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

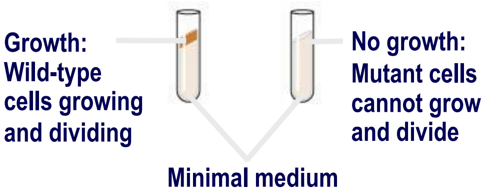
- mRNA carries the message. The linear amino acid sequence (primary) is encoded in the DNA. But the DNA does not make the proteins directly. mRNA is the link between gene and protein.
- The information contained in the mRNA is written in the genetic code. The genetic code is UNIVERSAL.
- The beginning 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

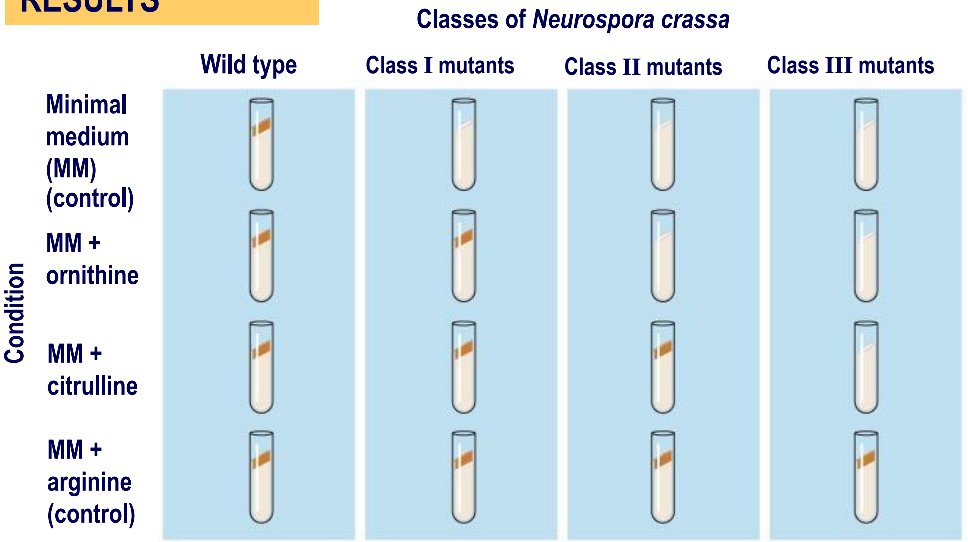


Growth: Wild-type cells growing and dividing

No growth: Mutant cells cannot grow and divide

Minimal medium

RESULTS



Classes of *Neurospora crassa*

Wild type

Class I mutants

Class II mutants

Class III mutants

Condition

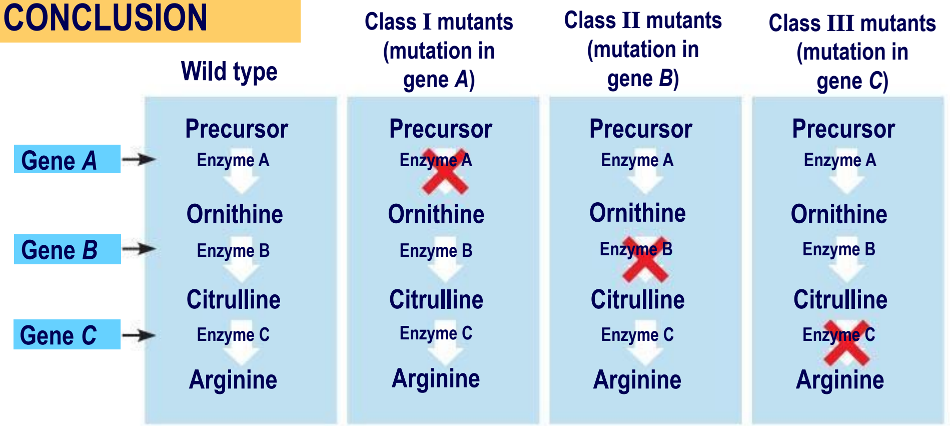
Minimal medium (MM) (control)

MM + ornithine

MM + citrulline

MM + arginine (control)

CONCLUSION



Wild type

Class I mutants (mutation in gene *A*)

Class II mutants (mutation in gene *B*)

Class III mutants (mutation in gene *C*)

Gene *A* →

Gene *B* →

Gene *C* →

Precursor

Enzyme A

Ornithine

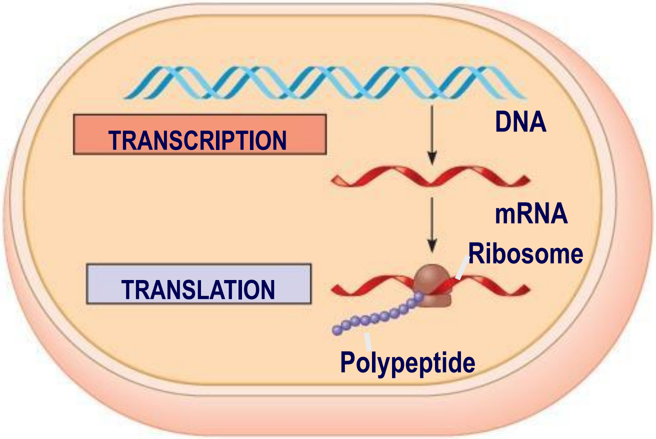
Enzyme B

Citrulline

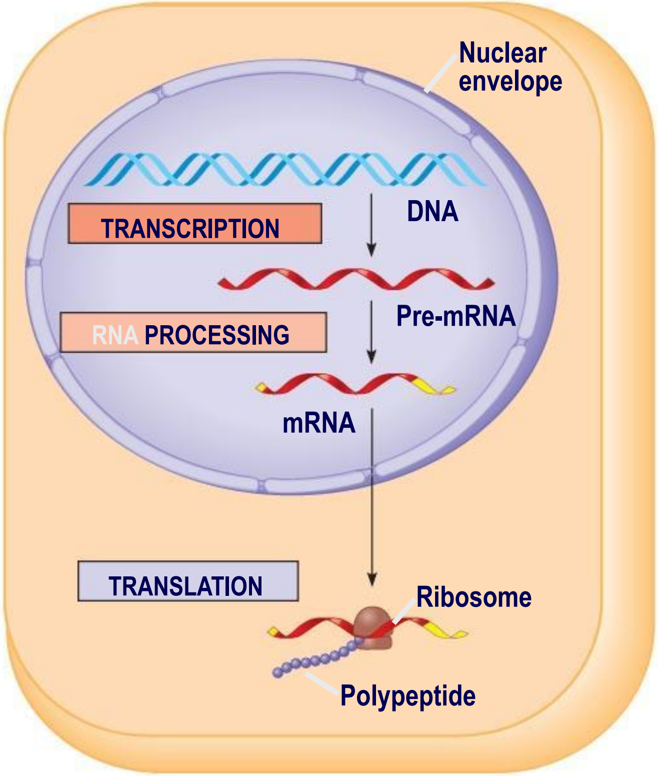
Enzyme C

Arginine

- 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



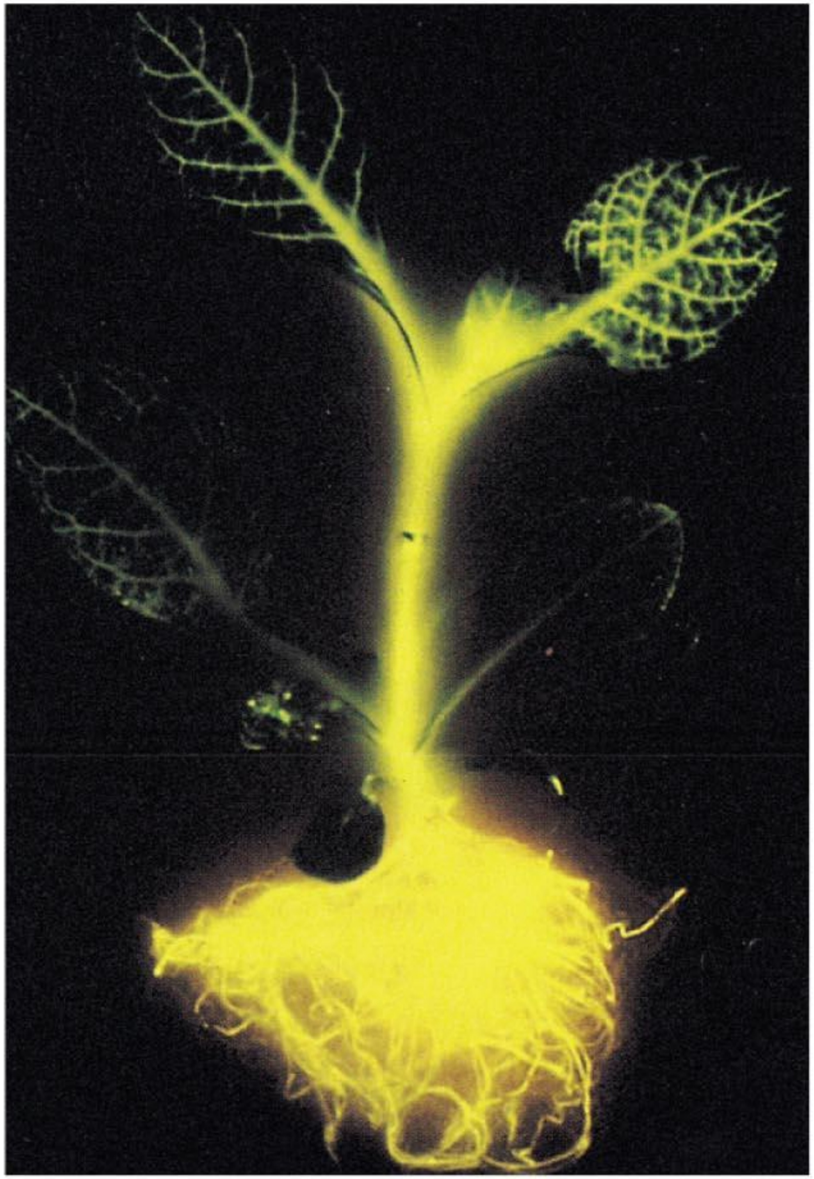
(a) Bacterial cell



(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

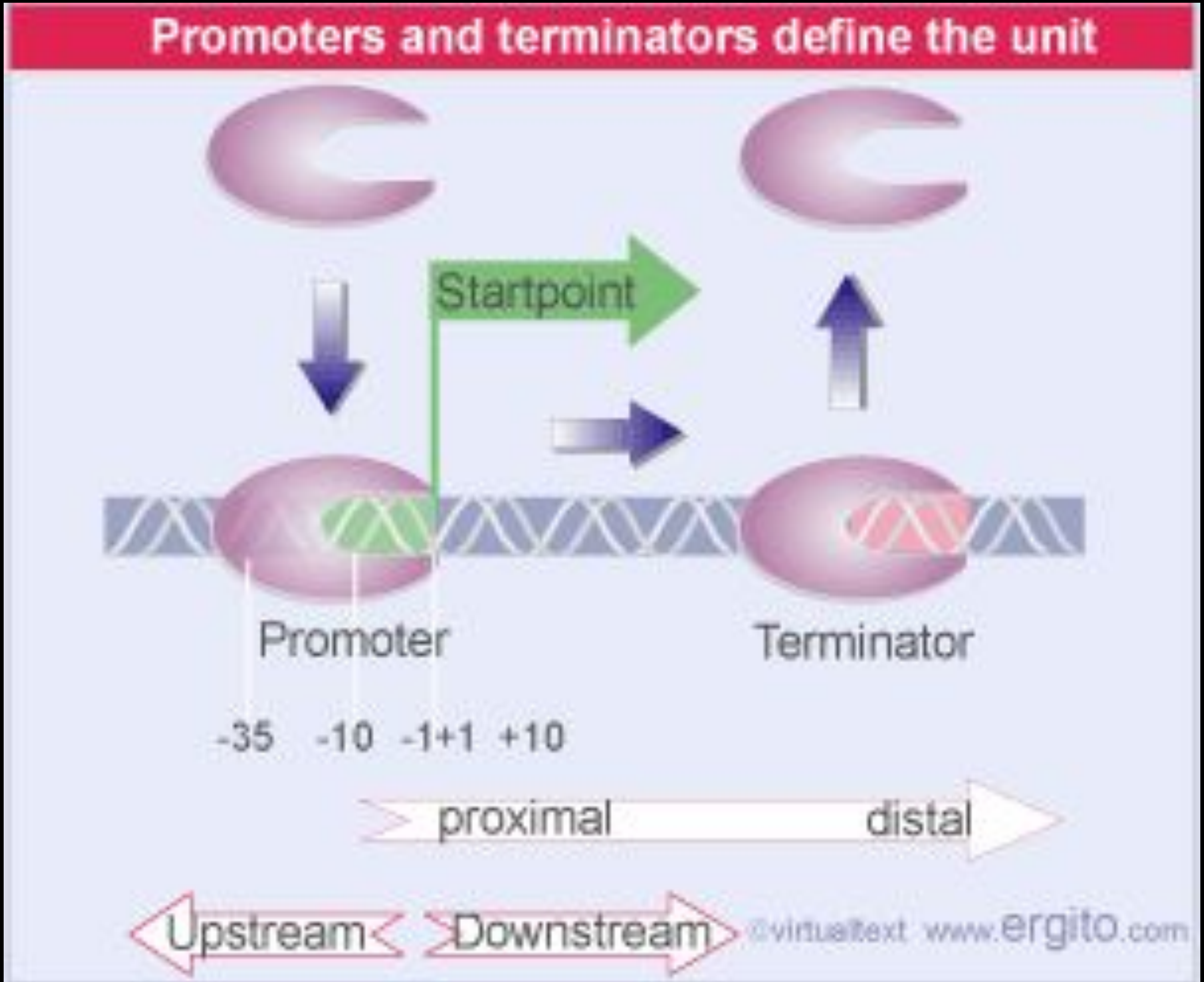
Key Words and definitions

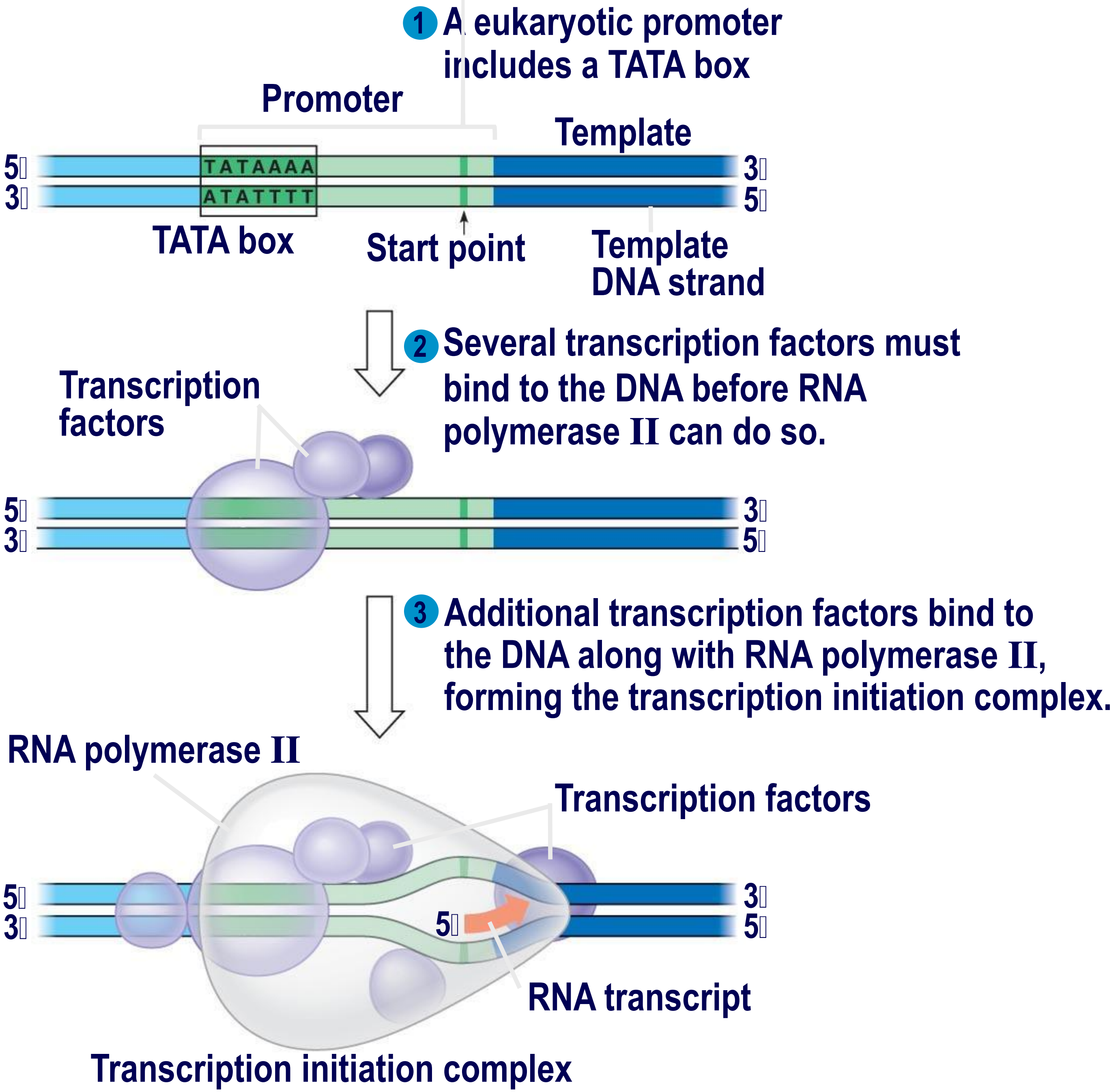
- **Transcription** describes the synthesis of RNA on a DNA template
- **Translation** is the synthesis of protein on a mRNA template
- **Coding region** of part of the gene that represents a protein sequence.
- **Codon** is a triplet of bases that represents an amino acid or a termination signal
- **The 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



