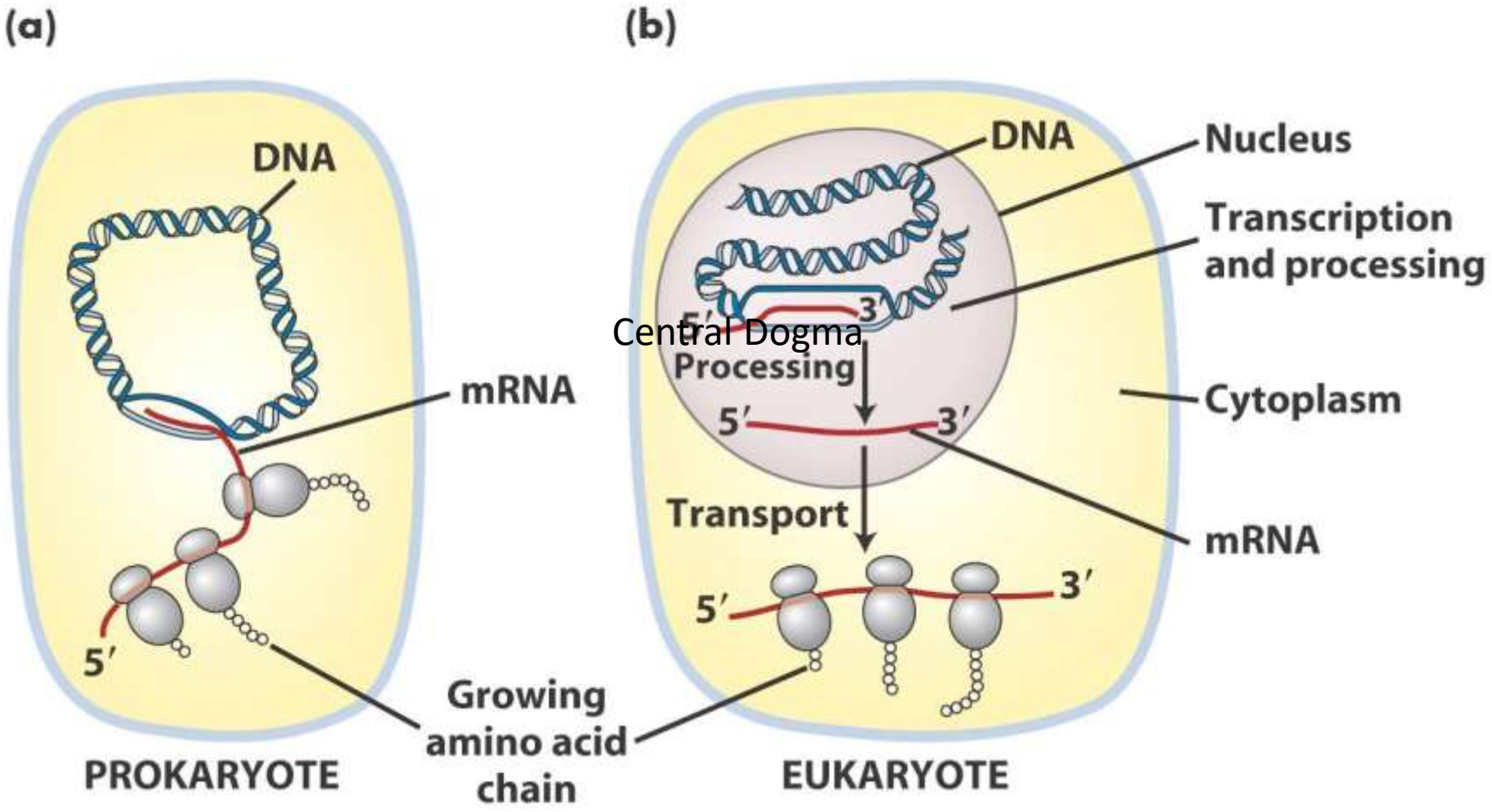
Central Dogma of Genetics

- Within each cell the genetic information flows from
  – **DNA to RNA to protein.**
- This flow of information is unidirectional and irreversible.
- The information carried within the DNA dictates the end product
  (protein) that will be synthesized.
  – This information is the **genetic code.**
- Conversion of DNA encoded information to RNA
  – is called **transcription.**
- The information from a mRNA is then **translated to an amino**
  acid sequence in the corresponding protein

