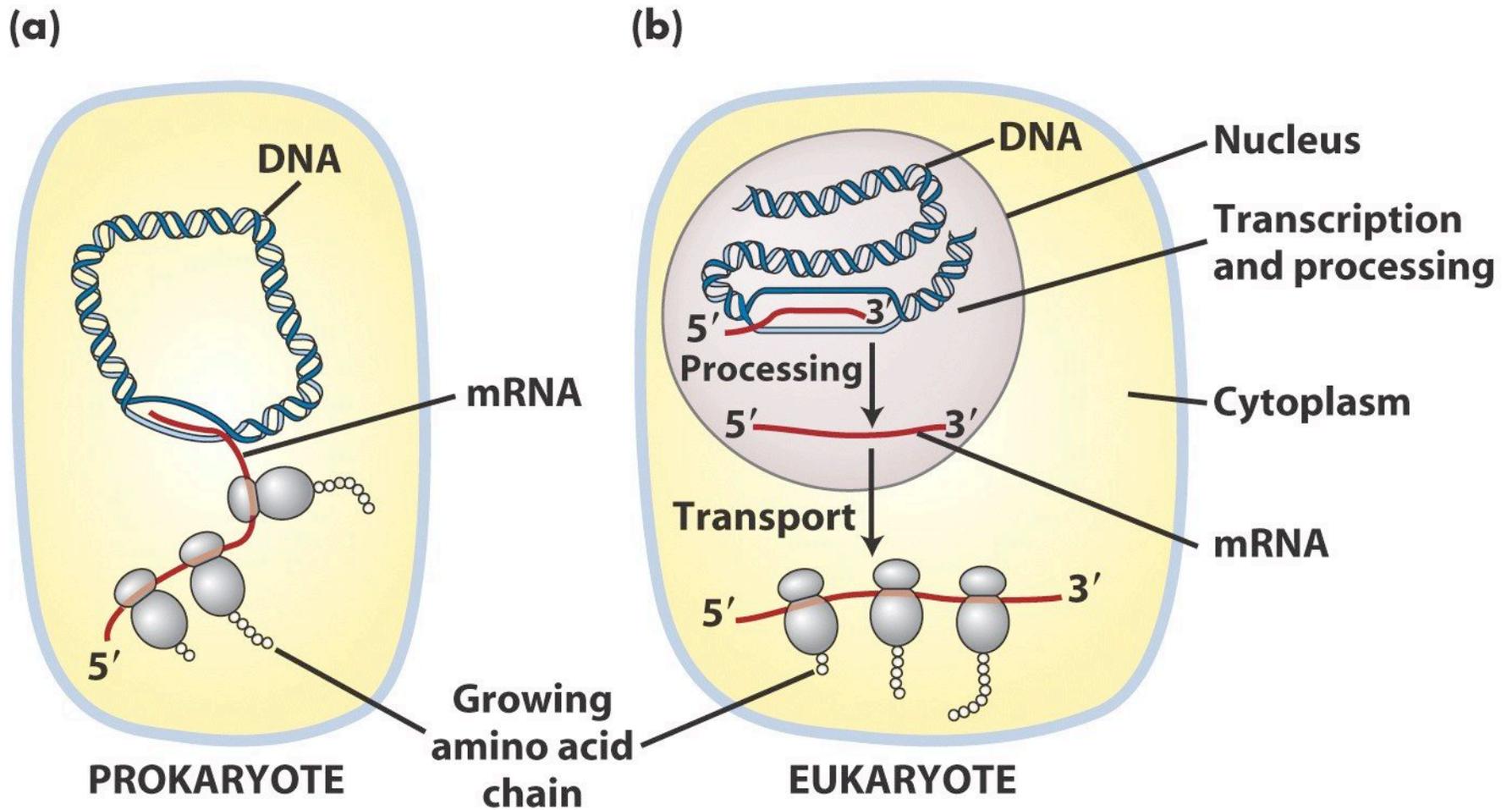


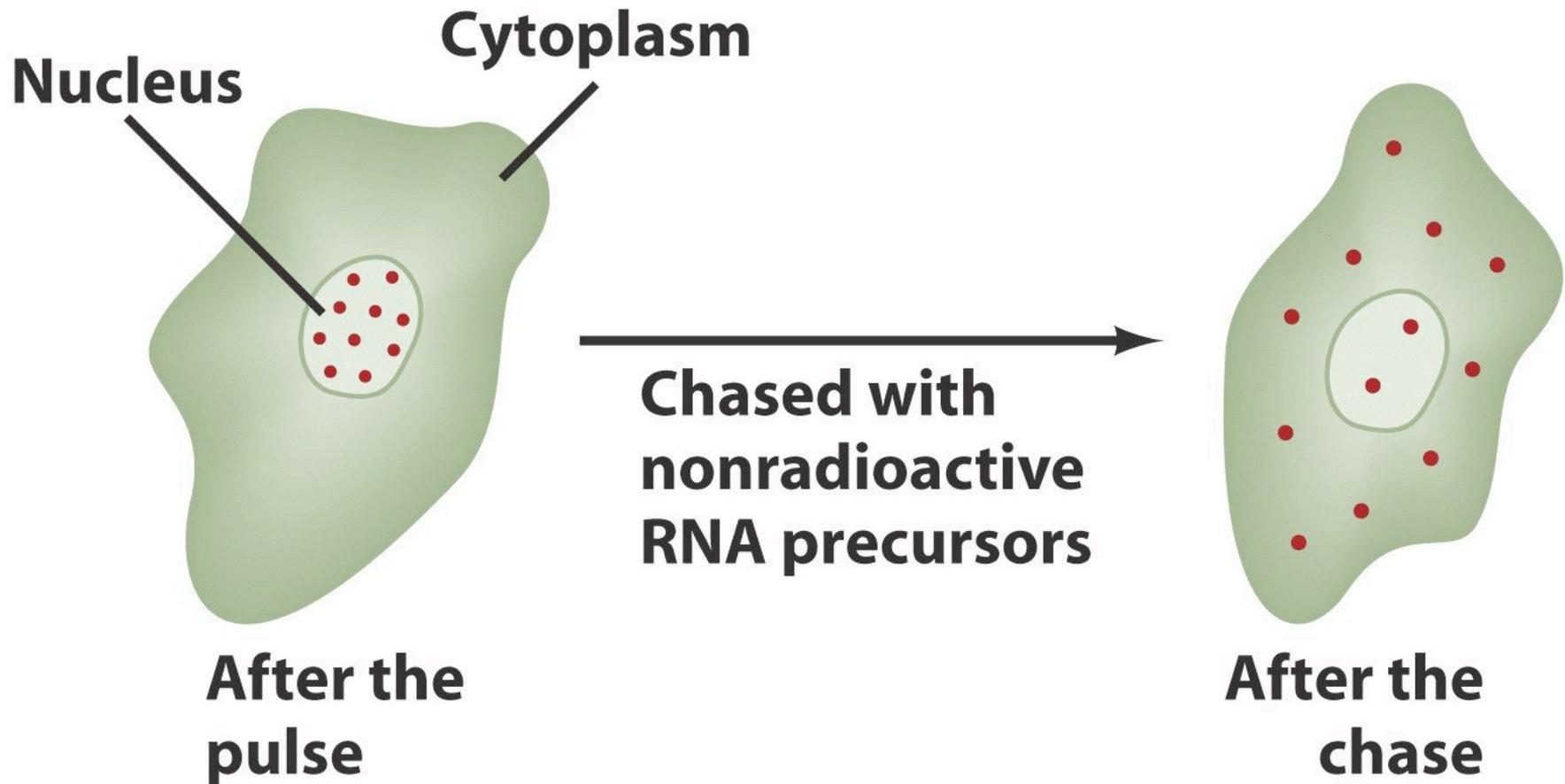
Central Dogma of Genetics

- 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



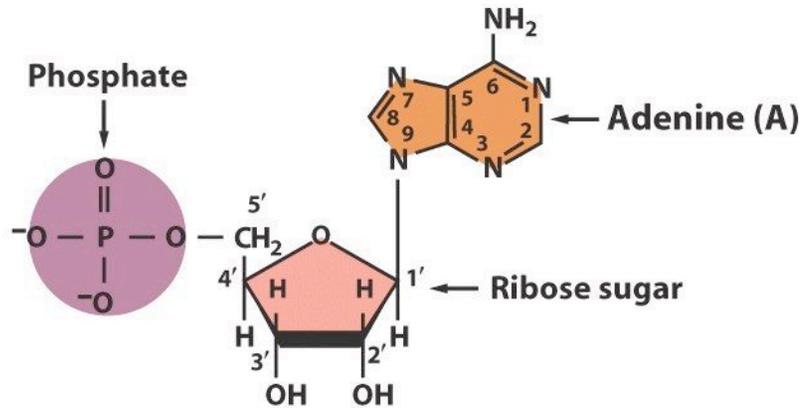
How do we know RNA is intermediate and moves to Cytoplasm in Euks?



