

Transcription

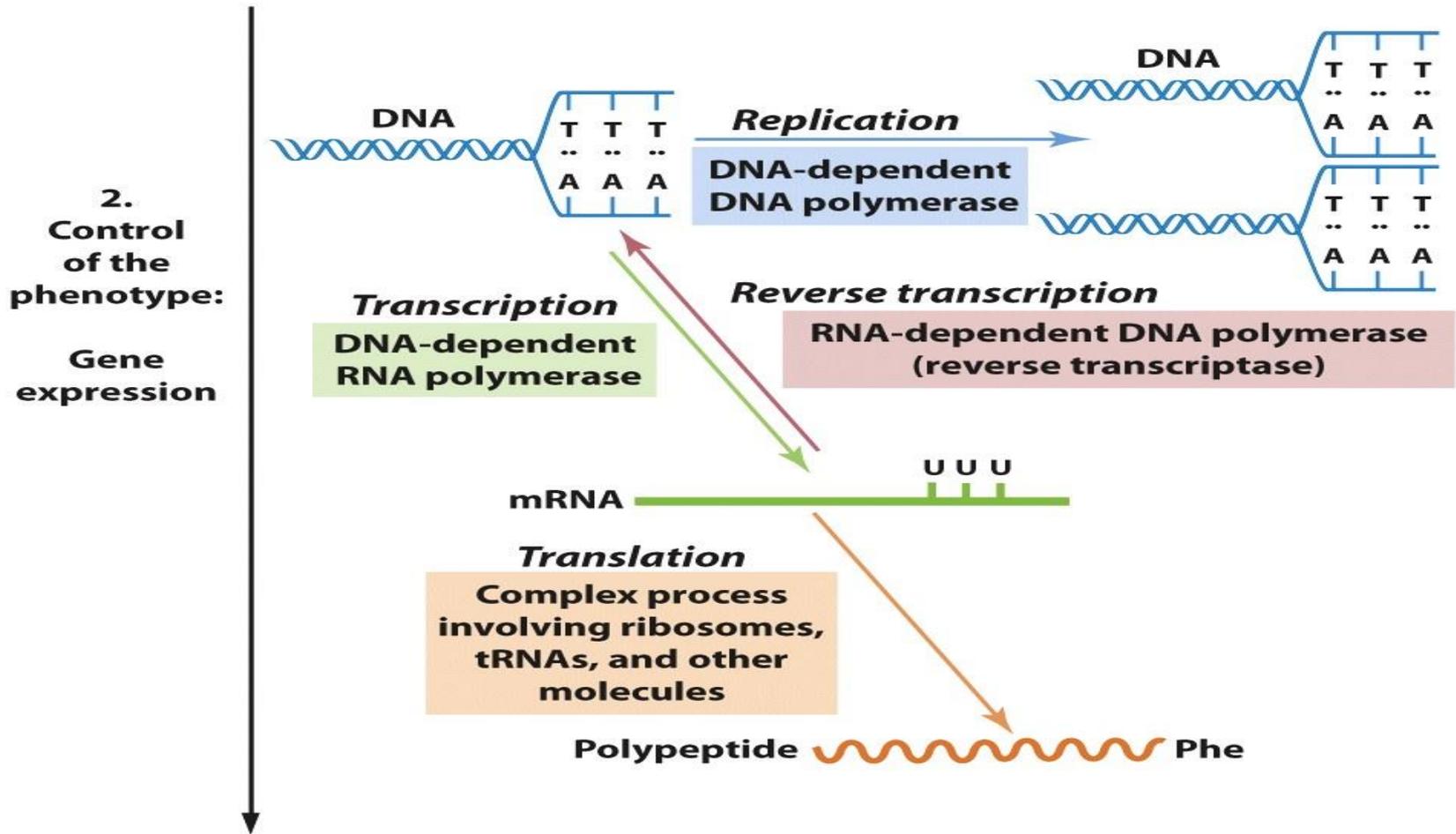
RNA Processing

The Central Dogma of life

The Central Dogma

Flow of genetic information:

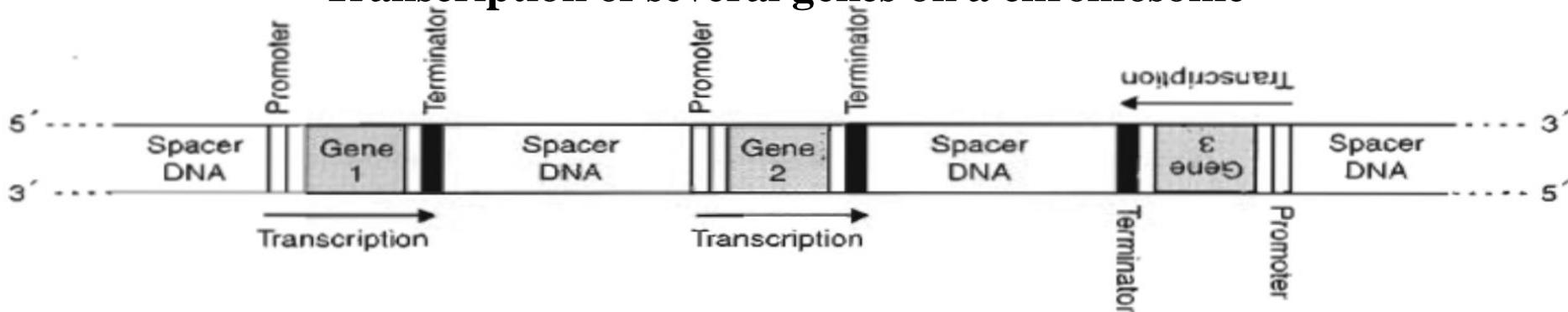
1. Perpetuation of genetic information from generation to generation



Overview of transcription

- The first stage in the expression of genetic information is transcription of the information in the DNA deoxyribonucleotides sequence into RNA ribonucleotides sequence.
- For any gene, only one strand of the DNA molecule, called the **template strand**, is transcribed by **RNA polymerase**.
- Because RNA polymerase moves in the 3' to 5' direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template.
- RNA polymerase recognizes start signals (**promoters**) and stop signals (**terminators**) for each of the thousands of transcription units in the genome of an organism.

Transcription of several genes on a chromosome



Promoter “Strength” (activity)

- Affects amount of RNA made, so, it affects level of expression for that gene.
- Not all promoters have same “strength”
- Promoters differ in DNA sequences and “strength”
- RNA polymerase binds differently to different sequences
“Strong promoters” initiate transcription more often than
“weak promoters”
- rRNA has strong promoter: ~1 initiation per second
- lacZ has a weak promoter: ~1 initiation per minute

Types of RNA

- RNA molecules play a variety of roles in the cell.

The types of RNA are:

Three main types

- 1- Ribosomal RNA (**rRNA**), which is the most abundant type of RNA in the cell.
 - 2- Transfer RNA (**tRNA**), which is the second most abundant type of RNA.
 - 3- Messenger RNA (**mRNA**) the only type of RNA that is translated, which carries the information specifying the amino acid sequence of a protein to the ribosome.
- The mRNA population in a cell is very heterogeneous in size and base sequence, as the cell has essentially a different mRNA molecule for each of the thousands of different proteins made by that cell.

N. B. Heterogeneous nuclear RNA (hnRNA or pre-mRNA) (the immediate product of gene transcription), which is found only in the nucleus of eukaryotic cells and it represents precursors of mRNA, 75% is degraded in the nucleus and 25% only is processed to mature RNA.

Three types for processing the main types of RNA:

4- Small nuclear RNA (**snRNA**) (RNA molecules with enzymatic activity), which is also only found in the nucleus of eukaryotes, small in size and complexed with proteins (forming ribonucleoproteins). One of its major functions is to participate in splicing (removal of introns) mRNA.

5- Small cytoplasmic RNA (scRNA), has catalytic activity in tRNA processing and acts as signal recognition particle.

6- Small nucleolar (snoRNA) acts in rRNA processing/ maturation/methylation.

Two types for regulating mRNA

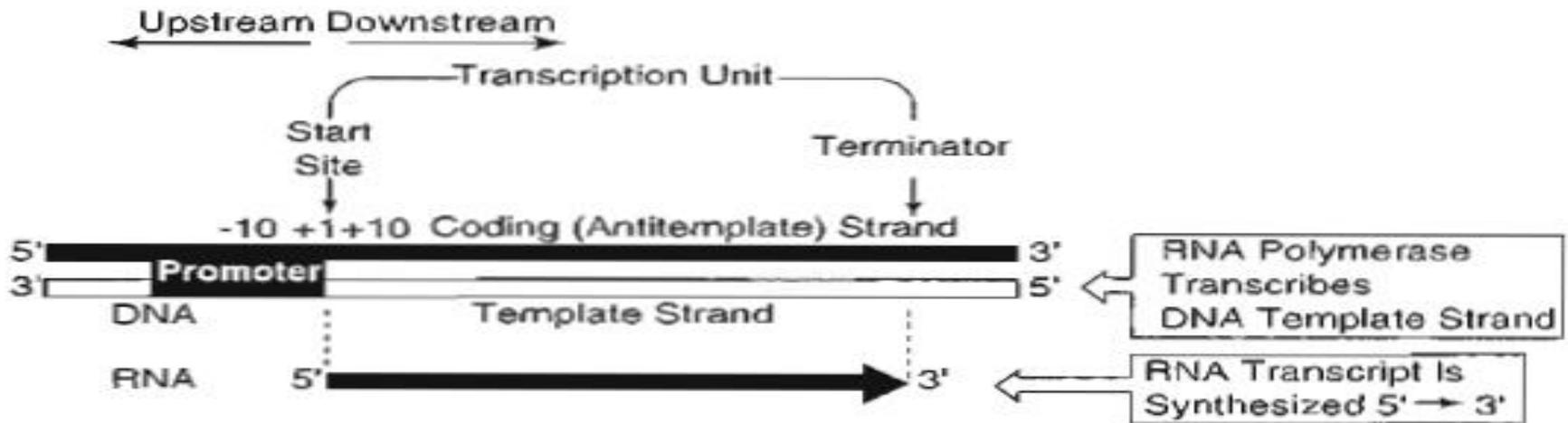
7- **Micro-RNA**, short, non-coding, ~ 22 nucleotide long, generated by nucleolytic processing of the **products of distinct genes** or **transcription unites**, some of which control the expression of other genes during development (mature micro RNA molecules can **hybridize** together to form **imperfect RNA-RNA duplex** within the **3' untranslated regions** of specific target mRNA causing unexplained gene expression regulation in **at least half of the human genes**).

