BHARATHIDASAN UNIVERSITY

Tiruchirappalli 620024

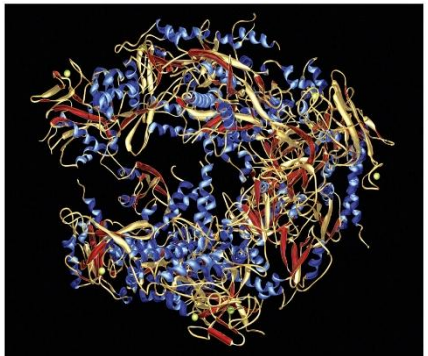
Tamilnadu, India

Program: M.Sc., Microbiology

Course Title : Microbial Genetics & Molecular Biology

Course Code: 24MICCC3

Unit IV: Transcription



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Professor

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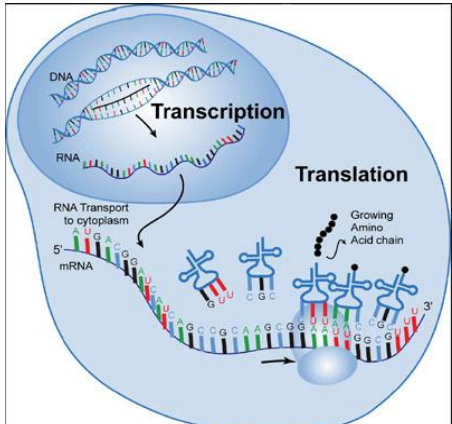


Transfer of Genetic Information: The Central Dogma

Transfer of Genetic Information:The Central Dogma

The central dogma of biology is that **information stored in DNA** is **transferred to RNA** molecules during ***transcription*** and to **proteins** during ***translation***.

DNA → **RNA** → **proteins**

Genotyping Phenotyping

RNA → **DNA/RNA** → **proteins**

virus

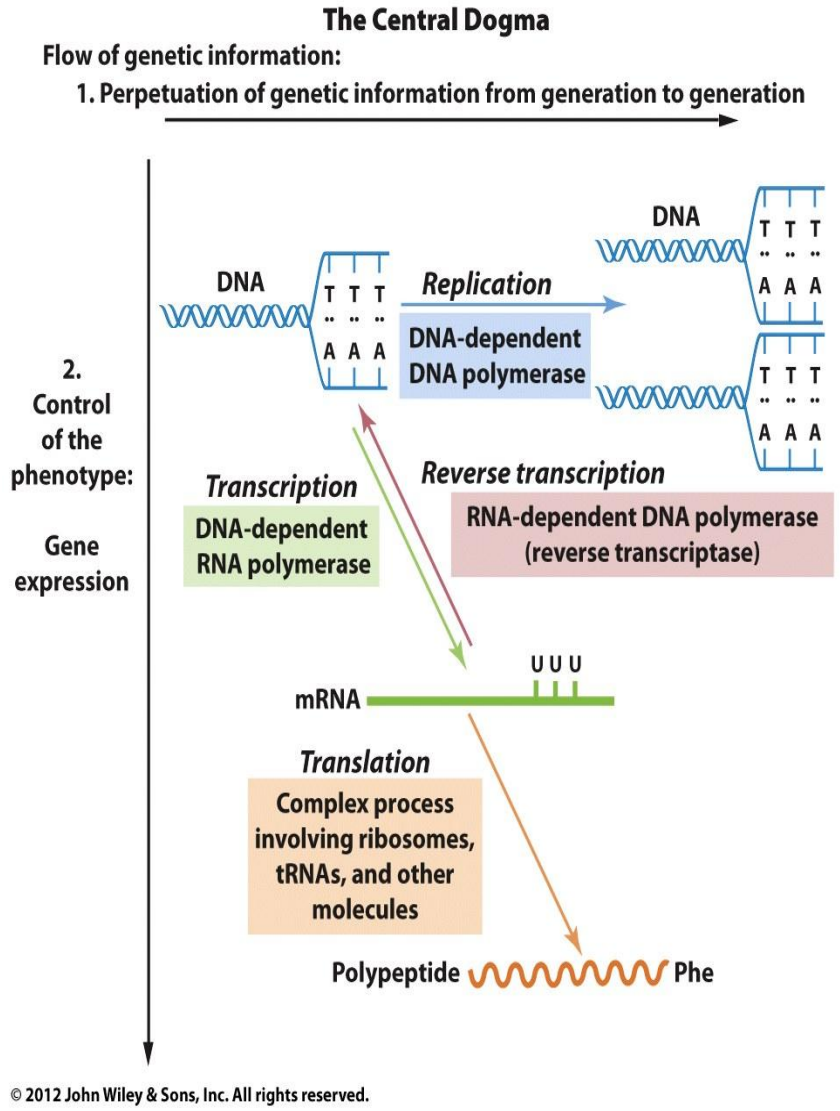
The Central Dogma

Transcription
--one strand of DNA to one complementary strand of RNA

--Uracil replaces Thymine (U-A bp)

--primary transcripts messenger RNA (mRNA)

-----chemical modification (splice=remove introns) (spliceosomes)



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The Central Dogma

Translation

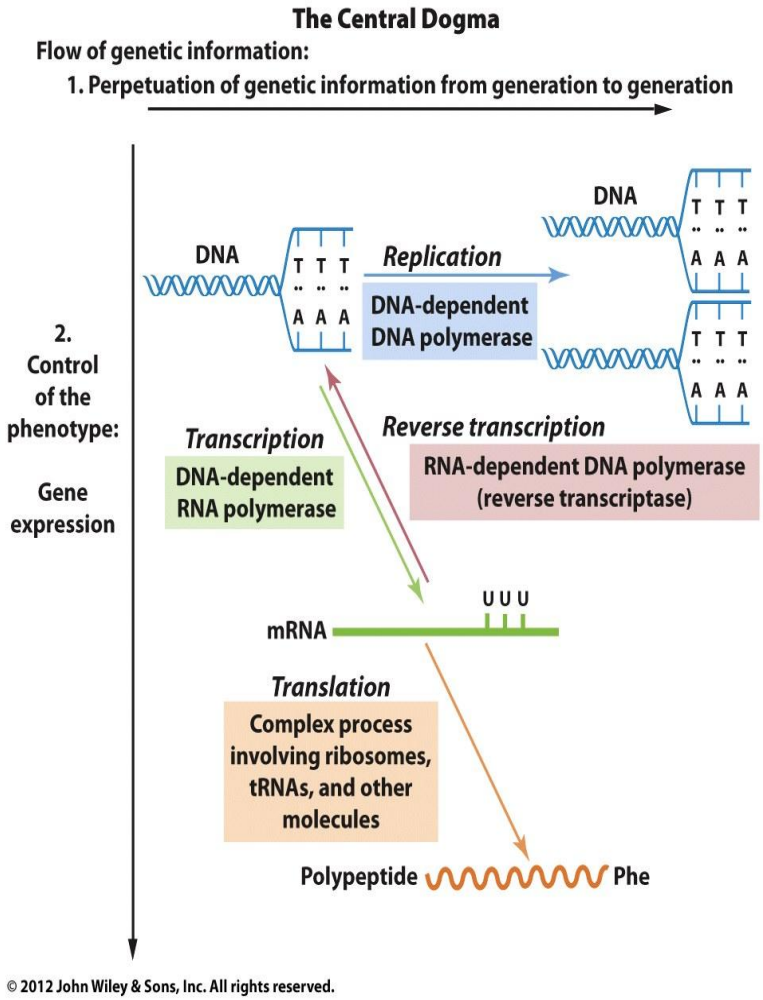
--RNA nucleotide into amino acids
UUU--Phe (F) phenylalanine(*)

--genetics code (codons)

--Ribosomes (ribonucleoproteins)

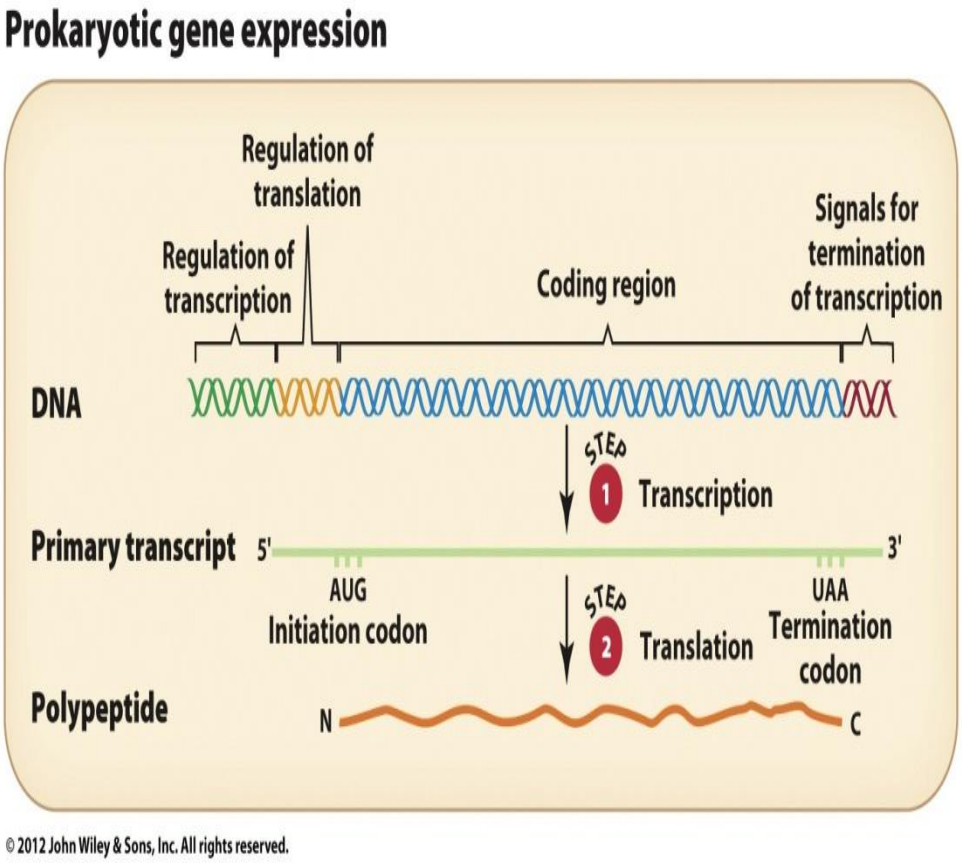
(*) sense codon

Non sense codon=stop codon



Transcription and Translation in Prokaryotes

- The primary transcript is equivalent to the mRNA molecule.
- The mRNA codons on the mRNA are translated into an amino acid sequence by the ribosomes.
- **Retroviruses**



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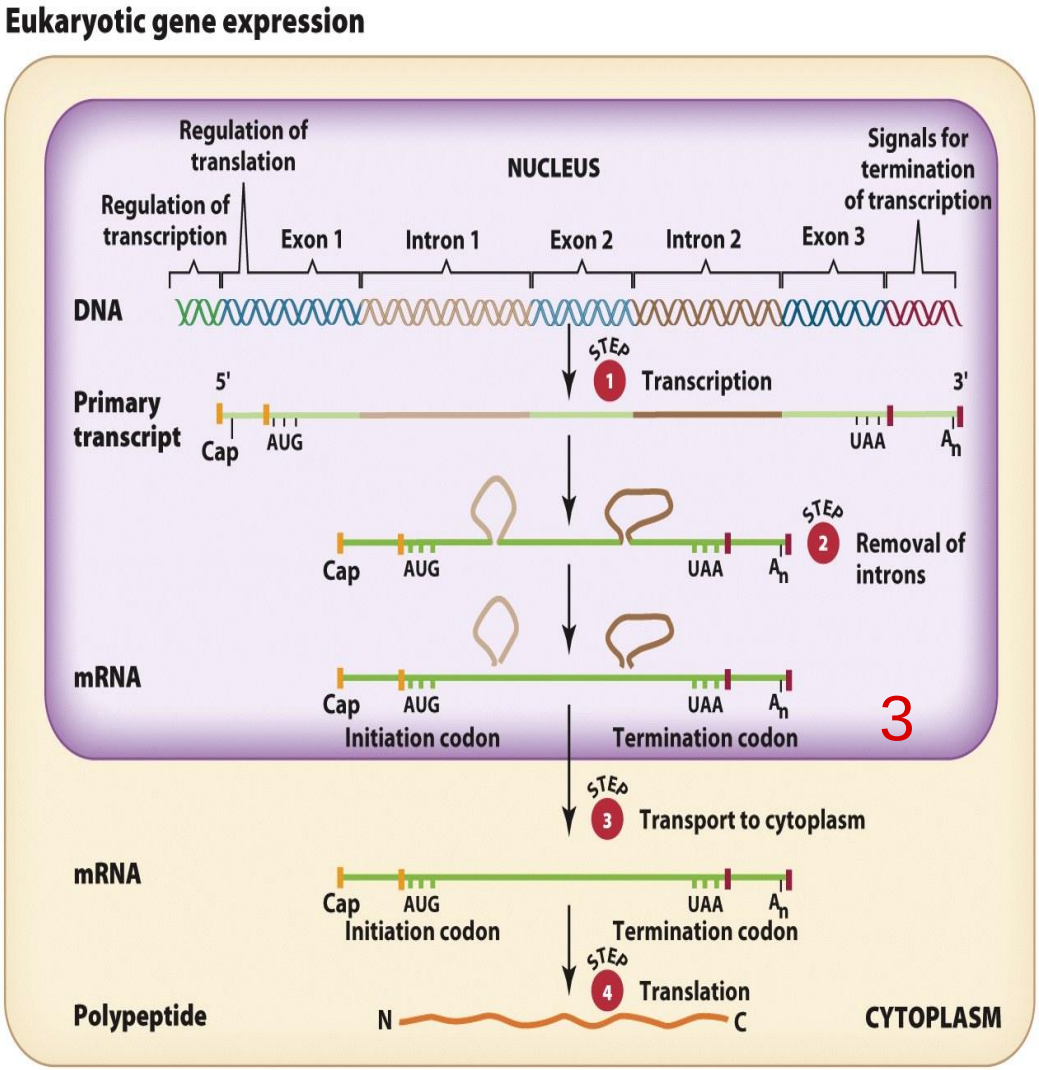
Transcription and Translation in Eukaryotes

- The primary transcript (pre-mRNA) is a precursor to the mRNA.
- The pre-mRNA is modified at both ends, and introns are removed to produce the mRNA.
- After processing, the mRNA is exported to the cytoplasm for translation by ribosomes.



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Types of RNA Molecules

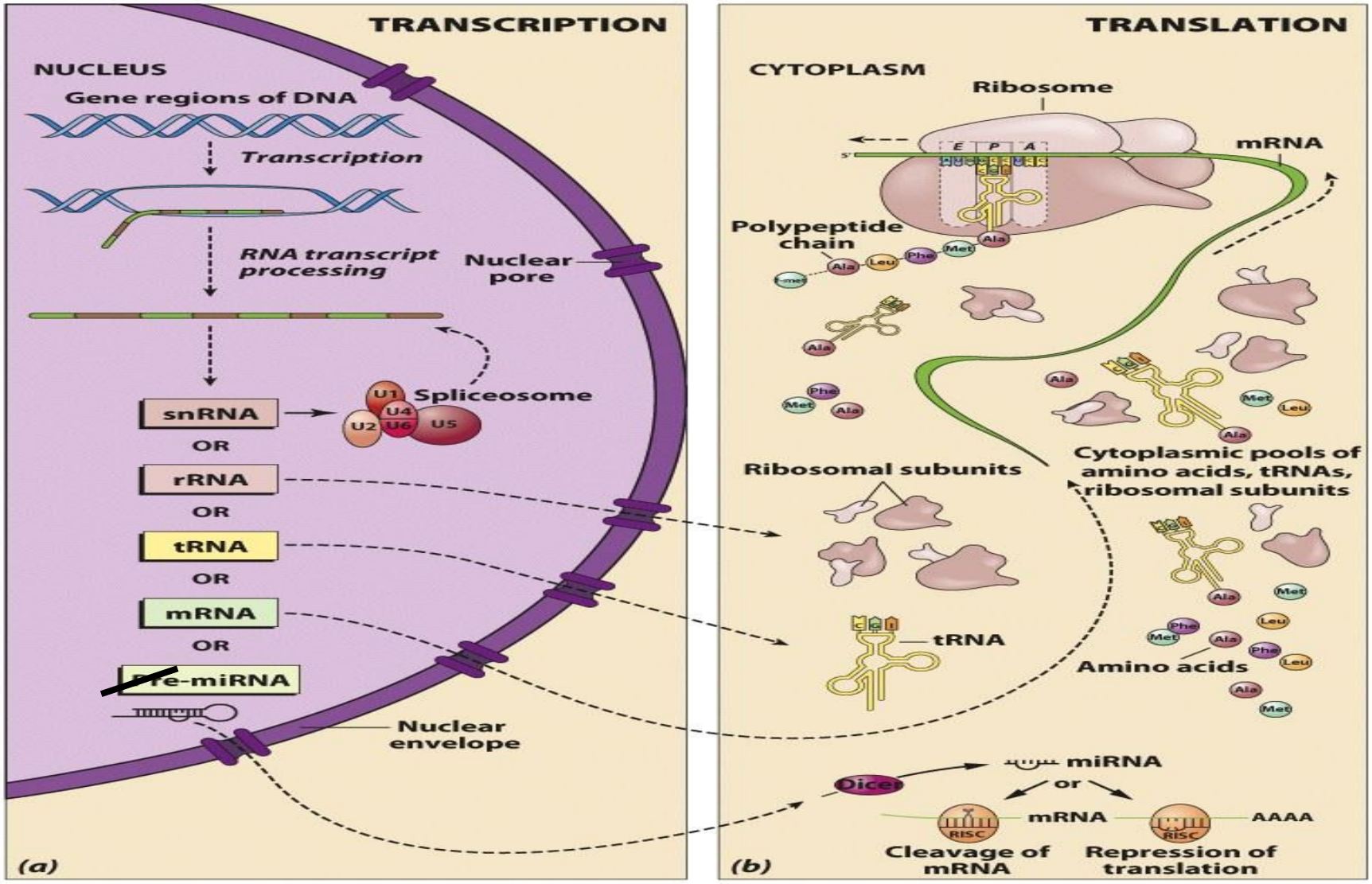
- **Messenger RNAs (mRNAs)**—intermediates that carry genetic information from DNA to the ribosomes.

- **Transfer RNAs (tRNAs)**—adaptors between amino acids and the codons in mRNA.

- **Ribosomal RNAs (rRNAs)**—structural and catalytic components of ribosomes.

Types of RNA Molecules

- **Small nuclear RNAs (snRNAs)**—structural components of spliceosomes.
- **Micro RNAs (miRNAs)**—short single-stranded RNAs (20 to 22 bp) that block expression of complementary mRNAs.
- **RNAi** is similar to **miRNA** (RNA interference, double strand RNA, plant) siRNA (small interference)
- piRNA (**Piwi-interacting RNA)** is a small non-coding RNA molecules



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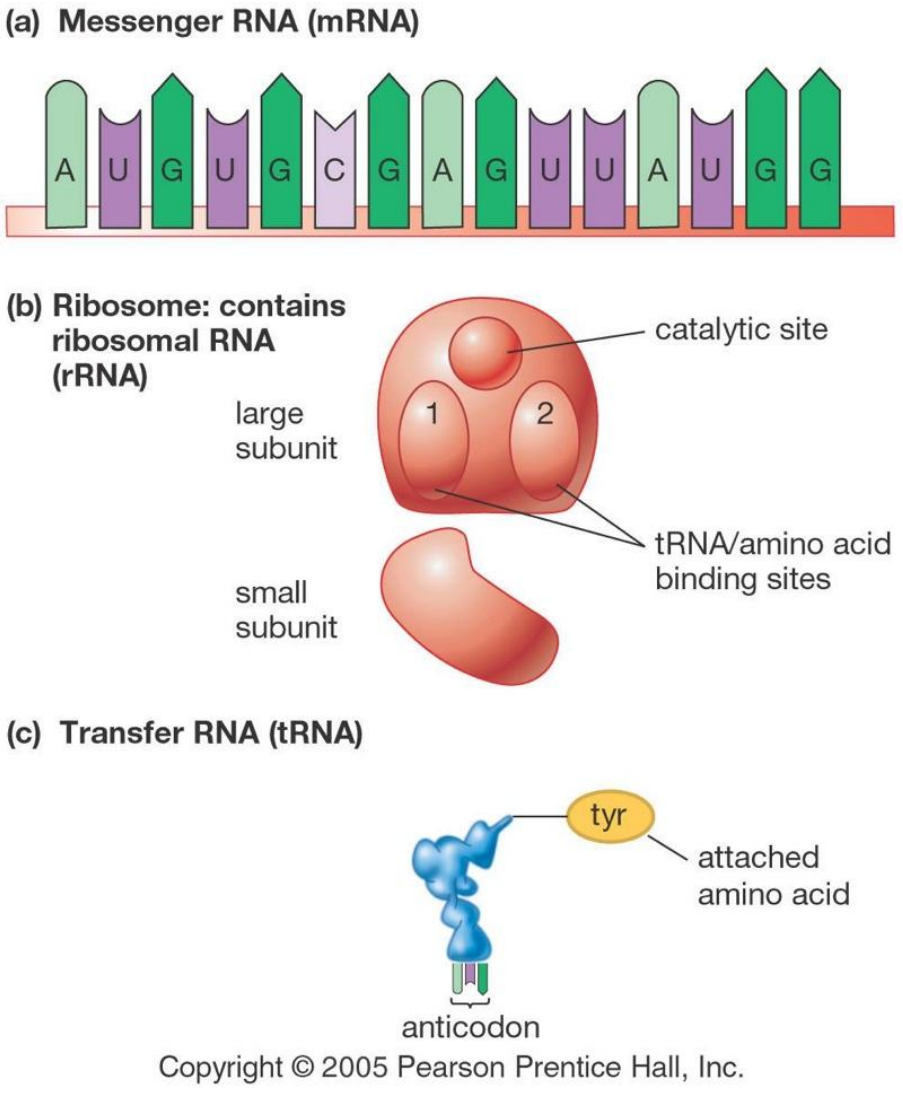
Key Players

- mRNA carries the information from a gene in DNA.
- Ribosomes, made of rRNA, consist of subunits and carry out an enzyme-like role.
- tRNA carries specific amino acids to the ribosome.



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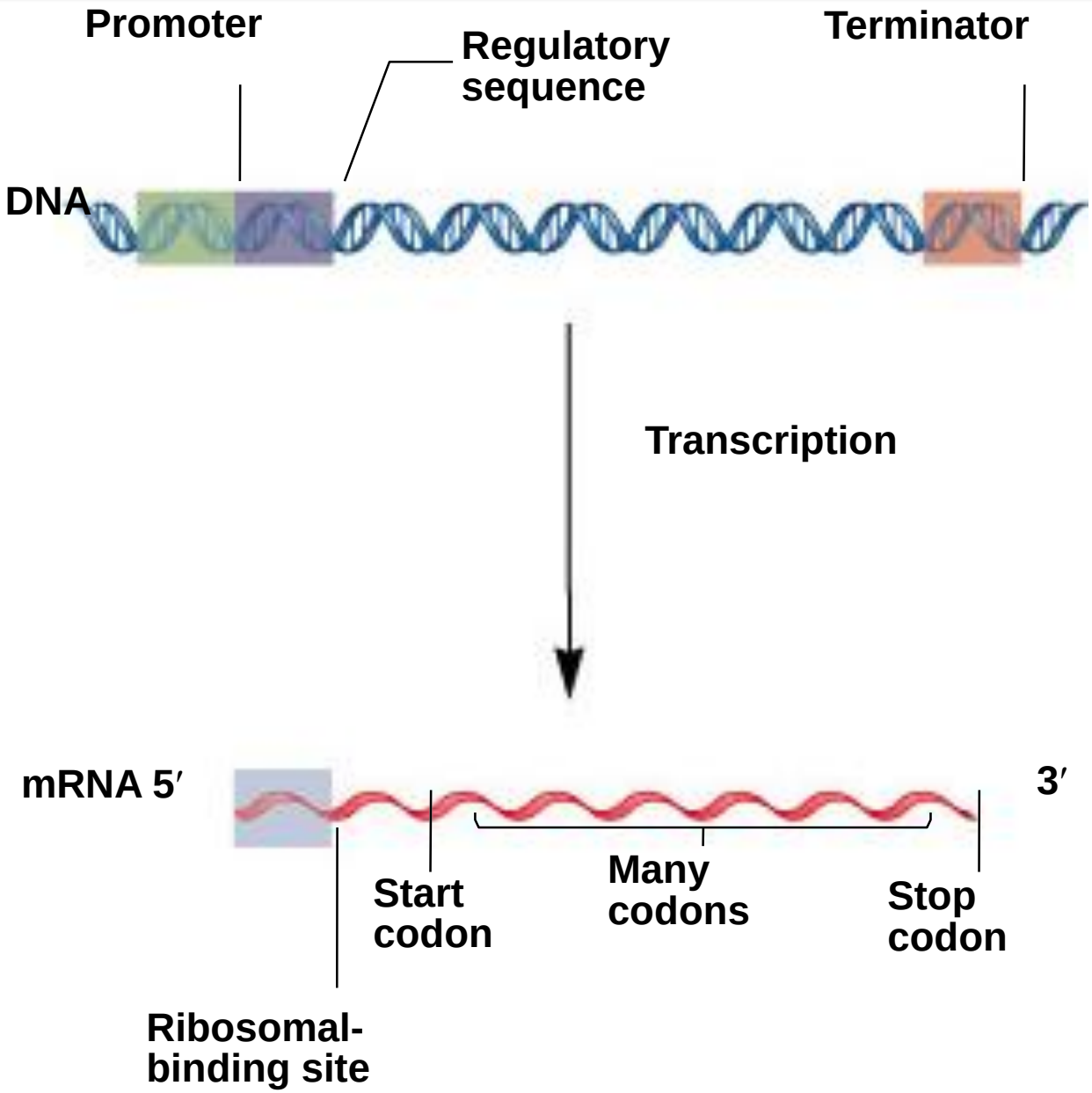
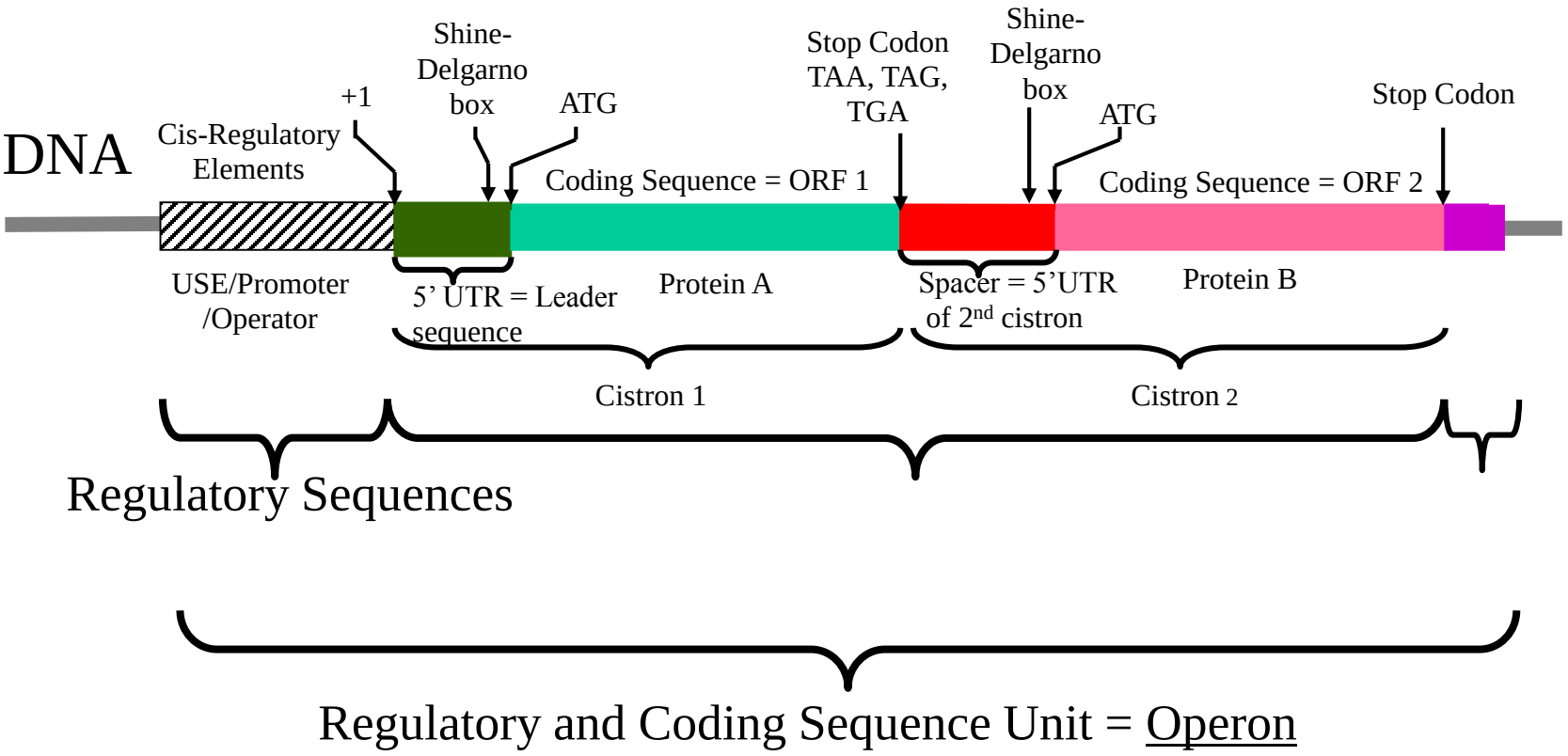
Gene Expression