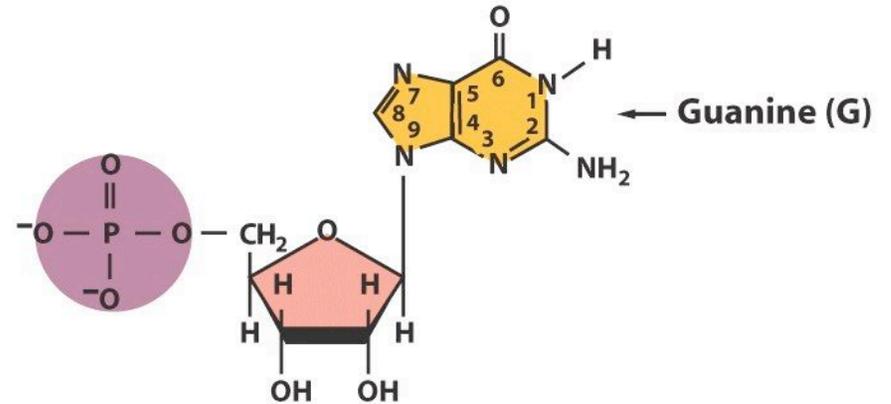
Grown briefly in Hot Uracil, spun out and grown in Cold Uracil.
Where does the radioactivity incorporated into RNA go?

RNA is Different than DNA

Purine ribonucleotides

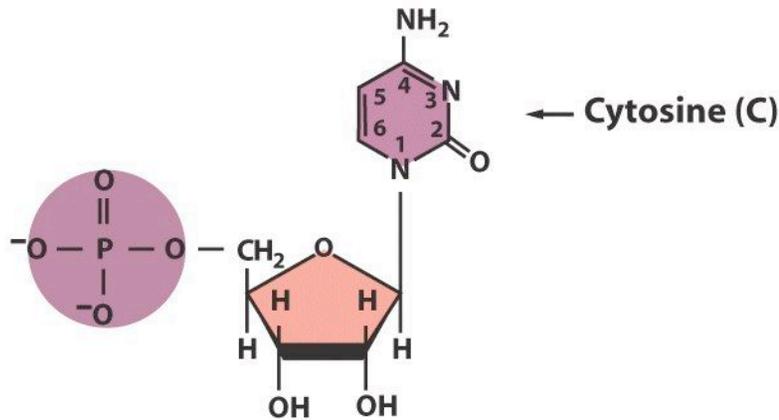


Adenosine 5'-monophosphate (AMP)

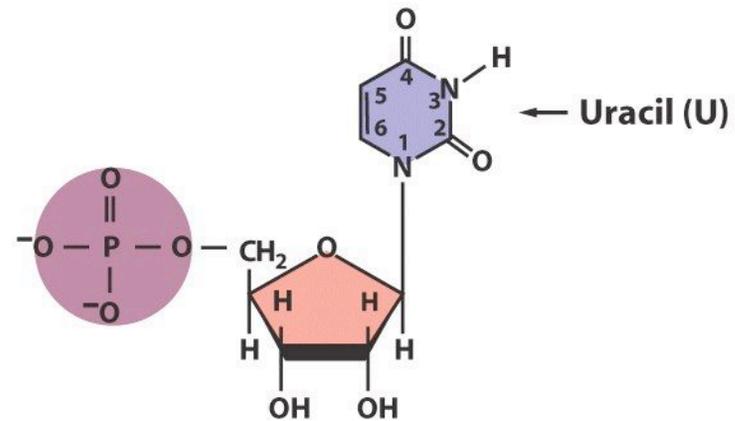


Guanosine 5'-monophosphate (GMP)

Pyrimidine ribonucleotides



Cytidine 5'-monophosphate (CMP)

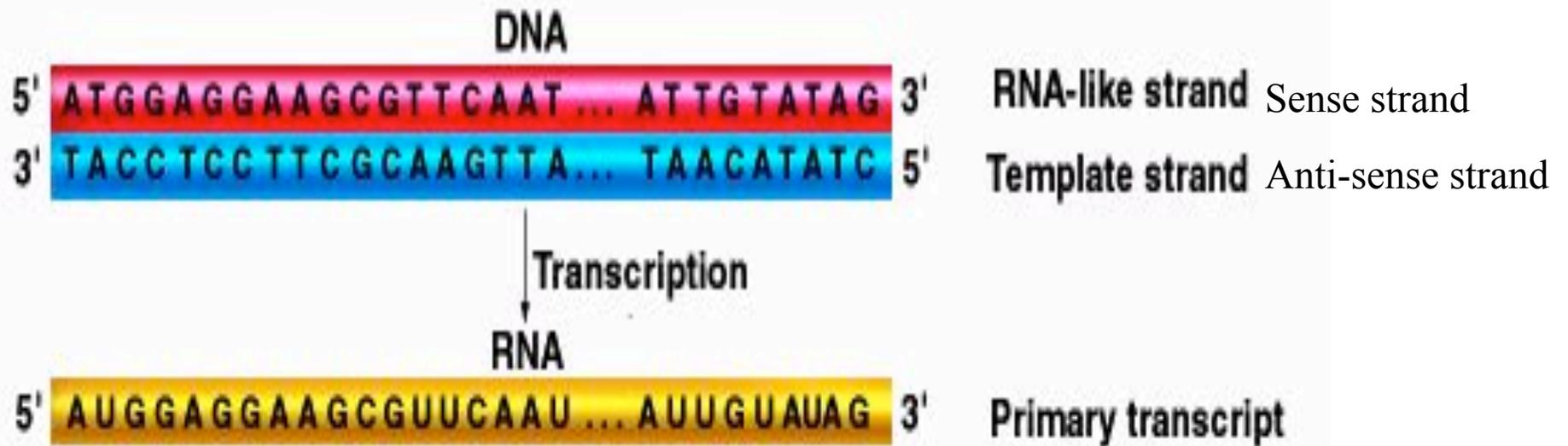


Uridine 5'-monophosphate (UMP)

