

Lecture 19

General Biology & Cytology **Course** **2301130**



Faculty of Dentistry, Mutah University

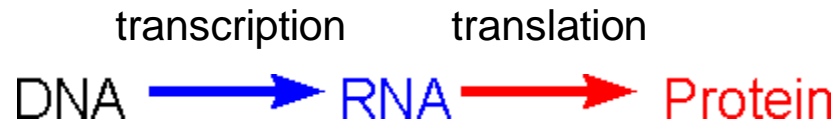
Dr. Samer Yousef Alqaraleh

From Gene to Protein

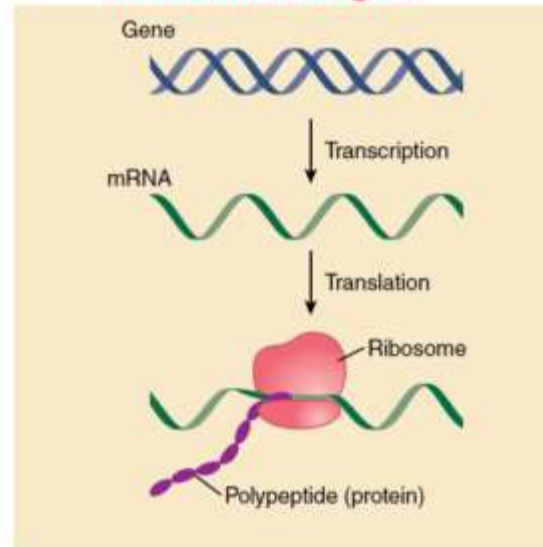
(Gene expression)

Overview of gene expression

Central Dogma of
Molecular Biology:



The Central Dogma



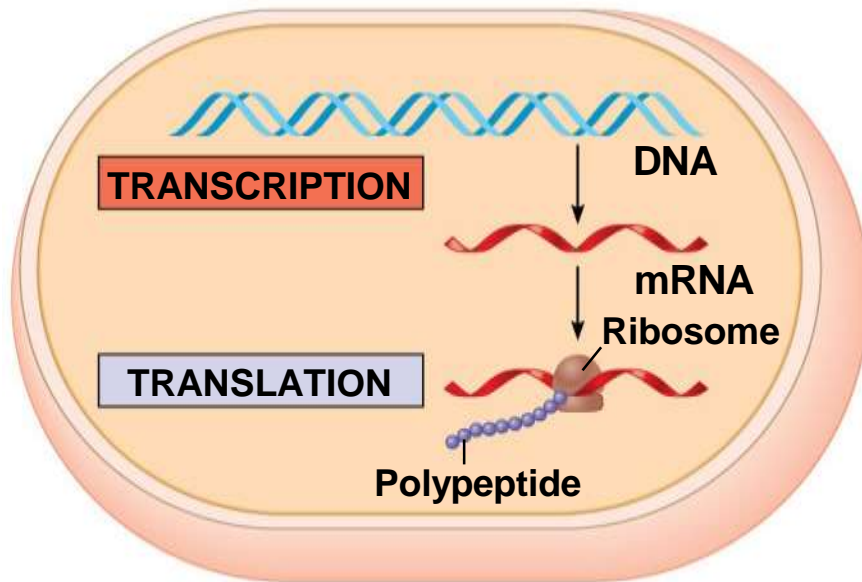
Gene expression: is the process our cells use to convert the instructions in our DNA into a functional product, such as a protein, or non-coding RNA, and ultimately affect a phenotype.

Basic Principles of Transcription and Translation

- **RNA** is the bridge 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, using information in the mRNA

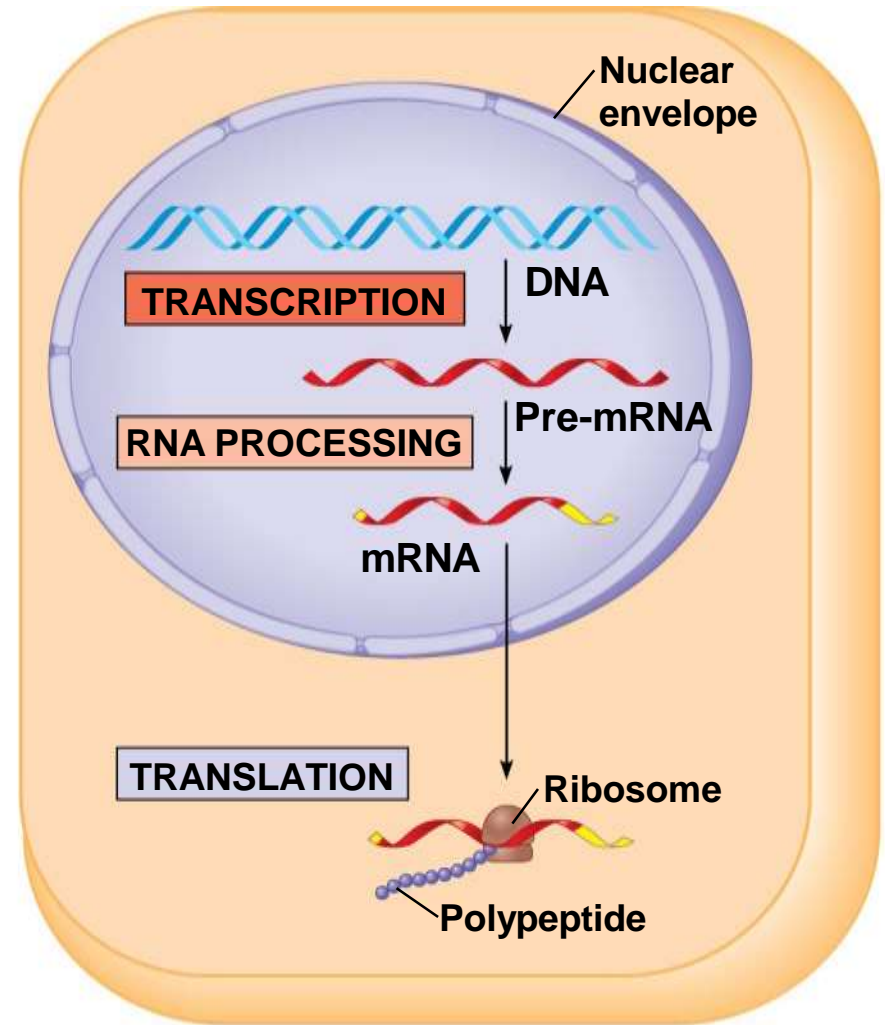
Where does translation occurs?

- A **primary transcript** is the initial RNA transcript from any gene prior to processing
- The *central dogma* is the concept that cells are governed by a cellular chain of command: DNA → RNA → protein



