Review: Chromatin Structure

H3K27 trimethylation (the addition of 3 methyl groups to the 27th amino acid residue (lysine; K) of histone H3) is known to affect the composition of chromatin

- What do you think the addition of these methyl groups is likely to do to the nearby chromatin?

- If you genetically engineer a histone H3 protein to have a modified lysine residue that is constitutively (always) trimethylated, what affect might this have on gene expression?

From DNA to Protein: How Cells Read the Genome

Dr. Matthew Ellis

Chapter 7 – Part 1

BIOL366

Learning Objectives for Chapter 7, Part 1

Upon completing this module, **you should be able to**:

- Describe the differences between RNA and DNA leading to the multiple roles of RNA in the body.
- Understand the steps involved in transcription that lead to the formation of a precursor mRNA
- Detail the components how RNA is processed into mature mRNA

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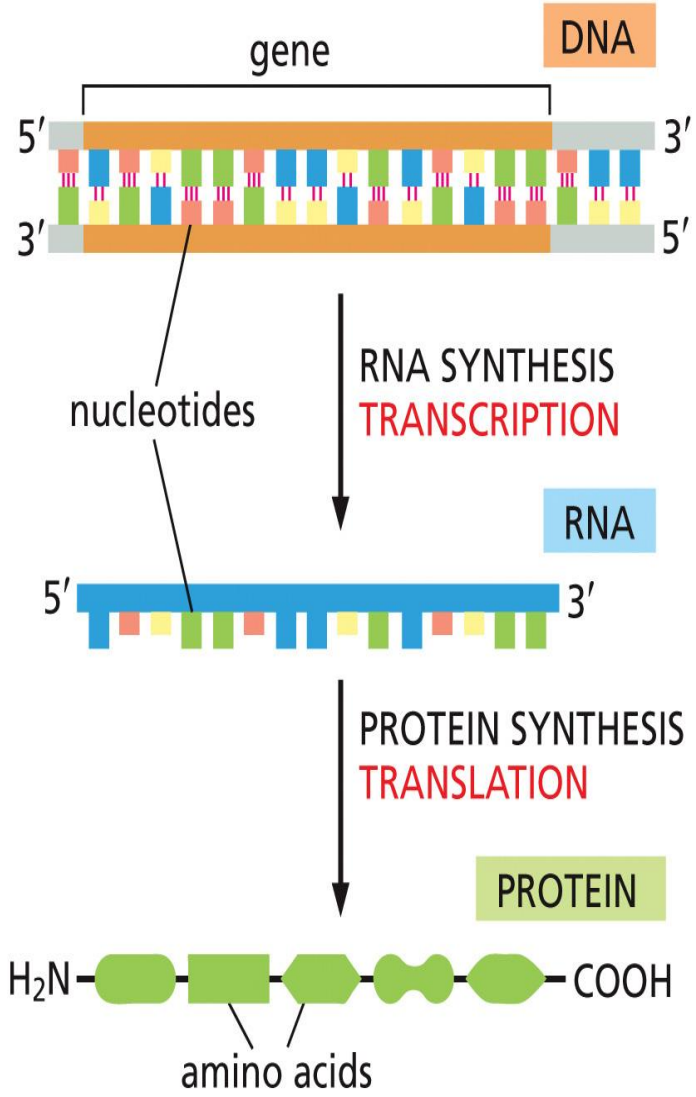
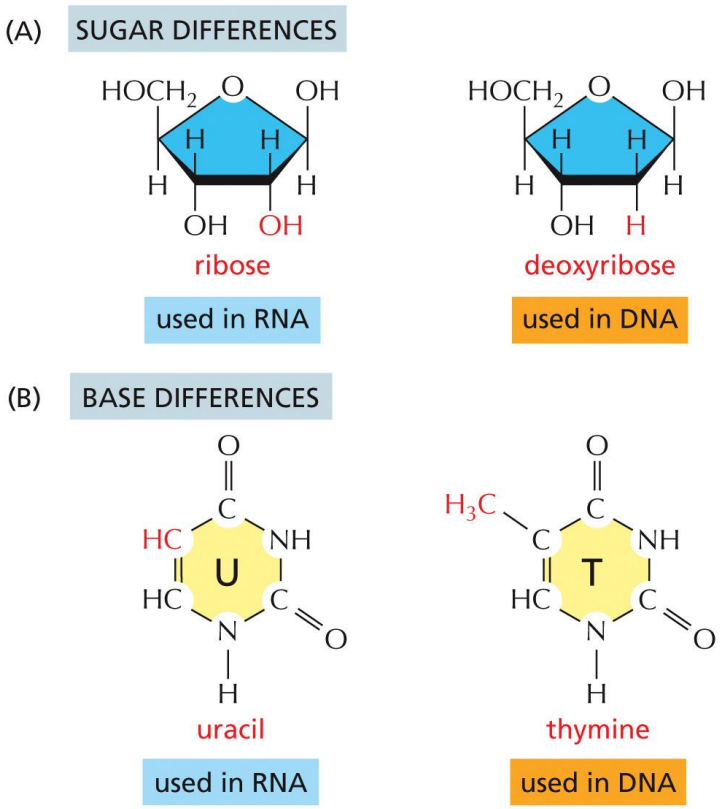
The Central Dogma

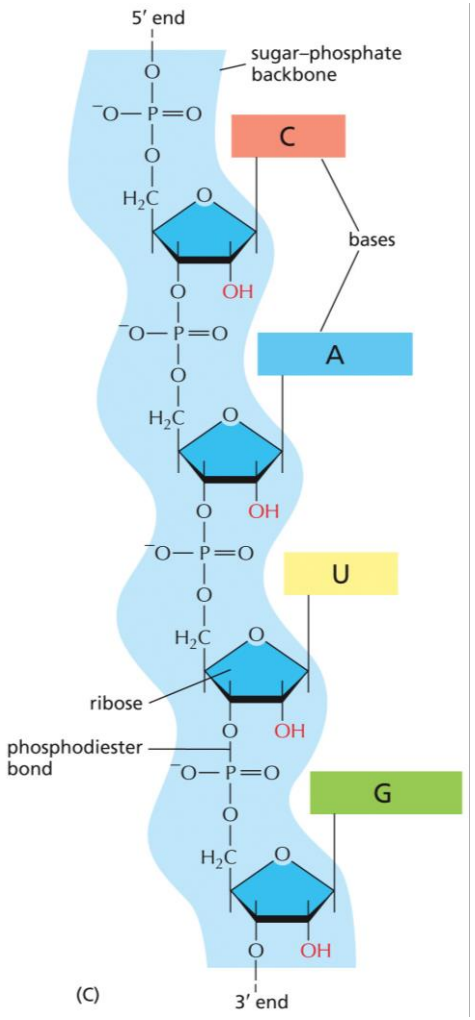
- *Gene Expression* is the process by which the information encoded in a DNA sequence is converted into a product.
- The synthesis of RNA molecules is called *transcription.*
- The synthesis of proteins is called *translation.*



Chemical structure of RNA differs slightly from that of DNA

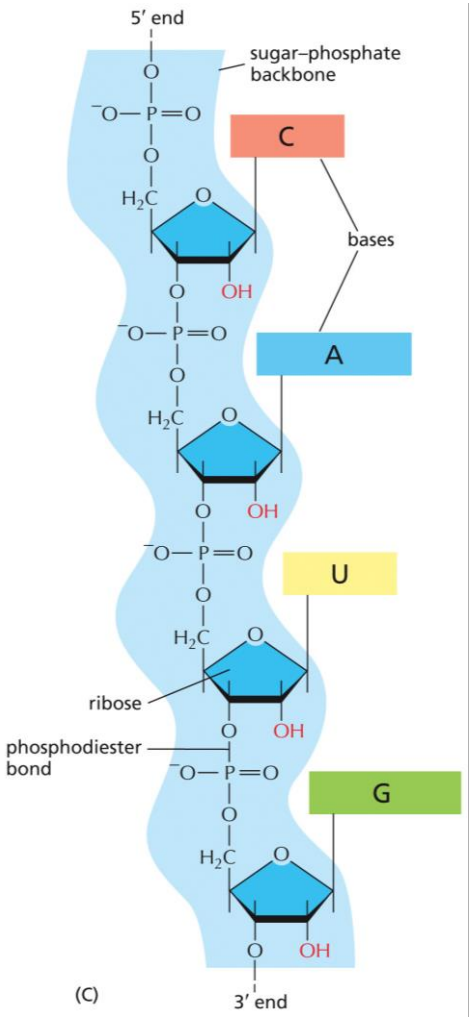
- Ribose sugar
- Uracil instead of thymine (adenine pairs with uracil)