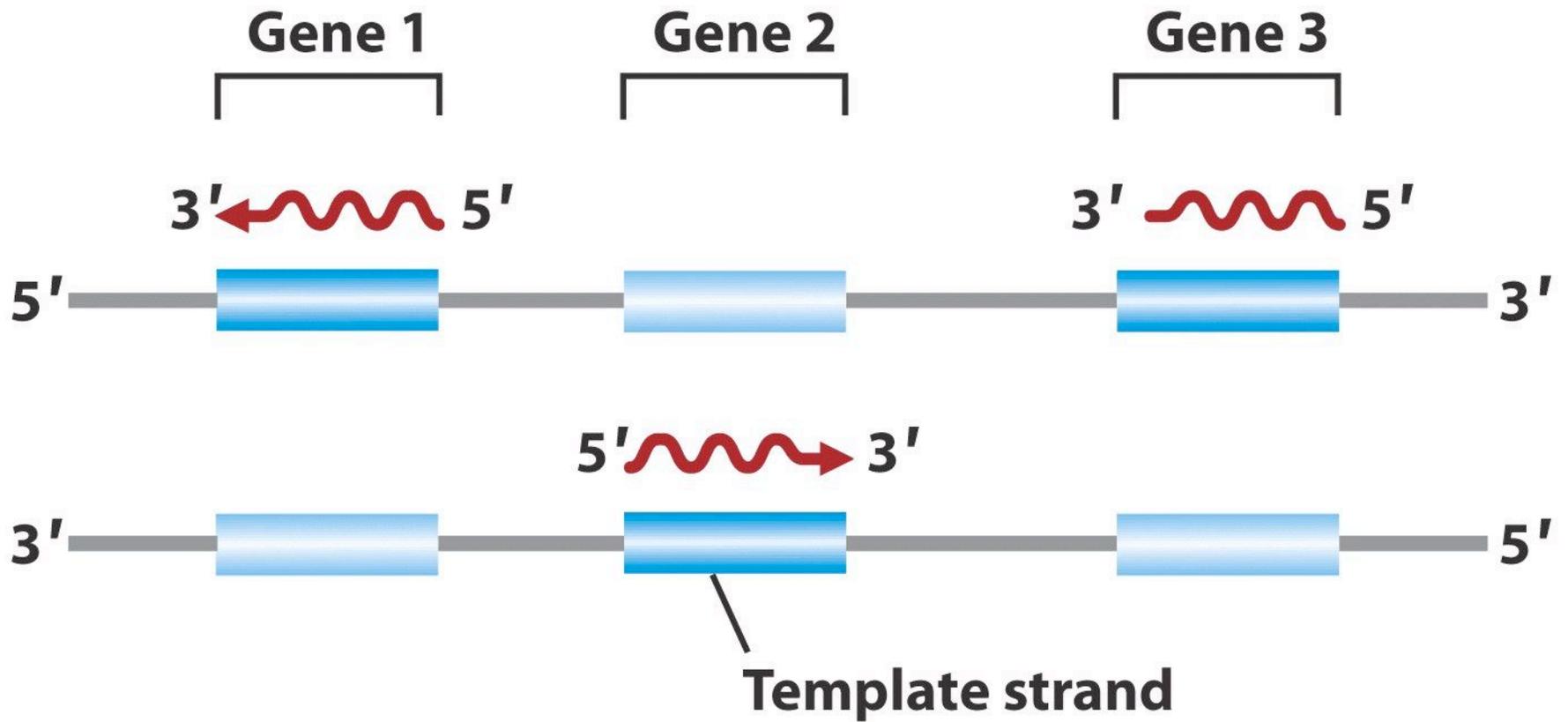
Transcription

- 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.

Designation of DNA strands



RNA is always 5' to 3'



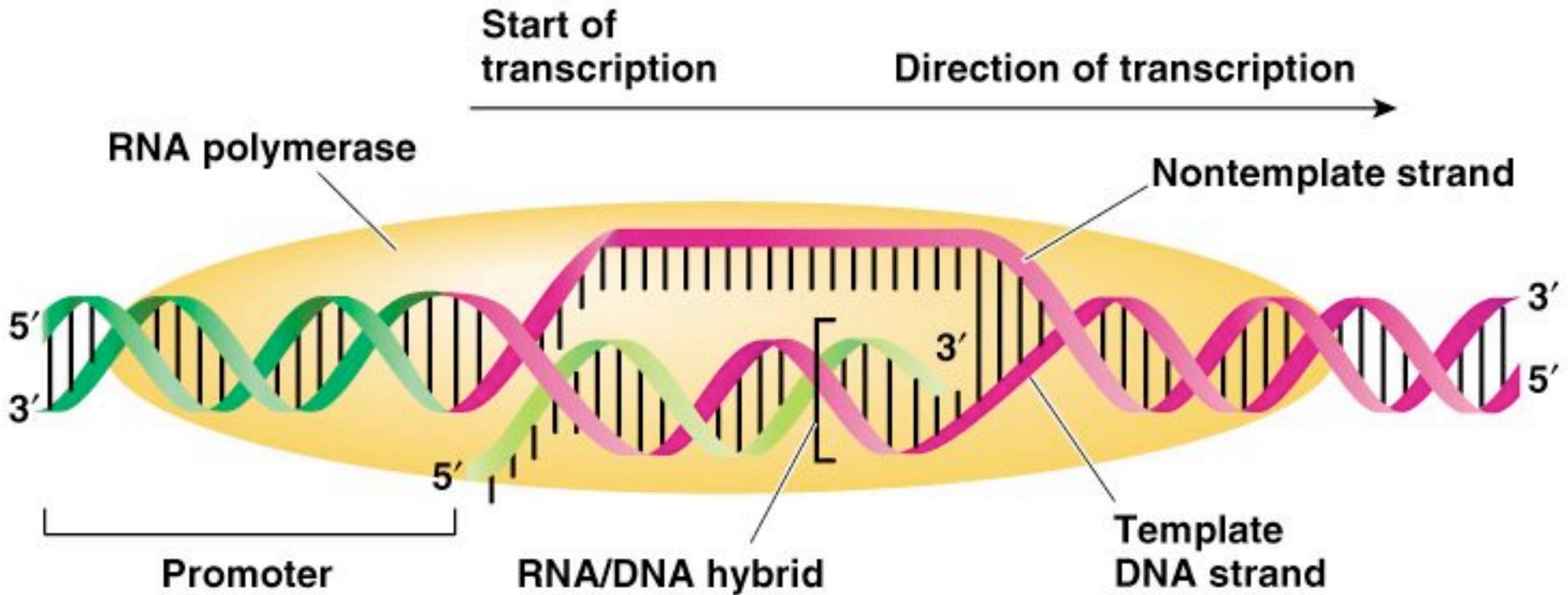
Salient Features of Transcription

- **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

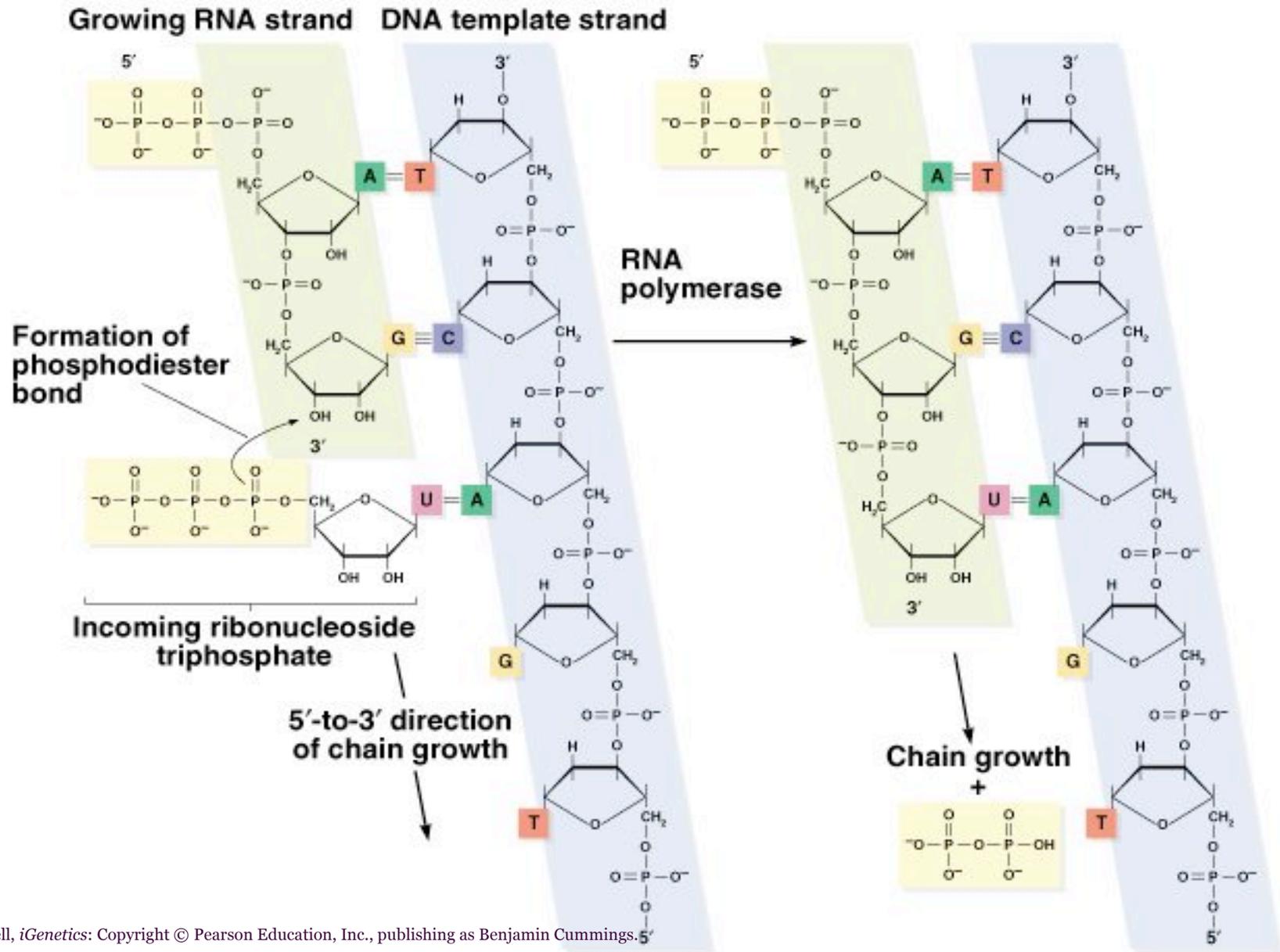
Fig. 13.1 Transcription process



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.