- There are 4 major events that occur during the process of gene expression
  - Transcription
  - RNA processing
  - Translation
  - Protein processing

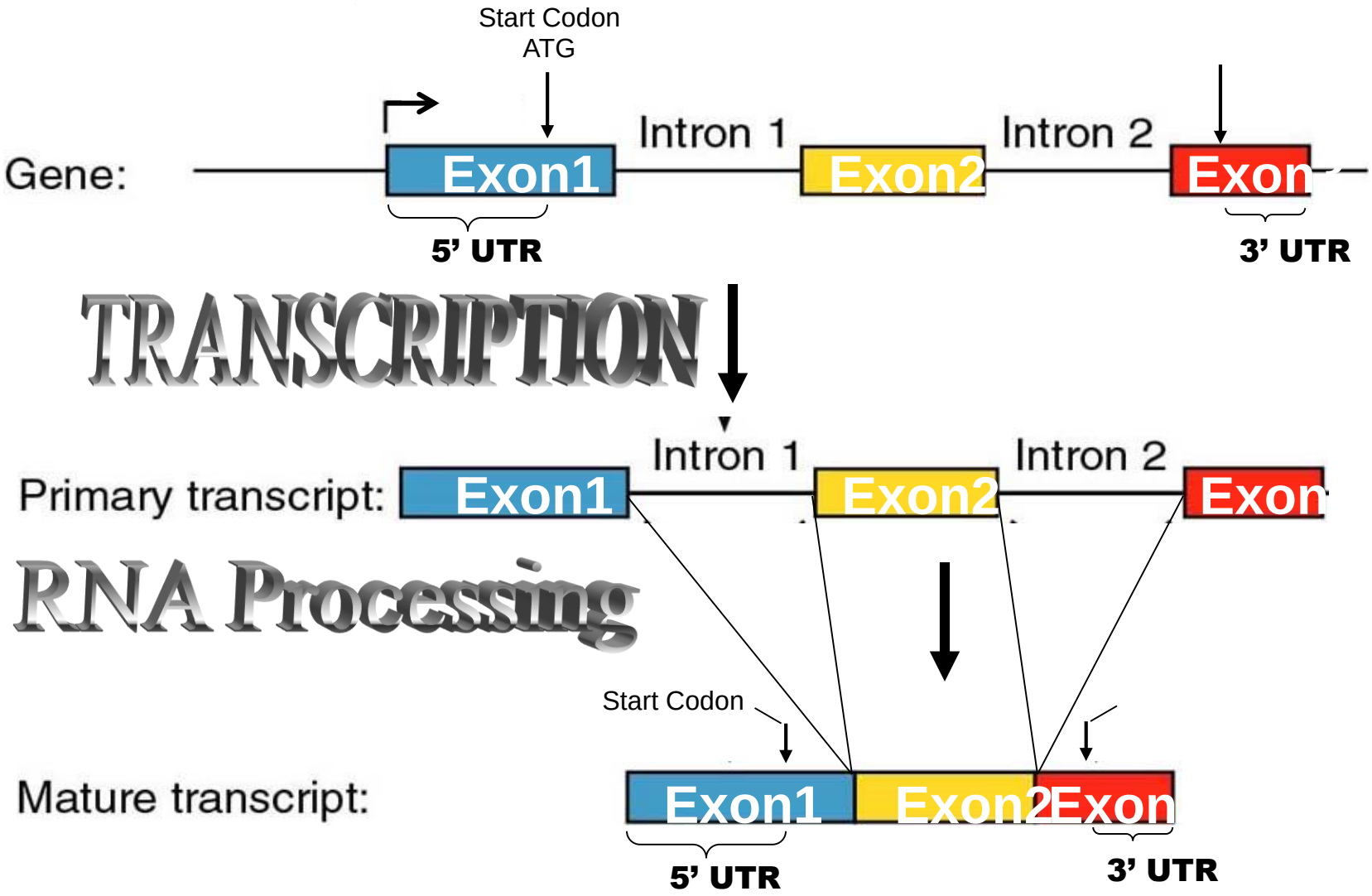
Gene is a Transcription Unit



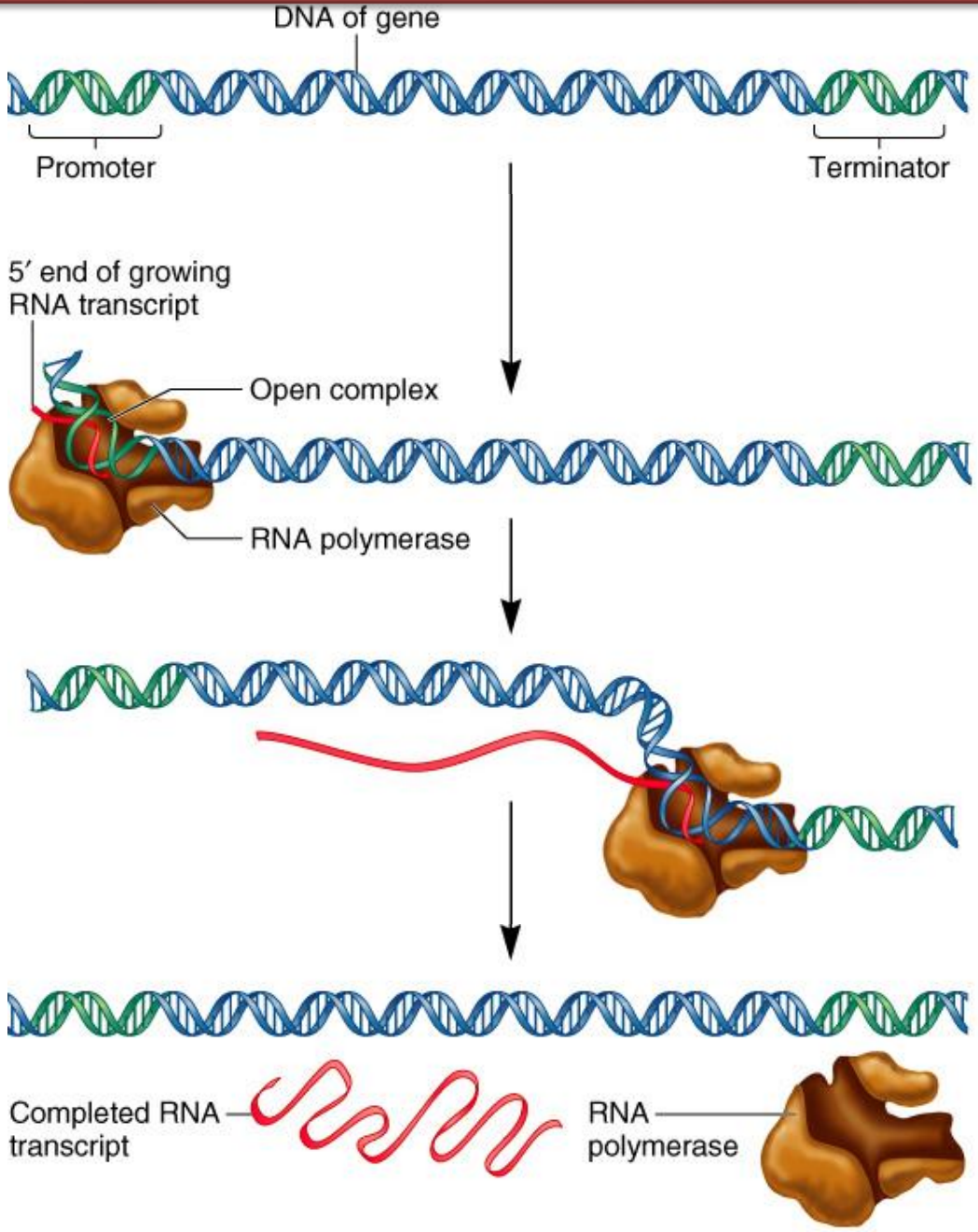
Prokaryotic Gene Structure



Eukaryotic Gene Structure

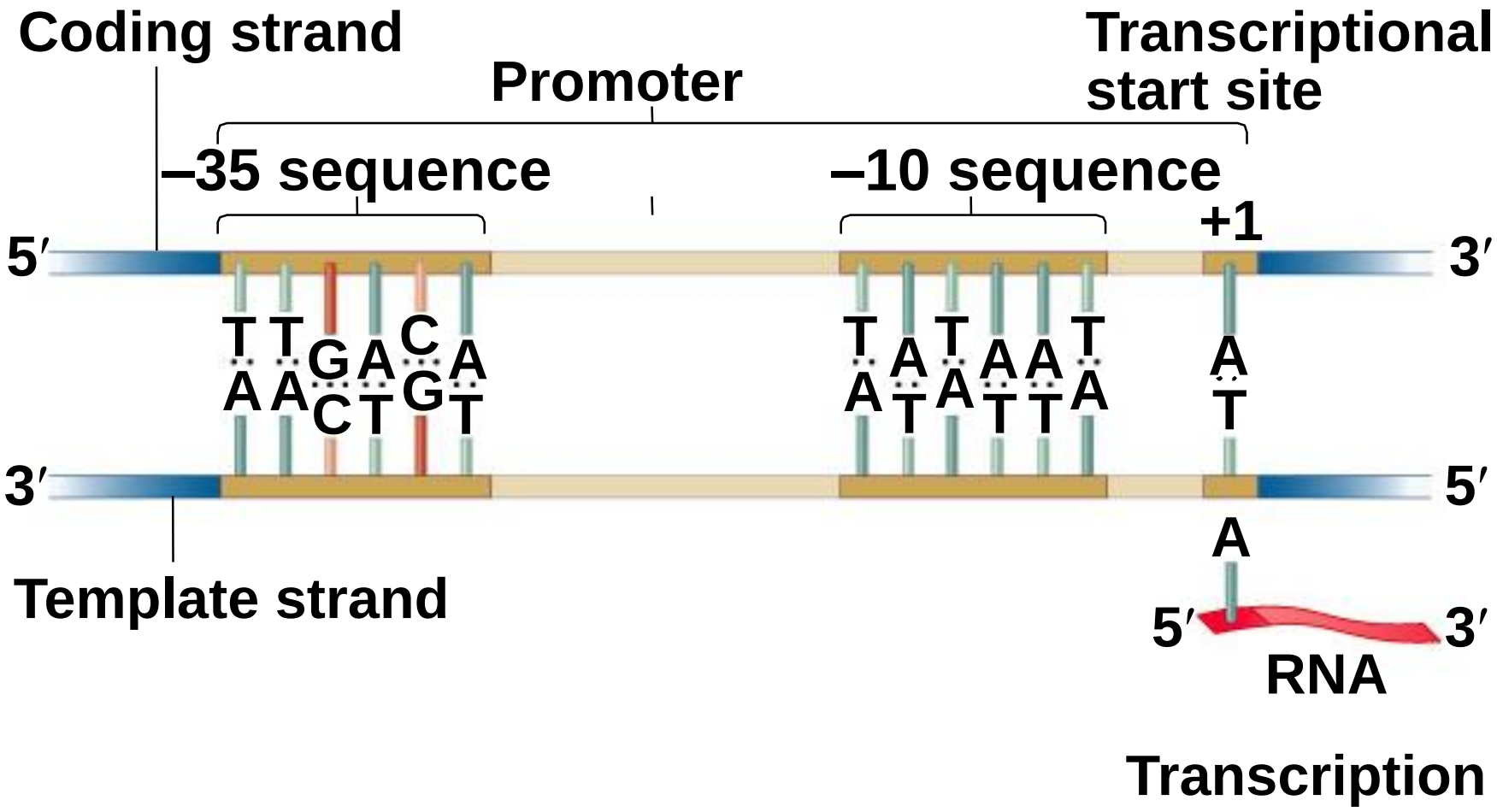


Transcription Proceeds Through 3 Steps



- **Transcription factors & RNA polymerase recognize & bind the promoter**
- **DNA adjacent to the promoter is denatured forming the open promoter complex**
- **RNA polymerase moves along the DNA in synthesizing a RNA transcript. Synthesis is 5'→3' – Only 1 strand of DNA is read as a template.**
- **A termination signal is reached causing RNA polymerase to dissociated from the DNA**

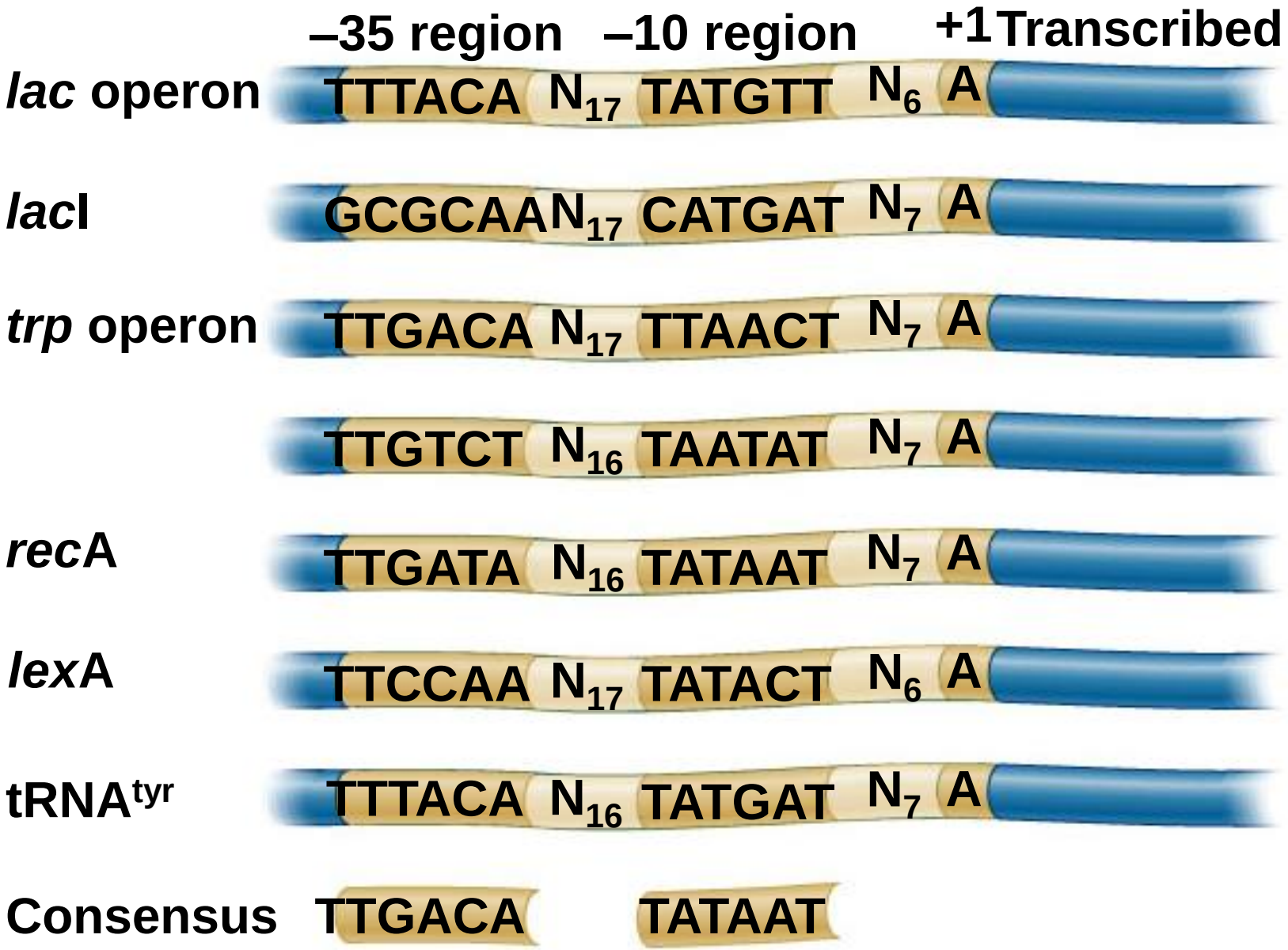
A Prokaryotic Promoter



Numbering of a Transcription Unit

- The transcript initiation site is +1 (A/T).
- Bases preceding the initiation site are given minus (–) prefixes and are referred to as **upstream sequences**.
- Bases following the initiation site are given plus (+) prefixes and are referred to as **downstream sequences**.
- **Consensus sequences**: highly conserved
- **Recognition sequences**: Sigma factor (σ)

Reaching A Consensus



General Features of RNA Synthesis

- Similar to DNA Synthesis except
  - The precursors are ribonucleoside triphosphates.
  - Only one strand of DNA is used as a template.
  - RNA chains can be initiated *de novo* (no primer required).
- The **RNA molecule** will be complementary to the **DNA template (antisense)** strand and identical (except that **uridine** replaces thymidine) to the DNA non-template **(sense)** strand.
- RNA synthesis is catalyzed by RNA polymerases and proceeds in the 5’ to 3’ direction.

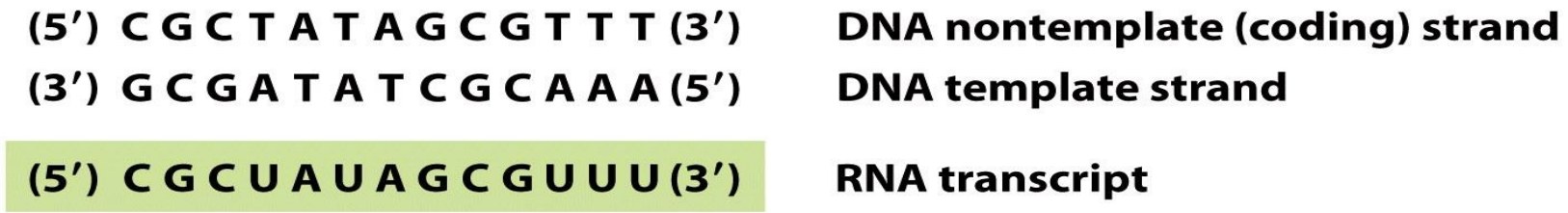


Figure 26-2
Lehninger Principles of Biochemistry, Fifth Edition
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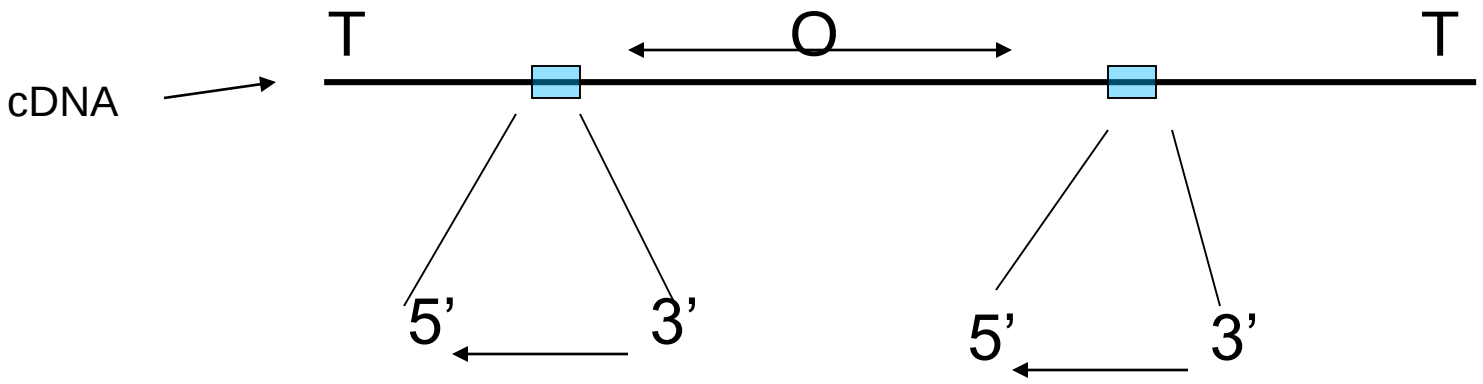
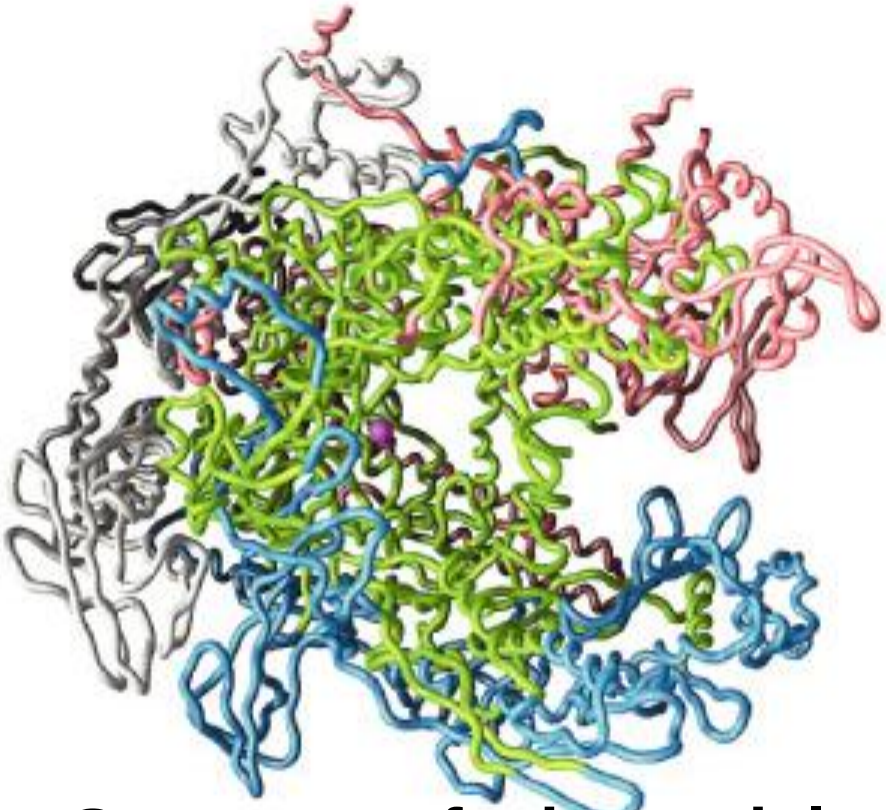
- ***RNA synthesis**, catalyzed by RNA polymerases, is similar to DNA synthesis in many respects.*

Prokaryotic:
OriC (245 bp)
AT-rich region (replication bubble)

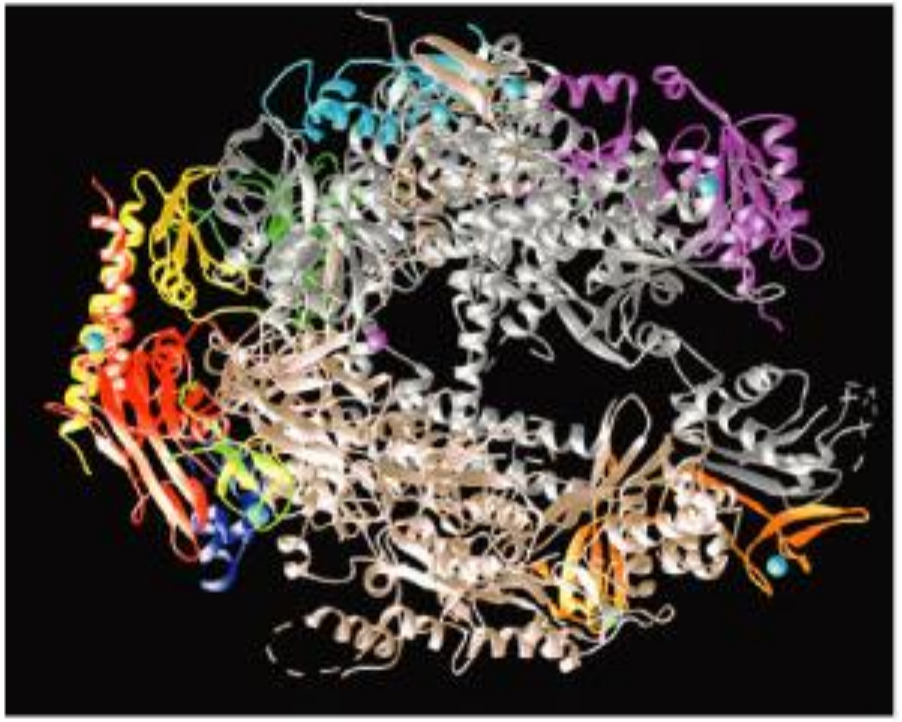
Eukaryotic:
ARS (Autonomously Replicating Sequences)
AT-rich region 11 bp



RNA Polymerases



Structure of a bacterial RNA polymerase



Structure of a eukaryotic RNA polymerase II

E. Coli RNA Polymerase

- Tetrameric core: $\alpha_2\ \beta\ \beta'$
- Holoenzyme: $\alpha_2\ \beta\ \beta'\ \sigma$
- (480,000 Daltons; bp~650 Daltons)

- Functions of the subunits:
  - α: assembly of the tetrameric core
  - β: ribonucleoside triphosphate binding site
  - β': DNA template binding region
  - σ (sigma factor): initiation of transcription (*)

(*) *in vivo*

In vitro: RNA polymerase works…just fine on both DNA strands

Eukaryotes Have Five RNA Polymerases

TABLE 11.1

Characteristics of the Five RNA Polymerases of Eukaryotes

Enzyme	Location	Products
RNA polymerase I	Nucleolus	Ribosomal RNAs, excluding 5S rRNA
RNA polymerase II	Nucleus	Nuclear pre-mRNAs
RNA polymerase III	Nucleus	tRNAs, 5S rRNA, and other small nuclear RNAs
RNA polymerase IV	Nucleus (plant)	Small interfering RNAs (siRNAs)
RNA polymerase V	Nucleus (plant)	Some siRNAs plus noncoding (antisense) transcripts of siRNA target genes.