Central Dogma



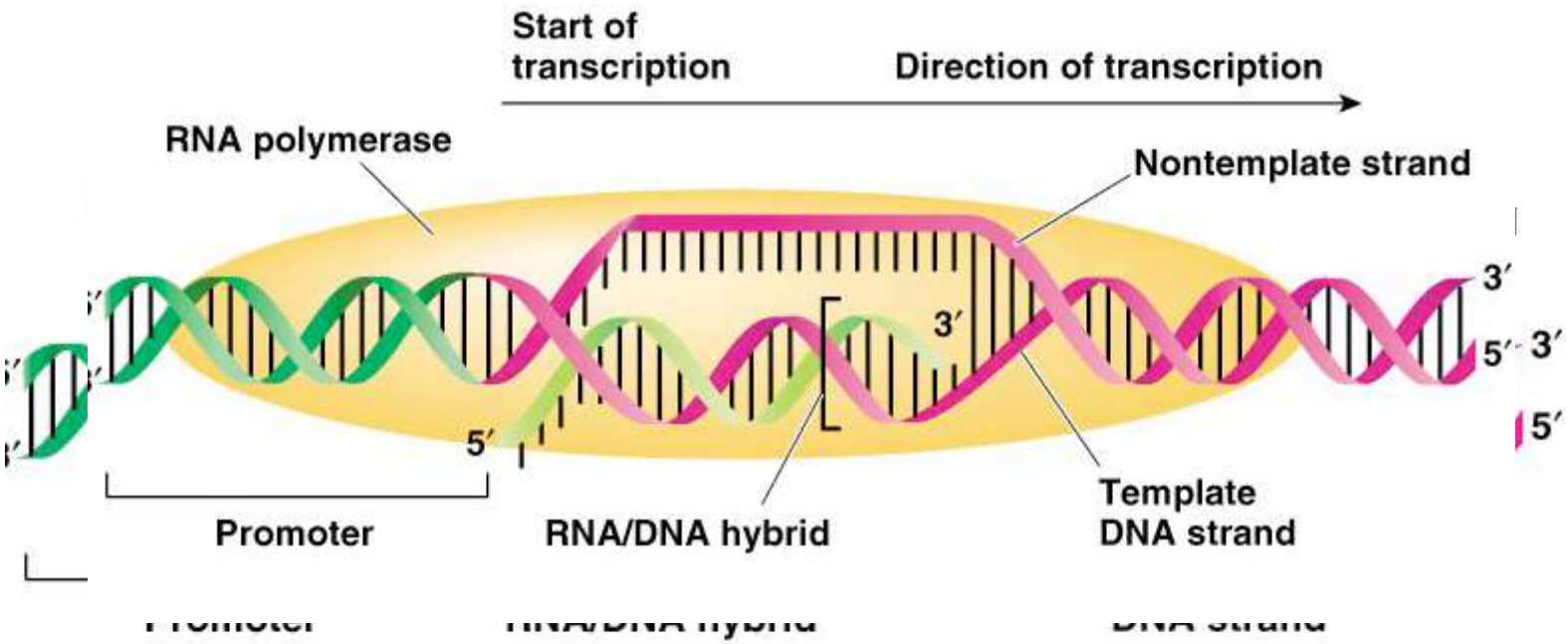
Central Dogma

- **Transcription**
- • Process by which the genetic information is conveyed
- from a double stranded DNA molecule to a single
- stranded RNA molecule.
- • Only one strand of DNA serves as a template:
- – this is the transcribed or anti-sense strand.
- • The complementary strand has a sequence identical to the
- RNA sequence (except for a U in place of a T),
- – is called the sense strand, or the RNA-like strand.