Fig. 5.2 Chemical reaction involved in the RNA polymerase catalyzed synthesis of RNA on a DNA template strand



Prokaryotic Transcription

The Transcription Process:

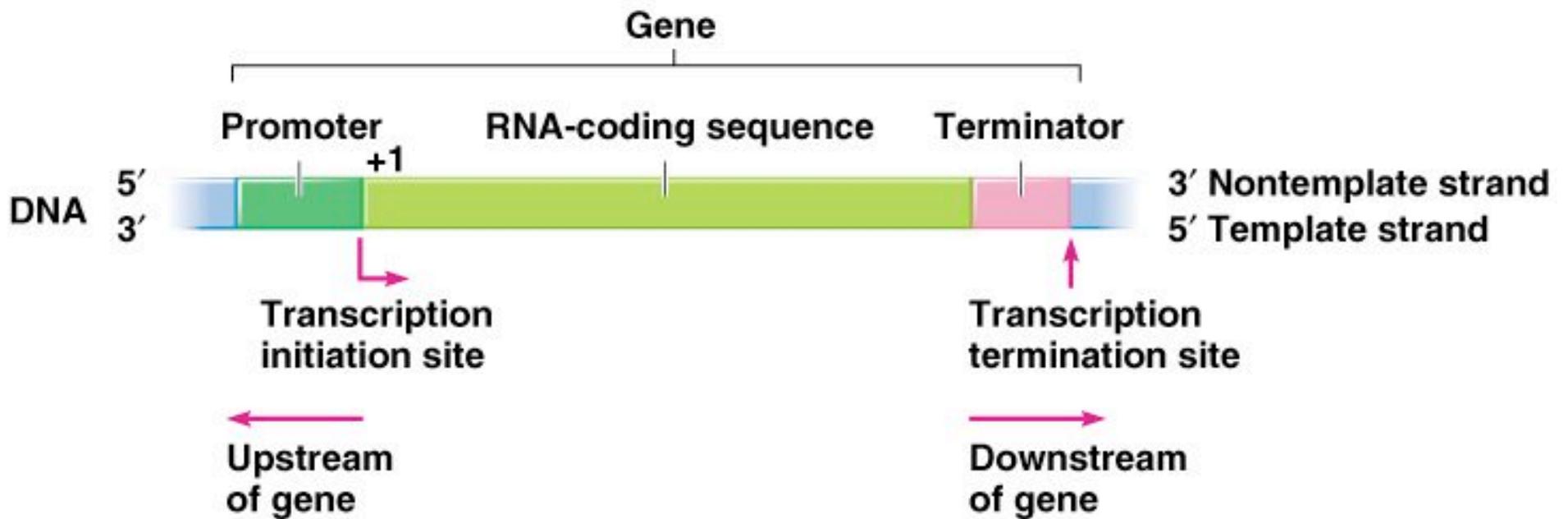
- Transcription is divided into three steps for both prokaryotes and eukaryotes. They are:
 1. Initiation
 2. Elongation
 3. Termination.
- The process of elongation is highly conserved between prokaryotes and eukaryotes, but initiation and termination are somewhat different.
- This section is about 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

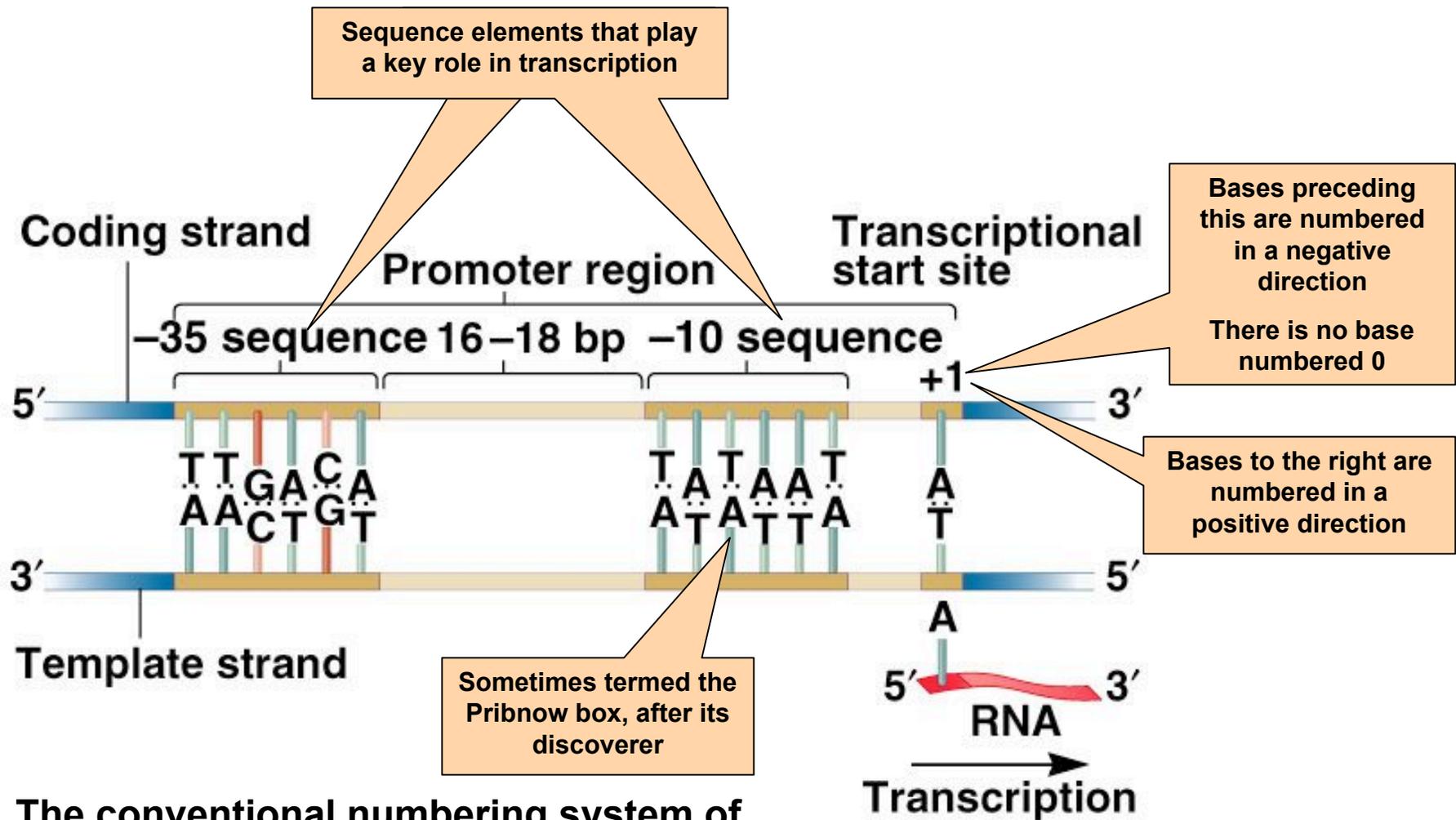
Promoter, RNA-coding sequence, and terminator regions of a gene