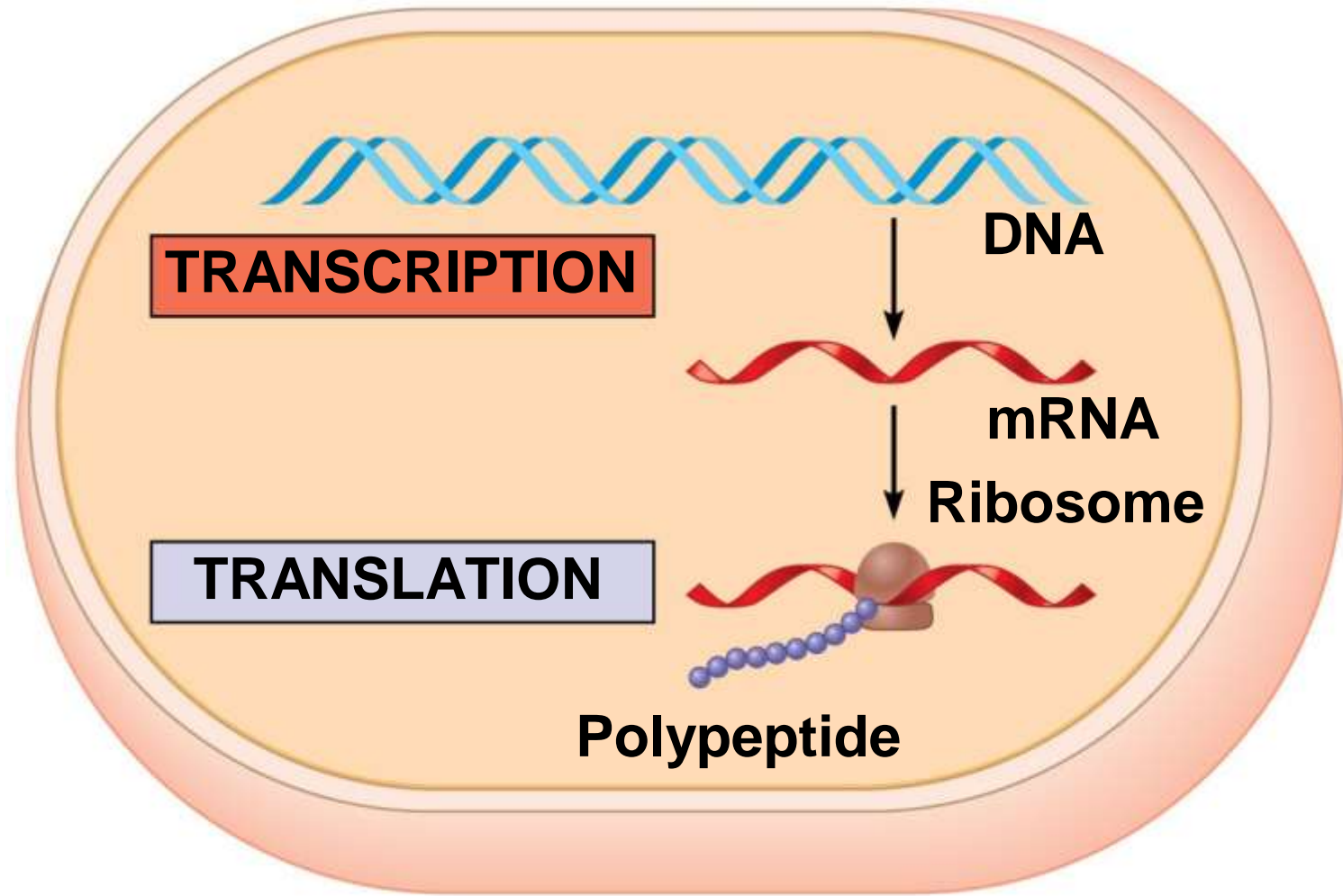
(a) Bacterial cell

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(b) Eukaryotic cell

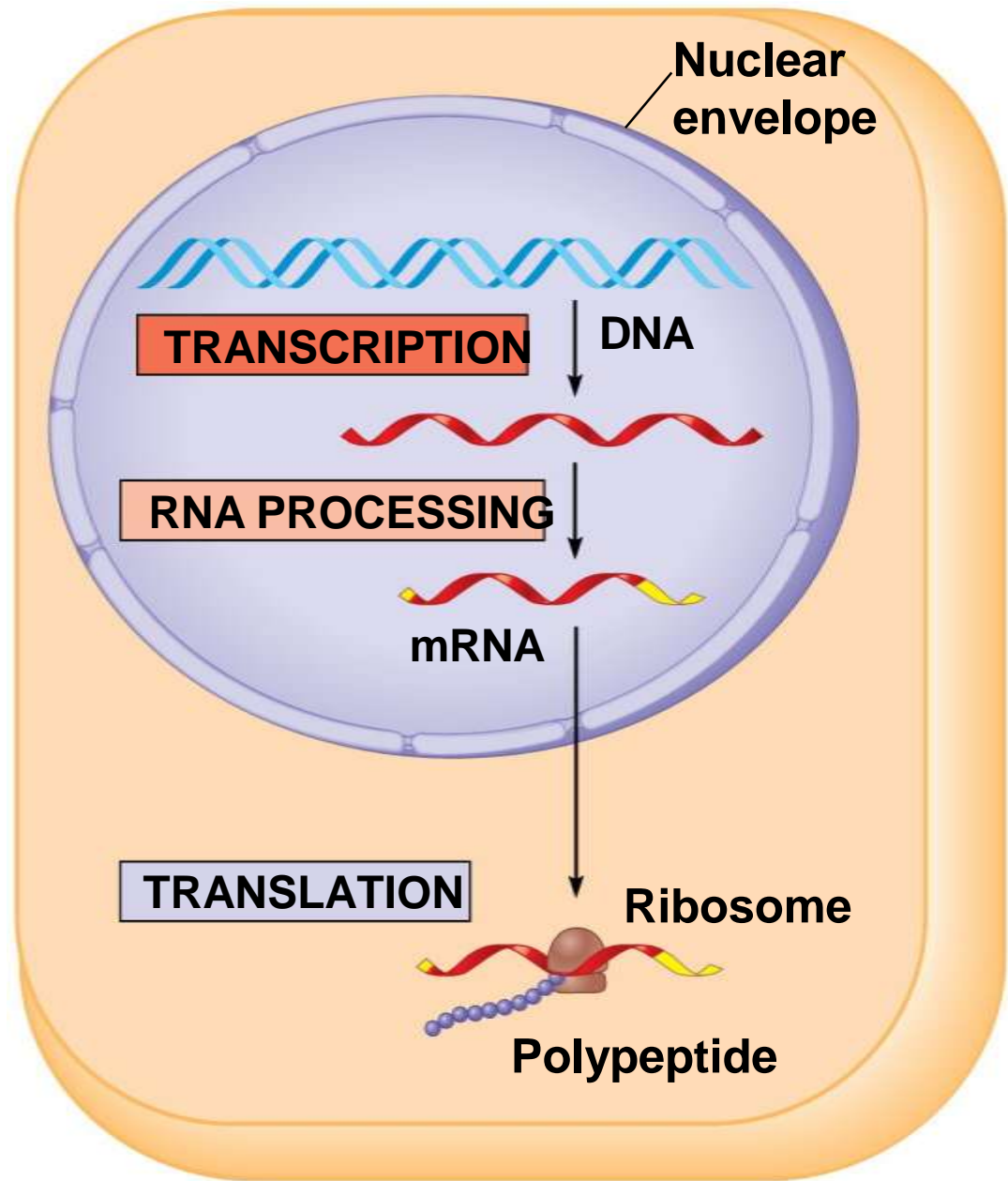
Transcription and Translation in Prokaryotes



- In prokaryotes, translation of mRNA can begin before transcription has finished

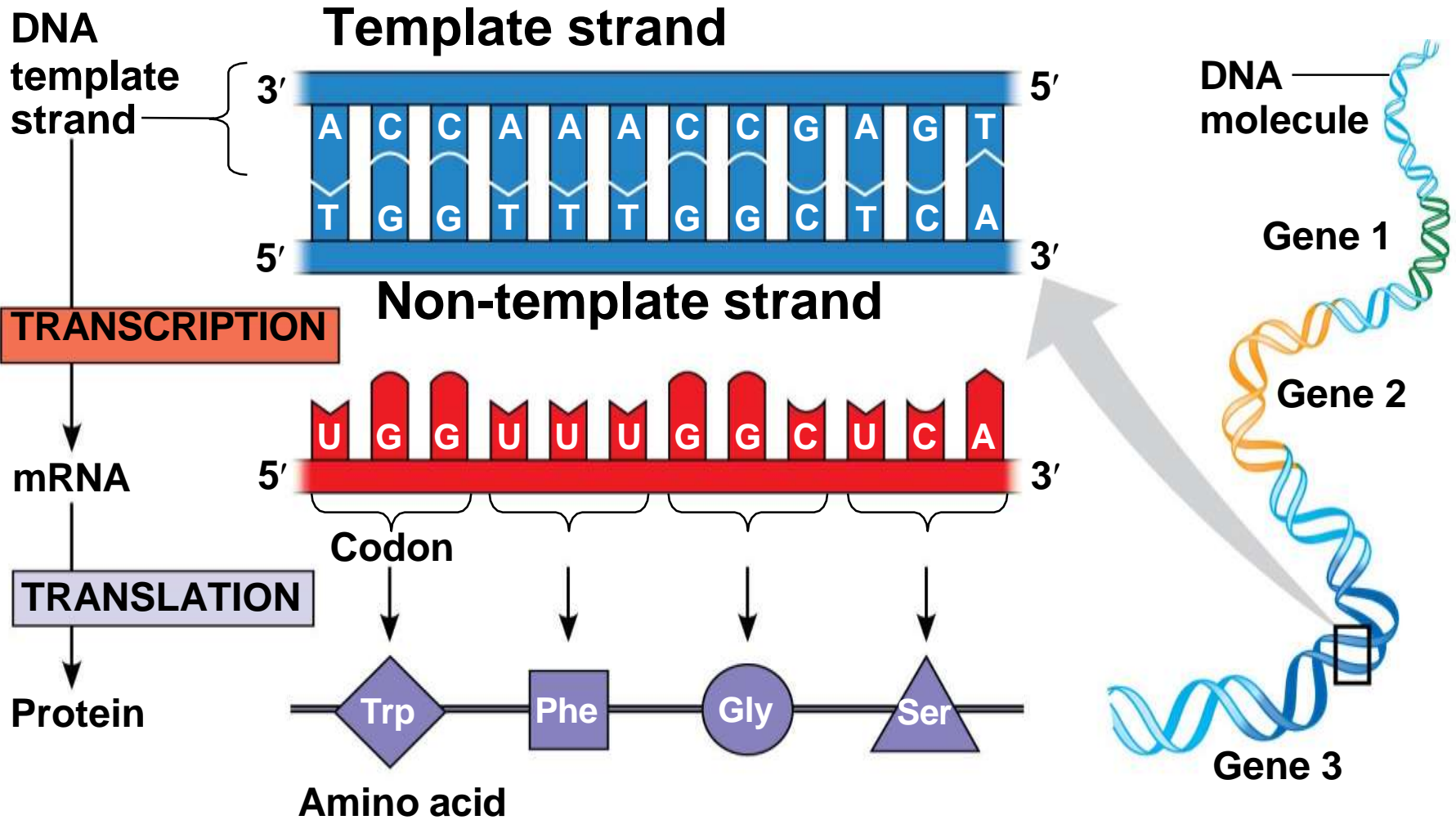
Transcription and Translation in Eukaryotes

- In a eukaryotic cell, the nuclear envelope separates transcription from translation
- Eukaryotic RNA transcripts are modified through RNA processing to yield finished mRNA



The Genetic Code

- The genetic code refers to the code (rules) that governs how a DNA sequence is converted to a protein (amino acid) sequence
- There are 20 amino acids, but only four nucleotide bases in DNA (and RNA)
 - What is the minimum number of nucleotides needed to code for a single amino acid?



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- The genetic code is a triplet code where a 3-nucleotide DNA word codes for a 3-nucleotide mRNA word (a **codon**) which codes for an amino acid

- During transcription, one of the two DNA strands, called the **template strand**, provides a template for ordering the sequence of complementary nucleotides in an RNA transcript.
- The template strand is always the same strand for a given gene
- During translation, the mRNA base triplets, called **codons**, are read in the 5' to 3' direction
- Each codon specifies the amino acid (one of 20) to be placed at the corresponding position along a polypeptide

Cracking the Code

- All 64 codons were deciphered by the mid-1960s
- Of the 64 triplets, 61 code for amino acids; 3 triplets are “stop” signals to end translation
- The genetic code is redundant (more than one codon may specify a particular amino acid) but not ambiguous; no codon specifies more than one amino acid
- Codons must be read in the correct **reading frame** (correct groupings) in order for the specified polypeptide to be produced

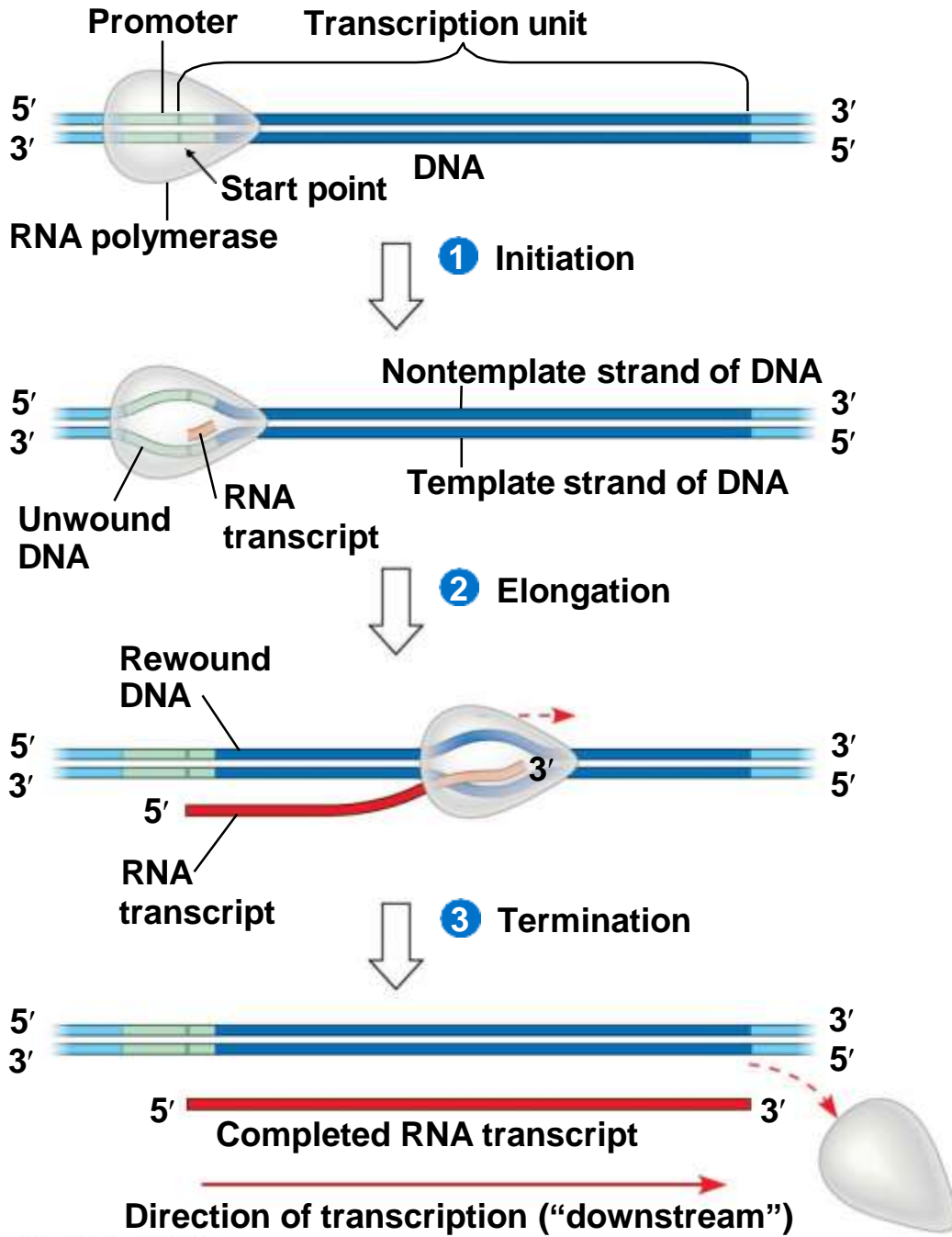
Properties of the Genetic Code

- 64 triplets
 - 61 code for amino acids
 - 3 triplets are “stop” codons
- The genetic code is redundant but not ambiguous

		Second mRNA base				
		U	C	A	G	
U	UUU	UCU	UAU	UGU	U C A G	
	UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys		
	UUA } Leu	UCA } Ser	UAA Stop	UGA Stop		
	UUG } Leu	UCG } Ser	UAG Stop	UGG Trp		
C	CUU	CCU	CAU	CGU	U C A G	
	CUC } Leu	CCC } Pro	CAC } His	CGC } Arg		
	CUA } Leu	CCA } Pro	CAA } Gln	CGA } Arg		
	CUG } Leu	CCG } Pro	CAG } Gln	CGG } Arg		
A	AUU	ACU	AAU	AGU	U C A G	
	AUC } Ile	ACC } Thr	AAC } Asn	AGC } Ser		
	AUA } Ile	ACA } Thr	AAA } Lys	AGA } Arg		
	AUG } Met or start	ACG } Thr	AAG } Lys	AGG } Arg		
G	GUU	GCU	GAU	GGU	U C A G	
	GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly		
	GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly		
	GUG } Val	GCG } Ala	GAG } Glu	GGG } Gly		

