

DNA Transcription and Translation

Comparing RNA and DNA

- ✴ DNA can replicate itself precisely and contain information in the specific sequence of its bases.
- ✴ RNA and DNA are very similar molecules.
- ✴ 5-carbon sugar in RNA is Ribose
- ✴ RNA contains Uracil instead of Thymine
- ✴ DNA is double stranded and RNA is single stranded with folded complex secondary and tertiary structures.
- ✴ DNA molecules are always longer than RNA molecules.
- ✴ DNA is more stable than RNA.
- ✴ There are several classes of RNA.

Messenger RNA

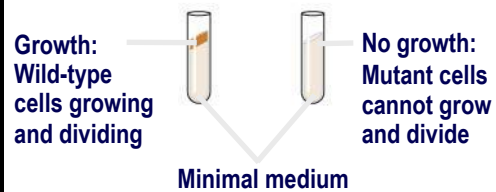
- ✱ mRNA carries the message. The linear amino acid sequence (primary) is encoded in the DNA. But the DNA does not make the proteins directly. mRNA is the link between gene and protein.
- ✱ The information contained in the mRNA is written in the genetic code. The genetic code is UNIVERSAL.
- ✱ The beginning of the mRNA is always on the 5' end and that is where the synthesis of the proteins starts.
- ✱ It takes three nucleotides to code for one amino acid.
- ✱ The mRNA has a 5' leader, a coding region, introns, and a 3' trailer.
- ✱ After transcription the mRNA is modified to go inside the cytoplasm. A 5' cap is added as well as a 3' poly A tail. Introns are spliced out. Only expressed regions (exons) are kept.

Basic Principles of Transcription and Translation

- ✦ RNA is the intermediate between genes and the proteins for which they code
 - ✦ **Transcription** is the synthesis of RNA under the direction of DNA
 - ✦ Transcription produces **messenger RNA (mRNA)**
 - ✦ **Translation** is the synthesis of a polypeptide, which occurs under the direction of mRNA
 - ✦ **Ribosomes** are the sites of translation
-

Fig. 17-2

EXPERIMENT

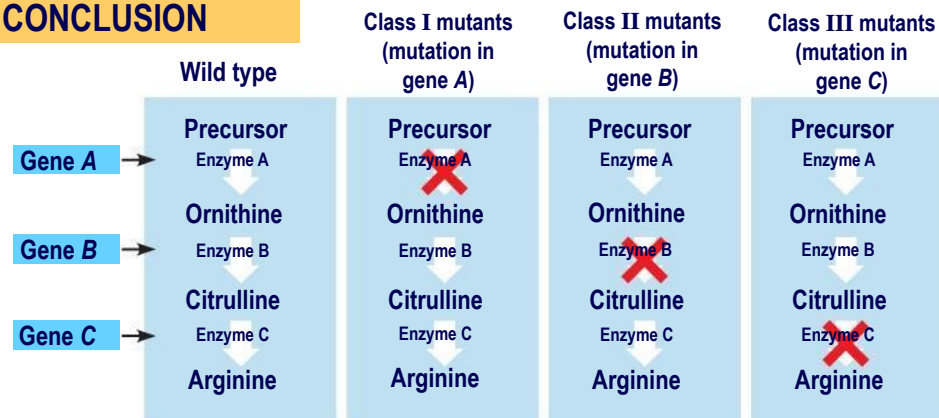


RESULTS

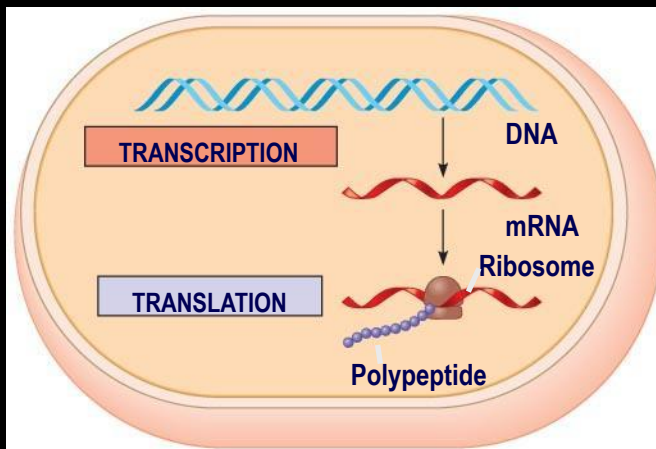
Classes of *Neurospora crassa*

	Wild type	Class I mutants	Class II mutants	Class III mutants
Minimal medium (MM) (control)				
MM + ornithine				
MM + citrulline				
MM + arginine (control)				

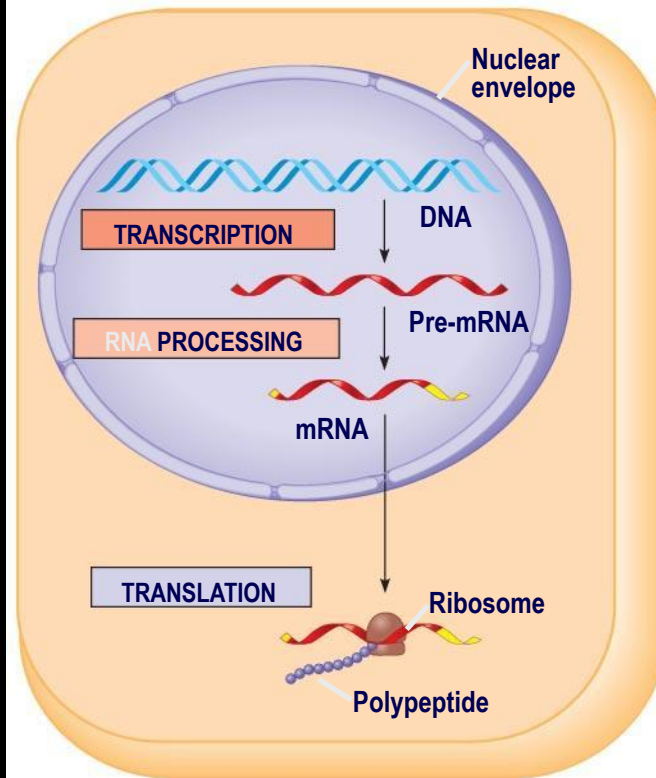
CONCLUSION



- In prokaryotes, mRNA produced by transcription is immediately translated without more processing
 - In a eukaryotic cell, the nuclear envelope separates transcription from translation
 - Eukaryotic RNA transcripts are modified through **RNA processing** to yield finished mRNA
-



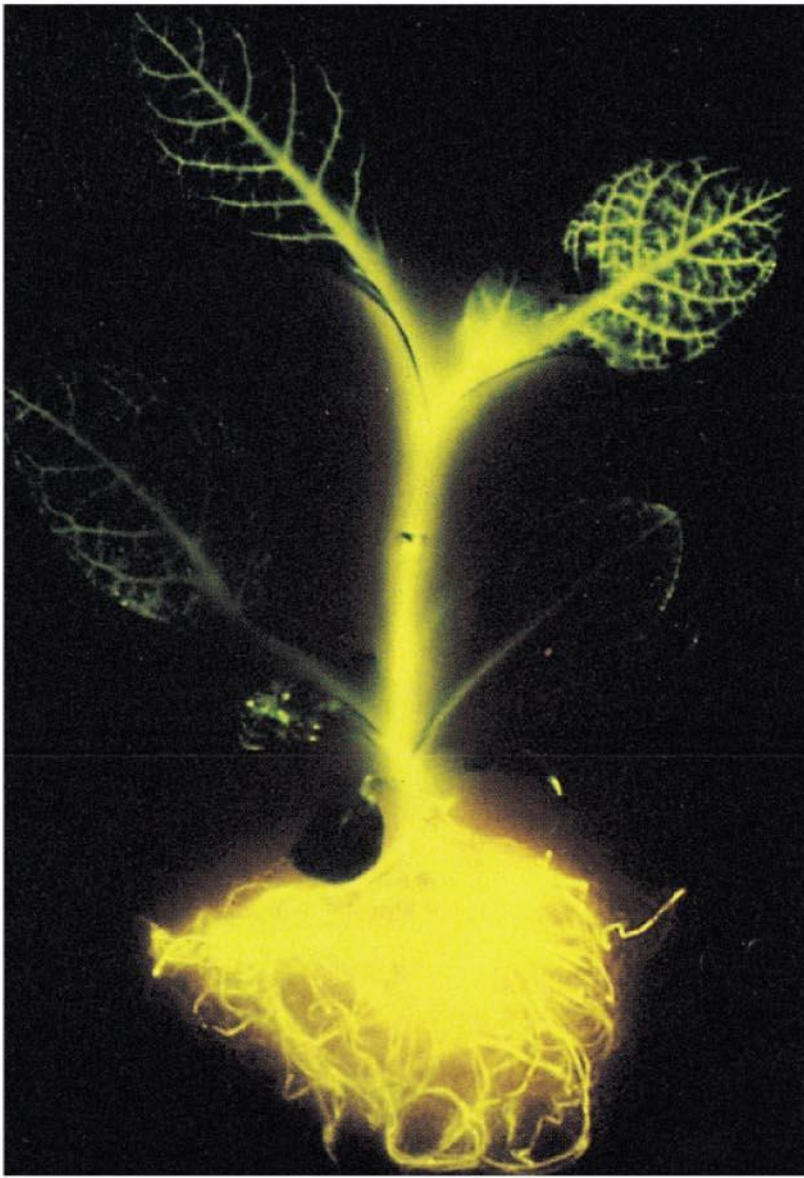
(a) Bacterial cell



(b) Eukaryotic cell

The Genetic Code

- ✴ The genetic code is the same for all organisms (universal)
- ✴ A codon is a “word” in DNA/RNA language. It is formed by three nucleotides.
- ✴ There are a lot of synonymous codons.
- ✴ The genetic code is “redundant”
- ✴ AUG is always the start codon for translation and there are three stop codons to end translation.



**(a) Tobacco plant expressing
a firefly gene**



**(b) Pig expressing a
jellyfish gene**

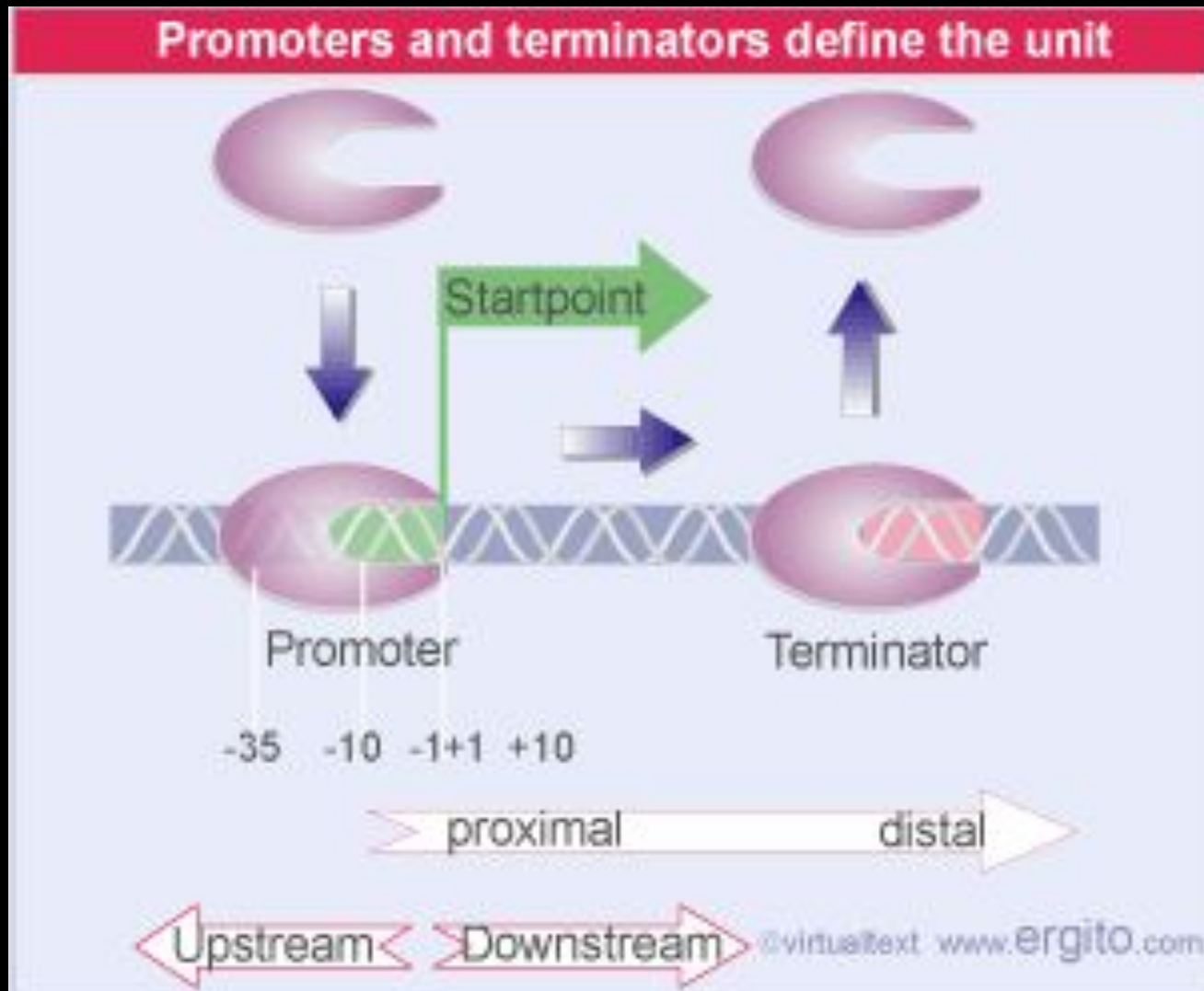
Key Words and definitions

- ✱ **Transcription** describes the synthesis of RNA on a DNA template
- ✱ **Translation** is the synthesis of protein on a mRNA template
- ✱ **Coding region** of part of the gene that represents a protein sequence.
- ✱ **Codon** is a triplet of bases that represents an amino acid or a termination signal
- ✱ **The antisense strand** (template) is complementary to the sense strand and is used as a template to synthesize RNA
- ✱ **The coding strand** (sense) has the same sequence as the mRNA and is related to the protein synthesized.