8- **Small interfering RNA (siRNA)** are derived by specific nucleolytic cleavage of larger double stranded RNAs to form small 21-25 long products. They form **perfect RNA-RNA hybrids** with their targets anywhere within the length RNA where the complementary sequence exists resulting in reduction of specific protein production because **siRNA-mRNA complexes** are degraded by nucleolytic machinery (interferes with the expression of specific gene by hybridizing to its corresponding RNA sequence in the target mRNA, then activates degradation of mRNA which can not be translated into proteins)

Transcription: important concepts and terminology

- RNA polymerase locates genes in DNA by searching for promoter regions.
- The promoter is the binding site for transcription factors and RNA polymerase.
- Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds.
- RNA polymerase moves along the template strand in the 3' to 5' direction as it synthesizes the RNA product in the 5' to 3' direction using NTPs (ATP, GTP, CTP, UTP) as substrates.
- RNA polymerase does not proofread its work.
- The RNA product is **complementary** and **antiparallel** to the **template strand**.

- The coding (**non-template**) strand is not used during transcription. It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.
- By convention, the base sequence of a gene is given from the coding strand (5' → 3').
- Transcription ends when RNA polymerase reaches a termination signal.



Transcription of DNA

RNA Polymerases

- There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell.
- The core polymerase has the subunit structure ($\alpha_2\beta\beta'$).
- A protein factor called sigma (σ) is required for the initiation of transcription at the promoter.
- σ factor is released immediately after transcription initiation.

Functions of the subunits:

α : assembly of the tetrameric core

β : ribonucleoside triphosphate binding site (link ribonucleotides together)

β' : DNA template binding region

σ (sigma factor): initiation of transcription

- Termination of transcription sometimes requires a protein called **rho (ρ) factor**.
- This enzyme is inhibited by rifampin and actinomycin D.

Eukaryotic RNA polymerases:

- Three types which can be distinguished by the particular types of RNA they produce:

1- **RNA polymerase I** is located in the nucleolus and synthesizes 28S, 18S, and 5.8S rRNAs.

2- **RNA polymerase II** is located in the nucleoplasm and synthesizes hnRNA/mRNA and some snRNA.

3- **RNA polymerase III** is located in the nucleoplasm and synthesizes tRNA, some snRNA, and 5S rRNA.

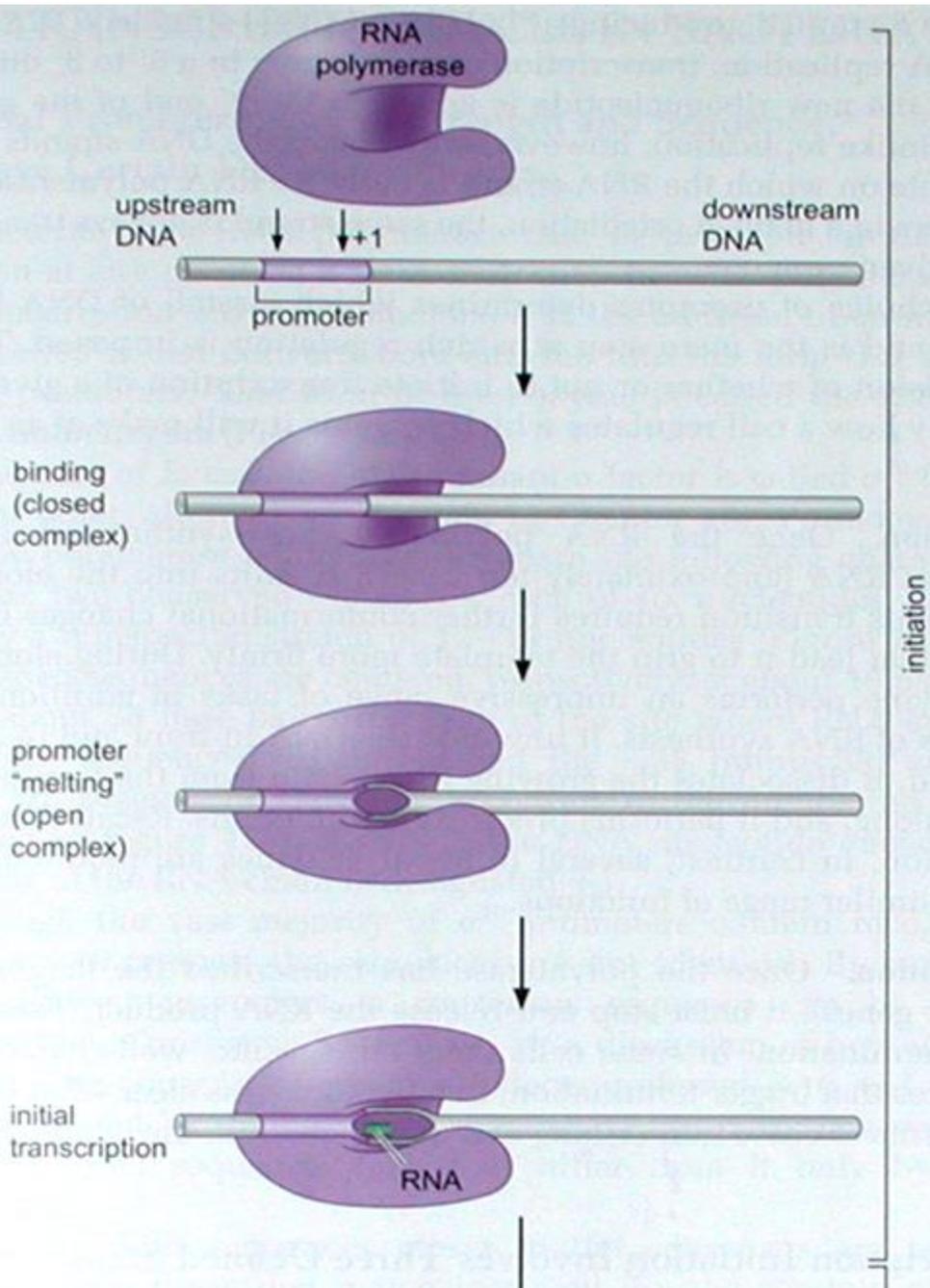
Prokaryotic	Eukaryotic
Single RNA polymerase ($\alpha_2\beta\beta'$)	RNAP 1: rRNA (nucleolus), except 5S rRNA RNAP 2: hnRNA/mRNA and some snRNA RNAP 3: tRNA, 5S rRNA
Requires sigma (σ) to initiate at a promoter	No sigma, but transcription factors (TFIID) bind before RNA polymerase
Sometimes requires rho (ρ) to terminate	No rho required
Inhibited by rifampin \rightarrow Actinomycin D	RNAP 2 inhibited by α -amanitin (mushrooms) Actinomycin D

- N. B. - α amanitin is a toxin from certain mushrooms.**
- It inactivates RNA pol II and can kill a person, while, RNA pol I and III are less affected by toxin**

Comparison of eukaryotic and prokaryotic RNA polymerases

- The following events occur during the expression of a prokaryotic gene:
- With the help of sigma factor, RNA polymerase recognizes and binds to the promoter, region.
- The bacterial promoter contains two "consensus" sequences, called the **Pribnow box** [TATA (**TATTAT**) box] and the **-35 sequence (TGTTGACA)**.
 - 1- The promoter identifies the start site for transcription and orients the enzyme on the template strand.
 - 2- Transcription begins at the + 1 base pair. Sigma factor is released as soon as transcription is initiated.
 - 3- The core polymerase continues moving along the template strand in the 3' to 5' direction, synthesizing the mRNA in the 5' to 3' direction.

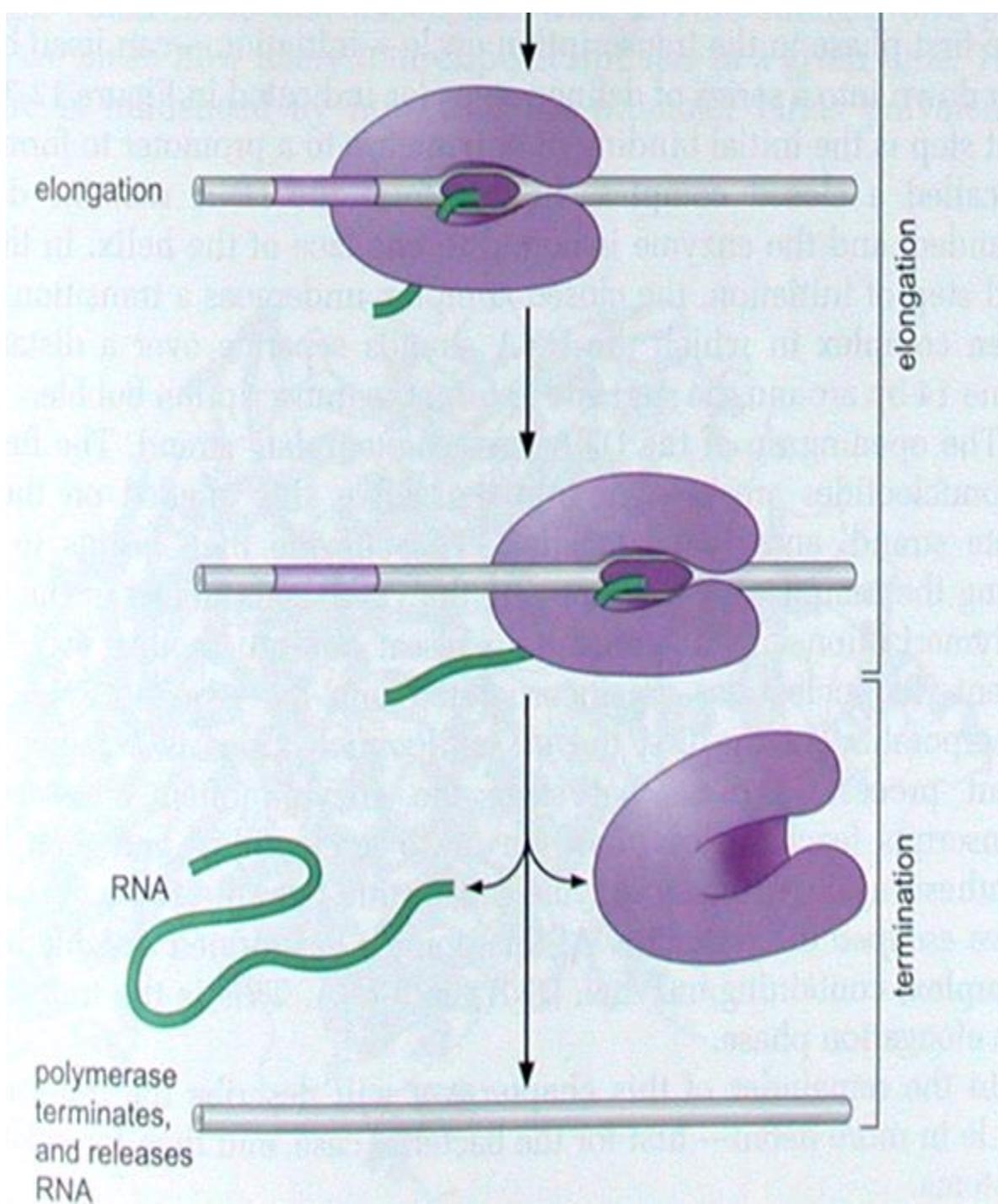
Initiation



A- Binding (closed complex)

