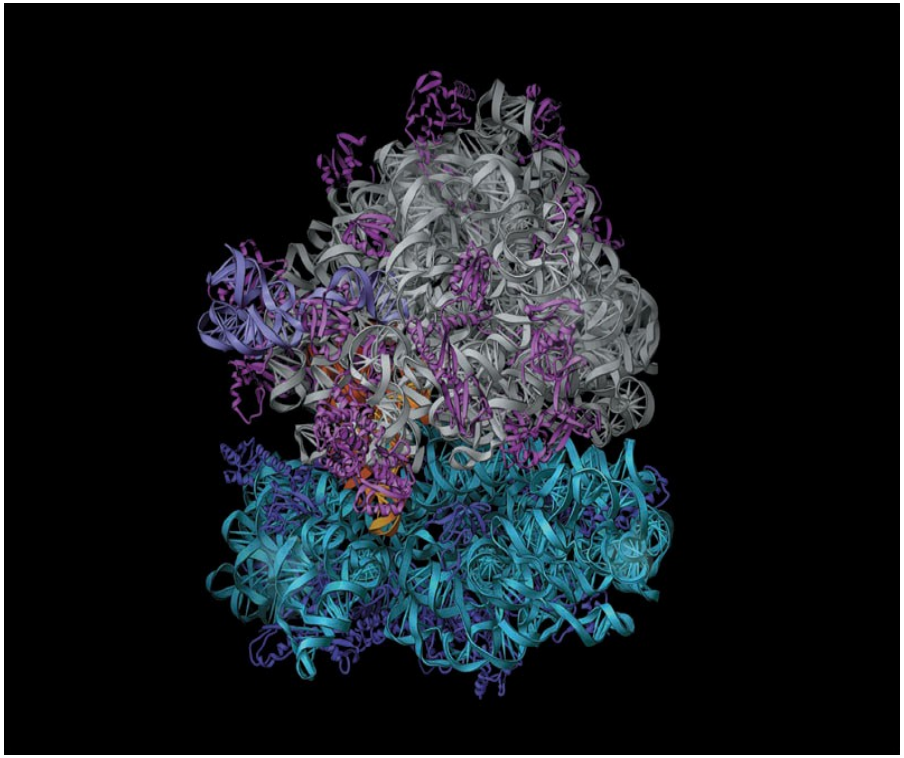
Protein Synthesis
From Gene to Protein

Unit 3

Protein synthesis

- The information content of DNA
  - Is in the form of specific sequences of nucleotides along the DNA strands
- The DNA inherited by an organism
  - Leads to specific traits by dictating the synthesis of proteins
- The process by which DNA directs protein synthesis, gene expression
  - Includes two stages, called transcription and translation

- The ribosome
  - Is part of the cellular machinery for translation, polypeptide synthesis



- Genes specify proteins via transcription and translation
- Transcription involves the transfer of genetic information from DNA into an RNA molecule while translation involves the transfer of the information in the RNA to the synthesis of a protein

Evidence from the Study of Metabolic Defects

- The relationship between genes and proteins was first proposed in 1909 by an English physician Archibald Garrod
  - He was the first to suggest that genes dictate phenotypes through enzymes which are proteins that catalyze specific chemical reactions in the cell.
  - He hypothesized that inherited diseases reflect a person's inability to make a particular enzyme.
  - Citing the disease alkaptonuria where urine appears dark red due to the presence of alkapton as an example, Garrod reasoned that normal individuals have an enzyme that breaks down alkapton while alkaptonuric individuals lack the enzyme
  - Garrod's hypothesis was ahead of its time but research decades later proved him right

Nutritional Mutants in Neurospora: Scientific Inquiry

- In 1940s, George Beadle and Edward Tatum proved the relationship between genes and enzymes by using the bread mold, *Neurospora crassa*.
- Beadle and Tatum studied strains of the mold that were unable to grow on the usual minimal growth medium. These strains were mutants created using X-ray radiation.
- Each of these mutants lacked an enzyme in a metabolic pathway and therefore were unable to produce a particular molecule such as an amino acid.
- They showed that each mutant was defective in a single gene and hypothesized that one gene controlled the production of one specific enzyme.
- This hypothesis has now been modified from one gene-one enzyme to one gene-one protein to one gene–one polypeptide.

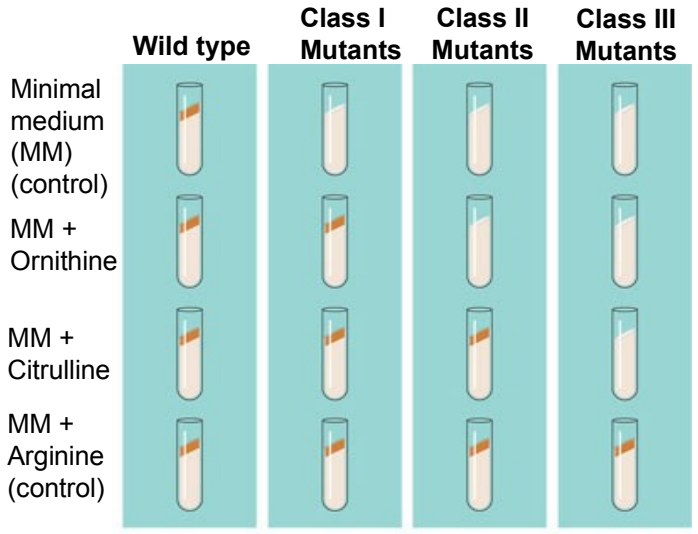
- Using genetic crosses
  - Tatum and Beadle determined that their mutants fell into three classes, each mutated in a different gene

EXPERIMENT

Working with the mold *Neurospora crassa*, George Beadle and Edward Tatum had isolated mutants requiring arginine in their growth medium and had shown genetically that these mutants fell into three classes, each defective in a different gene. From other considerations, they suspected that the metabolic pathway of arginine biosynthesis included the precursors ornithine and citrulline. Their most famous experiment, shown here, tested both their one gene–one enzyme hypothesis and their postulated arginine pathway. In this experiment, they grew their three classes of mutants under the four different conditions shown in the Results section below.

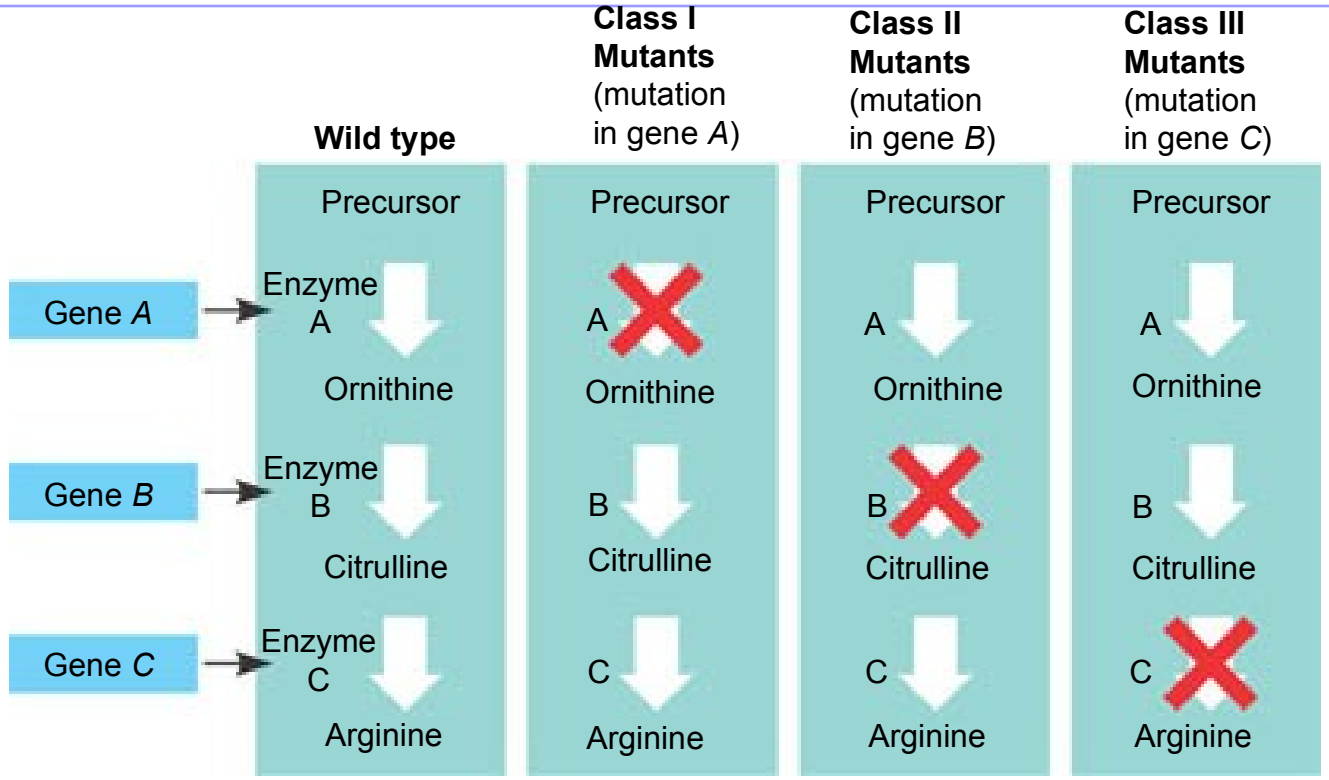
RESULTS

The wild-type strain required only the minimal medium for growth. The three classes of mutants had different growth requirements



CONCLUSION

From the growth patterns of the mutants, Beadle and Tatum deduced that each mutant was unable to carry out one step in the pathway for synthesizing arginine, presumably because it lacked the necessary enzyme. Because each of their mutants was mutated in a single gene, they concluded that each mutated gene must normally dictate the production of one enzyme. Their results supported the one gene–one enzyme hypothesis and also confirmed the arginine pathway.
(Notice that a mutant can grow only if supplied with a compound made *after* the defective step.)