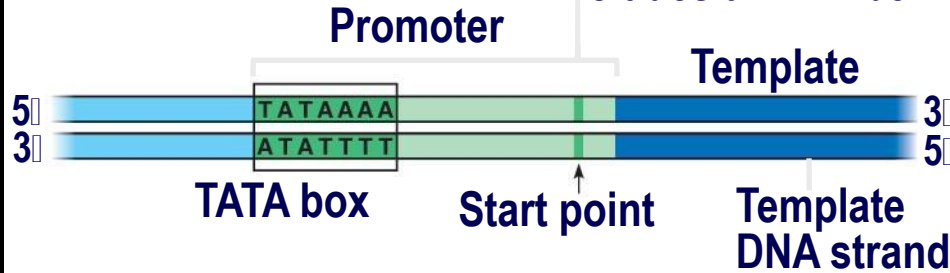
RNA Polymerase Binding and Initiation of Transcription

- ✱ Promoters signal the initiation of RNA synthesis
 - ✱ **Transcription factors** mediate the binding of RNA polymerase and the initiation of transcription
 - ✱ The completed assembly of transcription factors and RNA polymerase II bound to a promoter is called a **transcription initiation complex**
 - ✱ A promoter called a **TATA box** is crucial in forming the initiation complex in eukaryotes
-

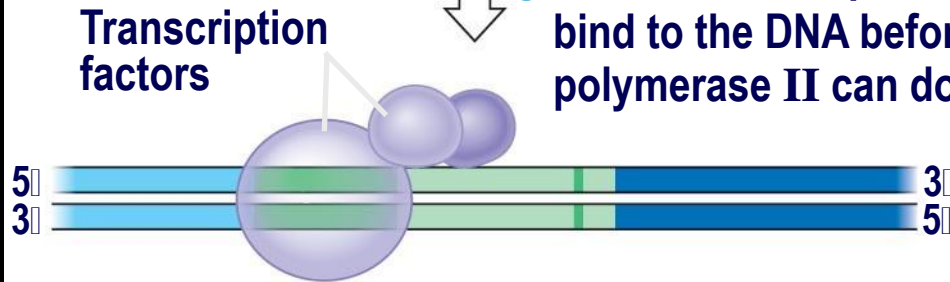
Promoters, terminators and start point



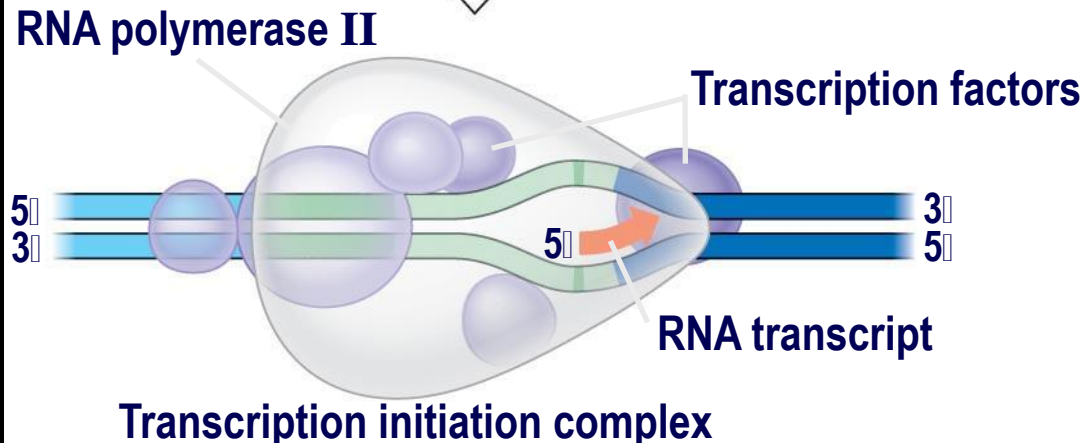
- 1 A eukaryotic promoter includes a TATA box



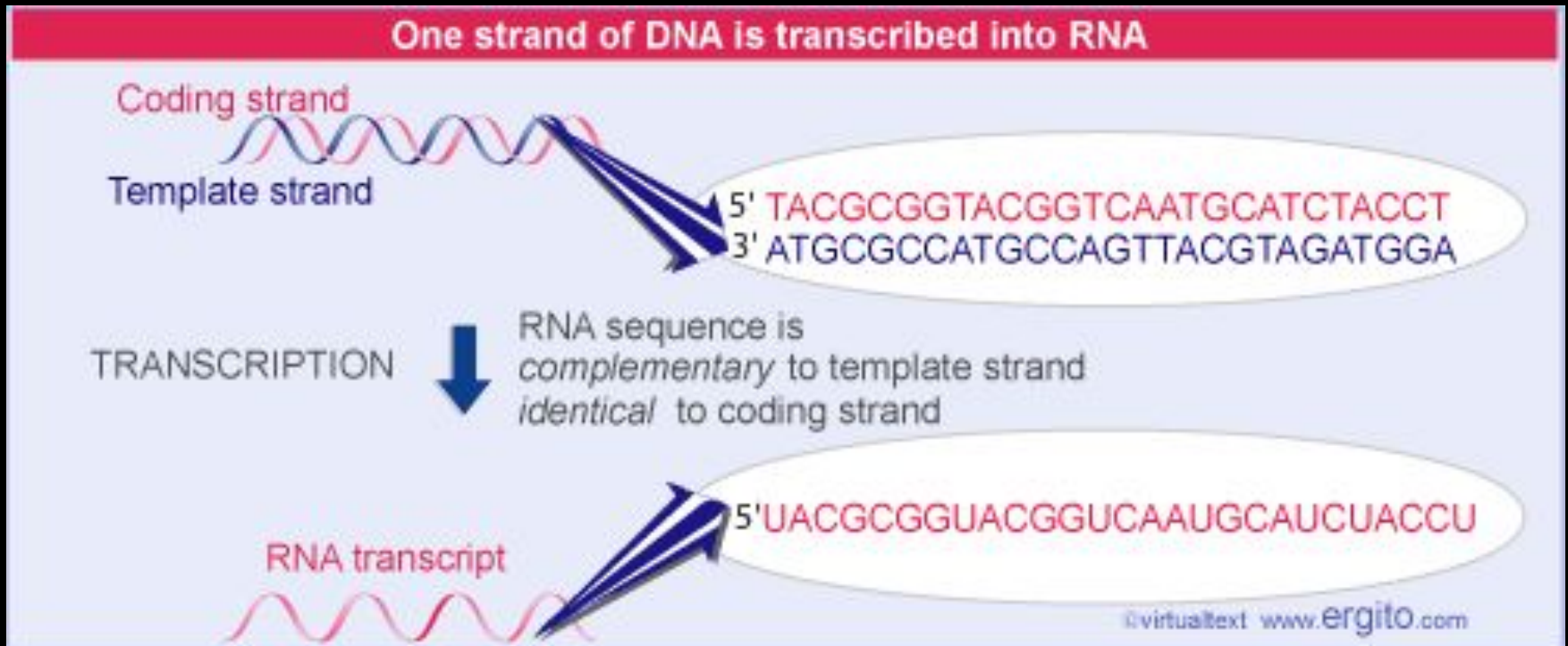
- 2 Several transcription factors must bind to the DNA before RNA polymerase II can do so.



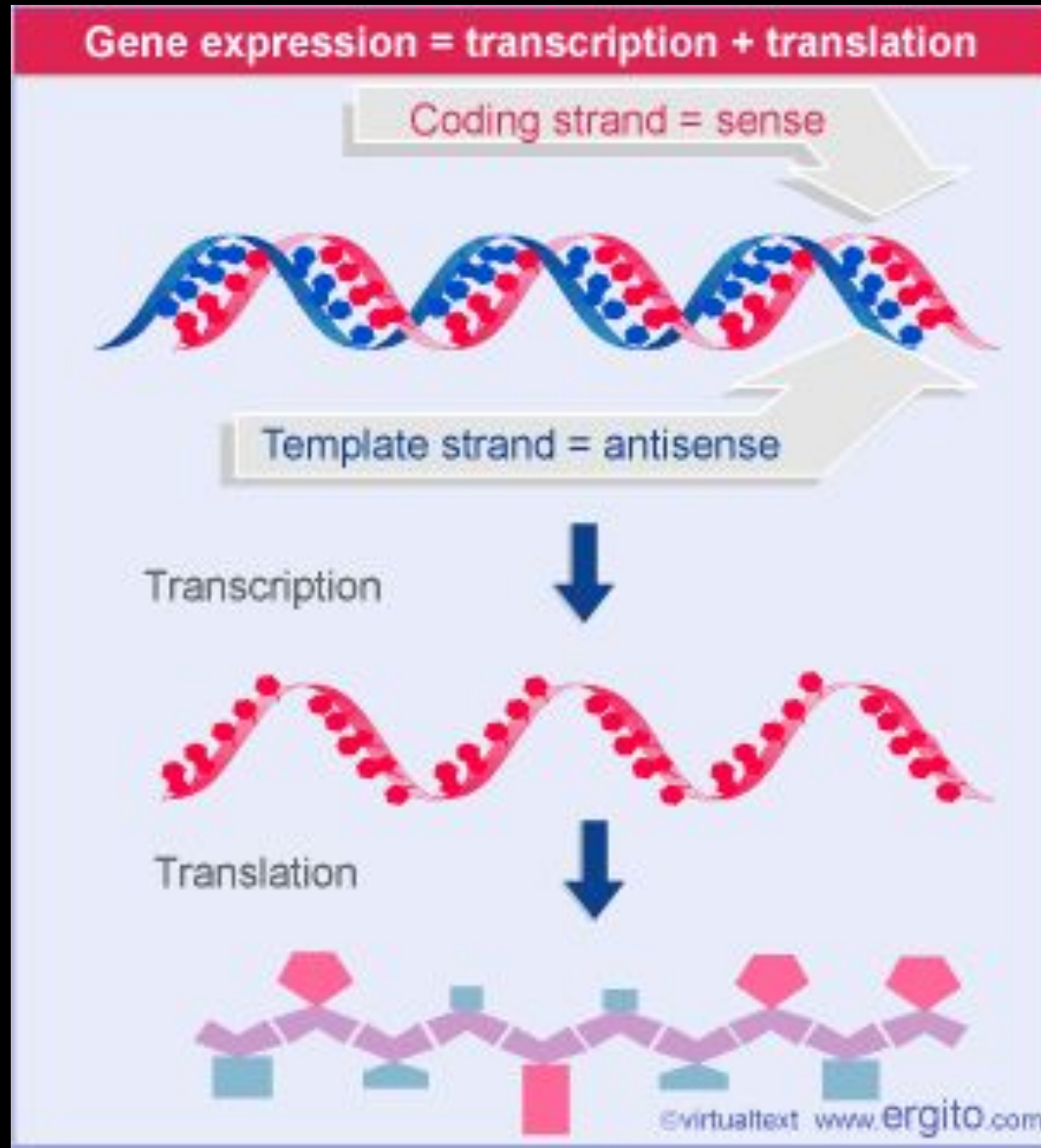
- 3 Additional transcription factors bind to the DNA along with RNA polymerase II, forming the transcription initiation complex.