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



The conventional numbering system of promoters

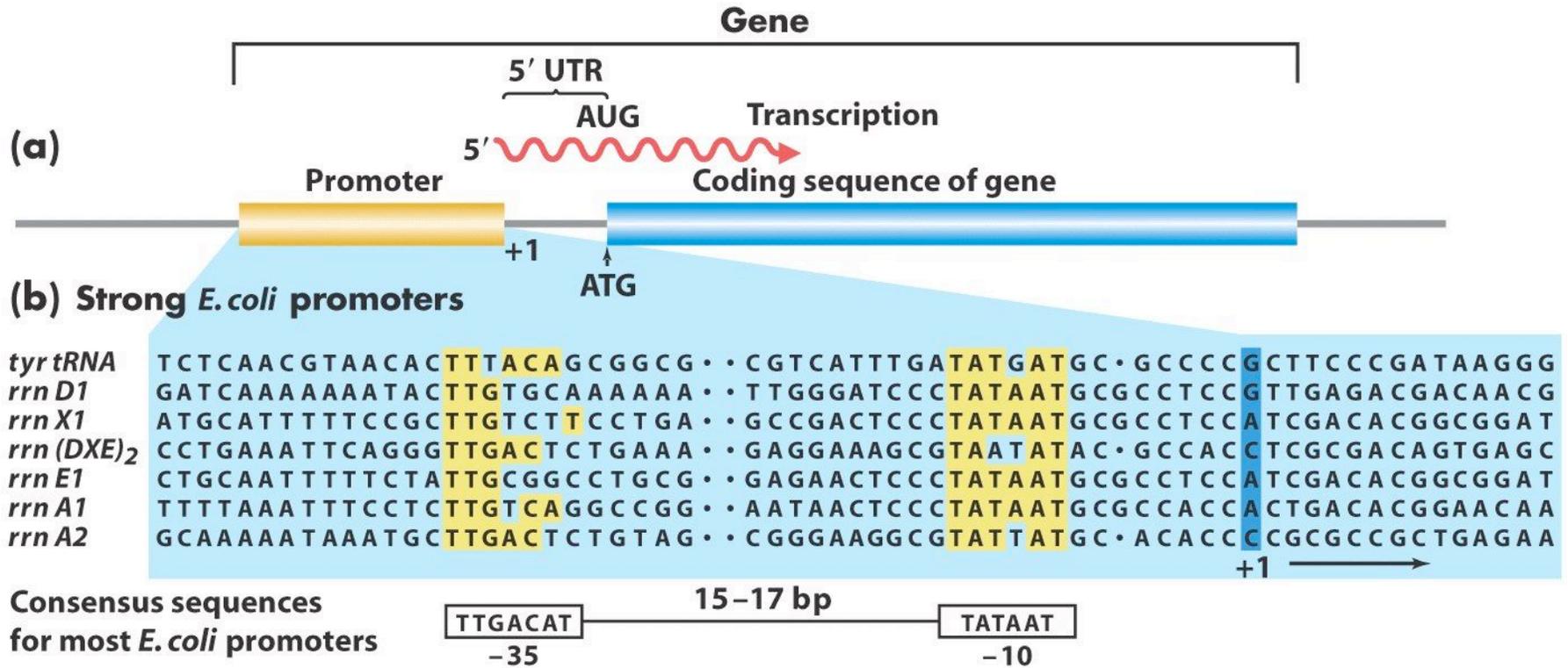
The Prokaryotic Transcription Process: Initiation of Transcription

- 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 α , one β , and one β').
 - Sigma factor (σ).
- 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.

E. Coli Promoters



Prokaryotic: RNA Polymerase-Promoter interactions.

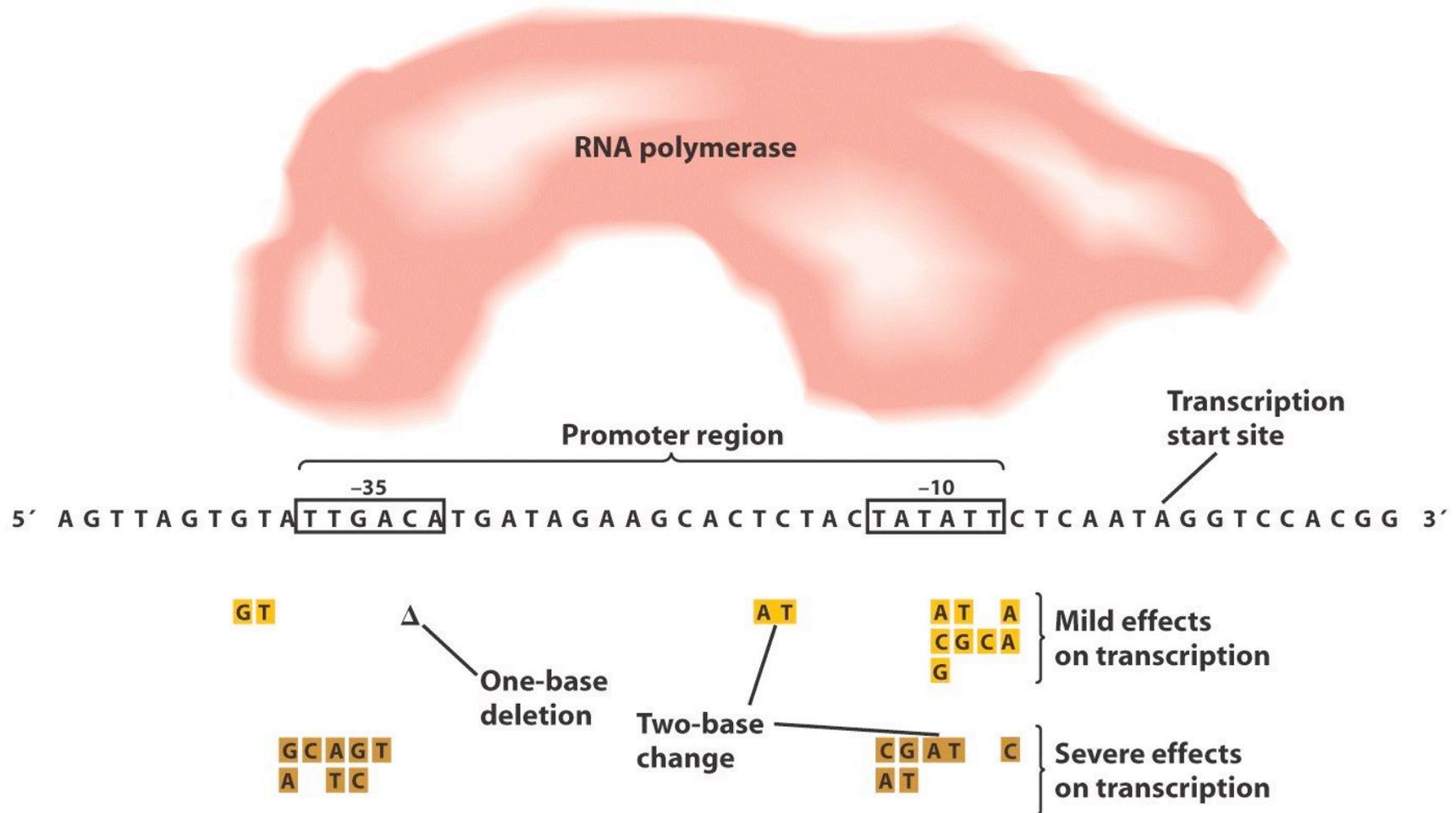
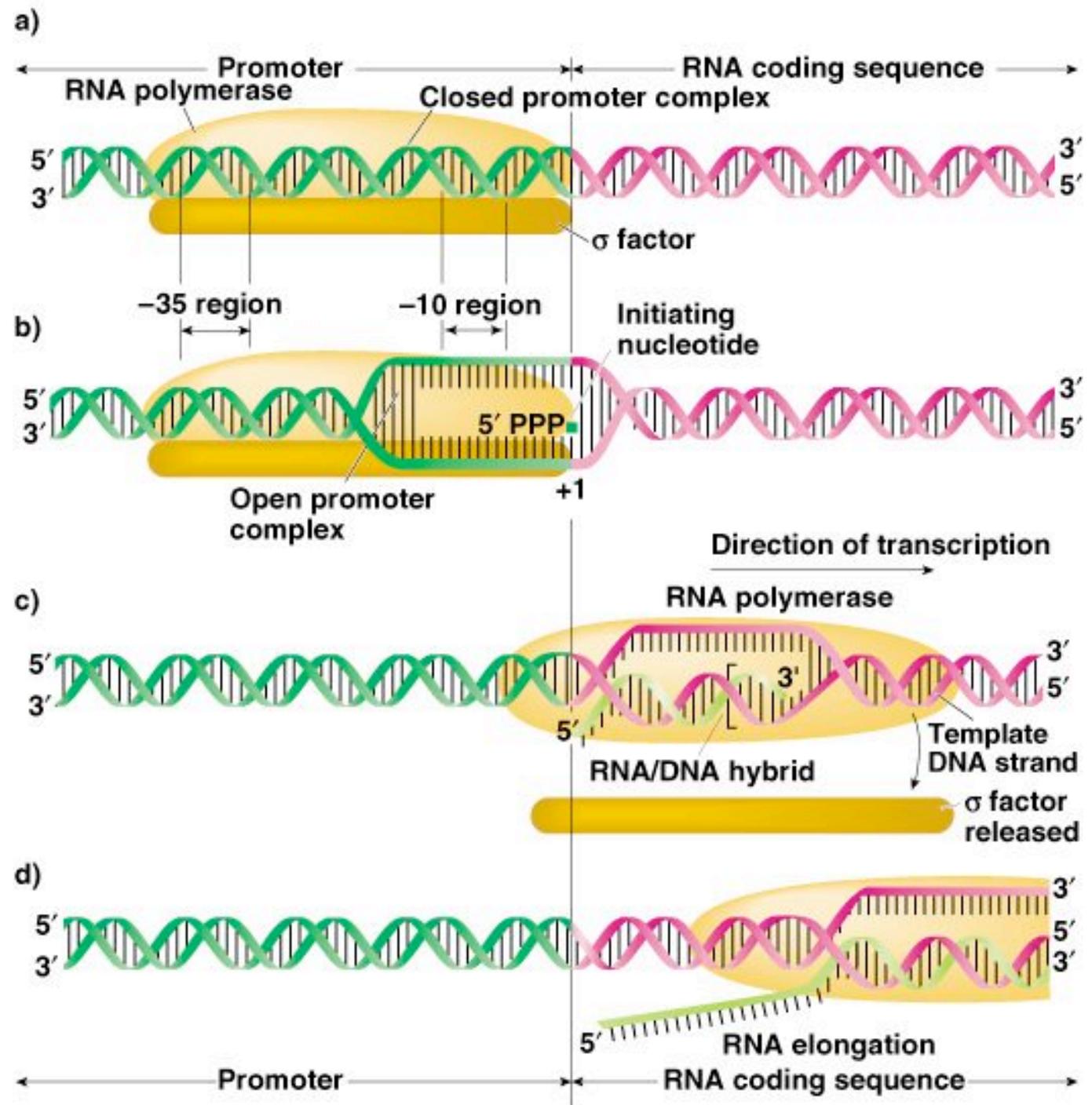