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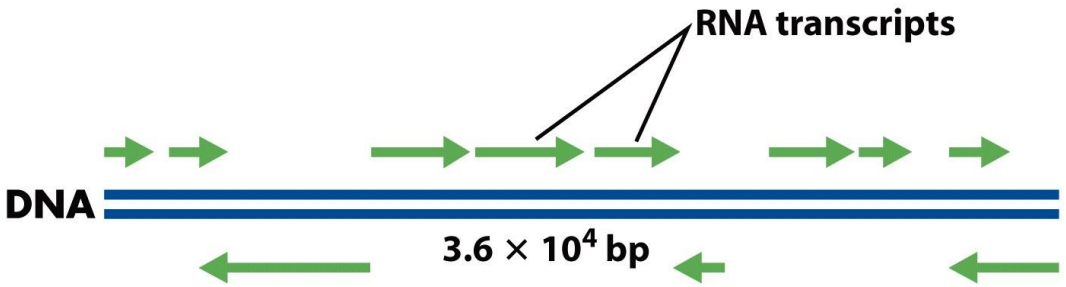
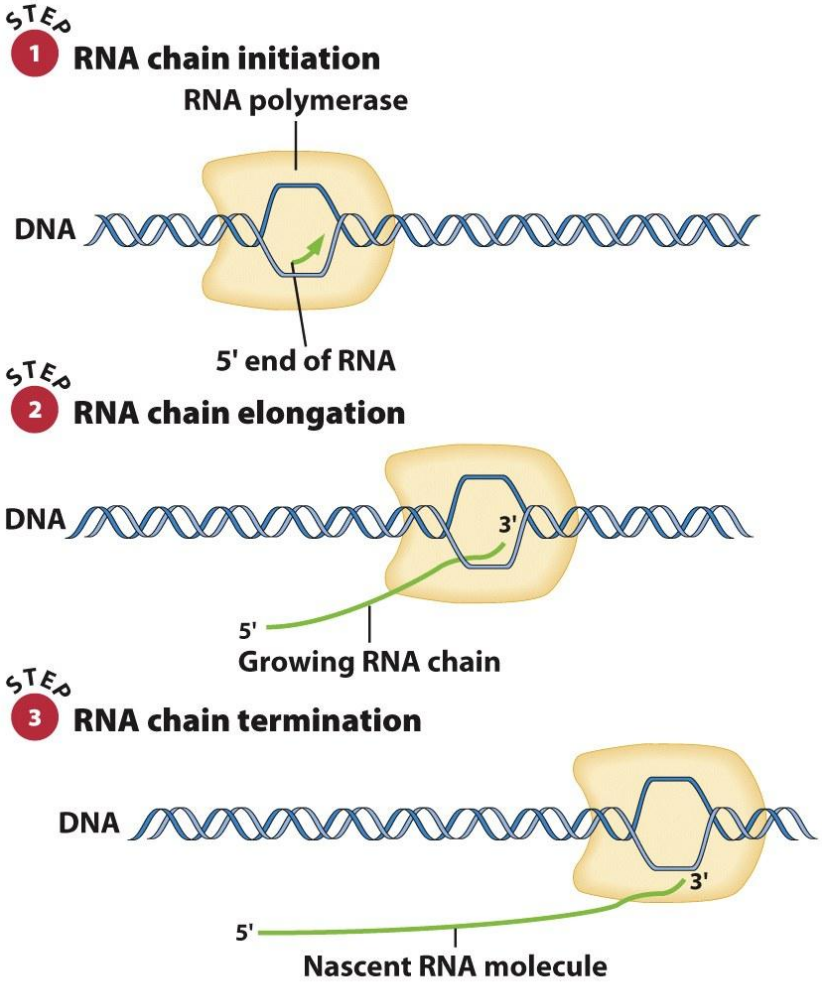
RNA polymerase II Nucleus miRNA

Pre-mRNA~Heterogeneous nuclear RNA (hnRNA)

The Process of Transcription

- ✓ Initiation
  - Where/when most regulation of gene expression occurs
  - Different between proks & euks
- ✓ Elongation
  - Essentially same between prokaryotes & eukaryotes
  - Some regulation, more in proks than euks
- ✓ Termination
  - Different between proks & euks
  - Some regulation





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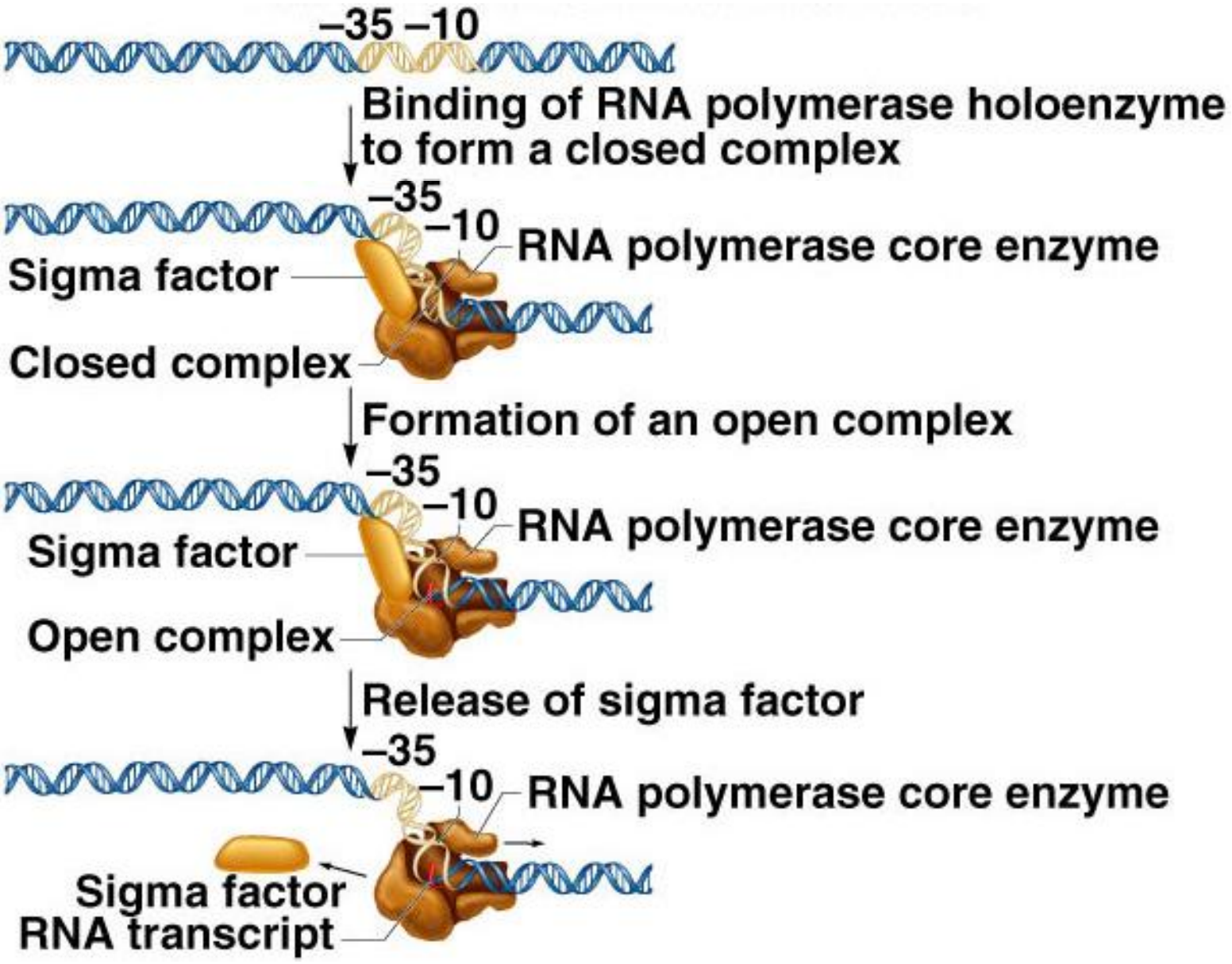
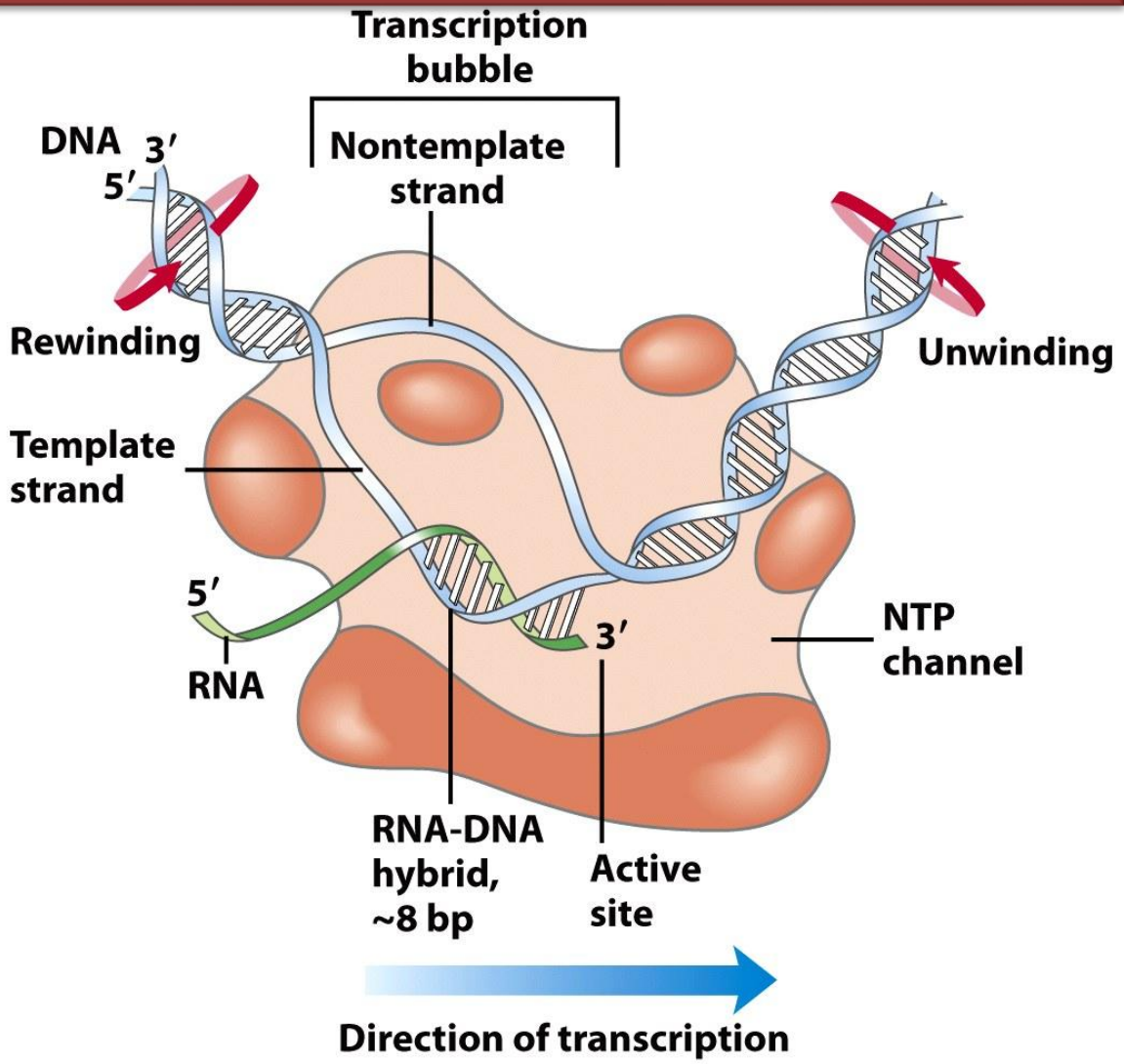
Prokaryotic Transcription Initiation



Transcription

- Open DNA at promoter
- Make RNA
  - 5'-> 3'
  - transcription bubble
    - Moves along gene
  - Prevent DNA knotting
    - DNA topo-isomerases



Elongation

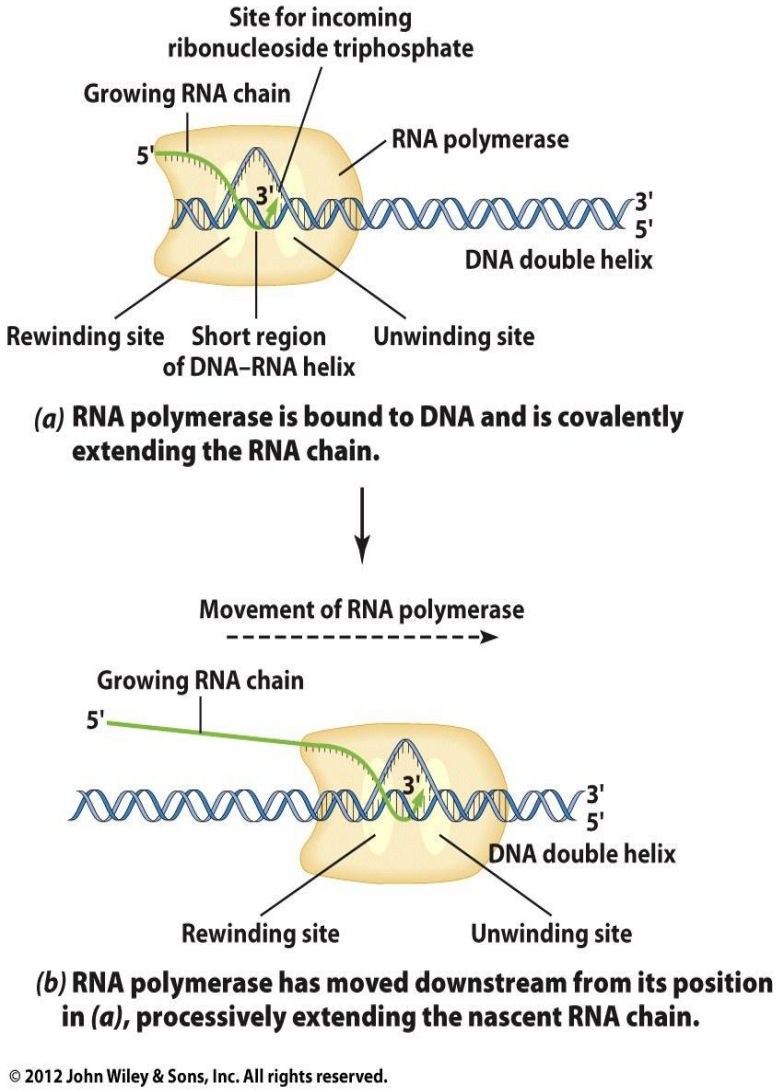
Sigma factor needs to be released

---Re- and Un-winding activities

-- Walk (literally) on the DNA
5’ to 3’

--growing RNA chain

RNA polymerase binds both
DNA template and growing RNA chain



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Termination Signals in *E. coli*

- Rho-dependent terminators—require a protein factor (ρ)

- Rho-independent terminators—do not require ρ

Termination Signals in *E. coli*

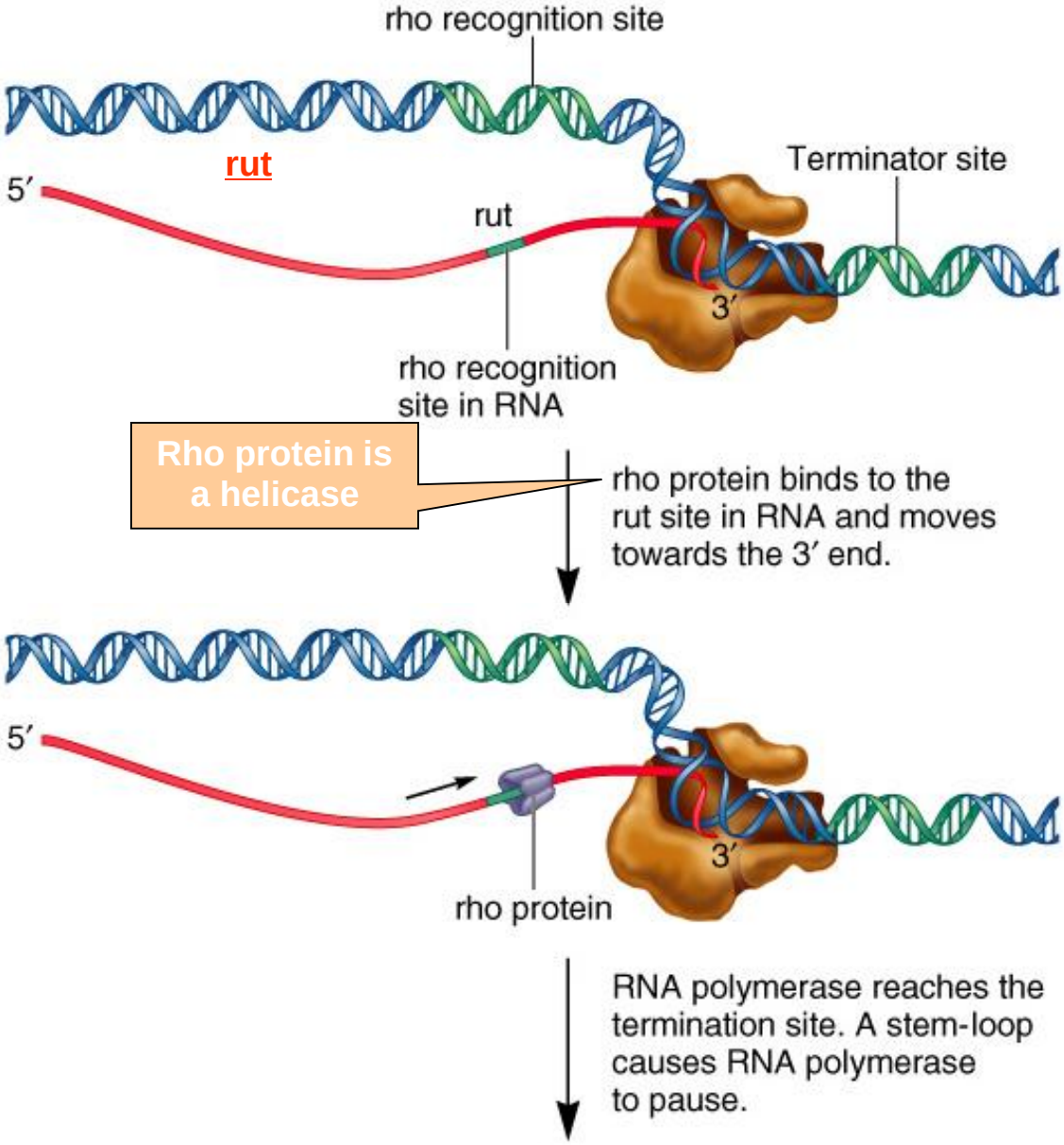
- Rho-dependent terminators (non-intrinsic) — require a protein factor (ρ) and ***rut* site**

- Rut proteins bind specific RNA sequences (>>Cs and <<<Gs)

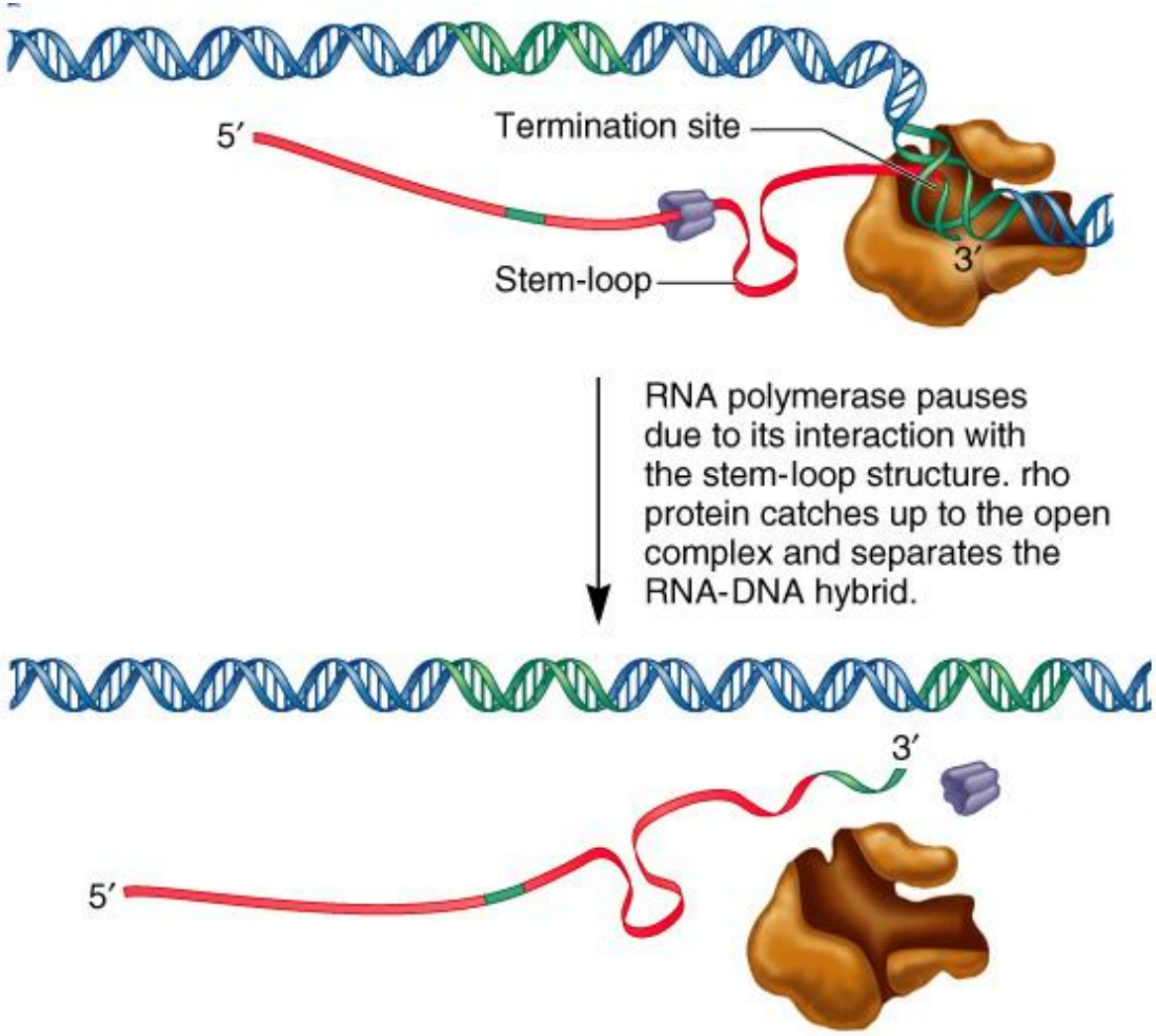
- Not hairpins or other secondary Structures

Rho utilization (rut)

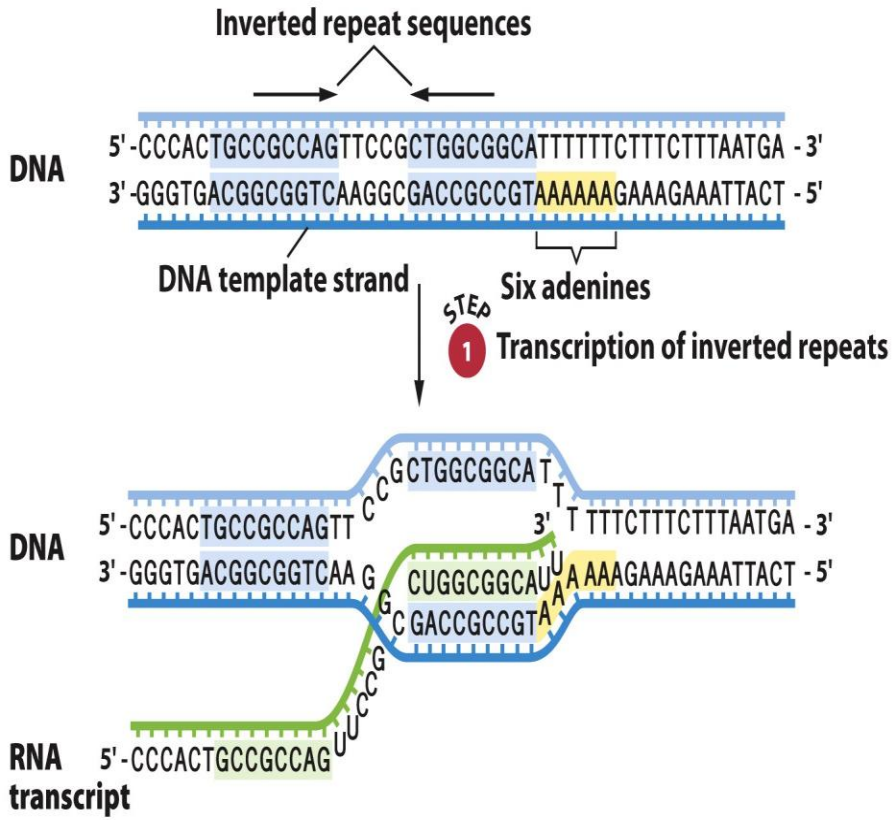
Rho Dependent Termination in Prokaryotes



Rho Dependent Termination in Prokaryotes



Rho-independent terminators—do not require ρ (**intrinsic termination**)