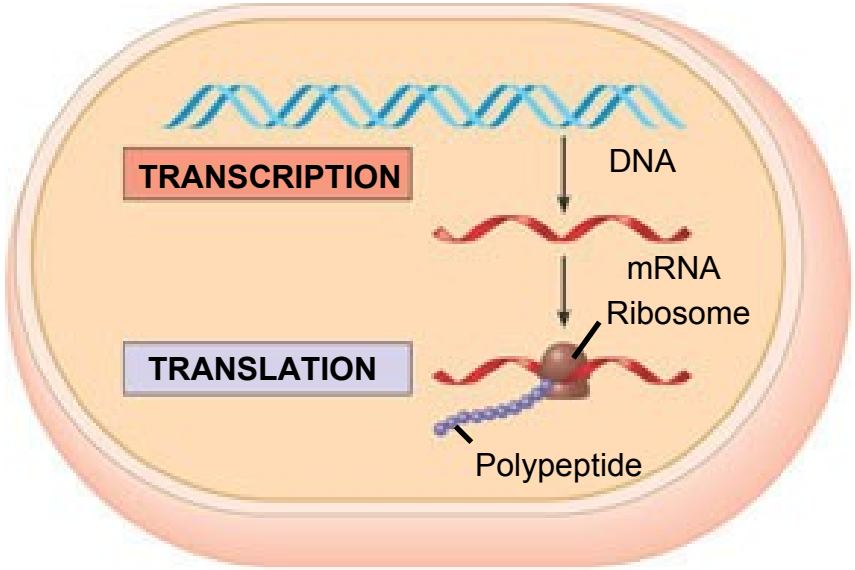
The Products of Gene Expression: A Developing Story

- Beadle and Tatum developed the “one gene–one enzyme hypothesis”
  - Which states that the function of a gene is to dictate the production of a specific enzyme
- As researchers learned more about proteins
  - They made minor revision to the one gene–one enzyme hypothesis
- Genes are now known to code for polypeptide chains or for RNA molecules.

Basic Principles of Transcription and Translation

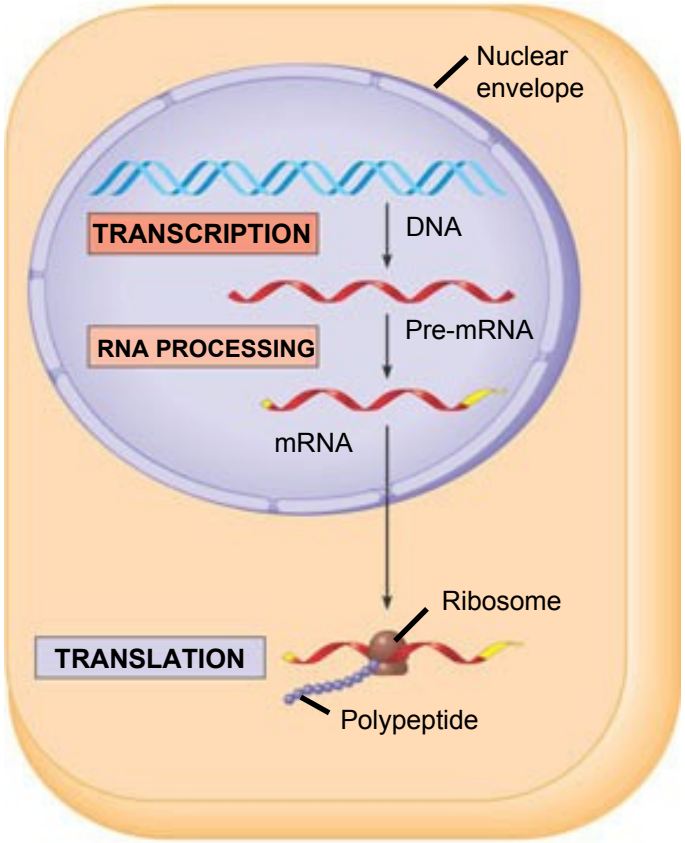
- Transcription
  - Is the synthesis of RNA under the direction of DNA
  - Produces messenger RNA (mRNA)
- Translation
  - Is the actual synthesis of a polypeptide, which occurs under the direction of mRNA
  - Occurs on ribosomes

- In prokaryotes
  - Transcription and translation occur together



(a) Prokaryotic cell. In a cell lacking a nucleus, mRNA produced by transcription is immediately translated without additional processing.

- In eukaryotes
  - RNA transcripts are modified before becoming true mRNA



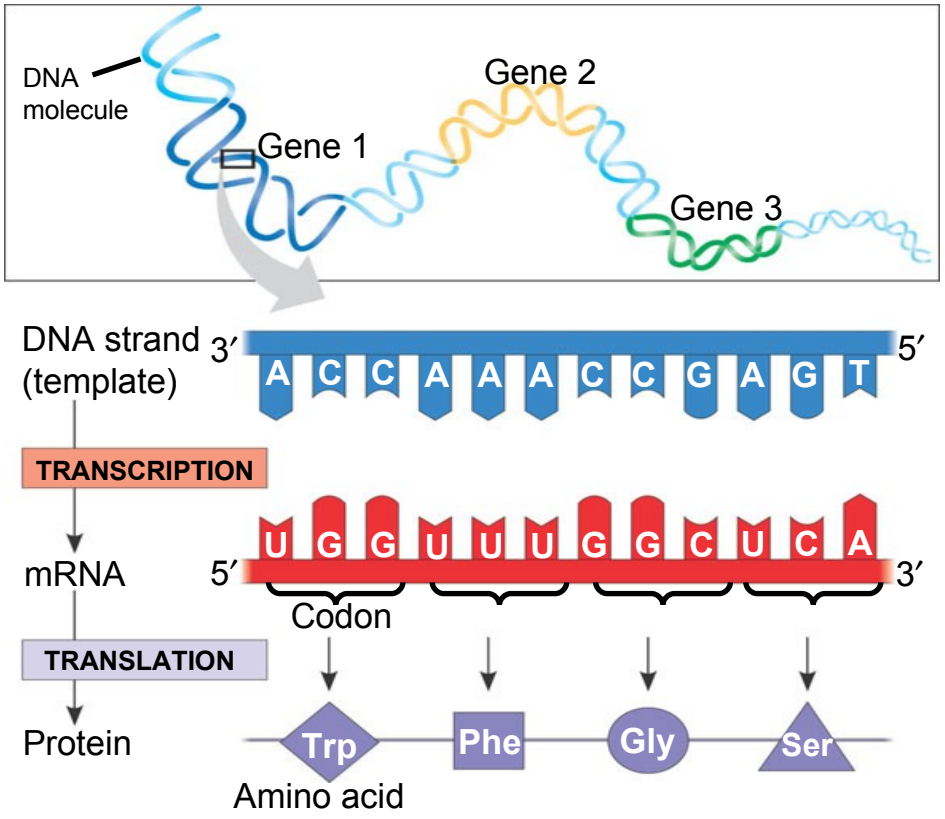
(b) Eukaryotic cell. The nucleus provides a separate compartment for transcription. The original RNA transcript, called pre-mRNA, is processed in various ways before leaving the nucleus as mRNA.

- Cells are governed by a cellular chain of command
  - DNA $\rightarrow$ RNA $\rightarrow$ protein

The Genetic Code

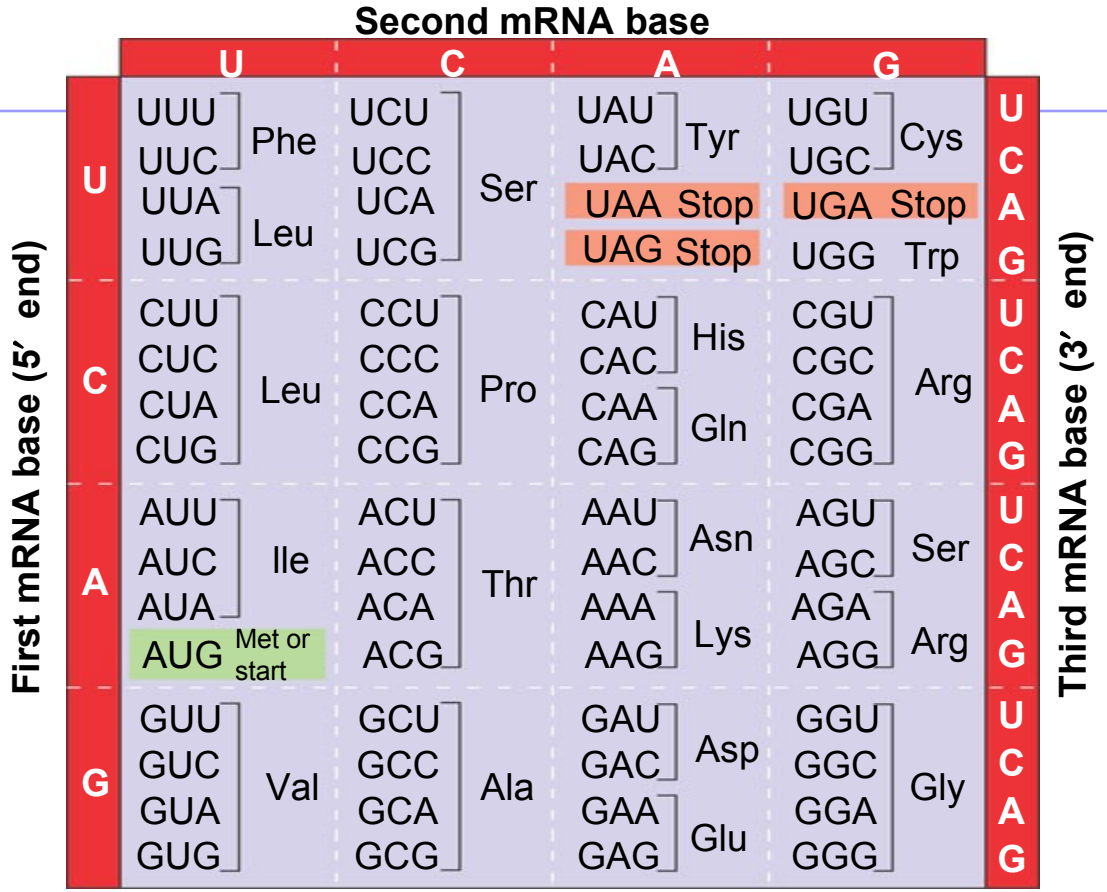
- How many bases correspond to an amino acid?
- A sequence of three bases known as a base triplet or a codon encode for one amino acid.
- Genetic information
  - Is encoded as a sequence of non-overlapping base triplets, or codons

- During transcription
  - The gene determines the sequence of bases along the length of an mRNA molecule



The Dictionary of the genetic code

- A codon in messenger RNA
  - Is either translated into an amino acid or serves as a translational stop signal



- Codons must be read in the correct reading frame from the 5’ end to the 3’ end without over-lapping
  - For the specified polypeptide to be produced
  - There are 64 codons out of which 61 codons encode for amino acids while the remaining 3 act as stop codons to terminate transcription and translation.
  - The stop codons are UAA, UGA, UAG
  - AUG is the start codon which also encodes for the amino acid Methionine.
  - The genetic code has redundancy whereby one amino acid can be encoded for by more than one codon. The maximum number of codons is 6 while the minimum is 1.