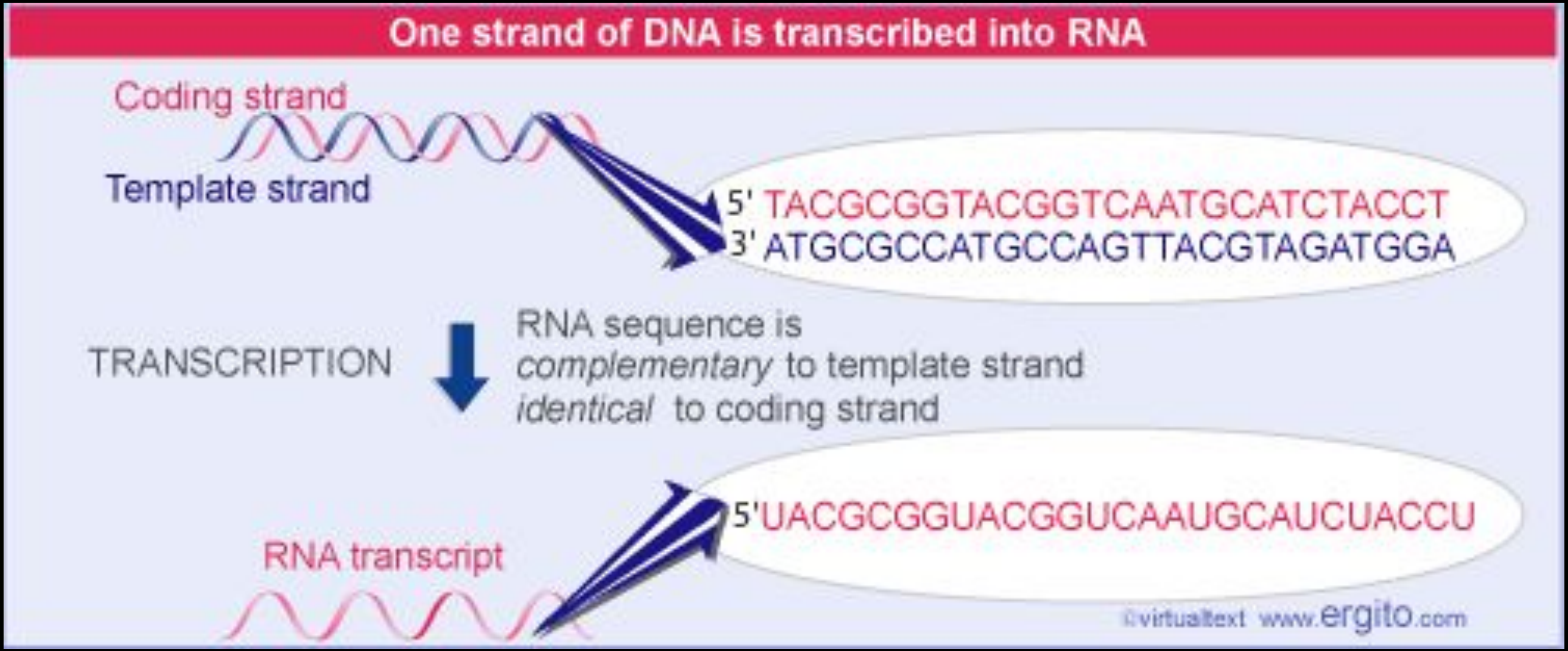


1 A eukaryotic promoter includes a TATA box

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



One strand of DNA is transcribed into RNA

5' TACGCGGTACGGTCAATGCATCTACCT
3' ATGCGCCATGCCAGTTACGTAGATGGA

RNA sequence is
complementary to template strand
identical to coding strand

5'UACGCGGUACGGUCAAUGCAUCUACCU

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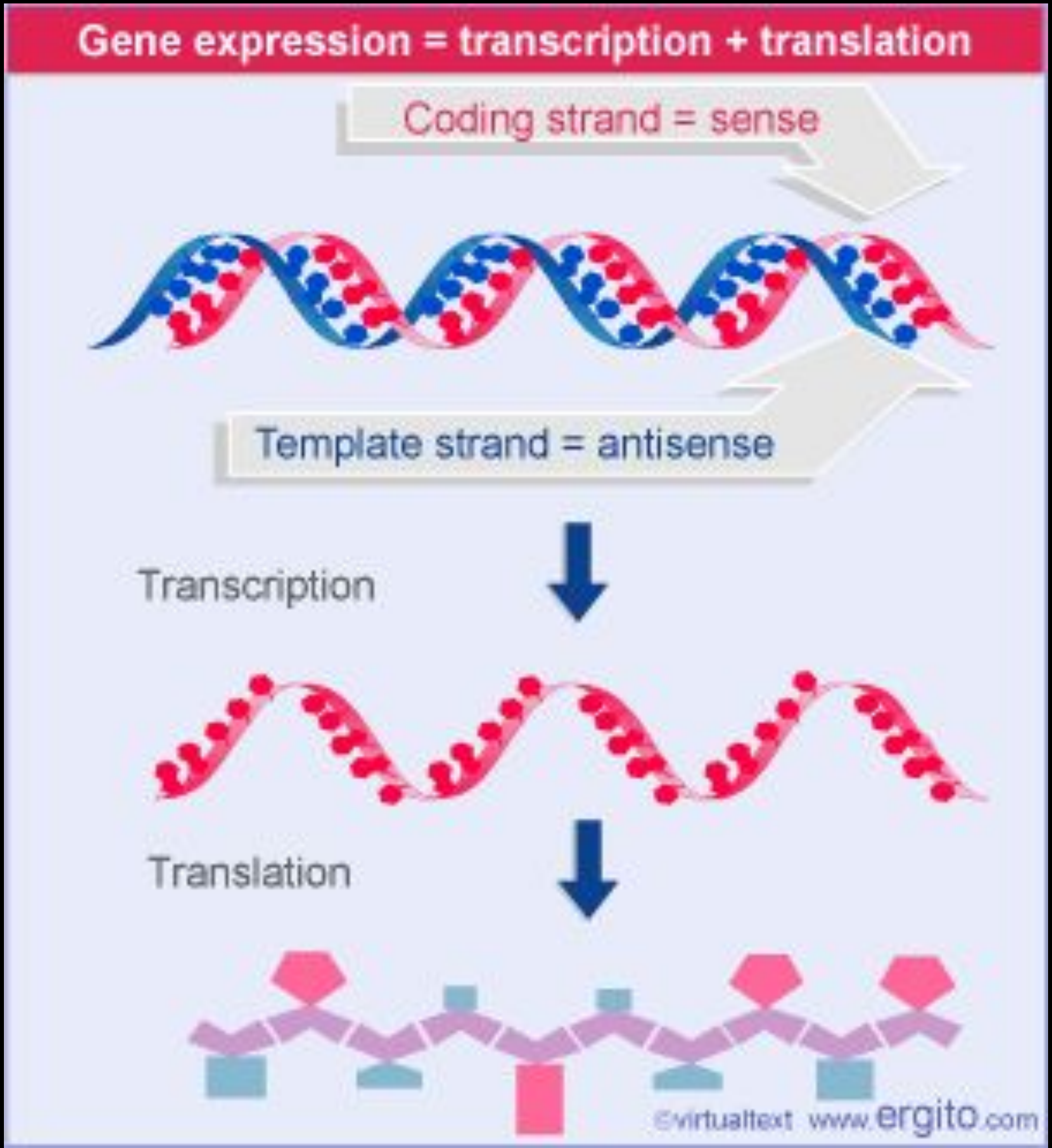
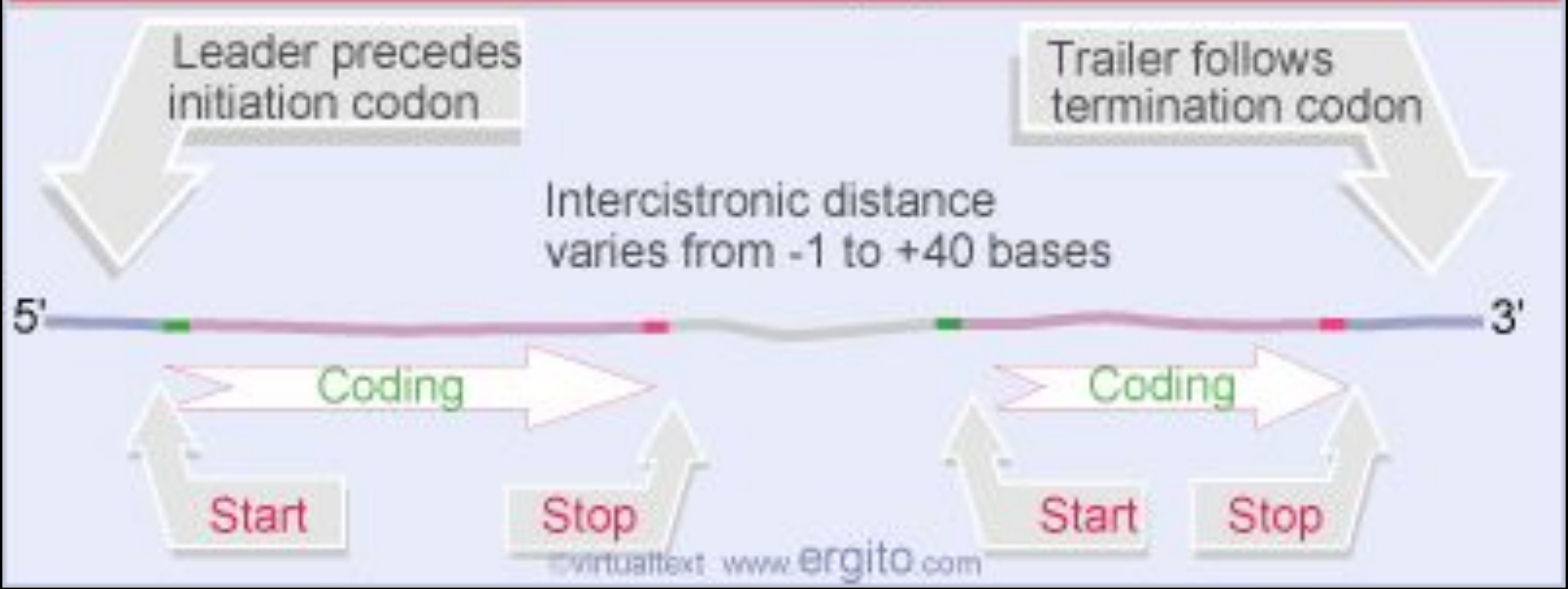
Sense and antisense strands



Bacterial mRNA is polycistronic



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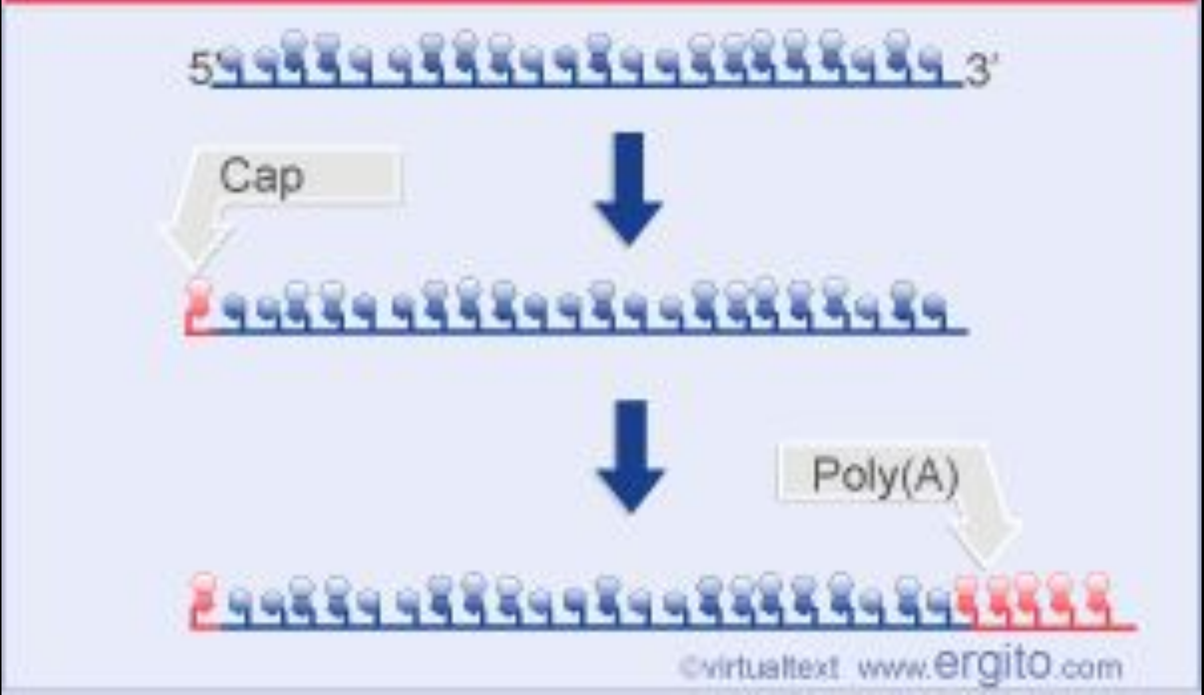
Eukaryotic mRNA is modified at both ends