Fig. 13.4
 Action of
E. coli RNA
 polymerase in
 the initiation
 and
 elongation
 stages of
 transcription



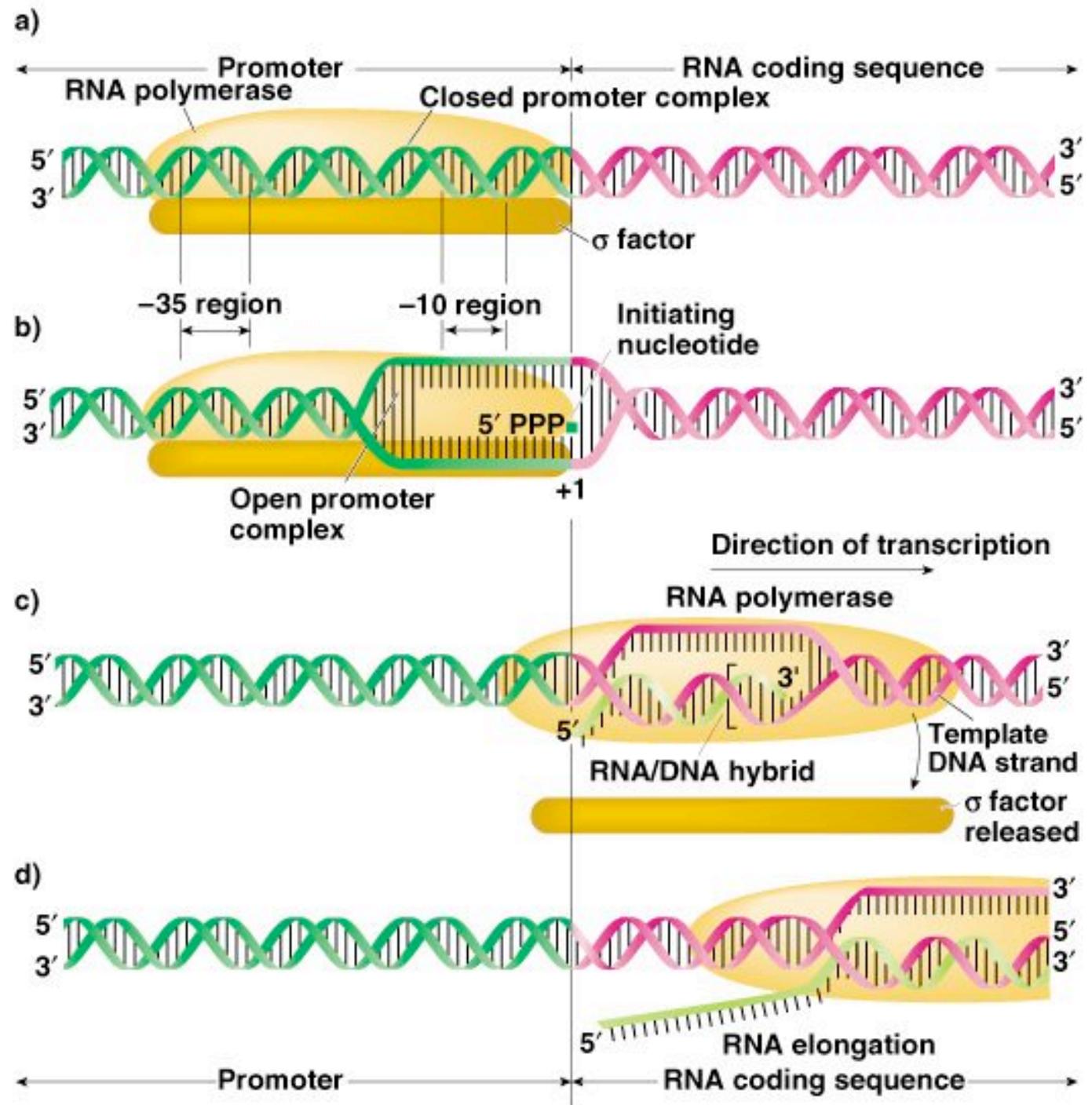
The Prokaryotic Transcription Process: Initiation of Transcription

- Most *E. coli* genes have a σ^{70} promoter, the most abundant sigma factor in the cell.
- Other sigma factors may be produced in response to changing conditions:
 1. σ^{70} recognizes the sequence TTGACA at -35, and TATAAT at -10.
 2. σ^{32} recognizes the sequence CCCC at -39 and TATAAATA at -15. Sigma³² arises in response to heat shock and other forms of stress.
 3. σ^{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.

Fig. 13.4
 Action of
E. coli RNA
 polymerase in
 the initiation
 and
 elongation
 stages of
 transcription



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.

Fig. 13.5

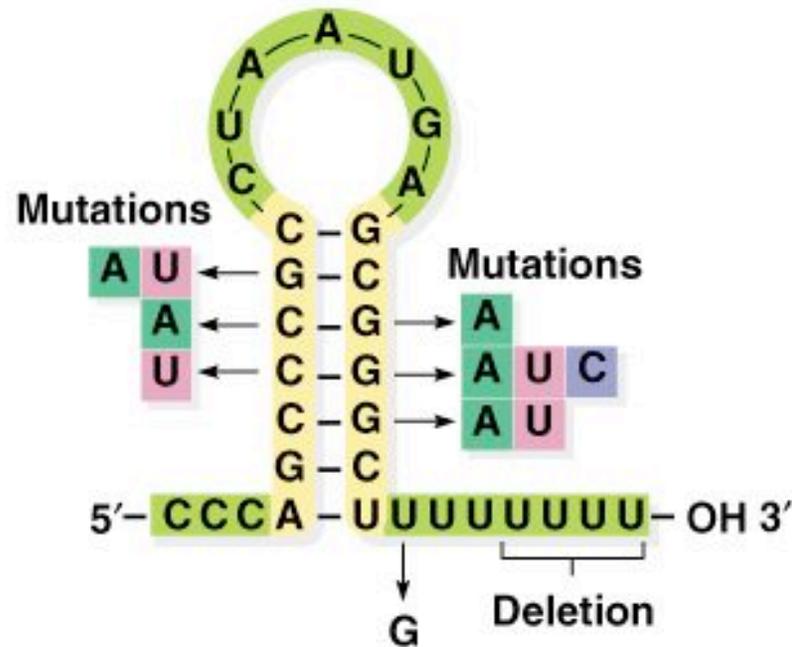
Sequence of a ρ -independent terminator and structure of the terminated RNA