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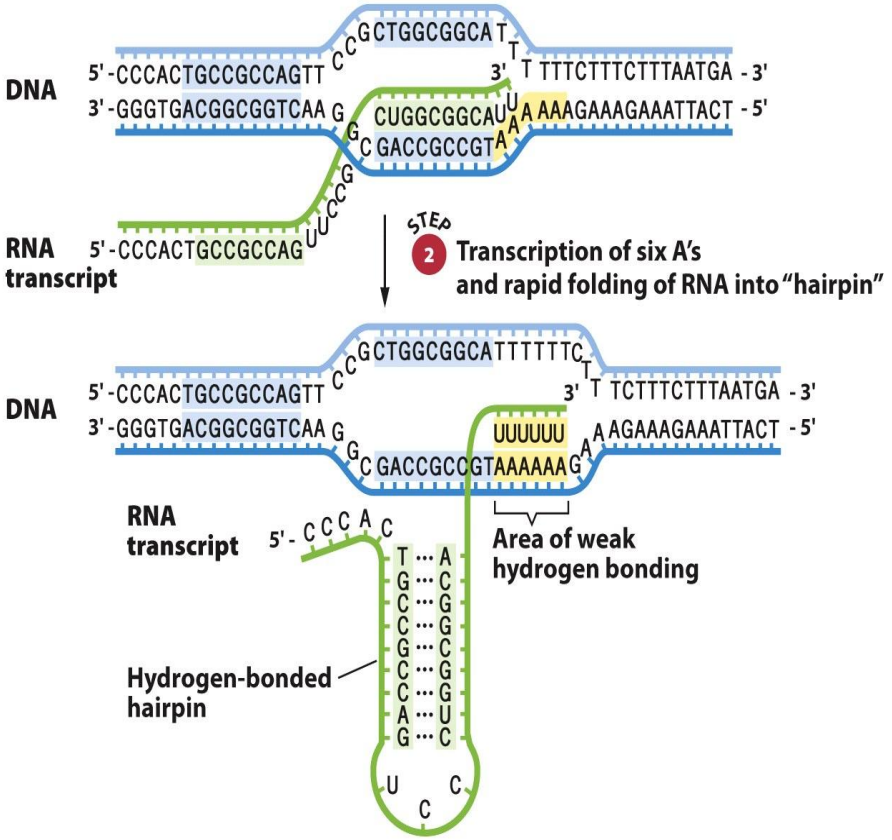

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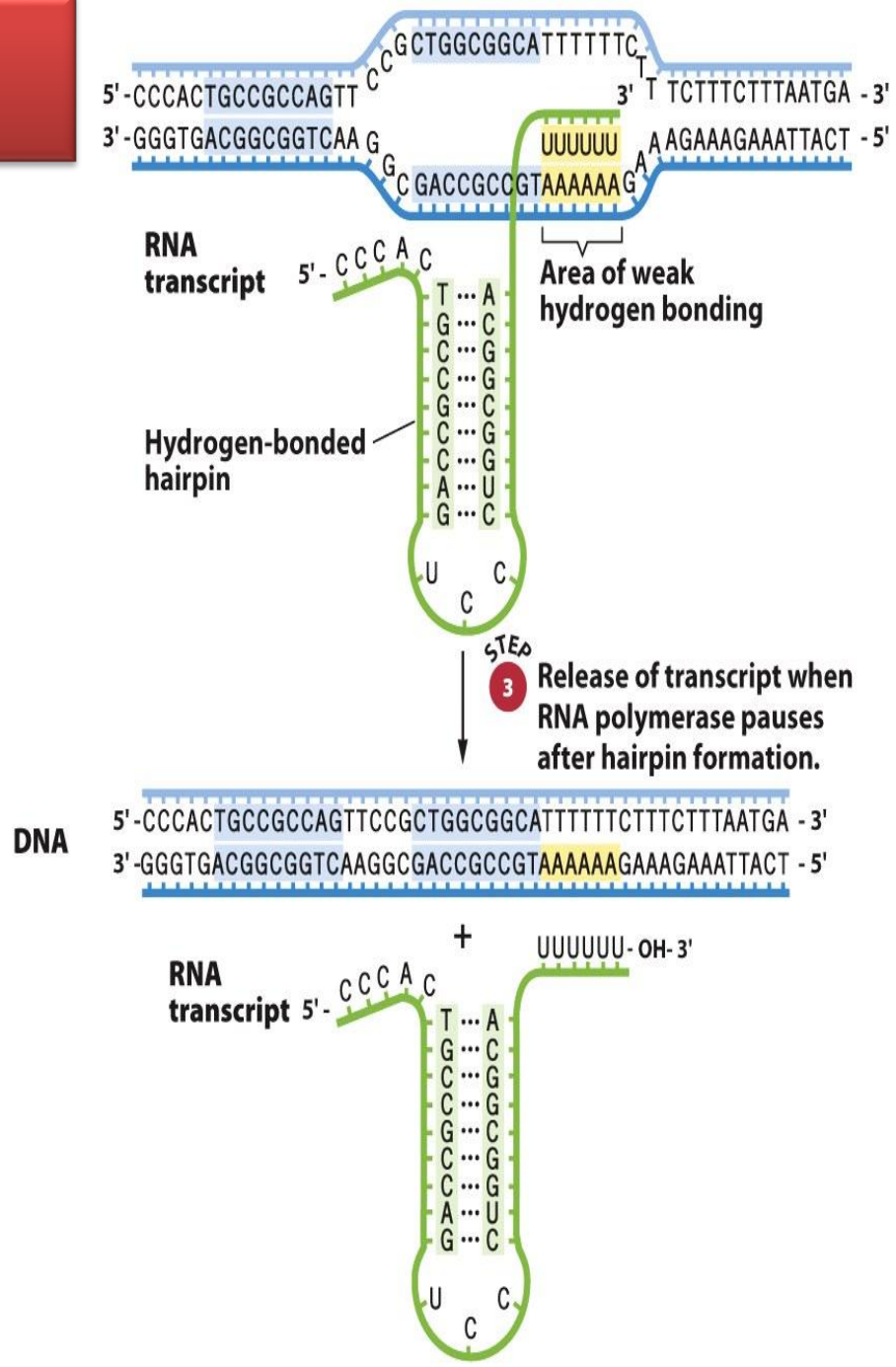
RNA transcription stops

--when the newly synthesized RNA molecule forms a **G-C**-rich hairpin loop
followed by a run of **As**

--Create a mechanical stress

--Pulls the poly-U transcript out of the
active site of the RNA polymerase

--A-U has very weak interaction



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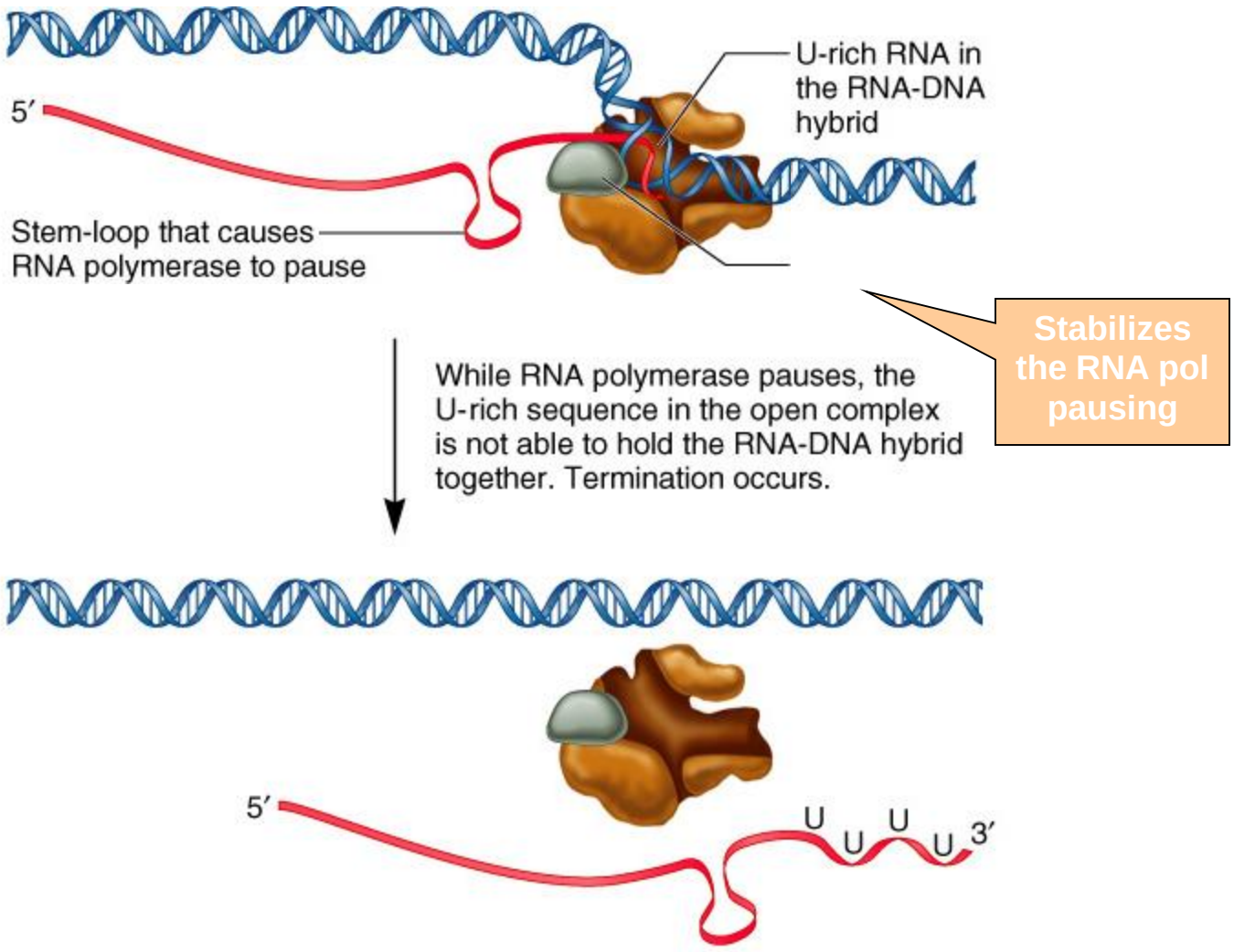
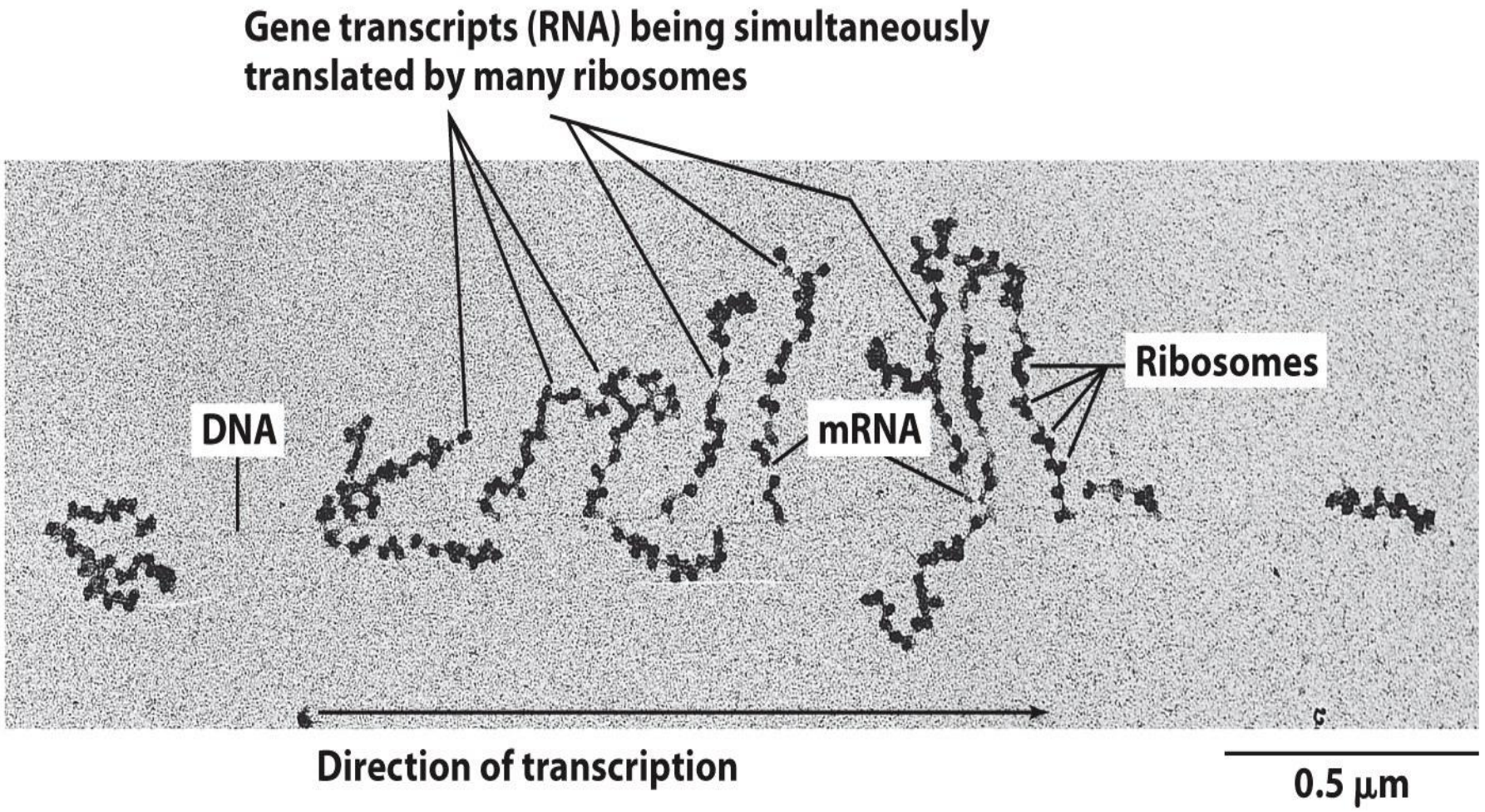
Rho Independent Termination in Prokaryotes

- ρ-independent termination requires two sequences in the RNA
  - A stem-loop structure upstream of 7-9 U residues



Coupled Transcription and Translation in *E. coli*



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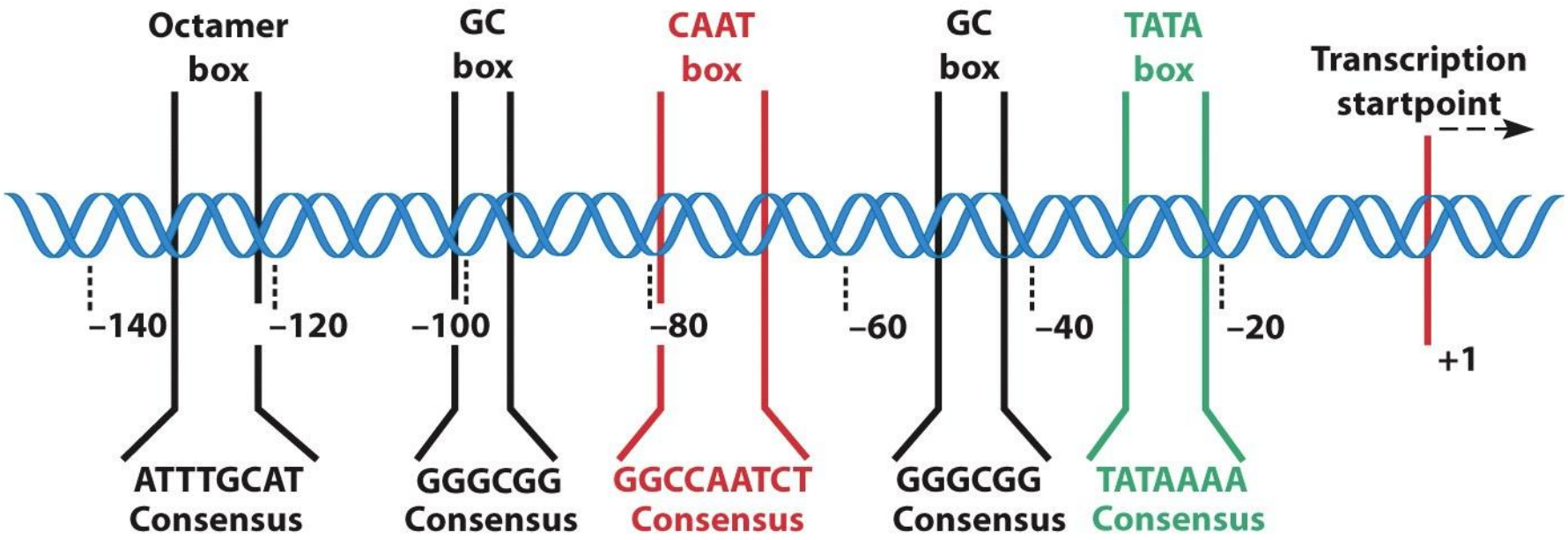
Eukaryotic Transcription

A Typical RNA Polymerase II Promoter (mRNA)



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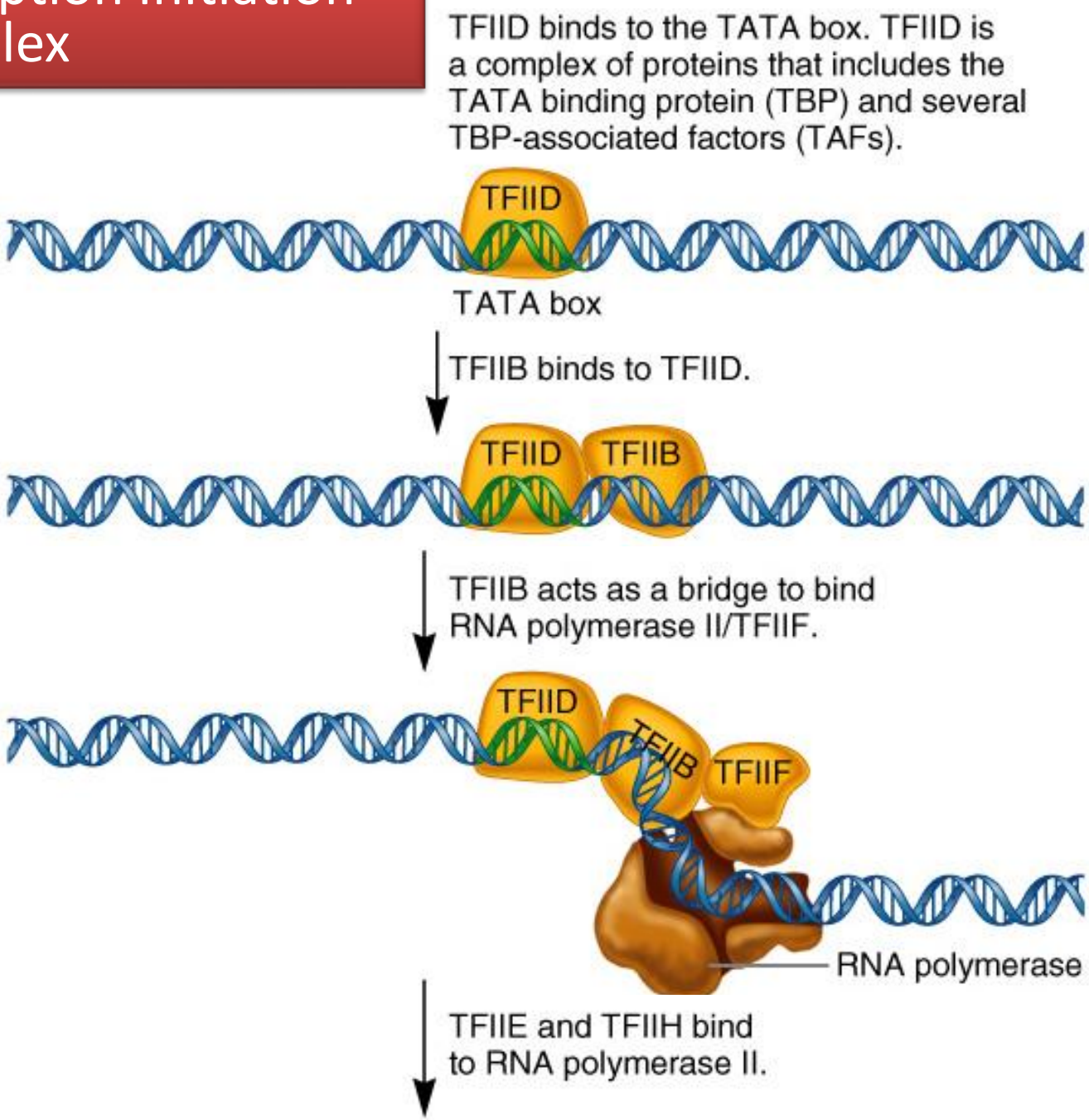
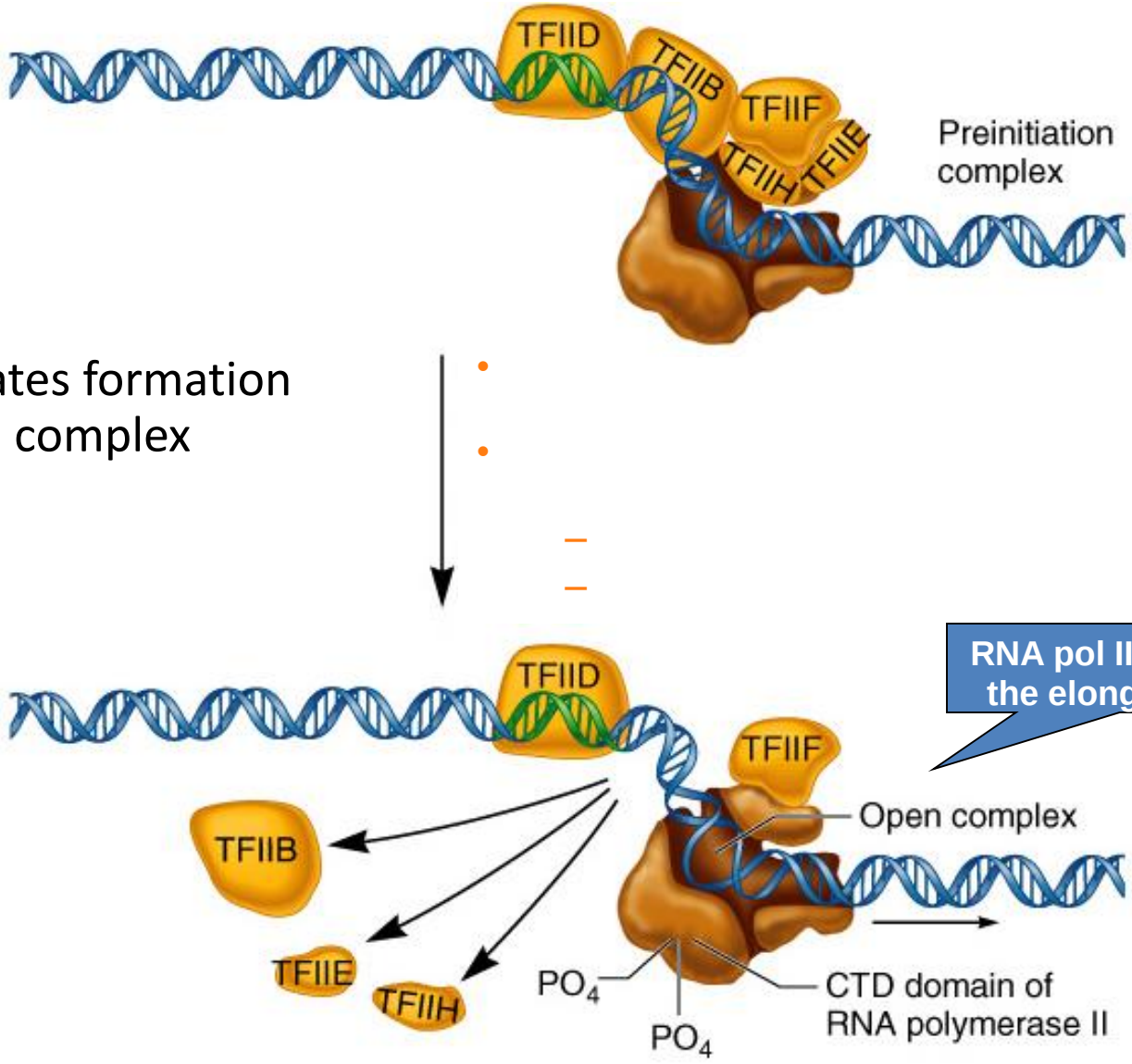
Promoter: short sequence of conserved elements (seq. of DNA) located upstream from the transcript starting point.

--~200 bp (DNA linear)
--~10 Kdp (DNA bending)

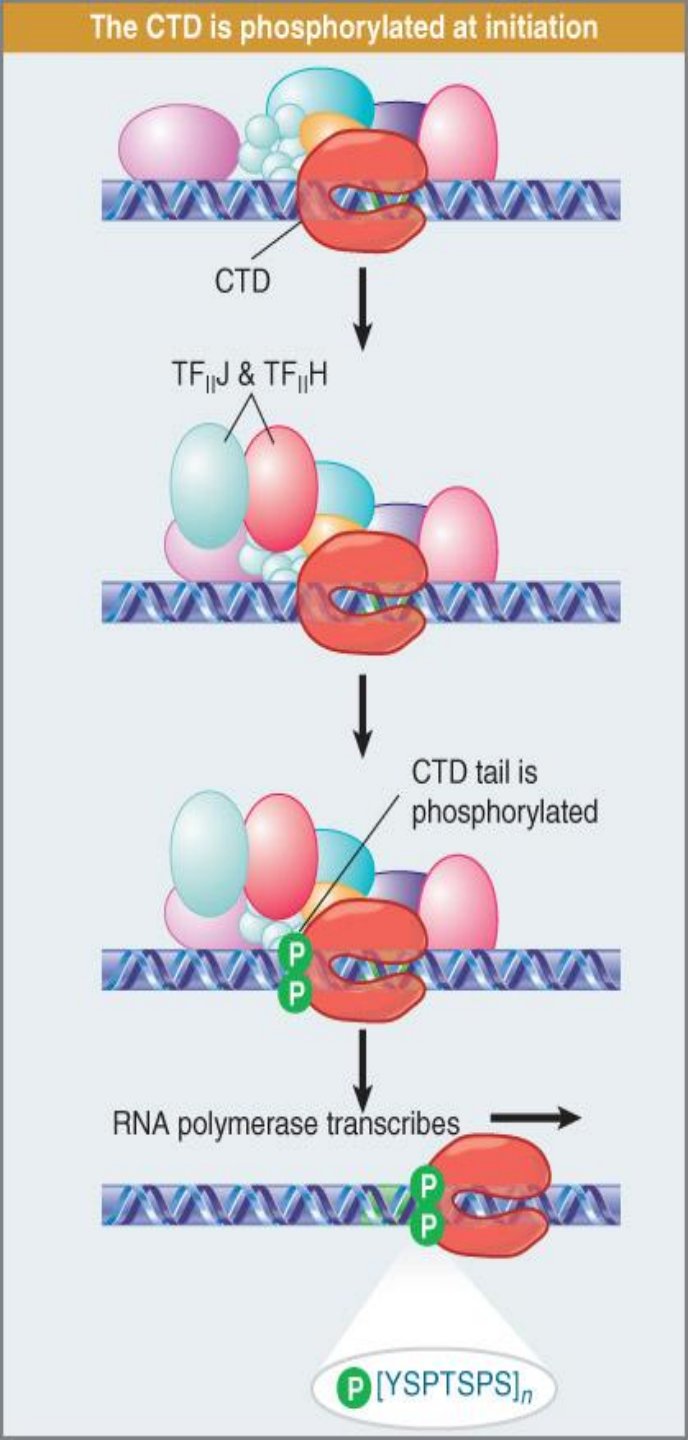
The Pol II Transcription Initiation Complex



Pol II Initiation Complex



- TFIIH regulates formation of the open complex



-TFIIH (helicase activity and kinase activity)

When RNA polymerase II binds to the complex, it initiates transcription.

