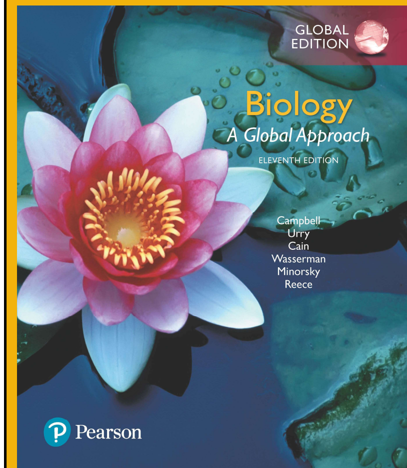


Chapter 17

Expression of Genes

Lecture Presentations by
Nicole Tunbridge and
Kathleen Fitzpatrick

The Flow of Genetic Information

- The information content of genes is in the specific sequences of nucleotides
- The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins
- Proteins are the links between genotype and phenotype
- **Gene expression**, the process by which DNA directs protein synthesis, includes two stages: transcription and translation

Figure 17.1

A single faulty gene result in the dramatic appearance of these albino Deer?

Figure 17.1a

An albino raccoon

Concept 17.1: Genes specify proteins via transcription and translation

- How was the fundamental relationship between genes and proteins discovered?

Evidence from the Study of Metabolic Defects

- In 1902, British physician Archibald Garrod first suggested that genes dictate phenotypes through enzymes that catalyze specific chemical reactions
- He thought symptoms of an inherited disease reflect an inability to synthesize a certain enzyme
- Cells synthesize and degrade molecules in a series of steps, a metabolic pathway

– Assignment –

Nutritional Mutants in Neurospora

- George Beadle and Edward Tatum exposed bread mold to X-rays, creating mutants that were unable to survive on minimal media
- The results of the experiments provided support for the one gene–one enzyme hypothesis
- The hypothesis states that the function of a gene is to dictate production of a specific enzyme

The Products of Gene Expression: A Developing Story

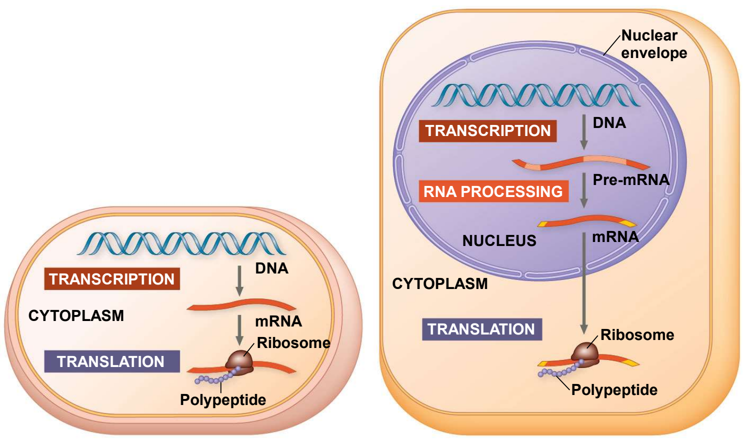
- Not all proteins are enzymes, so researchers later revised the hypothesis: one gene–one protein
- Many proteins are composed of several polypeptides, each of which has its own gene
- Therefore, Beadle and Tatum's hypothesis is now restated as the one gene–one polypeptide hypothesis
- It is common to refer to gene products as proteins rather than polypeptides

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 using information in DNA
- Transcription produces **messenger RNA (mRNA)**
- **Translation** is the synthesis of a polypeptide, using information in the mRNA
- **Ribosomes** are the sites of translation

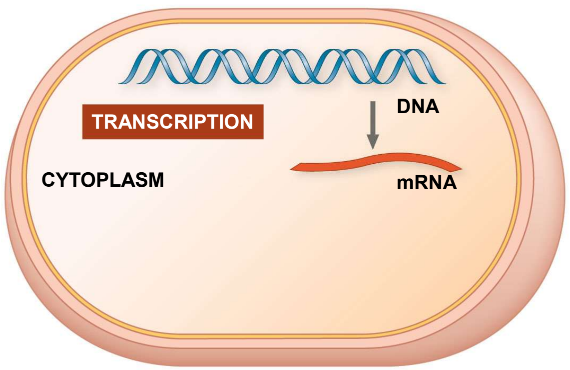
- In prokaryotes, translation of mRNA can begin before transcription has finished
- In a eukaryotic cell, the nuclear envelope separates transcription from translation
- Eukaryotic RNA transcripts are modified through RNA processing to yield the finished mRNA

