Prokaryotic transcription by Dr Sarmishtha Chanda

1. PROKARYOTIC TRANSCRIPTION

1. 2. PROKARYOTIC TRANSCRIPTION Bacterial transcription or prokaryotic transcription is the process in which messenger RNA transcripts of genetic material in prokaryotes are produced, to be translated for the production of proteins. Bacterial transcription occurs in the cytoplasm alongside translation. Unlike in eukaryotes, prokaryotic transcription and translation can occur simultaneously. This is impossible in eukaryotes, where transcription occurs in a membrane-bound nucleus while translation occurs outside the nucleus in the cytoplasm. In prokaryotes genetic material is not enclosed in a membrane-enclosed nucleus and has access to ribosomes in the cytoplasm.
2. 3. Transcription is known to be controlled by a variety of regulators in prokaryotes. Many of these transcription factors are homodimers containing helix- turn-helix DNA-binding motifs. Three steps in transcription ♣ Initiation ♣ Elongation ♣ Termination The following steps occur, in order, for transcription
3. 4. RNA polymerase RNA is synthesized by a single RNA polymerase enzyme which contains multiple polypeptide subunits. In E. coli, the RNA polymerase has five subunits: two α, one β, one β’ and one σ subunit (α2ββ’σ). This form is called the holoenzyme. The σ subunit may dissociate from the other subunits to leave a form known as the core enzyme.
4. 5. INITIATION RNA polymerase (RNAP) binds to one of several specificity factors, σ, to form a holoenzyme. In this form, it can recognize and bind to specific promoter regions in the DNA. The -35 region and the - 10 ("Pribnow box") region comprise the core prokaryotic promoter, and |T| stands for the terminator. The DNA on the template strand between the +1 site and the terminator is transcribed into RNA, which is then translated into protein. At this stage, the DNA is double-stranded ("closed"). This holoenzyme/wound-DNA structure is referred to as the closed complex.
5. 6. Pribnow box – it contain six nucleotide (TATAAT) located 8 to 10 nucleotide to the left of transcriptional start site, the initial base of mRNA. -35 region – a second sequence nucleotide (TTGAGA) located 35 nucleotide to the left of transcriptional start site. The DNA is unwound and becomes single- stranded ("open") in the vicinity of the initiation site (defined as +1). This holoenzyme/unwound-DNA structure is called the open complex.
6. 7. • The RNA polymerase transcribes the DNA (the beta subunit initiates the synthesis), but produces about 10 abortive (short, non-productive) transcripts which are unable to leave the RNA polymerase because the exit channel is blocked by the σ-factor. • The σ-factor eventually dissociates from the core enzyme and elongation proceeds. ELONGATION • Once the promoters region has been recognized by sigma factor of holoenzyme the enzyme begins to synthesis RNA sequence, sigma factor is released. This enzyme has no exo/endo nuclease activity and cannot repair the mistakes as DNA polymerase in replication.
7. 8. RNA polymerase add complementary base to the template strand of DNA. It adds Thiamine for Adenine (T =A), Guanine for Cytosine (G ≡ C), Cytosine for Guanine (C ≡ G) and Adenine for Uracil (A = U). Most transcripts originate using adenosine-5'-triphosphate (ATP) and, to a lesser extent, guanosine-5'-triphosphate (GTP) (purine nucleoside triphosphates) at the +1 site. Uridine-5'- triphosphate (UTP) and cytidine-5'-triphosphate (CTP) (pyrimidine nucleoside triphosphates) are disfavoured at the initiation site.]
8. 9. TERMINATION RNA synthesis will continue along the DNA template strand until the polymerase encounters a signal that tells it to stop, or terminate, transcription. In prokaryotes, this signal can take two forms, rho-independent and rho- dependent. Rho-independent Terminator Two termination mechanisms are well known: Intrinsic termination (also called Rho- independent transcription termination) involves terminator sequences

within the RNA that signal the RNA polymerase to stop. The terminator sequence is usually a palindromic sequence that forms a stem-loop hairpin structure that leads to the dissociation of the RNAP from the DNA template.

9. 10. Termination of transcription in vitro is classified as to its dependence on the protein factor, rho (ρ). Rho-independent terminators have a characteristic structure, which features (a) A strong G-C rich stem and loop, (b) a sequence of 4–6 U residues in the RNA, which are transcribed from a corresponding stretch of As in the template
10. 11. Rho-dependent termination uses a termination factor called ρ factor (rho factor) which is a protein to stop RNA synthesis at specific sites. This protein binds at a rho utilization site on the nascent RNA strand and runs along the mRNA towards the RNAP. A stem loop structure upstream of the terminator region pauses the RNAP, when ρ-factor reaches the RNAP, it causes RNAP to dissociate from the DNA, terminating transcription.