

Transcription II

1. Promoters in *E. coli* (RNA polymerase binding sites adjacent to genes) function with widely varying rates of efficiency - some stimulating initiation of transcription every few seconds, others only one or twice per life cycle. One way for a promoter to control such events is via variation in conserved sequences. *E. coli* genes have two conserved sequences - one at -10 relative to the transcription start site (TATAAT) and another at -35 relative to the transcription start site (TTGACA).
2. The more closely a given promoter's sequence matches the consensus sequence of the -10 sequence, the more active the promoter is at initiating transcription. RNA Polymerase slides along the DNA and when the sigma subunit identifies a promoter site, it stops.
3. Different sigma factors (such as the one made during heat shock) allow the cell to turn on sets of genes with different sequences in the -10/-35 sequences as needs arise.
4. Transcription occurs in a DNA "bubble" in which the RNA is partly base paired to the template strand, with the 5' end of the RNA "hanging" off. The bubble moves as the RNA polymerase moves. Ahead of the bubble, positive supercoils are created. This superhelical tension is relieved by DNA Gyrase. As the strands come back together behind the bubble, negative supercoiling occurs, and this is relieved by a topoisomerase.
5. The strand of DNA being copied is known as the template strand. It runs 3' to 5', since the RNA is being made 5' to 3' complementary to it.
6. The strand of DNA that is NOT copied in transcription is called the coding strand, since it has the same sequence as the RNA except for having T instead of U.
7. Transcription occurs in three phases - initiation, elongation, and termination. The initiation phase creates a open transcript complex and this phase can be inhibited in prokaryotes by rifampicin. During the initiation phase, the transcription bubble is formed and the first ten or so nucleotides are polymerized together by RNA polymerase. During the initiation phase, the sigma factor is released from RNA polymerase.
8. Transcription occurs under the 'control' of promoters. The more strongly RNA Polymerase (in prokaryotes) binds to the promoter, the more transcripts are made of that particular gene. The more transcripts are made, the promoter is referred to as being 'stronger.'
9. Transcription termination occurs by both factor dependent means (requires a protein factor) and factor independent means (no extra protein required). The factor independent method relies on formation of a duplex sequence of GC base pairs immediately ahead of a stretch of U's. The duplex destabilizes the RNA-DNA duplex and this is favored by the relatively weak hydrogen bonds of the U-A interactions.

10. The protein system involved in making FMN is controlled at the transcription level by the presence or absence of FMN. When FMN is abundant, it binds to the mRNA coding for the protein and favors formation of a transcriptional terminating stem-loop. When FMN is scarce, it does not bind, so that the mRNA does not form the stem loop and transcription of the entire gene progresses.
11. The factor involved in factor dependent transcription termination in *E. coli* is called rho. It binds to the 5' end of an RNA being made and (using ATP energy) "climbs" the RNA until it reaches the RNA polymerase. There it destabilizes the RNA/DNA duplex, favoring the release of the RNA polymerase from the DNA and the RNA from the DNA, as well.
12. In prokaryotes, tRNAs are the most altered (processed) RNAs. Modifications start with their being cleaved from a larger RNA containing both tRNAs and rRNAs. Ribonuclease P is a ribozyme (catalytic RNA) that cleaves the 5' end of tRNAs from the larger RNA. Ribonuclease III catalyzes excision of rRNAs from the larger molecule.
13. Eukaryotes and prokaryotes differ significantly in the relationship between transcription and translation. Prokaryotes have no nucleus. In them, translation starts oftentimes WHILE a message is being transcribed. There are no significant modifications to mRNAs in prokaryotes.
14. In eukaryotes, transcription and translation are spatially separated. Transcription occurs in the nucleus, whereas translation occurs in the cytoplasm. In addition, eukaryotic mRNAs are modified at the 5' end (capping), the 3' end (polyadenylation) and even in the middle (editing and splicing) (we'll talk about these later).
15. Eukaryotes have 3 specialized RNA polymerases. They differ in their sensitivity to alpha-amanitin (a poison from some mushrooms). RNA polymerase II (makes mRNAs) is the most sensitive. RNA polymerase III (makes tRNAs and small rRNA) has moderate sensitivity and RNA polymerase I (makes large rRNAs) has low sensitivity.
16. Sequence elements that affect transcription of eukaryotic genes. They include the TATA box (positioned approximately -30 to -100), and a CAAT box and GC box (-40 to -150).
17. The TATA box is not found in front of all eukaryotic genes, but is essential for strong transcription.