Figure 17.4



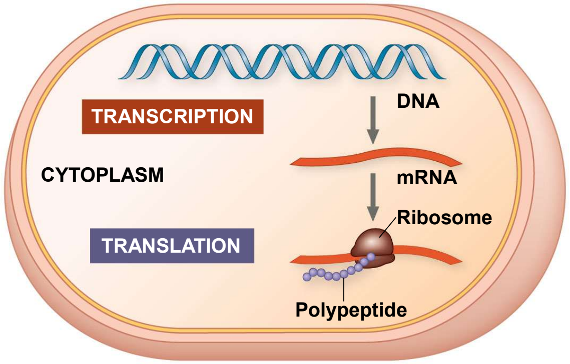
(a) Bacterial cell (b) Eukaryotic cell

Figure 17.4a_1



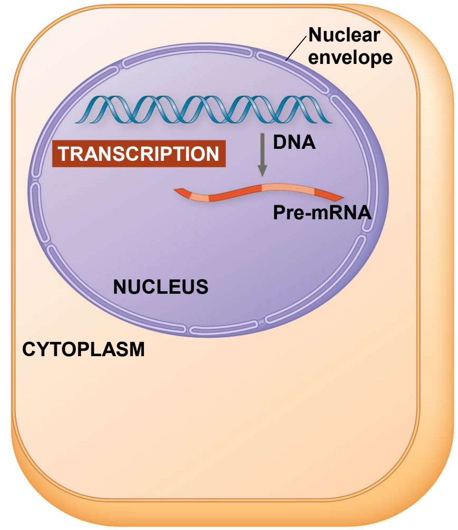
(a) Bacterial cell

Figure 17.4a_2



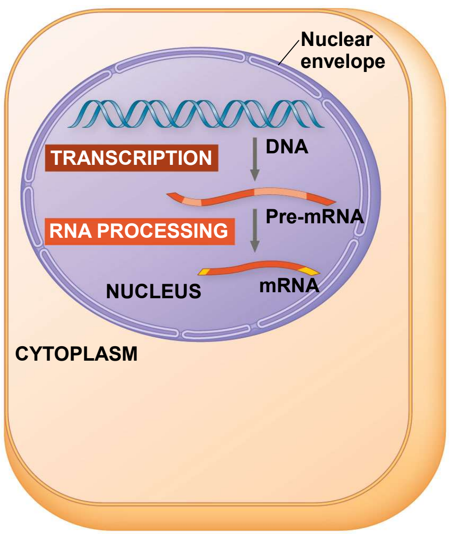
(a) Bacterial cell

Figure 17.4b_1



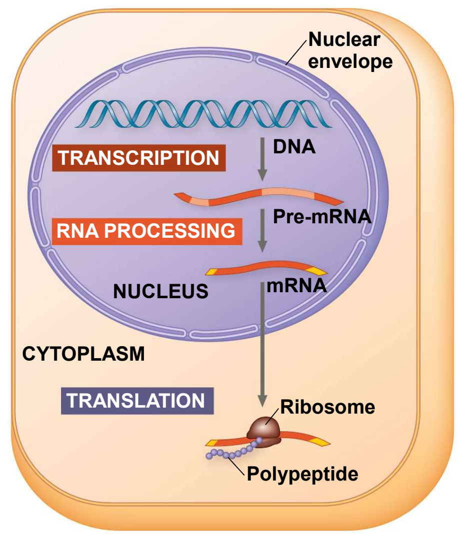
(b) Eukaryotic cell

Figure 17.4b_2



(b) Eukaryotic cell

Figure 17.4b_3



(b) Eukaryotic cell

- 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

Figure 17.UN01

The Genetic Code

- How are the instructions for assembling amino acids into proteins encoded into DNA?
- There are 20 amino acids, but there are only four nucleotide bases in DNA
- How many nucleotides correspond to an amino acid?