Phosphorylation of the CTD is required for elongation to begin.

CTD: carboxy-terminal domain

- All eukaryotic RNA polymerases have ~12 subunits and are aggregates of >500 kD. (nucleotide pair~0.660 kD)
- Some subunits are common to all three RNA polymerases.
- The largest subunit in RNA polymerase II has a CTD (carboxy-terminal domain) consisting of multiple repeats of a heptamer.

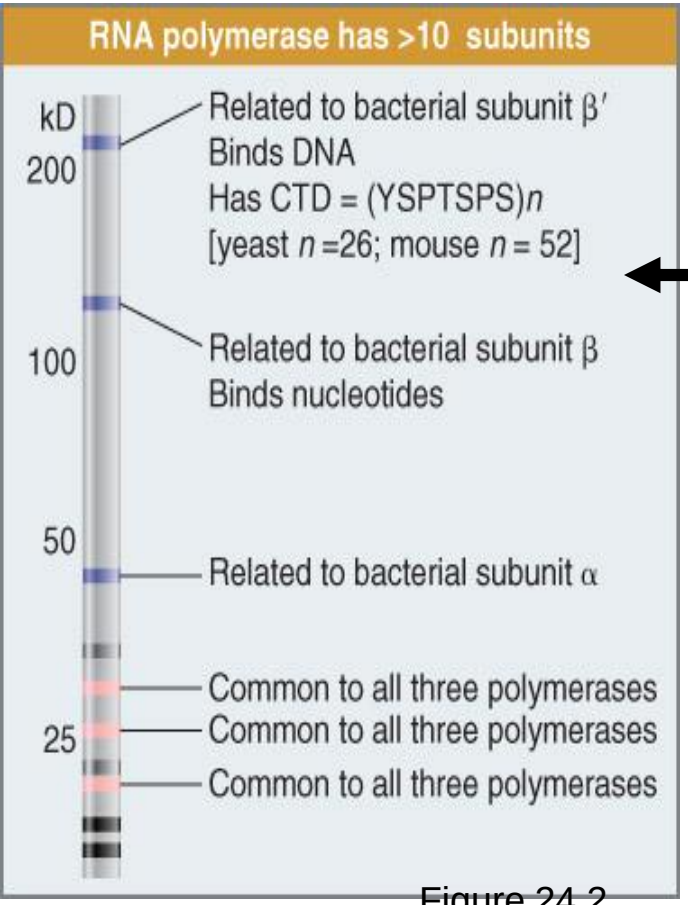


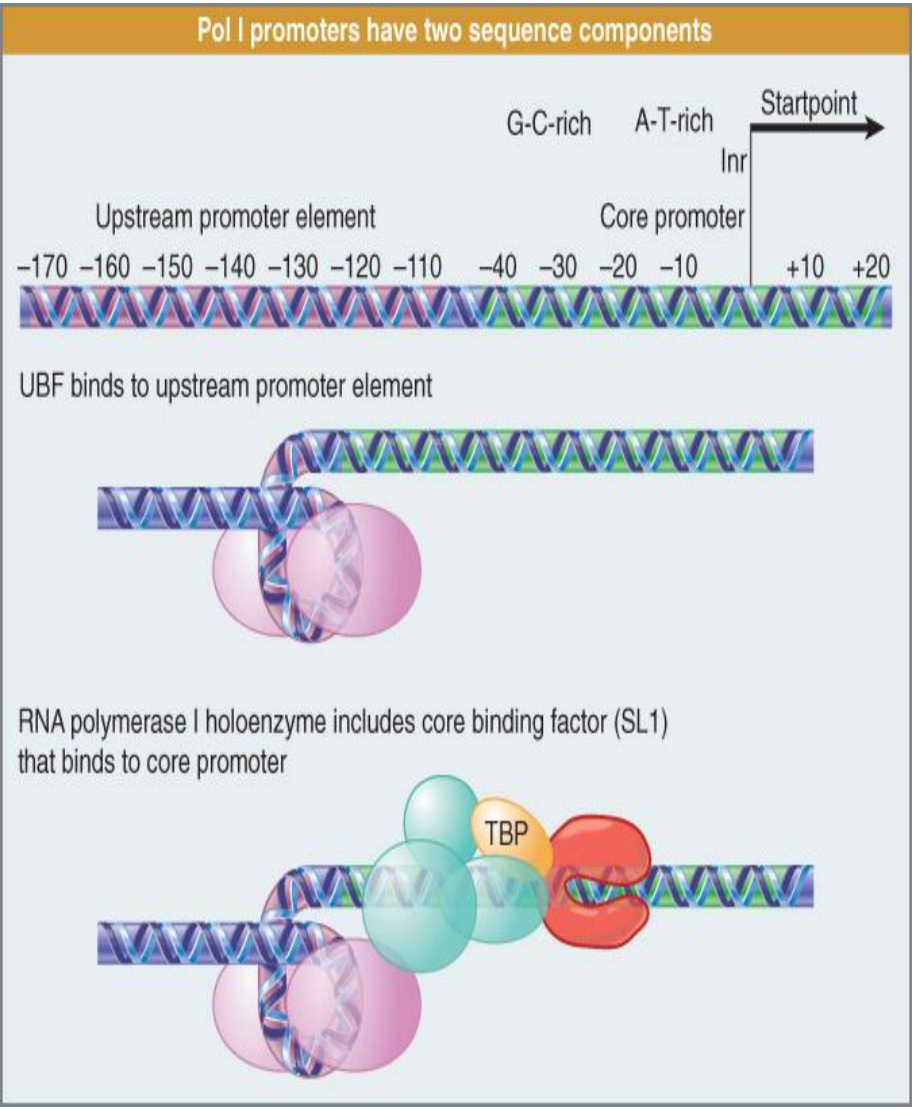
Figure 24.2

-Typical RNA polymerase isolated from yeast (S. cerevisiase) (α and β subunits)

- β subunits: CTD – carboxy-terminal domain, which consists in multiple repeats of 7 amino acids, unique and important of regulation (tyrosine (Try, Y), serine (Ser, S) and threonine (Thr, T) residues)

-Some subunits are common to all three polymerases.

RNA Polymerase I Has a Bipartite Promoter



- The RNA polymerase I promoter consists of:
- **--a core promoter**
- **--an upstream control element (UPE)**

RNA Pol I transcribes rRNA genes.

Core promoter: -45 to +20 seq.,

G-C-rich and **A-T**-rich (Inr-initiator) regions,

Binding factors - protein complexes formed by TFIs and **TBP**-*(TATA binding protein*)

Polymerase III Uses Both Downstream and Upstream Promoters

- RNA polymerase III has (3) types of promoters.

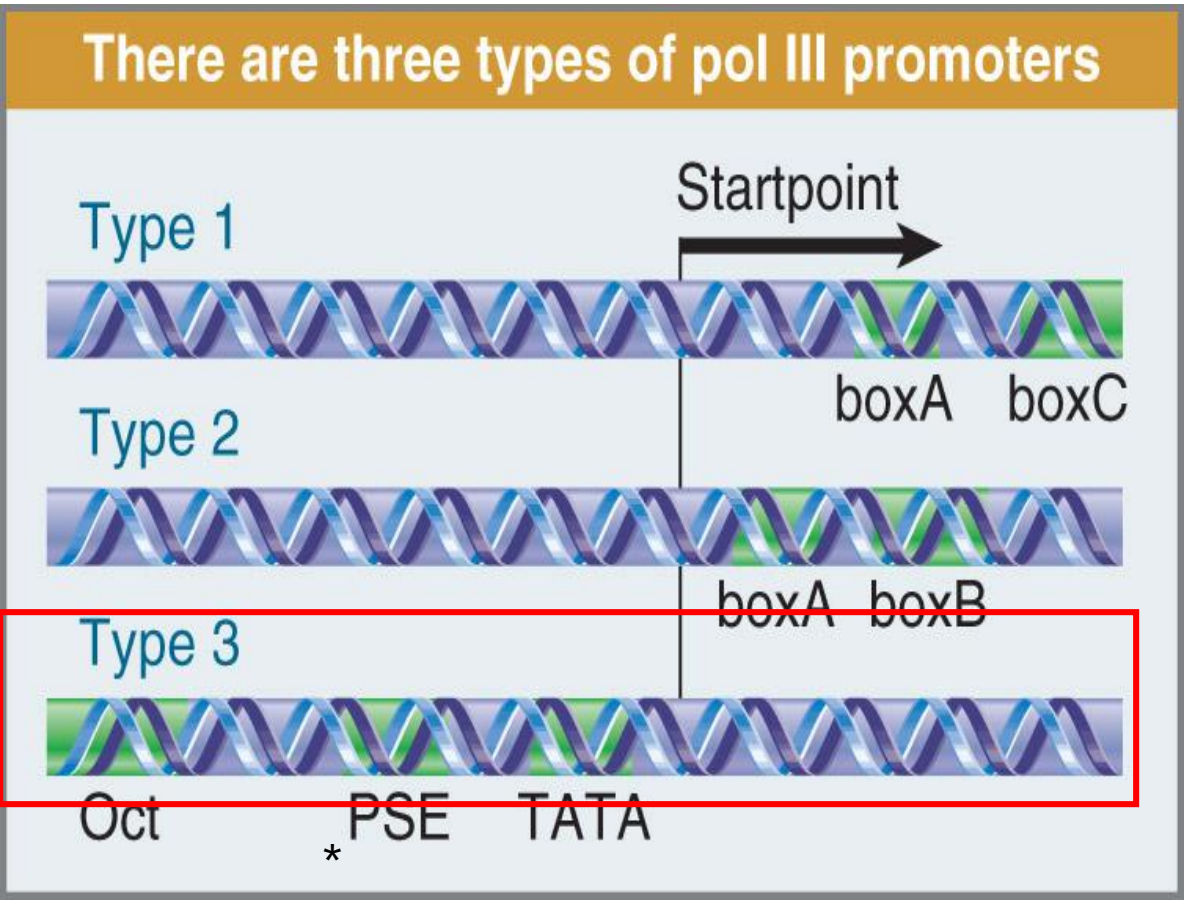


Figure 24.7

-RNA Pol III transcribes tRNA

-Core promoters (boxes)

-Transcriptional Factors(TF) III: general and specifics

*proximal sequence element

RNA Chain elongation

--Model

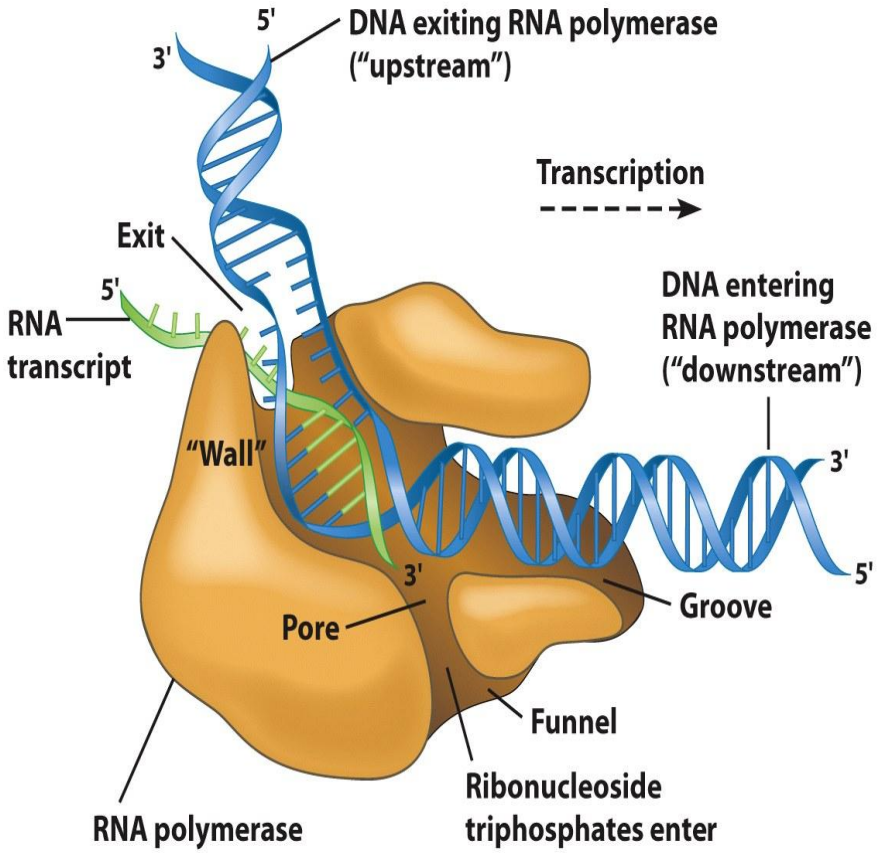


Diagram of the interaction between DNA and RNA polymerase based on crystal structures and other structural analyses.

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RNA Chain termination

Termination signal: specific DNA seq.
-1000 to 2000 nucleotides

--AAUAAA seq.
--GU-rich seq.

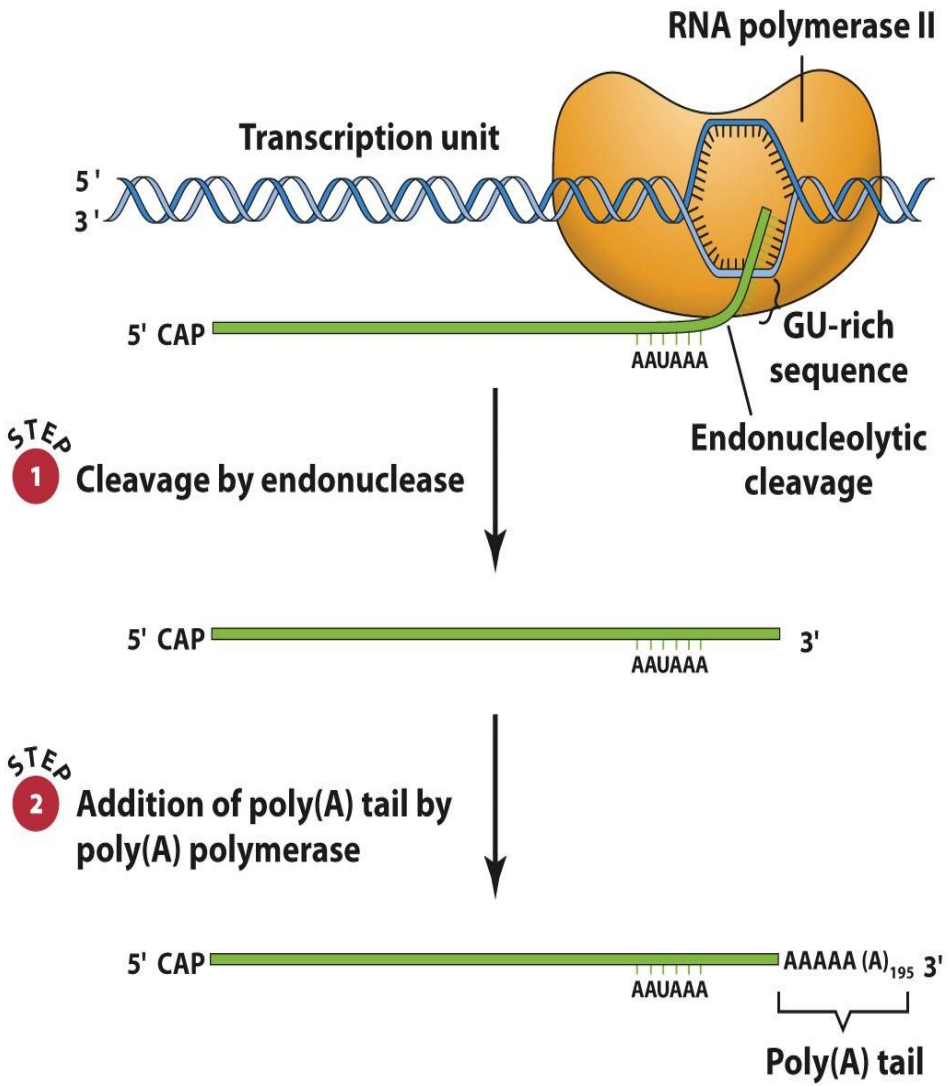
Endonuclease

--poly(A) polymerase

Pol-II vs Pol I and III

-Terminator proteins (Rho-indep. Terminator)

The 3’ Poly(A) Tail



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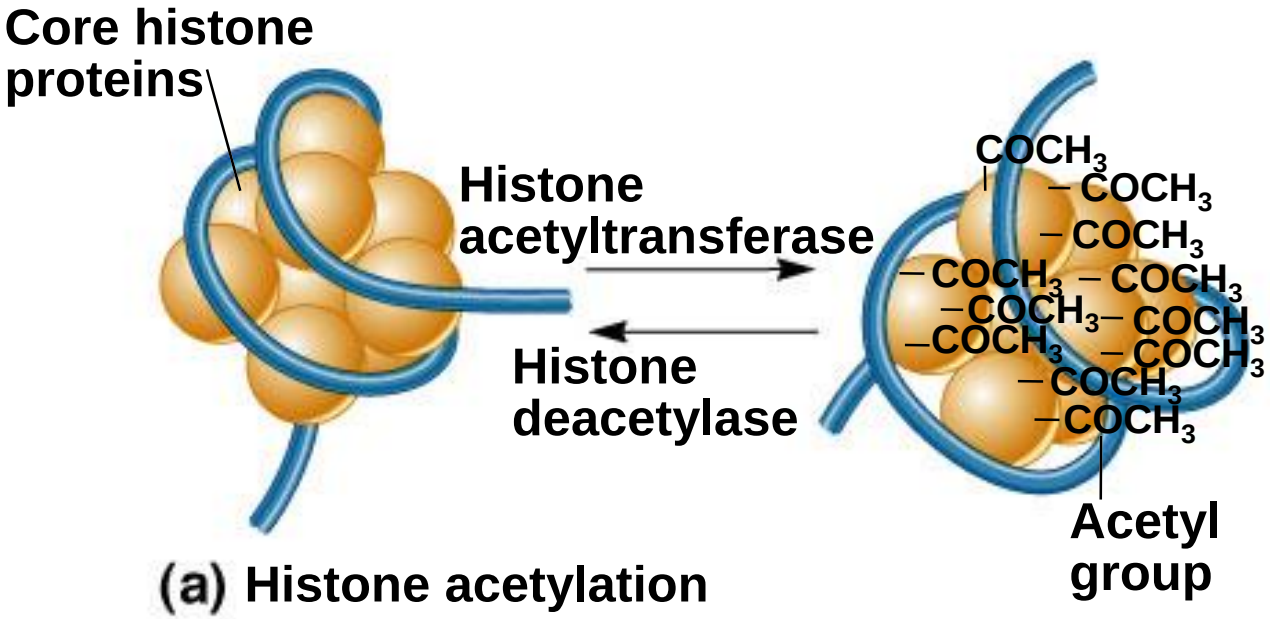
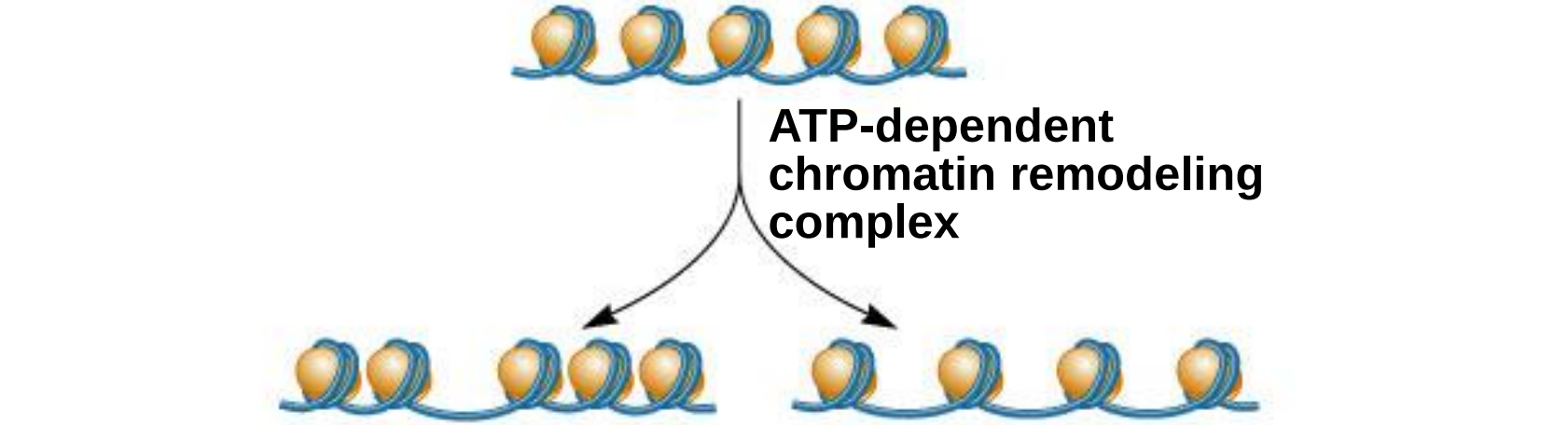
Chromatin Structure Affects Promoter Access

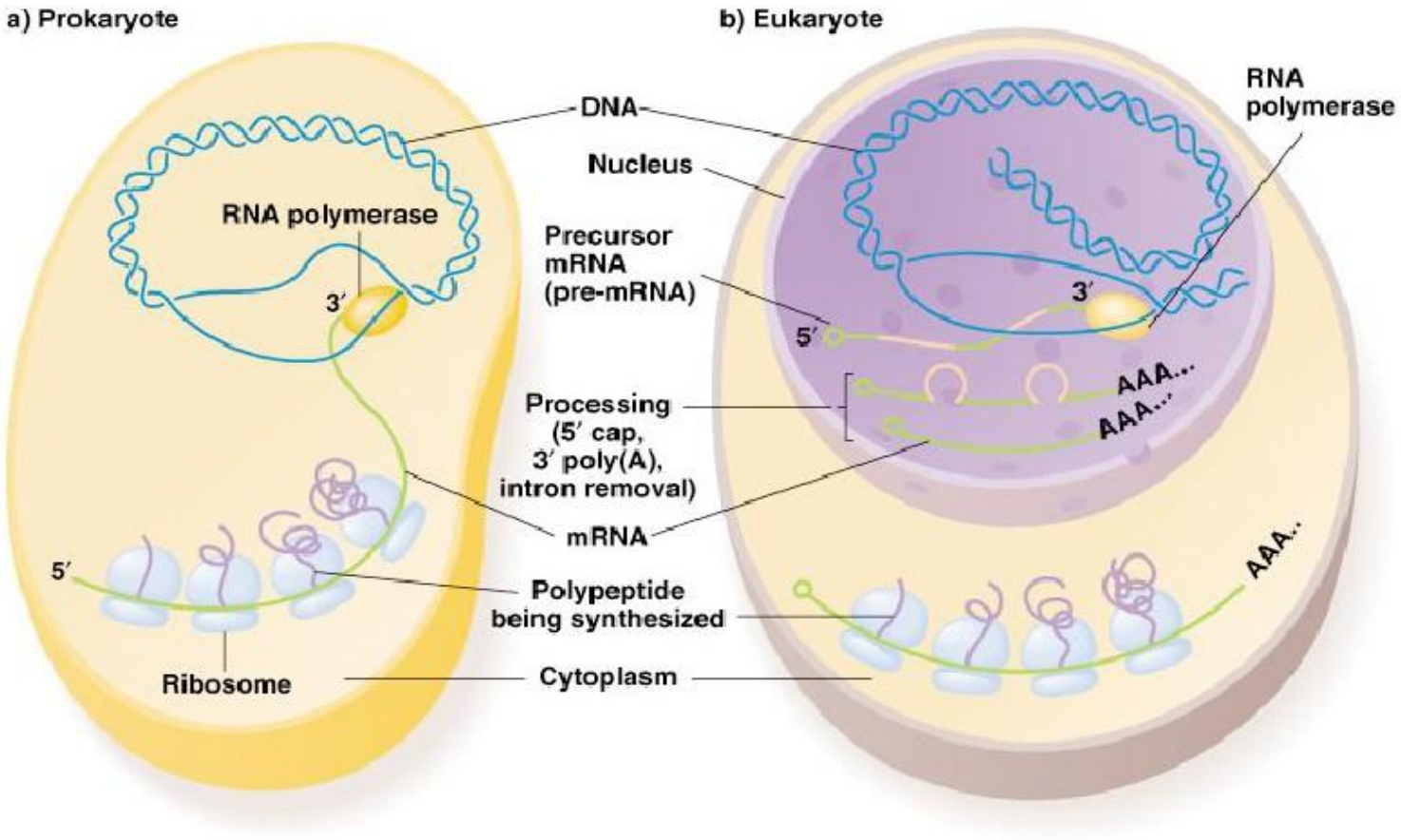


(a) Histone acetylation



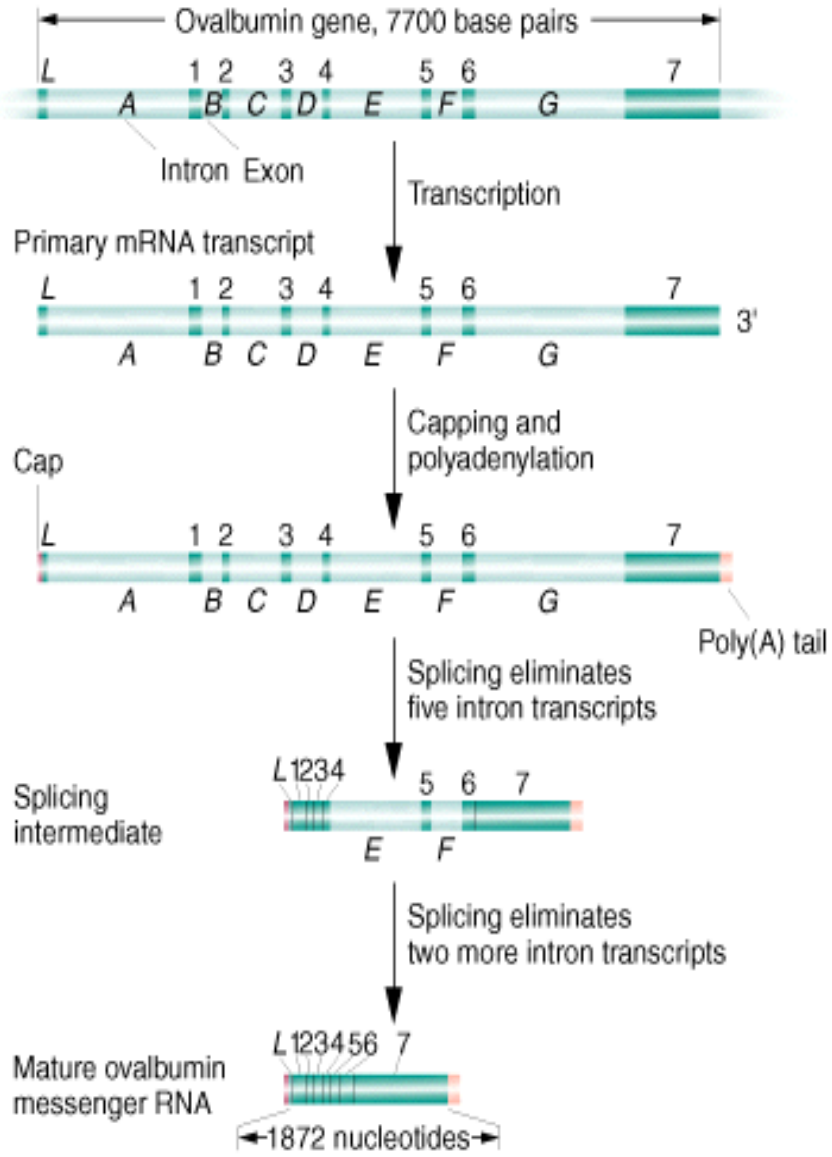
(b) Chromatin remodeling

Processes for synthesis of functional mRNA in prokaryotes and eukaryotes



RNA PROCESSING

RNA processing

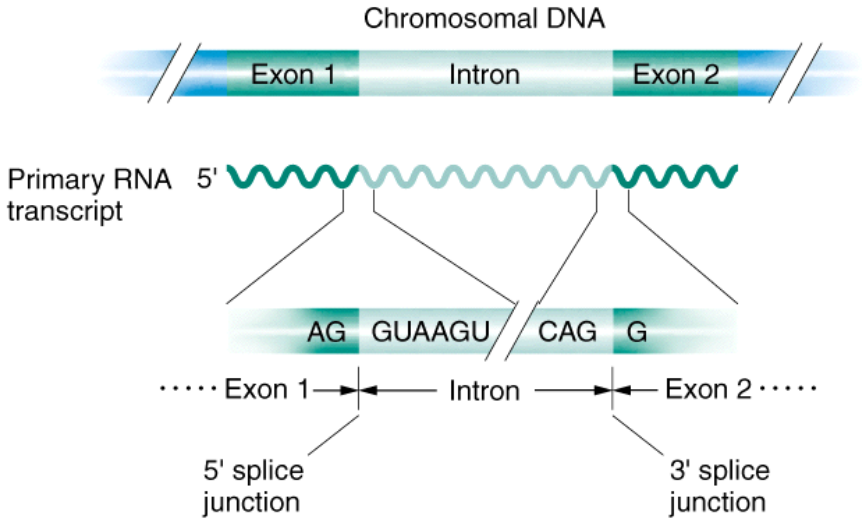


- Splicing and the mechanism of splicing of mRNA
- Capping and polyadenylation
- Alternative processing
- Processing of tRNA and rRNA
- Ribozymes
- RNA degradation