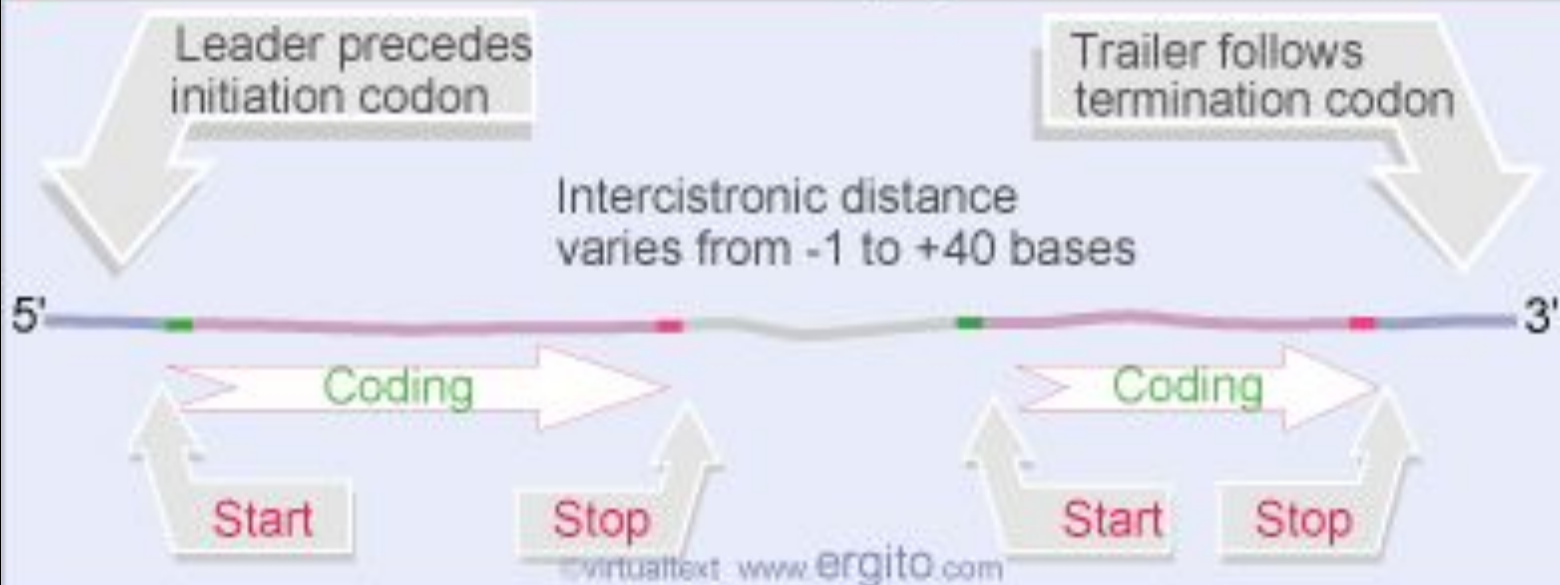
Only one strand of DNA is transcribed



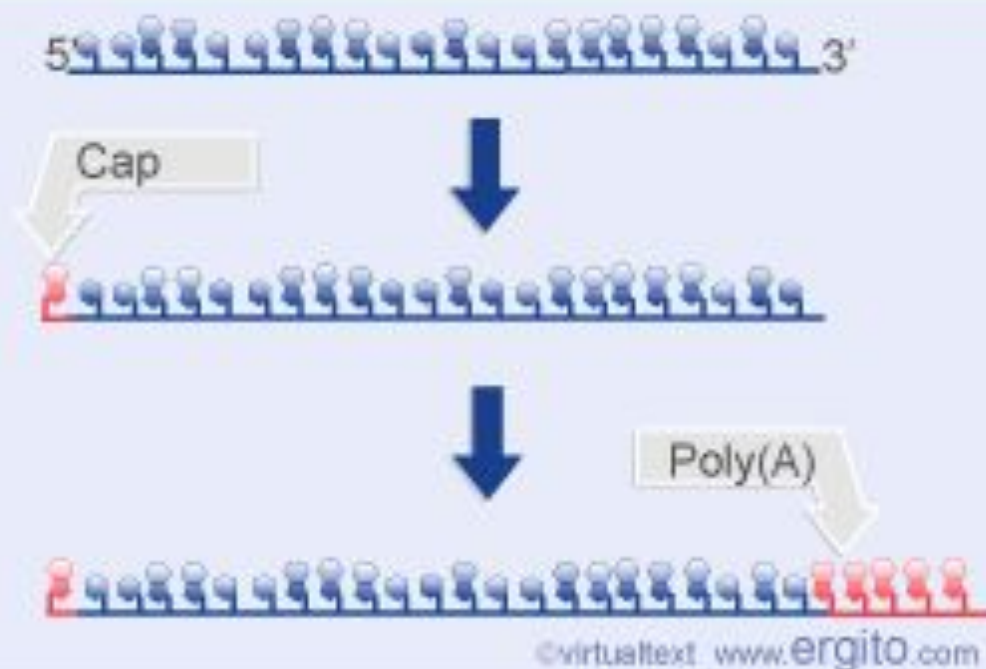
Sense and antisense strands

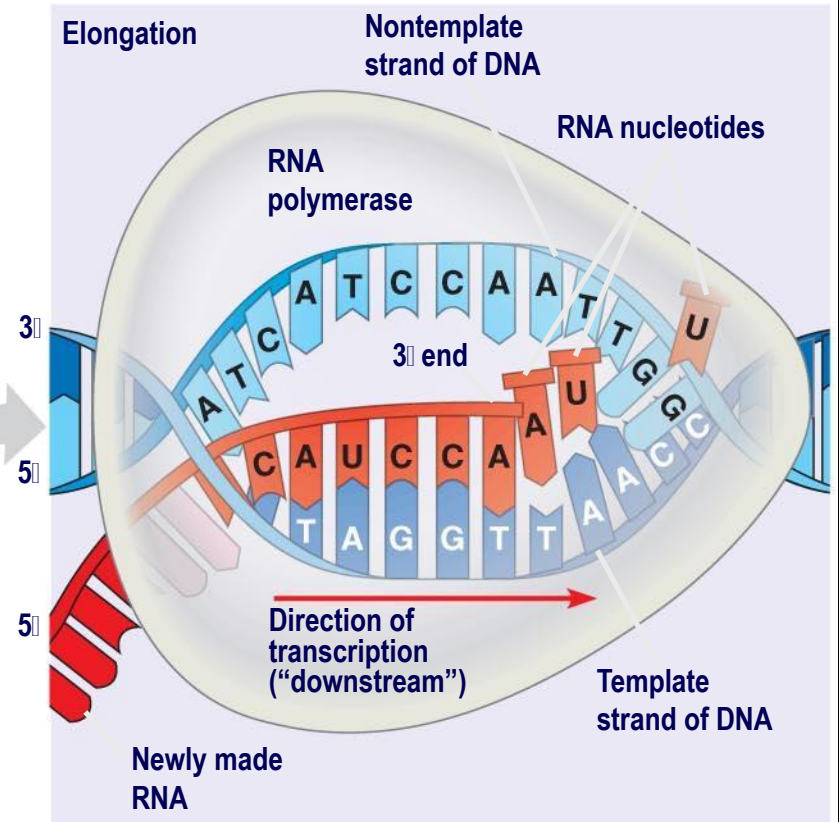
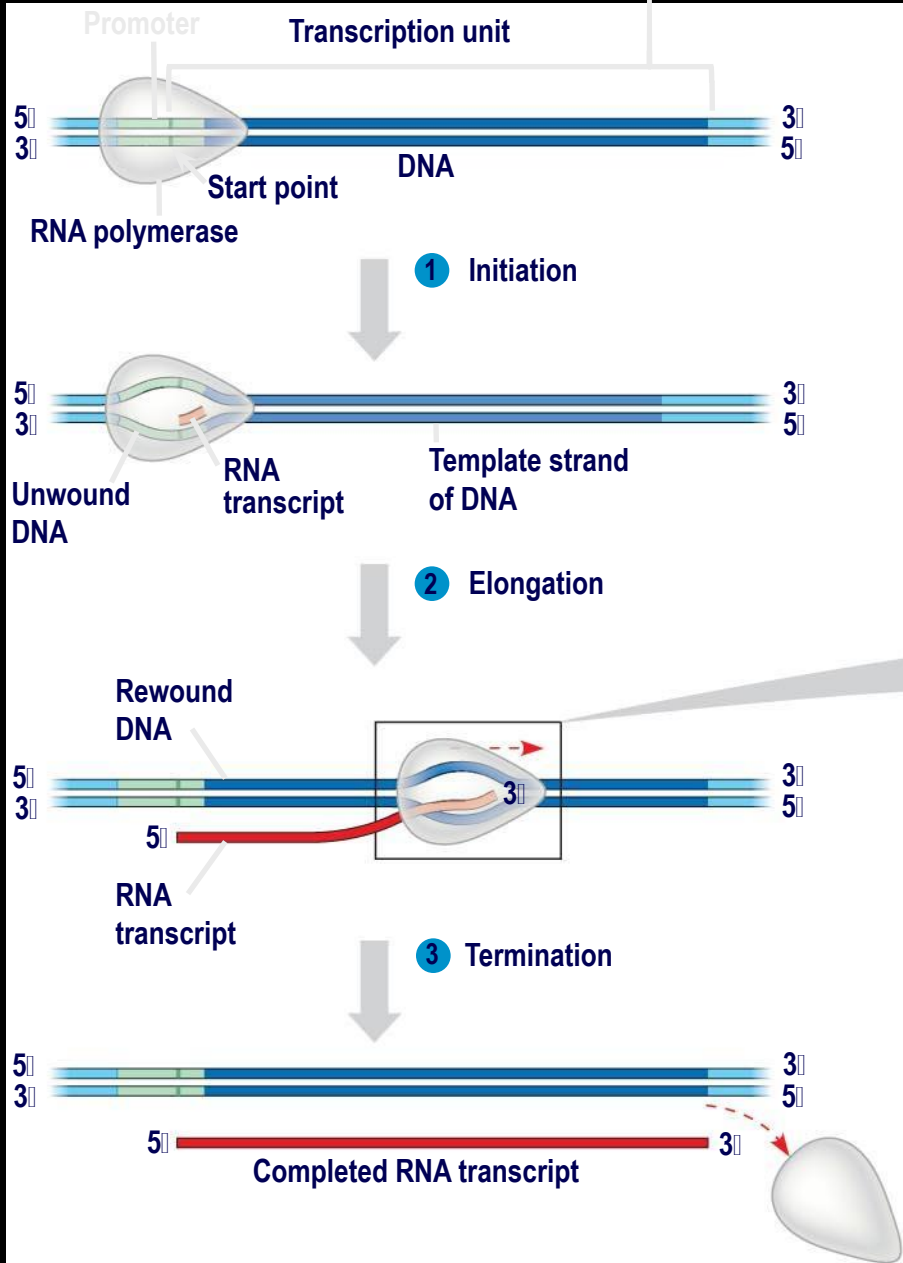


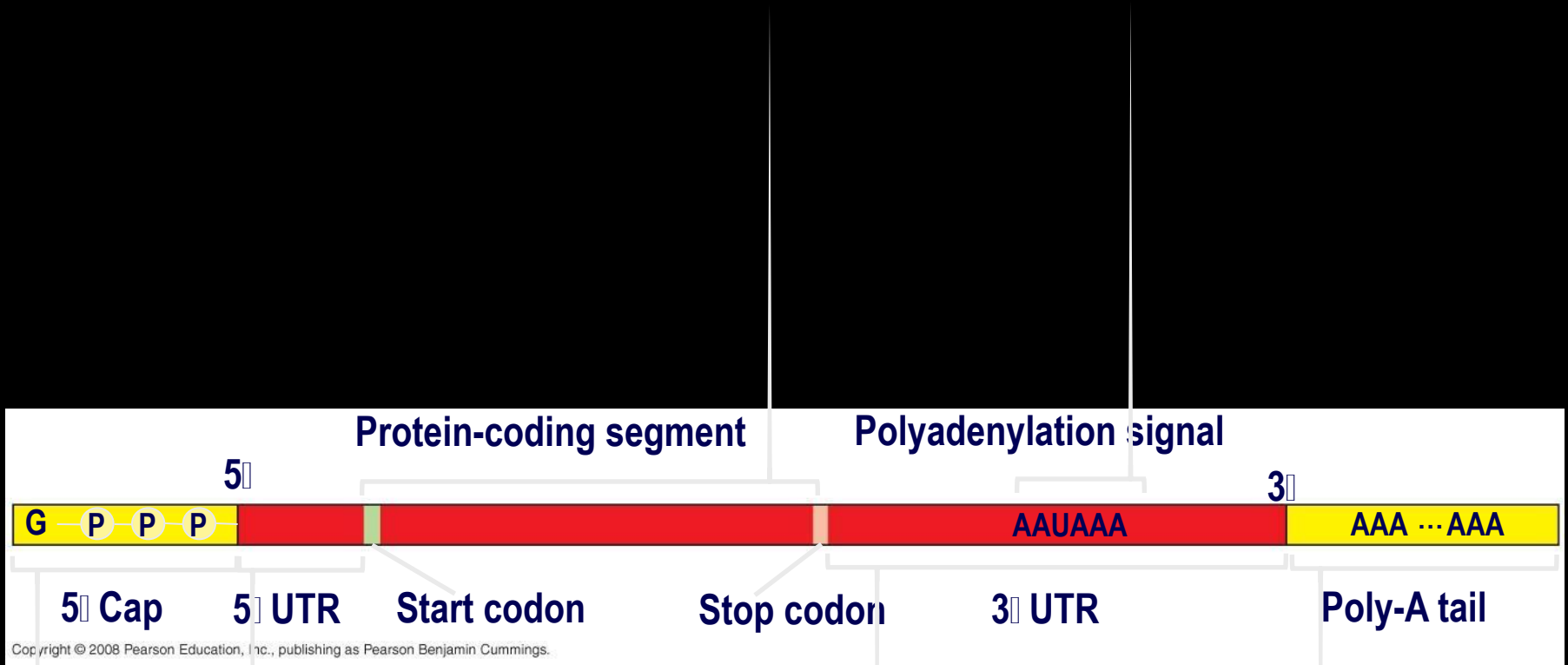
Bacterial mRNA is polycistronic



Eukaryotic mRNA is modified at both ends

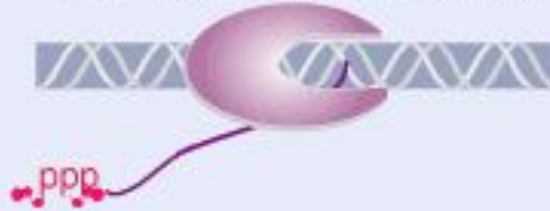




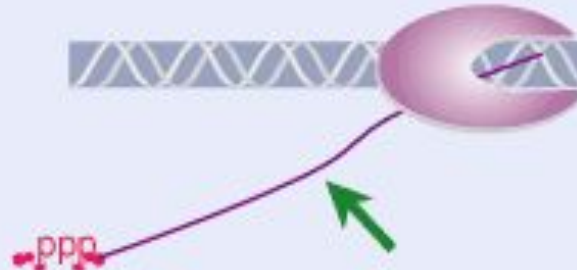


Eukaryotic mRNA is modified and exported

<1 min Transcription starts: 5' end is modified



6 min 3' end of mRNA is released by cleavage



20 min 3' end is polyadenylated



25 min mRNA is transported to cytoplasm

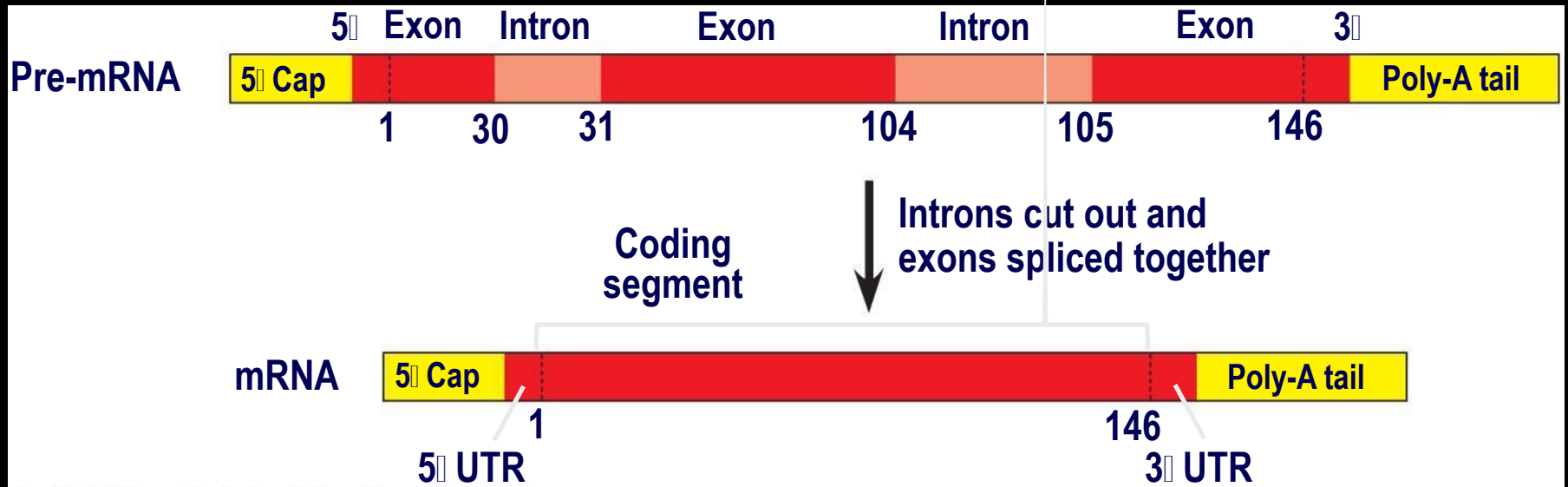


> 4 hr Ribosomes translate mRNA



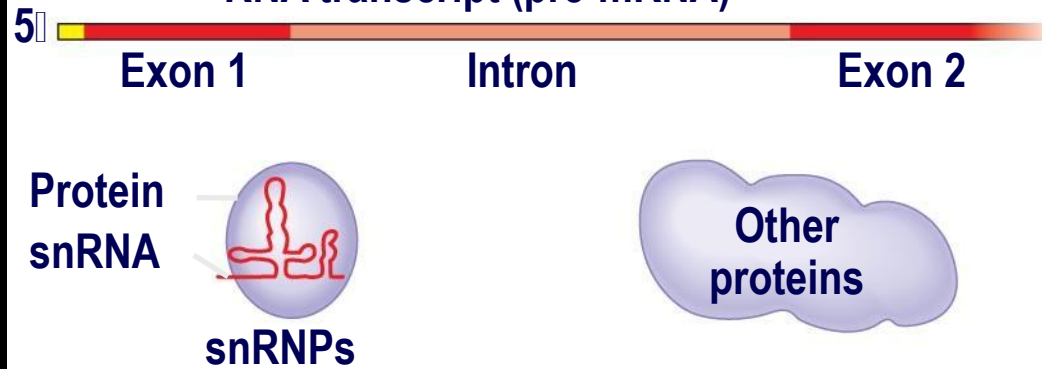
Split Genes and RNA Splicing

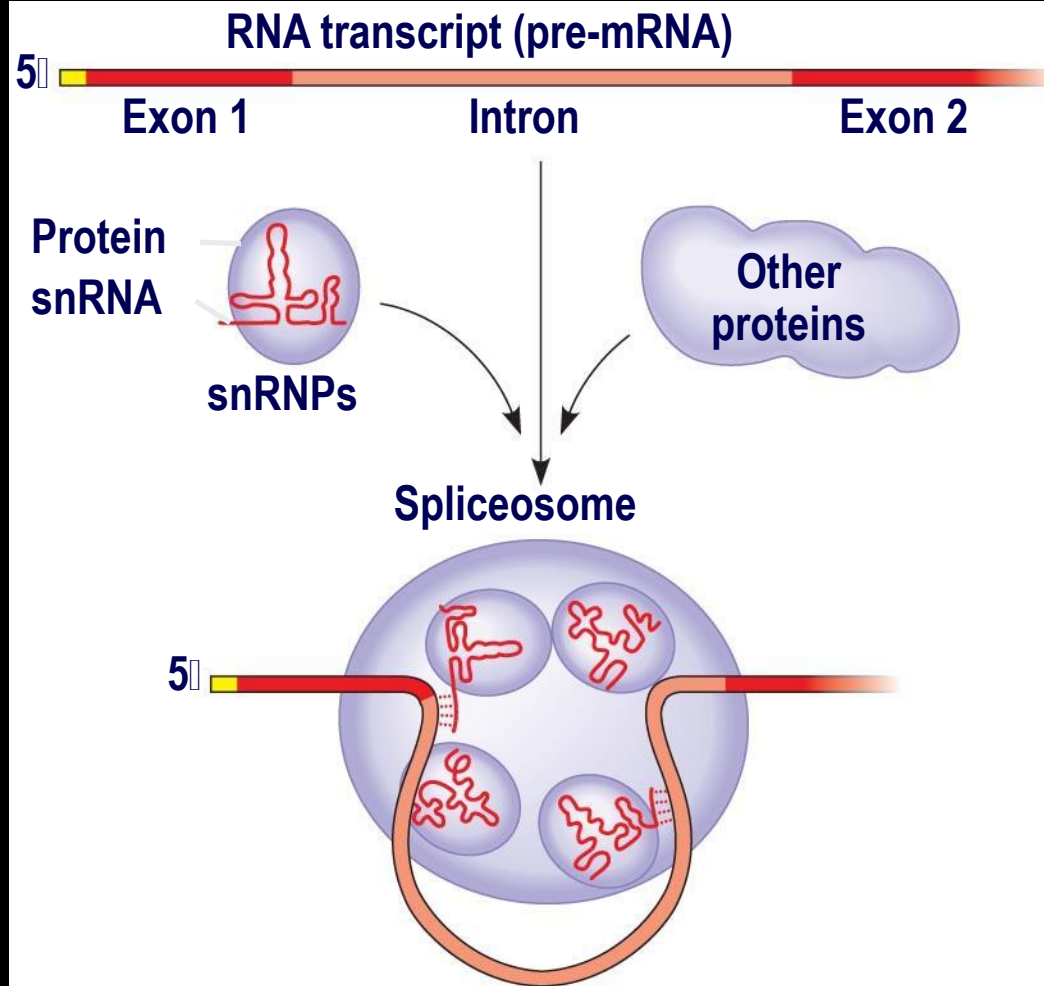
- Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions
 - These noncoding regions are called intervening sequences, or **introns**
 - The other regions are called **exons** because they are eventually expressed, usually translated into amino acid sequences
 - **RNA splicing** removes introns and joins exons, creating an mRNA molecule with a continuous coding sequence
-