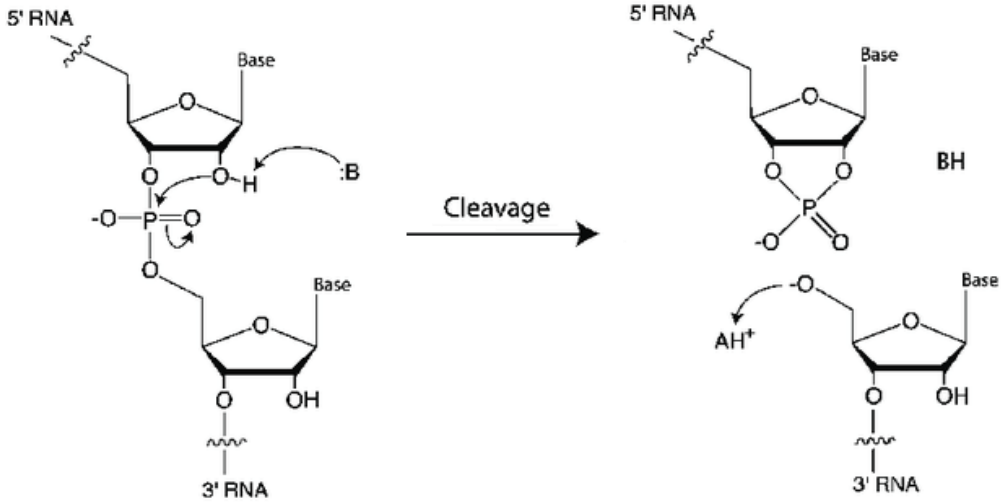




This additional –OH in RNA makes it a lot more volatile

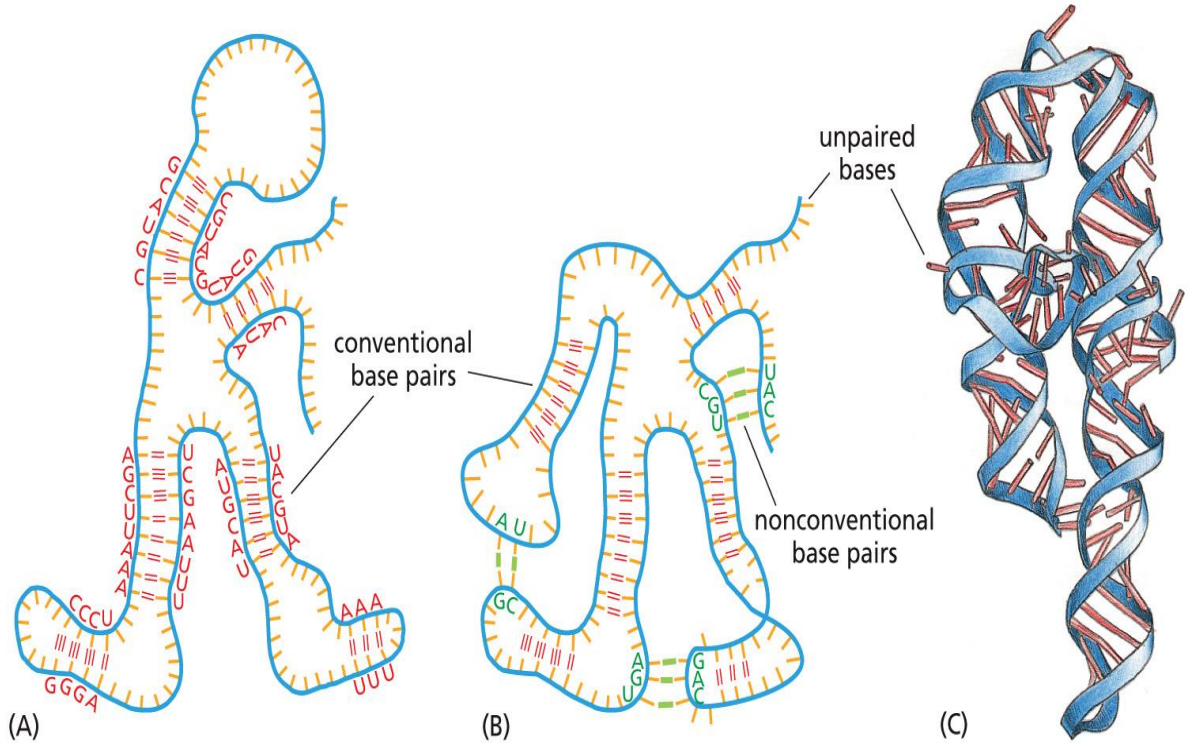
- In DNA the oxygen in the 3’ position “attacks” an incoming nucleotide triphosphate to elongate the polymer
- In RNA this additional 2’ oxygen creates at additional site of attack which could self-terminate the chain
  - Therefore, RNA is more reactive and less stable than DNA, allowing multi-functionality to RNAs





RNA molecules can fold into complex structures

- RNA is single-stranded and so can fold upon itself by base pairing with other regions of the molecule
- Additional hydrogen bonds afforded by the 2’ -OH stabilize these structures
- Nonconventional base pairing is also common in secondary RNA structures due to different 3D geometry than the DNA double helix favoring different hydrogen bonding energetically



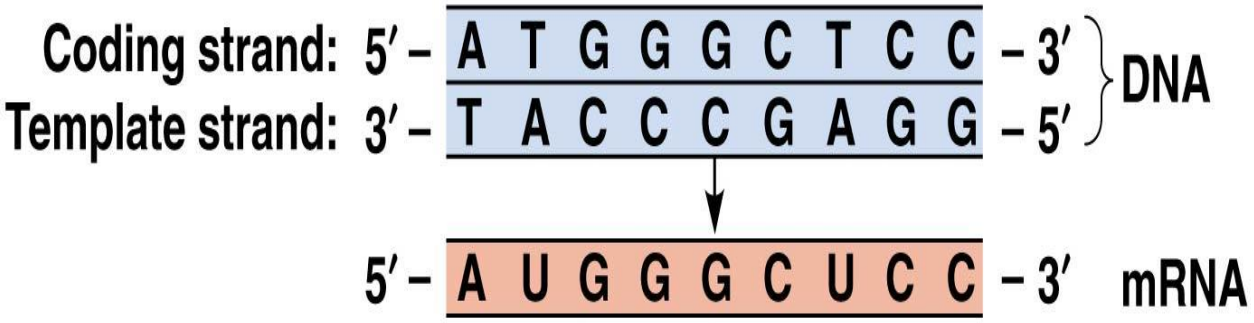
https://www.researchgate.net/figure/Mechanism-of-RNA-self-scission-and-ligation-by-general-acid-base-catalysis_fig1_352972739

Cells produce various types of RNA with a multitude of functions

TABLE 7–1 TYPES OF RNA PRODUCED IN CELLS	
Type of RNA	**Function**
messenger RNAs (mRNAs)	code for proteins
ribosomal RNAs (rRNAs)	form the core of the ribosome's structure and catalyze protein synthesis
microRNAs (miRNAs)	regulate gene expression
transfer RNAs (tRNAs)	serve as adaptors between mRNA and amino acids during protein synthesis
Other noncoding RNAs	used in RNA splicing, gene regulation, telomere maintenance, and many other processes

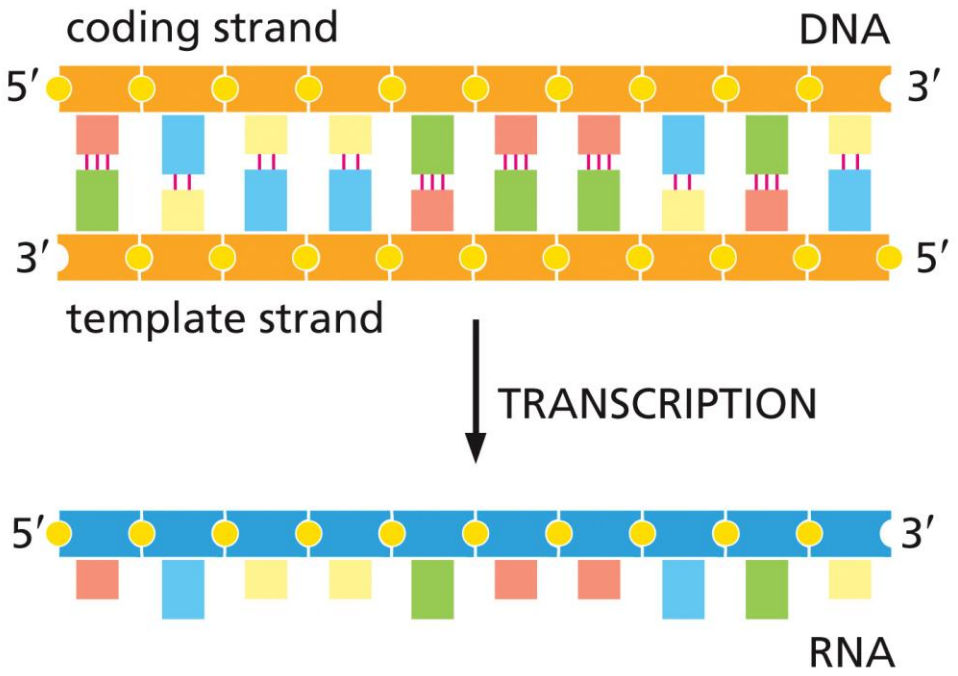
Transcription produces RNA that is complementary to one strand of DNA