Transcription is the DNA-directed synthesis of RNA

- Transcription consists of three steps
 - **Initiation**
 - The DNA sequence where RNA polymerase attaches is called the **promoter**
 - **Elongation**
 - **RNA polymerase** pries the DNA strands apart and hooks together the RNA nucleotides
 - The RNA is complementary to the DNA template strand, but uracil replaces thymine
 - **Termination**
 - in bacteria, the sequence signaling the end of transcription is called the **terminator**

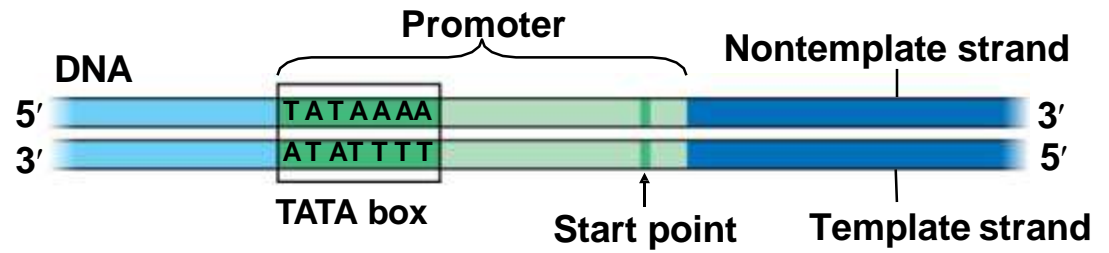


RNA Polymerase Binding and Initiation of Transcription

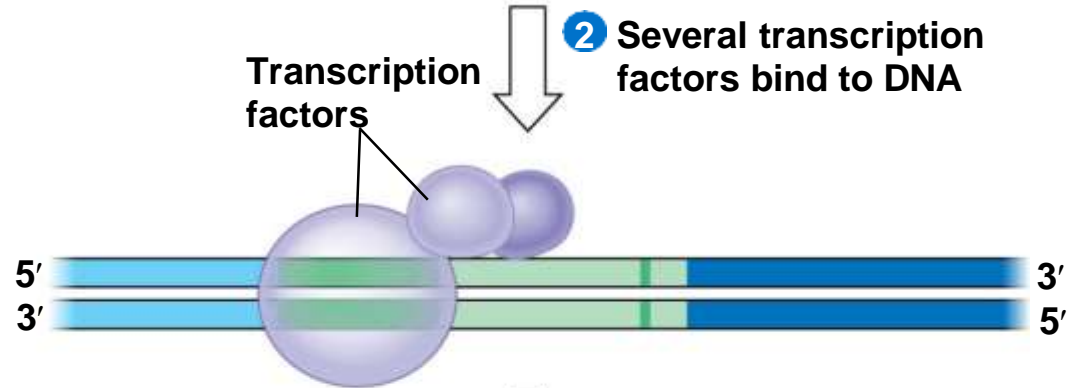
- Promoters signal the transcriptional **start point** and usually extend several dozen nucleotide pairs upstream of the start point
- **Transcription factors** mediate the binding of RNA polymerase and the initiation of transcription
- The completed assembly of transcription factors and **RNA polymerase 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

- Transcription in eukaryotes

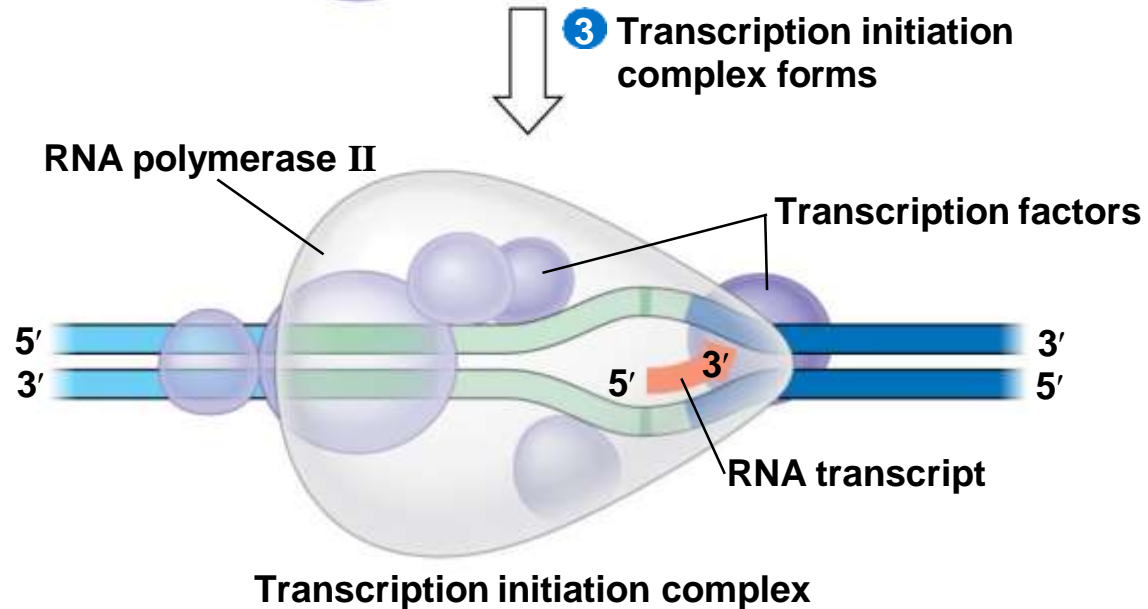
1 A eukaryotic promoter



2 Several transcription factors bind to DNA

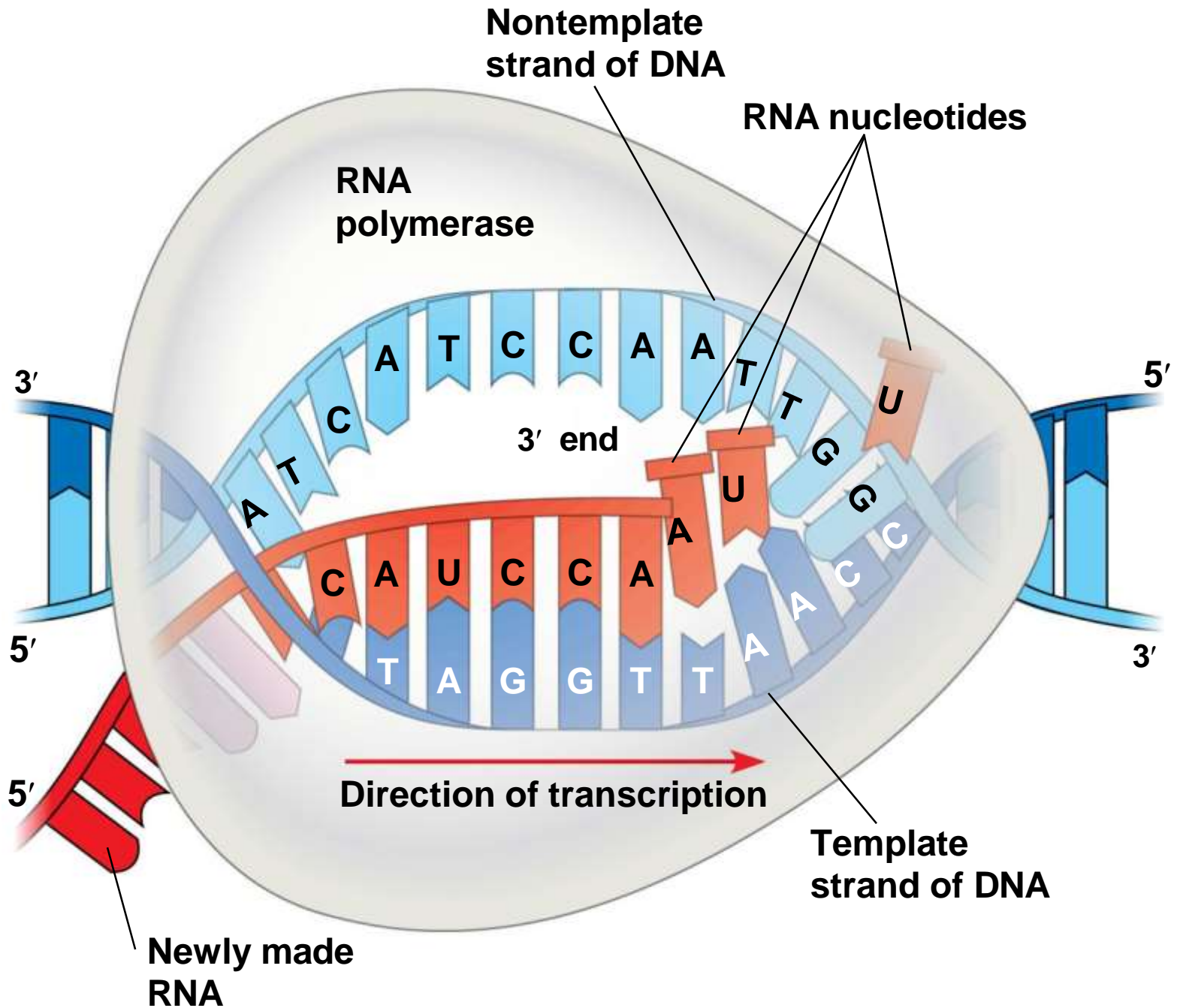


3 Transcription initiation complex forms



Elongation of the RNA Strand

- As RNA polymerase moves along the DNA, it untwists the double helix, 10 to 20 bases at a time
- Transcription progresses at a rate of 40 nucleotides per second in eukaryotes
- A gene can be transcribed simultaneously by several RNA polymerases
- Nucleotides are added to the 3' end of the growing RNA molecule



Termination of Transcription

- The mechanisms of termination are different in bacteria and eukaryotes
- In bacteria, the polymerase stops transcription after reaching a *terminator* sequence and the mRNA can be translated without further modification
- In eukaryotes, **RNA polymerase II** transcribes the polyadenylation signal sequence (AAUAAA); the RNA transcript is released 10–35 nucleotides past this polyadenylation sequence

Eukaryotic cells modify RNA after transcription

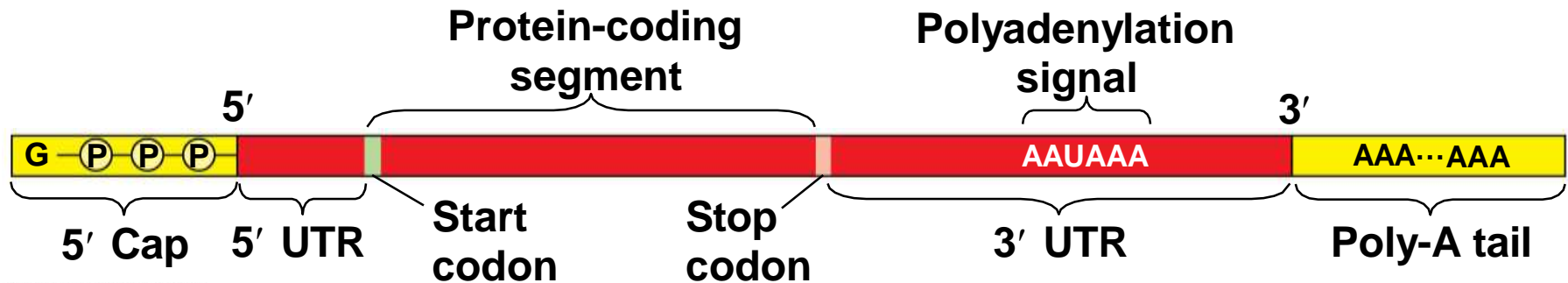
- Enzymes in the eukaryotic nucleus modify pre-mRNA (**RNA processing**) before the genetic messages are dispatched to the cytoplasm
 - RNA processing including:
 1. **Capping**: both ends of the primary transcript are usually altered
 2. **Polyadenylation**
 3. **Splicing**: Some interior parts of the molecule are cut out, and the other parts spliced together