B- Promoter "melting"
(open complex)

C- Initial transcription



Elongation

Termination

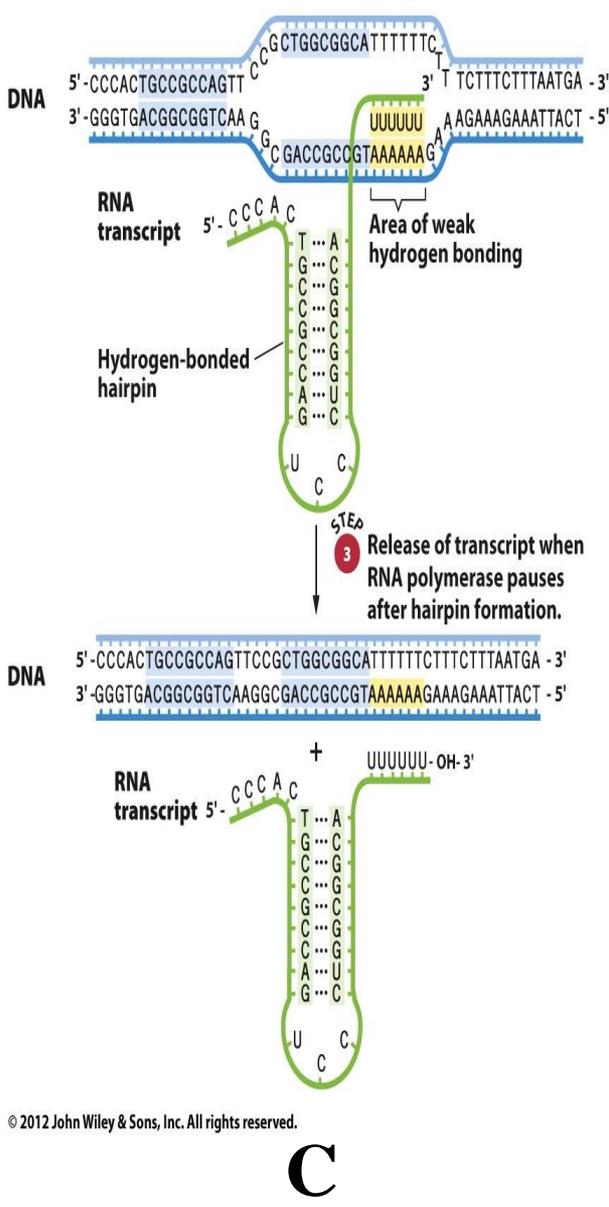
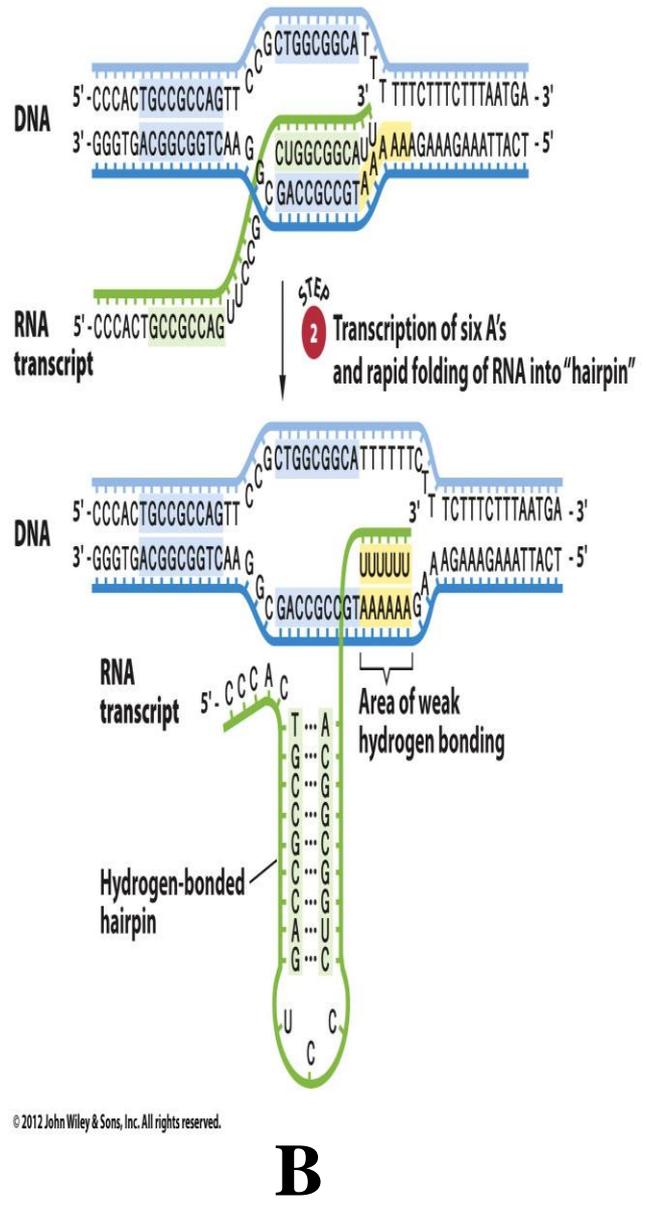
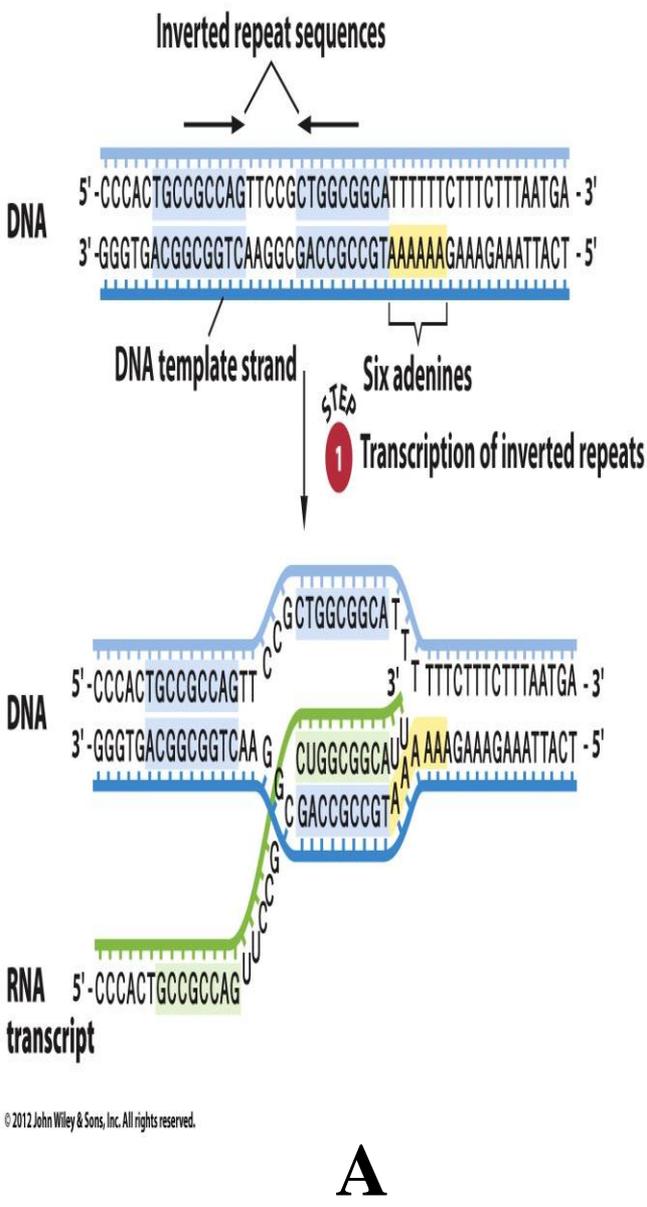
4. RNA polymerase eventually reaches a transcription termination signal, at which point it will stop transcription and release the completed mRNA molecule.

- There are two kinds of transcription terminators commonly found in prokaryotic genes:

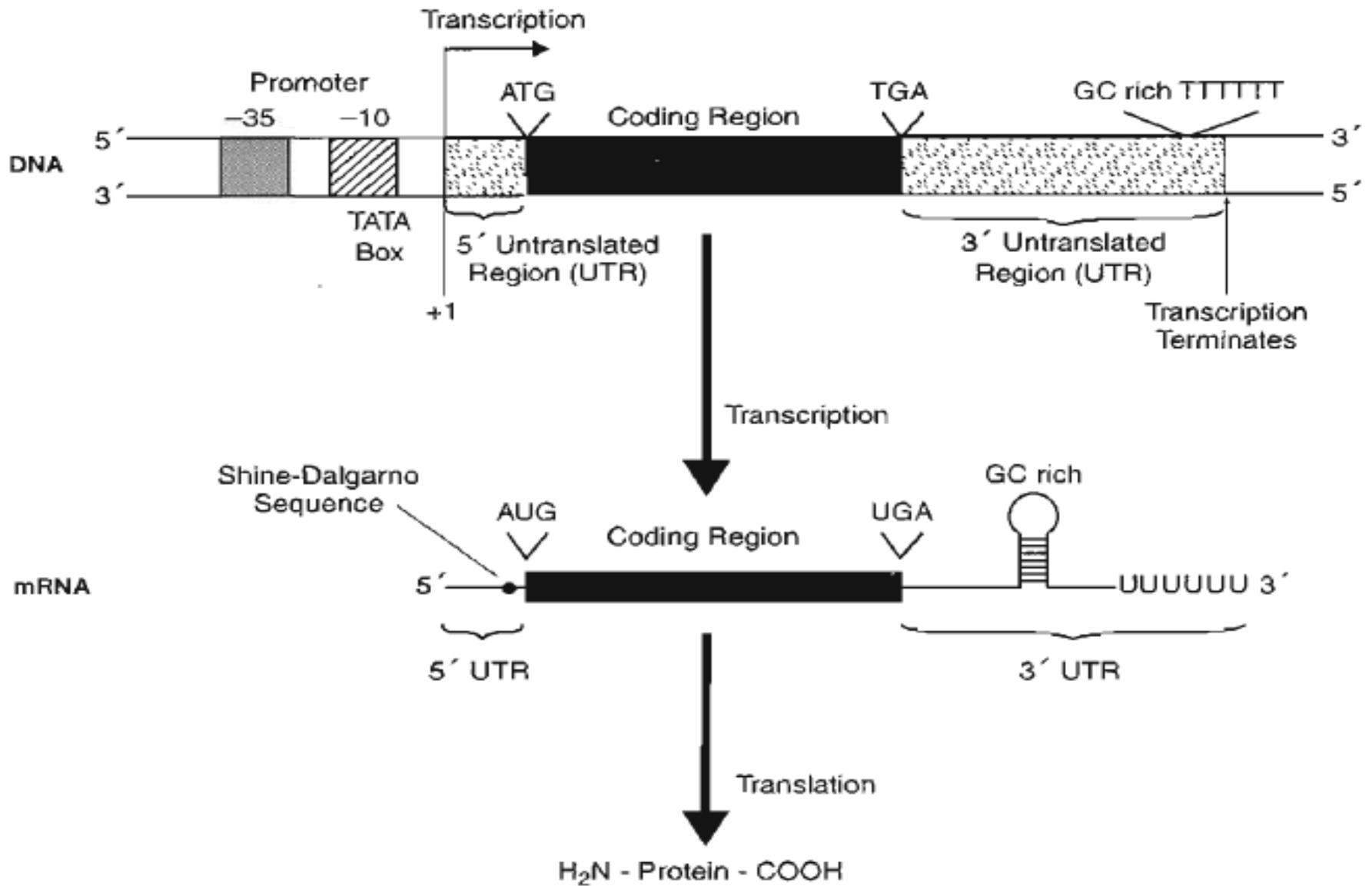
A- **Rho-independent termination** occurs when the newly formed RNA folds back on itself to form a GC-rich hairpin loop closely followed by **6-8 U** residues. These two structural features of the newly synthesized RNA promote dissociation of the RNA from DNA template.

B- **Rho-dependent termination** requires participation of rho factor. This protein binds to the newly formed RNA and moves toward the RNA polymerase that has paused at a termination site. Rho then displaces RNA polymerase from the 3' end of the RNA.

Transcription termination

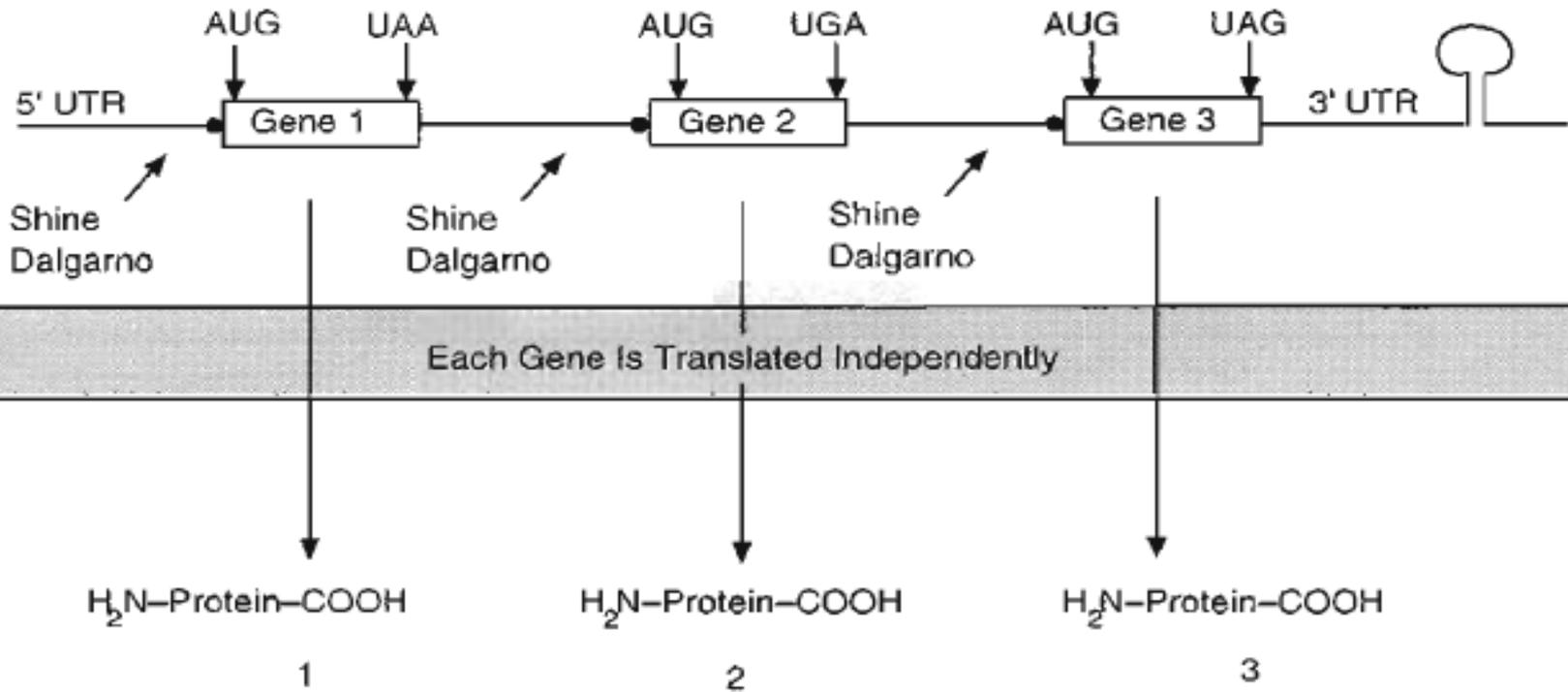


5. Transcription and translation can occur simultaneously in bacteria because there is no processing of prokaryotic mRNA (generally no introns), ribosomes can begin translating the message even before transcription is complete.
 - Ribosomes bind to a sequence called Shine-Dalgarno sequence in the 5' untranslated region (UTR) of the message
 - Protein synthesis begins at an AUG codon at the beginning of the coding region and continues until the ribosome reaches a stop codon at the end of the coding region.
6. The ribosome translates the message in the 5' to 3' direction, synthesizing the protein from amino terminus to carboxyl terminus.



A prokaryotic transcription unit.

- The mRNA produced by the gene shown above is a **monocistronic** message. That is, it is transcribed from a single gene and codes for only a single protein.
- The word **cistron** is another name for a gene. Some bacterial operons produce **polycistronic** messages. In these cases, related genes grouped together in the DNA are transcribed as one unit.
- The mRNA in this case contains information from several genes and codes for several different proteins

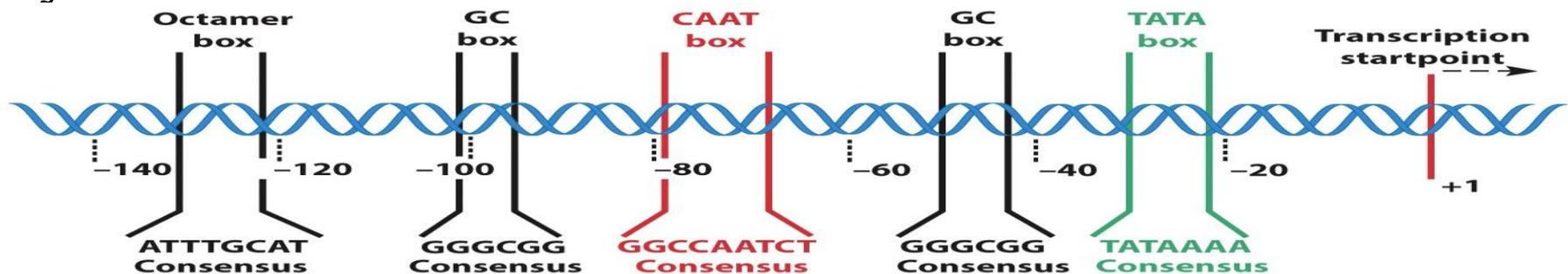


Prokaryotic polycistronic message codes for several different proteins

Production of eukaryotic mRNA

- In eukaryotes, most genes are composed of coding segments (**exons**) interrupted by noncoding segments (**introns**).
- Both exons and introns are transcribed in the nucleus.
- Introns are removed during processing of the RNA molecule in the nucleus.
- In eukaryotes, all mRNA is **monocistronic**.
- The mature mRNA is translated in the cytoplasm.

- Transcription of a typical eukaryotic gene occurs as follows:
1. With the help of proteins called transcription factors, RNA polymerase II recognizes and binds to the promoter region. The basal promoter region of eukaryotic genes usually has **two consensus sequences** called the **TATA box (also called Hogness box) and the CAAT box**.
 2. RNA polymerase II separates the strands of the DNA over a short region to initiate transcription and read the DNA sequence. The template strand is read in the 3' to 5' direction as the RNA product (the **primary transcript**) is synthesized in the 5' to 3' direction.