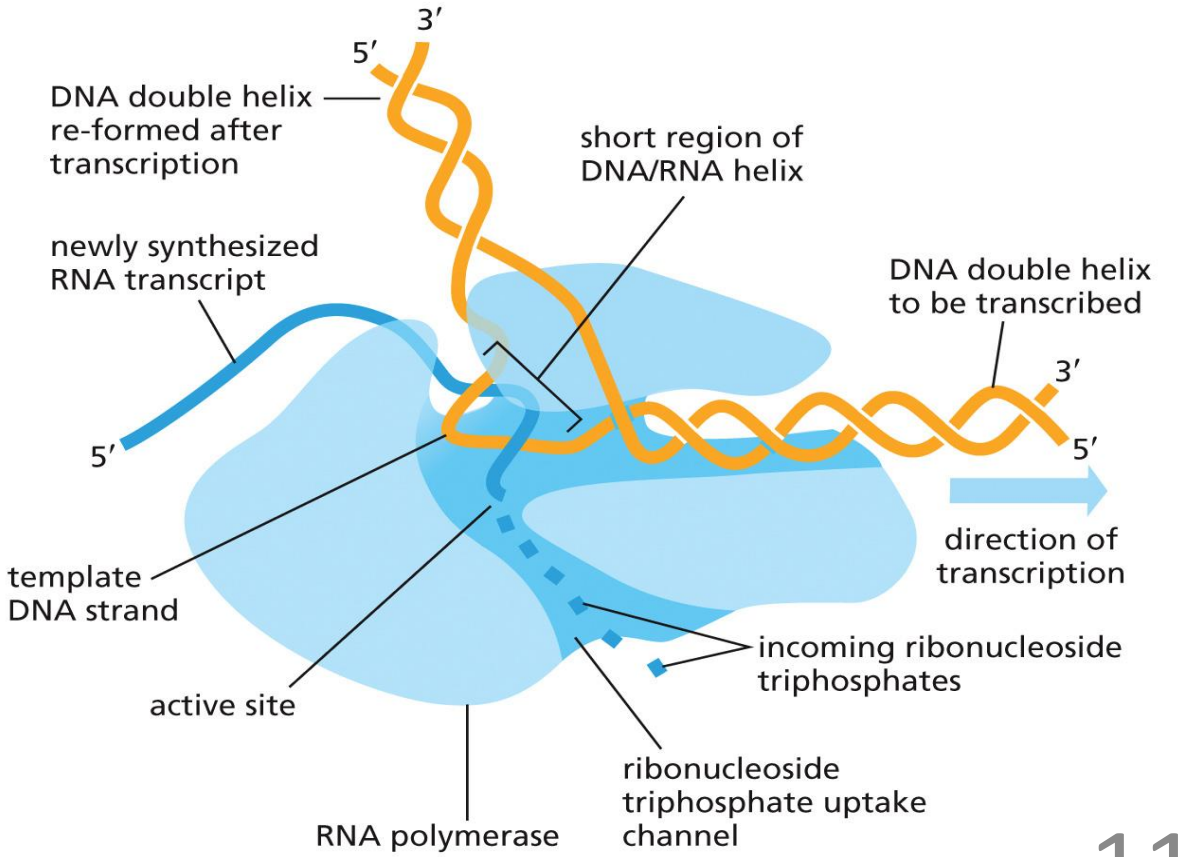
- Only one DNA strand is used for synthesizing RNA: **template strand (antisense)**
- Other strand is called the **coding strand (sense)** because its sequence is equivalent to the RNA product (aside from the change of T to U).



DNA is transcribed into RNA by the enzyme RNA polymerase

- **RNA polymerase**
  - Unwinds DNA and covalently links incoming ribonucleotide triphosphates to growing chain
  - Like DNA polymerase: synthesizes in the 5’ to 3’ direction
  - Unlike DNA polymerase: *minor* proofreading capability and does *not* require a primer





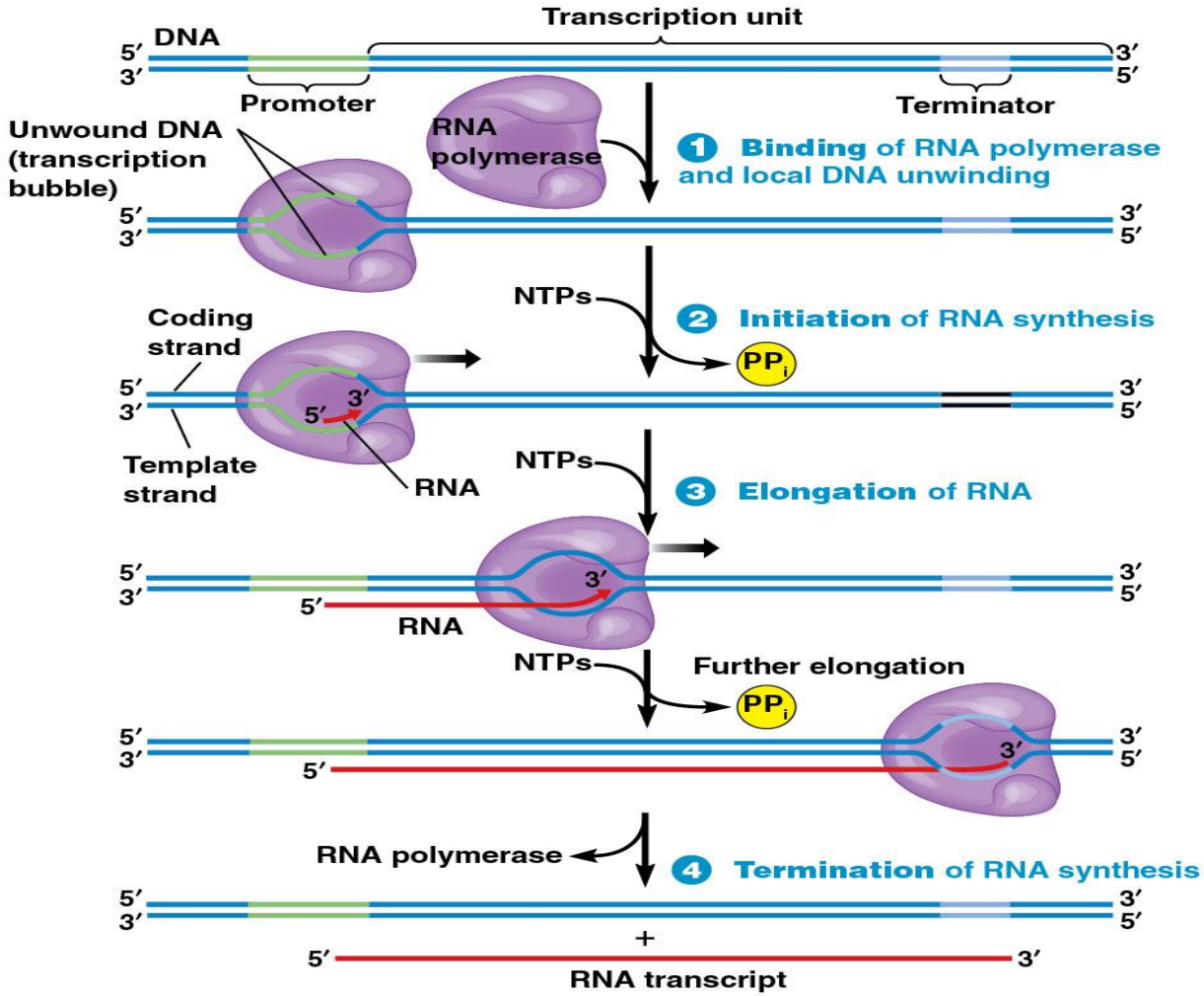
Transcription Involves Four Stages:

1) **Binding:** RNA polymerase binds to *promoter* and separates DNA strands

2) **Initiation:** RNA polymerase begins adding rNTPs

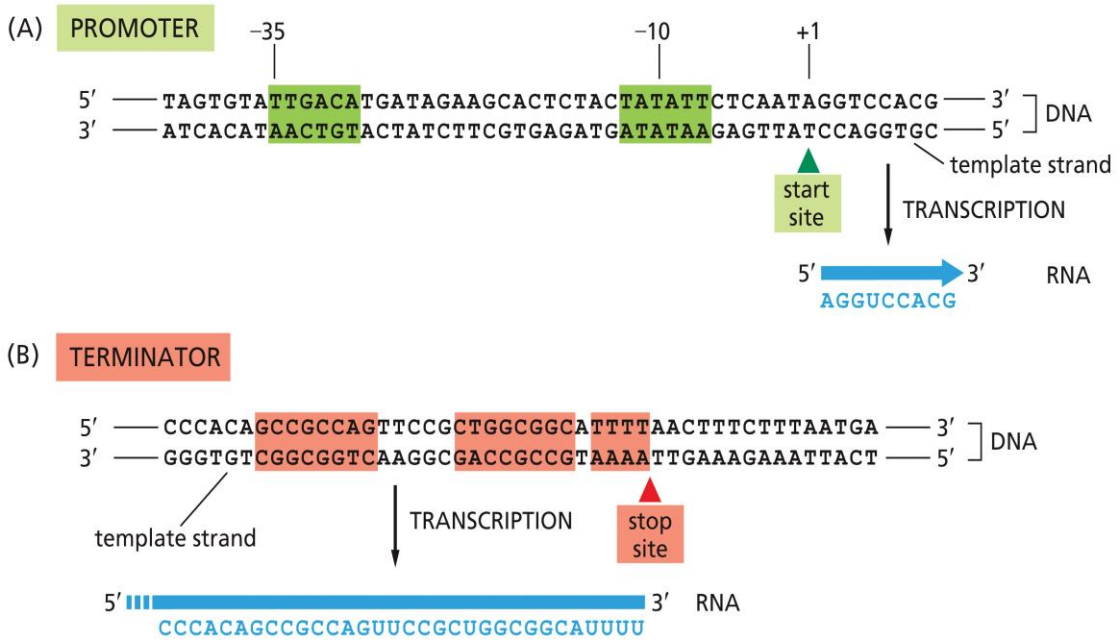
3) **Elongation:** Polymerase builds an RNA molecule that grows 5’ to 3’