- **Salient Features of Transcription**
- • **RNA polymerase:**
- – catalyzes the addition of one ribonucleotide at a time,
- – extending the RNA strand being synthesized in the 5’ to 3’ direction.
- • **Promoter:**
- – DNA sequences near the beginning of a gene.
- – These signal the RNA polymerase to begin transcription.
- • **Terminators:**
- – sequences within the RNA products,
- – which signal the RNA polymerase to stop transcription

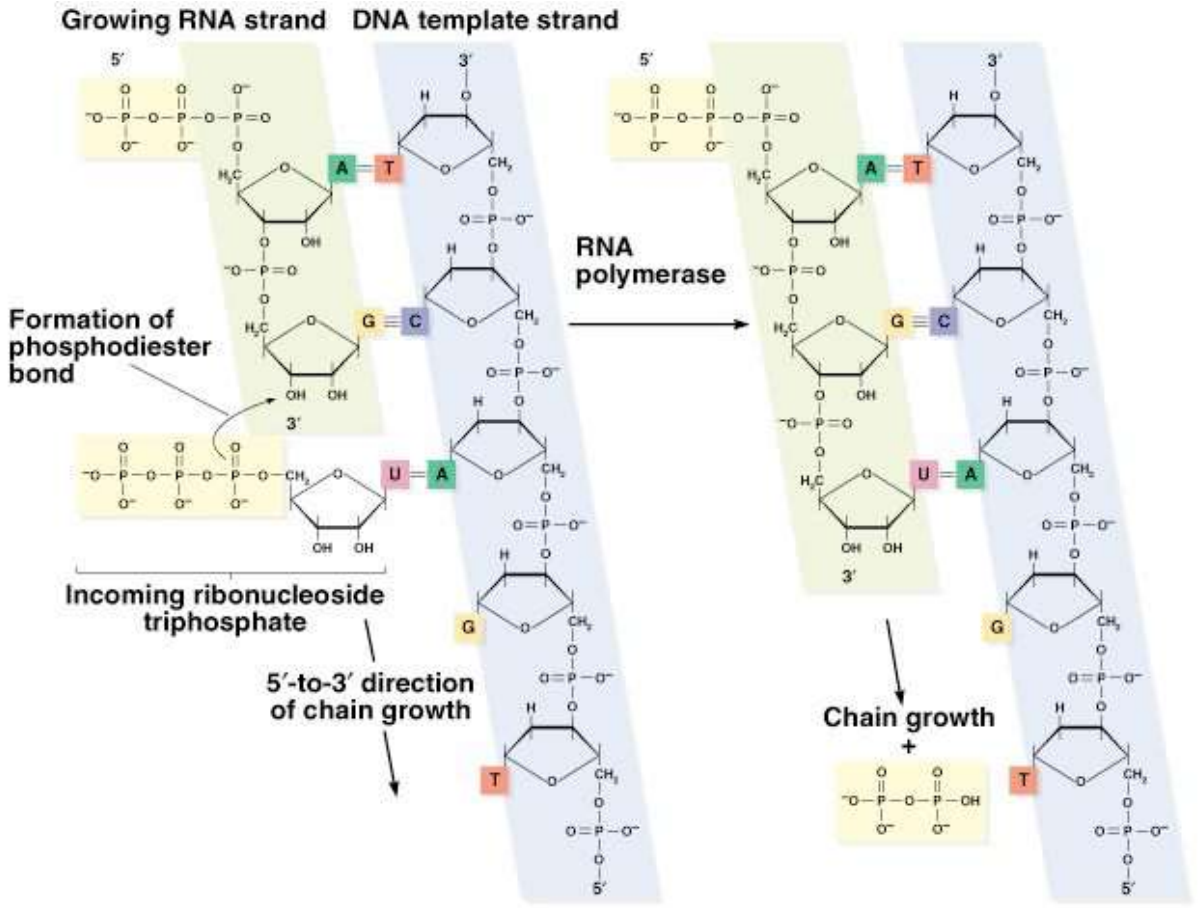
- Transcription in a Nutshell
- Purpose : make RNA
- Where does it happen: Nucleus of Euks (cyto of Proks)
- What is the template : Antisense strand
- How is it controlled : Promoter