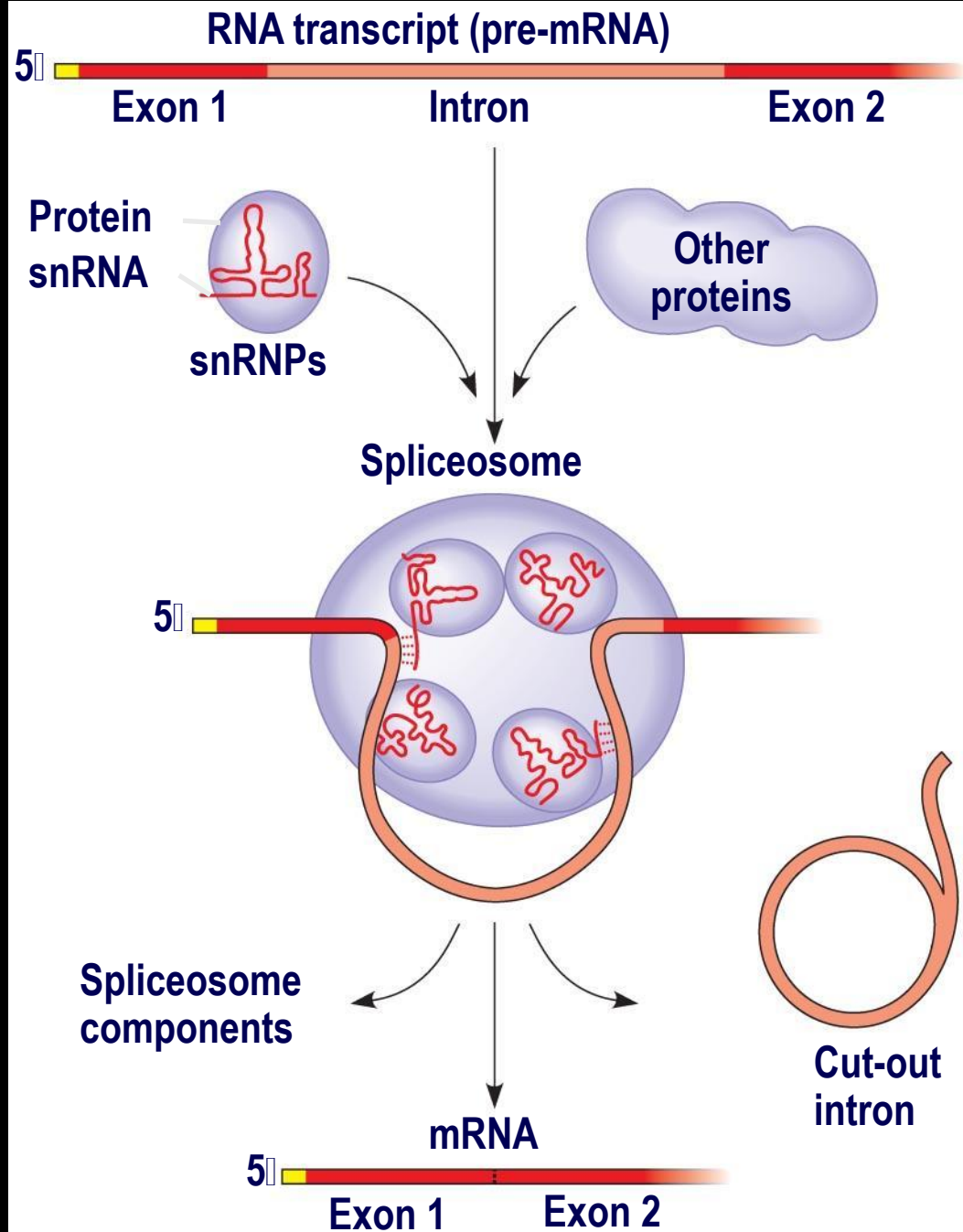


- ✴ In some cases, RNA splicing is carried out by spliceosomes
- ✴ **Spliceosomes** consist of a variety of proteins and several small nuclear ribonucleoproteins (snRNPs) that recognize the splice sites

RNA transcript (pre-mRNA)







Ribozymes

- ✦ **Ribozymes** are catalytic RNA molecules that function as enzymes and can splice RNA
- ✦ The discovery of ribozymes rendered obsolete the belief that all biological catalysts were proteins

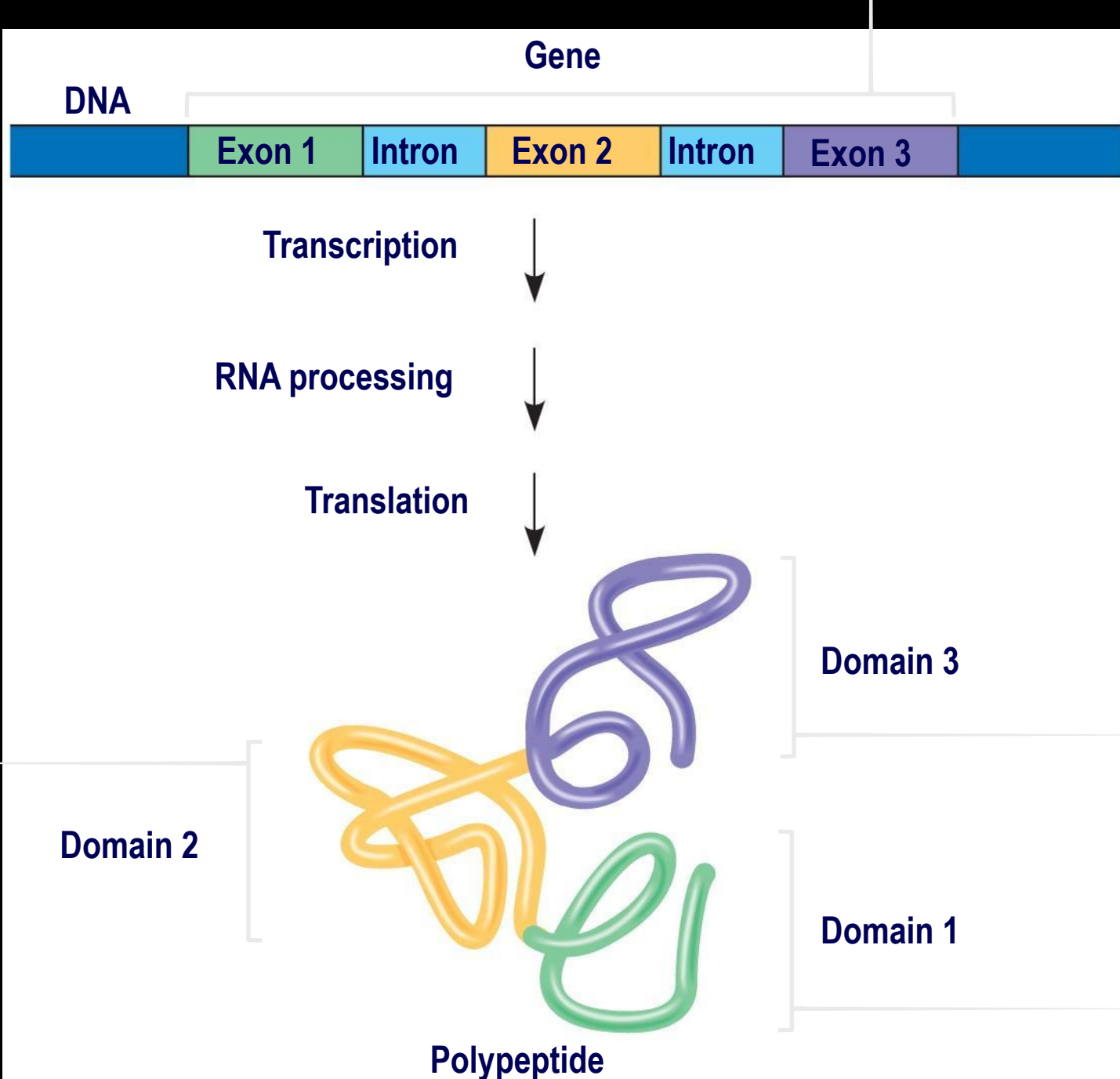
- ★ Three properties of RNA enable it to function as an enzyme
 - ★ It can form a three-dimensional structure because of its ability to base pair with itself
 - ★ Some bases in RNA contain functional groups
 - ★ RNA may hydrogen-bond with other nucleic acid molecules

The Functional and Evolutionary Importance of Introns

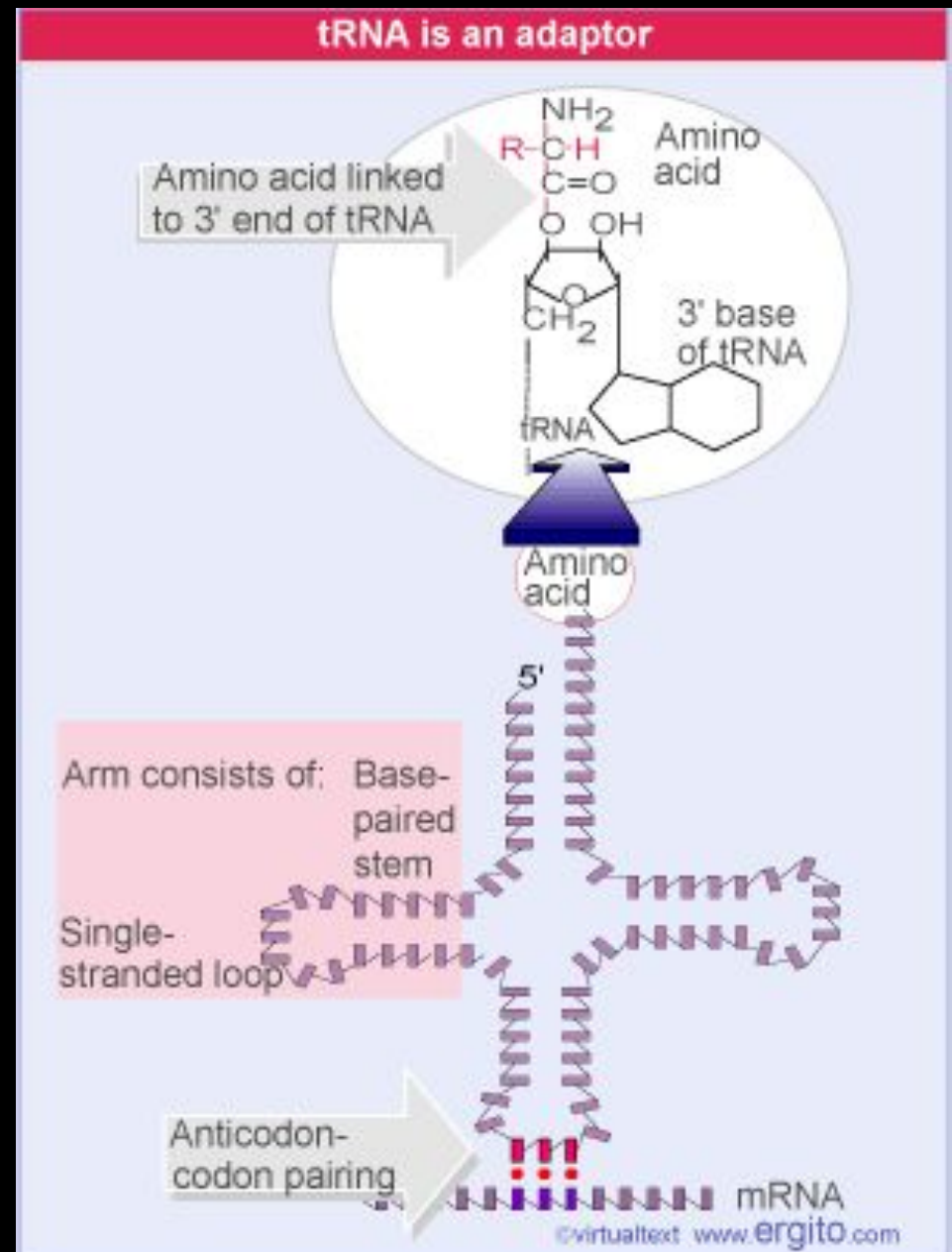
- ★ Some genes can encode more than one kind of polypeptide, depending on which segments are treated as exons during RNA splicing
 - ★ Such variations are called **alternative RNA splicing**
 - ★ Because of alternative splicing, the number of different proteins an organism can produce is much greater than its number of genes
-

- ✴ Proteins often have a modular architecture consisting of discrete regions called **domains**
 - ✴ In many cases, different exons code for the different domains in a protein
 - ✴ Exon shuffling may result in the evolution of new proteins
-