Codons: Triplets of Nucleotides

- The flow of information from gene to protein is based on a **triplet code**: a series of nonoverlapping, three-nucleotide words
- The words of a gene are transcribed into complementary nonoverlapping three-nucleotide words of mRNA
- These words are then translated into a chain of amino acids, forming a polypeptide

Figure 17.5

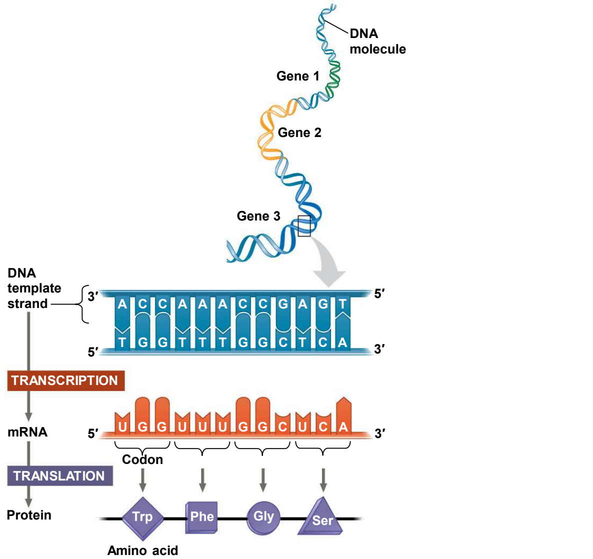
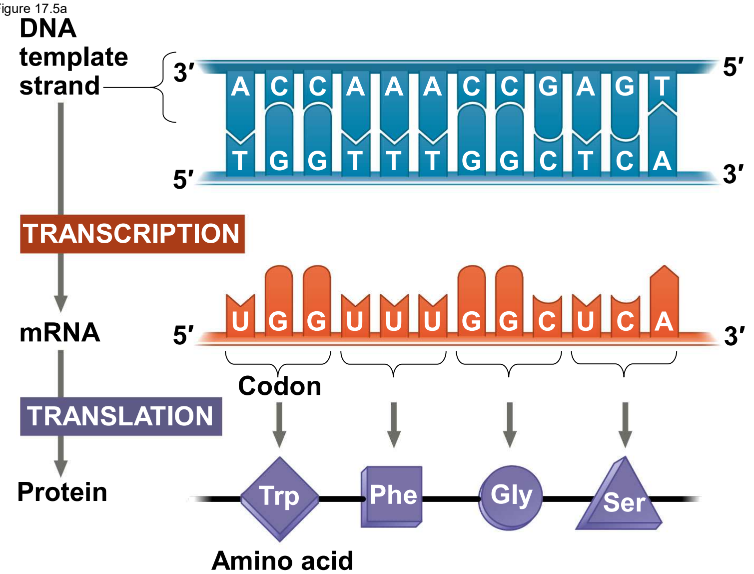


Figure 17.5a



- One of the two DNA strands, 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
- The strand used as the template is determined by the orientation of the enzyme that transcribes the gene
- This in turn, depends on the DNA sequences associated with the gene

- During translation, the mRNA base triplets, called **codons**, are read in the 5′ → 3′ direction
- The nontemplate strand is called the coding strand because the nucleotides of this strand are identical to the codons, except that T is present in the DNA in place of U in the RNA
- 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 in the early 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

Figure 17.6

Second mRNA base

First mRNA base (5' end of codon)	U	C	A	G	Third mRNA base (3' end of codon)
U	UUU Phe (F)	UCU Ser (S)	UAU Tyr (Y)	UGU Cys (C)	U
	UUC	UCC	UAC	UGC	C
	UUA Leu (L)	UCA	UAA Stop	UGA Stop	A
	UUG	UCG	UAG Stop	UGG Trp (W)	G
C	CUU Leu (L)	CCU Pro (P)	CAU His (H)	CGU Arg (R)	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Gln (Q)	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Ile (I)	ACU Thr (T)	AAU Asn (N)	AGU Ser (S)	U
	AUC	ACC	AAC	AGC	C
	AUA	ACA	AAA Lys (K)	AGA Arg (R)	A
	AUG Met (M) or start	ACG	AAG	AGG	G
G	GUU Val (V)	GCU Ala (A)	GAU Asp (D)	GGU Gly (G)	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glu (E)	GGA	A
	GUG	GCG	GAG	GGG	G

Evolution of the Genetic Code

- Not included

Concept 17.2: Transcription is the DNA-directed synthesis of RNA: *a closer look*