Fig. Transcription process



- The Transcription Process:
- RNA Synthesis
- • RNA polymerization is similar to DNA synthesis
- except:
- 1. The precursors are NTPs (not dNTPs).
- 2. No primer is needed to initiate synthesis.
- 3. Uracil is inserted instead of thymine

Chemical reaction involved in the RNA polymerase catalyzed synthesis of RNA on a DNA template strand



Prokaryotic Transcription

The Transcription Process:

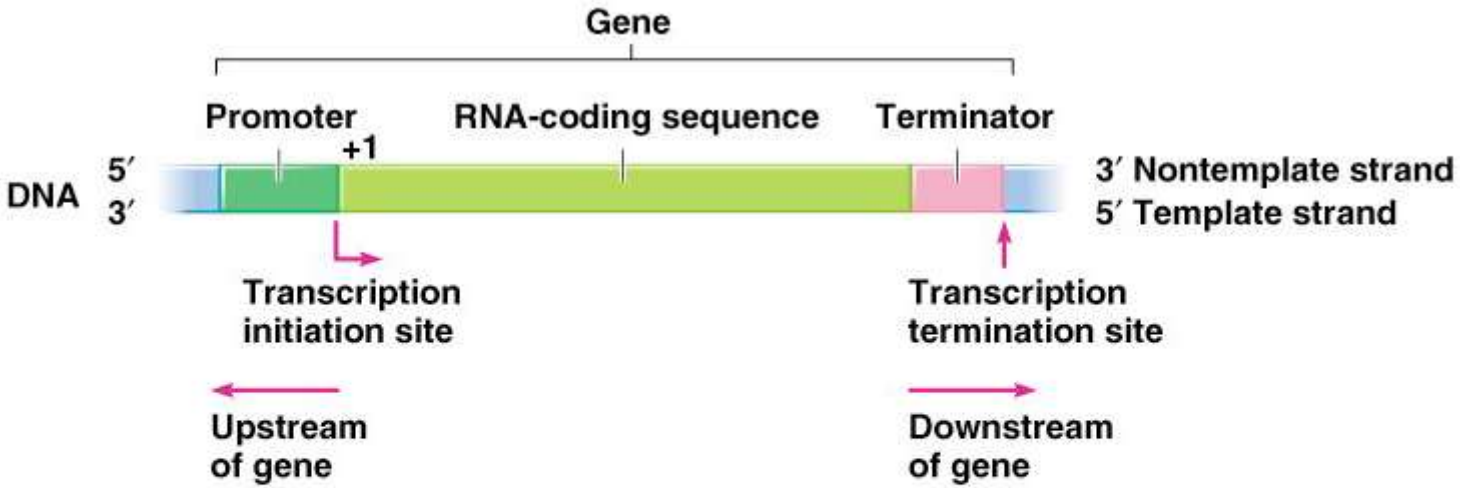
- Transcription is divided into three steps for both prokaryotes and eukaryotes. They are:

1. Initiation
2. Elongation
3. Termination.

- The process of elongation is highly conserved between prokaryotes and eukaryotes, but initiation and termination are somewhat different.
- This section is about initiation of transcription in prokaryotes. *E. coli is the model organism*

- The Transcription Process:
- Initiation of Transcription
- • A prokaryotic gene is a DNA sequence in the chromosome. The
- gene has three regions, each with a function in transcription
- (Figure 5.3):
- 1. A promoter sequence that attracts RNA polymerase to begin transcription at
- a site specified by the promoter.
- 2. The transcribed sequence, called the RNA-coding sequence. The sequence
- of this DNA corresponds with the RNA sequence of the transcript.
- 3. A terminator region that specifies where transcription will stop

Proks and Euks Fig. 13.3 Promoter, RNA-coding sequence, and terminator regions of a gene