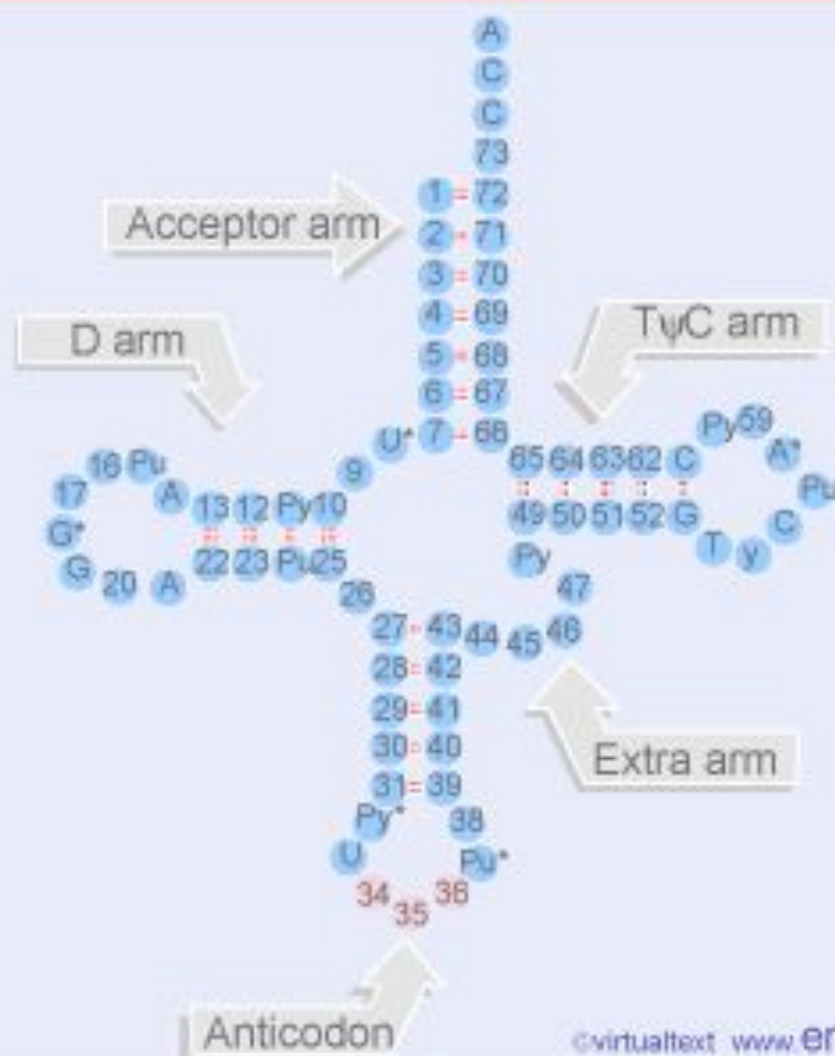
Fig. 17-12



tRNA

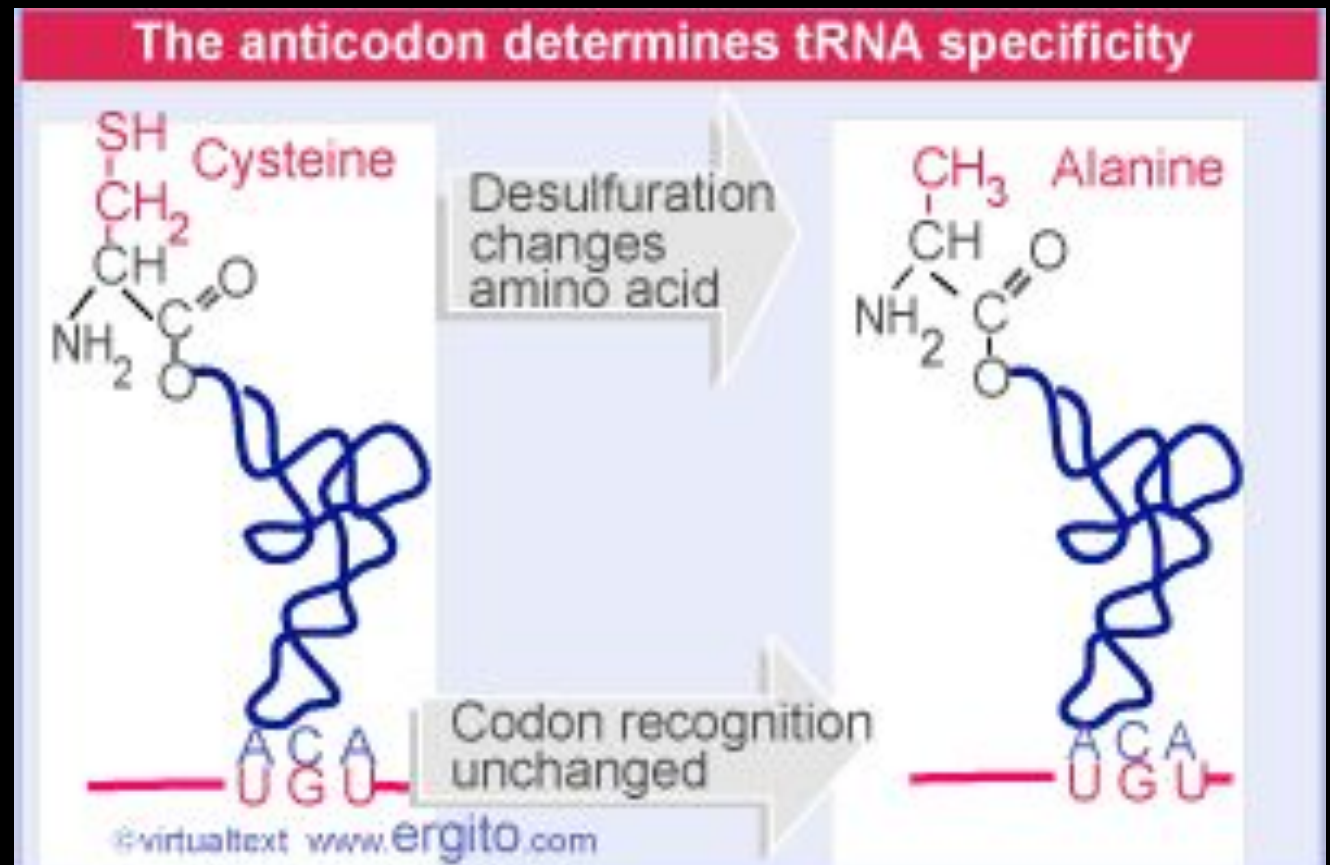


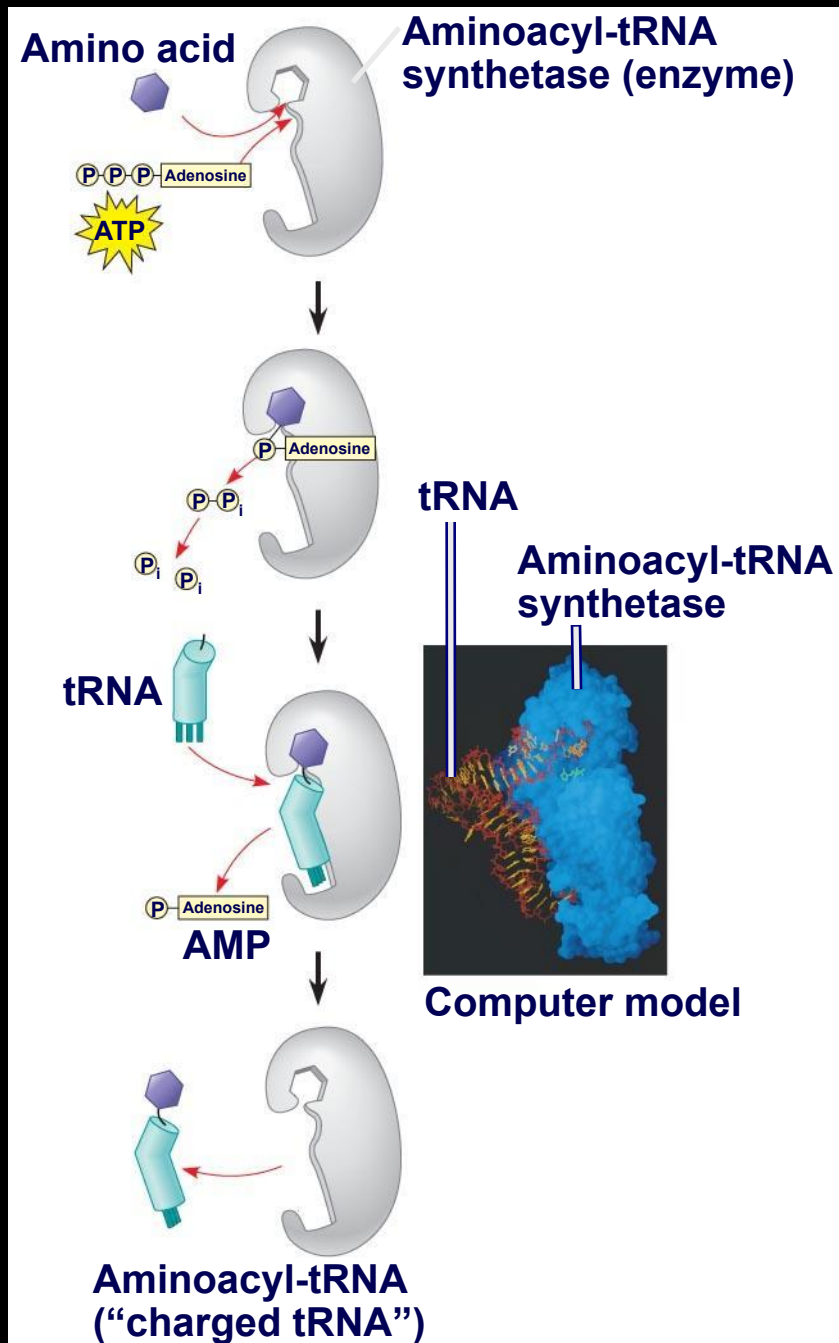
tRNA secondary structure is a cloverleaf



Aminoacyl-tRNA

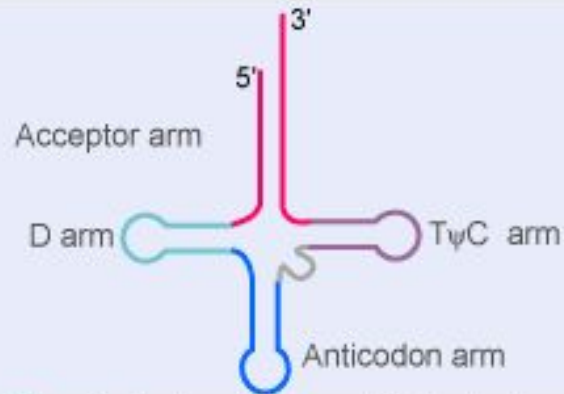
- ✦ The aminoacyl-tRNA synthase adds the correct amino acid to the corresponding tRNA.





All tRNAs share a tertiary structure

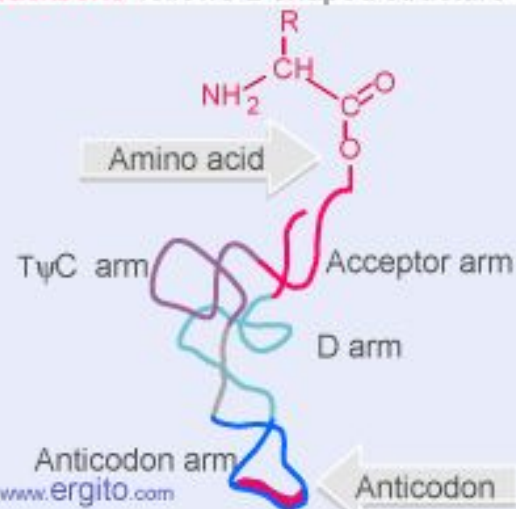
Cloverleaf has four arms



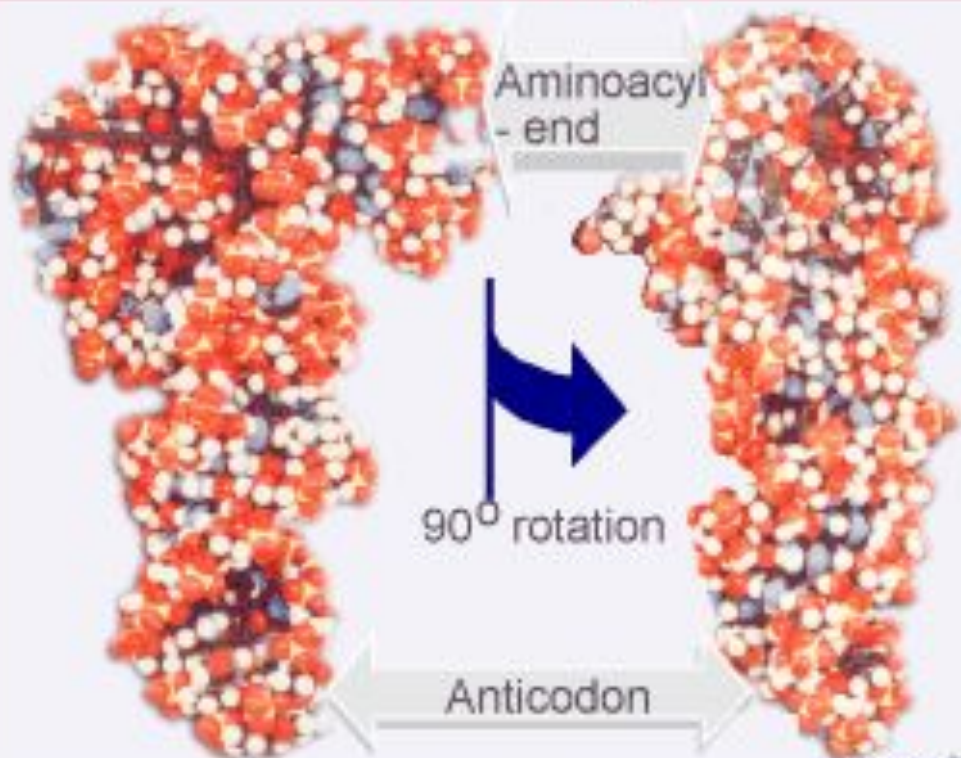
2D projection has 2 perpendicular duplexes



Backbone follows L-shaped structure

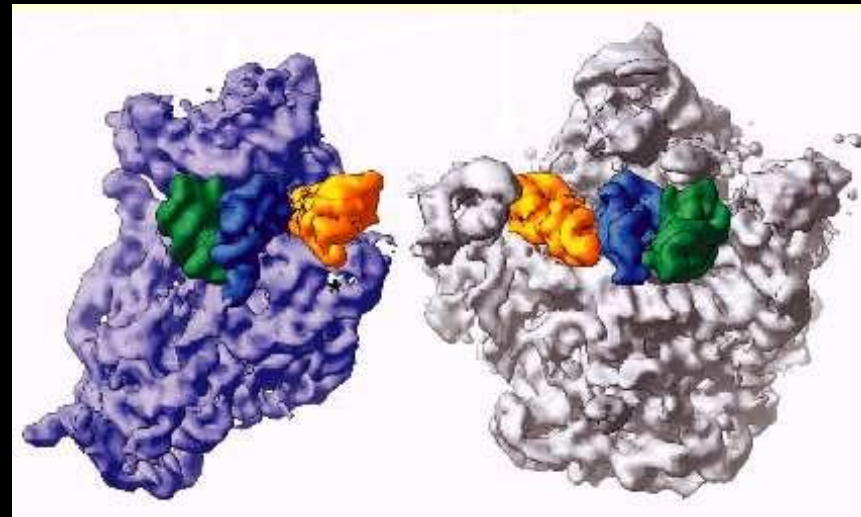
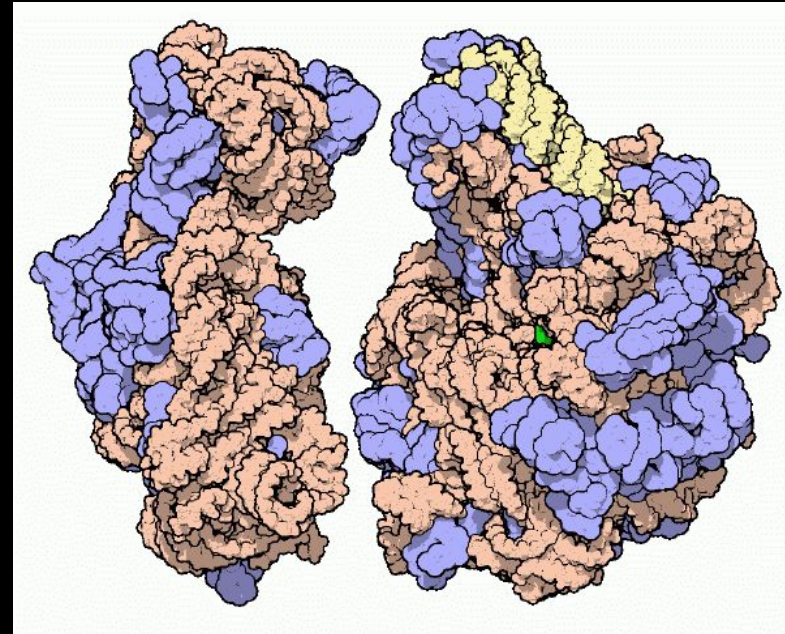