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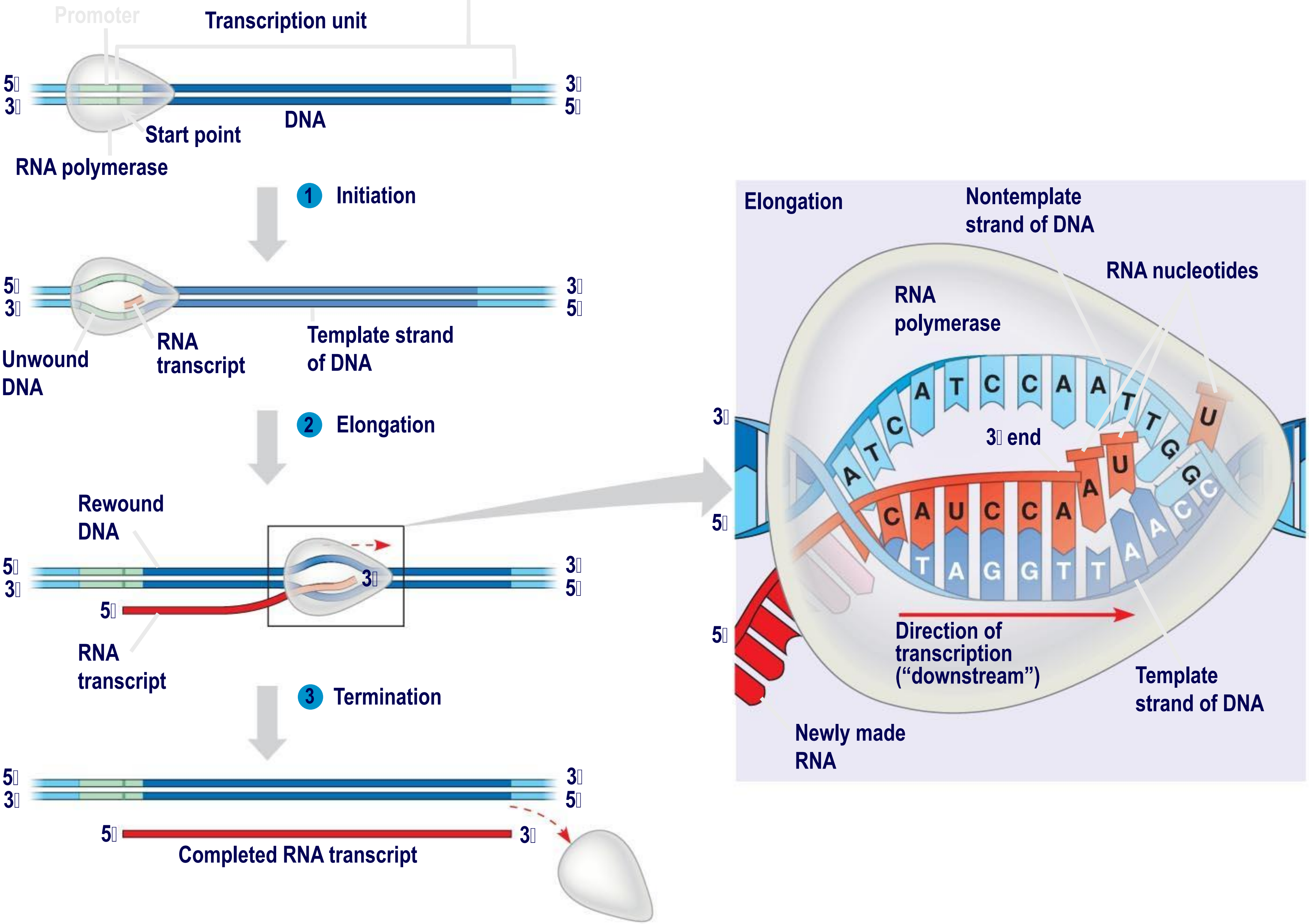
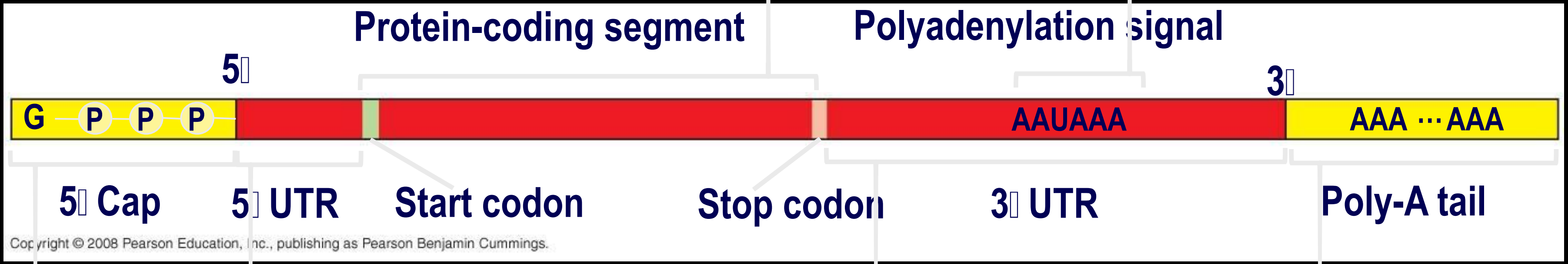
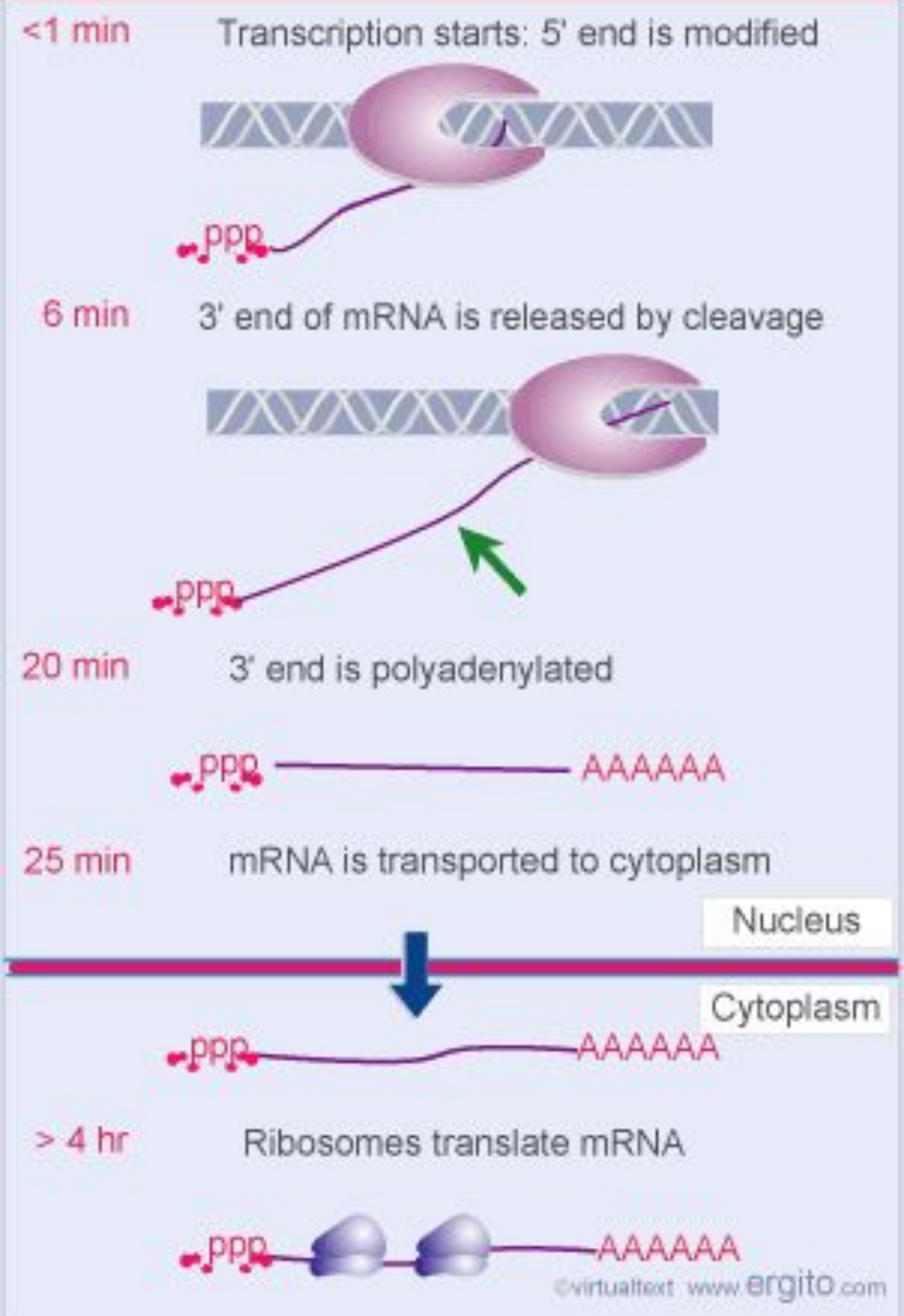

Promoter
Transcription unit
5′
3′
3′
5′
Start point
DNA
RNA polymerase
1 Initiation
5′
3′
3′
5′
Unwound DNA
RNA transcript
Template strand of DNA
2 Elongation
Rewound DNA
5′
3′
3′
5′
3′
5′
RNA transcript
3 Termination
5′
3′
3′
5′
5′
3′
Completed RNA transcript
Elongation
Nontemplate strand of DNA
RNA nucleotides
RNA polymerase
3′
5′
5′
A T C A T C C A A T T G G
U
3′ end
U
A
C A U C C A
T A G G T T A A C C
Direction of transcription (“downstream”)
Template strand of DNA
Newly made RNA


Protein-coding segment
Polyadenylation signal
5′
3′
G
P
P
P
AAUAAA
AAA ··· AAA
5′ Cap
5′ UTR
Start codon
Stop codon
3′ UTR
Poly-A tail

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

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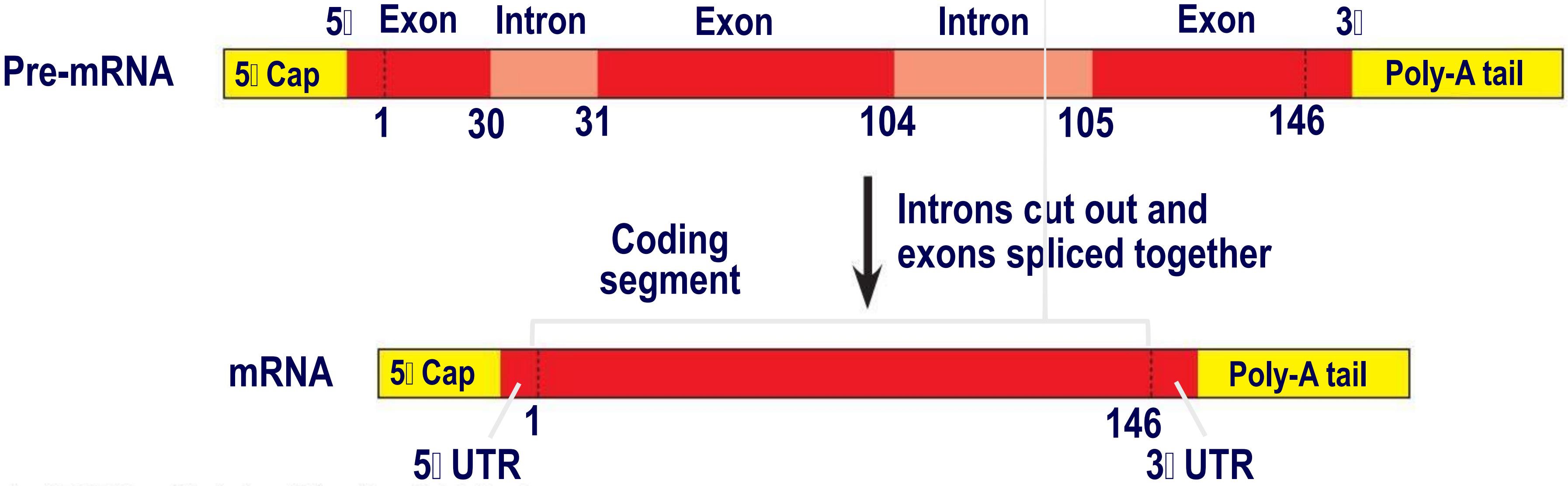
Split Genes and RNA Splicing

- 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



Pre-mRNA

5′ Exon Intron Exon Intron Exon 3′

5′ Cap

Poly-A tail

1 30 31 104 105 146

Introns cut out and exons spliced together

Coding segment

mRNA

5′ Cap

Poly-A tail

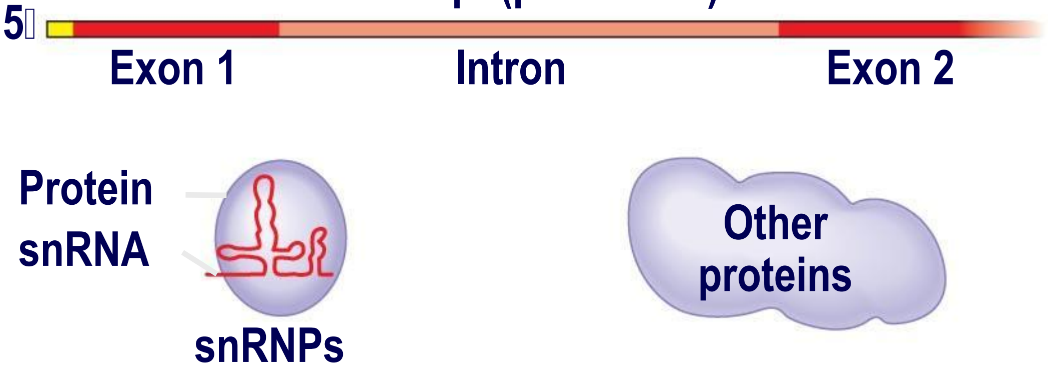
1 146

5′ UTR

3′ UTR

- 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)



5′

Exon 1

Intron

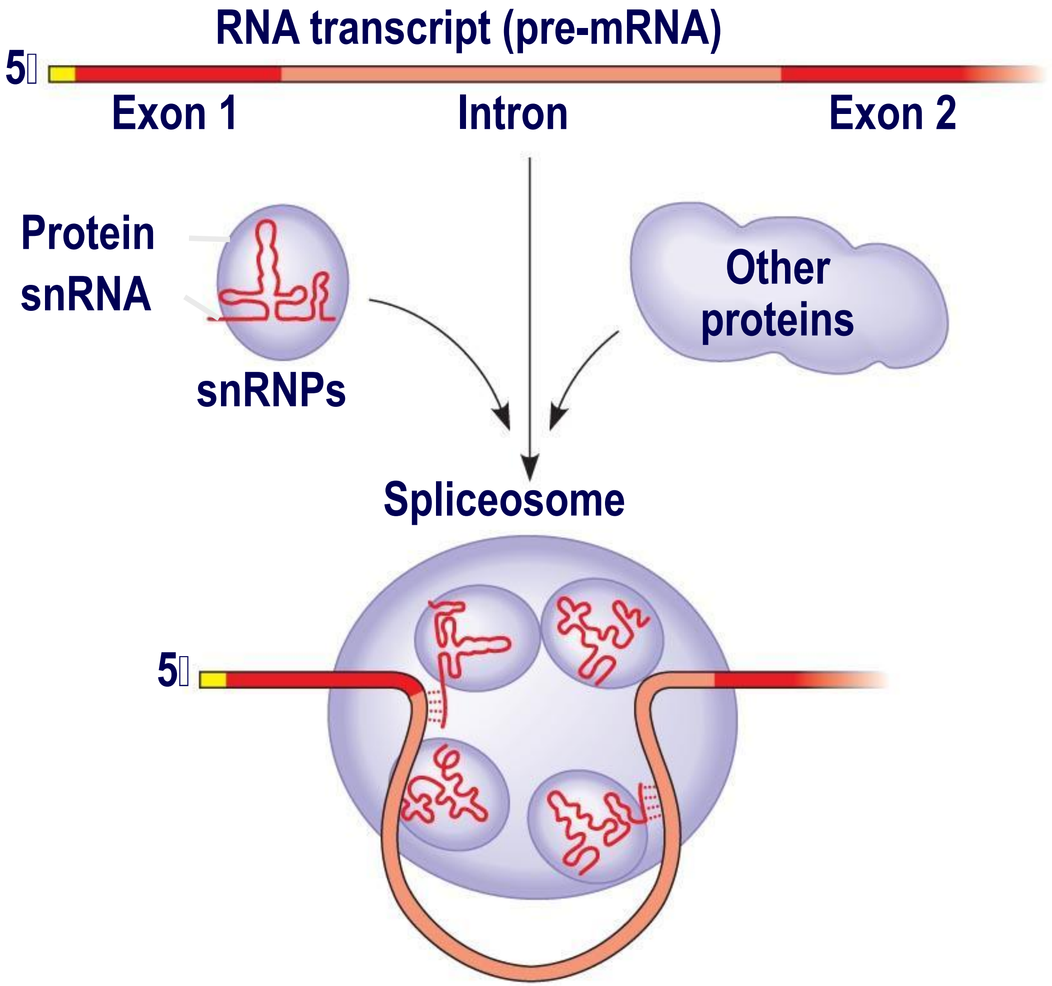
Exon 2

Protein

snRNA

snRNPs

Other proteins



RNA transcript (pre-mRNA)

