence, the better the binding, and hence the more RNA made.

Demonstrated in E. Coli

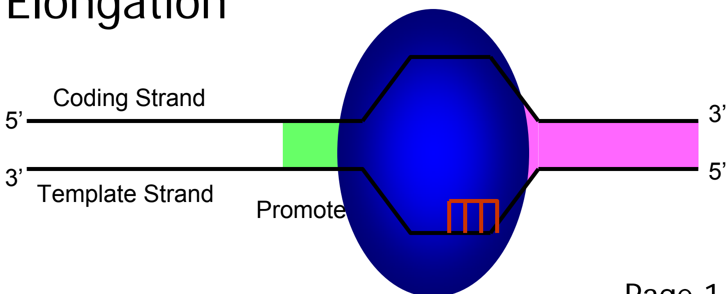


The RNA Polymerase core holoenzyme along with the sigma factor bind non-specifically to the DNA Template. They travel as a unit until the sigma factor recognizes the promoter.

Once the recognition takes place, the helicase within enzyme is activated to unwind the DNA at the promoter.

This forms the open promoter complex. Now the sigma factor is kicked off as the core continues down the DNA and begins to synthesize the RNA. The Template strand, 3′ -> 5′, is used to base pair the new RNA which, unlike DNA replication, requires no primer. The ribose based nucleotides are added to the free 3′ OH of the growing chain.

Elongation



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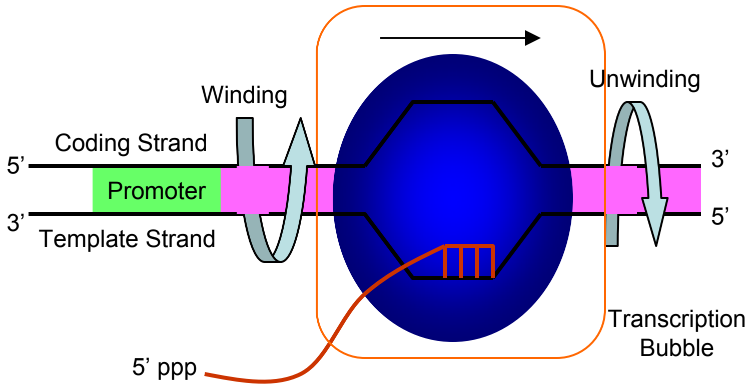
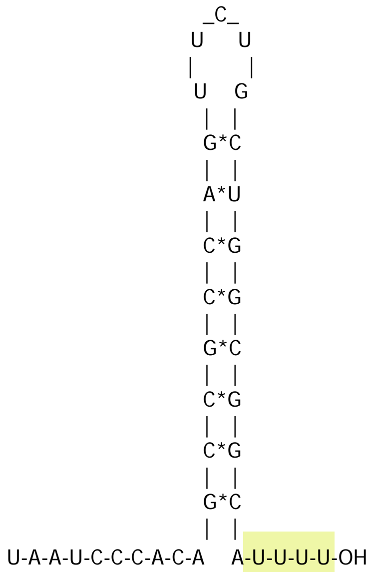
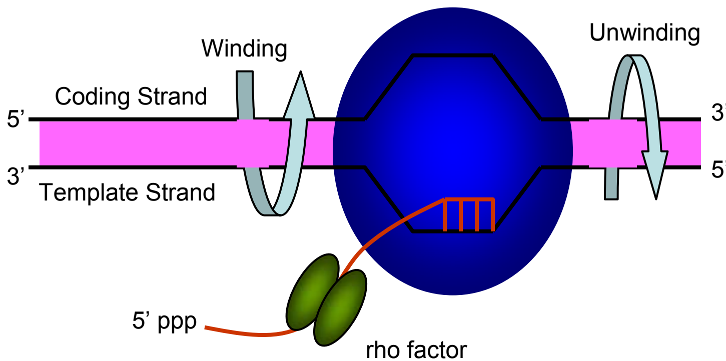


As the Polymerase progresses, the newly synthesized RNA trails behind it. The area containing the RNA Pol, DNA and nascent RNA is called the transcription bubble. During this time the DNA is continuously winding and unwinding. Also note that RNA Polymerase has no exonuclease activity, hence the error rate is quite high – 10^4 to 10^5.