tRNA is L-shaped

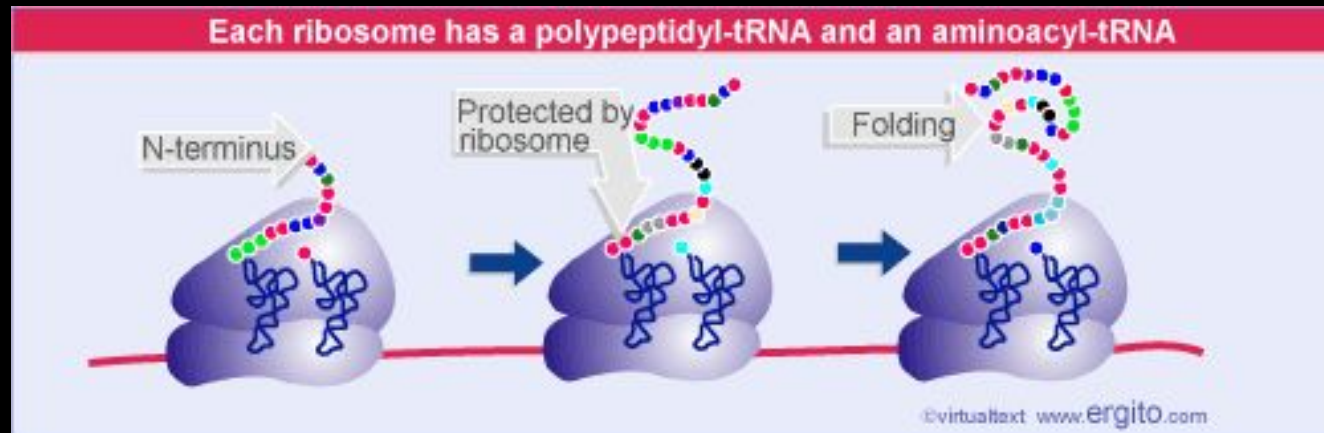


Ribosomal RNA

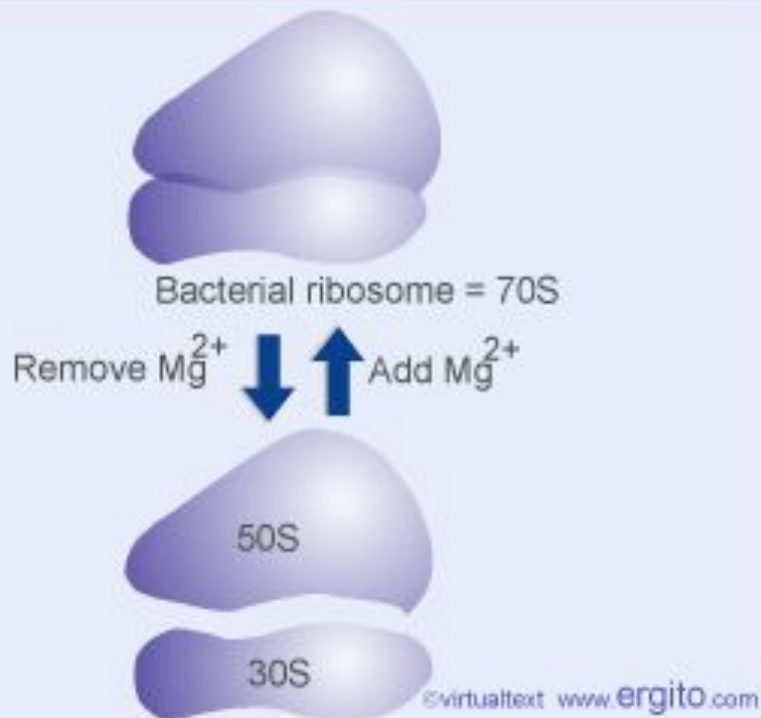
- ★ Ribosomal RNA: contributes to the structure of Ribosomes. In eukaryotes rRNA is transcribed exclusively in the nucleolus. The primary transcript is processed by an enzyme to produce the shorter rRNA constituting the ribosome.
- ★ Ribosomes are made of **40S** subunit (small) and **60S** subunit (large). Proteins make up about half of the ribosome. The complete ribosome is 80S.



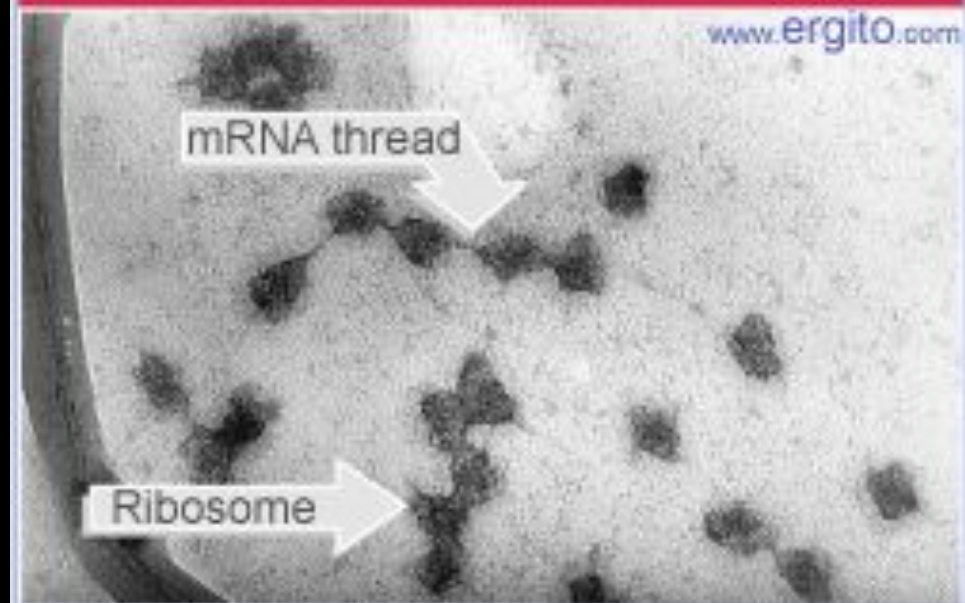
Ribosomes



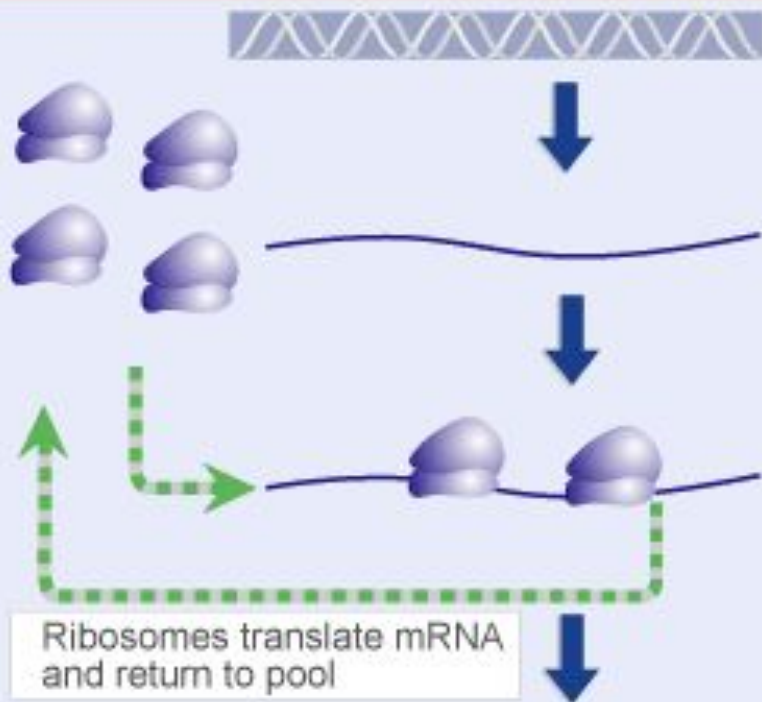
Ribosomes dissociate into subunits



Hemoglobin is synthesized on pentasomes



Ribosomes recycle for translation



©virtualtext www.ergito.com

~30% of bacterial dry mass is concerned with gene expression

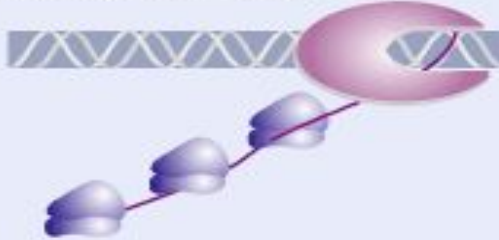
Component	Dry Cell Mass (%)	Molecules /cell	Different types	Copies of each type
Wall	10	1	1	1
Membrane	10	2	2	1
DNA	1.5	1	1	1
mRNA	1	1,500	600	2-3
tRNA	3	200,000	60	>3,000
rRNA	16	38,000	2	19,000
Ribosomal proteins	9	10^6	52	19,000
Soluble proteins	46	2.0×10^6	1,850	>1,000
Small molecules	3	7.5×10^6	800	

©virtualtext www.ergito.com

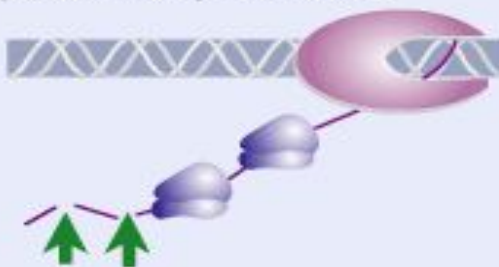
0 Transcription begins



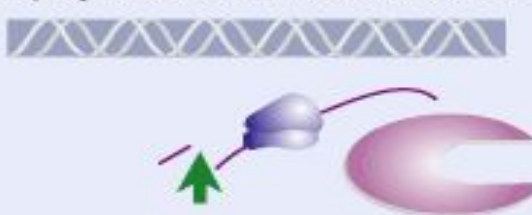
0.5 Ribosomes begin translation



1.5 Degradation begins at 5' end



2.0 RNA polymerase terminates at 3' end

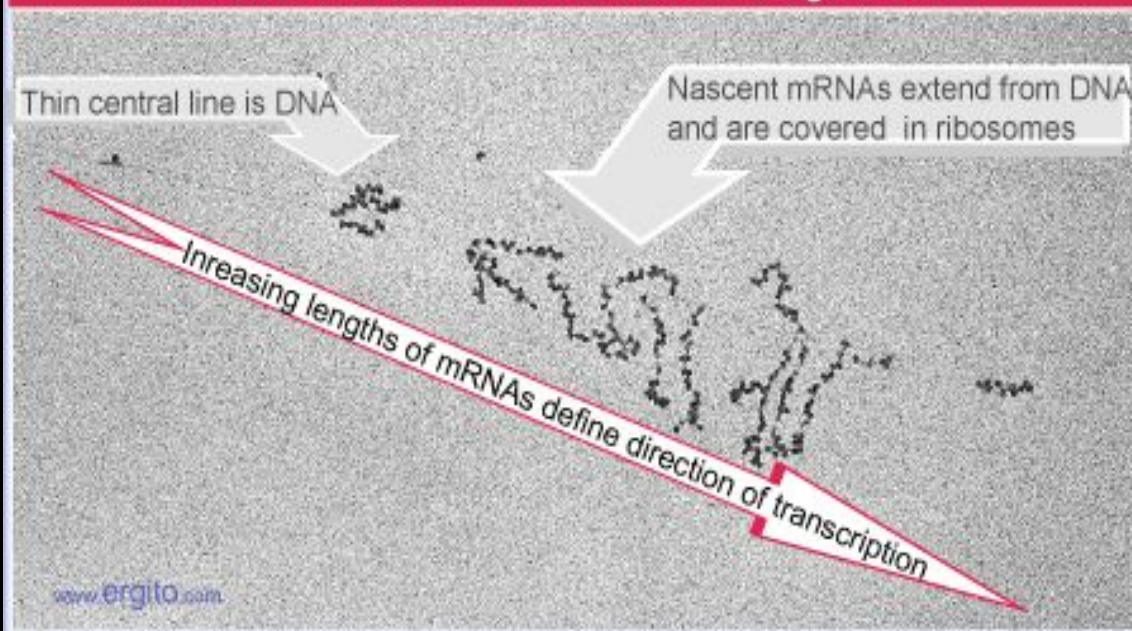


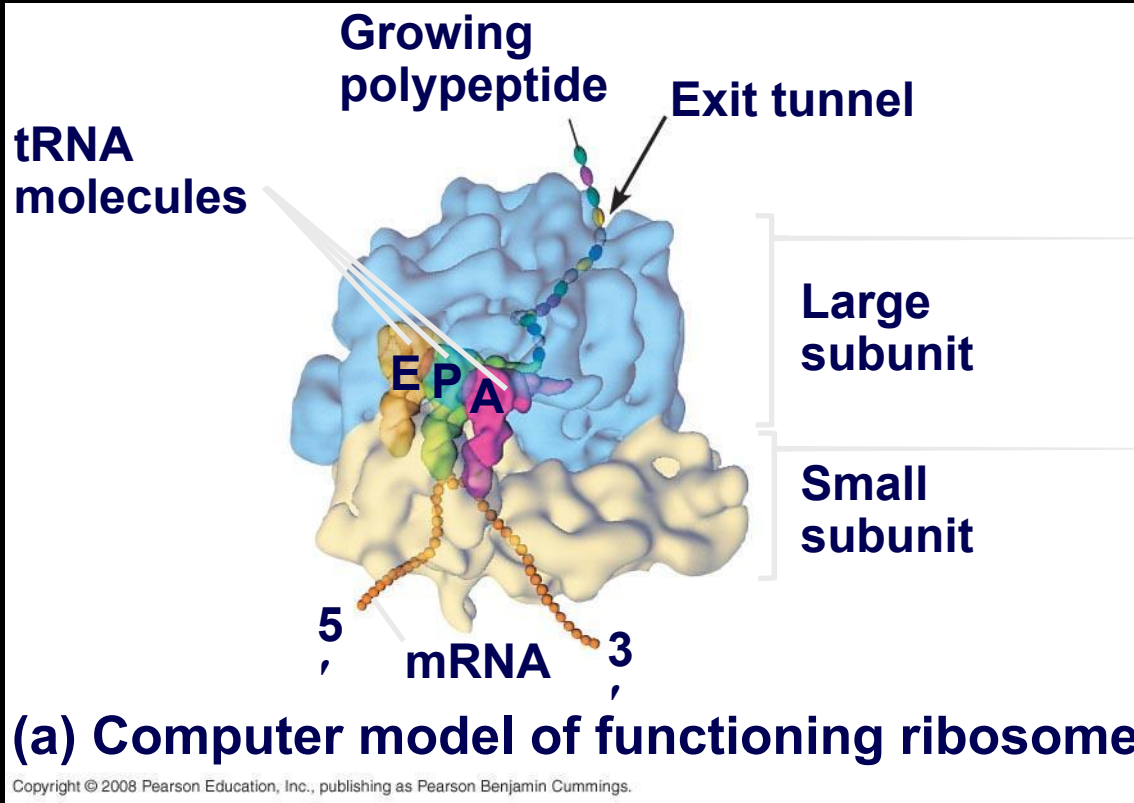
3.0 Degradation continues, ribosomes complete translation