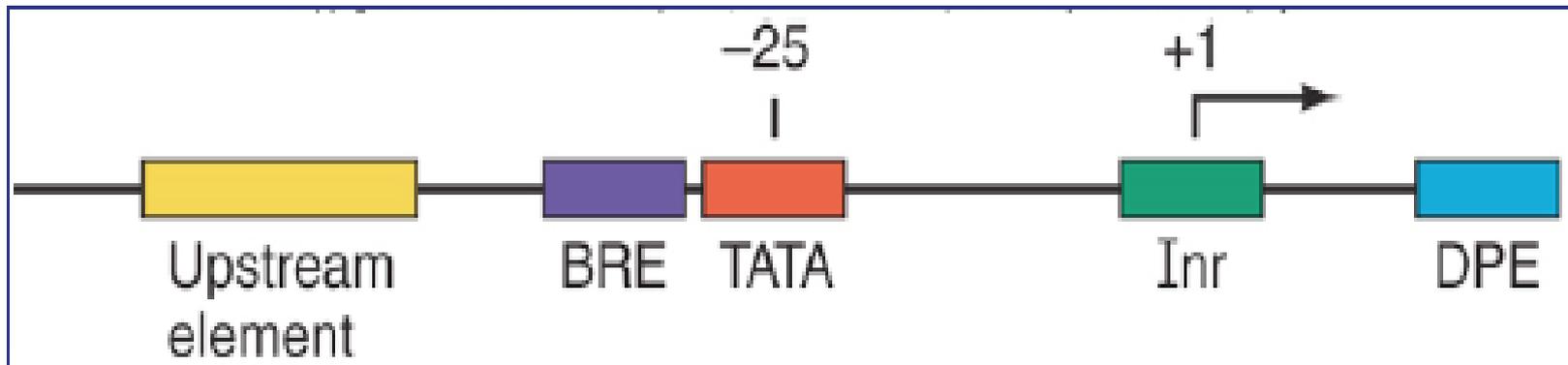
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A Typical RNA Polymerase II Promoter

Class II promoters (most similar to bacterial promoters)

- Common type of promoter (most genes use this)
- Many variations, but “consensus” has a “Core” “Upstream”
3 elements:
 1. “TATA box” (5’-TATA-3’)
 2. TFIIB recognition element (BRE)
 3. Initiator box with an “A” at +1, most common
- Downstream promoter element (DPE, less common)
- Core promoter is recognized by general TFs that associate with RNA pol to form a preinitiation complex at great majority of promoters
- At least one of these elements is missing in most promoters
e.g., highly expressed specialized genes tend to have TATA boxes, but promoters for housekeeping genes tend to lack them

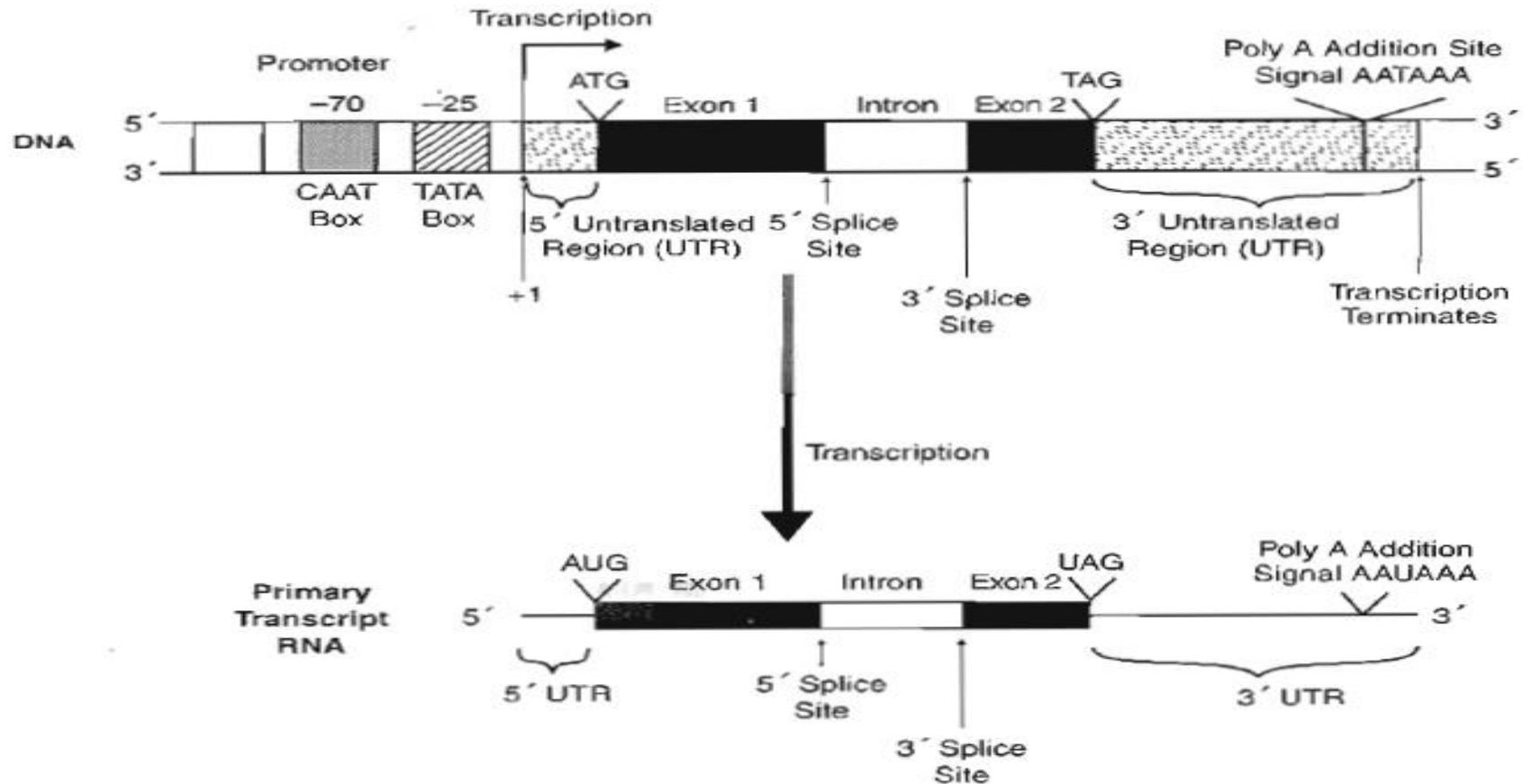
- Upstream elements: quite varied in number and can be orientation-independent (but relatively position-dependent) & recognized by other TFs (relatively gene-specific) that participate in initiation at smaller sub-sets of promoters
 1. GC box (GC rich)
 2. CAAT box (5'-CCAAT-3')



TFIIB recognition element; Inr: initiator box; DPE:
downstream promoter element

3. RNA polymerase II ends transcription when it reaches a termination signal.

- These signals are not well understood in eukaryotes.



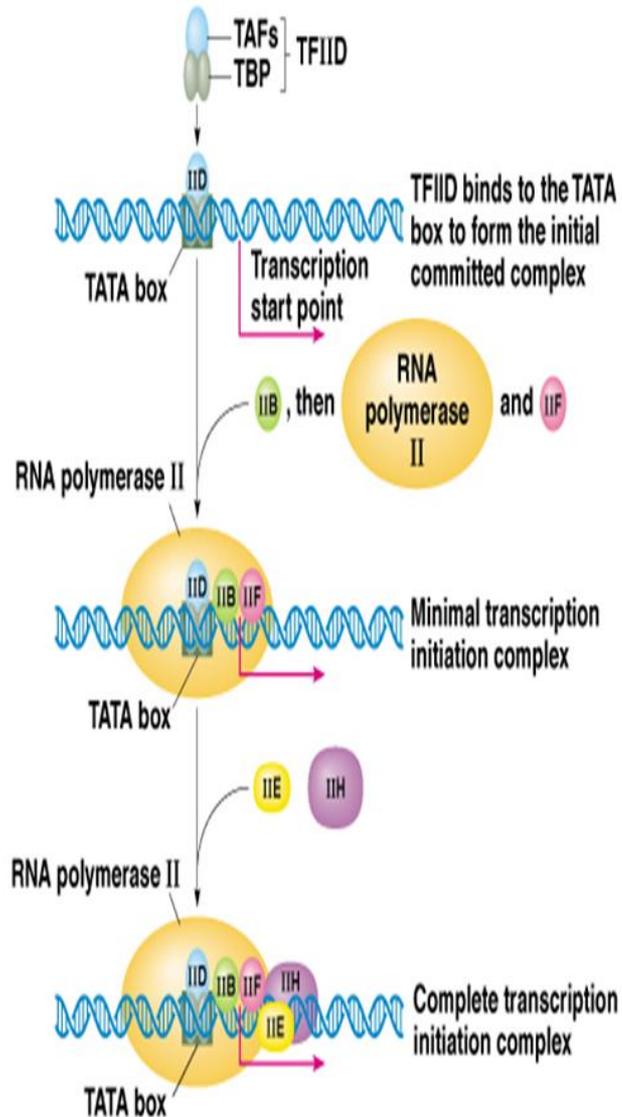
A eukaryotic transcription unit

Table 11.3

General Transcription Initiation Factors

Factor	Subunits	Size (kDa)	Function
TFIID-TBP	1	27	TATA box recognition, positioning of TATA box DNA around TFIIB and Pol II
TFIID-TAF _{II} s	14	15–250	Core promoter recognition (non-TATA elements), positive and negative regulation
TFIIA	3	12, 19, 35	Stabilization of TBP binding; stabilization of TAF–DNA binding
TFIIB	1	38	Recruitment of Pol II and TFIIF; start-site recognition for Pol II
TFIIF	3	156 total	Promoter targeting of Pol II
TFIIE	2	92 total	TFIIH recruitment; modulation of TFIIH helicase ATPase, and kinase activities; promoter melting
TFIIH	9	525 total	Promoter melting; promoter clearance via phosphorylation of CTD

a) Assembly of preinitiation complex



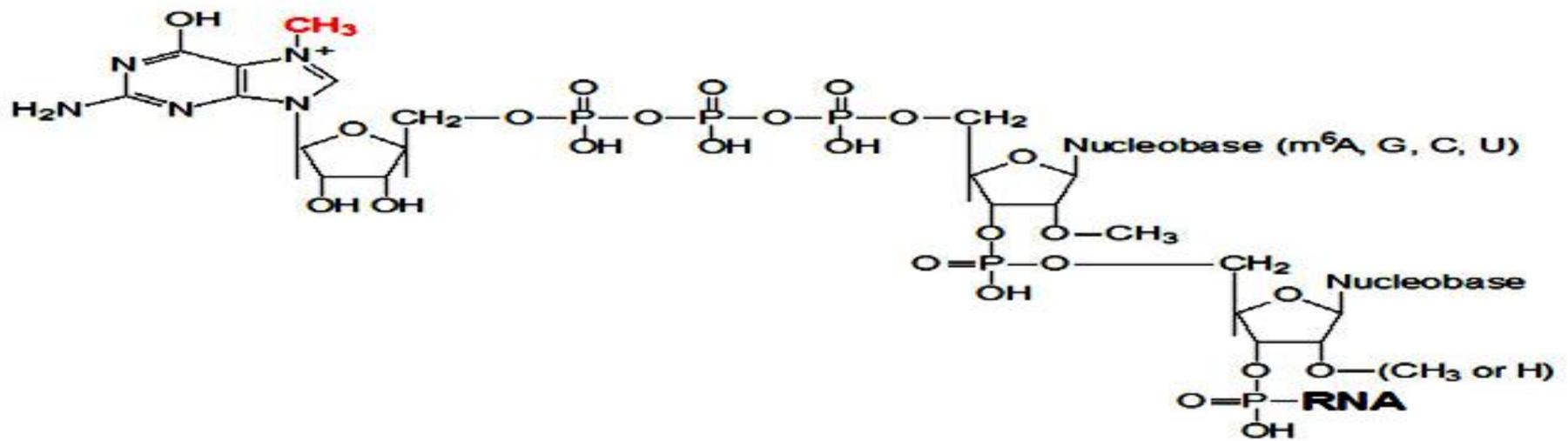
Transcription enhancers and silencers

- Both are binding sites for transcription factors (TF's)
- **Enhancers:** “non-promoter DNA elements that stimulate transcription” They interact with general transcription factors to promote formation of pre-initiation complex to increase the amount of Transcription from a nearby promoter (core + upstream elements)
- **Silencers:** Decrease amount of Transcription from nearby promoters
- Initially Defined as being “Position and orientation independent”
- Found upstream, within, or downstream of genes, they function in either orientation (not always true)
- Sometimes a DNA element can act as an enhancer or a silencer depending on what is bound to it.