The Prokaryotic Transcription Process: Initiation of Transcription

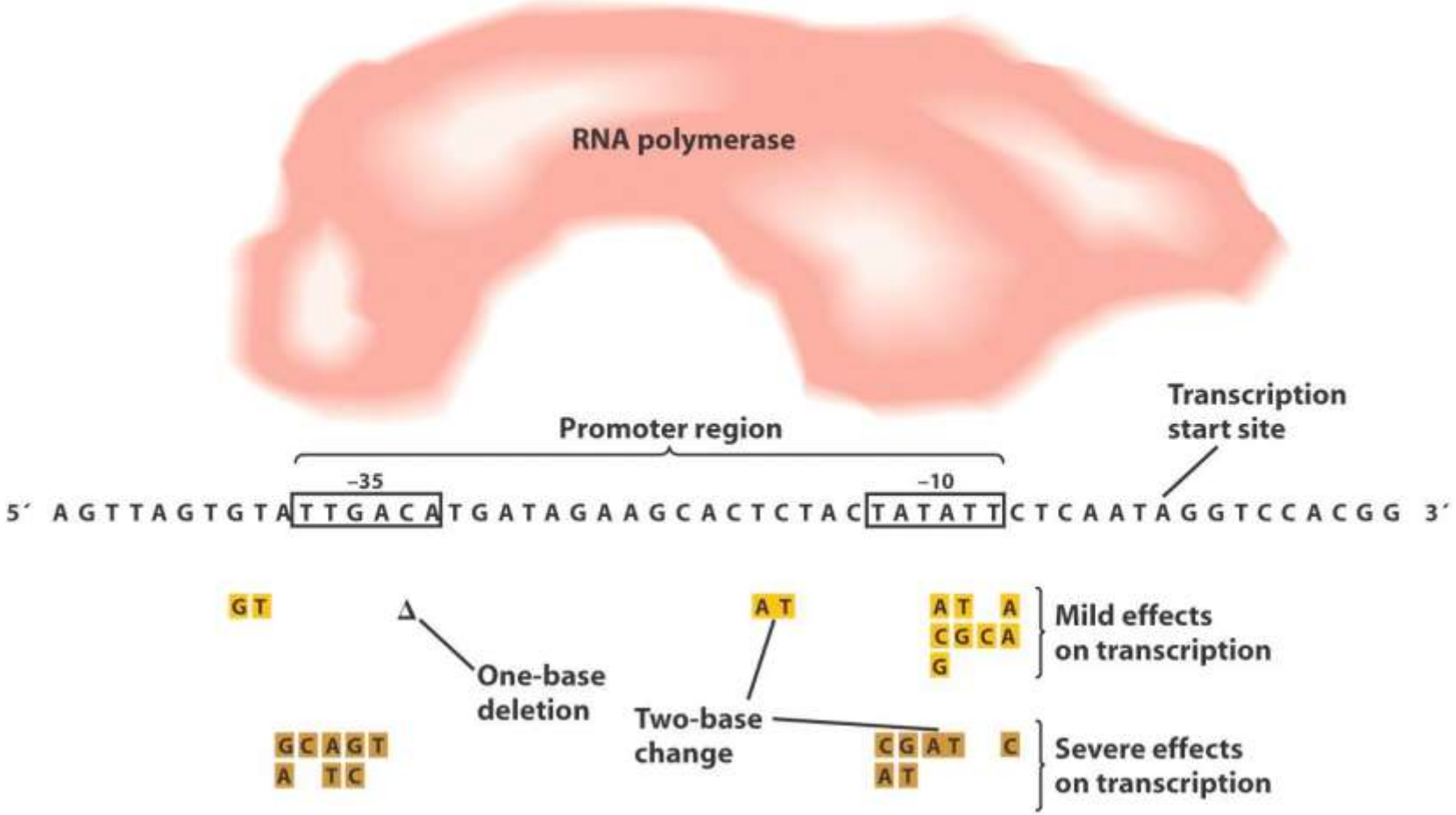
- • Promoters in *E. coli generally involve two DNA sequences,*
- centered at -35bp and -10bp upstream from the +1 start site of
- transcription.
- • The common *E. coli promoter that is used for most transcription*has these consensus sequences:– For the -35 region the consensus is
- • 5’-TTGACA-3’.
- – For the -10 region (previously known as a Pribnow box), the consensus is
- 5’-TATAAT-3’.