The primary transcript

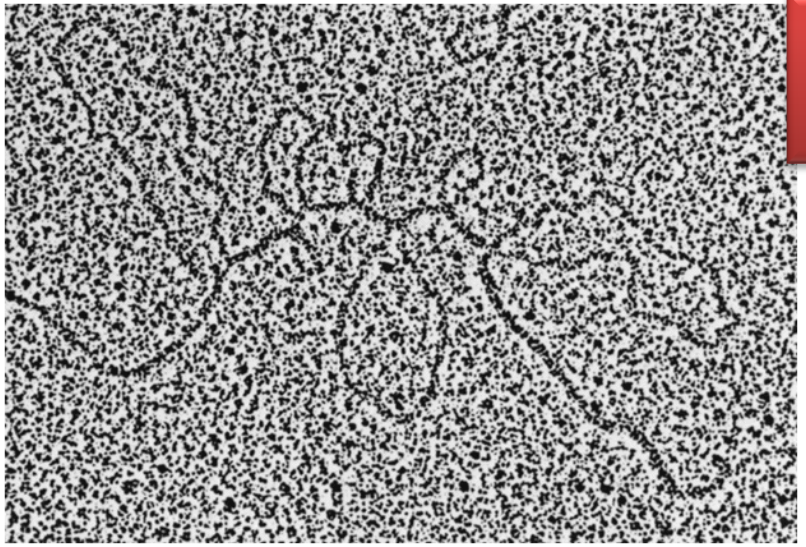
- This is an exact complementary copy of the template strand
  - ❑ mRNA modifications create an open reading frame and permit it to be translated
    - ○ Splicing removes non-functional regions of the primary transcript yielding mature message
    - ○ Capping and polyadenylation characterize mRNA processing
  - ❑ tRNA modifications include splicing, cleavage of sequences at the 5’ and 3’ end, and base modification
  - ❑ Mature rRNAs are cut out of a preribosomal primary transcript that includes one copy each of 18, 5.8 and 28S rRNA

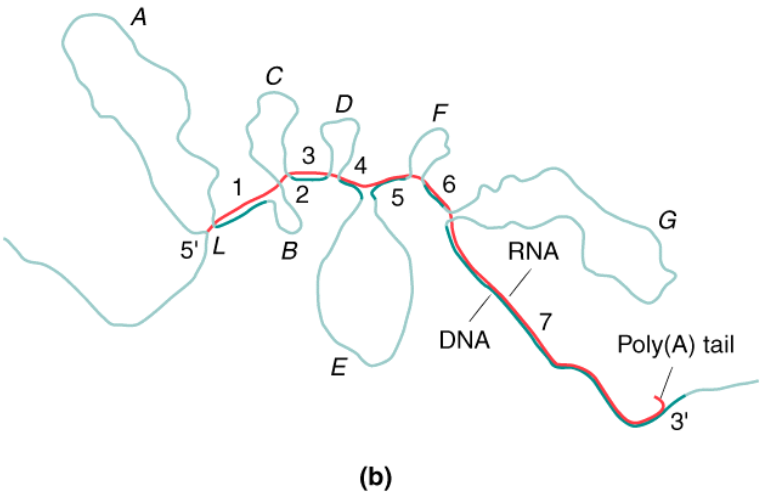
Introns



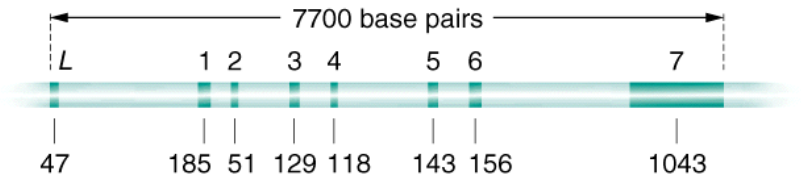
- RNA sequence not present in a mature RNA that is flanked by sequence that is present in the mature RNA
- Present in higher organisms more often than lower
  - Very few introns known in bacteria
  - The higher the organism,
    - the more likely introns are present
    - the more frequently they occur in a single gene
    - the larger they are
      - Represent the majority of sequence in most human genes
- A few genes are intron-less
  - They are regulated to yield very strong expression to specific signals
    - Histone genes
      - No CAP or tail

Initial description of introns





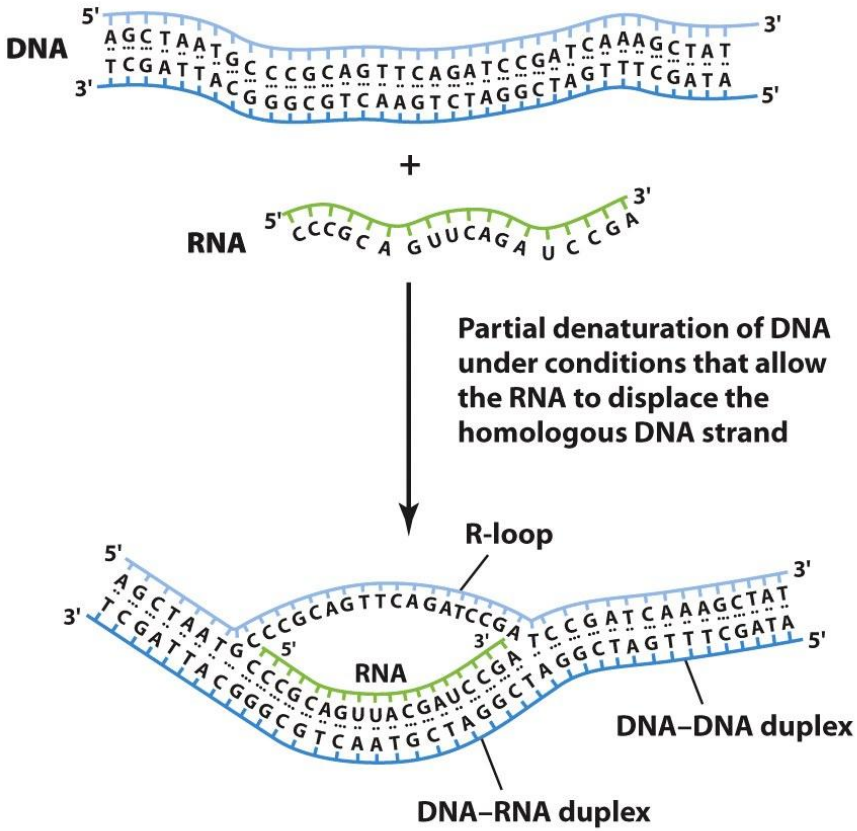
(b)



(c)

- Initially described in Adenovirus and later in the ovalbumin gene of the chicken
  - The isolated ovalbumin gene was denatured and rehybridized with mRNA from a chicken egg
  - The hybrids were examined using electron microscopy
  - D loops formed, representing single stranded regions of genomic DNA not present in the mature message

Hybridization: annealing



The RNA pairs with the complementary strand of DNA, forming a DNA–RNA duplex, leaving a single-stranded region of DNA called an R-loop.

The technique of R-loop hybridization.

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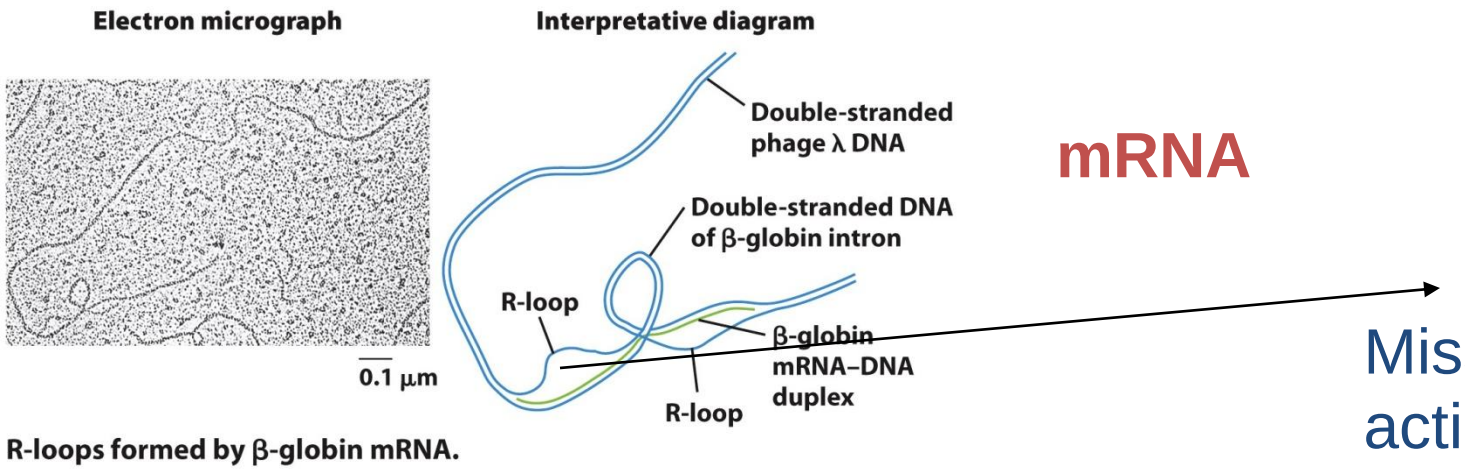
R-Loop Evidence of an Intron in the Mouse β-Globin Gene



R-loops formed by β-globin mRNA.

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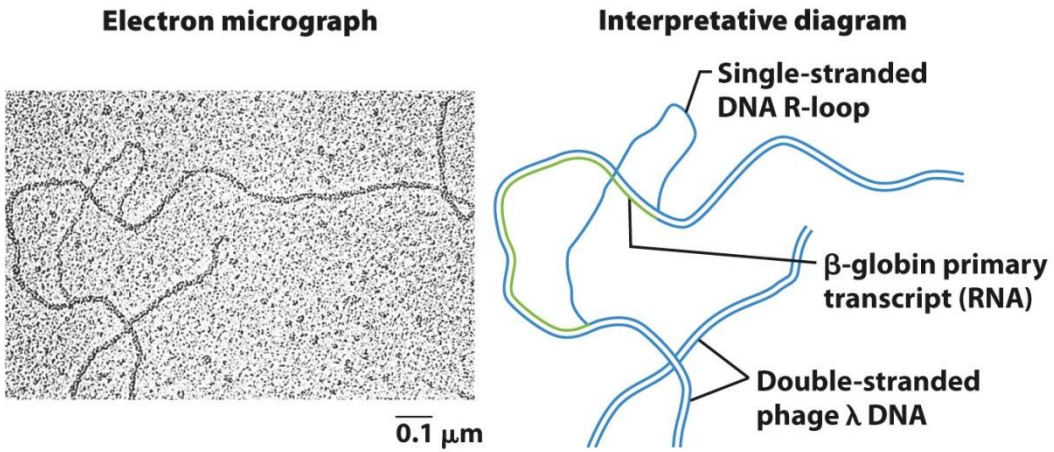
mRNA

Missing in actions

Pre-mRNA



R-loop formed by β-globin primary transcript (pre-mRNA).

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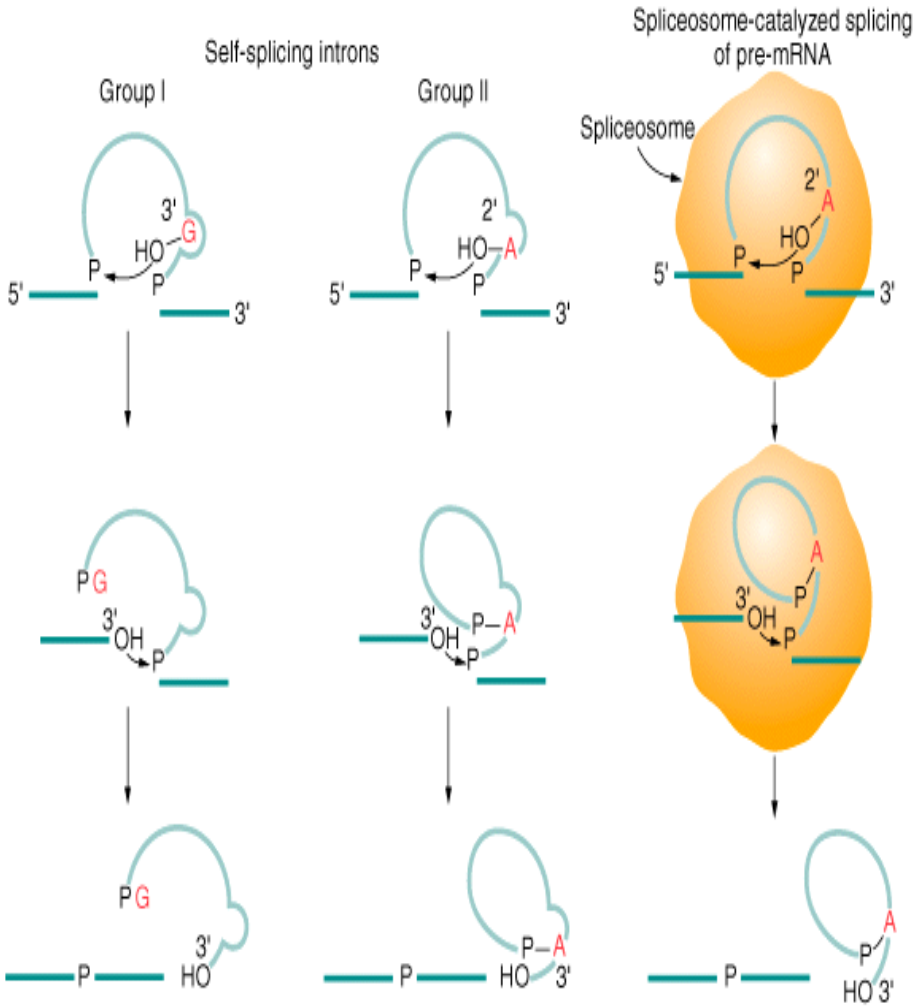
Introns



- Four classes differ in their distribution and reaction mechanisms
  - Group I – nuclear and non-nuclear rRNA, tRNA and mRNA genes
  - Group II non-nuclear, non-animal mRNA genes
  - Nuclear mRNA transcripts
  - ATP, endonuclease dependent tRNA splicing mechanism

What is the importance?

J. Microbiol. Biotechnol.

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

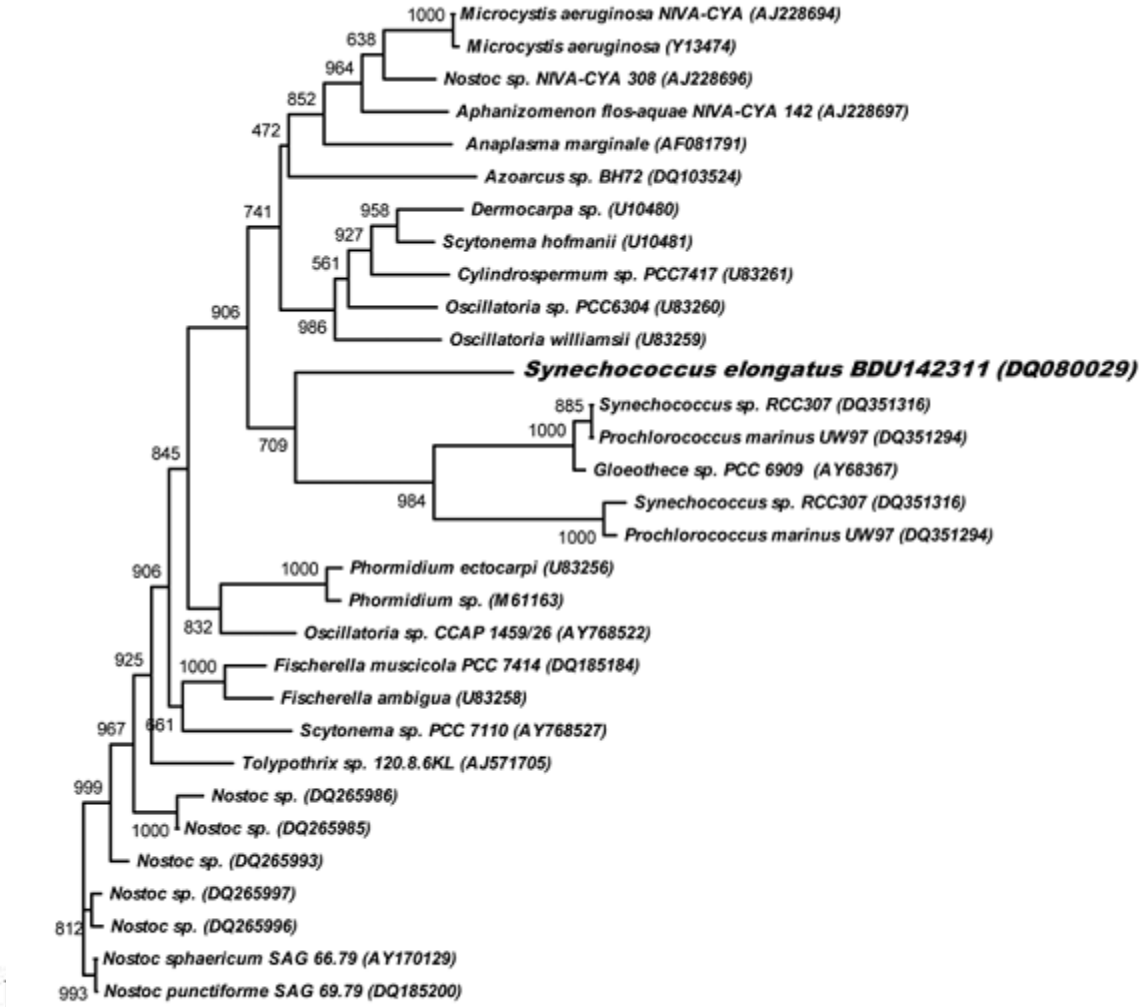
or Microbiology and Biotechnology

Evidence on **rine**
Cyanobacter

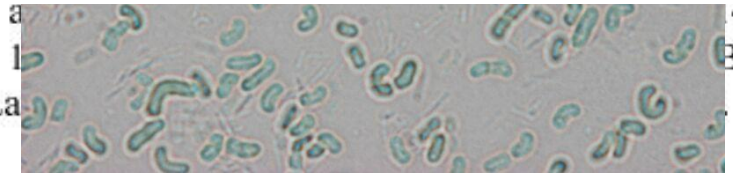
Muralitharan, Ga

Department of Microbi *palli 620 024, India*

Received: March 23, 2



Lanes 1 to 6 a 42311, BDU
30312, BDU 1 BDU 130912
respectively. La



Removal of Intron Sequences by **RNA Splicing**

The noncoding introns are excised from gene transcripts by several different mechanisms.

Eukaryotes
No prokaryotes (excepts a few a prokaryotes virus and others)

Excision of Intron Sequences

Conserved seq. for mRNA

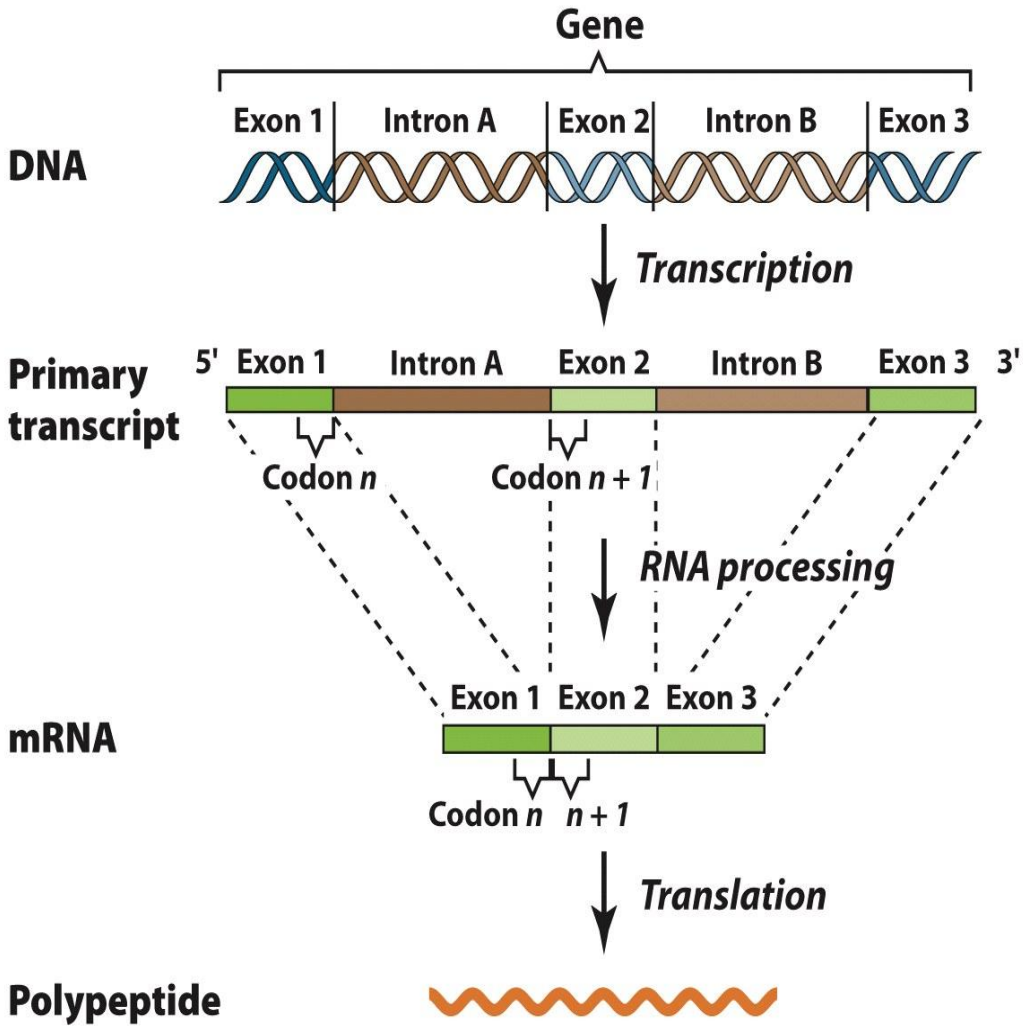
Exon-GT…AG-exon

intron

99%

Ribonucleoproteins:

Spliceosomes (1981)



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Mechanism of Splicing

- There is an intranuclear protein/RNA complex called the spliceosome that ensures proper splicing.
- Three types of short sequences dictate the precise cutting of the intron/exon boundaries - called splice junctions.
  - **Splice donor**: 5' end of intron: **exon-G-U**
  - **Splice Acceptor**: 3' end of intron: **A-G-exon**
  - **Branch site**: within the intron, about 30 nucleotides upstream of the splice acceptor, has an AT rich region with at least one A.
- Two sequential cuts:
  - splice donor site is cleaved,
  - attaches to the branch site to form a lariat or loop structure,
  - then the splice acceptor site is cleaved.
- The intron degrades, the two exons are ligated.