Alteration of mRNA Ends

- Each end of a pre-mRNA molecule is modified in a particular way
 1. The 5' end receives a modified nucleotide **5' cap** (7-Methyl guanosin) to be functional in protein synthesis
 2. The 3' end gets a **poly-A tail**
- These modifications share several functions
 - They seem to facilitate the export of mRNA
 - They protect mRNA from hydrolytic enzymes
 - They help ribosomes attach to the 5' end of the mRNA

mRNA in Eukaryotes

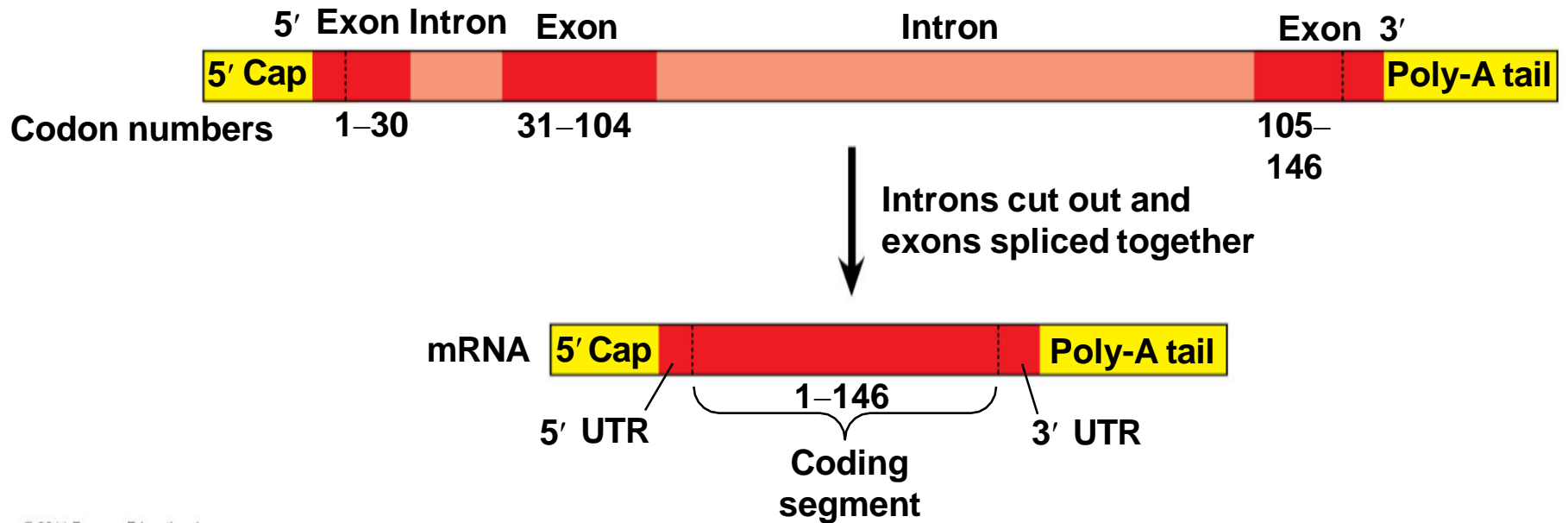
- Note that pre-mRNA (shown on subsequent slides)
 - does *not* contain the 5' Cap or Poly-A tail
 - does contain introns which are spliced out or removed
- mRNA has the following form:



3. 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

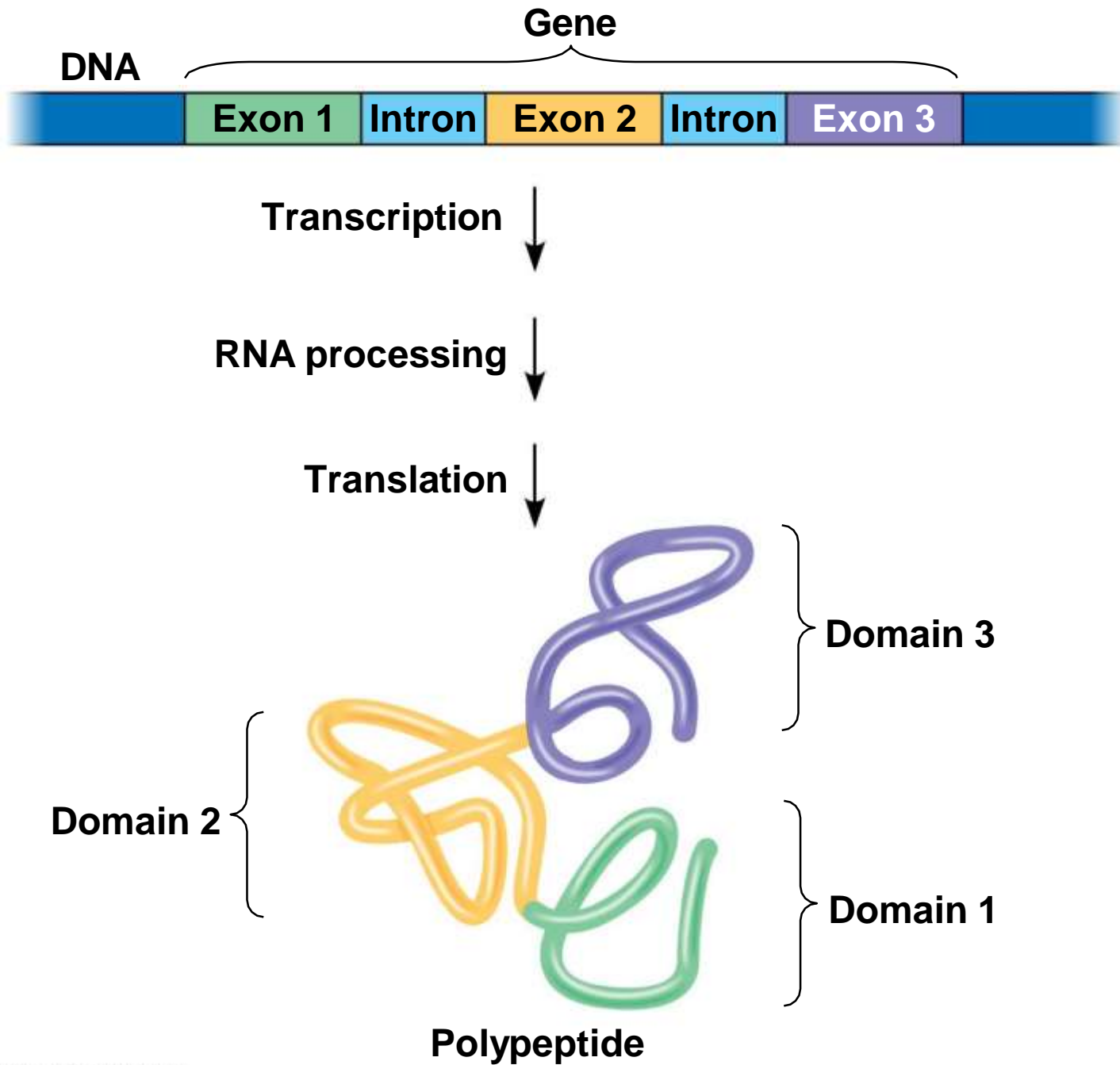
Pre-mRNA following addition of 5' Cap and Poly-A tail:



The Functional and Evolutionary Importance of Introns

- Some introns contain sequences that may regulate gene expression
- Some genes can encode more than one kind of polypeptide, depending on which segments are treated as exons during splicing
- This is called **alternative RNA splicing**
 - approximately 95% of genes with multiple exons are alternatively spliced!
 - 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



Mutations of one or a few nucleotides 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

Wild-type hemoglobin

Wild-type hemoglobin DNA



mRNA



Normal hemoglobin



Sickle-cell hemoglobin

Mutant hemoglobin DNA



mRNA



Sickle-cell hemoglobin



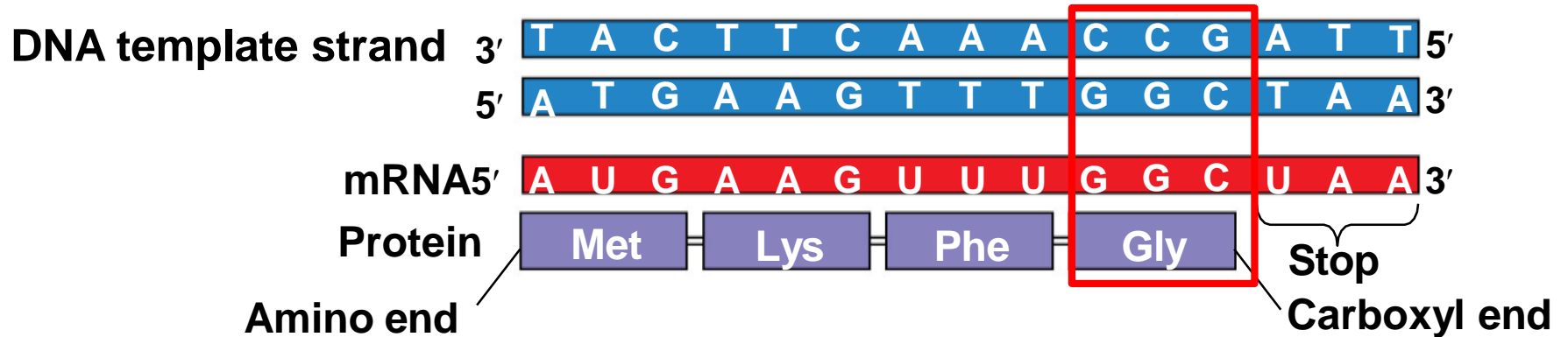
Types of Small-Scale Mutations

- Point mutations within a gene can be divided into two general categories
 - Nucleotide-pair substitutions
 - Nucleotide-pair insertions or deletions

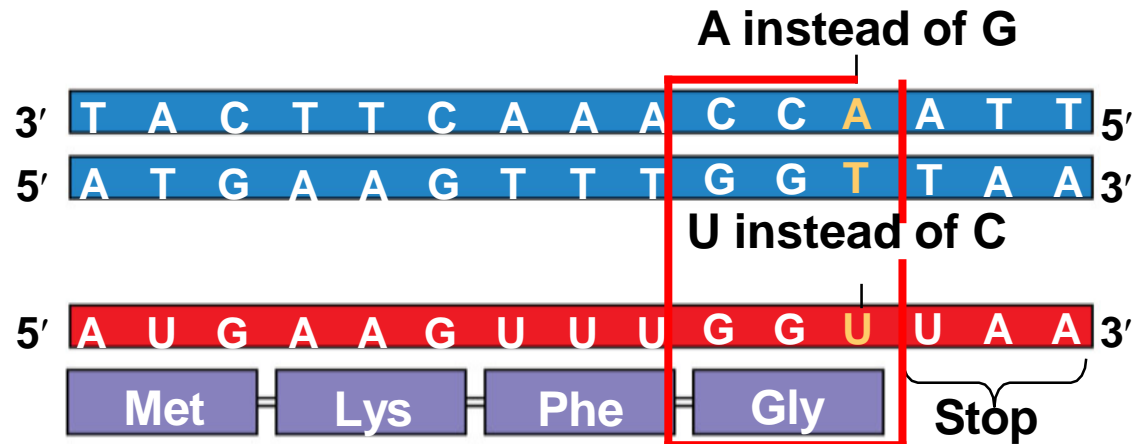
Nucleotide-Pair Substitutions

- A **nucleotide-pair substitution** replaces one nucleotide and its partner with another pair of nucleotides
- **Silent mutations** have no effect on the amino acid produced by a codon because of redundancy in the genetic code
- **Missense mutations** still code for an amino acid, but not the correct amino acid
- **Nonsense mutations** change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein

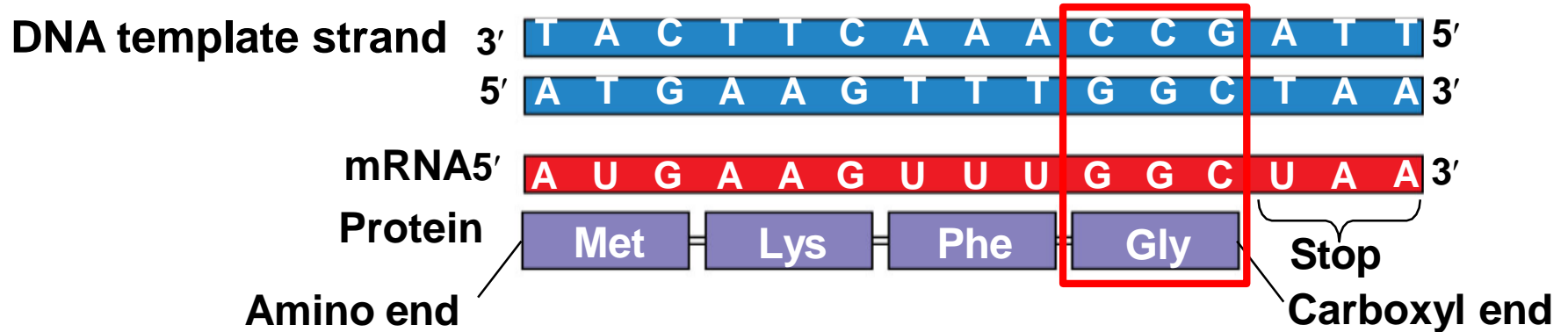
Wild type



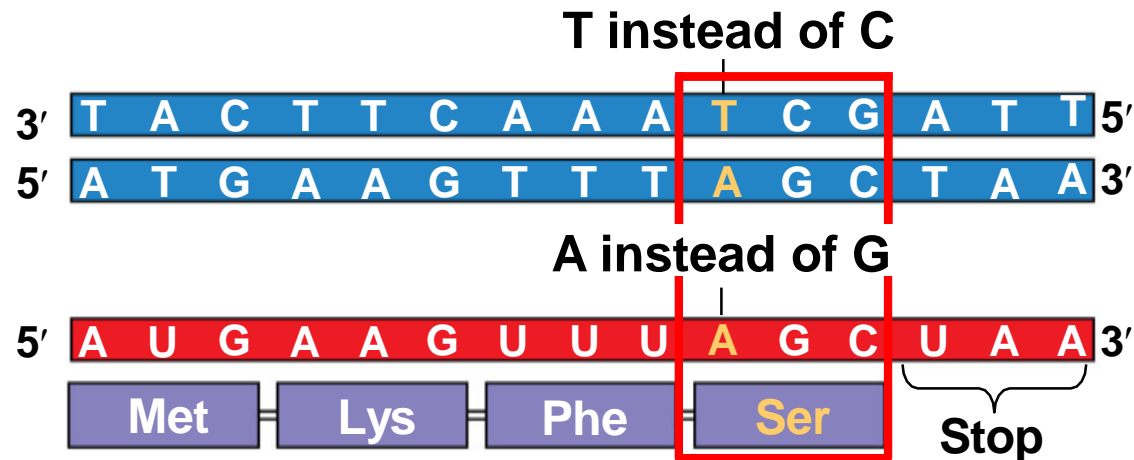
(a) Nucleotide-pair substitution: silent



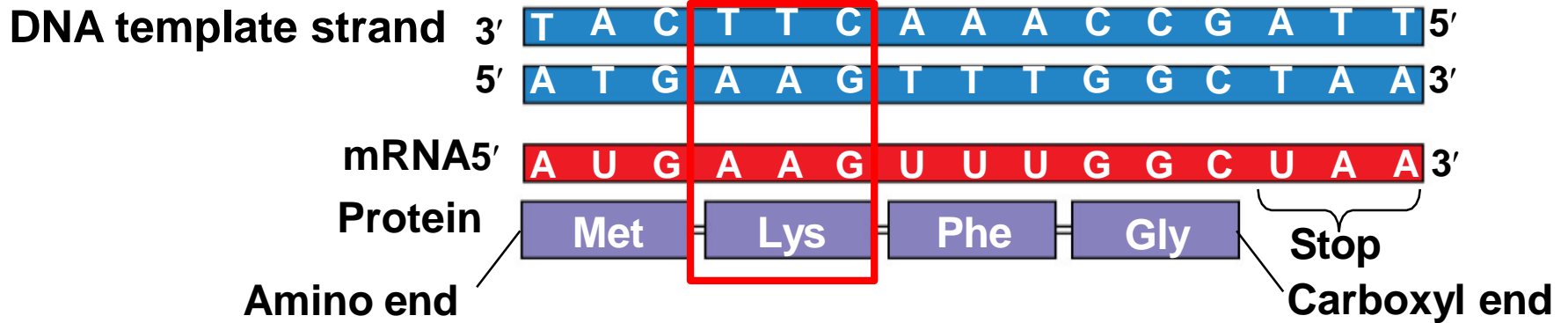
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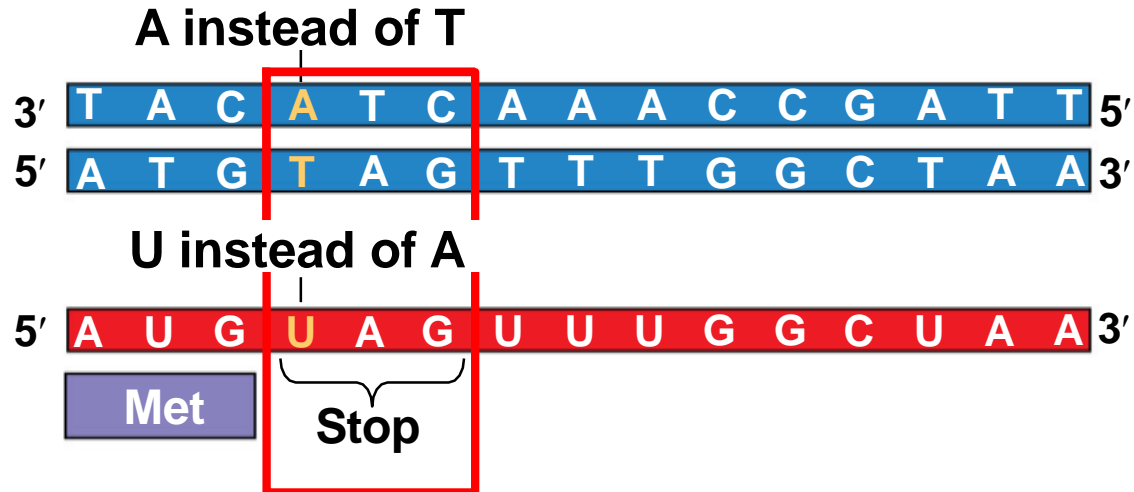
(a) Nucleotide-pair substitution: missense



Wild type



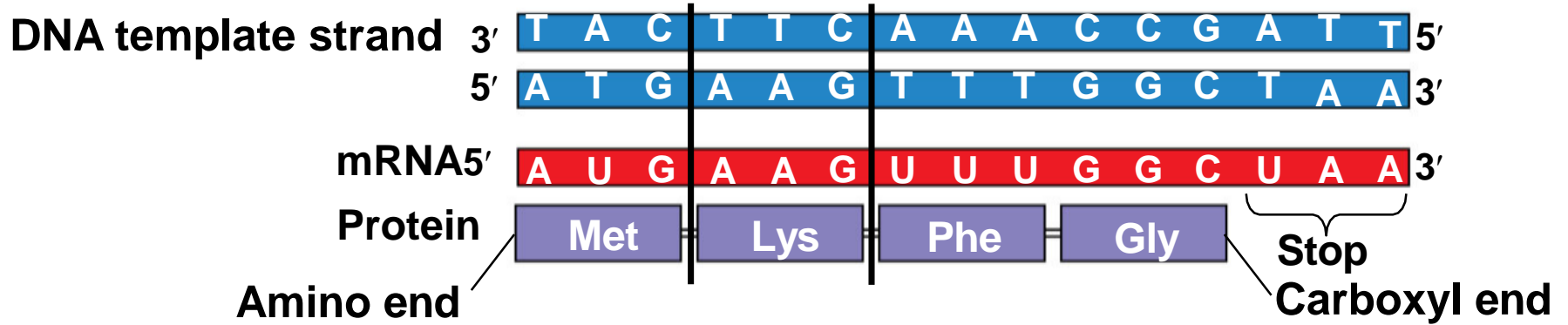
(a) Nucleotide-pair substitution: nonsense



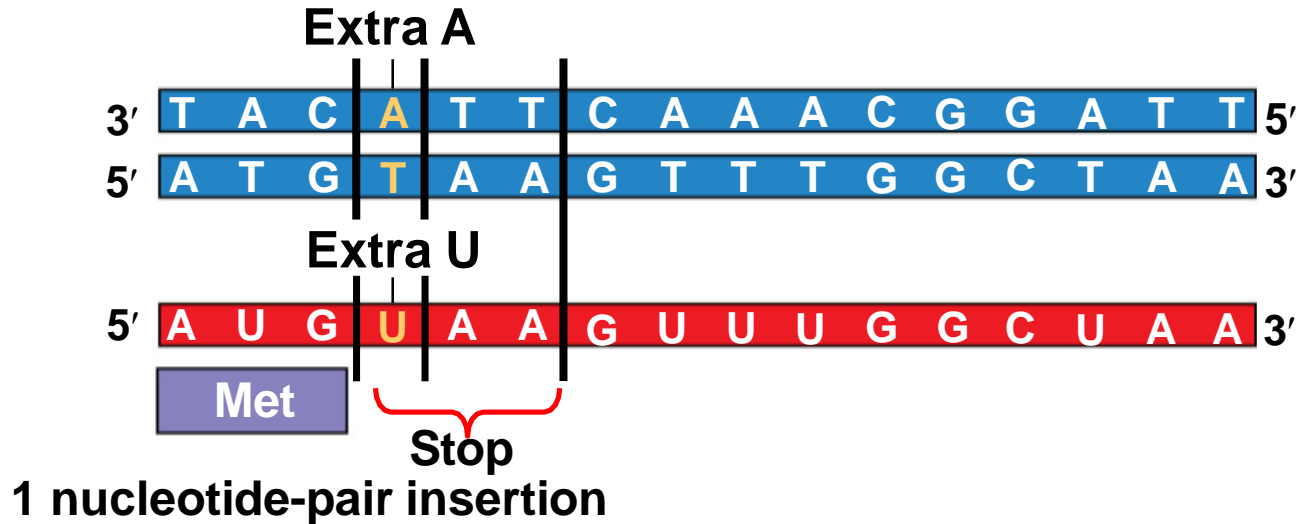
Insertions and Deletions

- **Insertions** and **deletions** are additions or losses of one or more nucleotide pairs in a gene
- These mutations often have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame, producing a **frameshift mutation**