The Prokaryotic Transcription Process: Initiation of Transcription

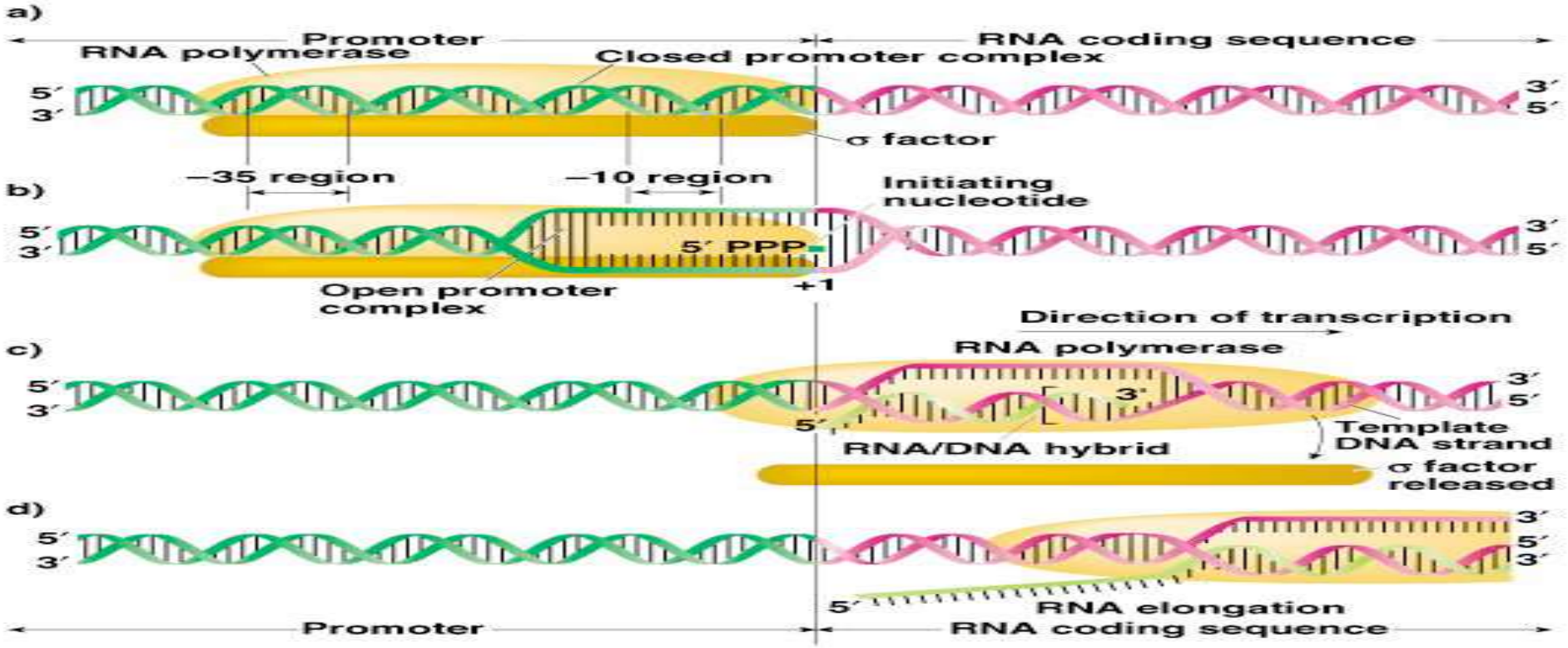
- • Transcription initiation requires the RNA polymerase holoenzyme
- to bind to the promoter DNA sequence.
- • Holoenzyme consists of:
- – Core enzyme of RNA polymerase, containing four polypeptides
- • (two !, one ", and one "#).
- – Sigma factor ($).
- • !Sigma factor binds the core enzyme, and confers ability to recognize promoters.

- The Prokaryotic Transcription Process:
- Initiation of Transcription
- • RNA polymerase holoenzyme binds promoter in two steps
- (Figure 5.4):
- 1. First, it loosely binds to the -35 sequence of dsDNA (closed promoter
- complex).
- 2. Second, it binds tightly to the -10 sequence, untwisting about 17bp of
- DNA at the site, and in position to begin transcription (open promoter
- complex).
- • Promoter sequences often deviate from consensus.
- – The associated genes will show different levels of transcription,
- corresponding with sigma's ability to recognize their sequences

Prokaryotic: RNA Polymerase-Promoter interactions.