5′

Exon 1

Intron

Exon 2

Protein

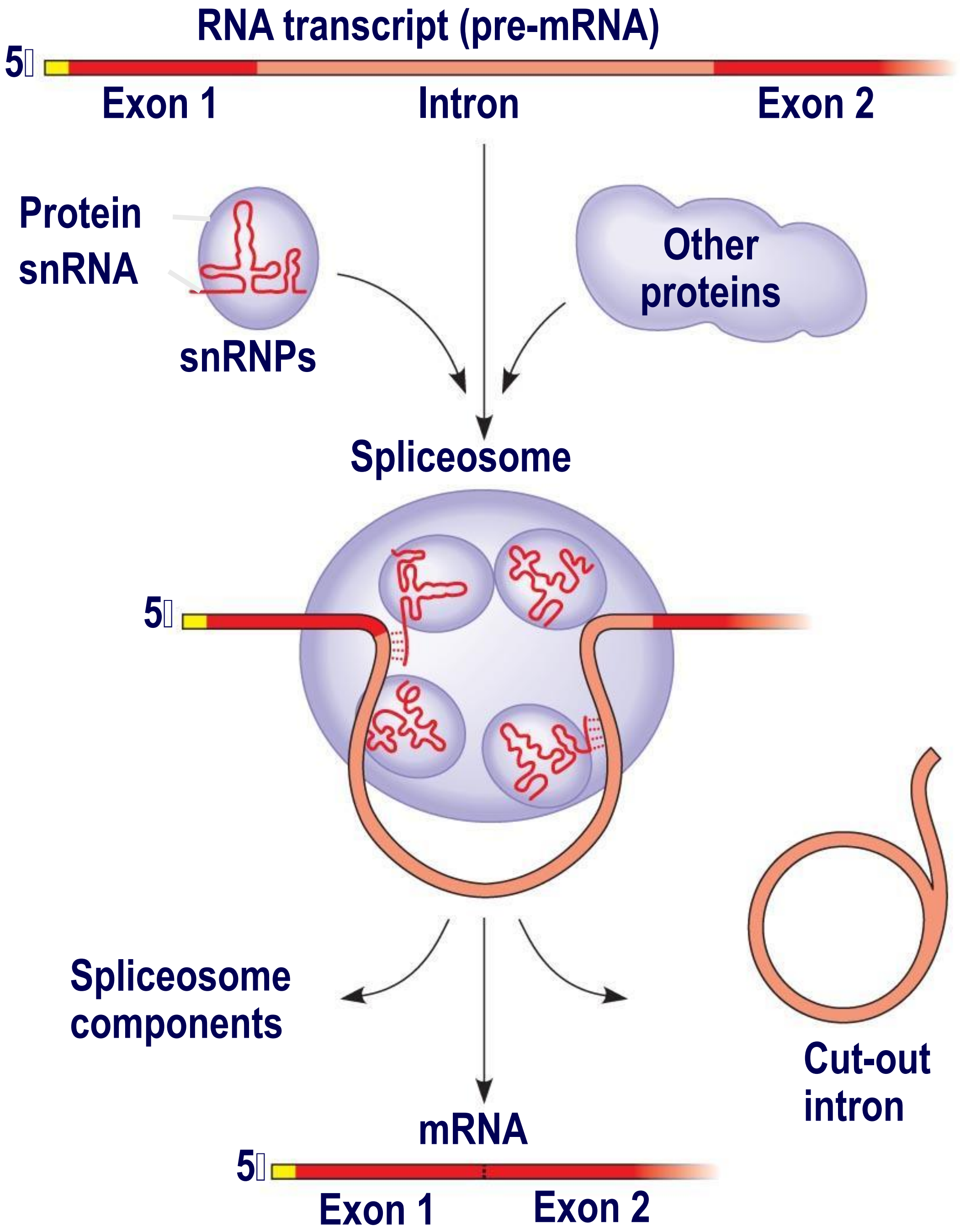
snRNA

snRNPs

Other proteins

Spliceosome

5′

RNA transcript (pre-mRNA)
5′
Exon 1
Intron
Exon 2
Protein
snRNA
snRNPs
Other proteins
Spliceosome
5′
Spliceosome components
Cut-out intron
mRNA
5′
Exon 1
Exon 2

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

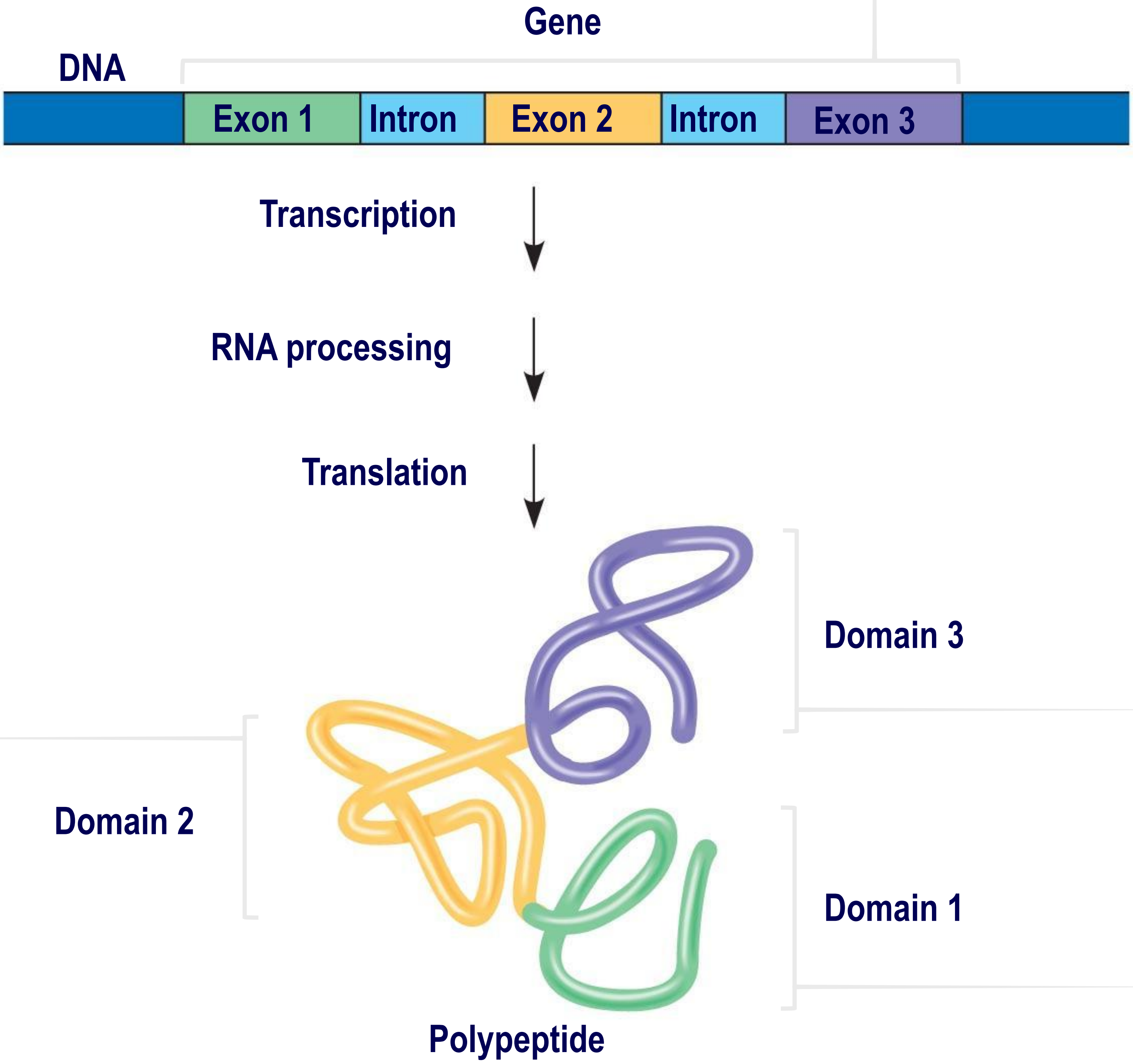
- Three properties of RNA enable it to function as an enzyme
  - It can form a three-dimensional structure because of its ability to base pair with itself
  - Some bases in RNA contain functional groups
  - RNA may hydrogen-bond with other nucleic acid molecules

The Functional and Evolutionary Importance of Introns

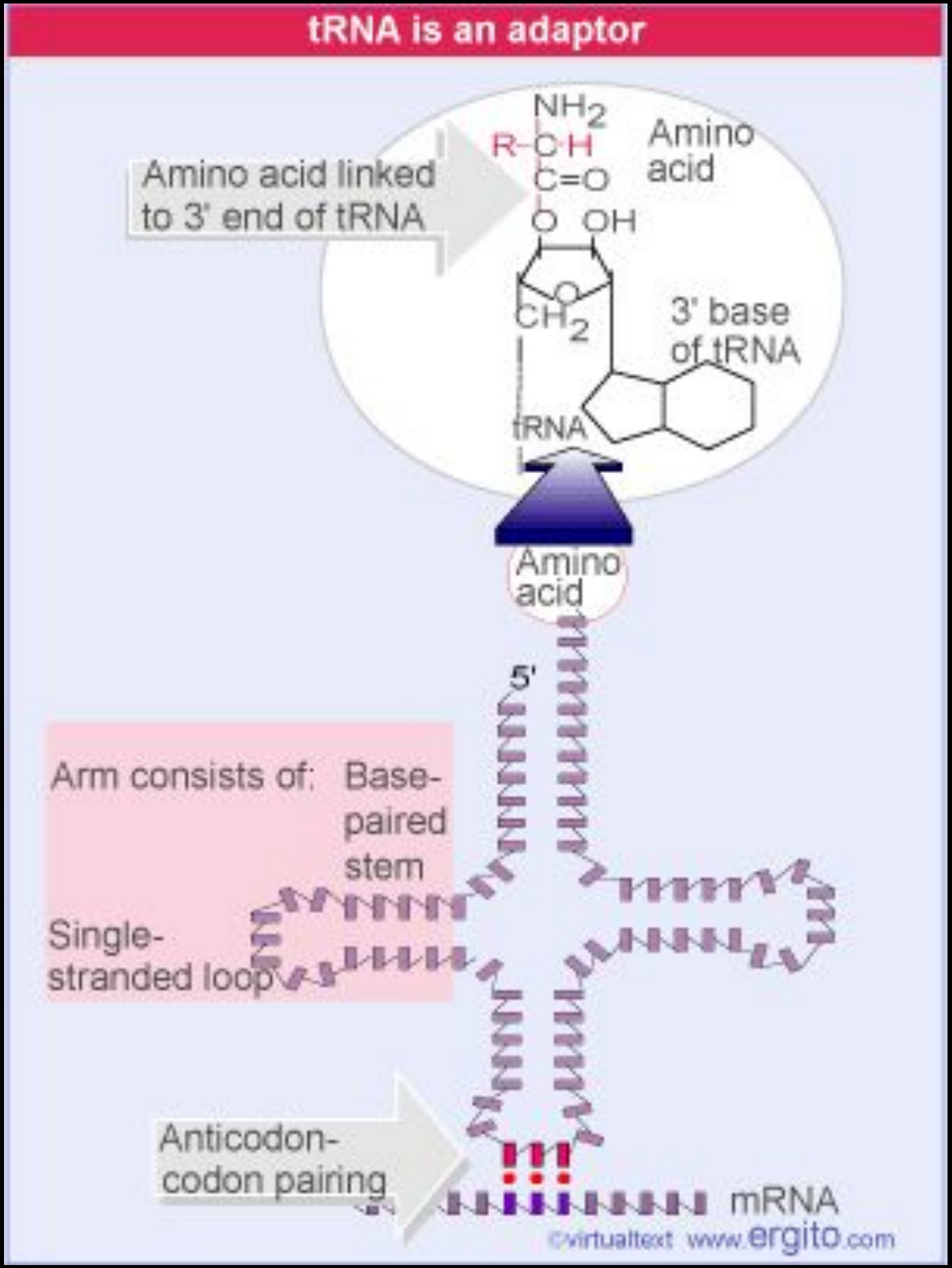
- Some genes can encode more than one kind of polypeptide, depending on which segments are treated as exons during RNA splicing
- Such variations are called **alternative RNA splicing**
- Because of alternative splicing, the number of different proteins an organism can produce is much greater than its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


Gene
DNA
Exon 1
Intron
Exon 2
Intron
Exon 3
Transcription
RNA processing
Translation
Domain 3
Domain 2
Domain 1
Polypeptide

tRNA


tRNA is an adaptor
Amino acid linked to 3' end of tRNA
NH2
R-C-H
C=O
O OH
CH2
Amino acid
3' base of tRNA
tRNA
Amino acid
5'
Arm consists of:
Base-paired stem
Single-stranded loop
Anticodon-codon pairing
mRNA
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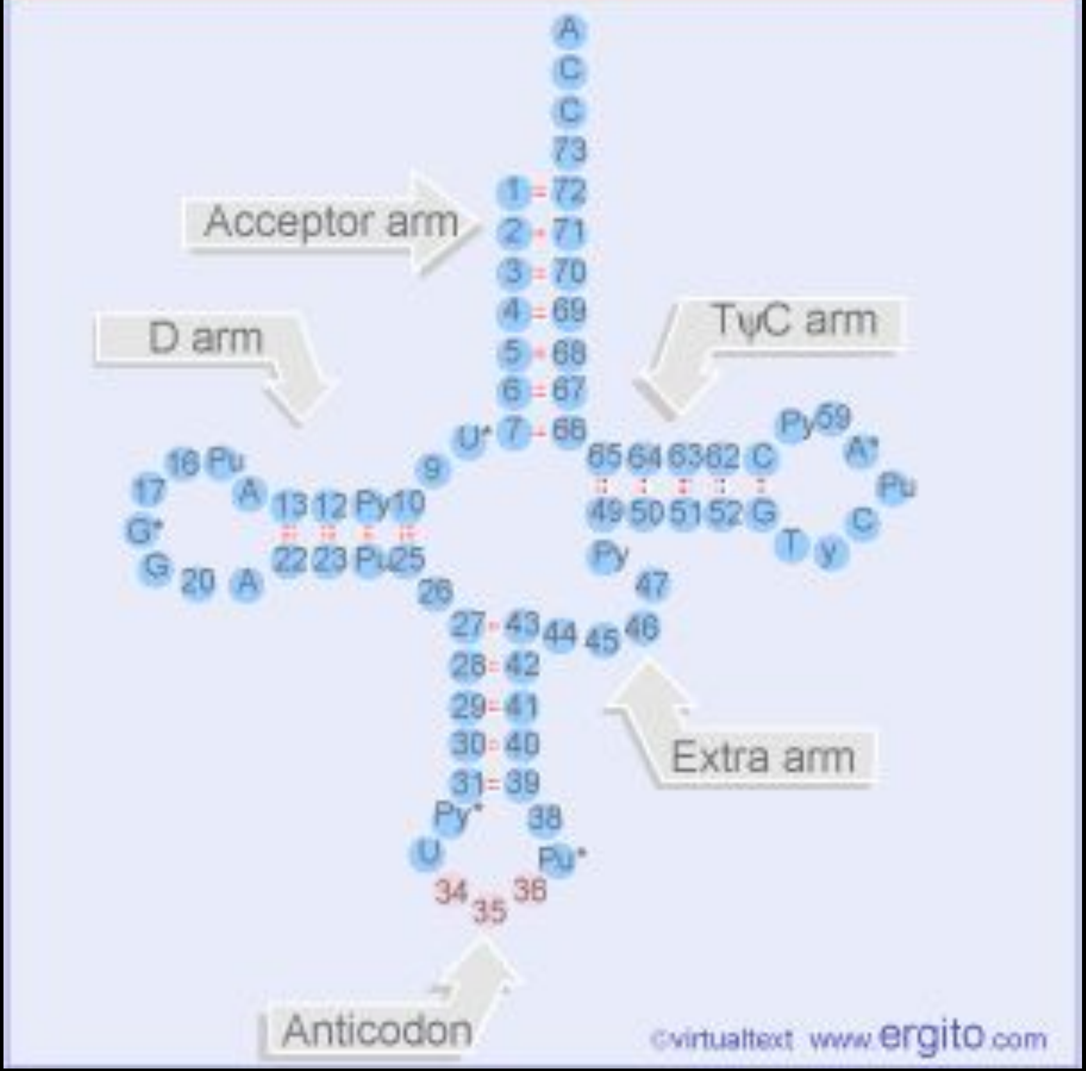
tRNA secondary structure is a cloverleaf