← Two-fold symmetry →

Template (DNA) 5' CCCAGCCCGCCTAATGAGCGGGCTTTTTTTTGAACAAA 3'
 3' GGGTCGGGCGGATTACTCGCCCGAAAAAACTTGTTTT 5'

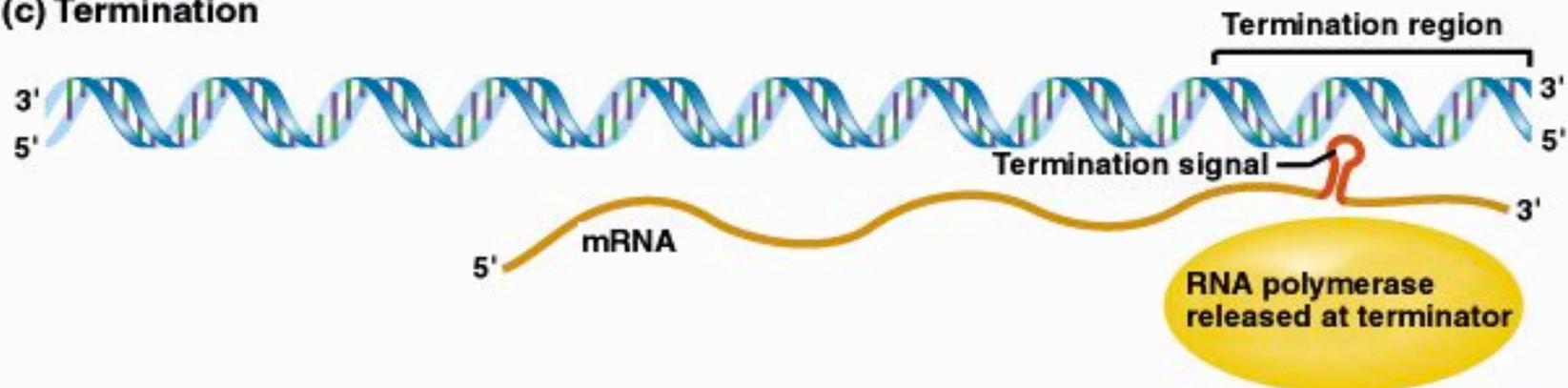
Transcript (RNA) 5' CCCAGCCCGCCUAAUGAGCGGGCUUUUUUUU-OH 3'

Transcript folded to form termination hairpin



Transcription, part 2

(c) Termination



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.
 - Holoenzyme consists of lots of proteins along with RNA pol II
 - TFIID = TBP and TAFs
 - TBP = TATA Binding Protein (functions analogous to sigma factor)
 - TAFs = TBP Associated Factors : there are hundreds of these
 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

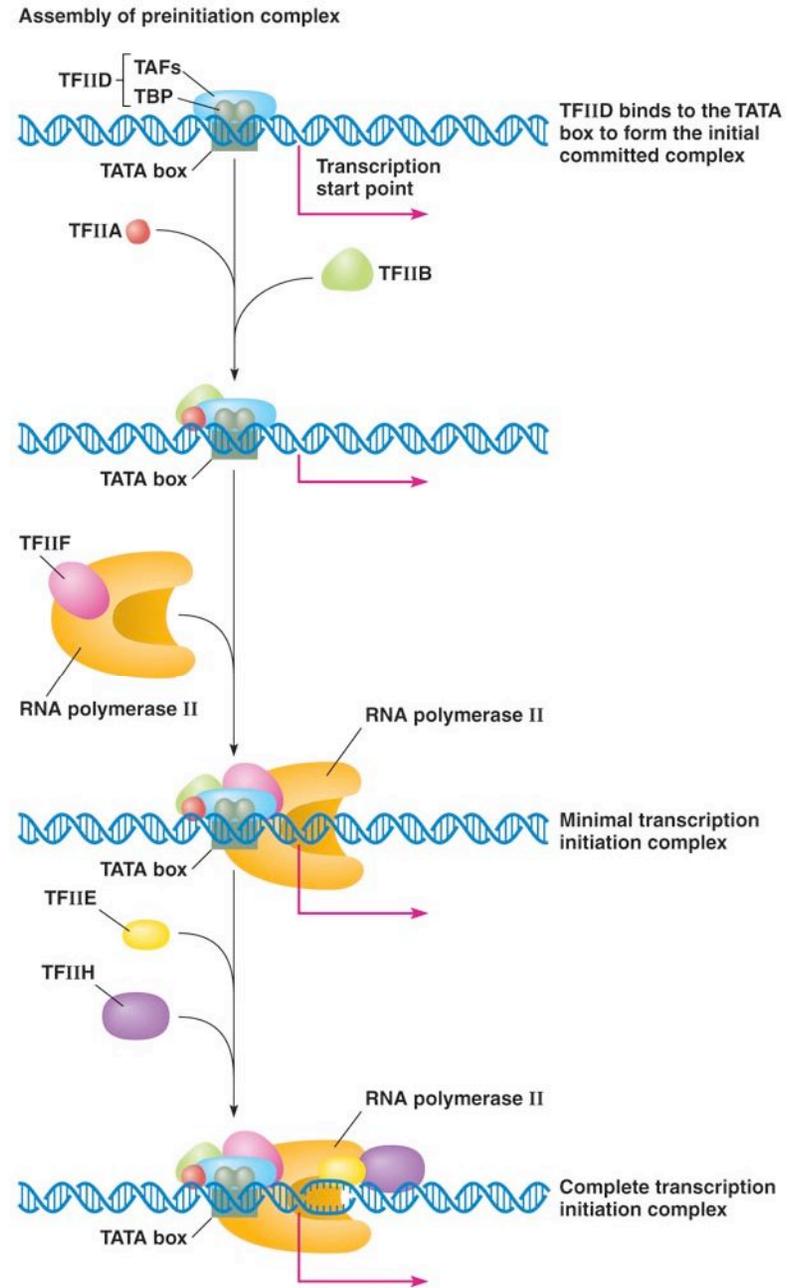
- When protein-coding genes are first transcribed by RNA pol II, the product is a precursor-mRNA (pre-mRNA or primary transcript).
- The primary transcript will be modified to produce a mature mRNA.
 1. Capping
 2. Splicing
 3. Tailing
- Promoters for protein-coding genes are analyzed in two ways:
 -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

None of this happens in PROKS!!!

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 RNA polymerase II: preinitiation complex



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Eukaryotic Transcription: Elongation