Action of *E. coli RNA* polymerase in the initiation and elongation stages oftranscription

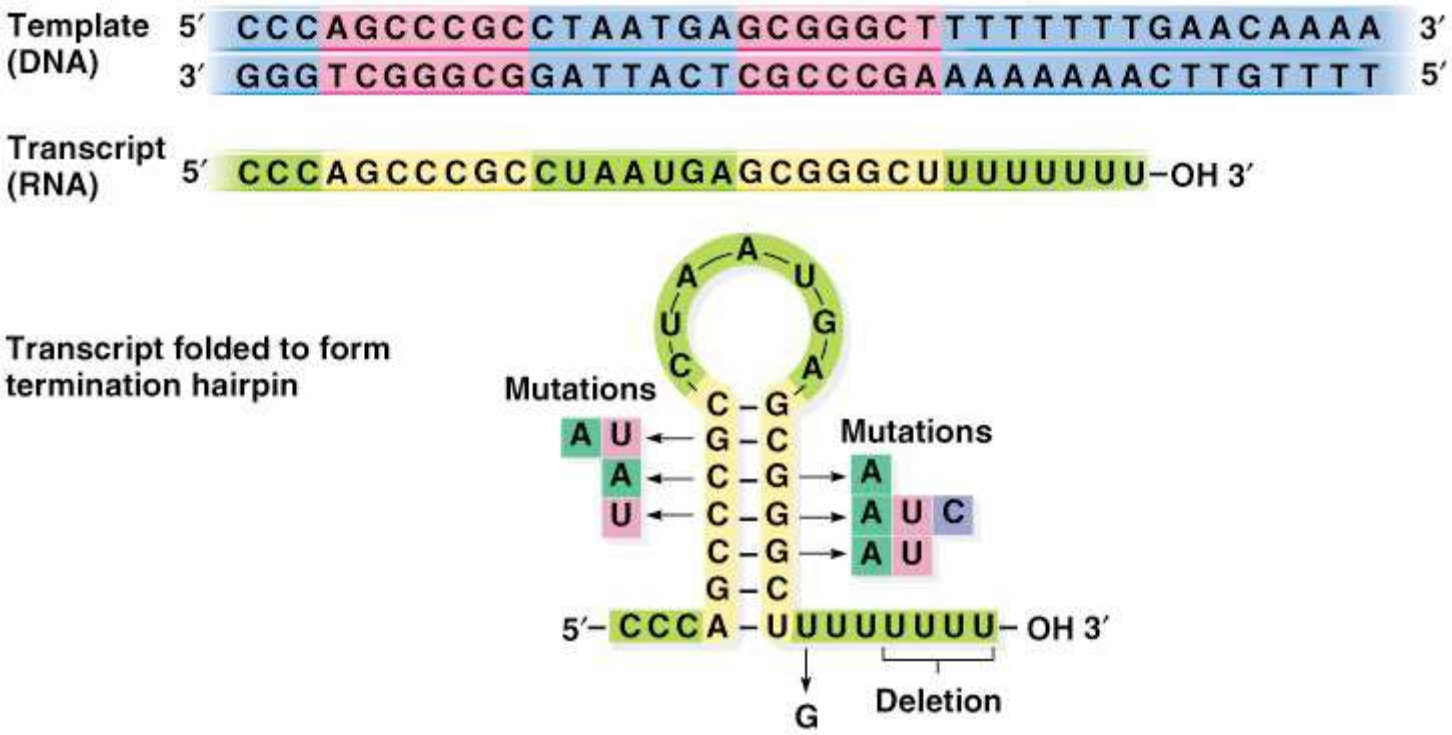


- The Prokaryotic Transcription Process:
- Initiation of Transcription
- • Most *E. coli genes have a $70 promoter, the most abundant*
- sigma factor in the cell.
- • Other sigma factors may be produced in response to
- changing conditions:
- 1. $70 recognizes the sequence TTGACA at %35, and TATAAT at %10.
- 2. $32 recognizes the sequence CCCCC at %39 and TATAAATA at %15.
- Sigma32 arises in response to heat shock and other forms of!stress.
- 3. $23 recognizes the sequence TATAATA at position %15. Sigma23 is
- present in cells infected with phage T4.