Posttranscriptional processing of RNAs:

- Bacterial rRNAs and tRNAs undergoes no additional processing, after being transcribed they are immediately ready for use in translation.
- Translation of bacterial mRNAs can begin even before transcription is completed due to the lack of the nuclear cytoplasmic separation that exists in eukaryotes and to afford a unique opportunity for regulating the transcription of certain genes.
- An additional feature of bacterial mRNAs is that most are **polycistronic** which means that multiple polypeptides can be synthesized from a single primary transcript.
- Polycistronic mRNAs are very rare in eukaryotic cells but have been identified.
- In addition, several viruses encode **polycistronic** RNAs.

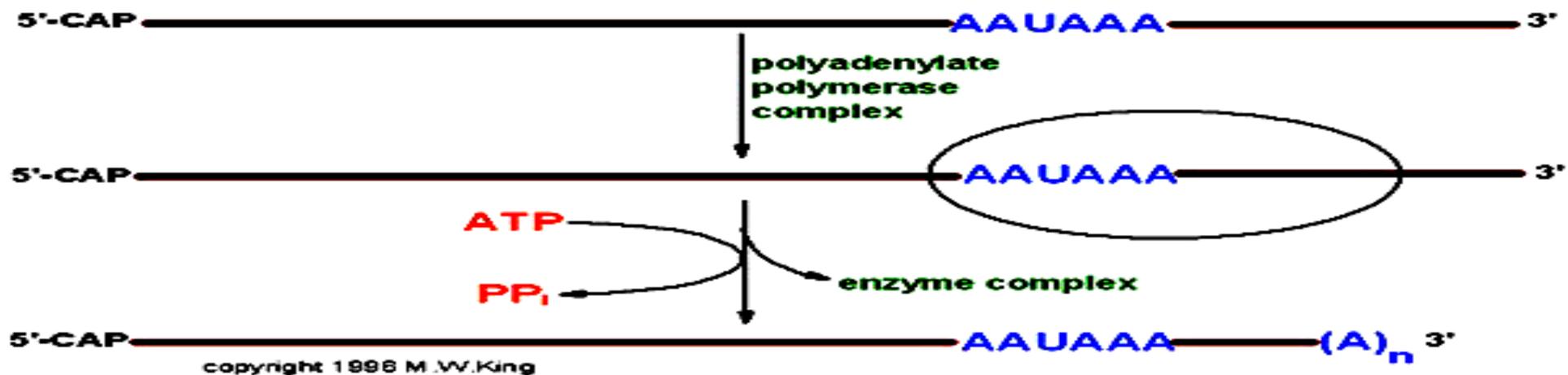
- In contrast to bacterial transcripts, eukaryotic RNAs (all 3 classes) undergo post-transcriptional processing.
- All 3 classes of RNA are transcribed from genes that contain introns.
- The sequences encoded by the intronic DNA must be removed from the primary transcript prior to the RNAs being biologically active, this process of intron removal is called **RNA splicing**, additional processing occurs to mRNAs, the 5' end of all eukaryotic mRNAs are capped with a unique 5'→5' linkage to a **7-methyl GTP**.
- The capped end of the mRNA is thus, protected from **exonucleases** and more importantly is recognized by specific proteins of the translational machinery.
- The capping process occurs after the newly synthesizing mRNA is around **20–30** bases long.



Structure of the 5'-cap of eukaryotic mRNAs

- Messenger RNAs also are polyadenylated at the 3' end.
- A specific sequence, AAUAAA, is recognized by the endonuclease activity of by **polyadenylate polymerase** which cleaves the primary transcript approximately 11–30 bases 3' of the sequence element.
- A stretch of 20–250 A residues is then added to the 3' end by the **polyadenylate polymerase** activity.

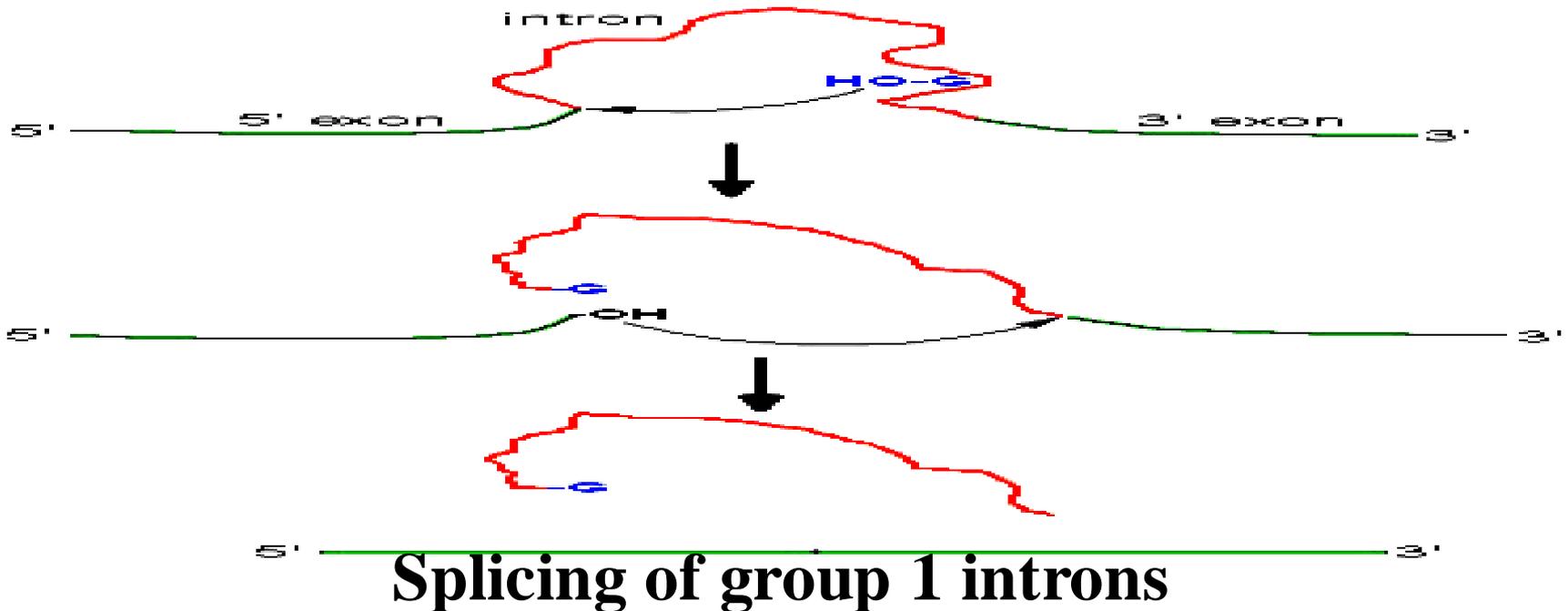
Polyadenylation of mRNAs



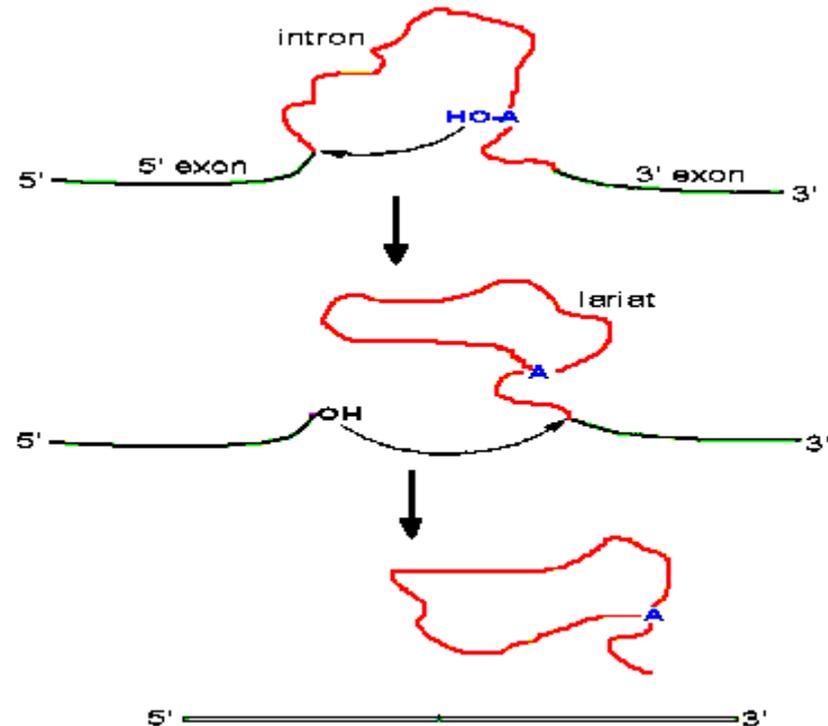
Splicing of RNAs

- There are several different classes of reactions involved in intron removal.
- The 2 most common are the **group I and group II introns**. **Group I introns** are found in nuclear, mitochondrial and chloroplast rRNA genes, group II in mitochondrial and chloroplast mRNA genes.
- Many of the **group I and group II introns** are self-splicing.
- **Group I introns** require an external guanosine as a cofactor.

- The 3'-OH of the guanosine nucleotide acts as a nucleophile to attack the 5'-phosphate of the 5' nucleotide of the intron.
- The resultant 3'-OH at the 3' end of the 5' exon then attacks the 5' nucleotide of the 3' exon releasing the intron and covalently attaching the two exons together.
- The 3' end of the 5' exon is termed the splice donor site and the 5' end of the 3' exon is termed the splice acceptor site.