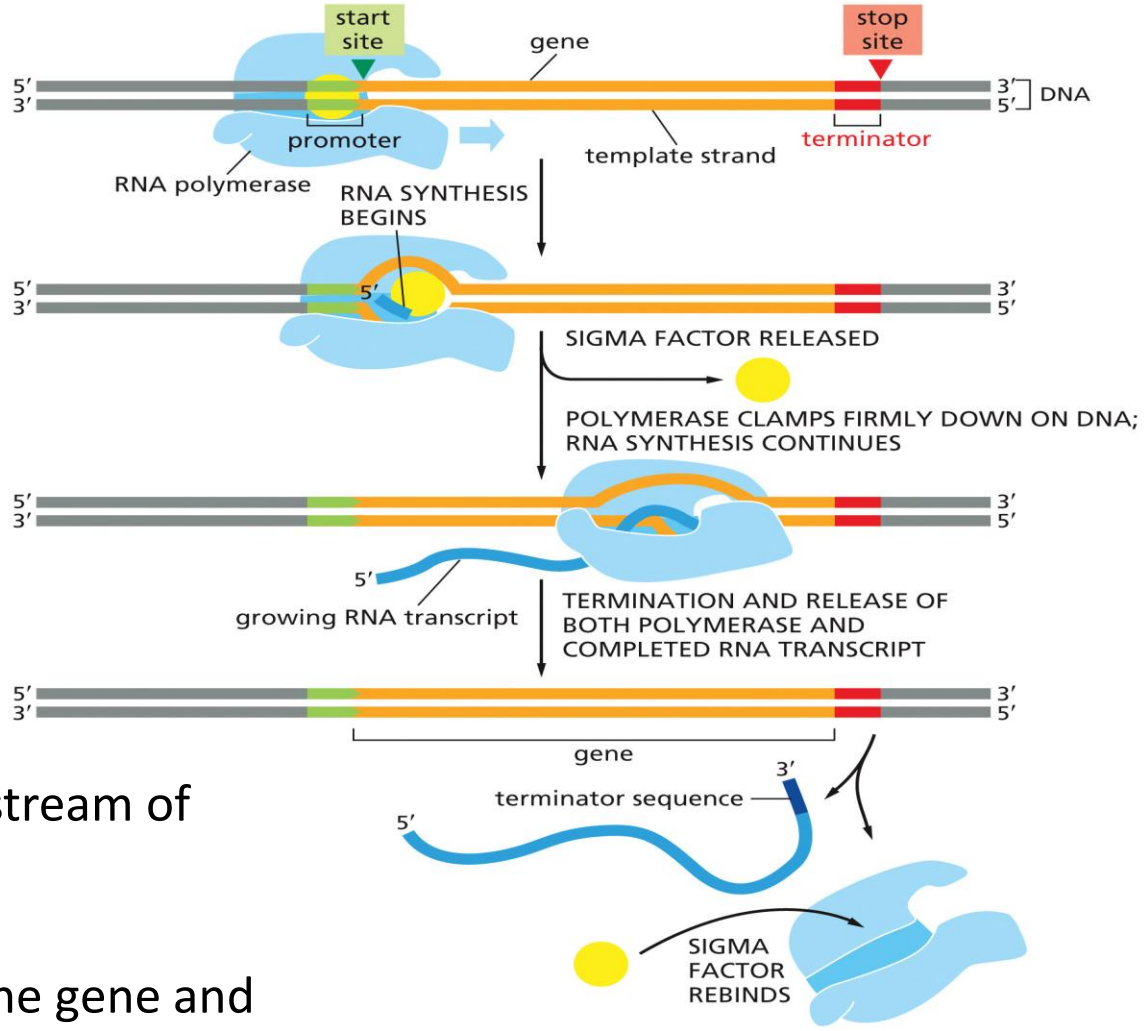
4) **Termination:** *Terminator* sequences transcribed causing RNA molecule to be released from polymerase



*NTPs are ribonucleoside triphosphates (rNTPs)

Signals in the DNA sequence tell the RNA Polymerase where to start and stop transcription





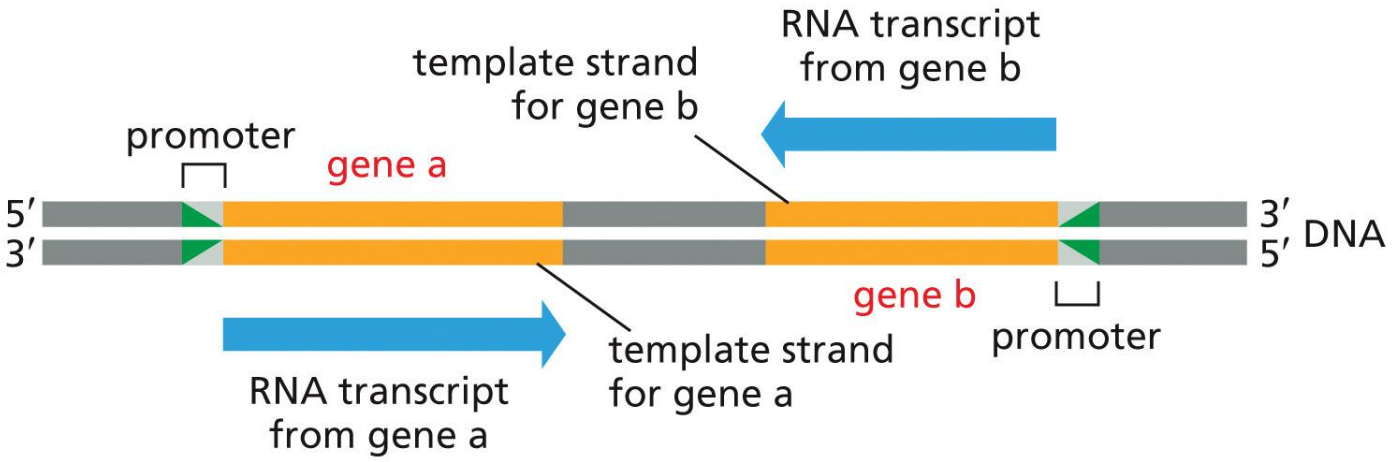
Promoters: specific DNA sequences that lie immediately upstream of genes and recognized by RNA polymerase

Terminators: specific DNA sequences that lie at the end of the gene and signal the polymerase to disengage from DNA strand

Orientation of promoter determines direction of RNA synthesis and thus template strand

- On an individual chromosome, some genes use one strand of DNA as a template, while other genes use the other strand

- RNA polymerase always moves 3’-to-5’ so whichever strand has promoter going from 3’-to-5’ will be recognized by polymerase as the template strand, thereby growing the RNA chain 5’-to-3’



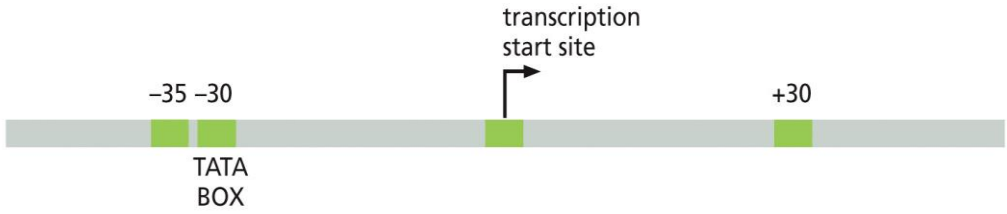
Transcription in eukaryotic cells has additional complexity compared to prokaryotes

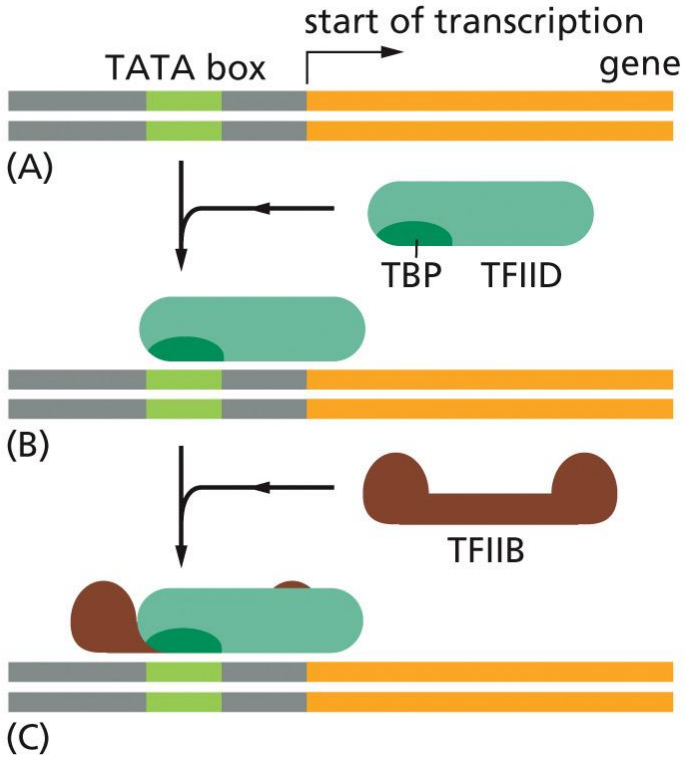
- Key differences:
  - Three different RNA polymerases transcribe one or more different classes of RNA (vs one in prokaryotes)
  - Eukaryotic promoters are more varied than bacterial ones with several different sequence motifs for *more highly regulated* transcriptional activity
  - RNA Polymerases in eukaryotes *require general transcription factors* to bind promoter first

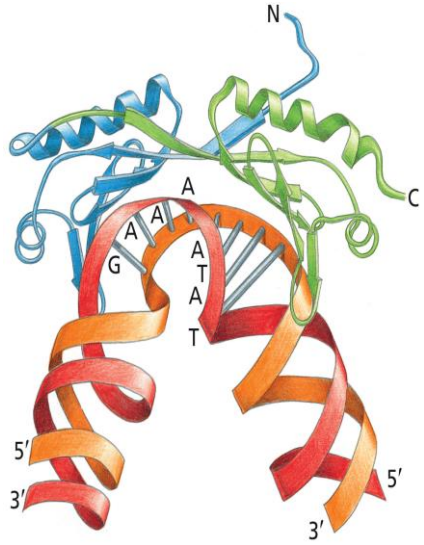
TABLE 7–2 THE THREE RNA POLYMERASES IN EUKARYOTIC CELLS

Type of Polymerase	Genes Transcribed
RNA polymerase I	most rRNA genes
RNA polymerase II	all protein-coding genes, miRNA genes, plus genes for other noncoding RNAs (e.g., those of the spliceosome)
RNA polymerase III	tRNA genes, 5S rRNA gene, genes for many other small RNAs

Eukaryotic promoters contain sequences that promote binding of general transcription factors