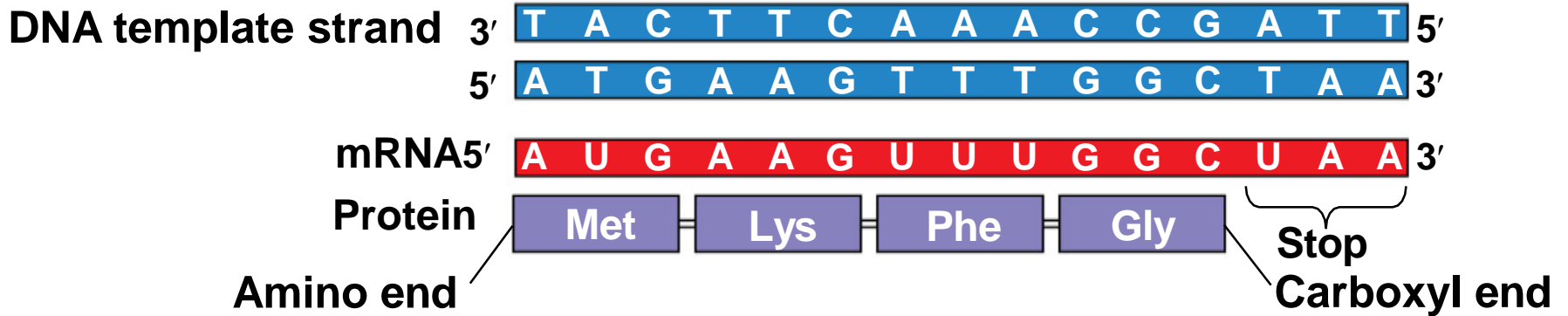
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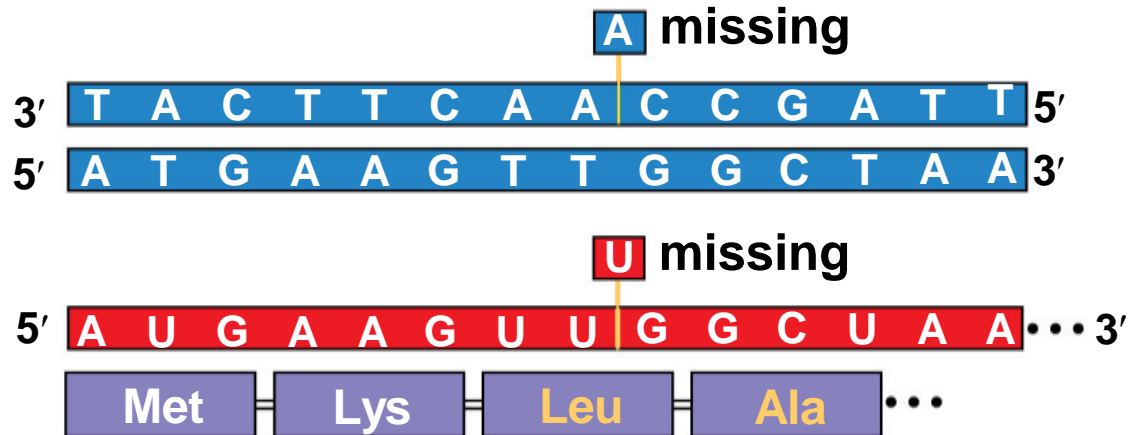
(b) Nucleotide-pair insertion or deletion: frameshift causing immediate nonsense



Wild type

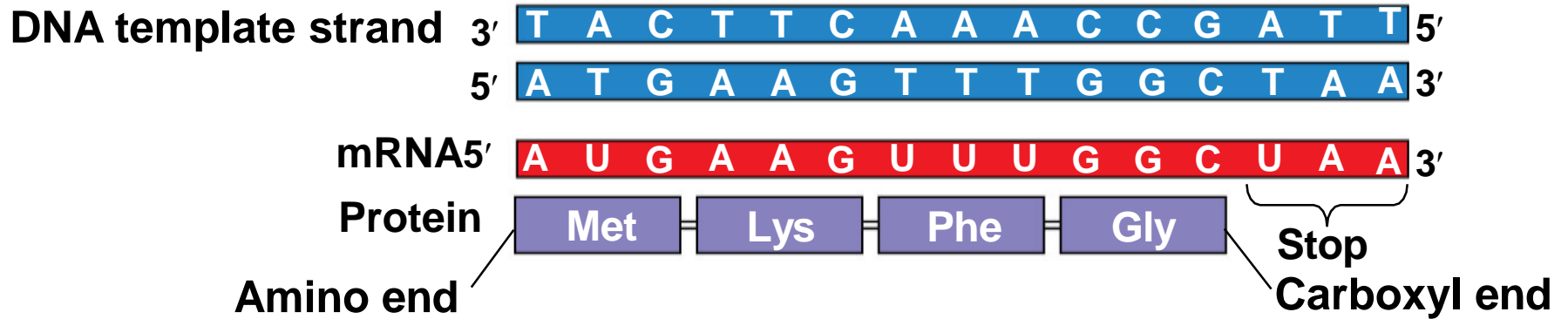


(b) Nucleotide-pair insertion or deletion: frameshift causing extensive missense

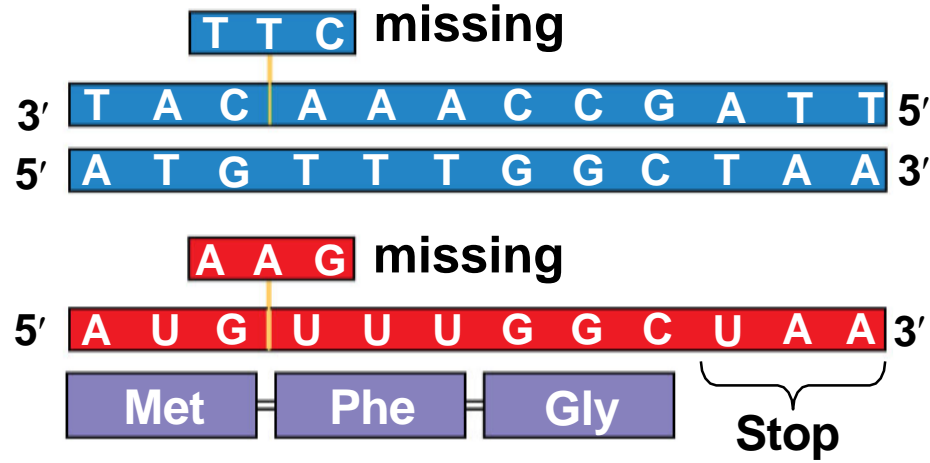


1 nucleotide-pair deletion

Wild type



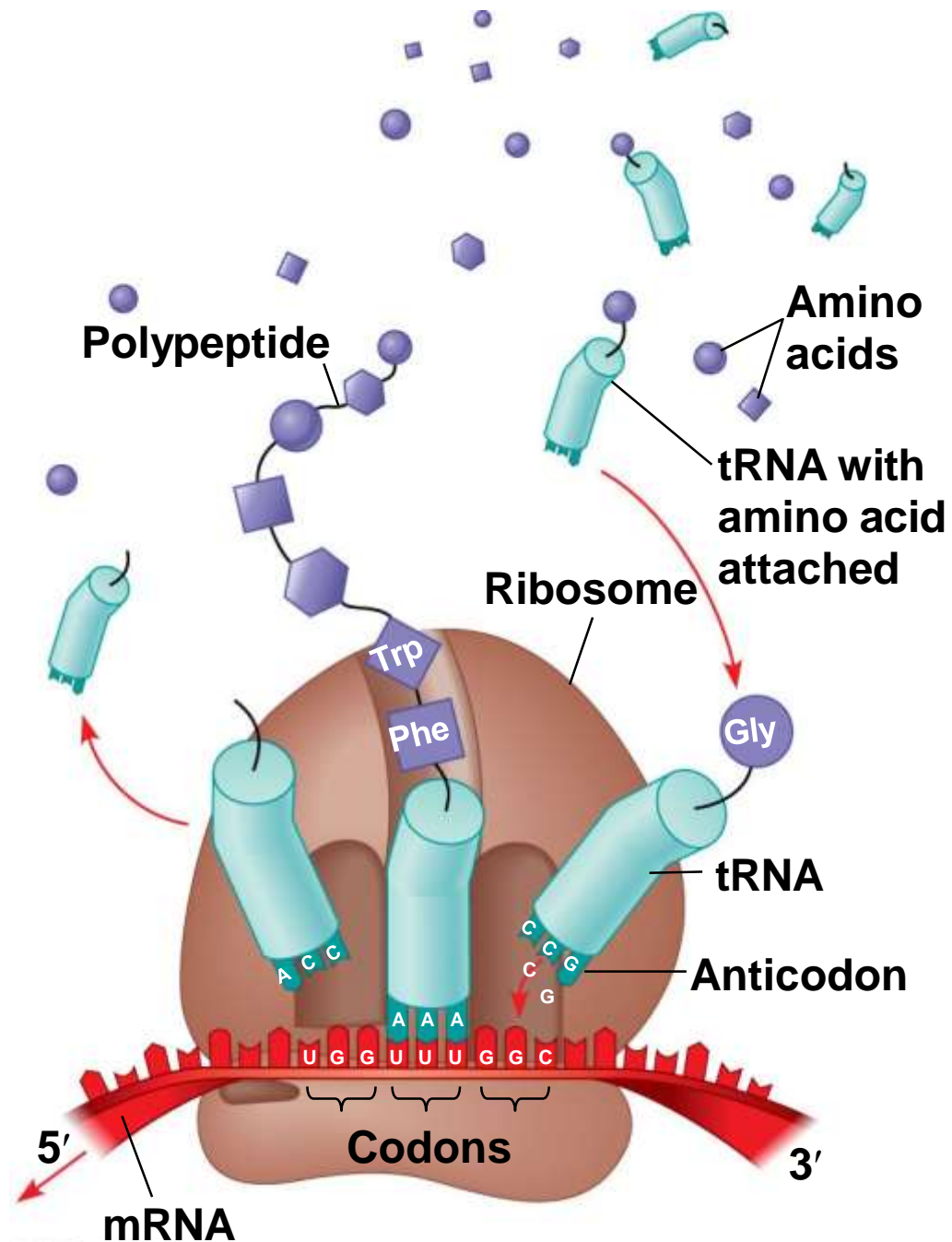
(b) Nucleotide-pair insertion or deletion: no frameshift, but one amino acid missing



3 nucleotide-pair deletion

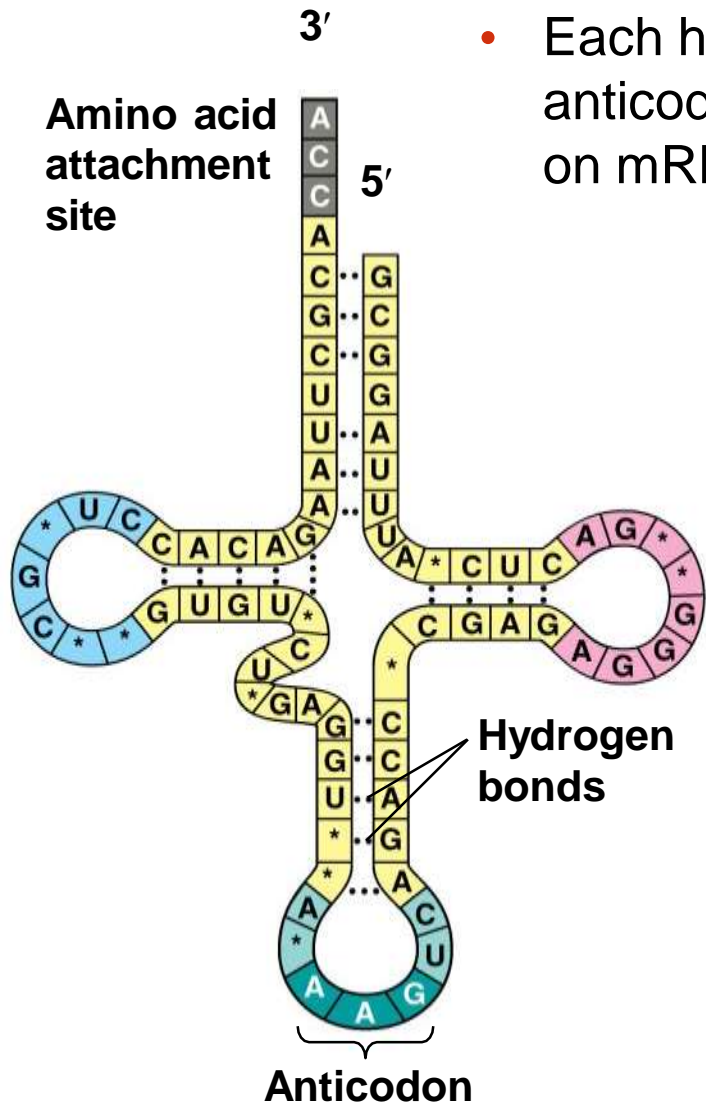
Translation

- A cell translates a mRNA message into protein with the help of **transfer RNA (tRNA)**
- tRNA transfer amino acids to the growing polypeptide in a ribosome
- Translation is a complex process in terms of its biochemistry and mechanics