- Transcription is the first stage of gene expression

Molecular Components of Transcription

- RNA synthesis is catalyzed by **RNA polymerase**, which pries the DNA strands apart and joins together the RNA nucleotides
- The RNA is complementary to the DNA template strand
- RNA polymerase does not need any primer
- RNA synthesis follows the same base-pairing rules as DNA, except that uracil substitutes for thymine

Figure 17.8_1

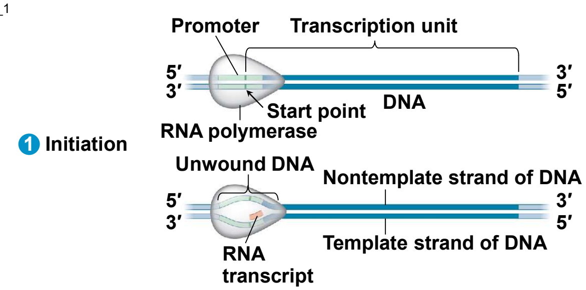


Figure 17.8_2

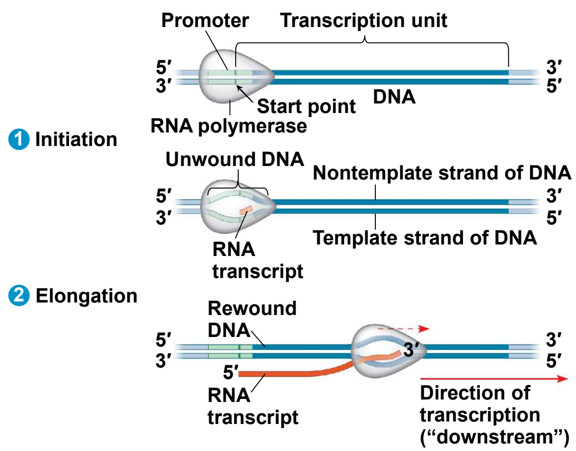
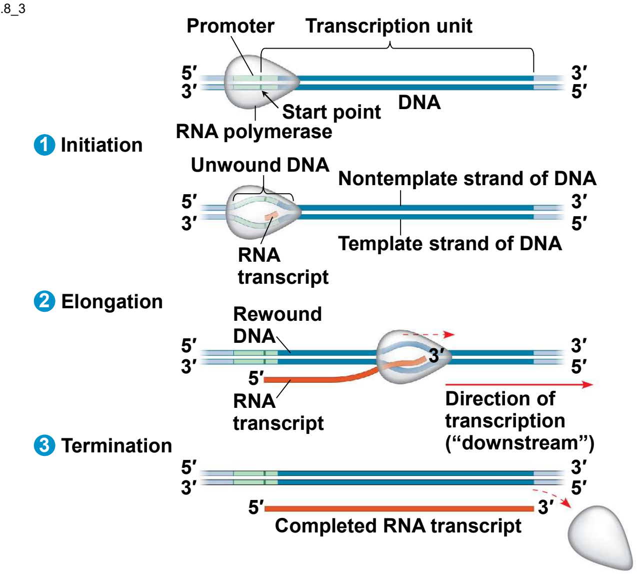
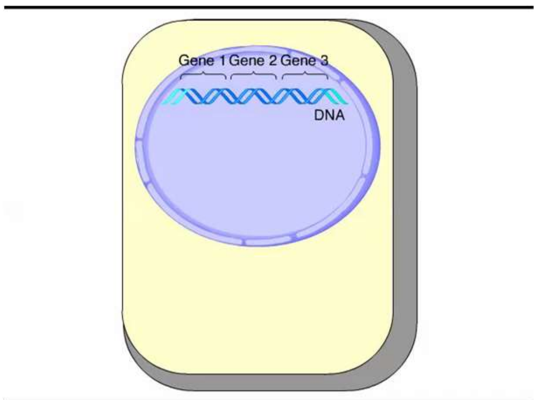


Figure 17.8_3



Animation: Transcription



- The DNA sequence where RNA polymerase attaches is called the **promoter**
- In bacteria, the sequence signaling the end of transcription is called the **terminator**
- The stretch of DNA that is transcribed is called a **transcription unit**