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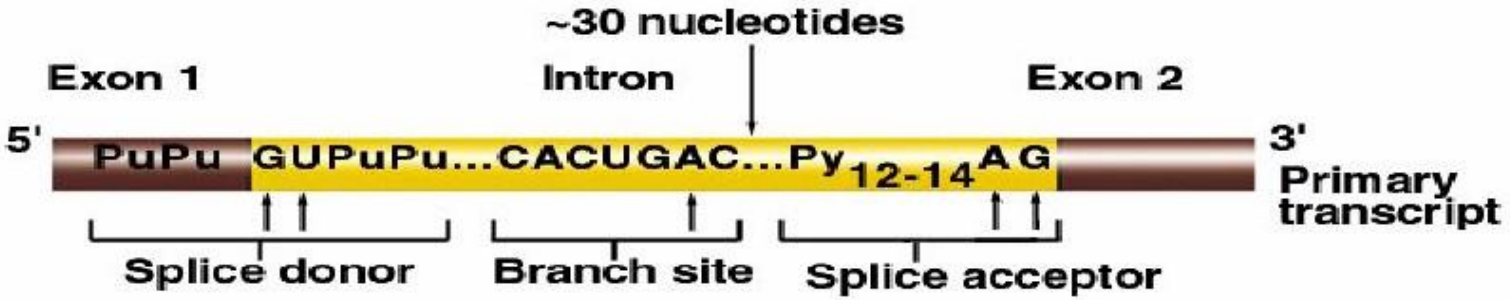
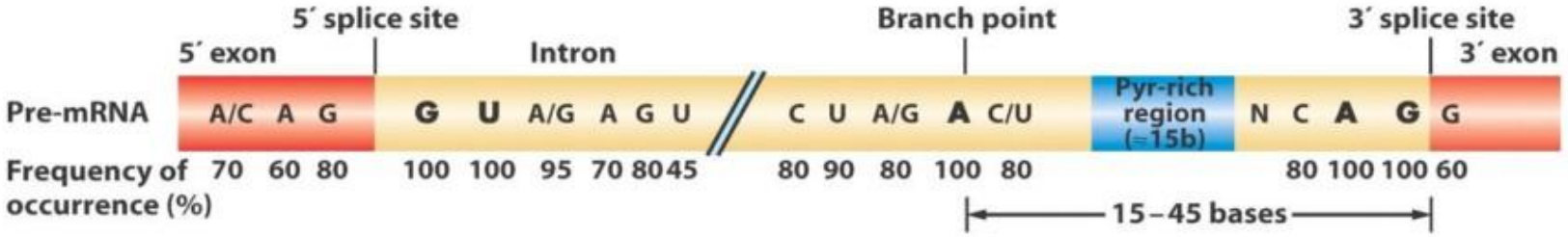
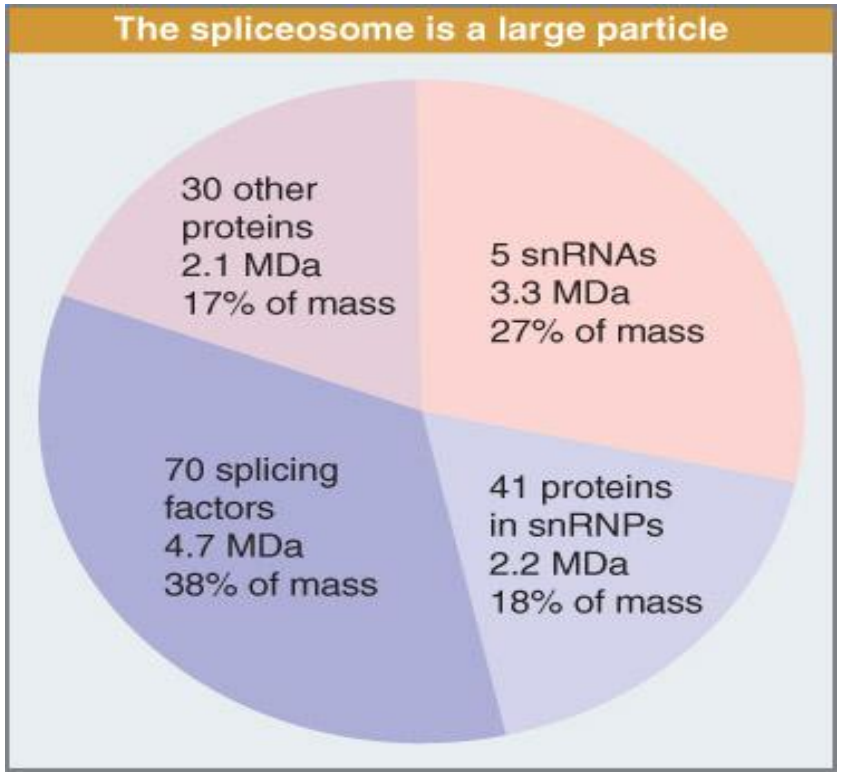
Mechanism of splicing

Short sequences dictate the sites of splicing



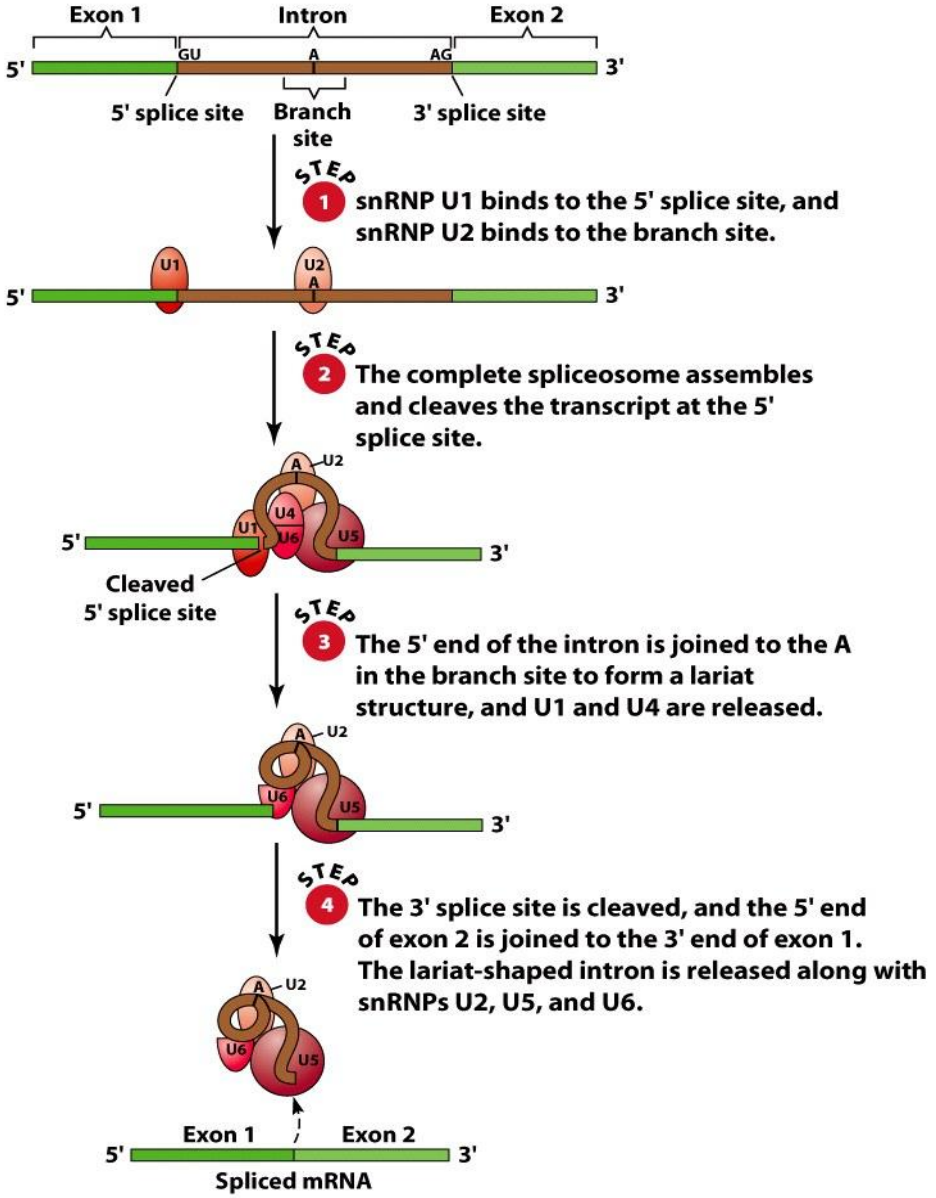




Spliceosomes: snRNA plus ~40 proteins

1%: CG…AG

AT…AC



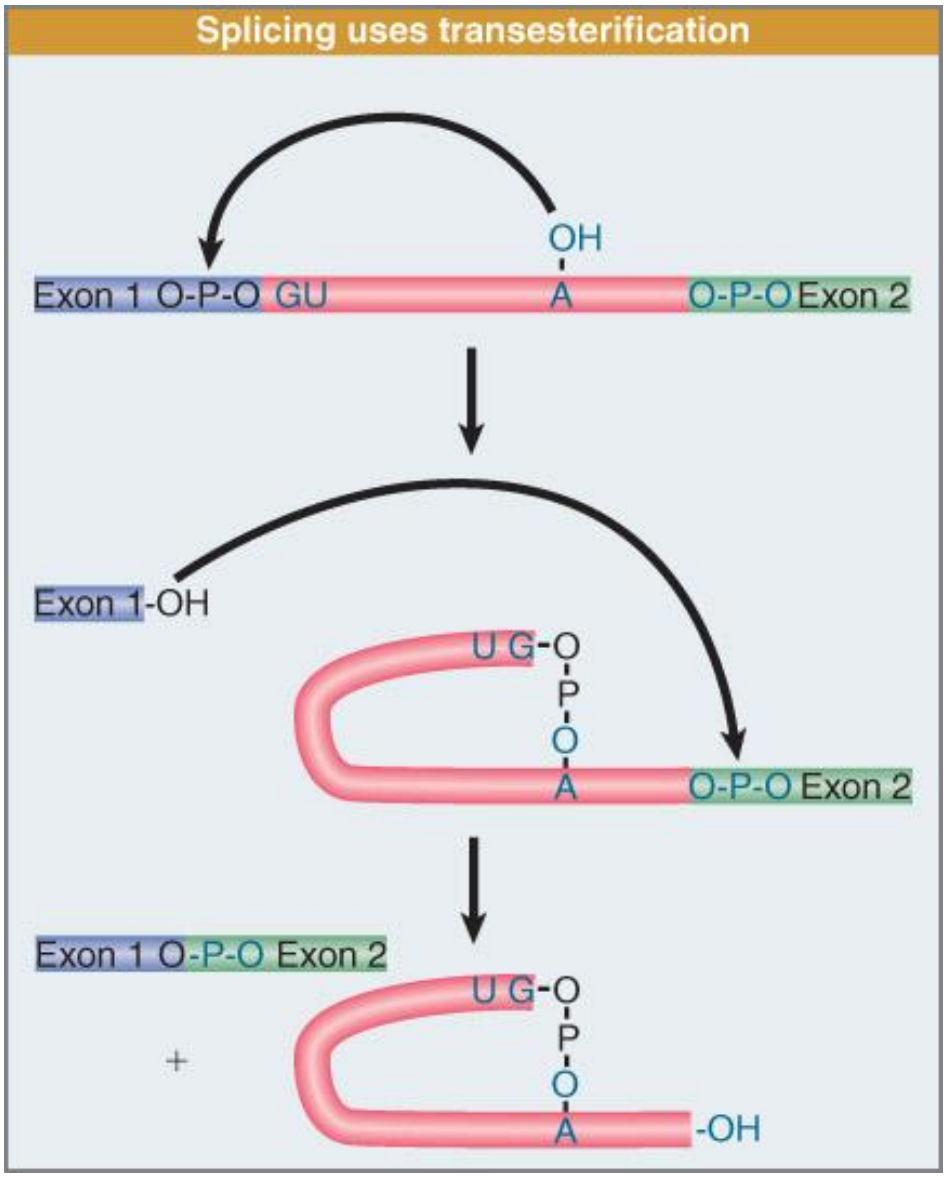


Figure 26.7

Nuclear splicing involves **trans-esterification**

GU…**UACUAAC**….AG

"Branch site"

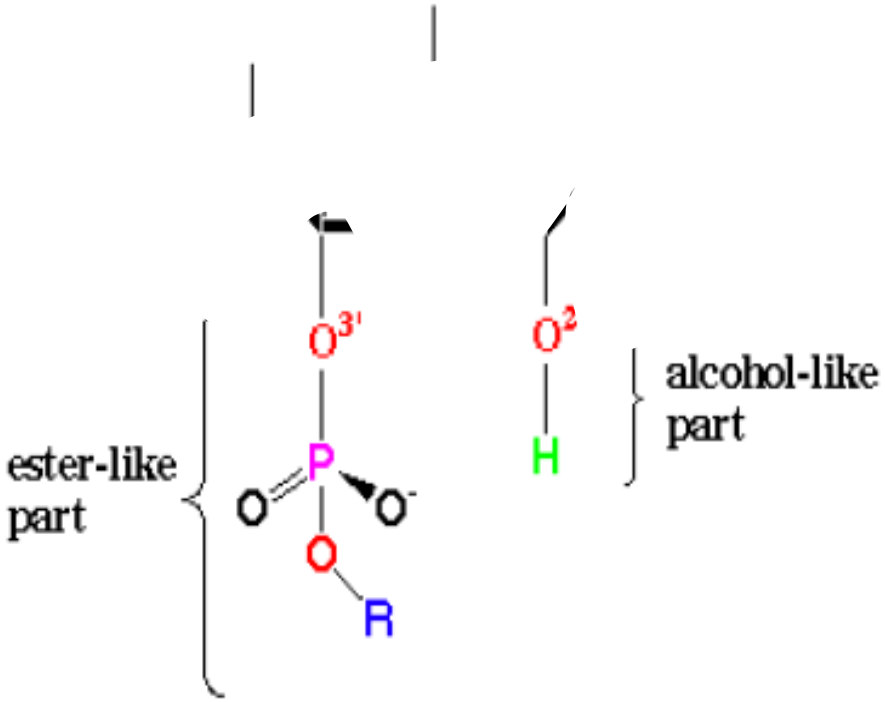
Does splicing require energy?

Does splicing ever occur in DNA?

Transesterifi-what ?

A process in which an ester and alcohol react to give another ester with a different alkoxy group is referred to as transesterification

- At the beginning the alcohol part starts the *nucleophilic attack* to the **ester** part
- The phosphodiester can be activated by a proton transfer either prior to or simultaneously with the nucleophilic attack



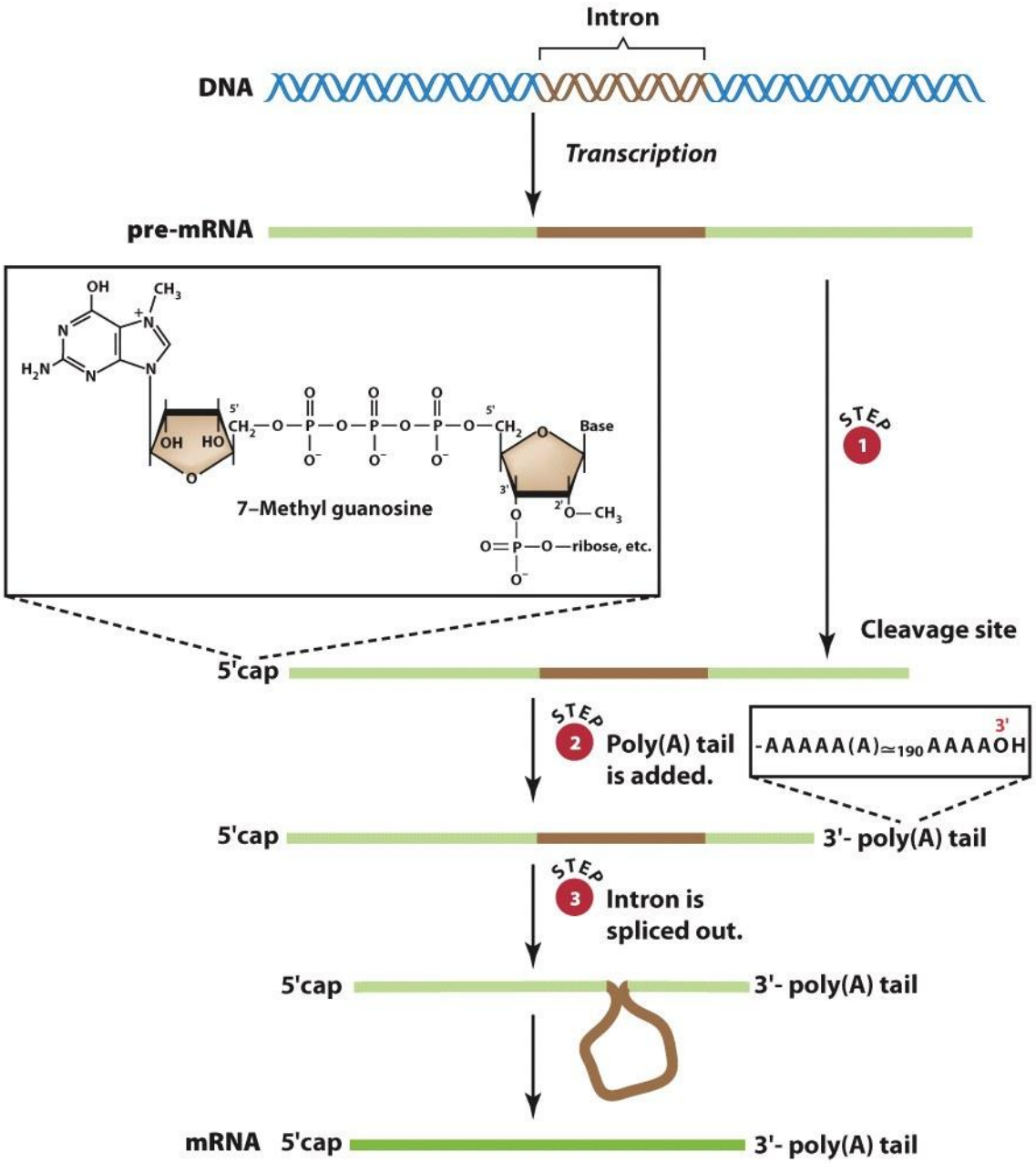
Modifications to Eukaryotic pre-mRNAs

- A 7-Methyl guanosine **cap** is added to the 5’ end of the primary transcript by a 5’-5’ phosphate linkage.

  (stability and protection)

- A **poly(A) tail** (a 20-200 nucleotide polyadenosine tract, As) is added to the 3’ end of the transcript. The 3’ end is generated by cleavage rather than by termination. (stability and protection)

- When present, intron sequences are spliced out of the transcript. (stability)



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Eukaryotic RNA Processing: Capping

- When the RNA chain is about 30 nucleotides long, the 5' ends are modified by the addition of a guanine group in the opposite orientation:
  - involves a 5'-5' triphosphate linkage.
  - Happens before transcription is finished = co-transcriptionally

- Methyl transferases then add methyl groups in the 7 position to that and a couple more nucleotides.

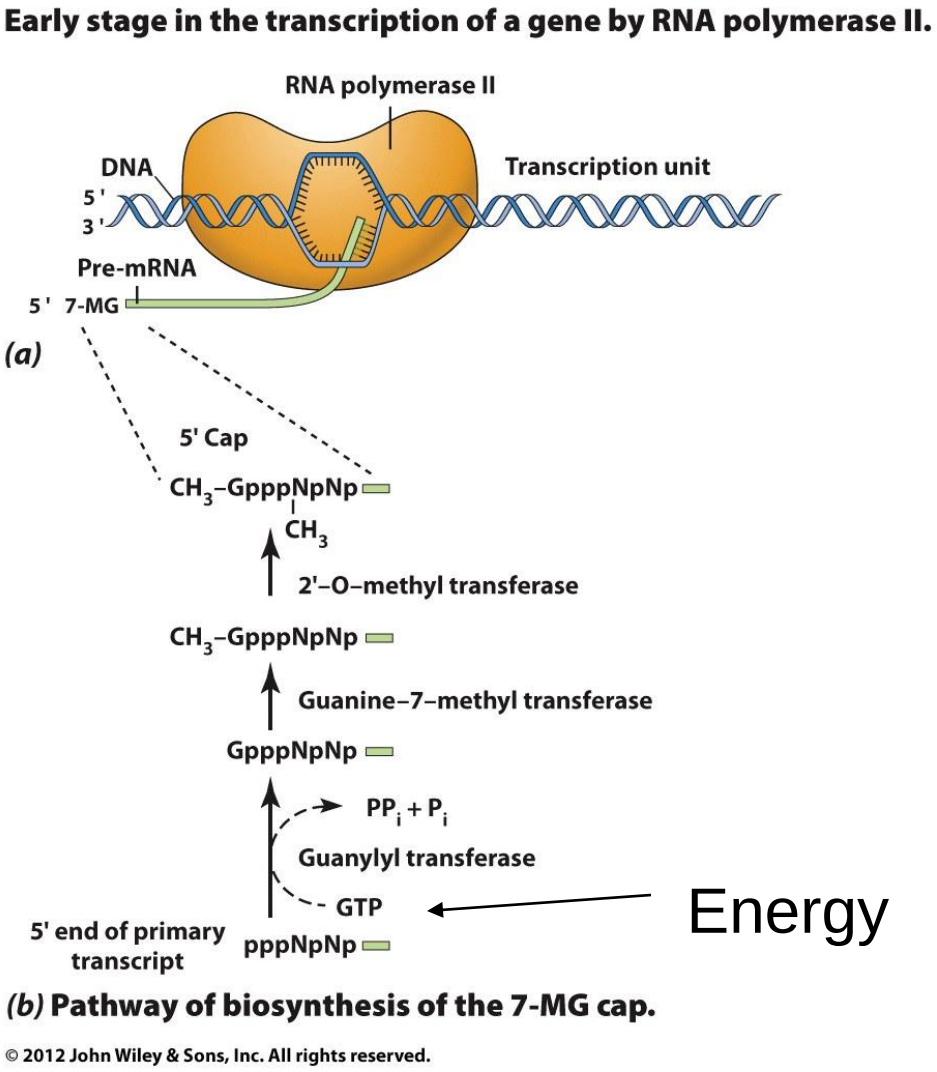
- The caps are recognized by the translation machinery.

- They protect the growing RNA chain from degradation by nucleases.

The 7-Methyl Guanosine (7-MG) Cap

Histones:?

FACT (facilitates chromatin transcriptional)



Energy

Polyadenylation

• nascent RNA is cleaved downstream from the AAUAAA conserved sequence.

- By ribonuclease

• The enzyme poly(A) polymerase adds adenine ribonucleotides

- up to 200 bases long at the 3’ end of the RNA.

• The poly(A) tail

- enhances the stability of eukaryotic mRNA and
- regulates its transport to the cytoplasmic compartment.

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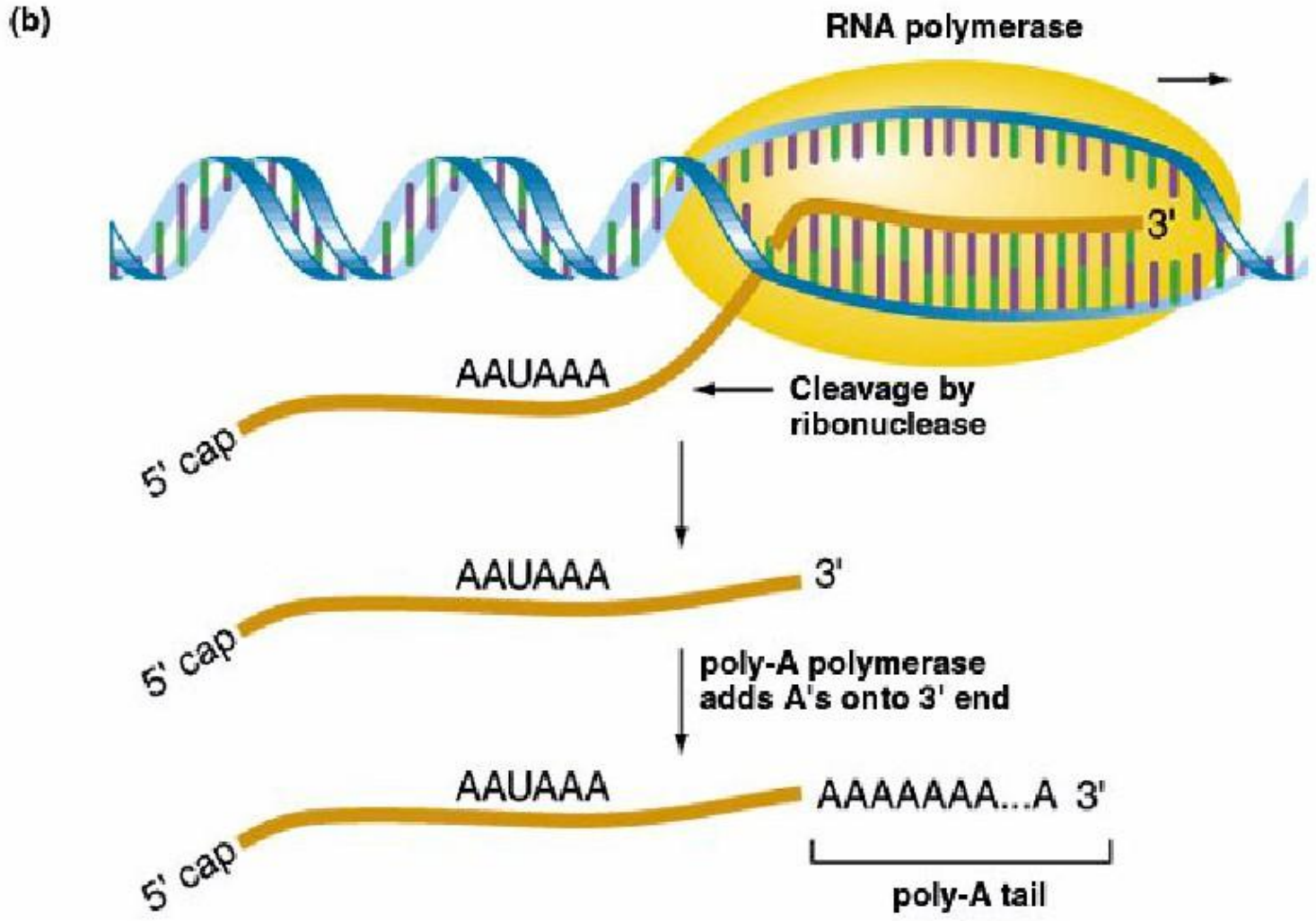
The ends of eukaryotic mRNAs