Termination



Your book gives you two ways to terminate. Factor Independent: The first is by forming an RNA Hairpin structure due to a few Uracil Residues. The DNA region is a palindromic GC region followed by an AT rich region. Once the RNA is made, it literally sticks to itself and blocks the RNA Pol from moving on, forcing the complex to disassociate.



Factor Dependant: The second method involves the rho protein. This protein, similar in structure to the ATP Synthase, uses an ATP while literally traveling along the newly synthesized RNA scanning it for signals. We know very little about what it actually is looking for and finds. There appears to be no consensus sequence indicating it has the ability to detect noncontiguous structural features. It literally yanks the RNA away from the polymerase.

Transcription in a Nutshell

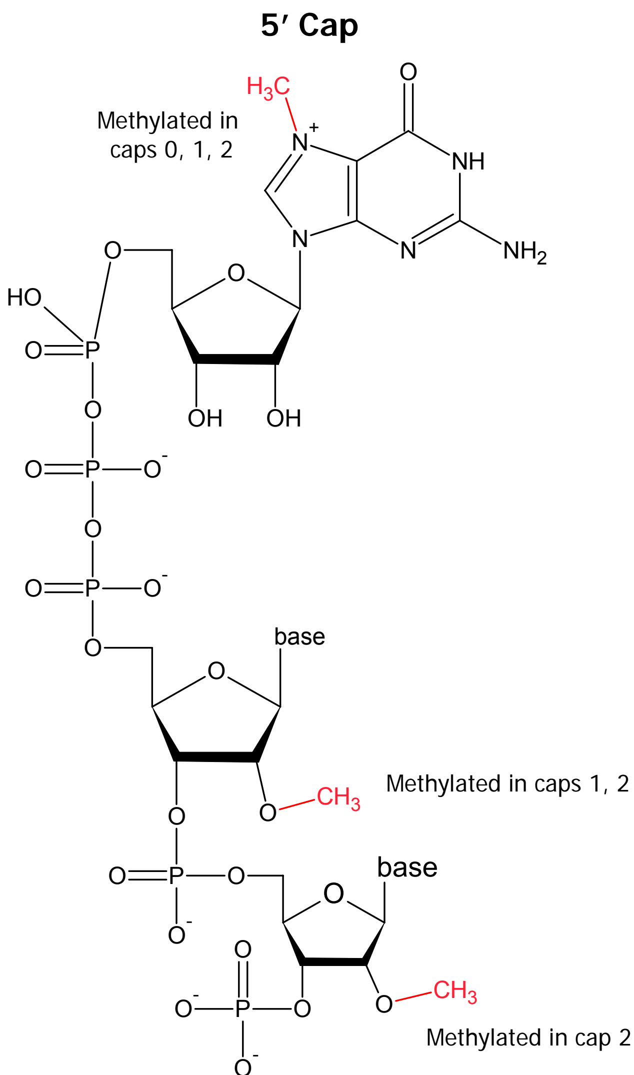
RNA Processing

Prokaryotic

5'

Translation – often at the same time as there is no nucleus to keep the translation machinery away from the RNA.

5′ Cap



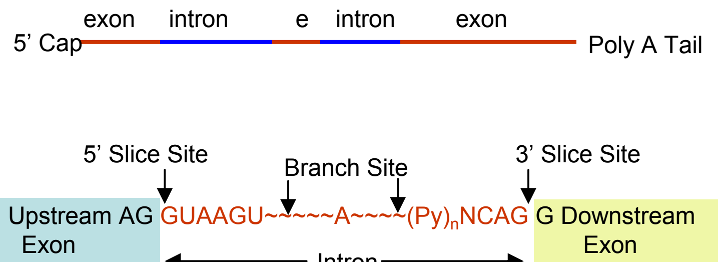
The 3 Caps: 0, 1, 2. Note that only one methyl group is used in Cap 0, two in Cap 1 and three in Cap 2. Also note the usage of the 7-methylguanylate.