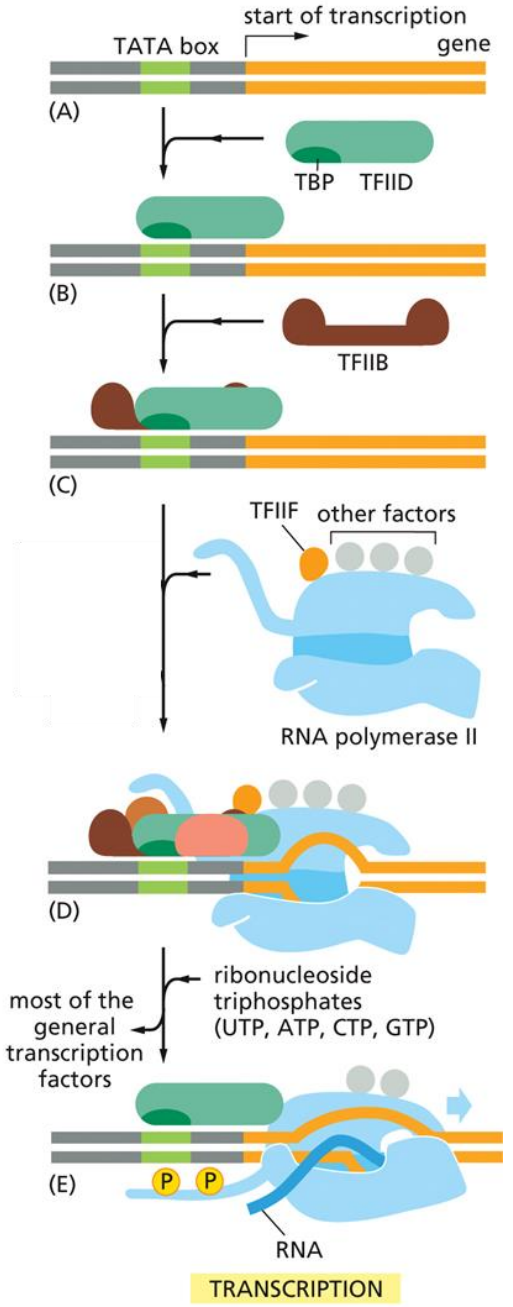




- TBP: TATA-binding protein
  - A subunit of the transcription factor TFIID that recognizes a string of A-T base pairs (TATA box)
  - Binds DNA double helix and distorts shape creating a “landmark” for assembly of other factors

Eukaryotic RNA polymerases require general transcription factors that assemble on a promoter

Fig. 7-12



- TFIID recognizes and binds TATA, inducing binding of TFIIB, recruiting RNA polymerase II to form an initiation complex
- Phosphorylation of the RNA pol II tail liberates enzyme for elongation activities.
- When transcription is done, enzyme dissociates, and tail is dephosphorylated to re-initiate RNA synthesis on a new promoter

Squarecap Q#1-3

Learning Objectives for Chapter 7, Part 1

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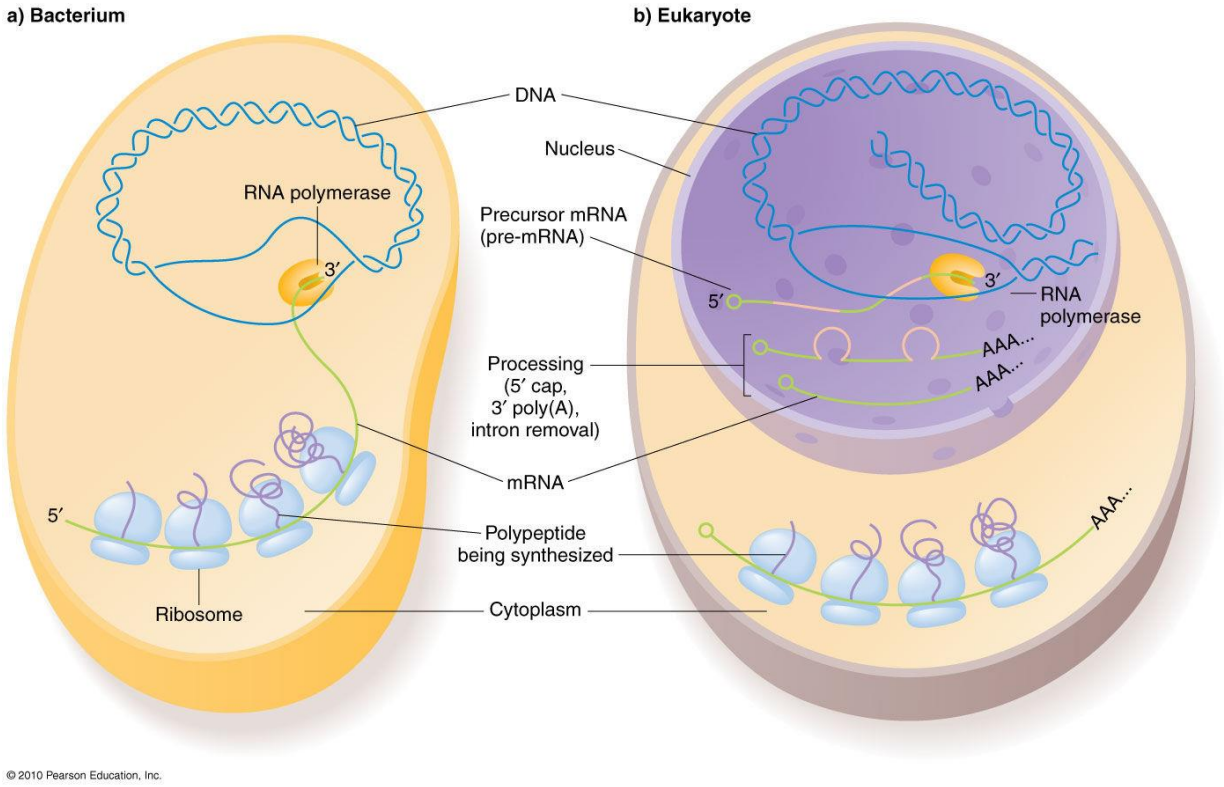
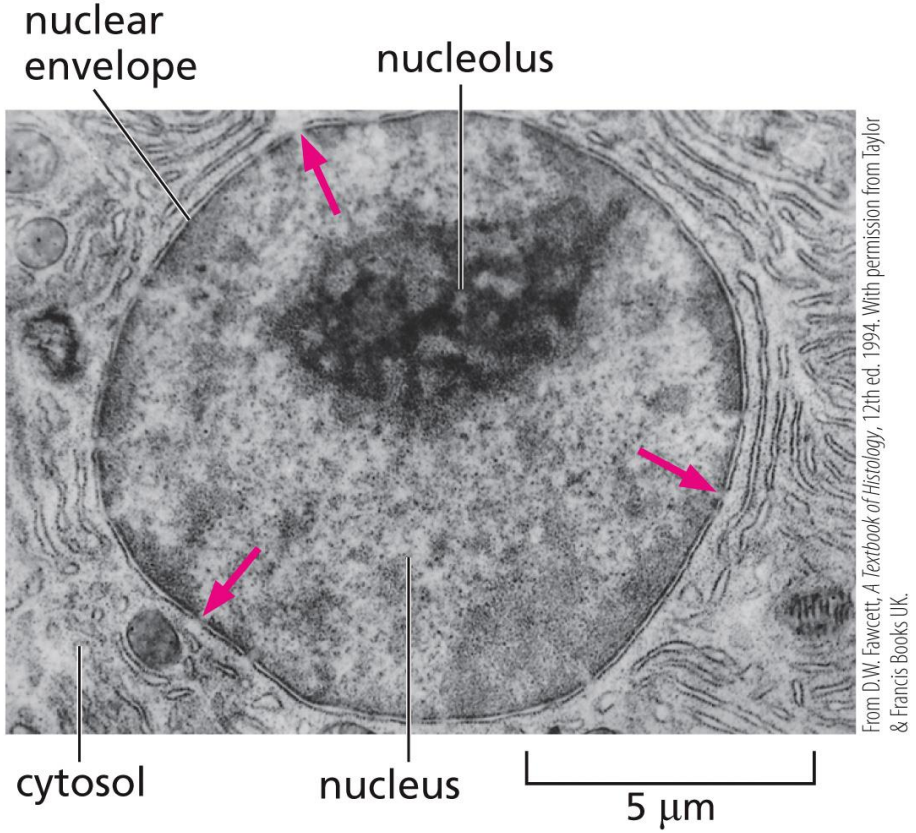
- Detail the components how RNA is processed into mature mRNA