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Aminoacyl-tRNA

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

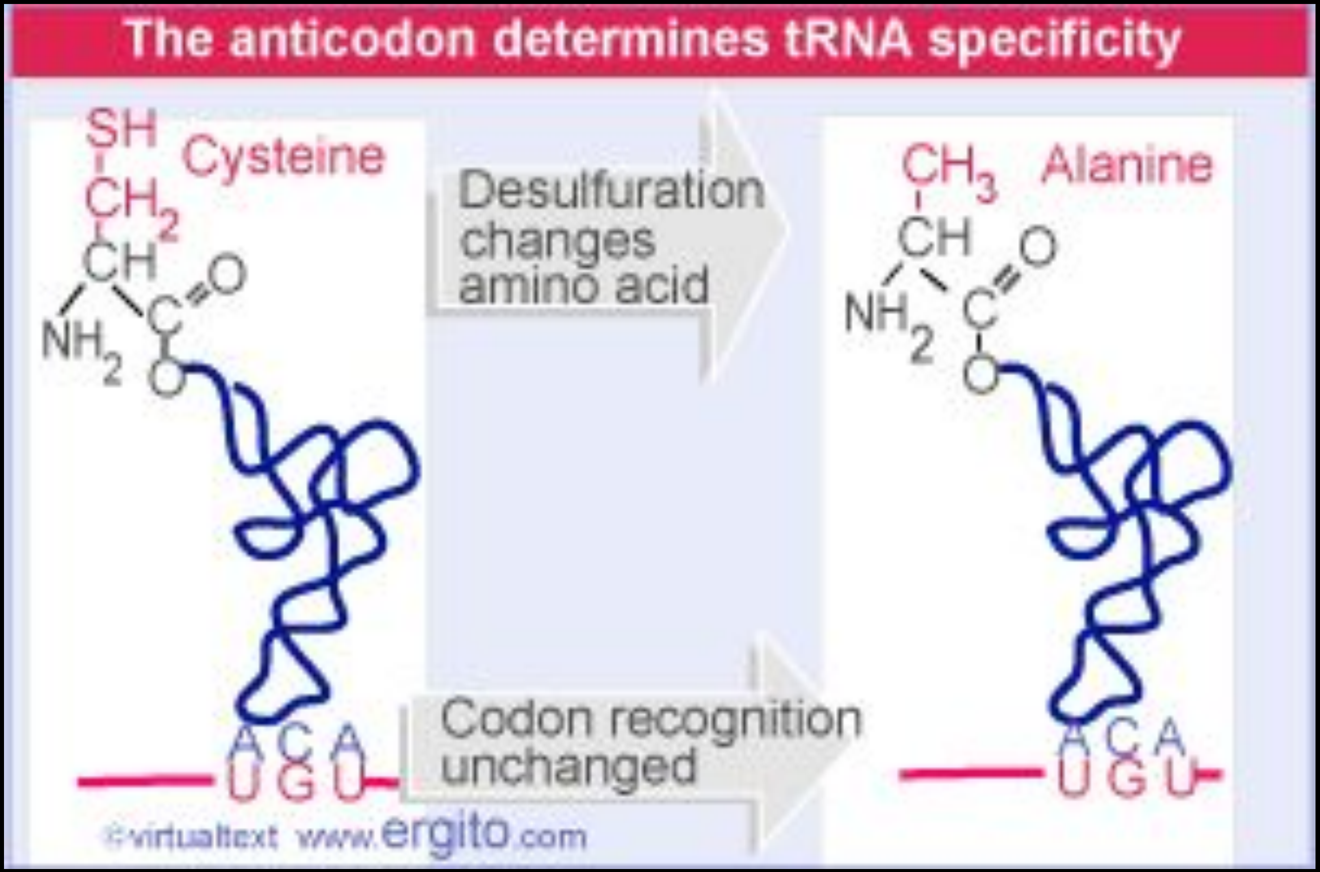
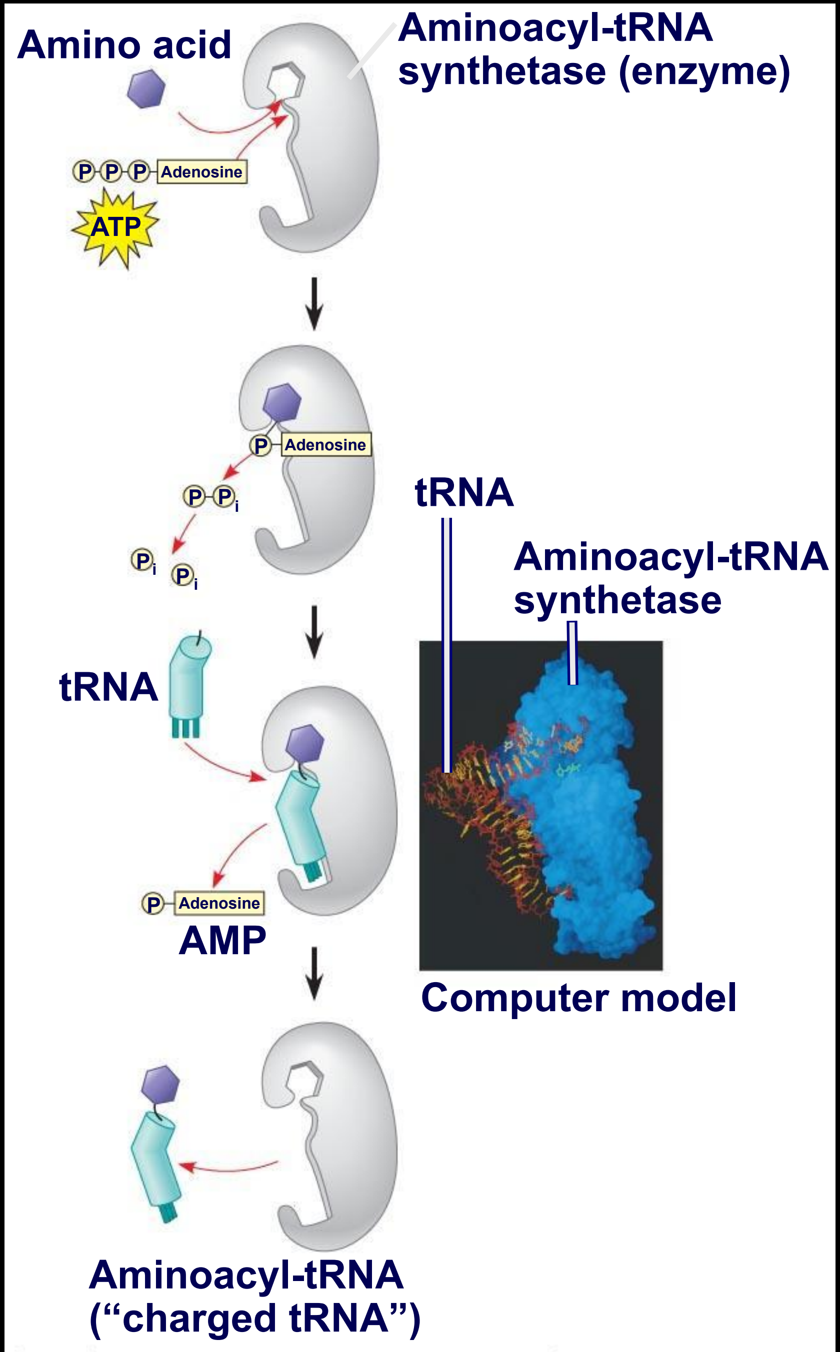
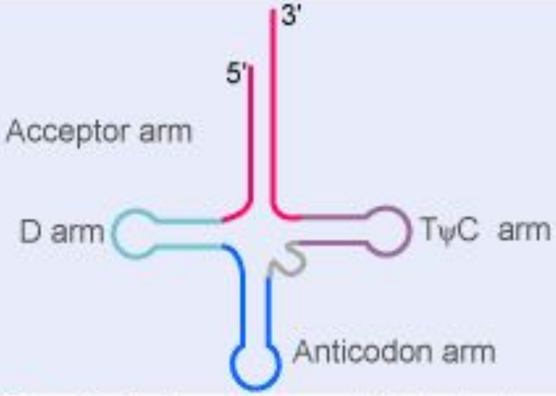


Fig. 17-15-4


Amino acid
Aminoacyl-tRNA synthetase (enzyme)
P P P Adenosine
ATP
P Adenosine
P P$_i$
P$_i$
P$_i$
tRNA
P Adenosine
AMP
tRNA
Aminoacyl-tRNA synthetase
Computer model
Aminoacyl-tRNA ("charged tRNA")

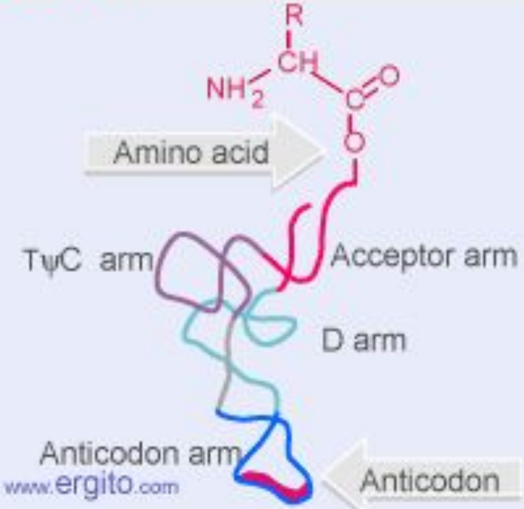
All tRNAs share a tertiary structure

Cloverleaf has four arms

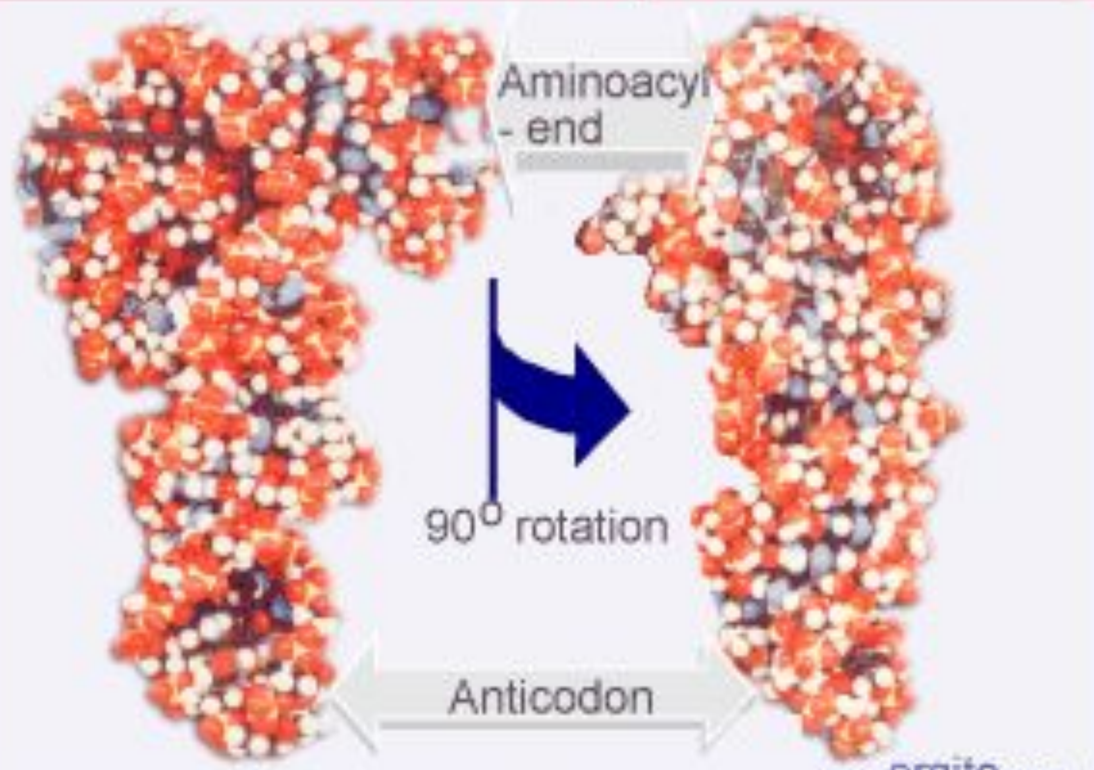


2D projection has 2 perpendicular duplexes

Backbone follows L-shaped structure



tRNA is L-shaped



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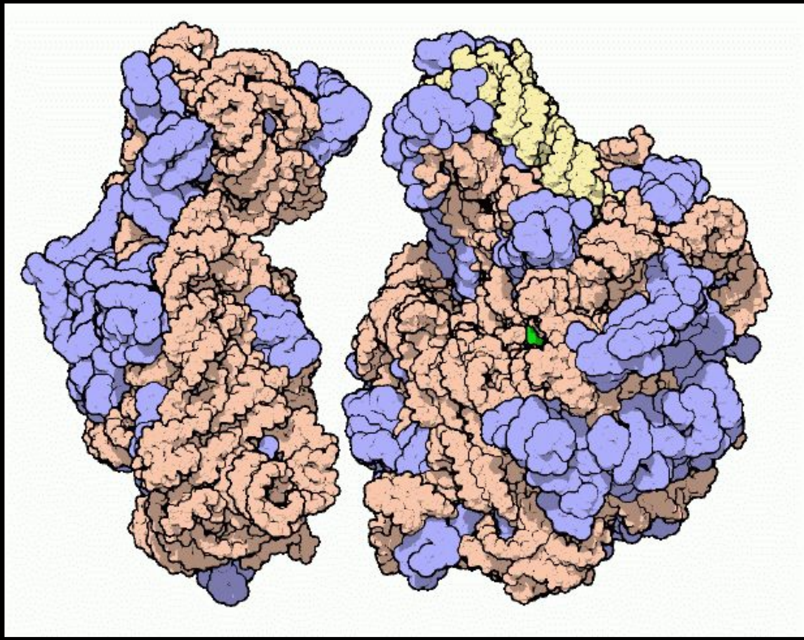
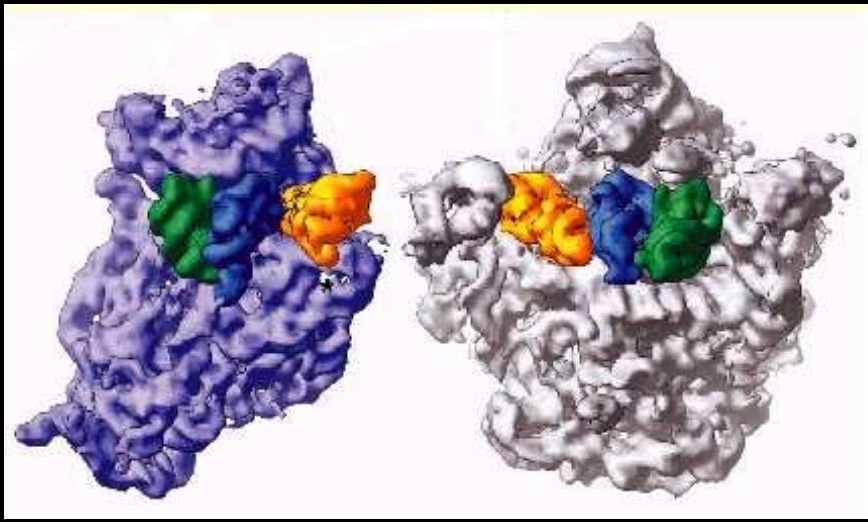
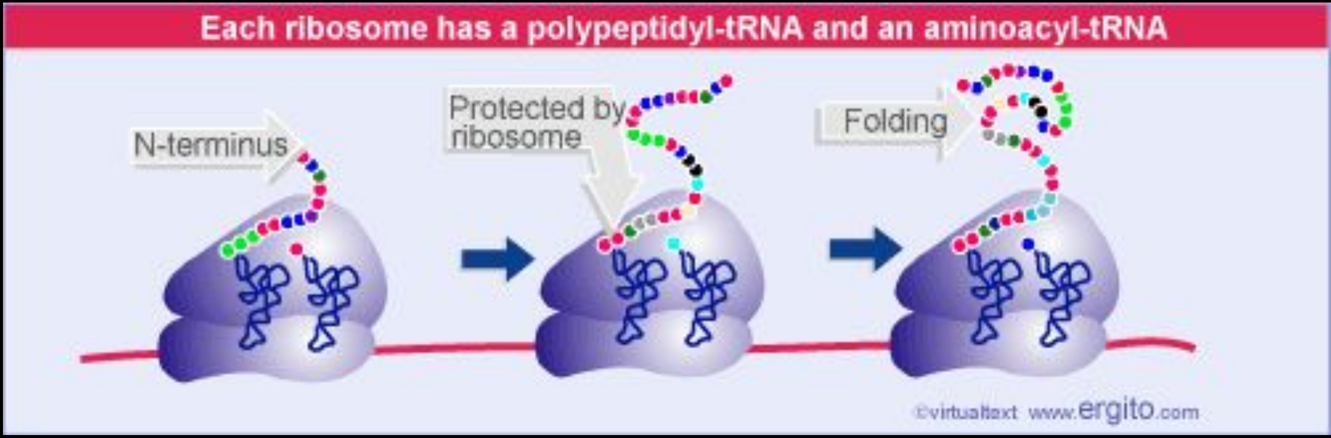
Ribosomal RNA

- 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.