- Group II introns are spliced similarly except that instead of an external nucleophile, the 2'-OH of an adenine residue within the intron is the nucleophile.
- This residue attacks the 3' nucleotide of the 5' exon forming an internal loop called a lariat structure.
- The 3' end of the 5' exon then attacks the 5' end of the 3' exon as in group I splicing releasing the intron and covalently attaching the two exons together.

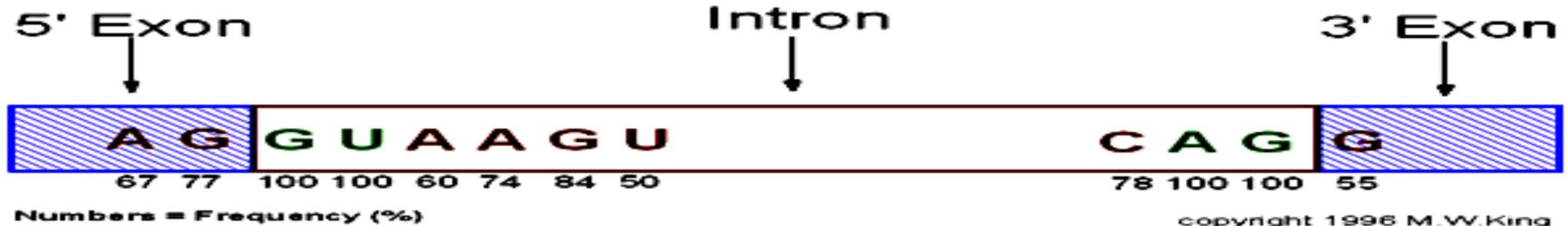


Splicing of group 2 introns

- The third class of introns is also the largest class found in nuclear mRNAs, that undergoes a splicing reaction similar to group II introns in that an internal lariat structure is formed.
- However, the splicing is catalyzed by specialized RNA–protein complexes called small nuclear ribonucleoprotein particles (snRNPs).
- The RNAs found in snRNPs are identified as U1, U2, U4, U5 and U6.
- Analysis of a large number of mRNA genes has led to the identification of highly conserved consensus sequences at the 5' and 3' ends of essentially all mRNA introns.
- The U1 RNA has sequences that are complimentary to sequences near the 5' end of the intron, its binding allows distinguishing the GU at the 5' end of the intron from other randomly placed GU sequences in mRNAs.

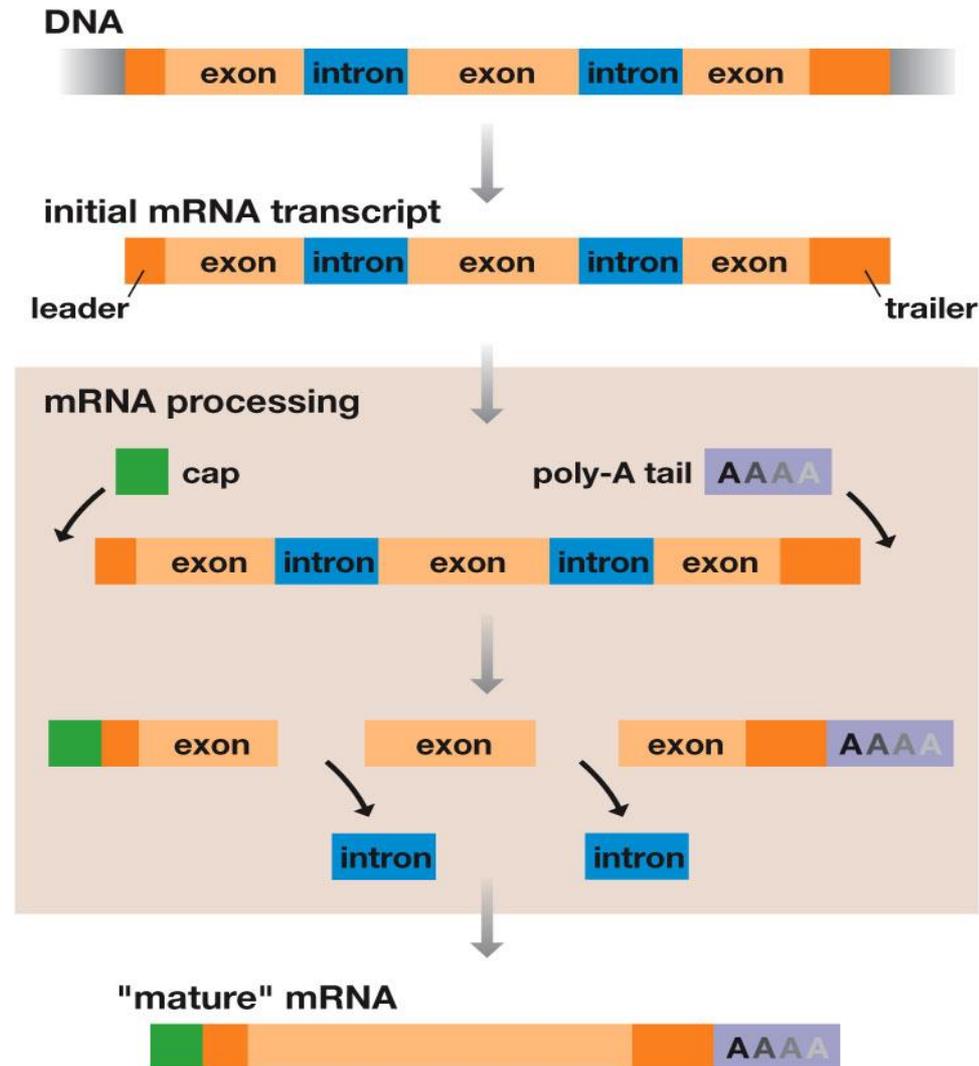
- The U2 RNA also recognizes sequences in the intron, in this case near the 3' end.
- The addition of U4, U5 and U6 RNAs forms a complex identified as the spliceosome (snRNA plus ~40 proteins) that removes the intron and joins the two exons together.
- U7 is involved in the production of the correct 3' ends of histone mRNA which lacks poly (A) tail.

Splicing Consensus Sequences



- An additional mechanism of intron removal is the process of tRNA splicing.
- These introns are spliced by a specific splicing endonuclease that involves a cut-and-paste mechanism.

- In order for tRNA intron removal to occur the tRNA must first be properly folded into its characteristic cloverleaf shape.
- Misfolded precursor tRNAs are not processed which allows the splicing reaction to serve as a control step in the generation of mature tRNAs.



RNA processing: pre-mRNA → mRNA

Alternative splicing (eukaryotes only)

- Exons are “coding” regions
- Introns are removed
- Different combinations of exons form different mRNA resulting in multiple proteins from the same gene
- Humans have 30 to 50 thousand genes but are capable of producing 100,000 proteins.

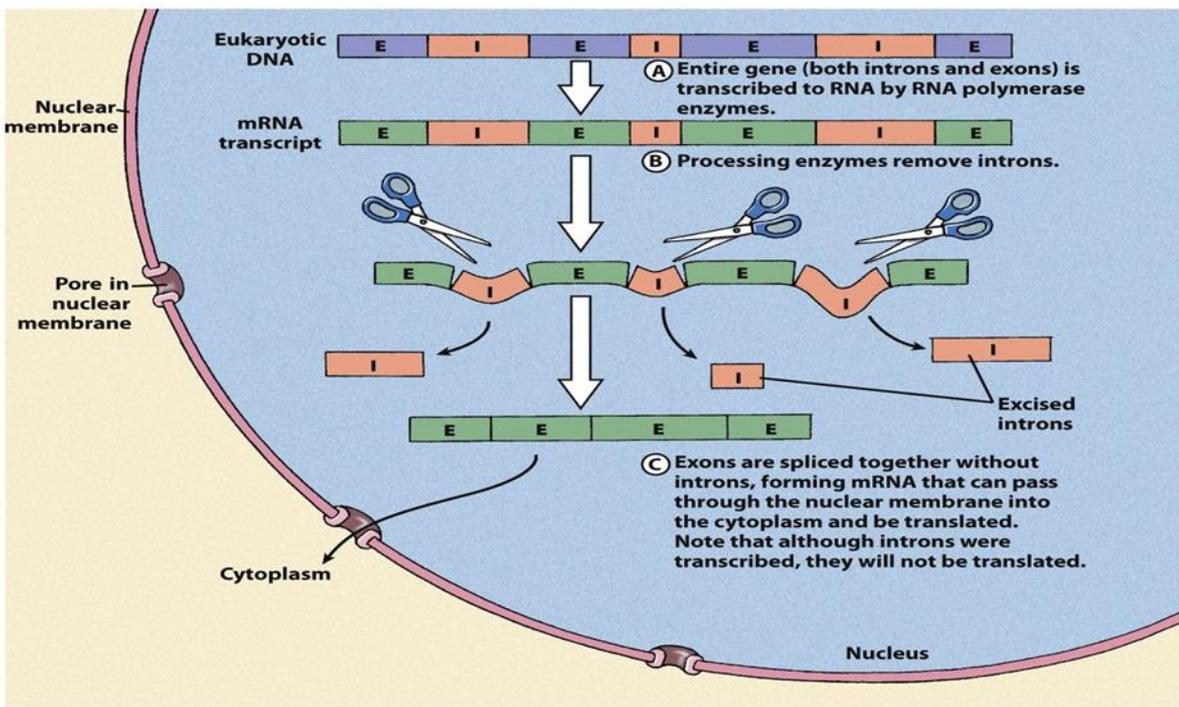
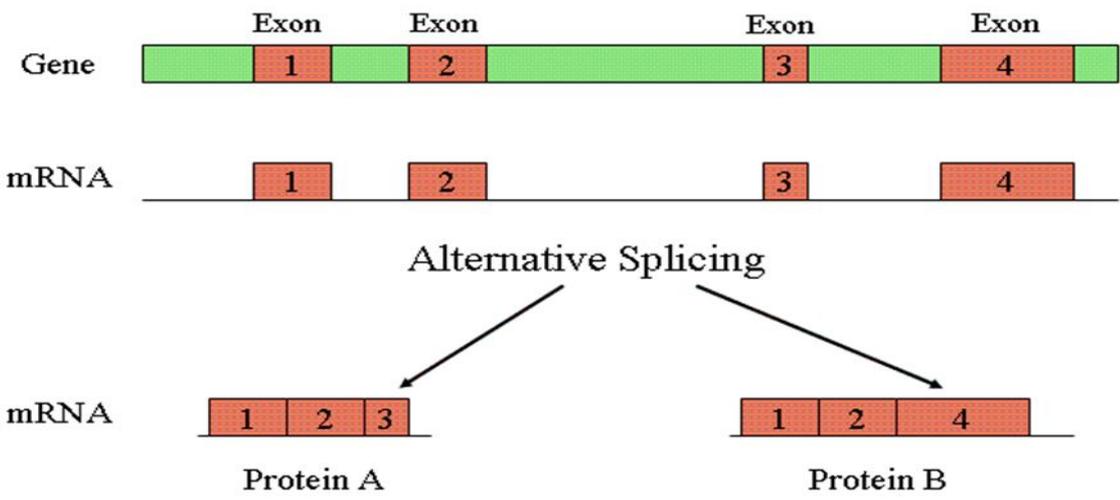


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rRNA is used to construct ribosomes

- Eukaryotic ribosomal RNA is transcribed in the nucleolus by RNA polymerase I as a single piece of 45S RNA, which is subsequently cleaved to yield 28S rRNA, 18S rRNA, and 5.8S rRNA.
- RNA polymerase III transcribes the 5S rRNA unit from a separate gene. The ribosomal subunits assemble in the nucleolus as the rRNA pieces combine with ribosomal proteins.
- Eukaryotic ribosomal subunits are 60S and 40S. They join during protein synthesis to form the whole 80S ribosome.