Evolution of the Genetic Code

- The genetic code is nearly universal
  - Shared by organisms from the simplest bacteria to the most complex animals
- In laboratory experiments
  - Genes can be transcribed and translated after being transplanted from one species to another

Transcription

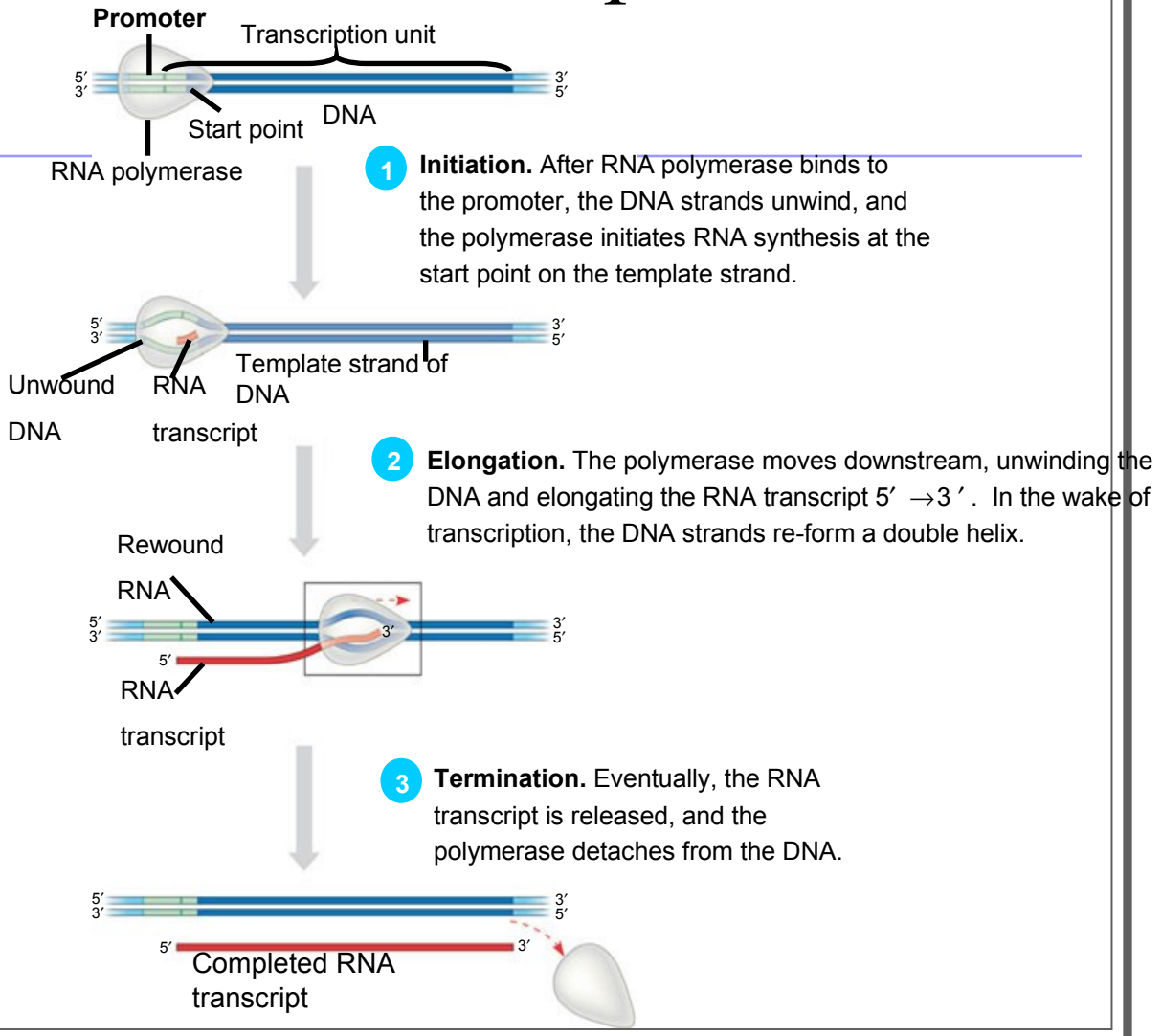
- Transcription is the DNA-directed synthesis of mRNA: *a closer look*

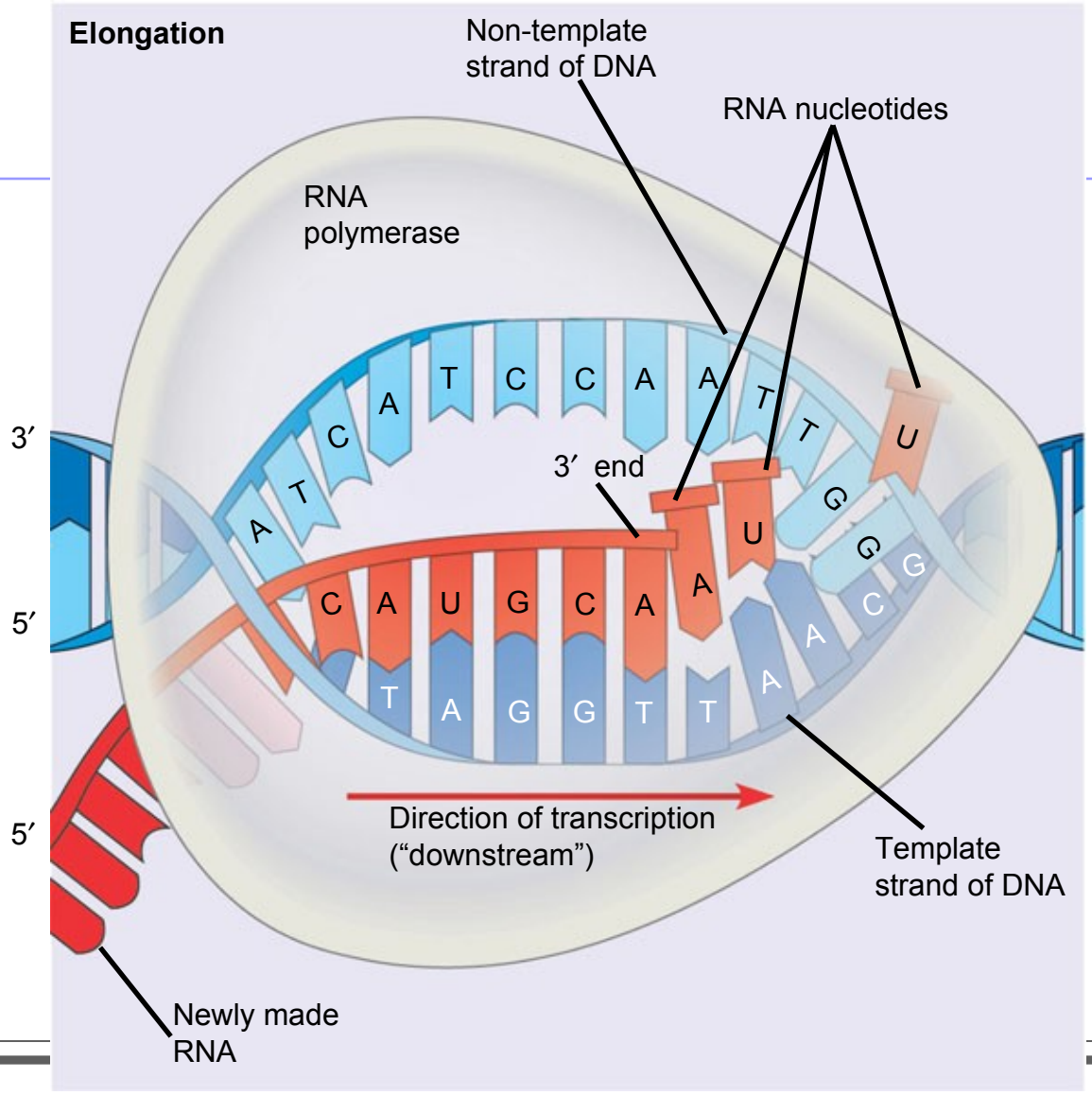
Molecular Components of Transcription

- mRNA synthesis
  - Is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
  - Follows the same base-pairing rules as DNA, except that in RNA, uracil substitutes for thymine

Synthesis of an mRNA Transcript

- The stages of transcription are
  - Initiation
  - Elongation
  - Termination




Elongation
Non-template strand of DNA
RNA nucleotides
RNA polymerase
3′
5′
3′ end
5′
Direction of transcription ("downstream")
Template strand of DNA
Newly made RNA

Elongation of the RNA Strand

- As RNA polymerase moves along the DNA
  - It continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides

Termination of Transcription

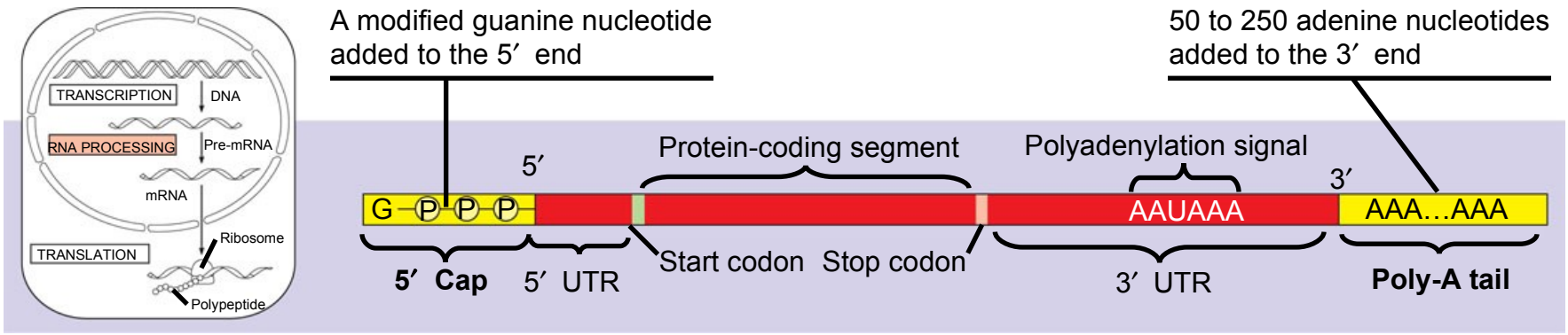
- The mechanisms of termination
  - Are different in prokaryotes and eukaryotes

RNA Processing/Post-Transcriptional Modification

- Eukaryotic cells modify RNA after transcription
- Enzymes in the eukaryotic nucleus
  - Modify pre-mRNA in specific ways before the genetic messages are dispatched to the cytoplasm
  - They modify the 5’ and 3’ ends and also remove the introns to splice the exons together to form a continuous reading frame.