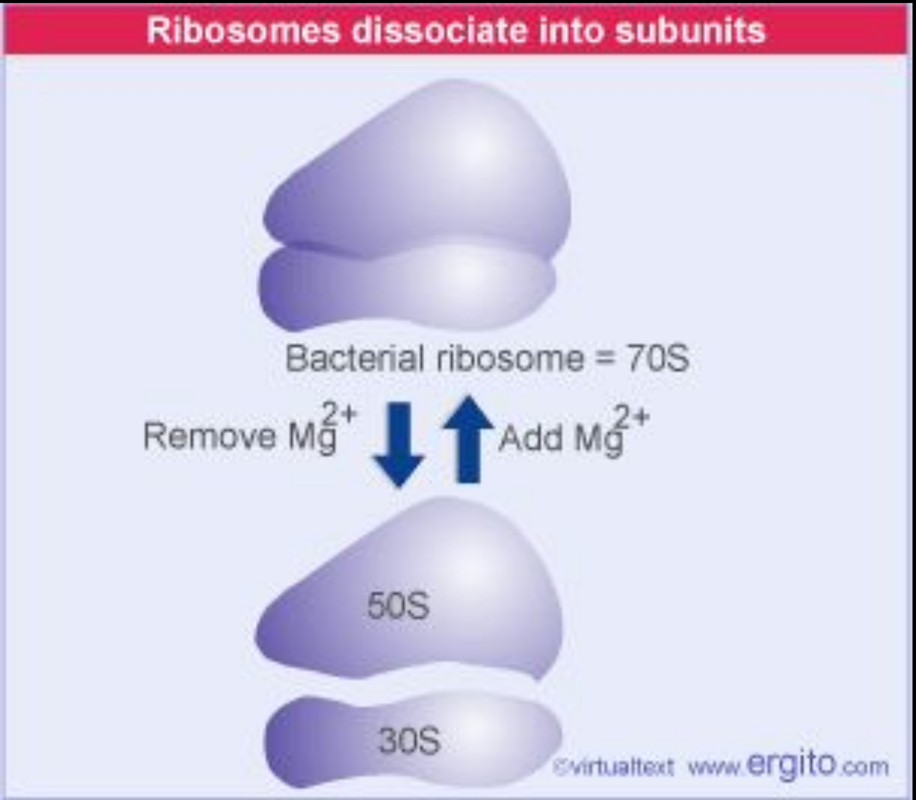




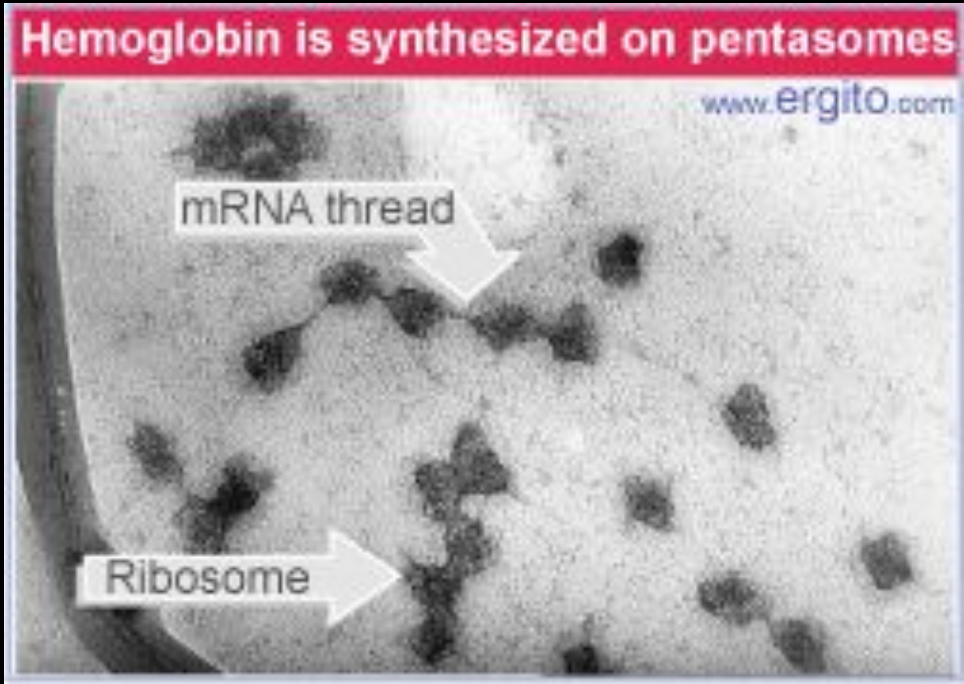
Ribosomes



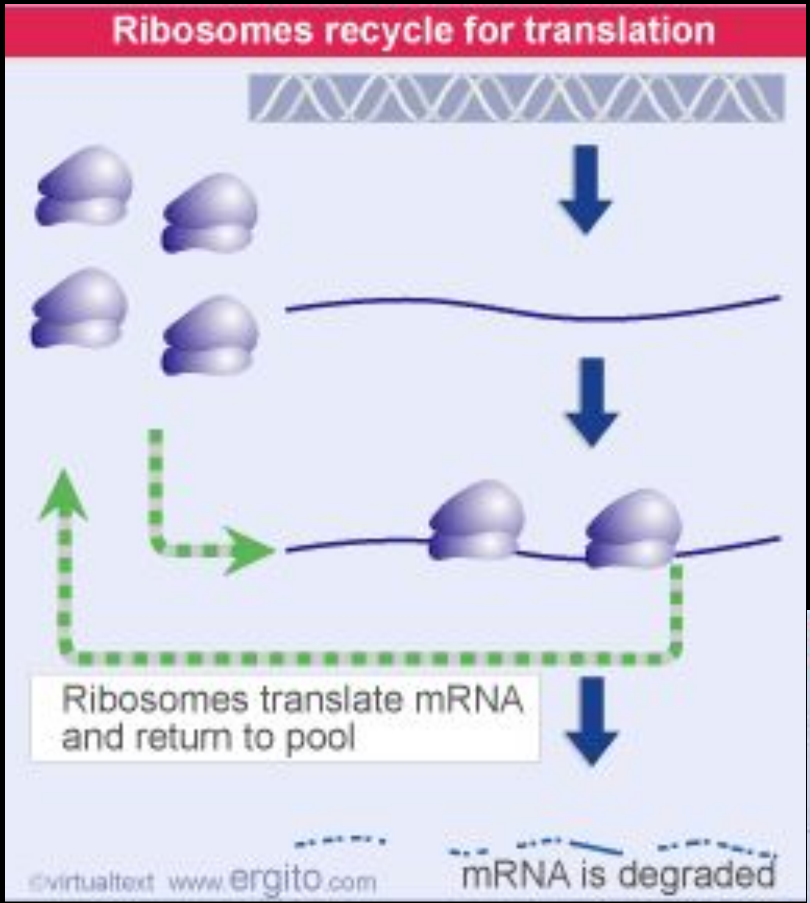
Each ribosome has a polypeptidyl-tRNA and an aminoacyl-tRNA



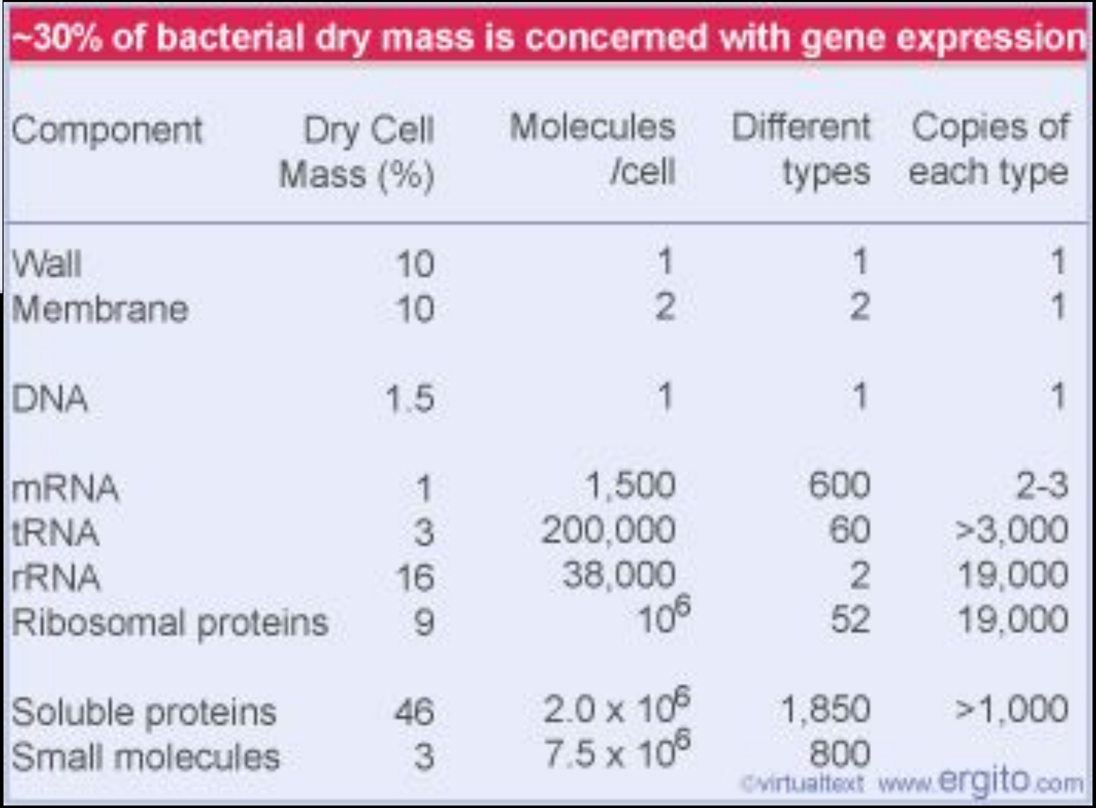
Ribosomes dissociate into subunits



Hemoglobin is synthesized on pentasomes



Ribosomes recycle for translation



~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	

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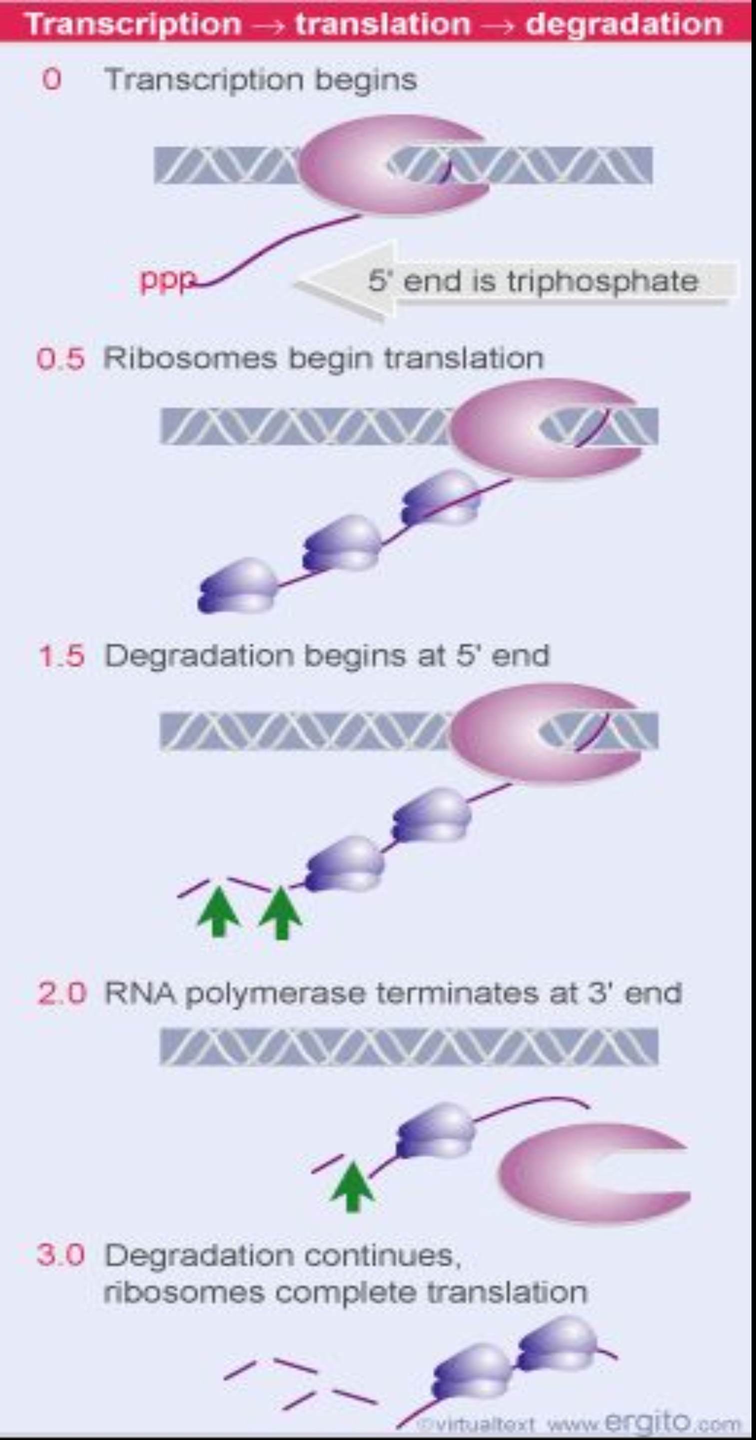
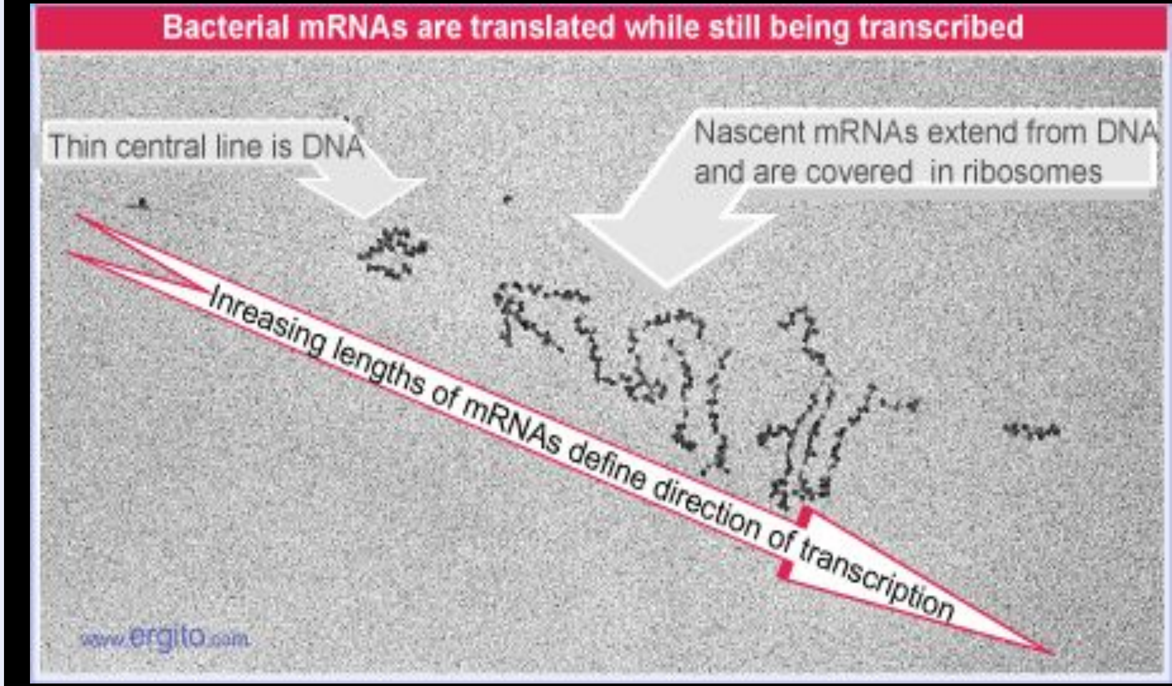
In Prokaryotes