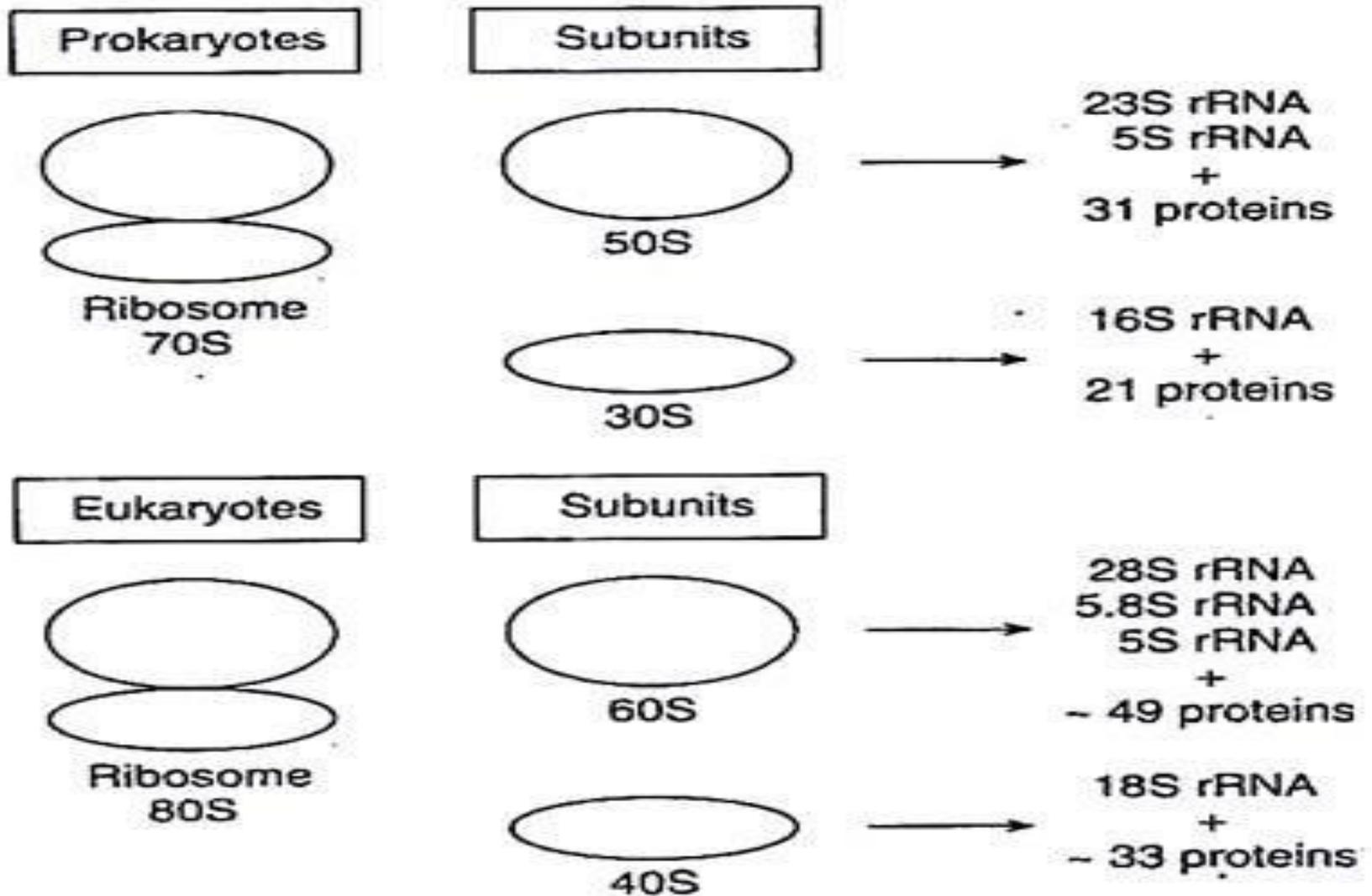


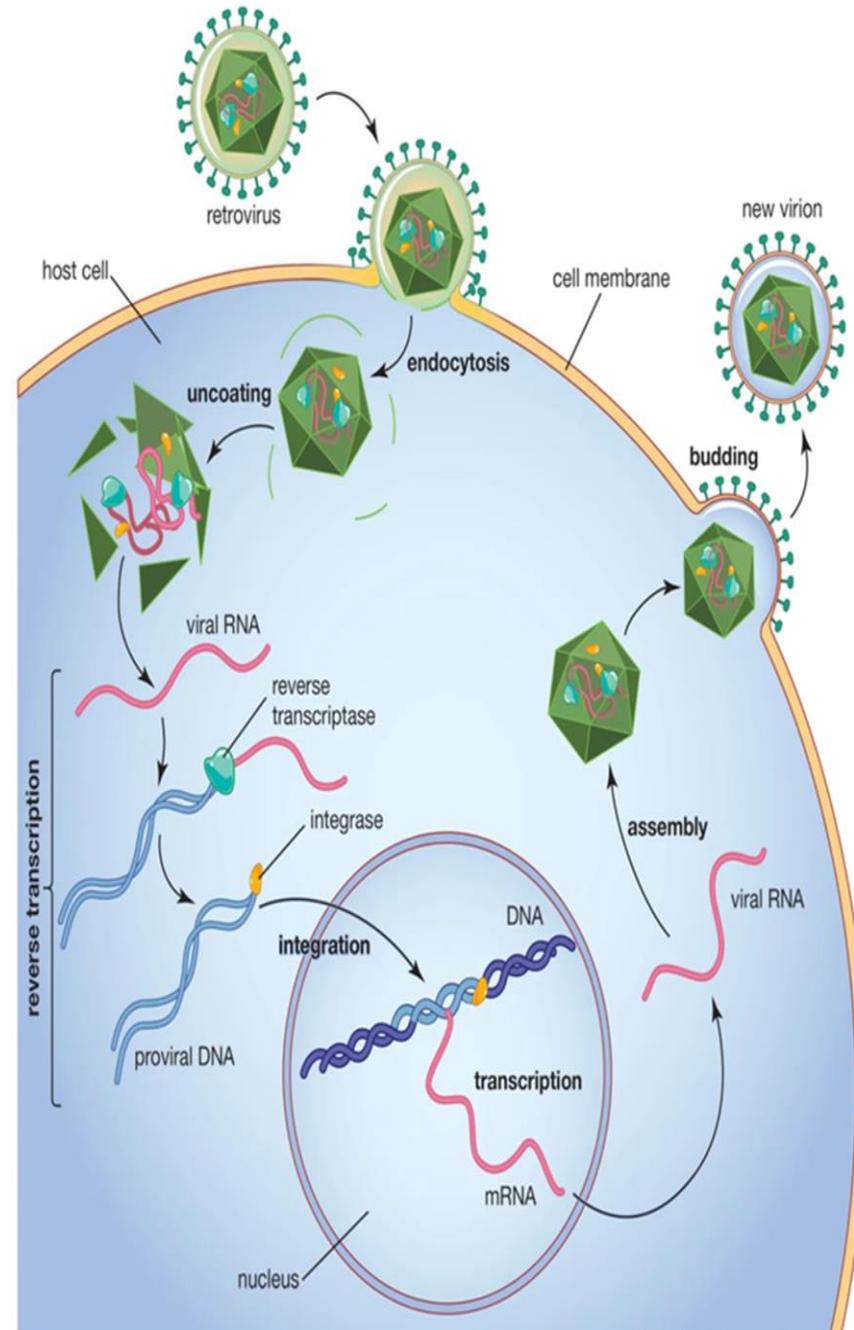
Fig. 2.47: Composition of typical prokaryotic and eukaryotic ribosomes

Reverse transcription

Reverse transcription

- The genetic information carrier of some biological systems is **ssRNA** instead of **dsDNA** (such as ssRNA viruses such as HIV).
- The information flow is from RNA to DNA (called reverse transcription) to be merged with the DNA genome of the host cell by **reverse transcriptase** enzyme which has the following activities:
 - 1- RNA-dependent DNA polymerase
 - 2- RNase
 - 3- DNA-dependent DNA polymerase

Retrovirus infection and reverse transcription



- In the case of HIV, **reverse transcriptase** is responsible for synthesizing a complementary DNA strand (cDNA) to the viral RNA genome.
- An associated enzyme, **ribonuclease H**, digests the RNA strand, and **reverse transcriptase** synthesizes DNA complementary strand to form a double helix DNA structure.
- This DNA is integrated into the host cell's genome by **integrase** enzyme causing the host cell to generate viral proteins that reassemble into new viral particles.
- However, in retroviruses, the host cell remains intact as the virus buds out of the cell but in the case of HIV, the host cell undergoes apoptosis.
- Some eukaryotic cells contain an enzyme with reverse transcription activity called **telomerase**.

- Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes.
- Telomerase carries an RNA template from which it synthesizes DNA **repeating sequence**, or "**junk**" DNA.
- This repeated sequence of DNA is important because, every time a linear chromosome is duplicated, it is shortened in length.
- Telomerase** is often activated in cancer cells to enable cancer cells to duplicate their genomes indefinitely without losing important **protein-coding DNA sequence** (the discovery of **RT** enriches the understanding about the **cancer-causing theory of viruses**, where cancer genes in RT viruses, and HIV having RT function).
- Activation of telomerase could be part of the process that allows cancer cells to become immortal.