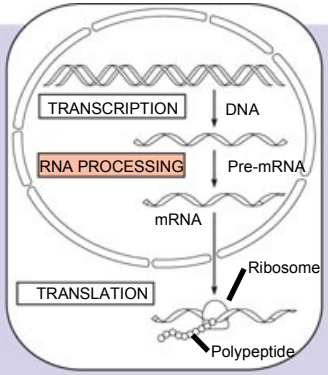
Alteration of mRNA Ends

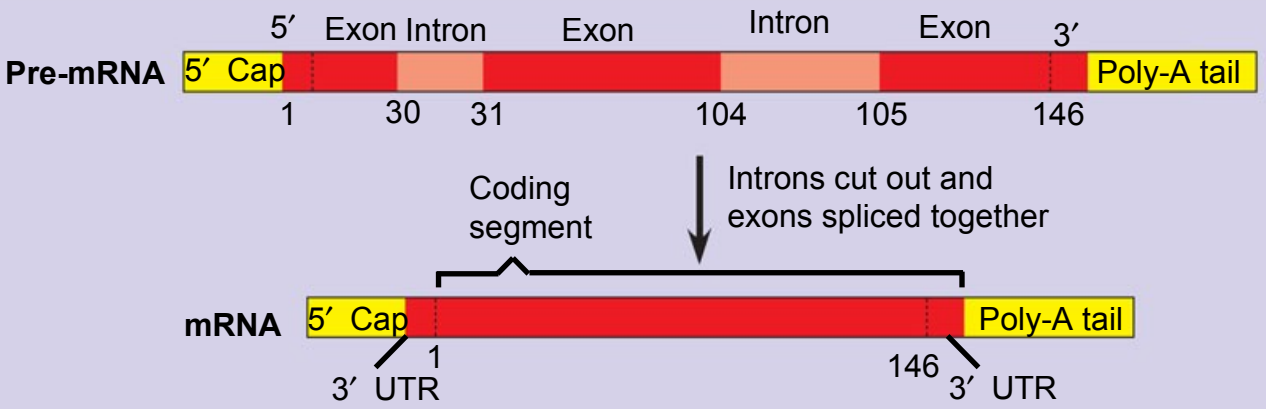
- Each end of a pre-mRNA molecule is modified in a particular way
  - The 5′ end receives a modified nucleotide cap
  - The 3′ end gets a poly-A tail



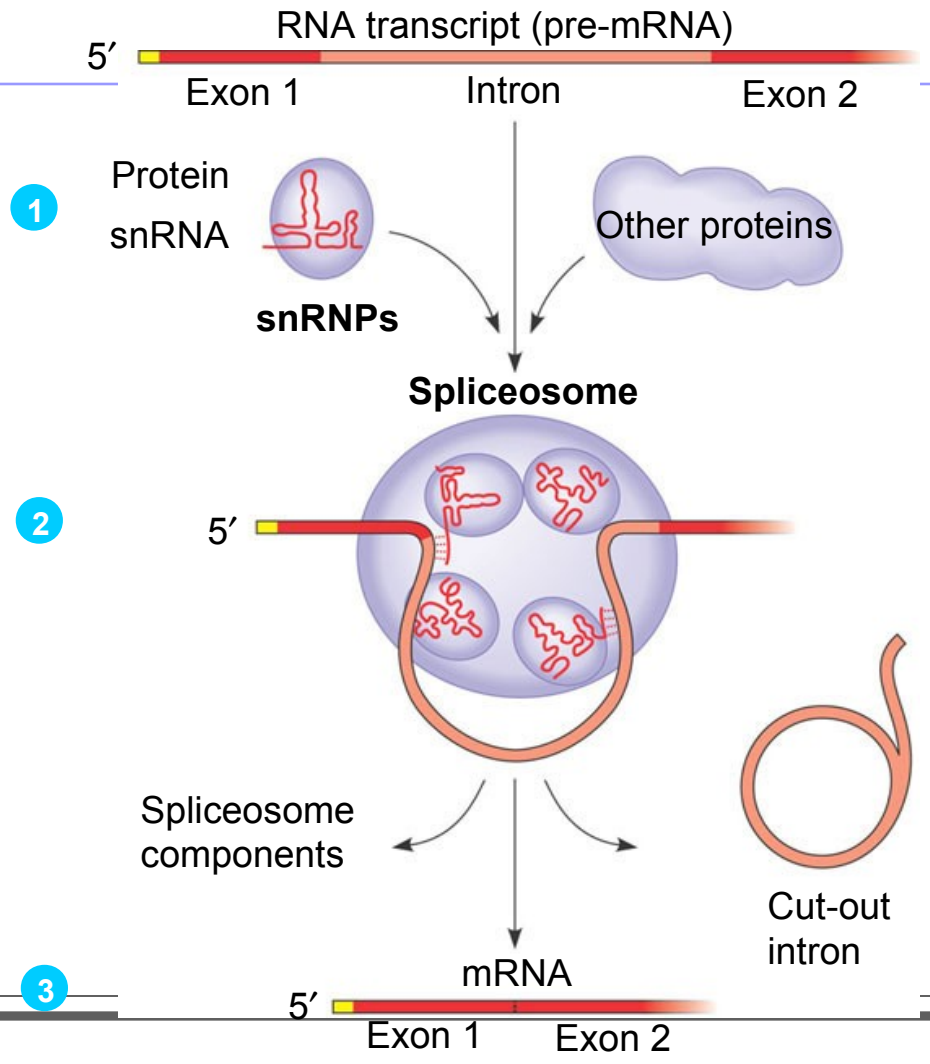
RNA Splicing

- Removes introns and joins exons to produce a continuous reading frame





- Is carried out by spliceosomes in some cases



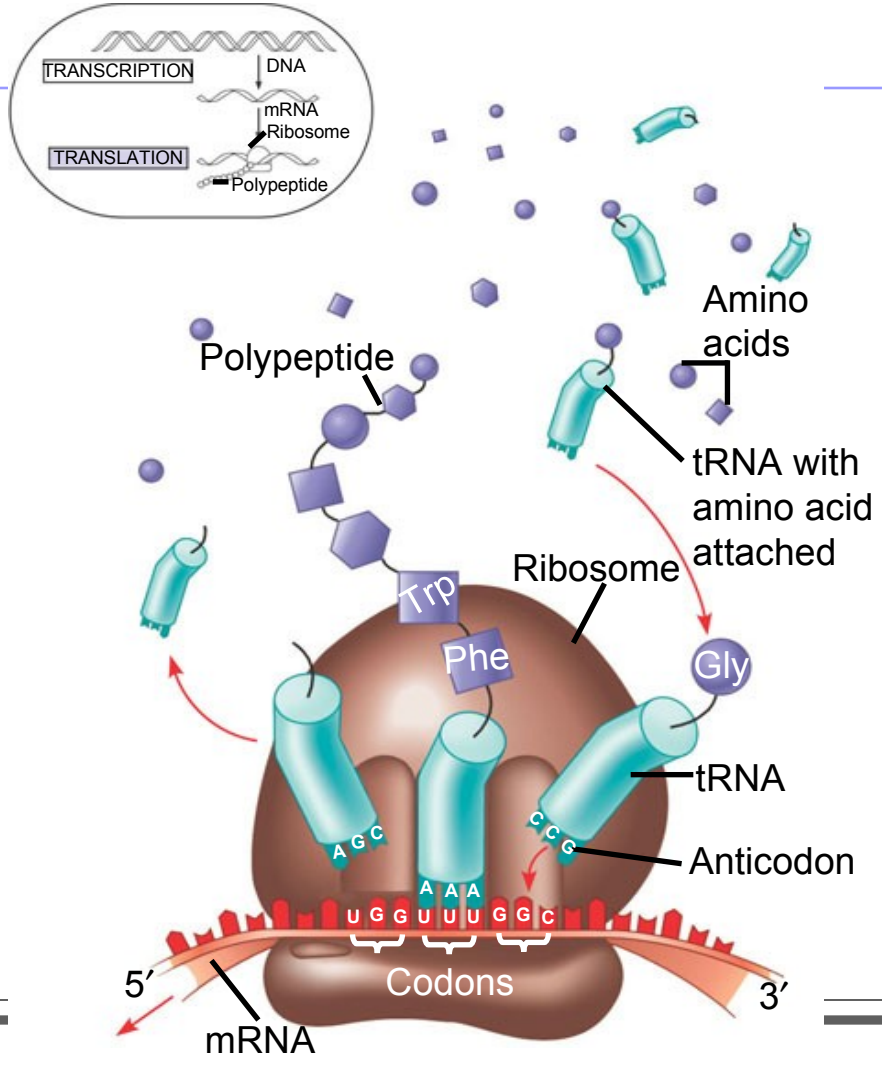
Translation

- Translation is the RNA-directed synthesis of a polypeptide: *a closer look*

Molecular Components of Translation

- A cell translates an mRNA message into protein
  - With the help of transfer RNA (tRNA)