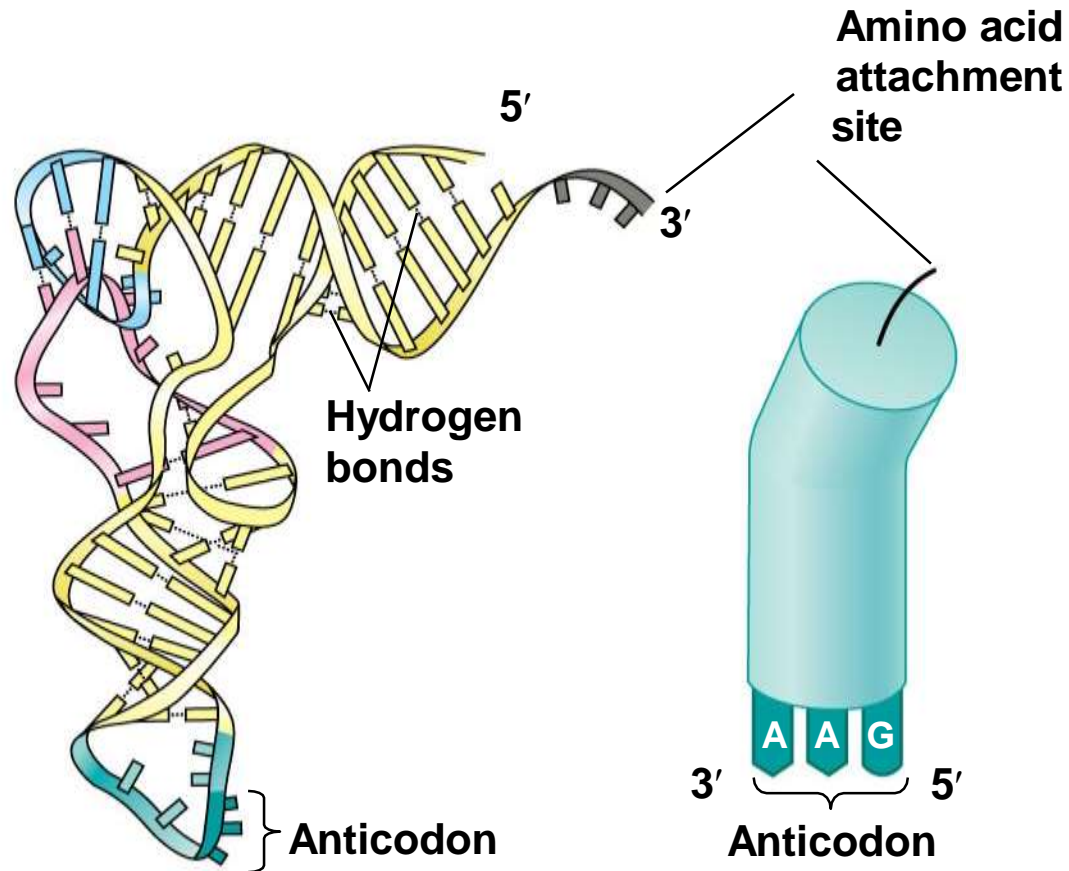


tRNA structure

- Each carries a specific amino acid on one end
- Each has an **anticodon** on the other end; the anticodon base-pairs with a complementary codon on mRNA

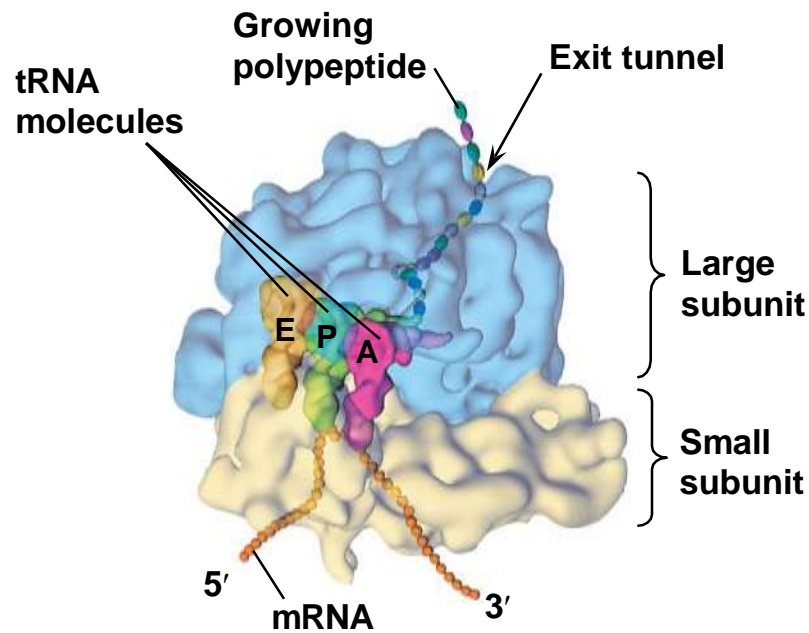


(a) Two-dimensional structure

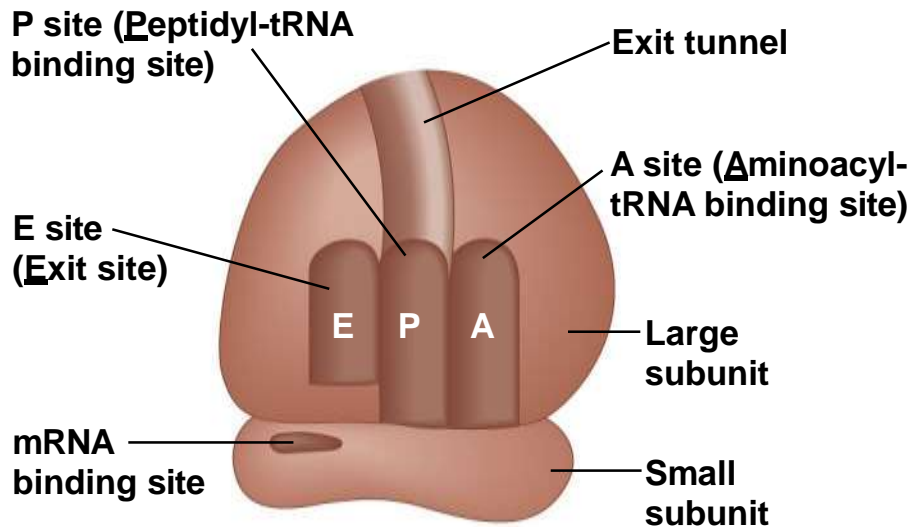


Ribosomes

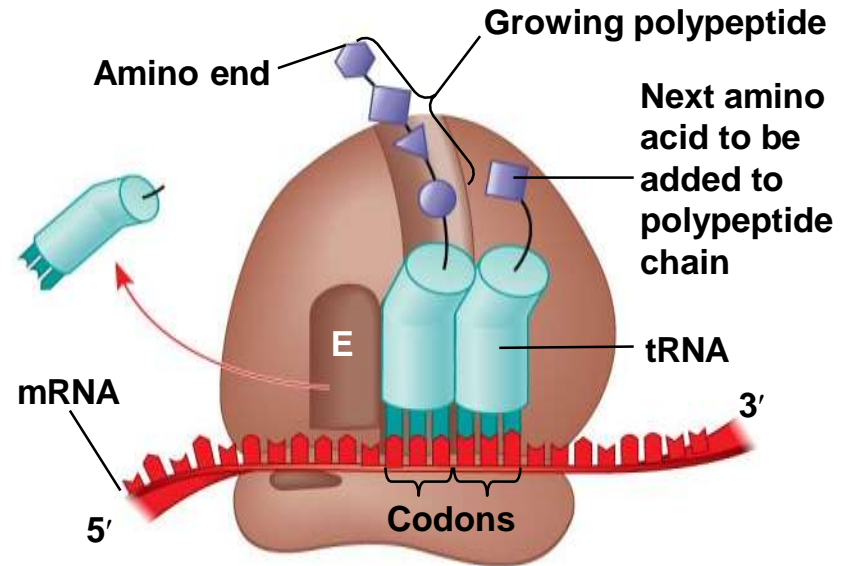
- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis
- The two ribosomal subunits (large and small) are made of proteins and **ribosomal RNA (rRNA)**
- Bacterial and eukaryotic ribosomes are somewhat similar but have significant differences: some antibiotic drugs specifically target bacterial ribosomes without harming eukaryotic ribosomes



(a) Computer model of functioning ribosome



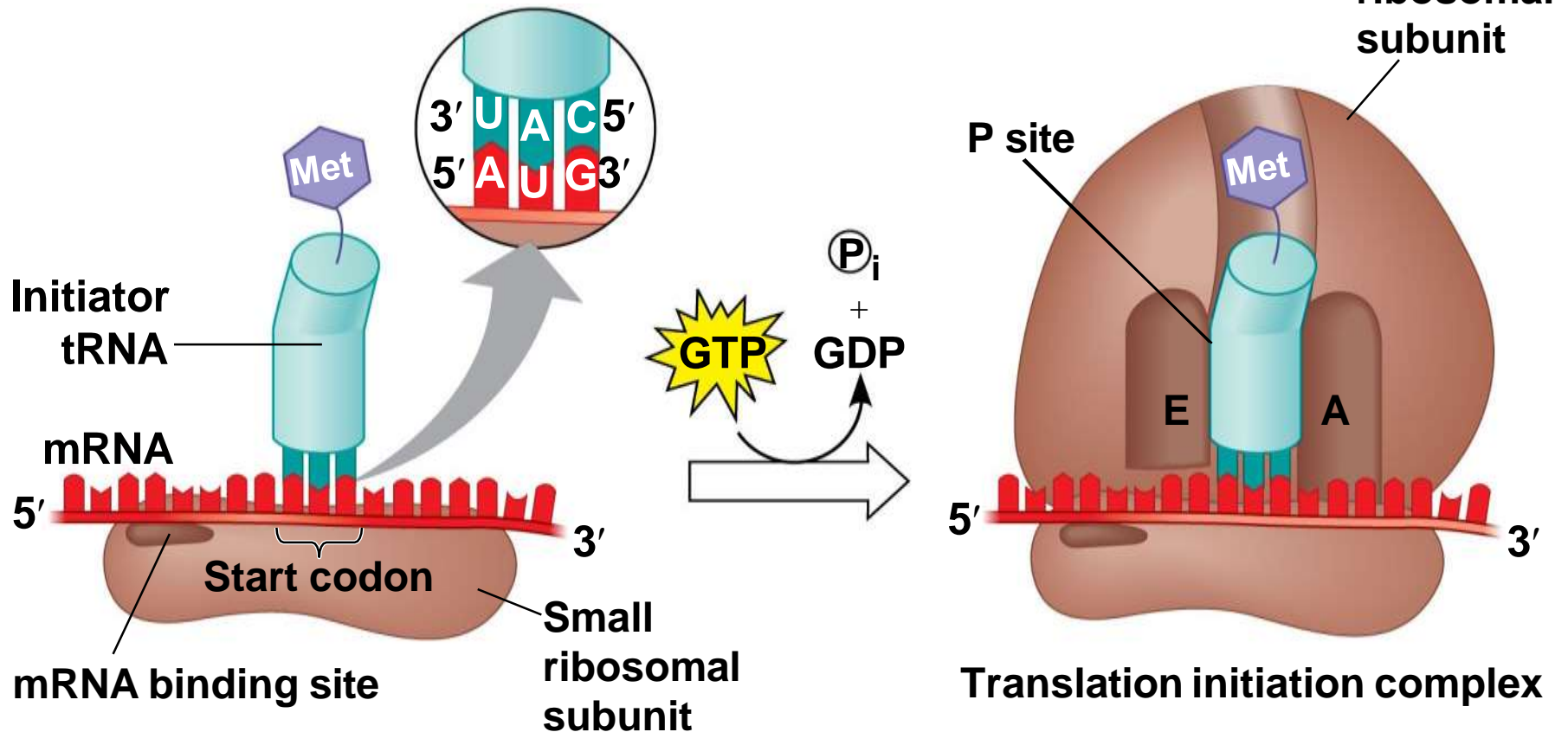
(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