Eukaryotic

5'

5' Cap Poly A Tail

One of three 5′ Caps is added (see bottom left) as well as a 3′ Poly-Adenosine Tail. For the latter case, an endonuclease recognizes the sequences AAUAAA, cleaves 3′ to it and then a poly(A) polymerase adds dozens to hundreds of adenylate residues. It is thought that this helps the stability of the mRNA.



At this point, all the introns are spliced out of the RNA. You will need to know the chemical basis for this splicing and the major mechanisms. This is covered on the next page.

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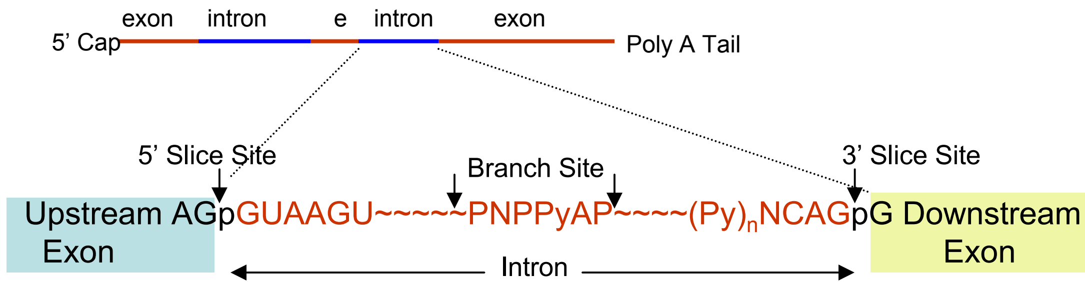
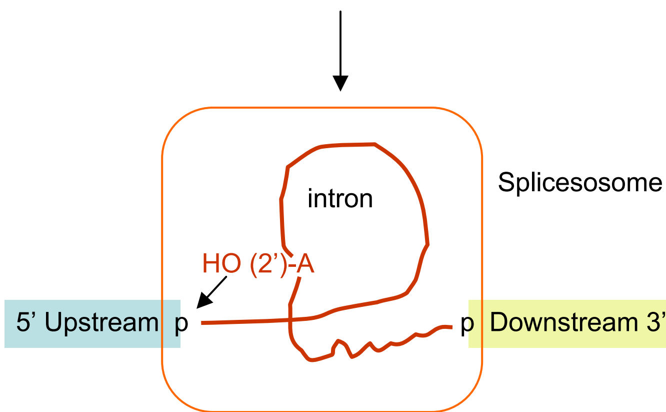
RNA Splicing

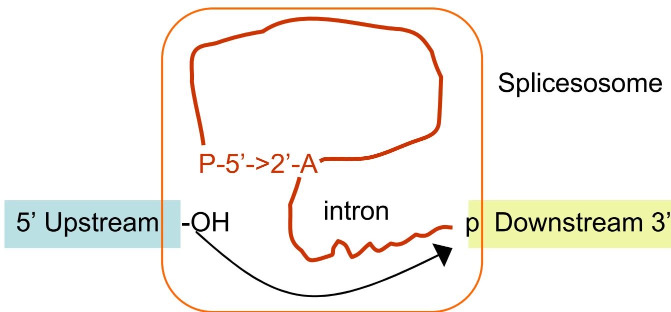
Eukaryotic Only



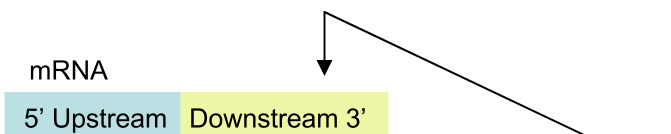


5 snRNPs and other proteins come together to form the splicesosome and the RNA is looped over itself.

The 2′OH on the Adenosine residue in the branch site attacks the phosphate at the 5′ Splice Site and snaps the 5′ exon off while forming the 5′ -> 2′ lariat structure.



Once this is complete the 3′ OH on the 5′ exon attacks the phosphate at the 3′ Splice Site which effectively splices out the intron. At this point the splicesosome disassociates.



Intron lariat

P-5'->2'-A

3'

Degraded by cell machinery

Repeated with all introns

5' Cap mRNA Poly A Tail

Translation – after being transferred to the cytoplasm (aka, another handout)