- The Prokaryotic Transcription Process: Elongation
- • Once initiation is completed, RNA synthesis begins.
- – After 8–9 NTPs have been joined in the growing RNA chain,
- – sigma factor is released and reused for other initiations.
- – Core enzyme completes the transcript (Figure 13.4).
- • Core enzyme untwists DNA helix, allowing a small region to
- denature.
- – Newly synthesized RNA forms an RNA-DNA hybrid,
- – but most of the transcript is displaced as the DNA helix reforms.
- • The chain grows at 30–50nt/second.
- • RNA polymerase has two types of proofreading:
- – Similar to DNA polymerase editing, newly inserted nucleotide
- is removed by reversing synthesis reaction.
- – Enzyme moves back one or more nucleotides, cleaves RNA,
- then resumes synthesis in forward direction

- The Prokaryotic Transcription Process:
- Termination
- • Terminator sequences are used to end transcription.
- • In prokaryotes there are two types:
- 1. Rho-independent or type I terminators have twofold symmetry
- – allows a hairpin loop to form (Figure 13.5).
- 2. Rho-dependent or type II terminators lack the poly(U) region.
- – The protein Rho is required for termination.
- – It has two domains
- – one binding RNA and the other binding ATP.
- – ATP hydrolysis provides energy for rho to move along the
- transcript and destabilize the RNA-DNA hybrid at the termination region.

Sequence of a &-independent terminator and structure of the terminated RNA



Eukaryotic Transcription RNA Polymerases

- Eukaryotes contain three different RNA polymerases:
- 1. RNA polymerase I:
- – located in the nucleolus, transcribes the three major rRNAs
- 2. RNA polymerase II:
- – located in the nucleoplasm, transcribes mRNAs and some snRNAs..
- – Holloenzyme consiistts of llotts of protteiins allong wiitth RNA poll II
- – TFIID = TBP and TAFS
- – TBP = TATA Biindiing Protteiin (functtiions anallogous tto siigma facttor)
- – TAFs = TBP Associiatted Facttors :: tthere are hundreds of tthese
- 3. RNA polymerase III:
- – located in the nucleoplasm, transcribes tRNAs, 5S rRNA, and the remaining snRNAs

Transcription of Protein-Coding Genes by RNA Polymerase II

- When protein-coding genes are first transcribed by RNA pol II, the
- product is a precursor-mRNA (pre-mRNA or primary transcript).
- • The primary transcript will be modified to produce a mature
- mRNA.
- 1. Capping
- 2. Splicing
- 3. Tailing
- • Promoters for protein-coding genes are analyzed in two ways:
- – Directed mutation.
- – Comparison of sequences from known genes

Eukaryotic Promoters

- Results of promoter analysis reveal two types of elements:
- – Core promoter elements are located near the transcription start
- site
- • specify where transcription begins. Examples include:
- – TATA-less promoters have a yet to be identified element
- – The initiator element (Inr), a pyrimidine-rich sequence that spans
- the transcription start site.
- – The TATA box at -30ish
- » full consensus sequence is TATAAAA.
- » aids in local DNA denaturation
- » sets the start point for transcription.
- » Is bound by TBP (TATA binding protein)

Eukaryotic Transcription: Elongation

- RNA pol II adds complementary ribonucleotides to the
- template strand/anti-sense strand
- – 3’ Ends only!
- – New RNA chain grow in the 5’ to 3’ direction
- – Does not require a primer (unlike replication that does)
- – Keeps going until termination

Eukaryotic Terminator Sequences

- In eukaryotes, the transcript ends at various sites beyond
- the final 3’ end of the RNA
- – (AAUAAA sequence),
- – later precisely cut during RNA processing.
- – RNA pol II mysteriously falls off after cutting….

Overview of Transcription