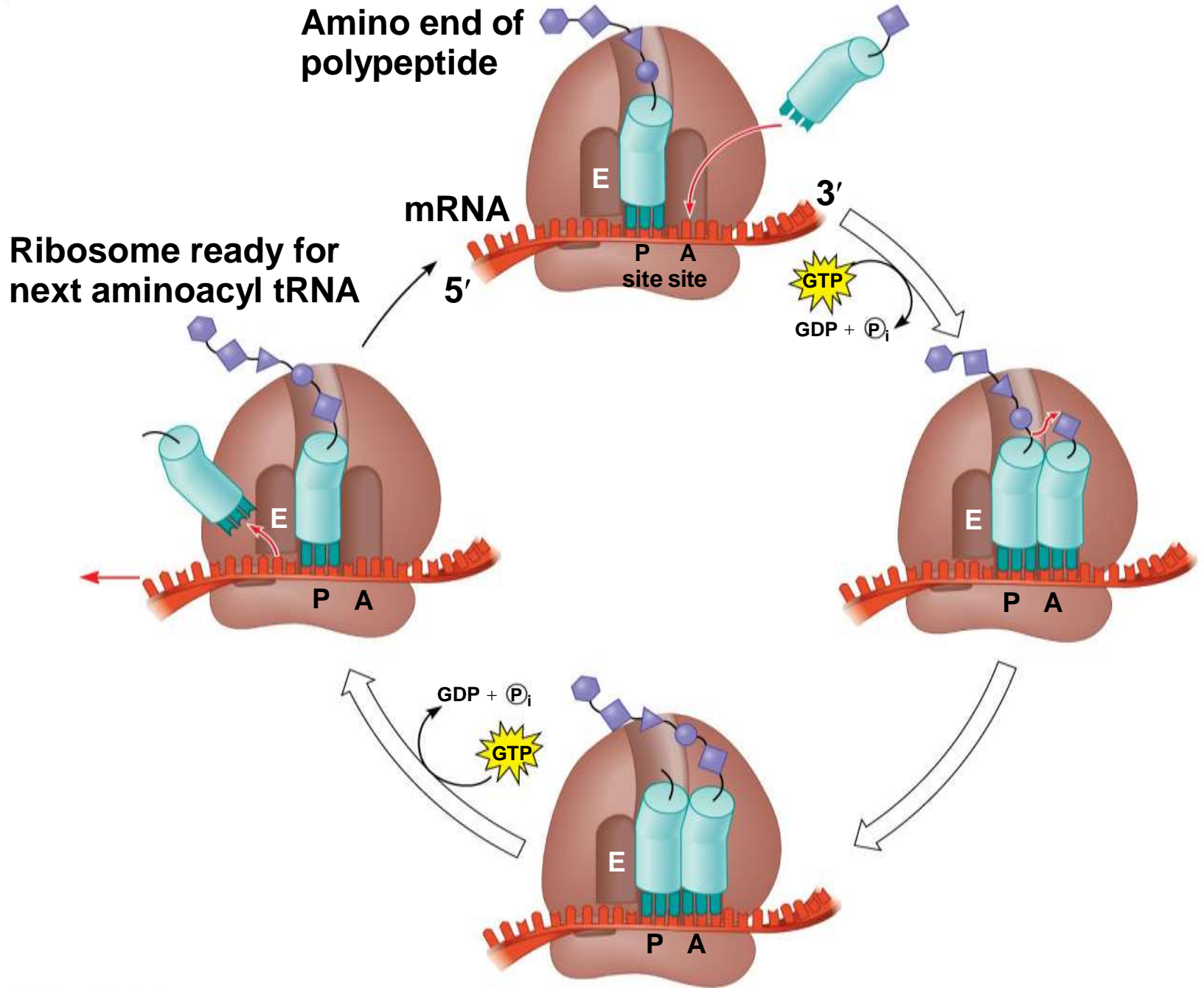
- A ribosome has three binding sites for tRNA
 - The **P site** holds the tRNA that carries the growing polypeptide chain
 - The **A site** holds the tRNA that carries the next amino acid to be added to the chain
 - The **E site** is the exit site, where discharged tRNAs leave the ribosome
- As with transcription, there are three steps to translation
 - Initiation
 - Elongation
 - Termination

Initiation of Translation



Elongation of the Polypeptide Chain

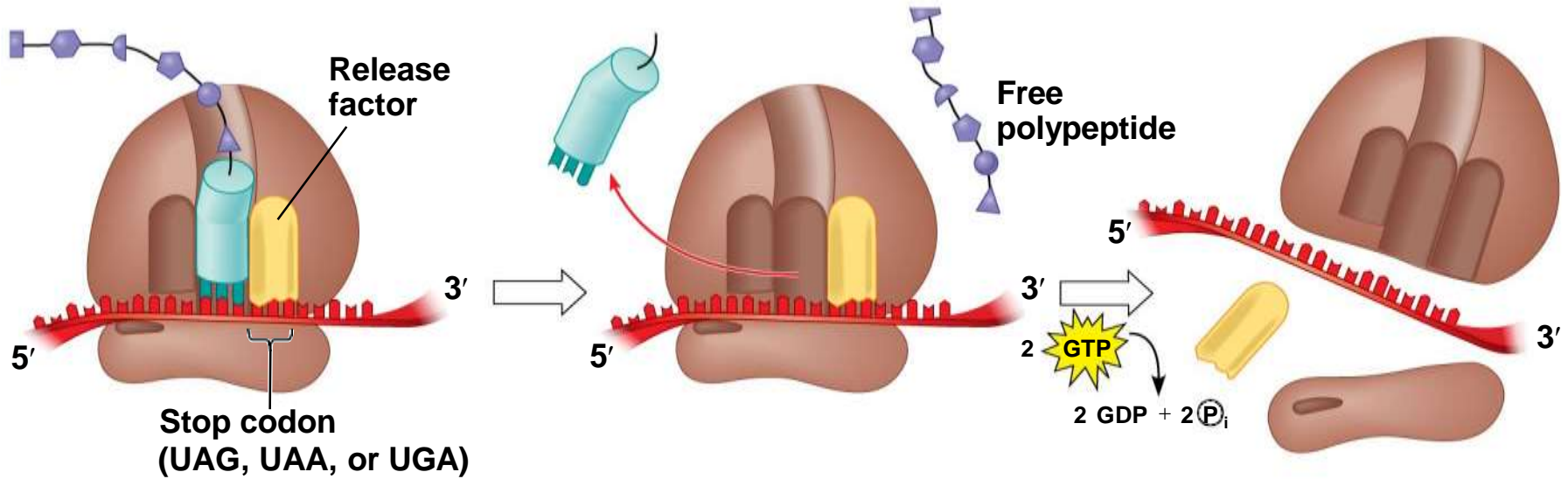
- During the elongation stage, amino acids are added one by one to the preceding amino acid at the C-terminus of the growing chain
- Each addition involves proteins called elongation factors and occurs in three steps:
 1. codon recognition,
 2. peptide bond formation,
 3. and translocation
- Translation proceeds along the mRNA in a 5 to 3 direction



Termination of Translation

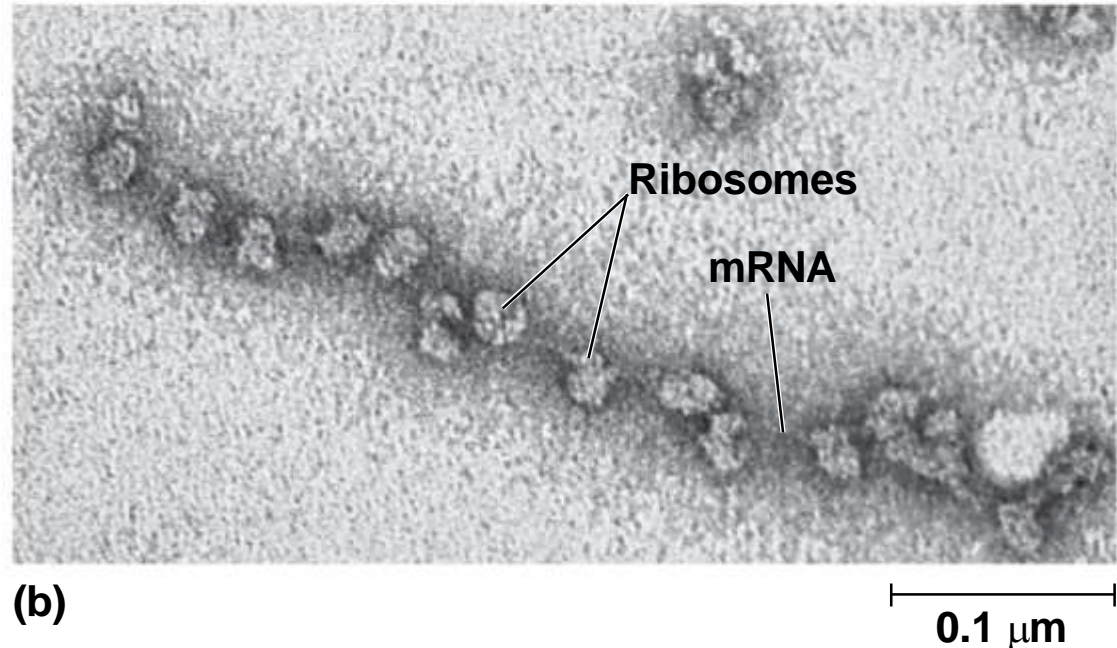
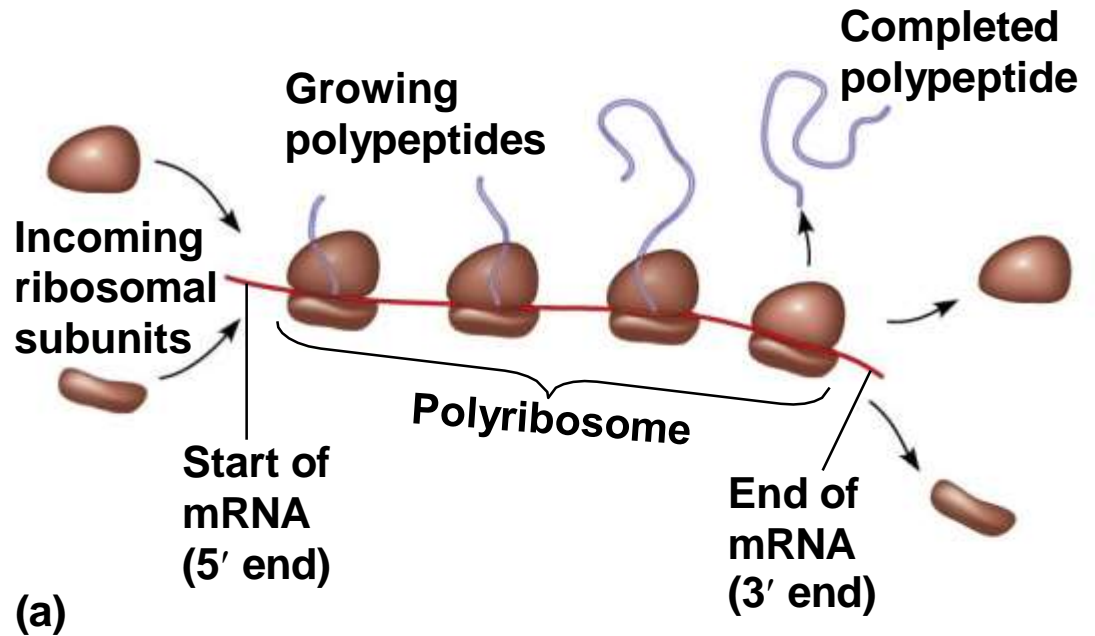
- Termination occurs when a stop codon in the mRNA reaches the A site of the ribosome
- The A site accepts a protein called a release factor
- The release factor causes the addition of a water molecule instead of an amino acid
- This reaction releases the polypeptide, and the translation assembly then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

Termination of Translation



Polyribosomes

- A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**)
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly



What is a gene?

- a region of DNA that can be expressed to produce a final functional product, either
 - a polypeptide or
 - an RNA molecule