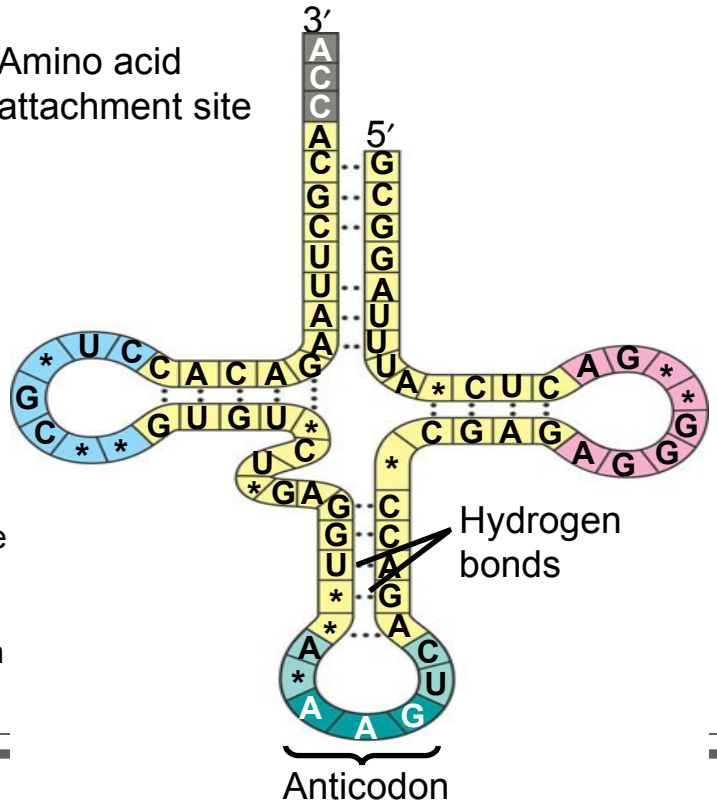
Translation: the basic concept



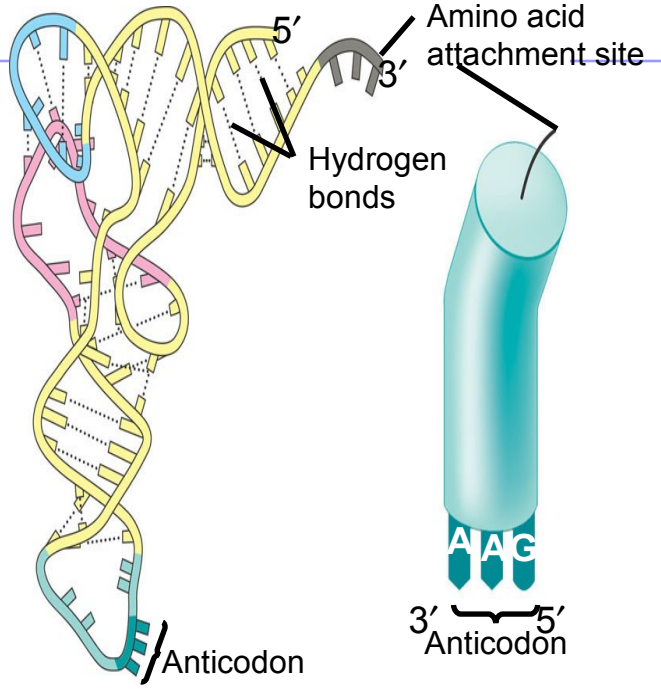
- Molecules of tRNA are not all identical
  - Each carries a specific amino acid on one end (3’ end)
  - Each has an anticodon on the other end which is complementary to a codon of mRNA

The Structure and Function of Transfer RNA

- A tRNA molecule
  - Consists of a single RNA strand that is only about 80 nucleotides long
  - Is roughly L-shaped



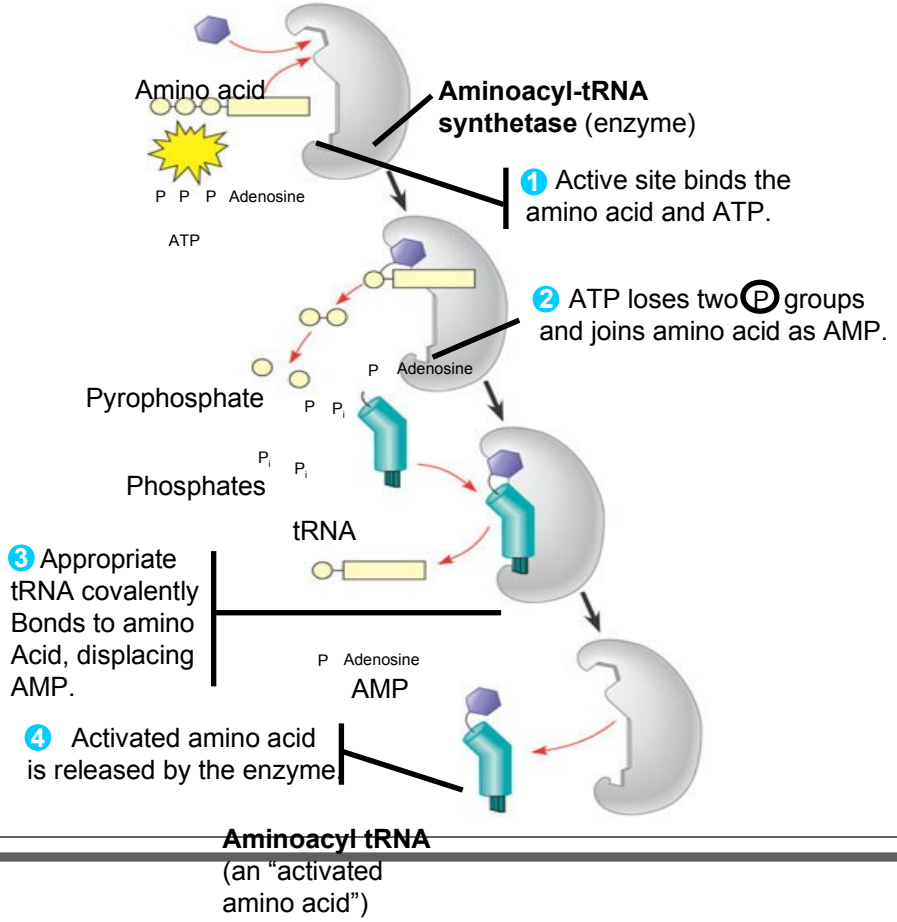
(a) **Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3′ end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)



(b) Three-dimensional structure

(c) Symbol used in this book

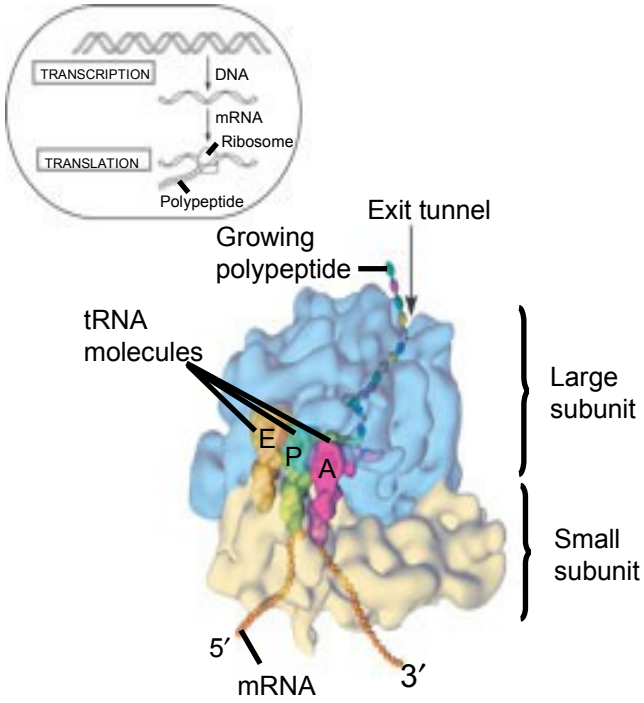
- A specific enzyme called an aminoacyl-tRNA synthetase
  - Joins each amino acid to the correct tRNA



Ribosomes

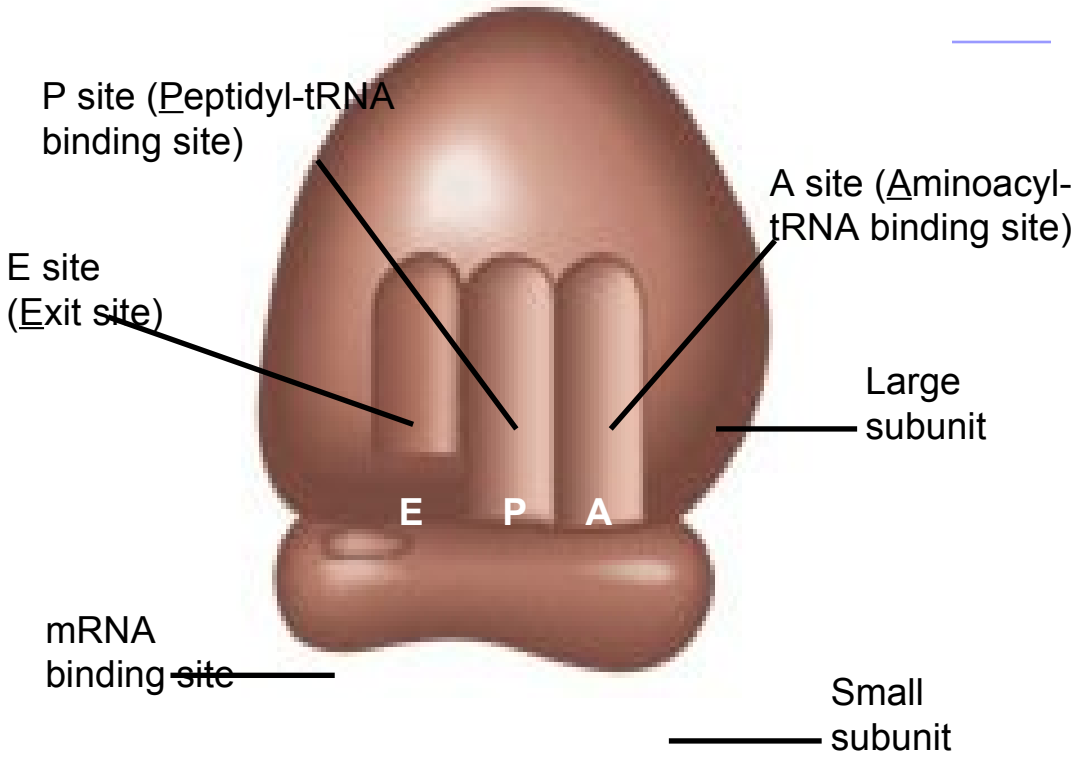
- Facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis

- The ribosomal subunits
  - Are constructed of proteins and RNA molecules named ribosomal RNA or rRNA

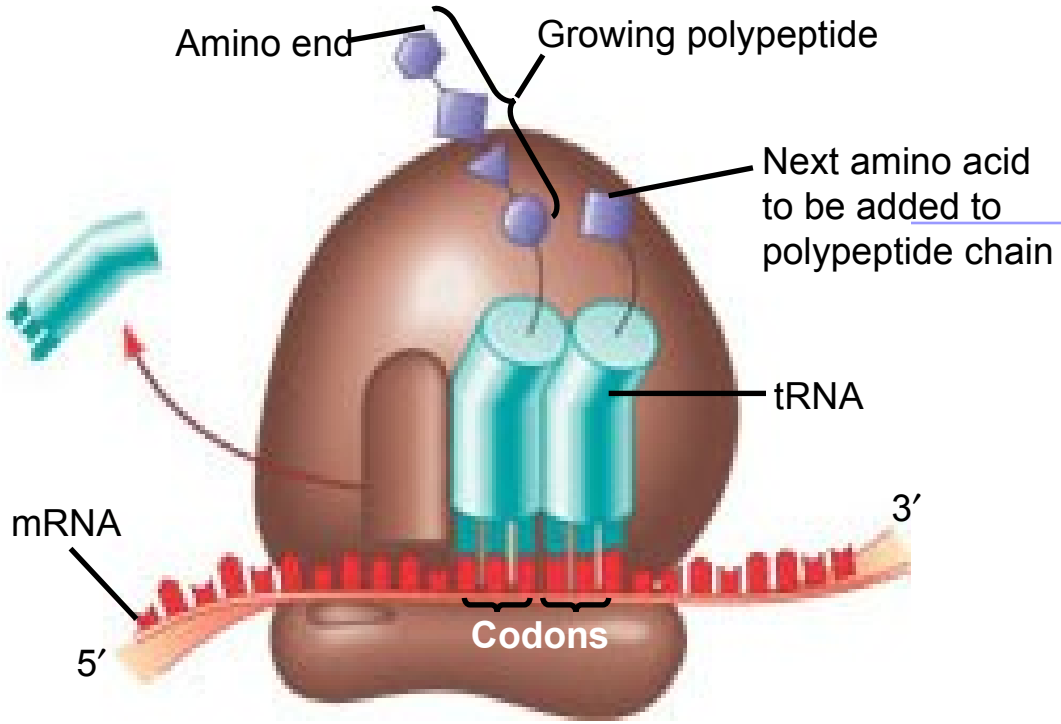


(a) Computer model of functioning ribosome. This is a model of a bacterial ribosome, showing its overall shape. The eukaryotic ribosome is roughly similar. A ribosomal subunit is an aggregate of ribosomal RNA molecules and proteins.

- The ribosome has three binding sites for tRNA
  - The P site
  - The A site
  - The E site



(b) Schematic model showing binding sites. A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.



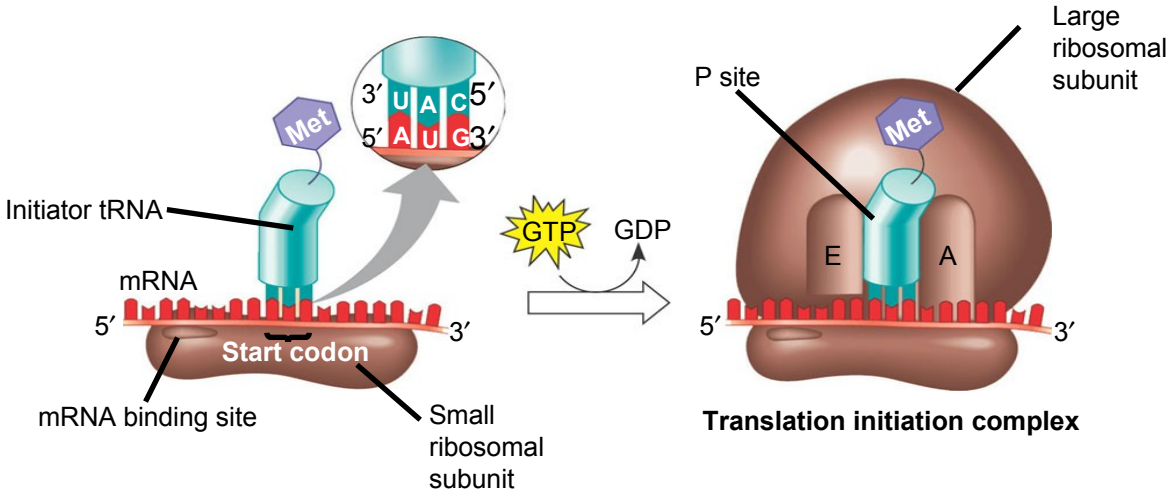
(c) **Schematic model with mRNA and tRNA.** A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.

Building a Polypeptide

- We can divide translation into three stages
  - Initiation
  - Elongation
  - Termination

Initiation

- The initiation stage of translation
  - Brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome

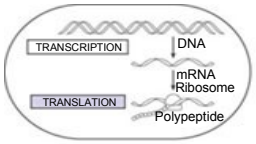


1. A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

2. The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.

Elongation

- In the elongation stage of translation
  - Amino acids are added one by one to the preceding amino acid



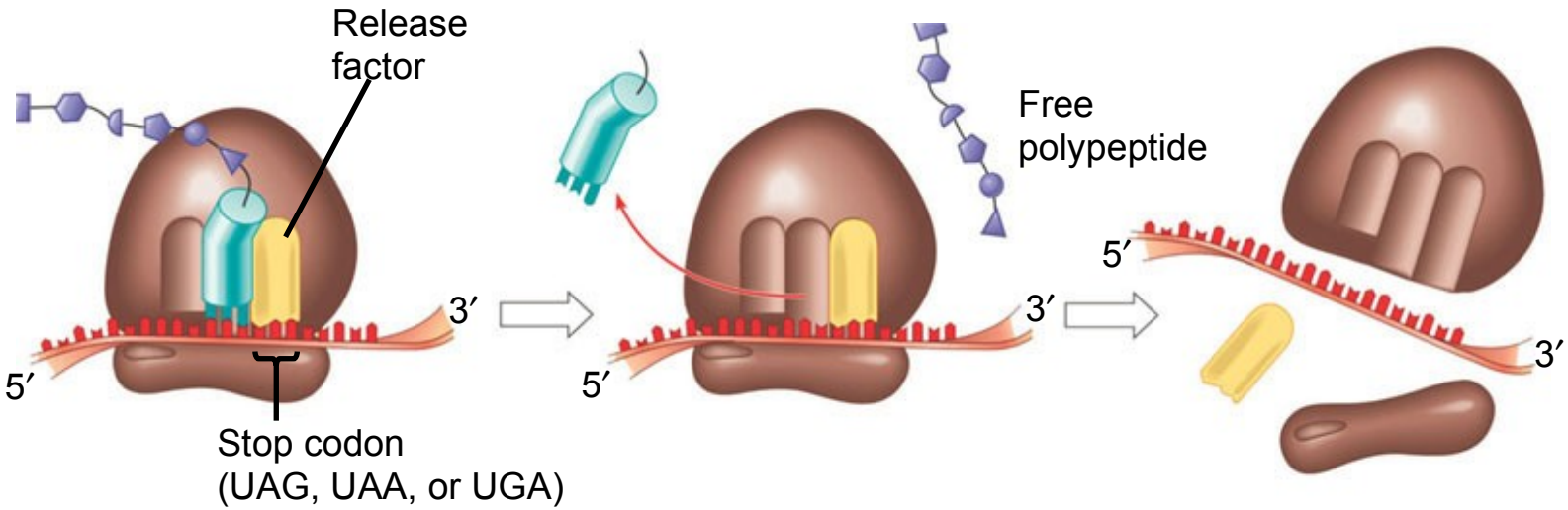
1 Codon recognition. The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step.

2 Peptide bond formation. An rRNA molecule of the large subunit catalyzes the formation of a peptide bond between the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step attaches the polypeptide to the tRNA in the A site.

3 Translocation. The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bringing the next codon to be translated into the A site.