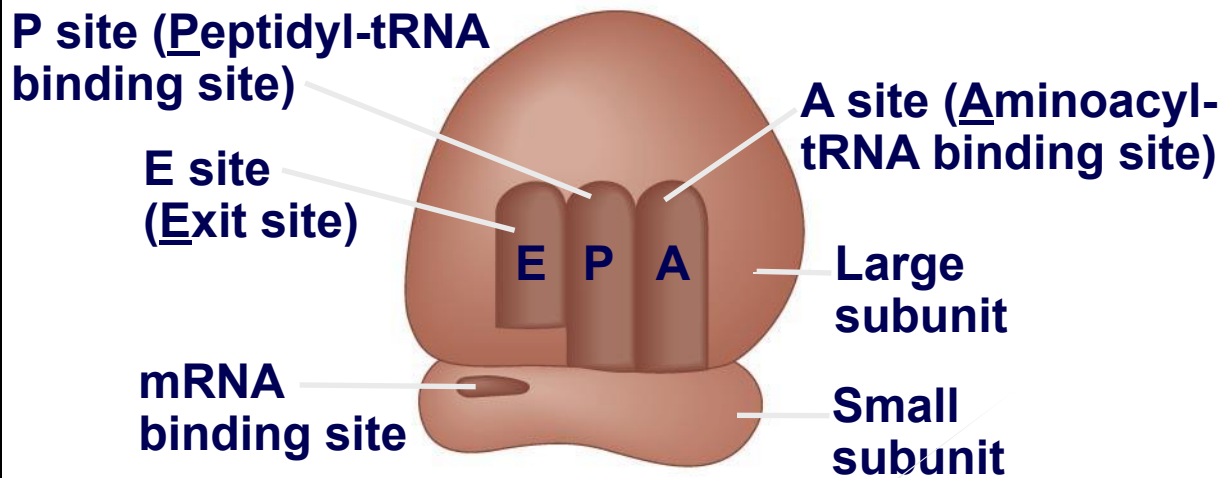


In Prokaryotes

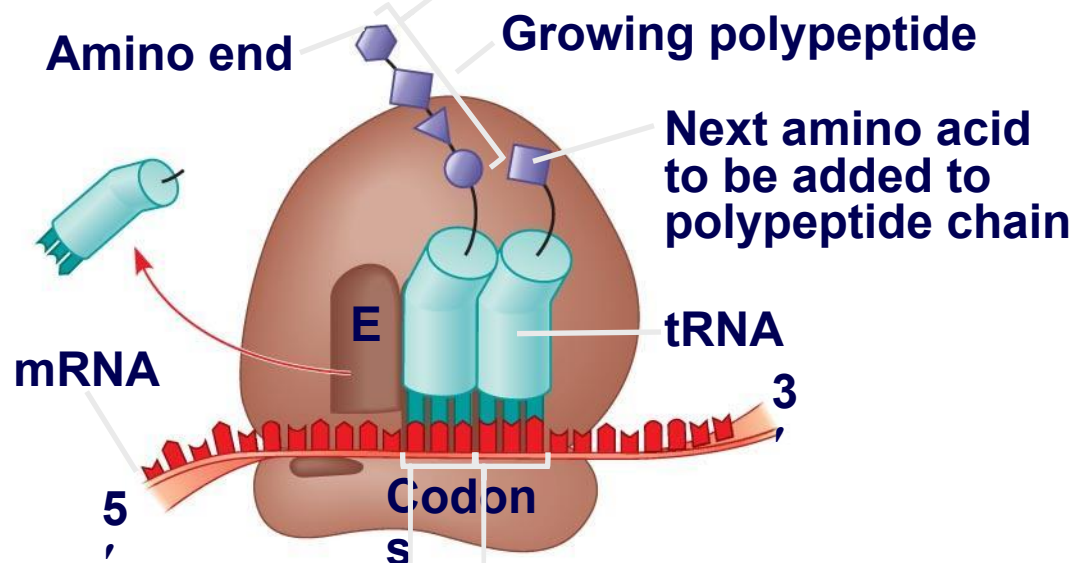
Bacterial mRNAs are translated while still being transcribed







(b) Schematic model showing binding sites



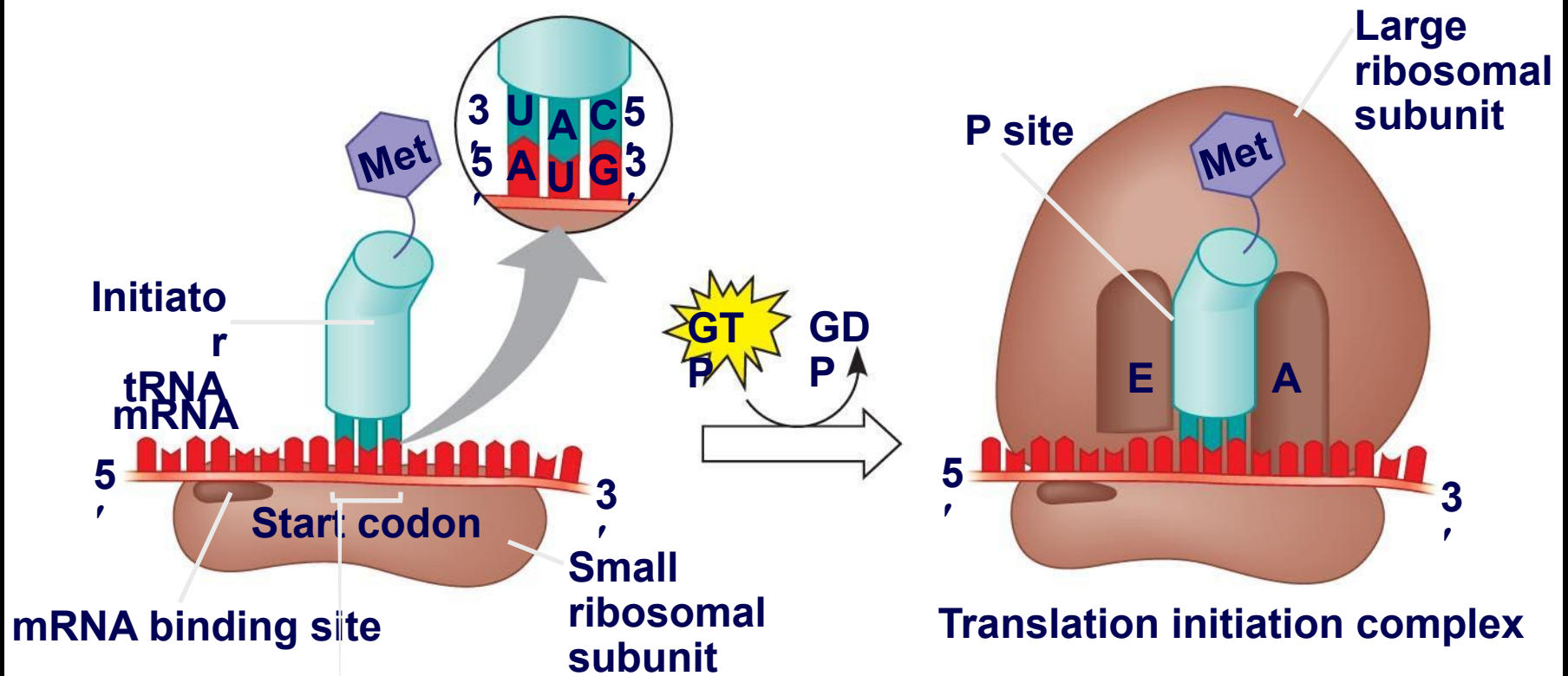
(c) Schematic model with mRNA and tRNA

✱ A ribosome has three binding sites for tRNA:

- ✱ The **P site** holds the tRNA that carries the growing polypeptide chain
 - ✱ The **A site** holds the tRNA that carries the next amino acid to be added to the chain
 - ✱ The **E site** is the exit site, where discharged tRNAs leave the ribosome
-

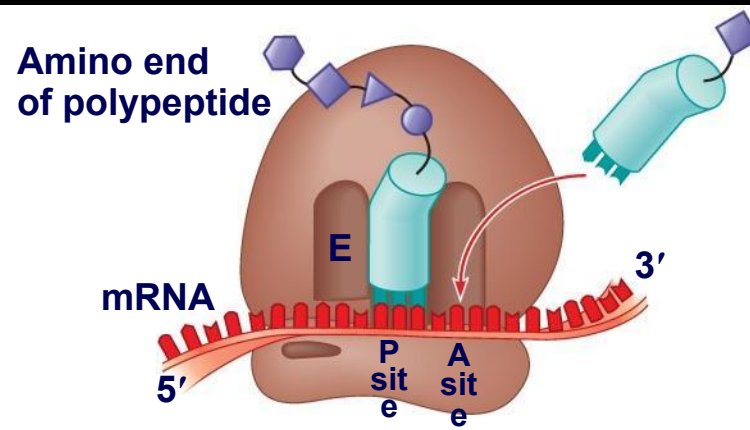
Ribosome Association and Initiation of Translation

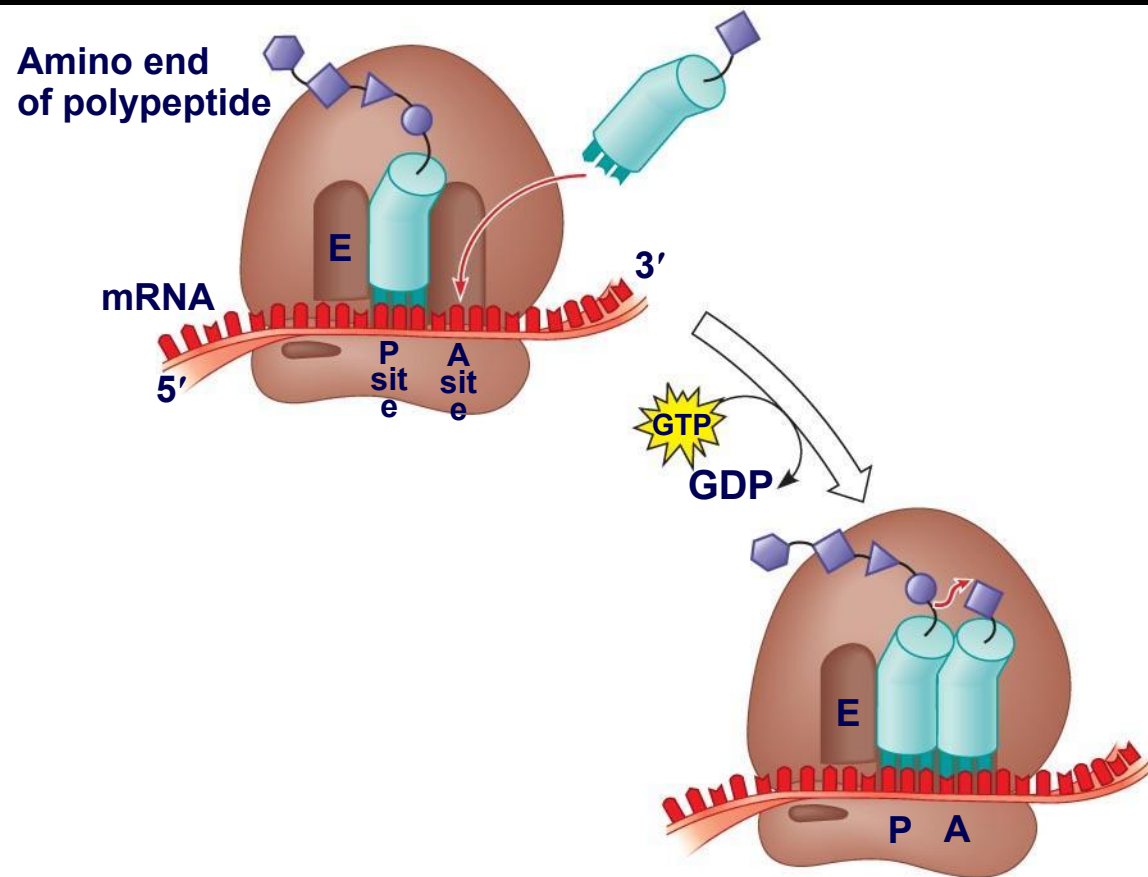
- ✱ The initiation stage of translation brings together mRNA, a tRNA with the first amino acid, and the two ribosomal subunits
 - ✱ First, a small ribosomal subunit binds with mRNA and a special initiator tRNA
 - ✱ Then the small subunit moves along the mRNA until it reaches the start codon (AUG)
 - ✱ Proteins called initiation factors bring in the large subunit that completes the translation initiation complex
-