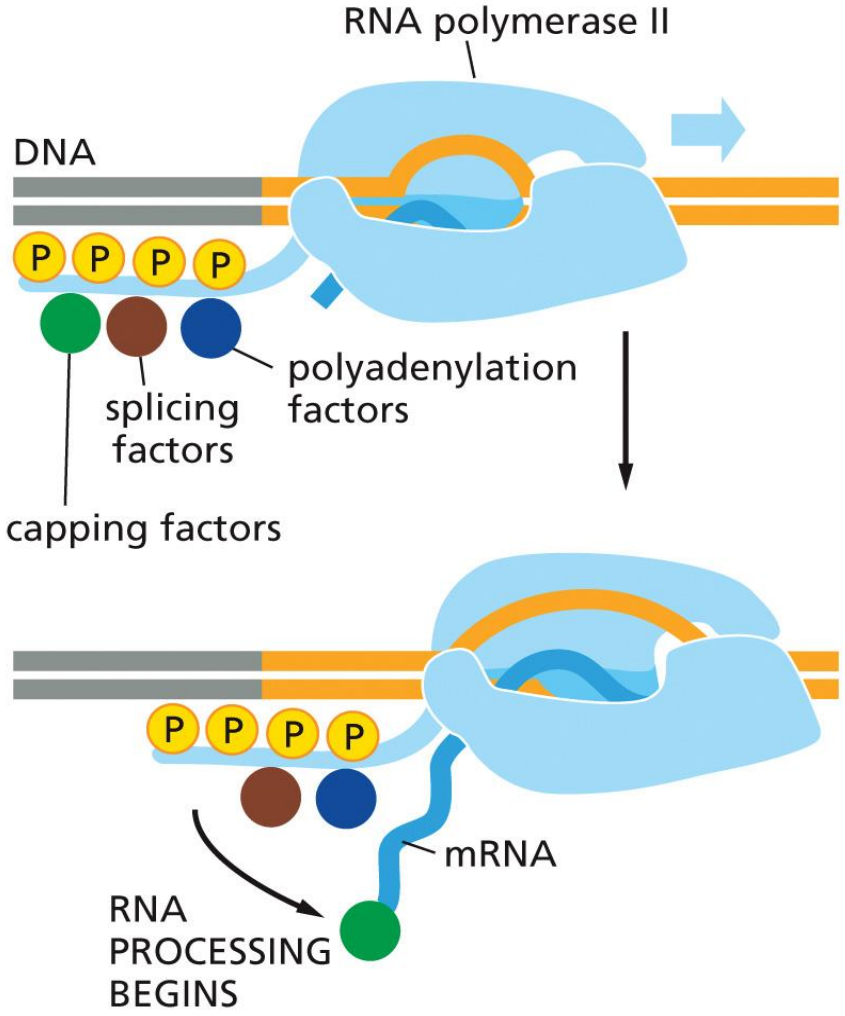
Eukaryotic mRNAs are processed in the nucleus before being exported into the cytosol for translation





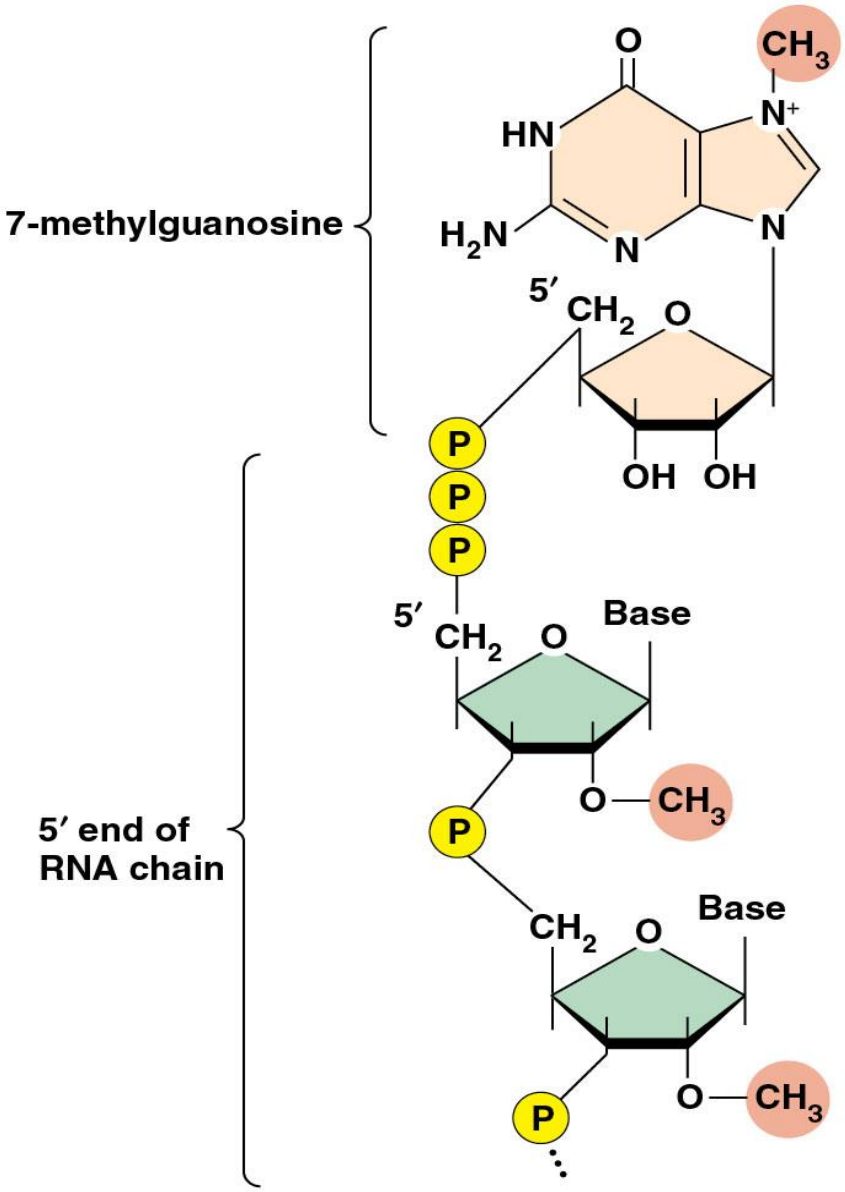
RNA molecules undergo RNA processing via chemical modifications during and after transcription

- Phosphorylation of the RNA polymerase II tail additionally allows RNA-processing proteins to assemble
  - **5’ Capping** (all mRNAs)
  - **Polyadenylation** of 3’ end (all mRNAs)
  - **Splicing** of exons and introns (most mRNAs)
- Processing occurs *as the RNA is being synthesized*.



5’ Caps

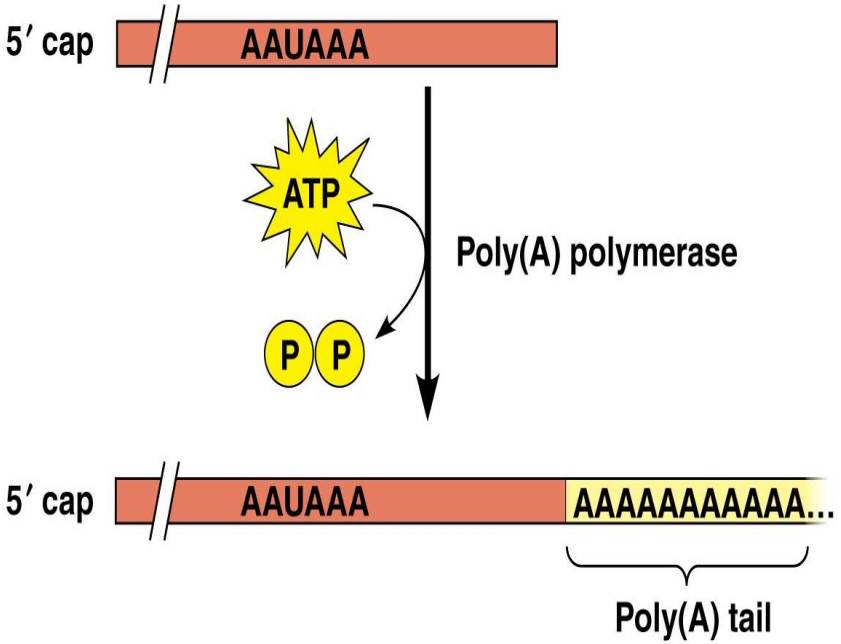
- Eukaryotic mRNAs have a modified nucleotide at their 5’ end.
- The 5’ cap (guanine bearing an extra methyl group) is added soon after transcription is initiated, and connects via a triphosphate linkage
- The cap contributes to mRNA export, stability by protecting the RNA from nucleases, and positioning the RNA on the ribosome for initiation of translation



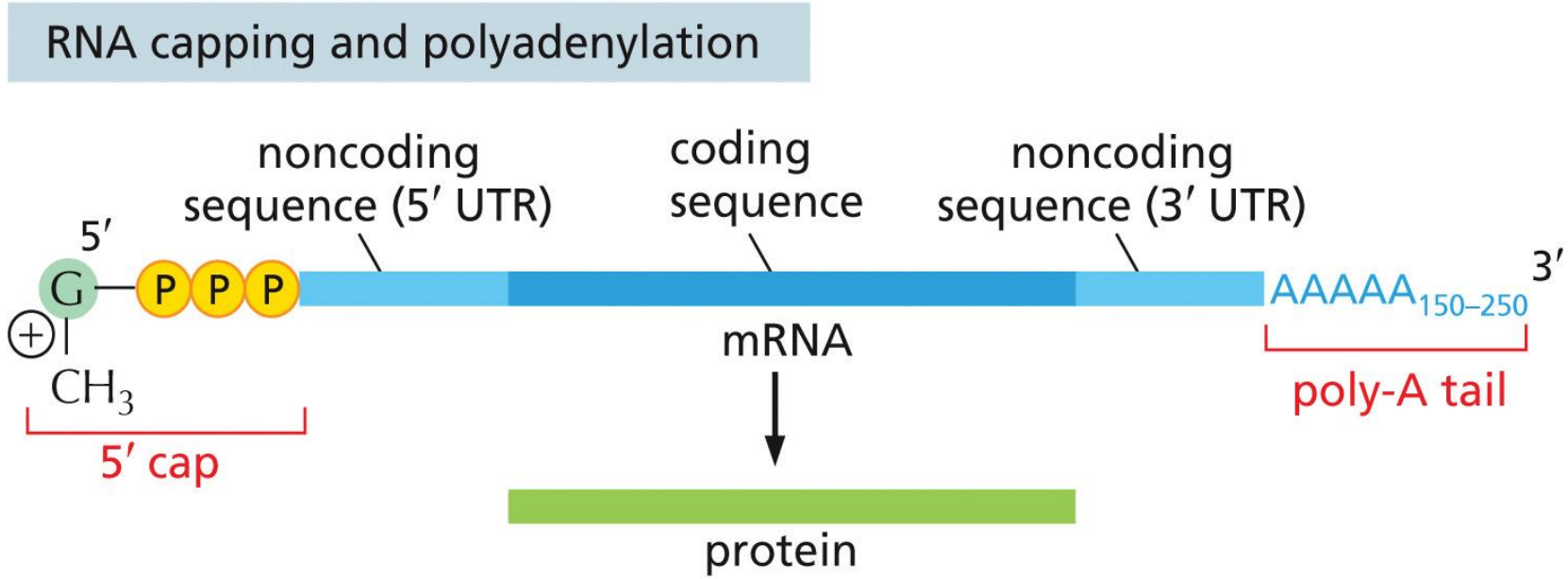
The Poly(A) Tail at 3’ end

- Eukaryotic mRNAs have a long stretch of adenines called the **poly**(**A**) **tail** synthesized at their 3’ ends
  - Poly (A) polymerase adds 50 to 250 adenosines.