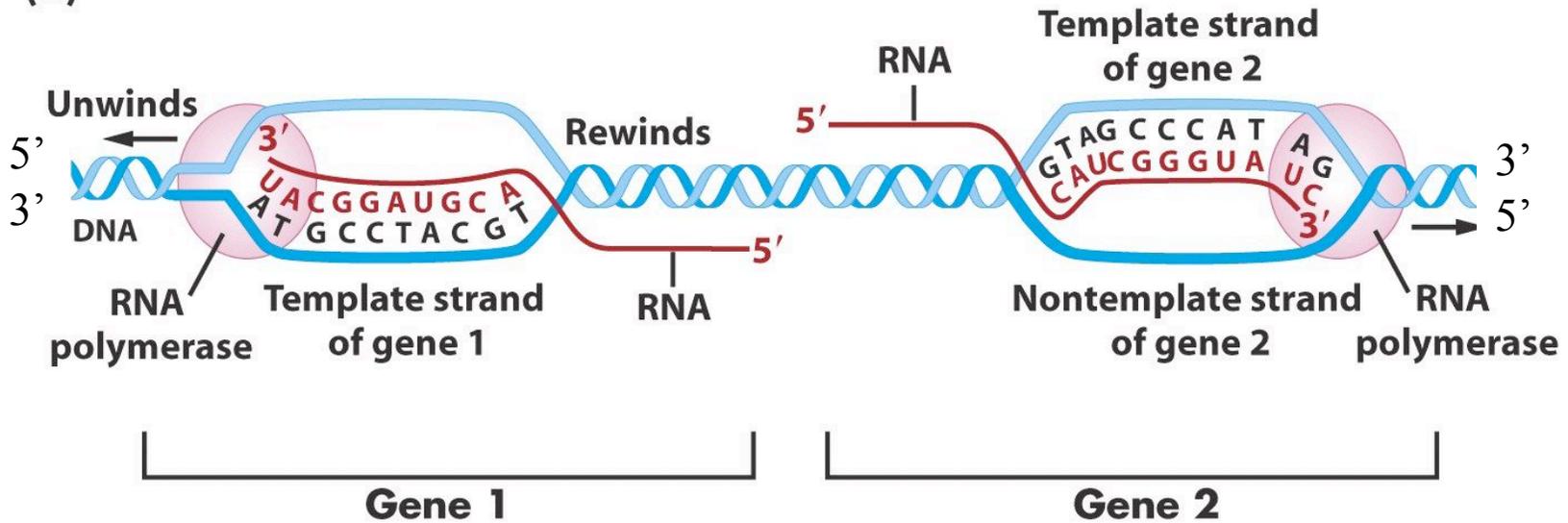
- RNA pol II adds complementary ribonucleotides to the template strand/anti-sense strand
 - 3' Ends only!
 - New RNA chain grow in the 5' to 3' direction
 - Does not require a primer (unlike replication that does)
 - Keeps going until termination....

Eukaryotic Terminator Sequences

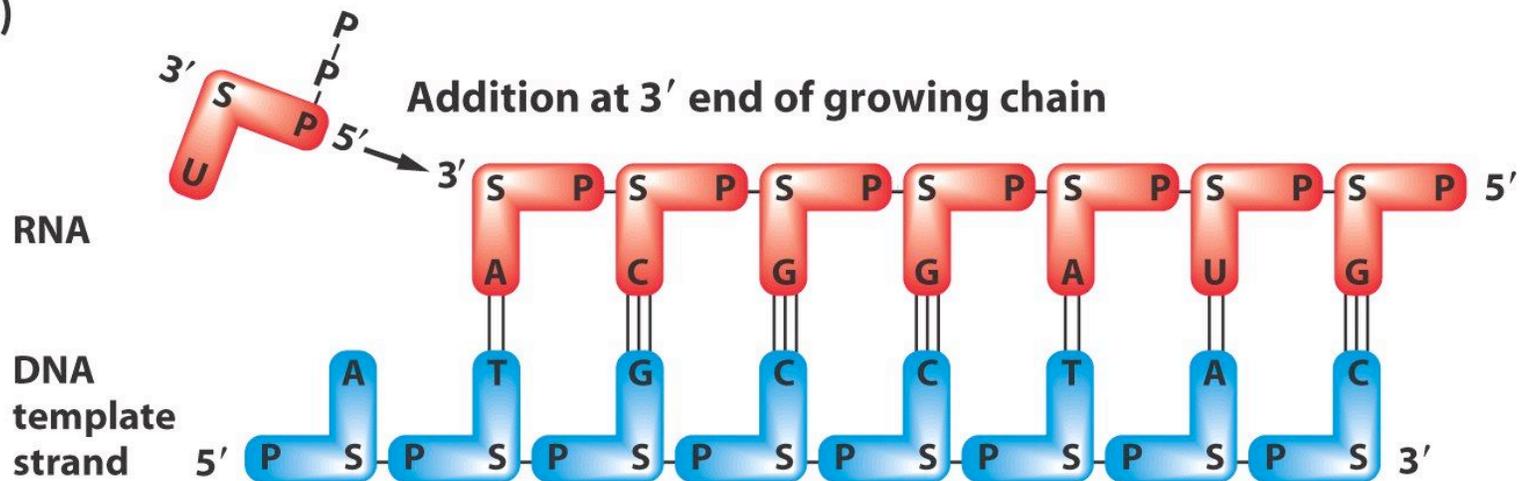
- In eukaryotes, the transcript ends at various sites beyond the final 3' end of the RNA
 - (AAUAAA sequence),
 - later precisely cut during RNA processing.
 - RNA pol II mysteriously falls off after cutting....

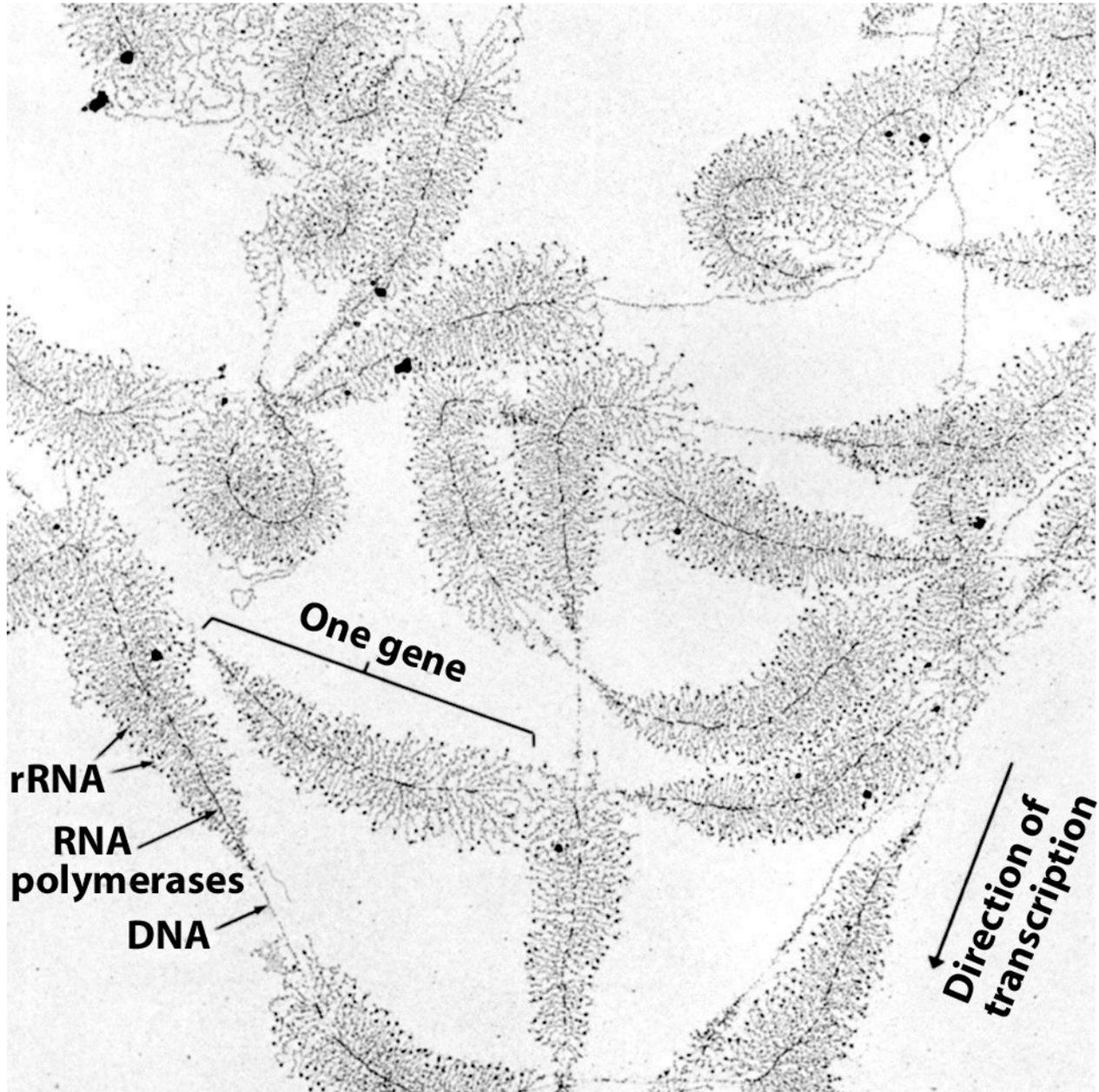
Overview of Transcription

(a)



(b)





Central Dogma

- DNA---> RNA---> protein
 - Transcription translation
- DNA to DNA is?
 - replication

Homework Problems

Chapter 13

7, 8