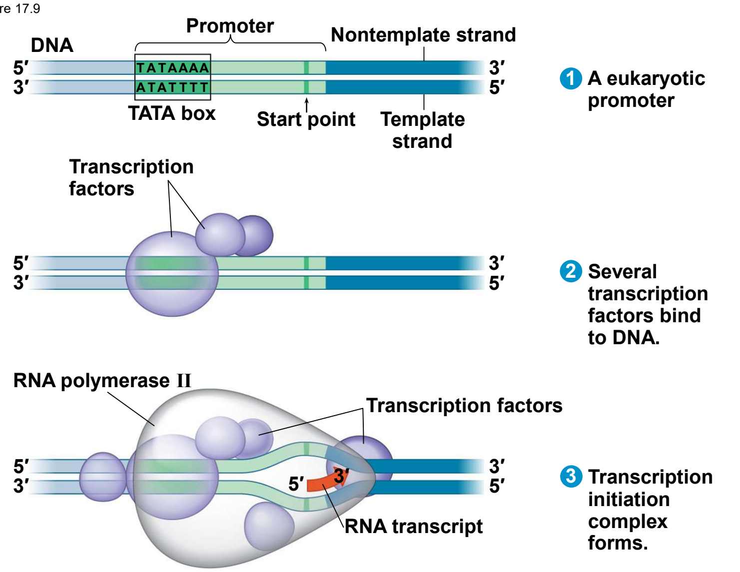
Synthesis of an RNA Transcript

- The three stages of transcription:
  - Initiation
  - Elongation
  - Termination

RNA Polymerase Binding and Initiation of Transcription

- Promoters signal the transcription **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

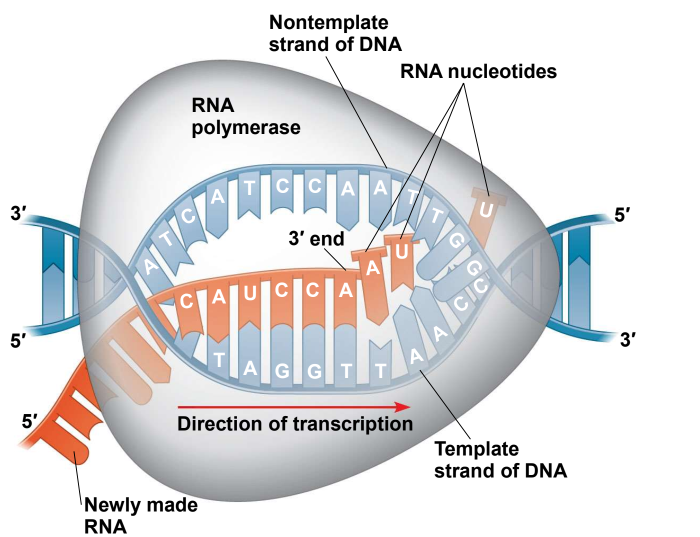
Figure 17.9



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

Figure 17.10



Termination of Transcription

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

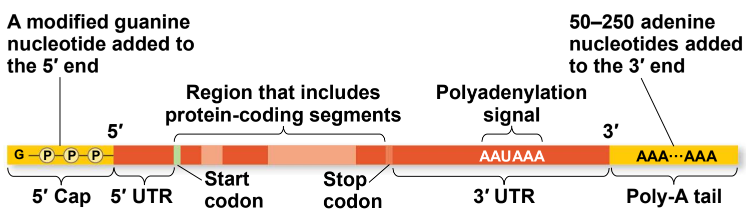
Concept 17.3: Eukaryotic cells modify RNA after transcription

- Enzymes in the eukaryotic nucleus modify pre-mRNA (**RNA processing**) before the genetic messages are dispatched to the cytoplasm
- During RNA processing, both ends of the primary transcript are altered
- Also, in most cases, certain interior sections of the molecule are cut out and the remaining parts spliced together

Alteration of mRNA Ends

- Each end of a pre-mRNA molecule is modified in a particular way
  - The 5′ end receives a modified nucleotide **5′ cap**
  - The 3′ end gets a **poly-A tail**
- These modifications share several functions
  - They seem to facilitate the export of mRNA to the cytoplasm
  - They protect mRNA from hydrolytic enzymes
  - They help ribosomes attach to the 5′ end