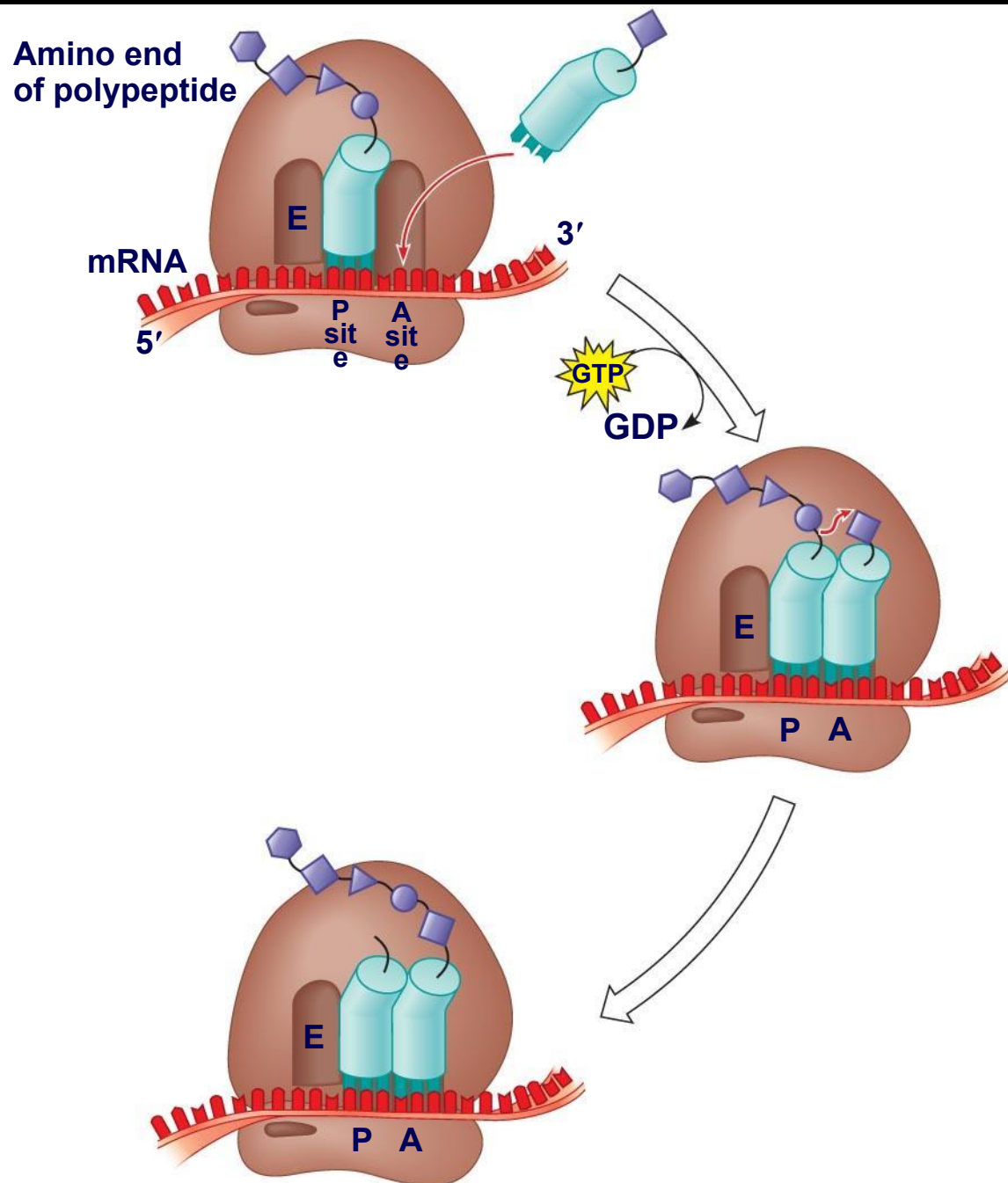


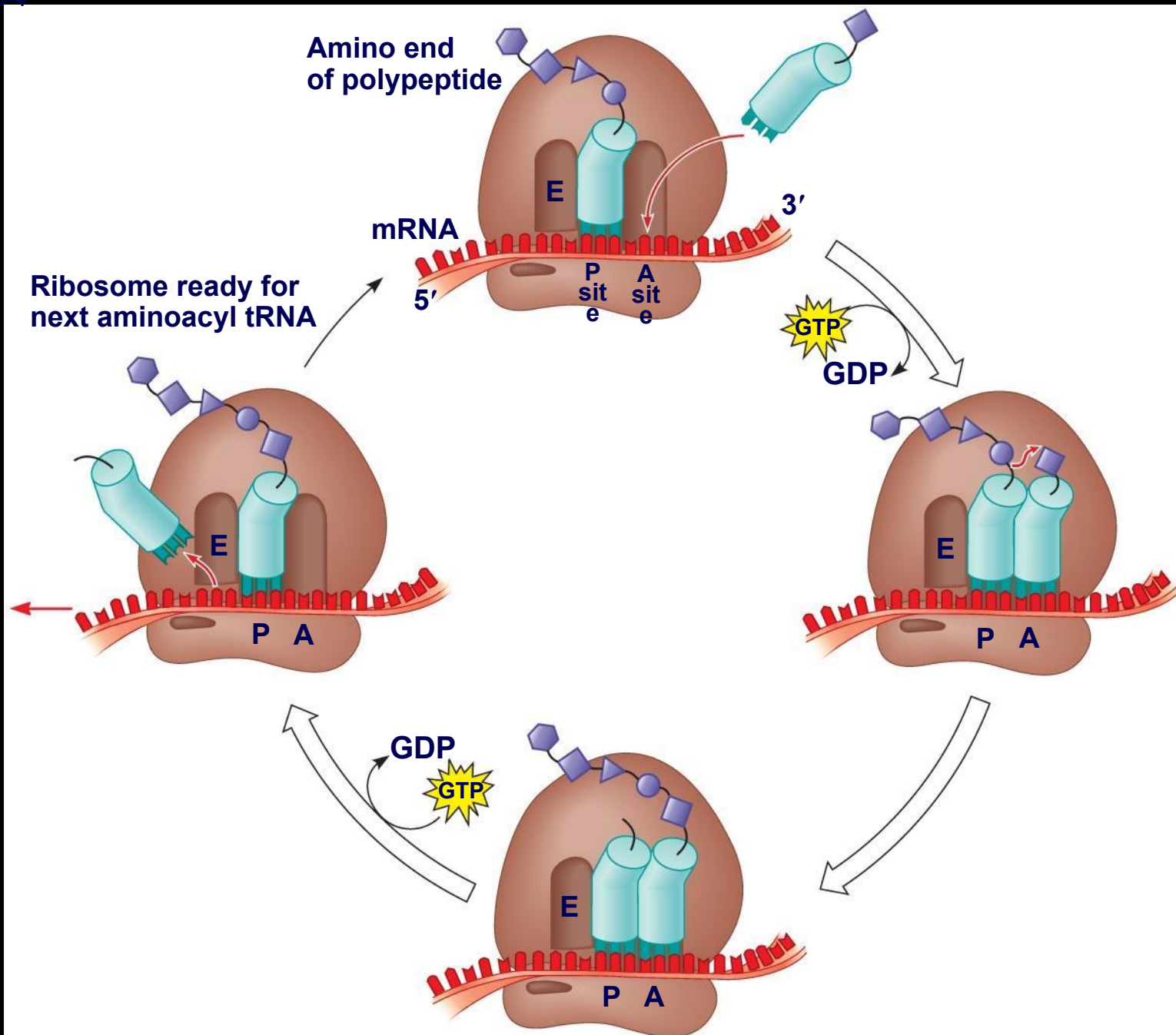
Elongation of the Polypeptide Chain

- ✦ During the elongation stage, amino acids are added one by one to the preceding amino acid
 - ✦ Each addition involves proteins called elongation factors and occurs in three steps: codon recognition, peptide bond formation, and translocation
-



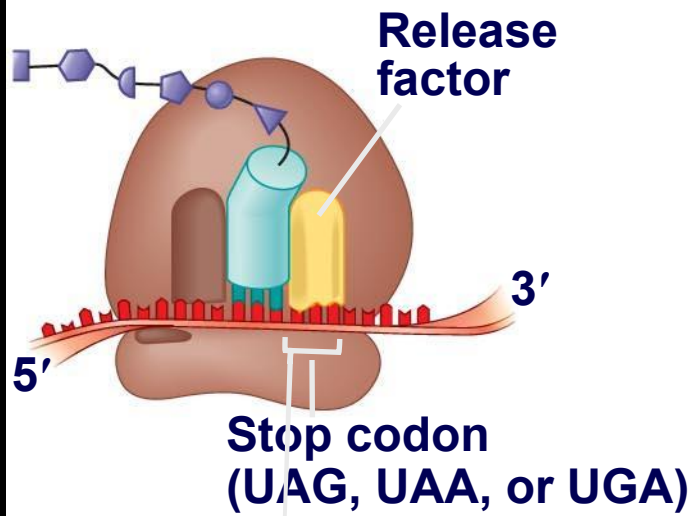


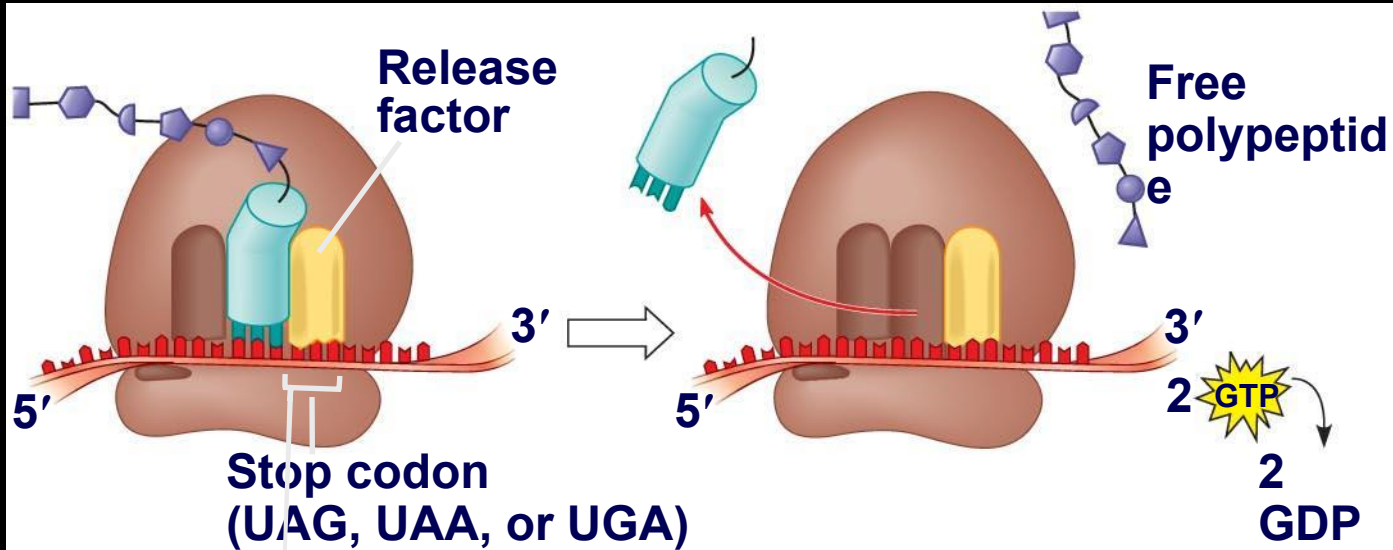


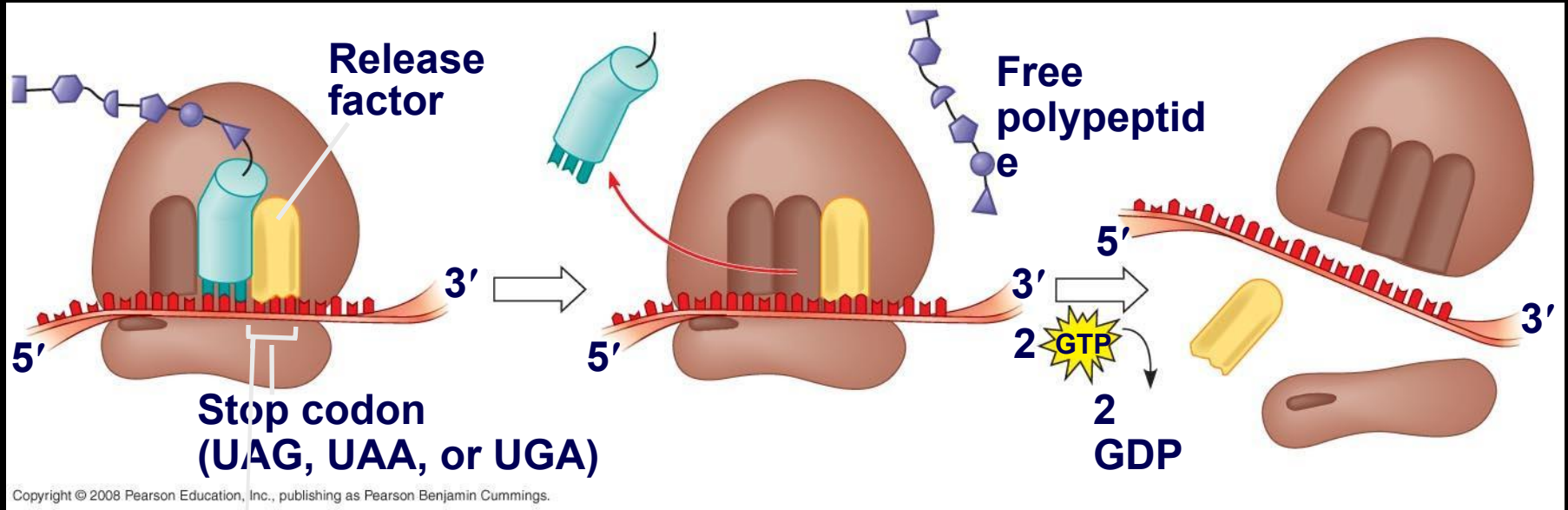


Termination of Translation

- ✱ Termination occurs when a stop codon in the mRNA reaches the A site of the ribosome
 - ✱ The A site accepts a protein called a release factor
 - ✱ The release factor causes the addition of a water molecule instead of an amino acid
 - ✱ This reaction releases the polypeptide, and the translation assembly then comes apart
-







Completing and Targeting the Functional Protein

- ✦ Often translation is not sufficient to make a functional protein
 - ✦ Polypeptide chains are modified after translation
 - ✦ Completed proteins are targeted to specific sites in the cell
-

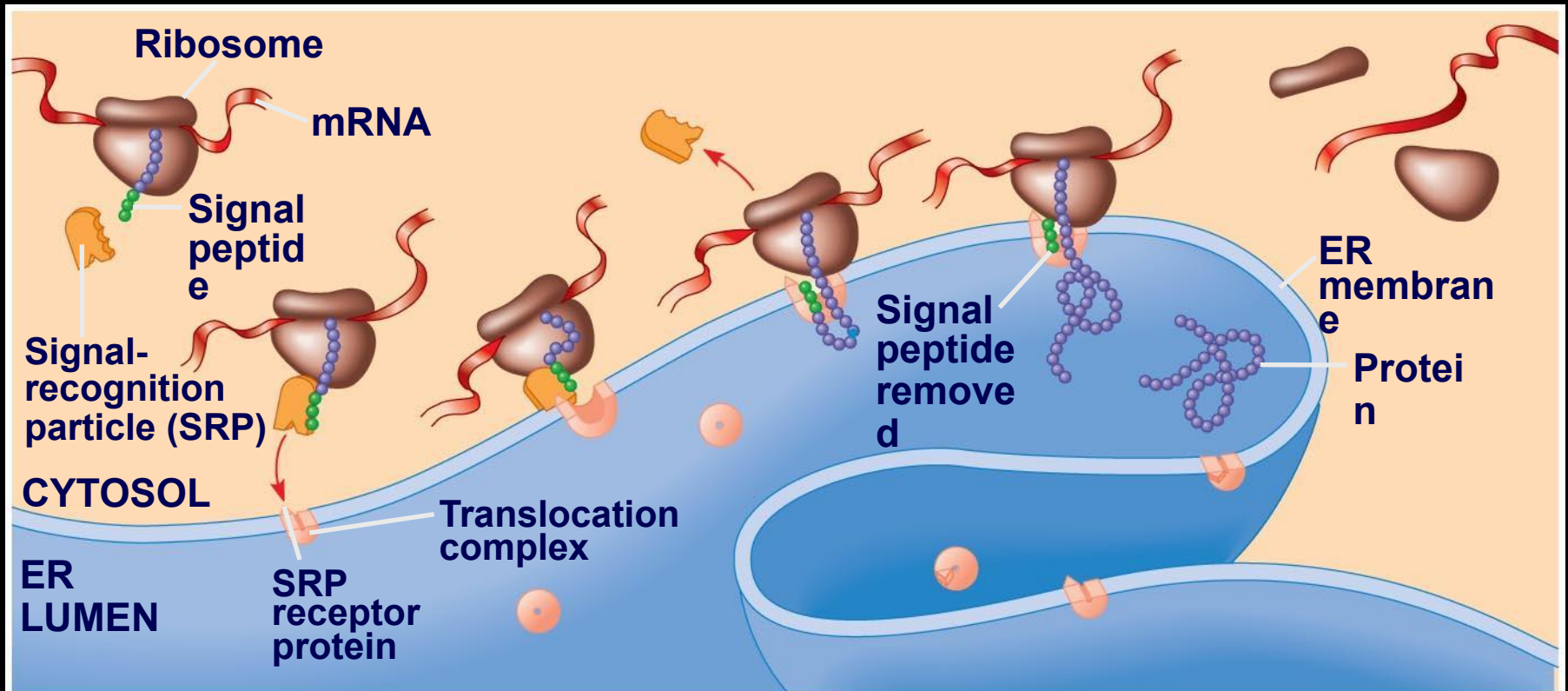
Protein Folding and Post-Translational Modifications

- ✱ During and after synthesis, a polypeptide chain spontaneously coils and folds into its three-dimensional shape
 - ✱ Proteins may also require post-translational modifications before doing their job
 - ✱ Some polypeptides are activated by enzymes that cleave them
 - ✱ Other polypeptides come together to form the subunits of a protein
-

Targeting Polypeptides to Specific Locations

- ✱ Two populations of ribosomes are evident in cells: free ribosomes (in the cytosol) and bound ribosomes (attached to the ER)
 - ✱ Free ribosomes mostly synthesize proteins that function in the cytosol
 - ✱ Bound ribosomes make proteins of the endomembrane system and proteins that are secreted from the cell
 - ✱ Ribosomes are identical and can switch from free to bound
-

- Polypeptide synthesis always begins in the cytosol
 - Synthesis finishes in the cytosol *unless* the polypeptide signals the ribosome to attach to the ER
 - Polypeptides destined for the ER or for secretion are marked by a **signal peptide**
 - A **signal-recognition particle (SRP)** binds to the signal peptide
 - The SRP brings the signal peptide and its ribosome to the ER
-



Point mutations can affect protein structure and function

- ✦ **Mutations** are changes in the genetic material of a cell or virus
 - ✦ **Point mutations** are chemical changes in just one base pair of a gene
 - ✦ The change of a single nucleotide in a DNA template strand can lead to the production of an abnormal protein
-