- The poly(A) tail:
  - Protects the mRNA from nuclease attack (longer = more stable)
  - Required for export to the cytoplasm
  - Helps ribosomes recognize and bind mRNAs for translation



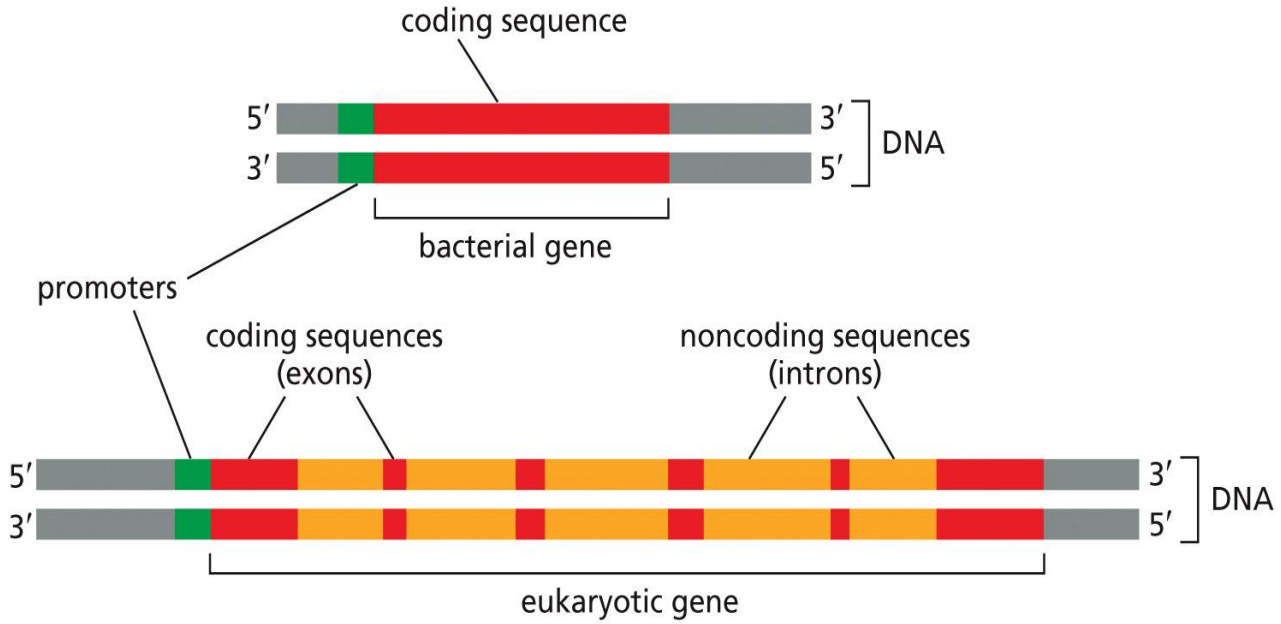
Most mRNAs also have non-coding sequences (untranslated regions; UTRs)



- 5′ UTR helps ribosome recruitment and recognition of start codon to begin translation
- 3′ UTR helps in post-transcriptional regulation of gene expression, including mRNA stability, localization, and translation efficiency via binding sites within its sequence

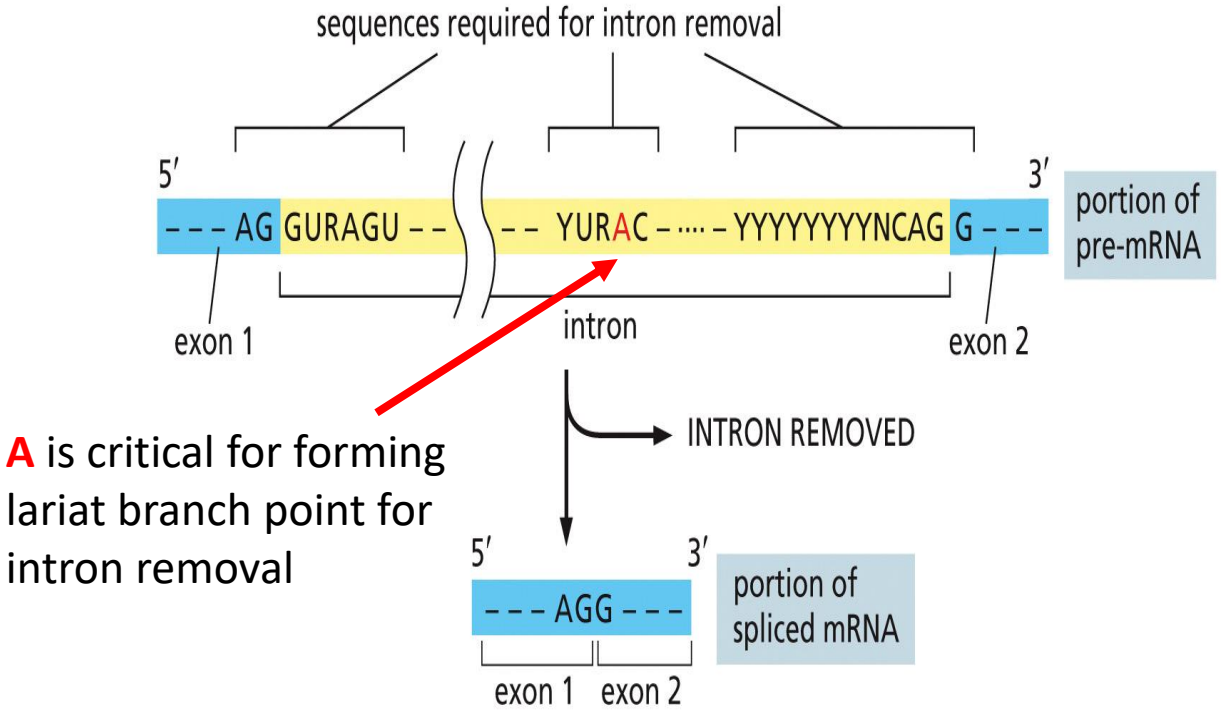
RNA Splicing removes introns from pre-mRNAs

- The precursors for most mRNAs and some rRNAs and tRNAs contain non-coding sequence **introns**, which can make up >90% of the primary transcript
- **Exons** are typically shorter sections of the coding sequence which are joined together through splicing
- About 15% of inherited human diseases involve splicing errors (e.g. muscular dystrophy)



Specific nucleotide sequences determine sites of intron removal

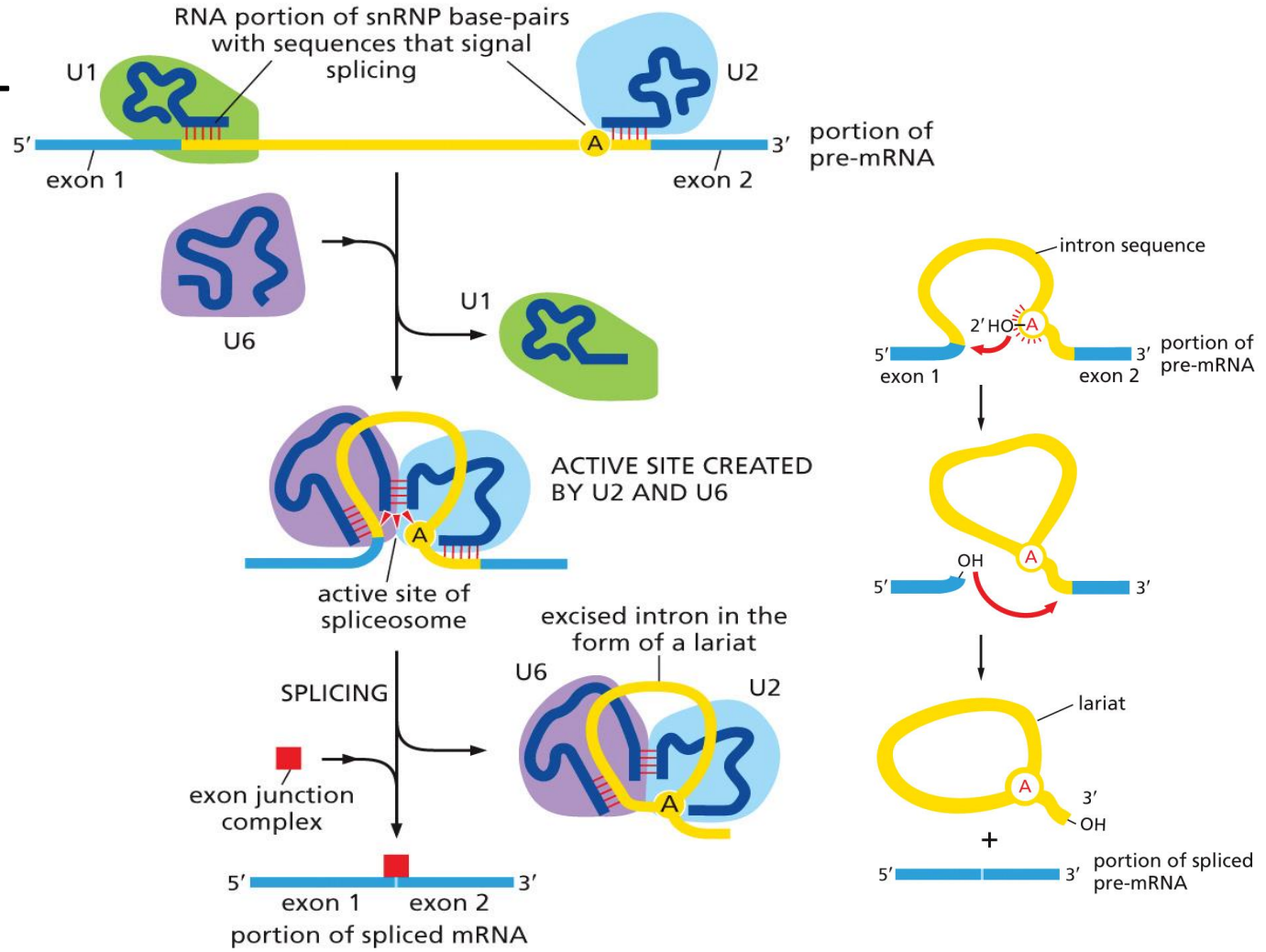
- Sequences commonly found at the intron-exon boundaries determine the *5′ and 3′ splice sites:*
  - the 5′ end of an intron typically starts with **GU** and terminates with **AG** at the 3′ end.
  - One additional sequence near the 3′ end of the intron is called the *branch point*.