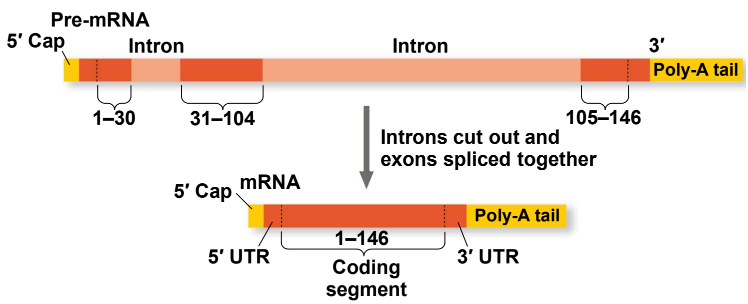
Figure 17.11



Split Genes and RNA Splicing

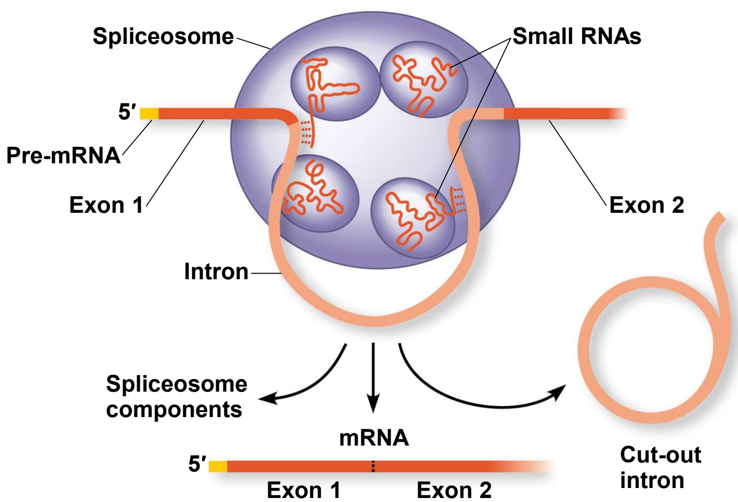
- Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions
- These noncoding regions are called intervening sequences, or **introns**
- The other regions are called **exons** because they are eventually expressed, usually translated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

Figure 17.12



- In some cases, RNA splicing is carried out by spliceosomes
- **Spliceosomes** consist of a variety of proteins and several small RNAs that recognize the splice sites
- The RNAs of the spliceosome also catalyze the splicing reaction

Figure 17.13



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 that may participate in catalysis
  - RNA may hydrogen-bond with other nucleic acid molecules

The Functional and Evolutionary Importance of Introns

- Not included

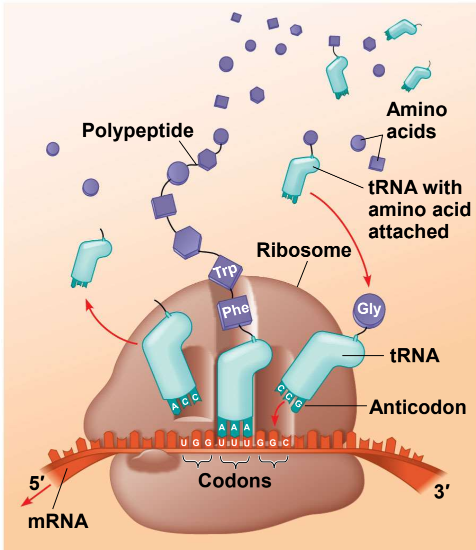
Concept 17.4: Translation is the RNA-directed synthesis of a polypeptide: *a closer look*

- Genetic information flows from mRNA to protein through the process of translation

Molecular Components of Translation

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

Figure 17.15



The Structure and Function of Transfer RNA

- Each tRNA molecule enables translation of a given mRNA codon into a certain amino acid
  - 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 tRNA molecule consists of a single RNA strand that is only about 80 nucleotides long
- Flattened into one plane to reveal its base pairing, a tRNA molecule looks like a cloverleaf

- Because of hydrogen bonds, tRNA actually twists and folds into a three-dimensional molecule
- tRNA is roughly L-shaped with the 5' and 3' ends both located near one end of the structure
- The protruding 3' end acts as an attachment site for an amino acid