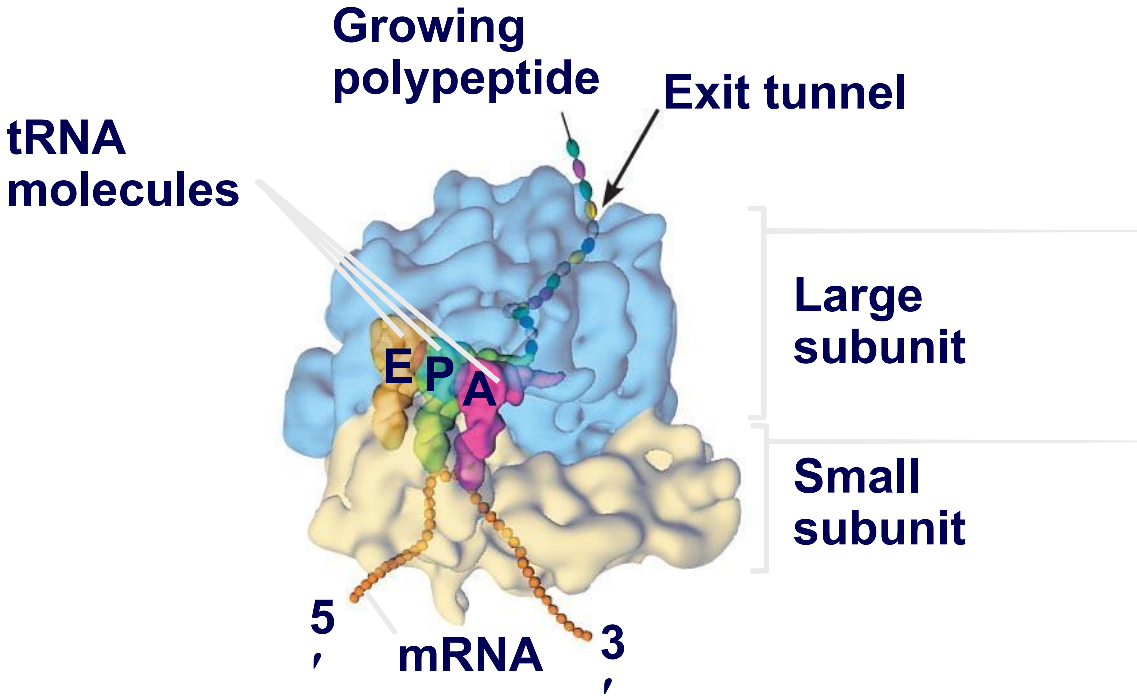


Transcription → translation → degradation



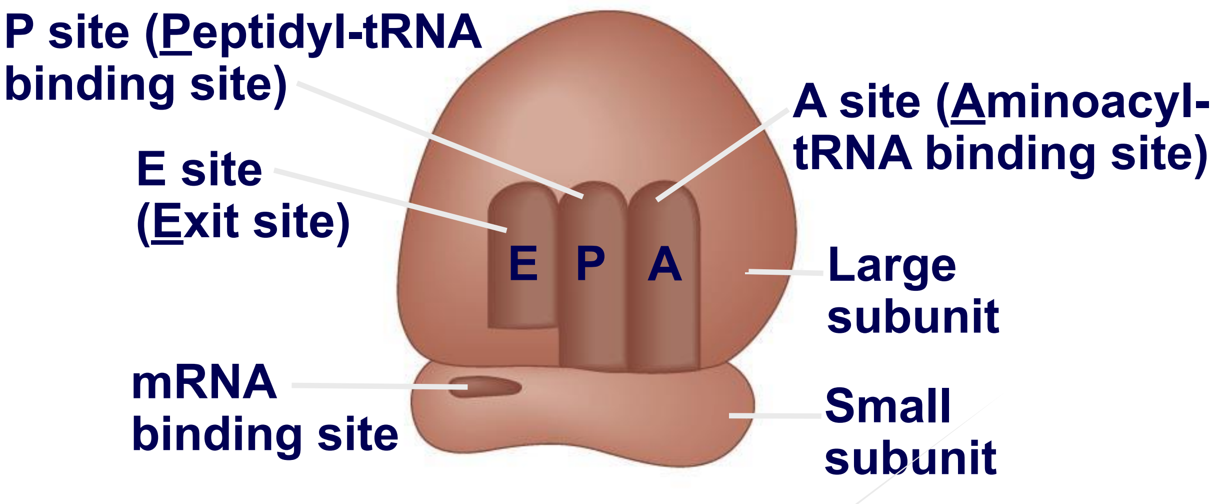
Bacterial mRNAs are translated while still being transcribed

Fig. 17-16a

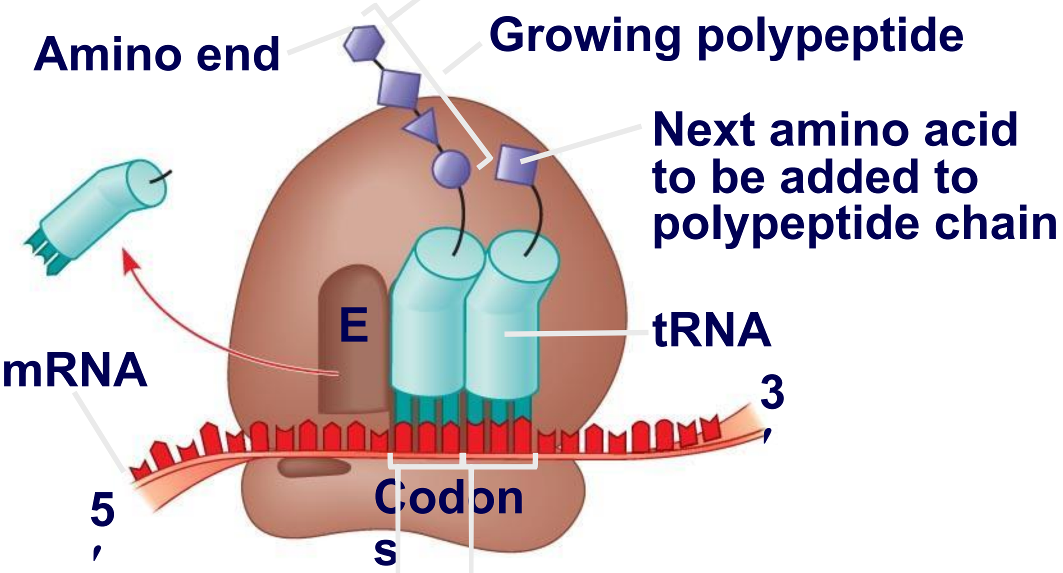


(a) Computer model of functioning ribosome

Fig. 17-16b



(b) Schematic model showing binding sites



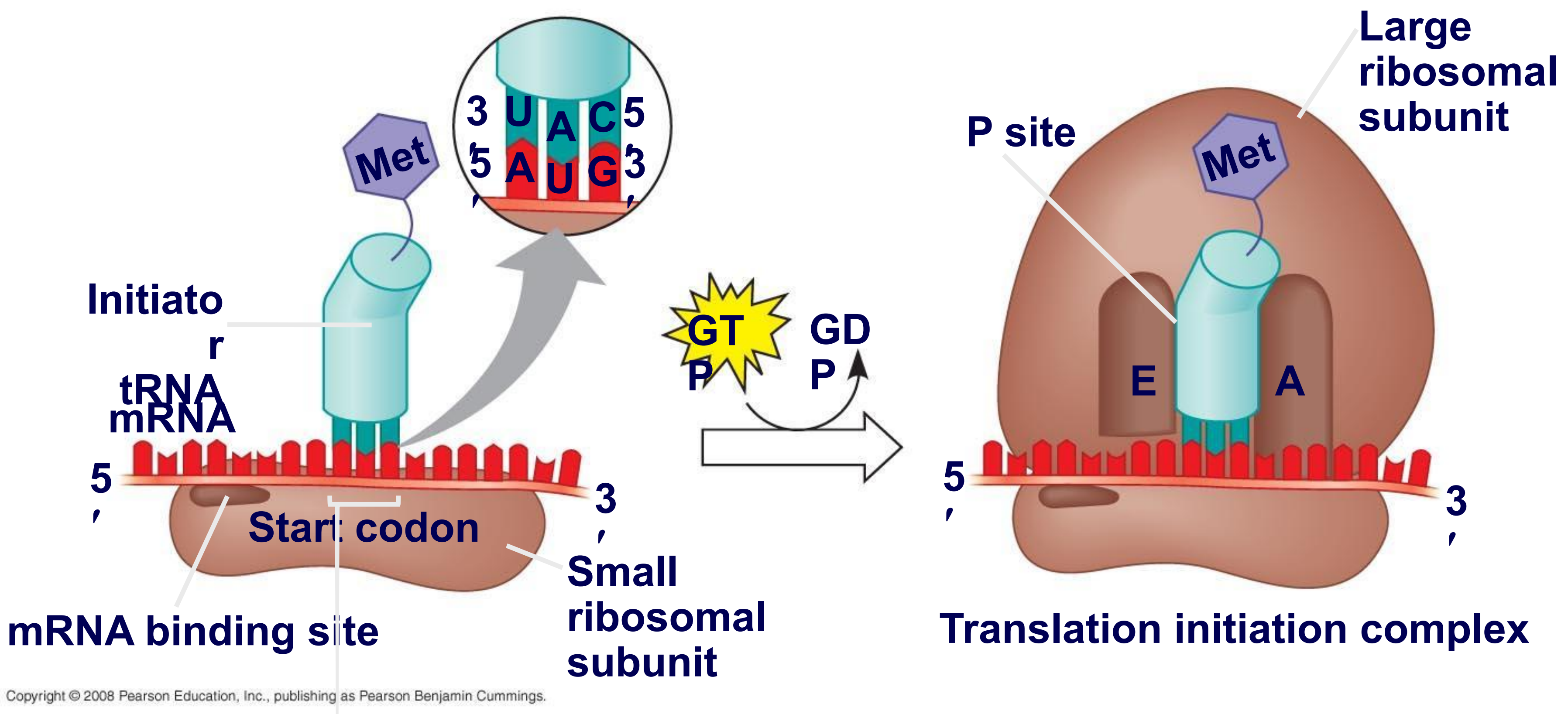
(c) Schematic model with mRNA and tRNA

- 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

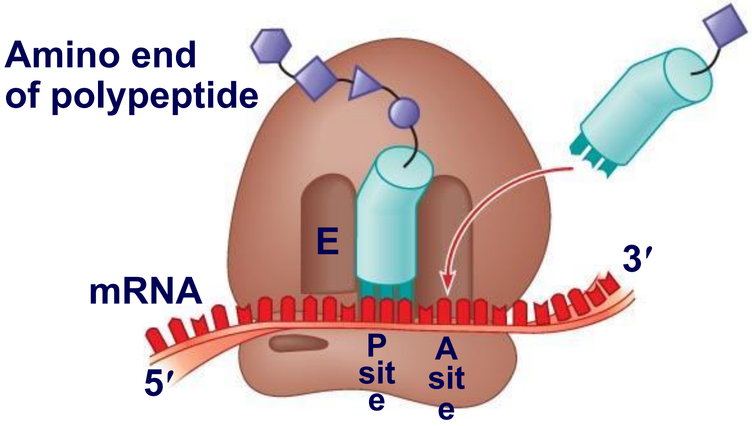
- The initiation stage of translation brings together mRNA, a tRNA with the first amino acid, and the two ribosomal subunits
- First, a small ribosomal subunit binds with mRNA and a special initiator tRNA
- Then the small subunit moves along the mRNA until it reaches the start codon (AUG)
- Proteins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

Fig. 17-17


3′
U
A
C
5′
5′
A
U
G
3′
Met
Initiator tRNA
mRNA
5′
3′
Start codon
mRNA binding site
Small ribosomal subunit
GTP
GDP
P site
Large ribosomal subunit
Met
E
A
5′
3′
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