Termination

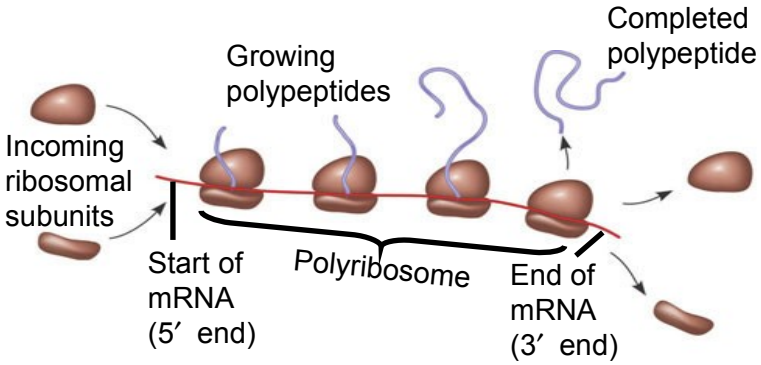
- The final stage of translation is termination
  - When the ribosome reaches a stop codon in the mRNA



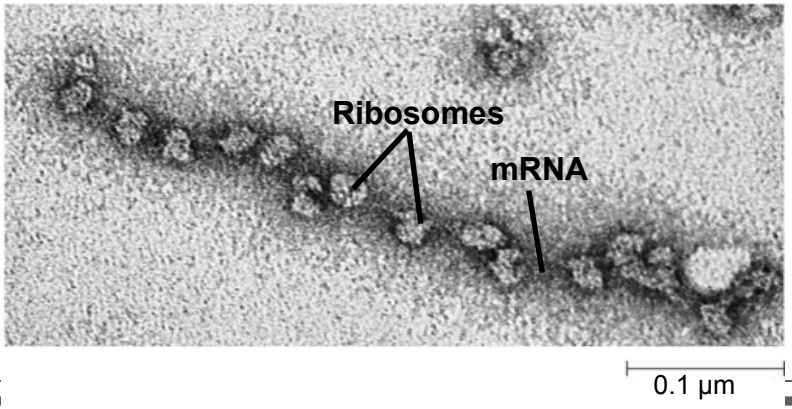
1. When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a protein called a release factor instead of tRNA.
2. The release factor hydrolyzes the bond between the tRNA in the P site and the last amino acid of the polypeptide chain. The polypeptide is thus freed from the ribosome.
3. The two ribosomal subunits and the other components of the assembly dissociate.

Polyribosomes

- A number of ribosomes can translate a single mRNA molecule simultaneously
  - Forming a polyribosome



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Completing and Targeting the Functional Protein

- Polypeptide chains
  - Undergo modifications after the translation process

Protein Folding and Post-Translational Modifications

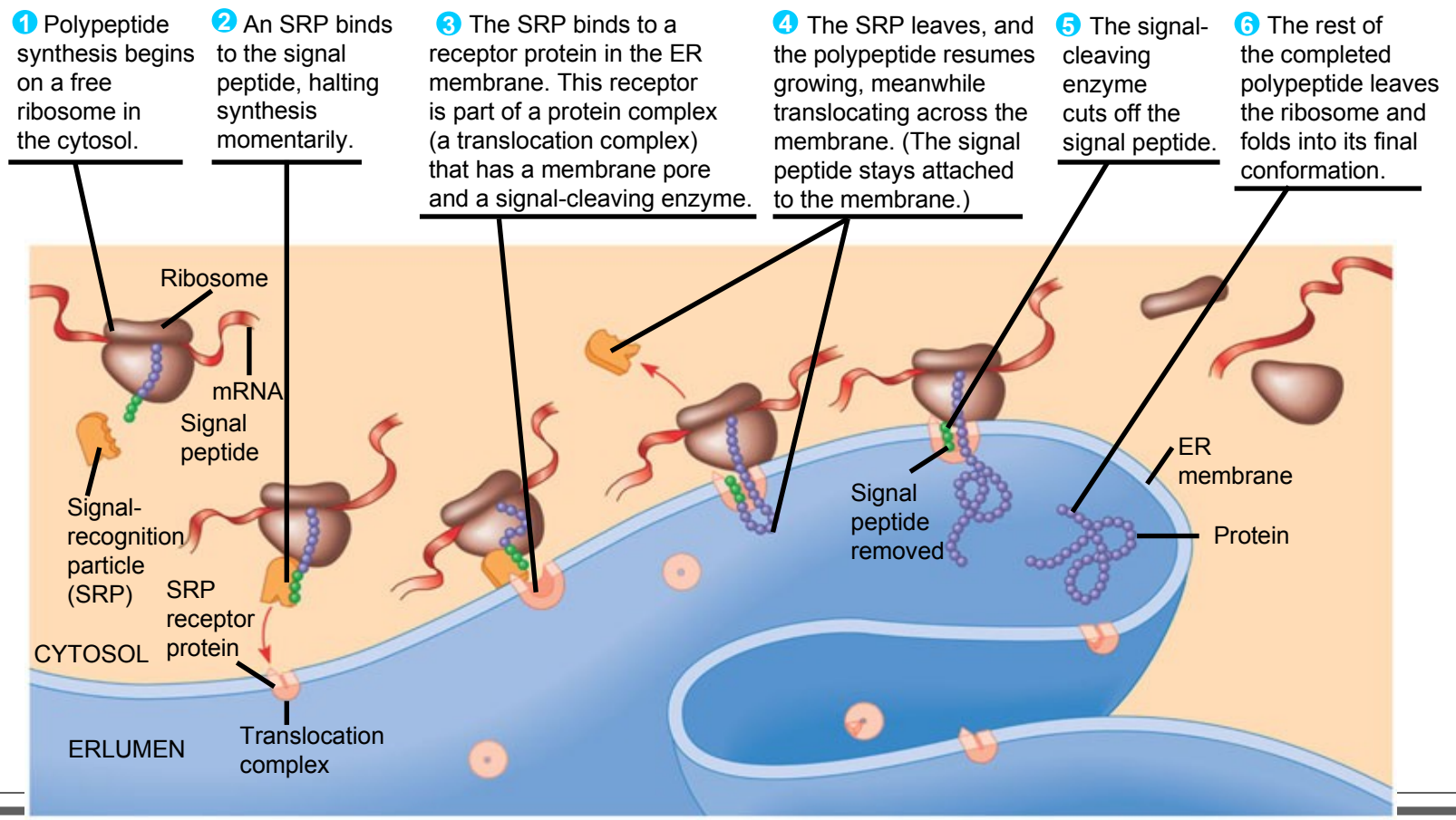
- After translation
  - Proteins may be modified in ways that affect their three-dimensional shape

Targeting Polypeptides to Specific Locations

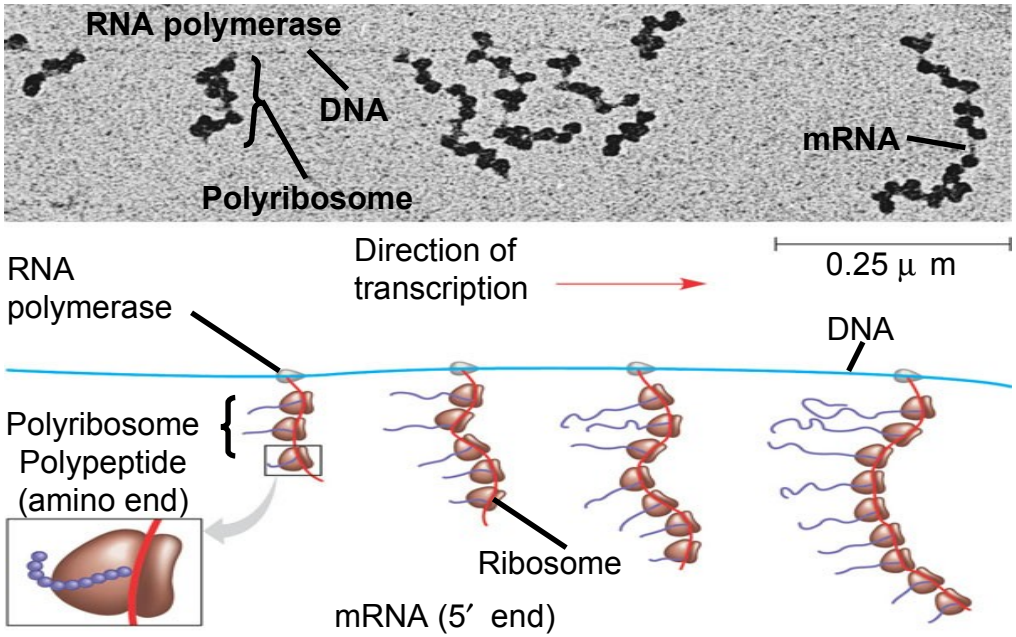
- Two populations of ribosomes are evident in cells
  - Free and bound
- Free ribosomes in the cytosol
  - Initiate the synthesis of all proteins

- Proteins destined for the endomembrane system or for secretion
  - Must be transported into the ER
  - Have signal peptides to which a signal-recognition particle (SRP) binds, enabling the translation ribosome to bind to the ER

The signal mechanism for targeting proteins to the ER



- Comparing gene expression in prokaryotes and eukaryotes reveals key differences
- Prokaryotic cells lack a nuclear envelope
  - Allowing translation to begin while transcription is still in progress

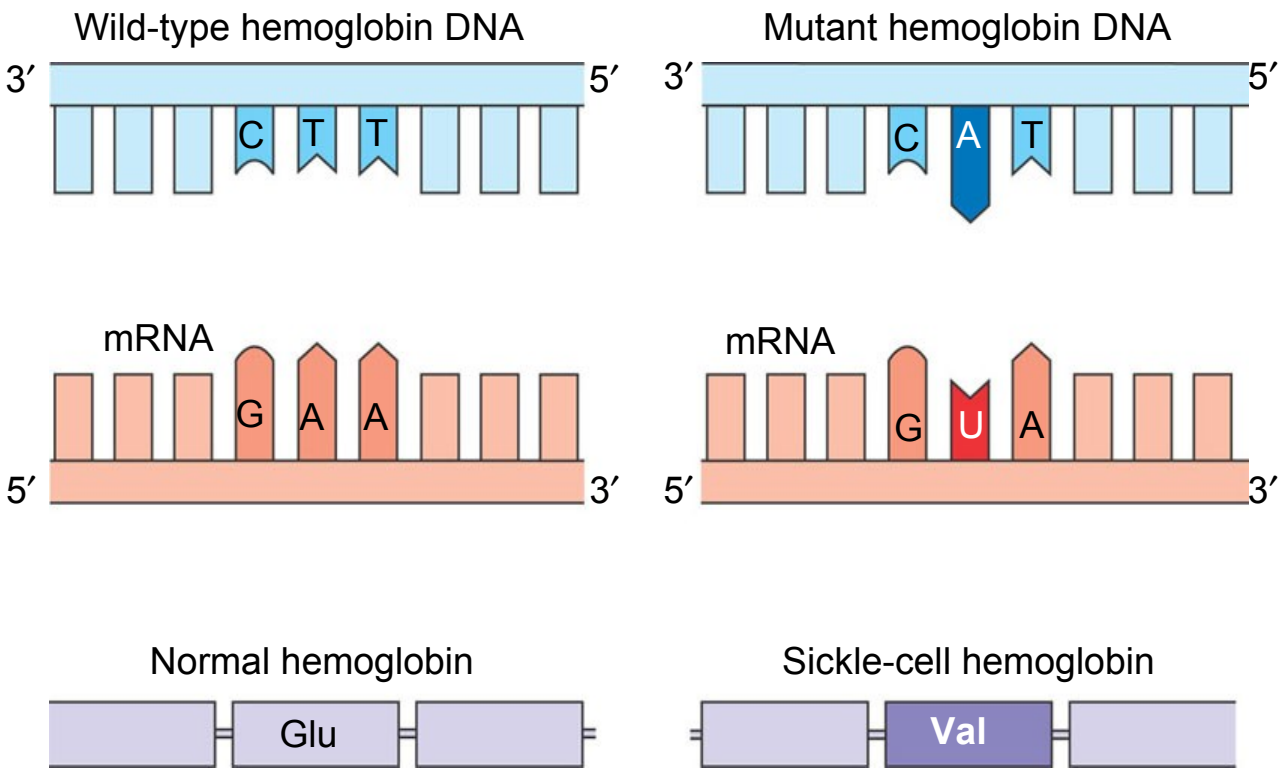


- In a eukaryotic cell
  - The nuclear envelope separates transcription from translation
  - Extensive RNA processing occurs in the nucleus