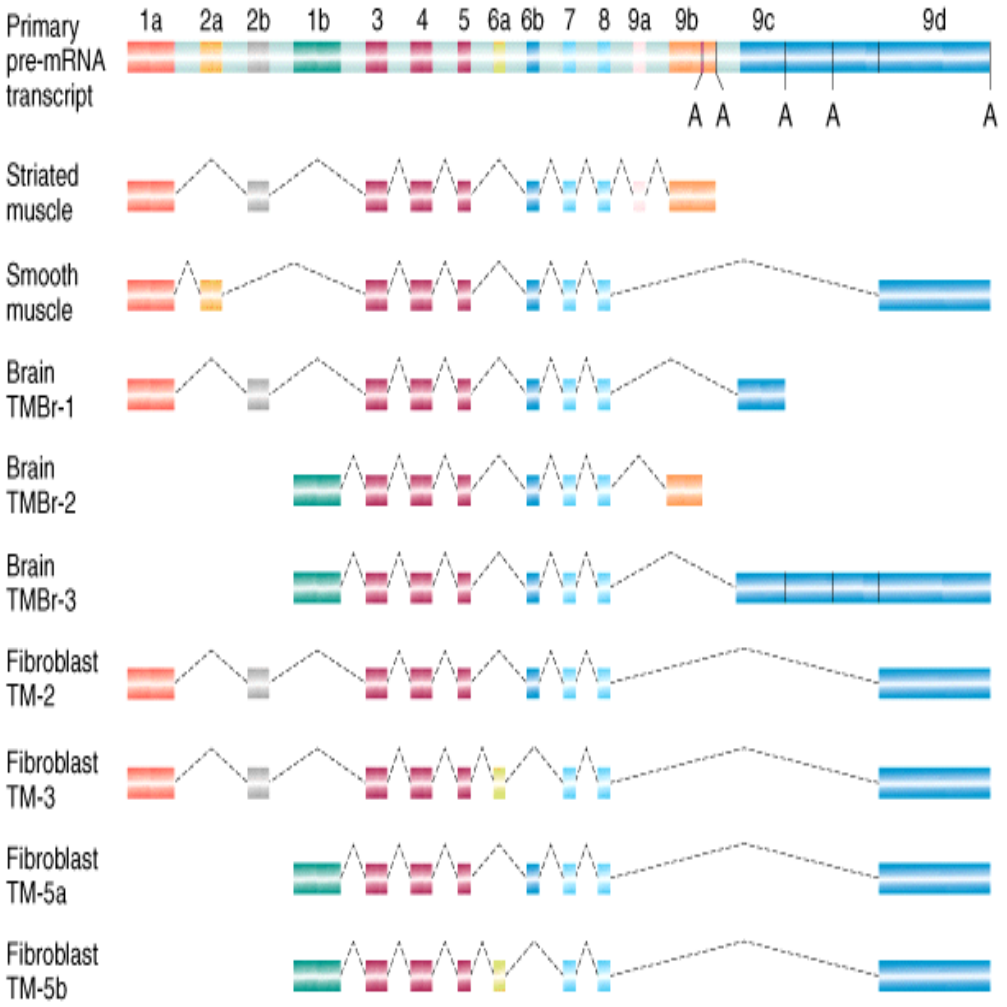


The functional domains of a protein

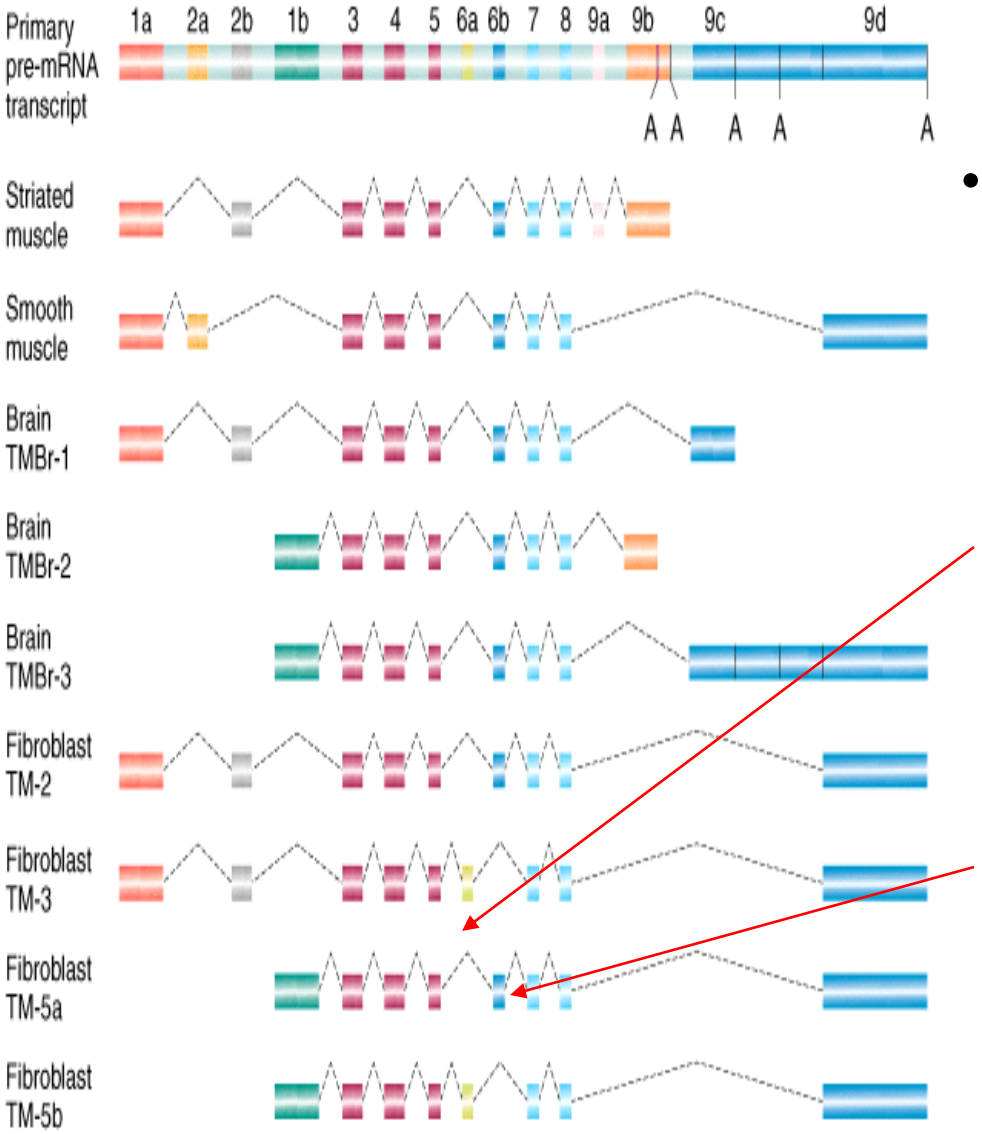
- The function of a protein may be divided into domains
  - Simple examples are the 5'-3' exonuclease, 3'-5' exonuclease and polymerase domains of DNA polymerase I
- Some eukaryotic genes may have evolved by switching functional domains into other genes
  - Evolving domains is easier than evolving a complete protein
  - Domains are sometimes reflected in exons
    - For example, the immunoglobulin domain that embeds IgM into the plasma membrane is coded for by a specific exon at the end of the gene
    - This results in a protein domain at the end of IgM that attaches it to the membrane
    - The cell can produce an IgM that is free in the serum by not including that exon in the mature message

Alternative splicing

- This is a method for producing alternative messages from one gene
  - A primary transcript is made
  - Different splice products are made that are cell type specific
    - Cell type specific means that one cell, such as an epithelial cell, will make a different form than another cell, even though the gene making the primary transcript is the same
    - This happens because the snRNP's or components of the spliceosomes are different in the two cells



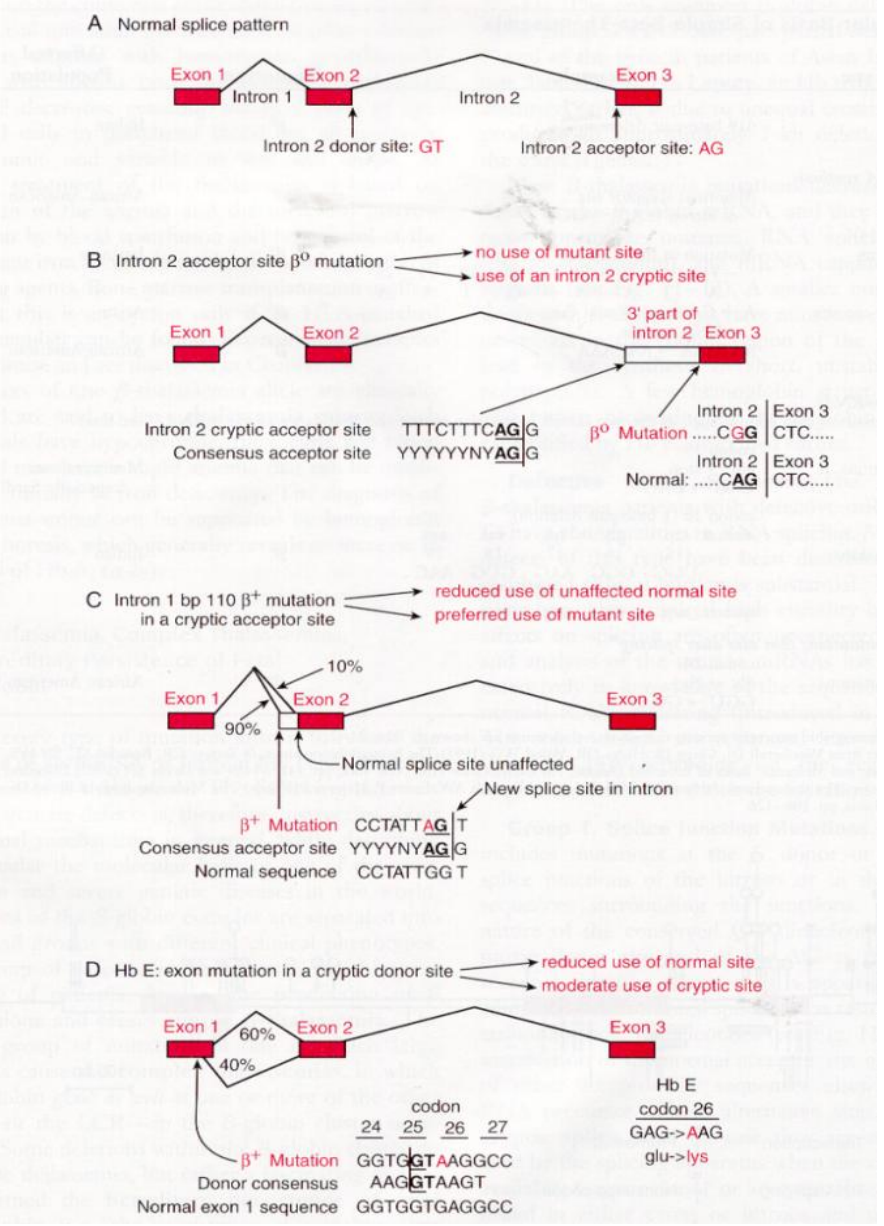
Altenative splicing: Exon skipping



- Splice site choices can exclude an entire exon internal to the message
- Myosin heavy chain gene expression skips exons during fly development
  - Exclusion of a splice junction causes exon skipping
  - One cell recognizes the downstream (3') splice junction of the next exon in line
    - So the 5' donor site is added to the 3' acceptor site
  - In another cell, the first downstream site is not recognized and the next 3' acceptor site is recognized
    - This skips both the 3' acceptor site and the 5' donor site of the skipped exon

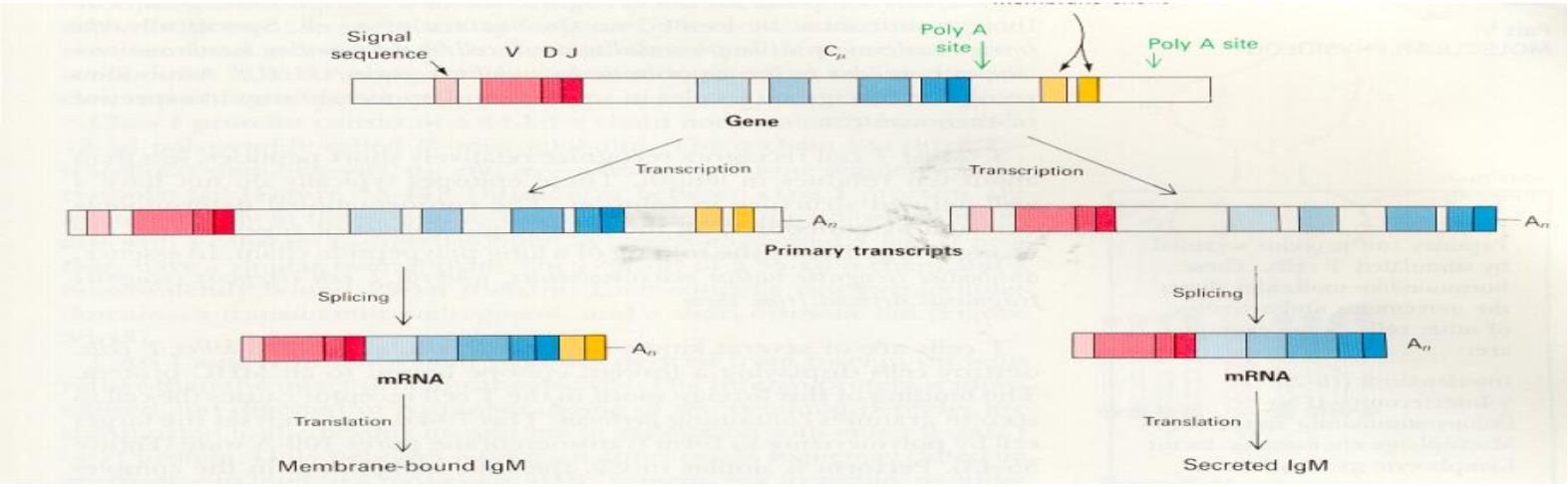
Alternative splicing: cryptic splice sites

- Alternative splicing can also add exons
  - The alternative exon is within a gene but not normally recognized
    - Normal mechanisms can be at work to add the exon in a cell type specific manner
    - Mutations can also destroy splice junction sequences
      - Without a normal splice site, the cell may choose a sequence that is similar within an intron or exon that is not normally used
        - » This is a cryptic splice site
      - A cryptic splice site can result in a less than functional protein
        - » But sometimes having a damaged protein is better than having no protein at all

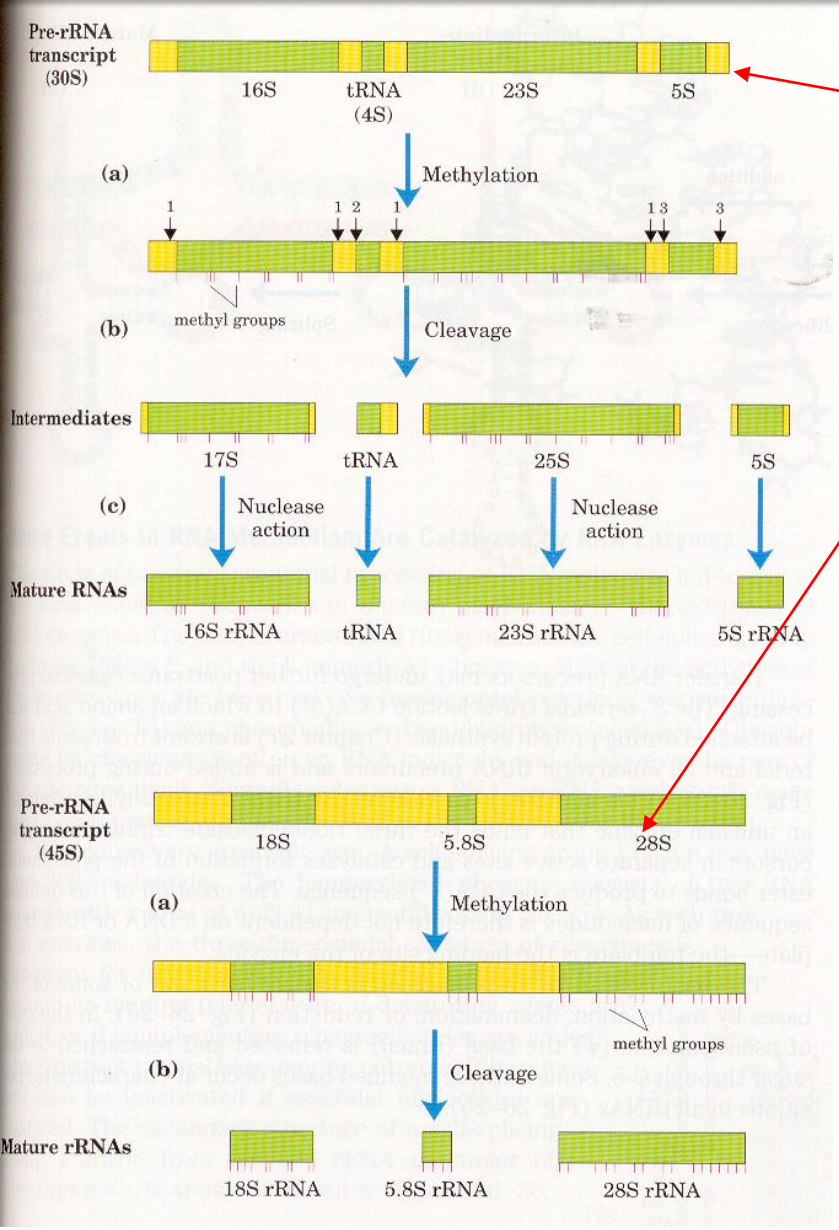


Alternative cleavage

- This is at work with IgM expression
  - At one stage of the immune response, IgM makes a membrane bound form of an IgM antibody
  - Upon receiving a signal, the cell converts to making the exact same protein, but lacking the carboxyterminal peptide holding it to the membrane
  - The conversion occurs because cleavage and polyadenylation exclude the last exon of the primary transcript



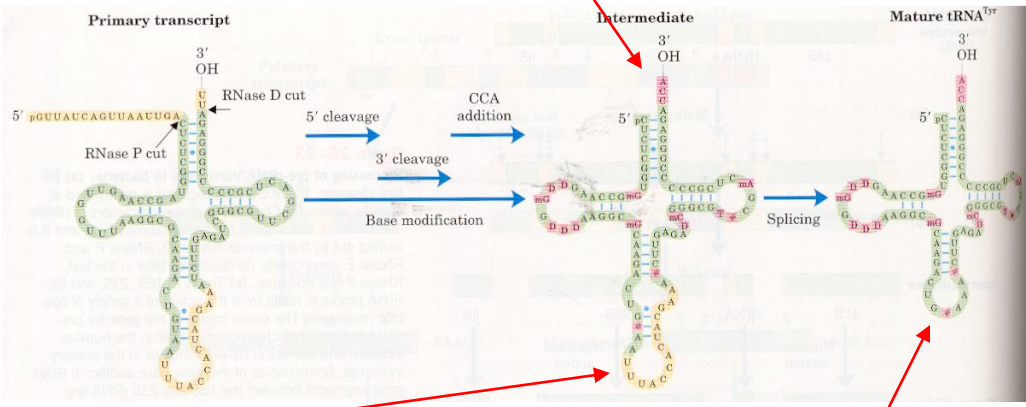
Post transcriptional processing of tRNA and rRNA



- Prokaryotes
  - Shown above
- Eukaryotes
- 18S, 5.8S and 28 S rRNA is made as one long transcript by RNA polymerase I from a gene
  - There are multiple copies of these genes and transcription is almost continuously occurring
- Processing is enzymatic, cleaving a final product from the large precursor

tRNA processing

- This requires enzymatic cleavage of sequences on the ends of the primary transcript
  - RNAse P (a ribozyme) cleaves the 5’ end, and RNAse D the 3’ end
  - Following RNAse D cleavage, a CCA sequence is enzymatically polymerized onto the 3’ end of the tRNA
  - This sequence is necessary for the tRNA to accept and bond to its specific amino acid
- This is followed by splicing a specific segment out of the tRNA to produce a mature anticodon loop
- Base modification occurs during this process



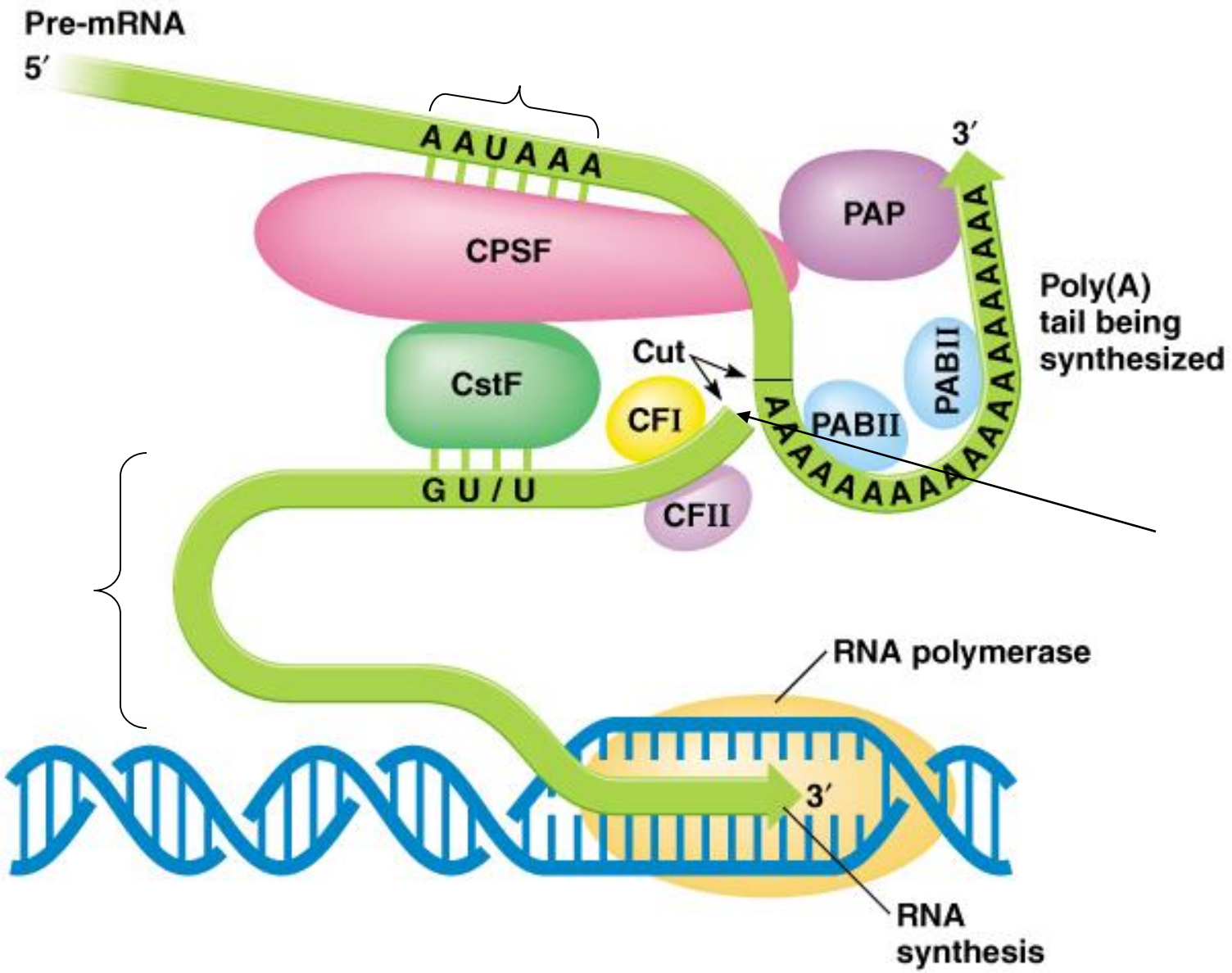
Ribozymes

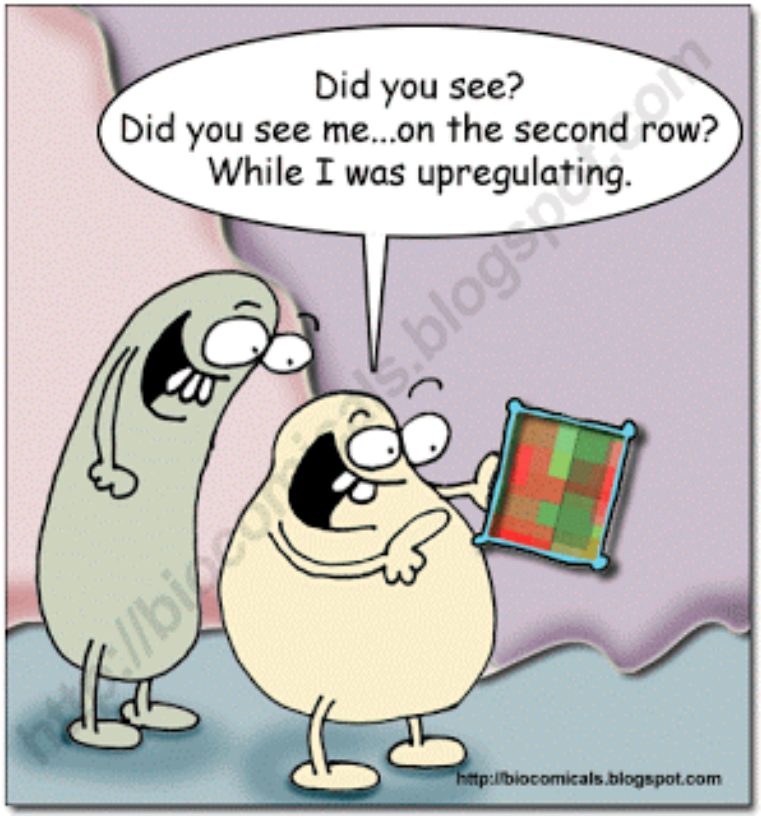
- These are catalytic RNAs that mainly participate in the cleavage of RNA
  - All self-splicing mechanisms are examples of ribozymes
  - They are not true catalysts because they alter their own structure as a result of catalysis
    - However some group I introns that are excised can continue to catalyze simple transesterification reactions
- The may act as free catalytic agents, however, able to cleave RNA in a sequence specific manner
  - The hammerhead ribozyme can, in theory, be designed and synthesized in a gene machine to degrade any specific RNA sequence
  - Ribozymes are, though, unstable and subject to degradation by RNAse *in vivo*

RNA degradation

- The amount of any substance present depends on its rate of synthesis and degradation
- RNA (and protein) levels are controlled at the level of degradation as well as synthesis
  - Less RNA means less resulting protein from translation
- Degradation in eukaryotes proceeds by
  - Endonucleolytic attack on the poly A tail
  - Decapping
  - Exonucleolytic from the 5’ end
- The rate of degradation is determined by the sequence and structure of the RNA
  - Exonucleases attack RNA
  - Exonuclease attack can be inhibited by
    - Hairpin loops
    - Poly A tails

Termination of RNA synthesis in (eukaryotic) RNA Pol II

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Did you see me...on the second row?
While I was upregulating.
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THANK U