Amino end
of polypeptide
E
3′
mRNA
P site
A site
5′

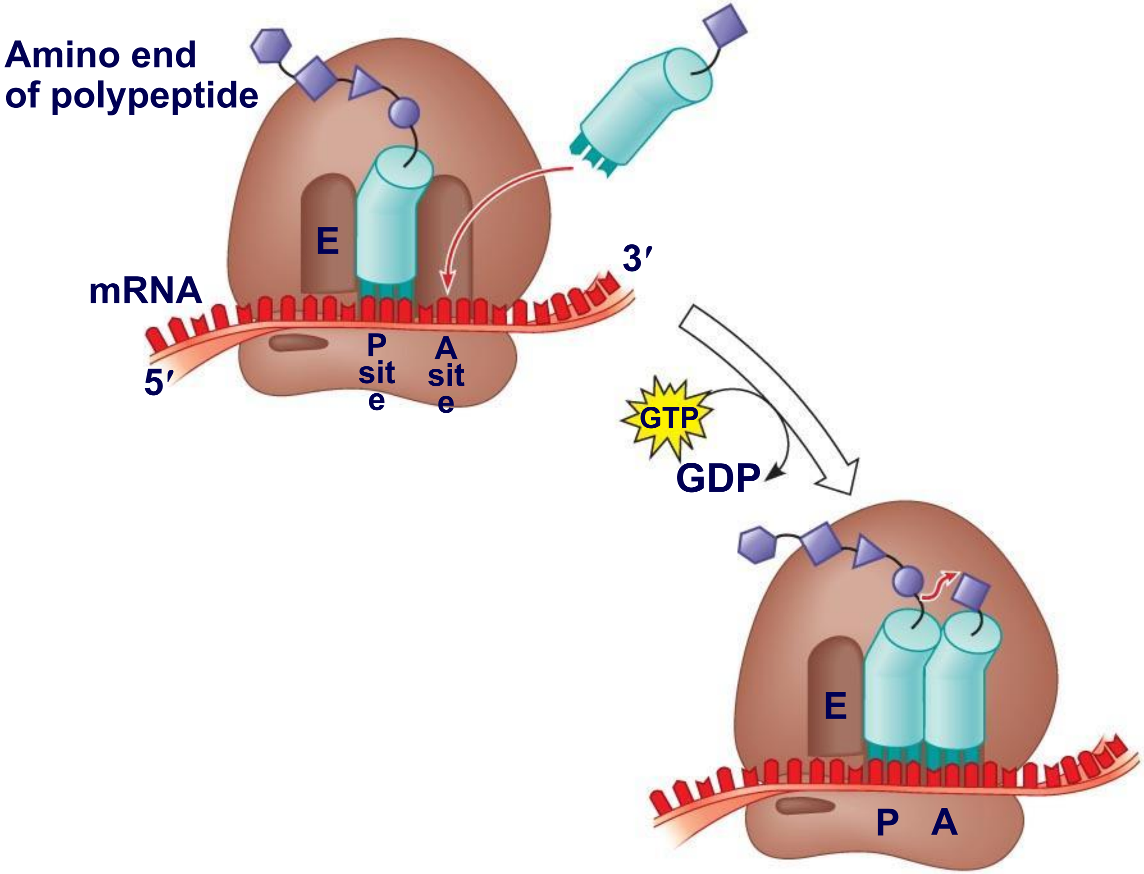

Amino end of polypeptide
mRNA
E
3′
5′
P site
A site
GTP
GDP
E
P
A

Fig. 17-18-3

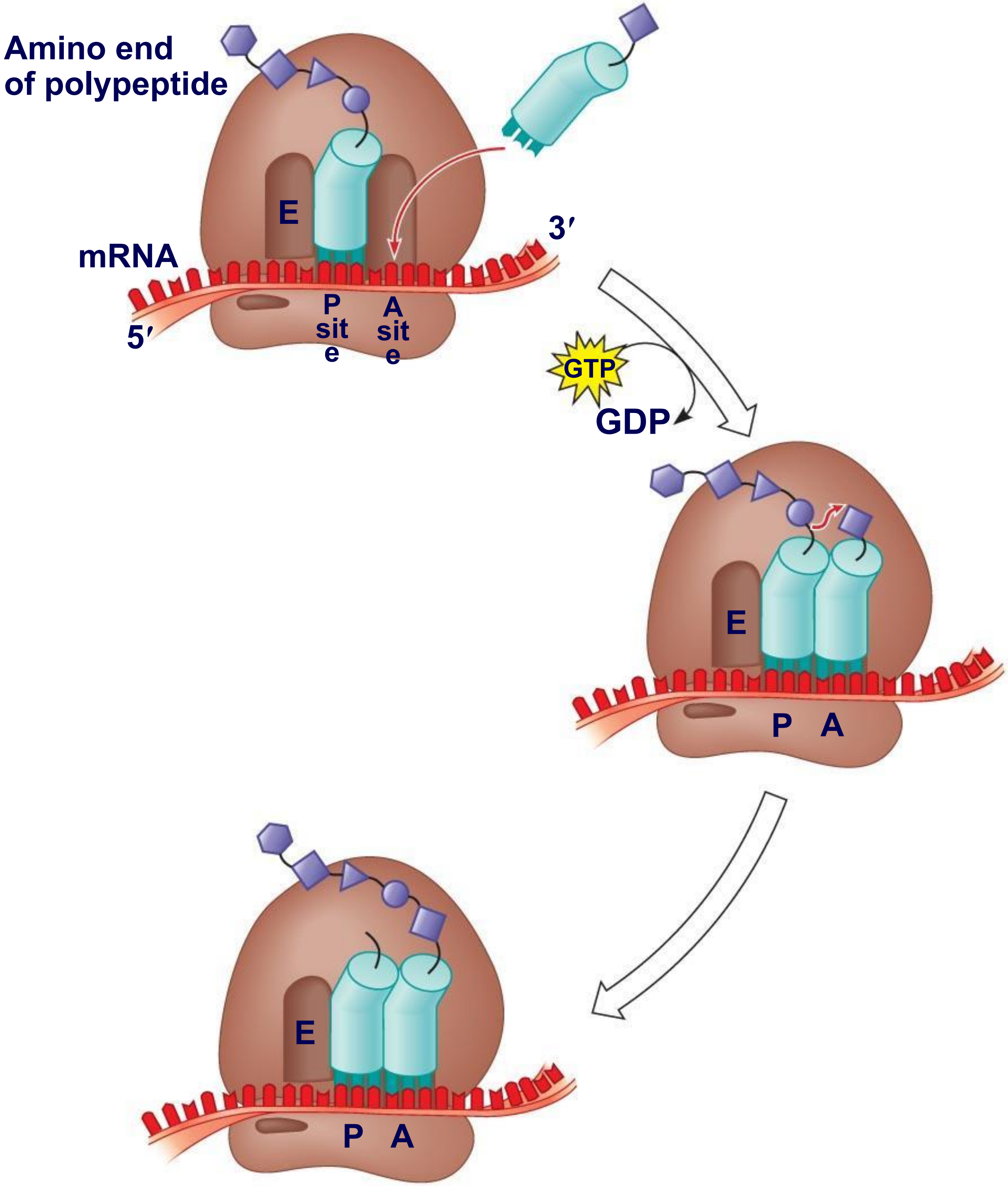
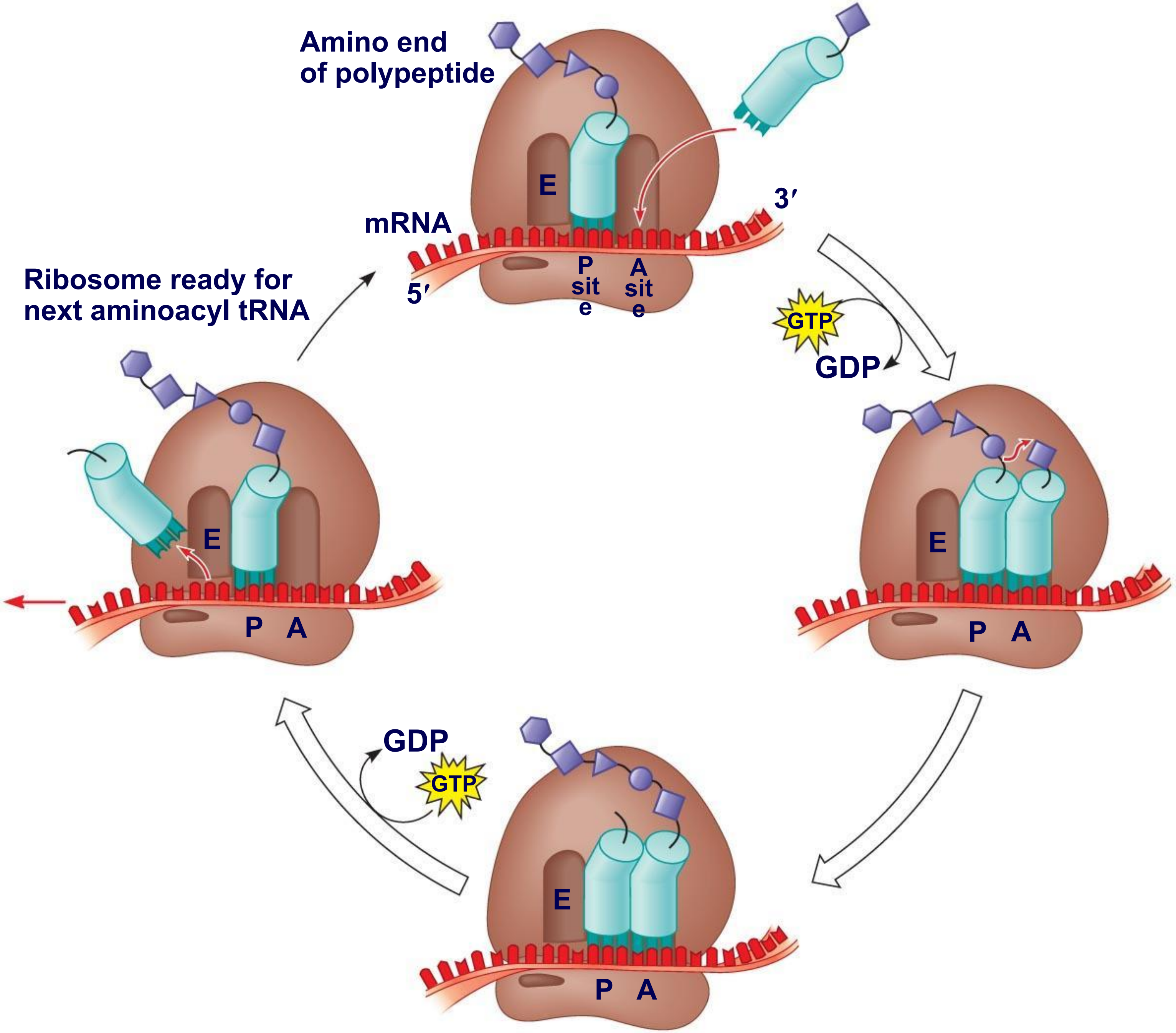

Amino end
of polypeptide
E
mRNA
3′
5′
P
site
A
site
GTP
GDP
E
P
A
E
P
A

Fig. 17-18-4


Amino end of polypeptide
mRNA
E
P site
A site
3′
5′
GTP
GDP
E
P
A
E
P
A
GDP
GTP
Ribosome ready for next aminoacyl tRNA
E
P
A

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

Fig. 17-19-1

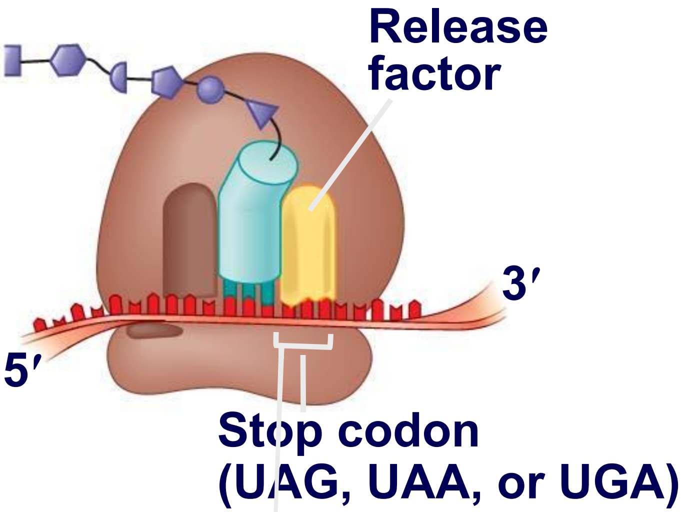


Fig. 17-19-2

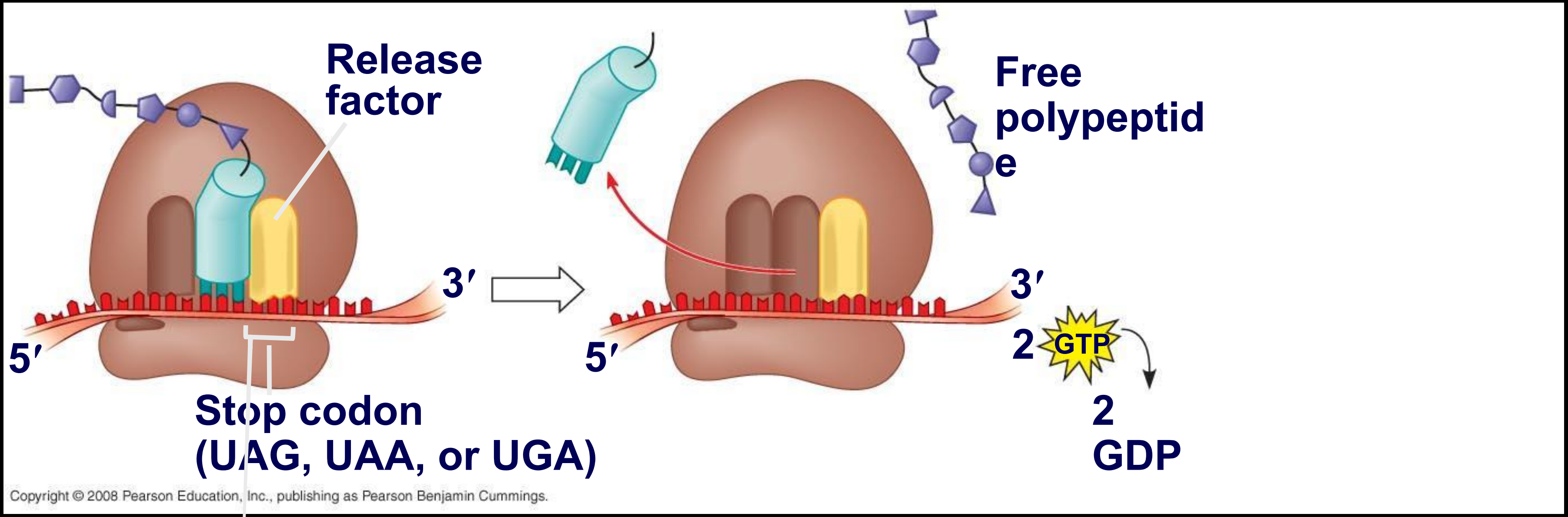
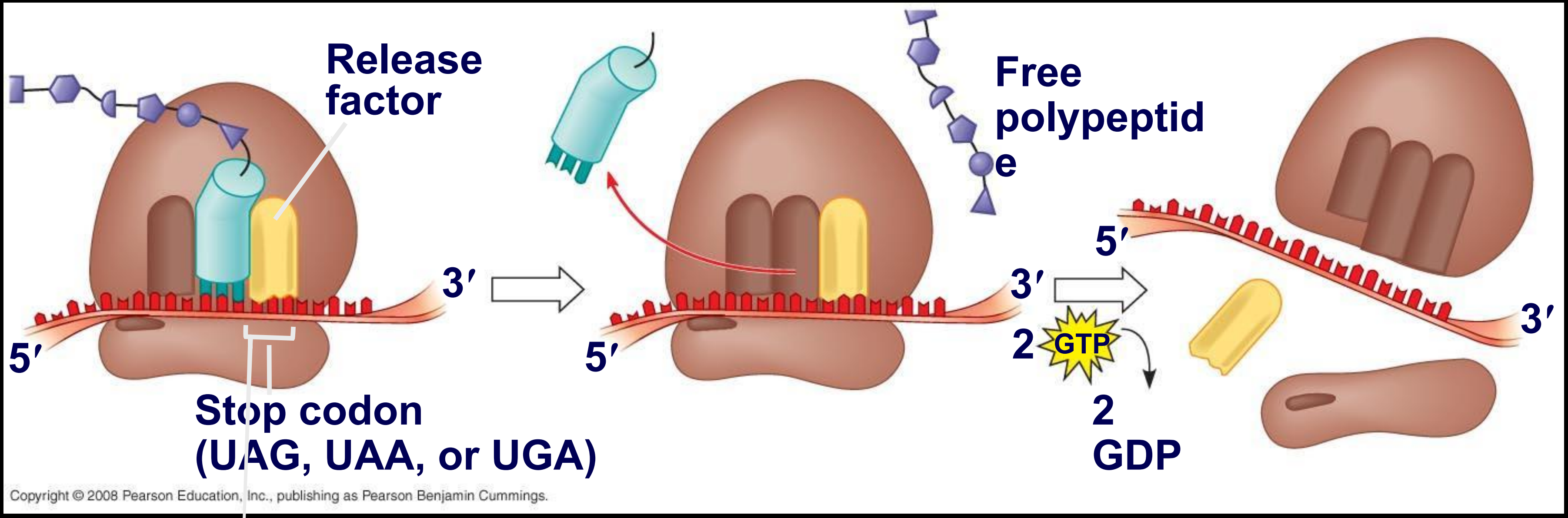

Release factor
Free polypeptide
3′
5′
3′
5′
2 GTP
2 GDP
Stop codon (UAG, UAA, or UGA)

Fig. 17-19-3


Release
factor
3′
5′
Stop codon
(UAG, UAA, or UGA)
5′
Free
polypeptid
e
3′
5′
3′
2
GTP
2
GDP

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

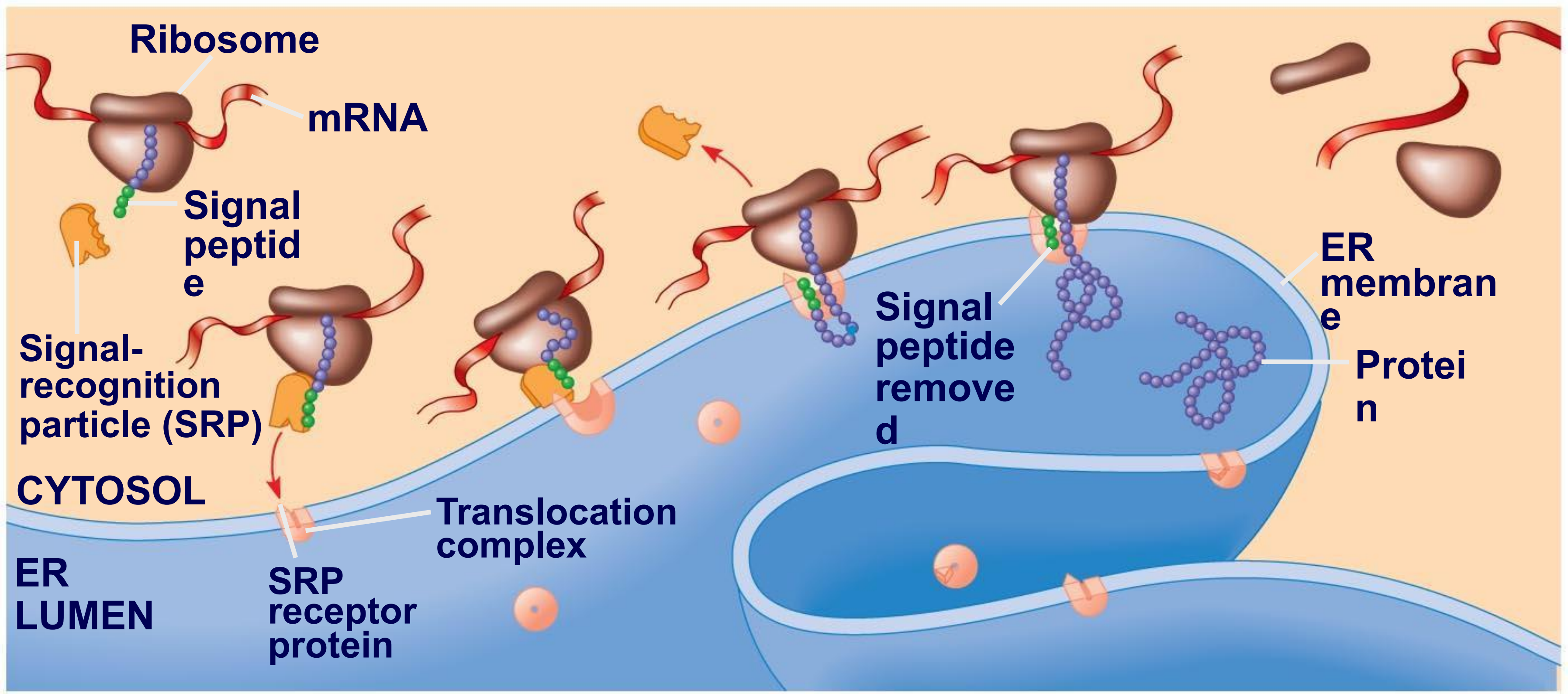
- During and after synthesis, a polypeptide chain spontaneously coils and folds into its three-dimensional shape
- Proteins may also require post-translational modifications before doing their job
- Some polypeptides are activated by enzymes that cleave them
- Other polypeptides come together to form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

Targeting Polypeptides to Specific Locations

- Two populations of ribosomes are evident in cells: free ribsomes (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

Fig. 17-21


Ribosome
mRNA
Signal peptide
Signal-recognition particle (SRP)
CYTOSOL
ER LUMEN
SRP receptor protein
Translocation complex
Signal peptide removed
ER membrane
Protein

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