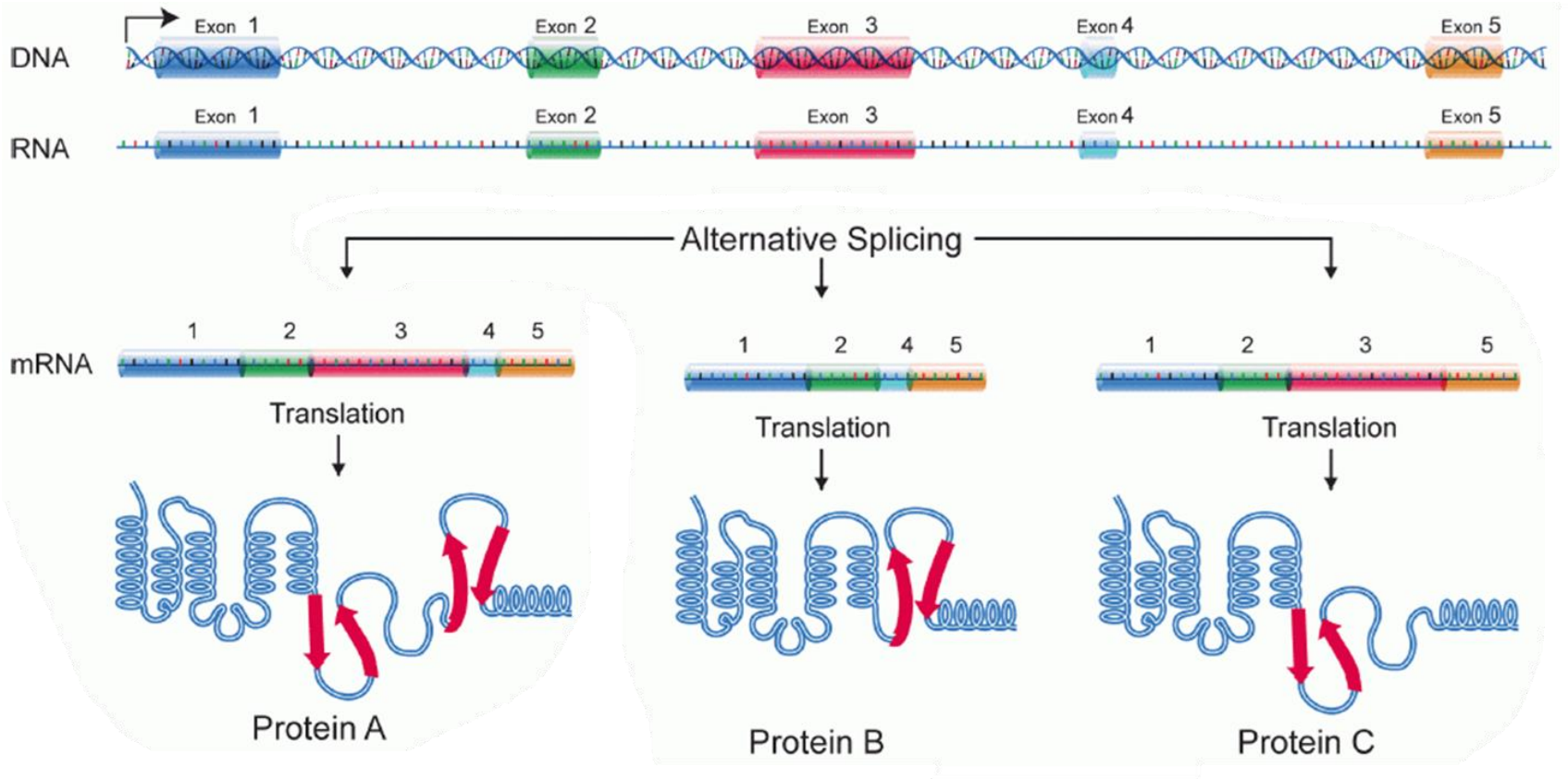
Standard symbols for nucleotides (A, C, G, U)
R stands for purine
Y stands for pyrimidine
N stands for any nucleotide

Introns are removed as “lariat loops” by the spliceosome

- Splicing is carried out by a collection of **RNA–protein complexes** called **snRNPs**
- snRNPs (U1 and U2) recognize splice site through complementary base pairing. U6 displaces U1 to “double check” before splicing
- Conformational changes in U6 and U2 drive formation of *spliceosome active site*
- With assistance of RNA binding proteins (red) the two exons are joined together into a continuous coding sequence.

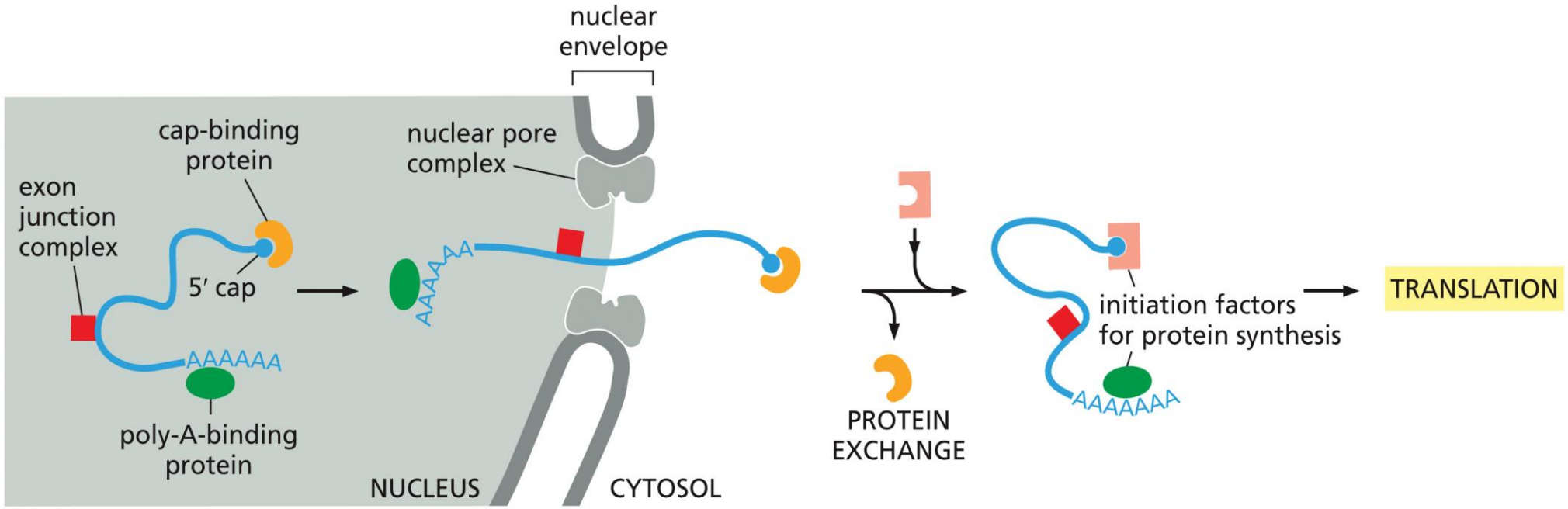


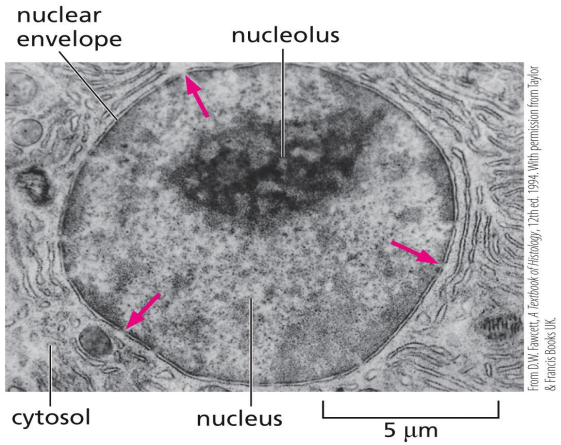
The existence of introns permits *alternative splicing* to produce different mRNAs and proteins from the same gene



https://en.wikipedia.org/wiki/Alternative_splicing

Once fully processed mature mRNA molecules are exported to the cytosol via nuclear pore complexes for translation





Nuclear pore complexes

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Feedback/Reflection

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