Point Mutations

- Point mutations can affect protein structure and function
- Mutations
  - Are changes in the genetic material of a cell
- Point mutations
  - Are changes in just one base pair of a gene

- The change of a single nucleotide in the DNA's template strand
  - Leads to the production of an abnormal protein



In the DNA, the mutant template strand has an A where the wild-type template has a T.

The mutant mRNA has a U instead of an A in one codon.

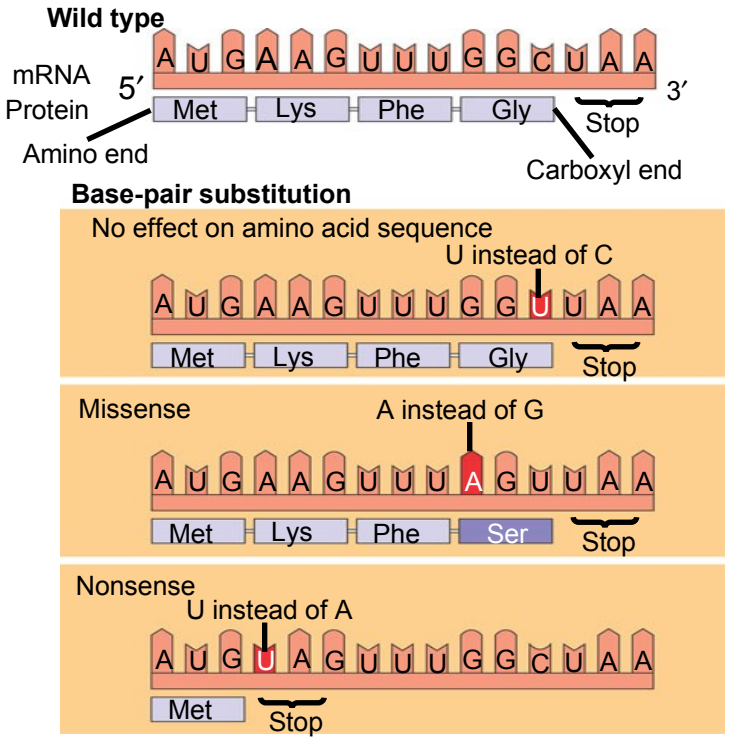
The mutant (sickle-cell) hemoglobin has a valine (Val) instead of a glutamic acid (Glu).

Types of Point Mutations

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

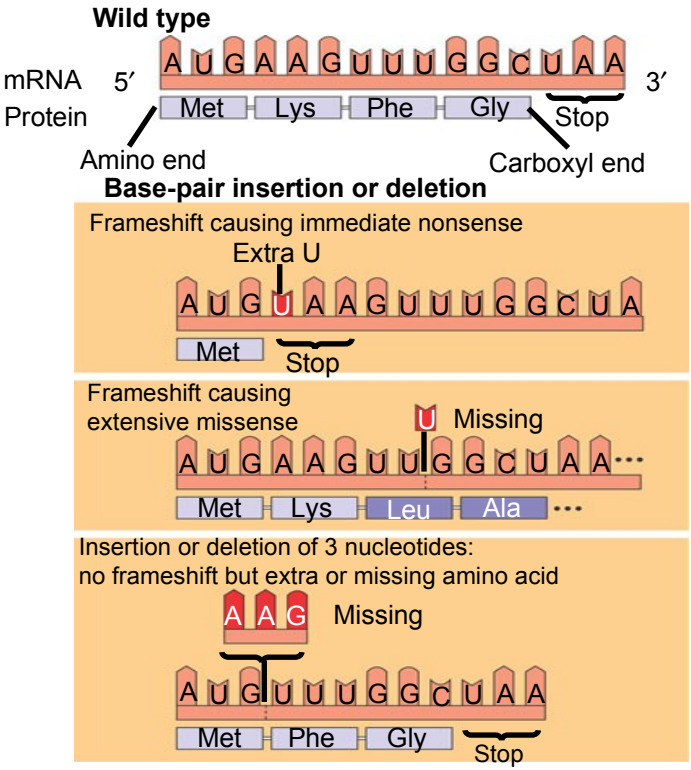
Substitutions

- A base-pair substitution
  - Is the replacement of one nucleotide and its partner with another pair of nucleotides
  - Can cause mis-sense or nonsense



Insertions and Deletions

- Insertions and deletions
  - Are additions or losses of nucleotide pairs in a gene
  - May produce frameshift mutations



Mutagens

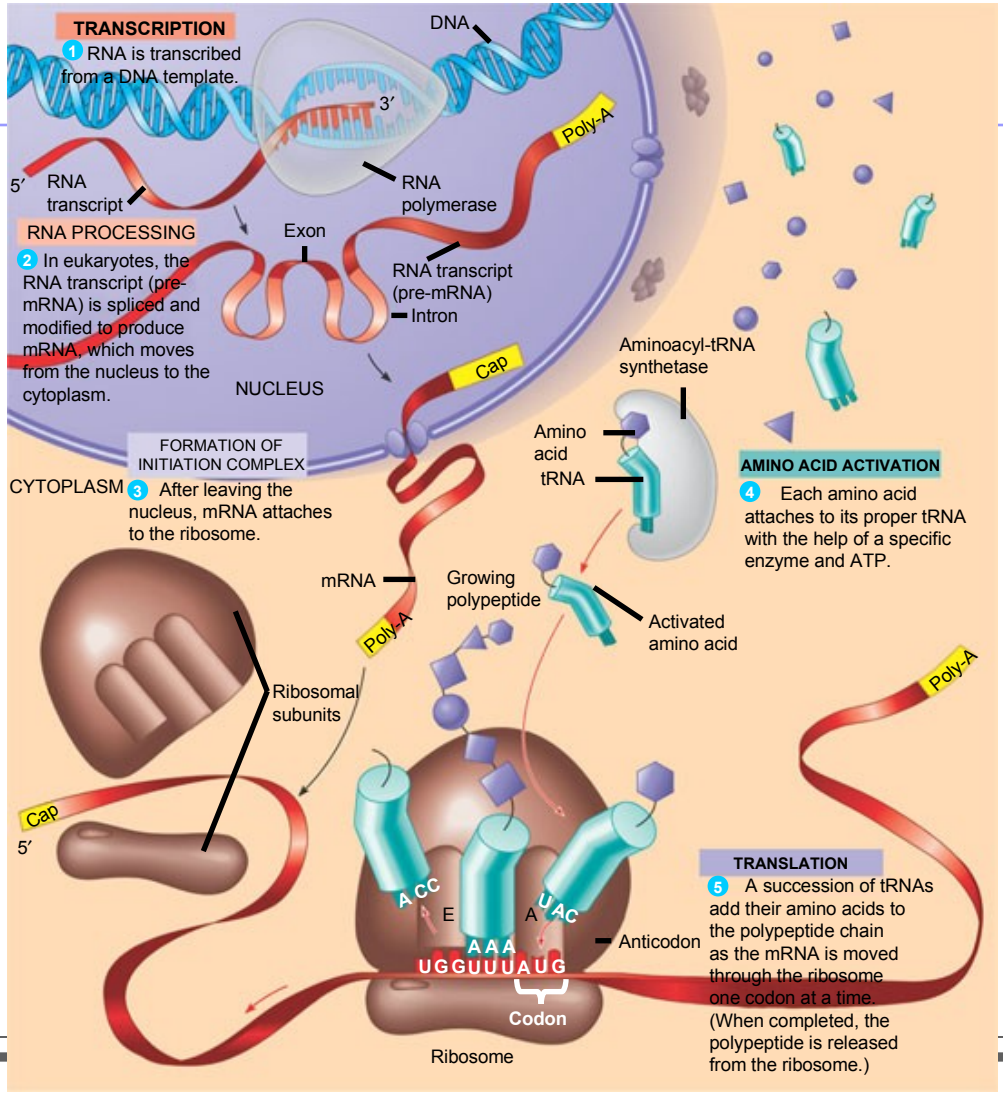
- Spontaneous mutations
  - Can occur during DNA replication, recombination, or repair

- Mutagens
  - Are physical or chemical agents that can cause mutations

What is a gene? *revisiting the question*

- A gene
  - Is a region of DNA whose final product is either a polypeptide or an RNA molecule

- A summary of transcription and translation in a eukaryotic cell



Try this!

- 1. What are transcription and translation?
- 2. How many nucleotides are necessary to code for a polypeptide that is 100 amino acids long?
- 3. An mRNA molecule contains the nucleotide sequence CCAUUUACG. Using the dictionary of the genetic code, translate this sequence into the corresponding amino acid sequence.
- 4. What is an anticodon?
- 5. What is the function of the ribosome in protein synthesis?
- 6. Which of the following does not participate directly in translation: ribosomes, tRNA, mRNA, DNA, enzymes and ATP?