Figure 17.16

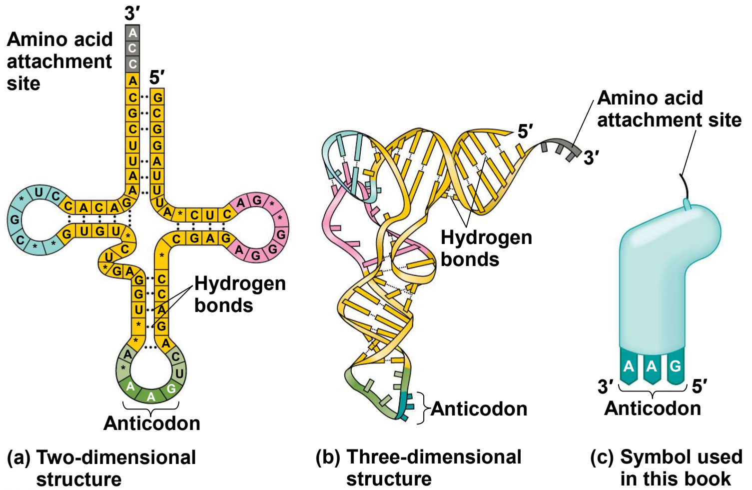


Figure 17.16a

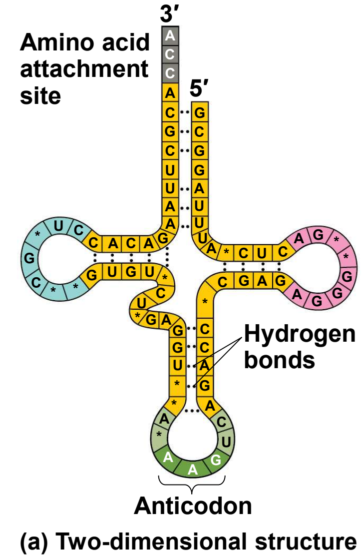
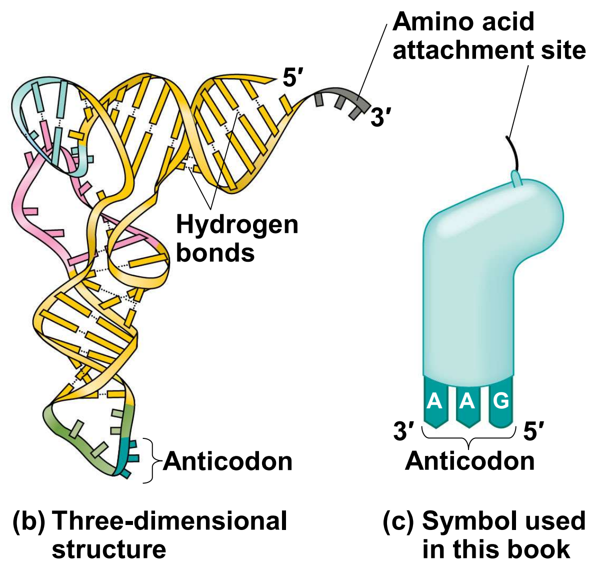
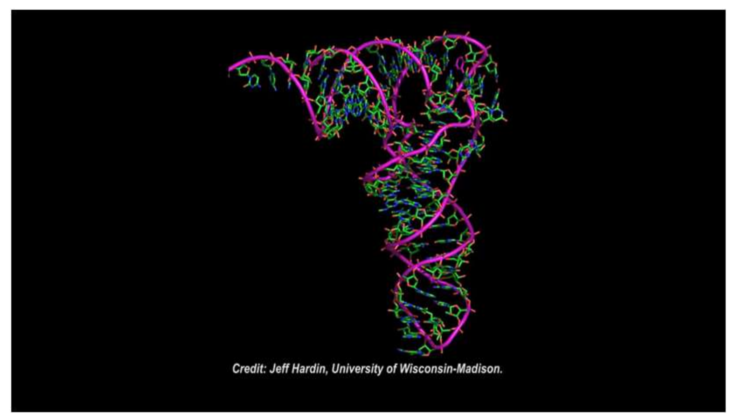


Figure 17.16b



Video: Stick and Ribbon Rendering of a tRNA



- Accurate translation requires two steps
  - First: a correct match between a tRNA and an amino acid, done by the enzyme **aminoacyl-tRNA synthetase**
  - Second: a correct match between the tRNA anticodon and an mRNA codon
- Flexible pairing at the third base of a codon is called **wobble** and allows some tRNAs to bind to more than one codon

Figure 17.17_1

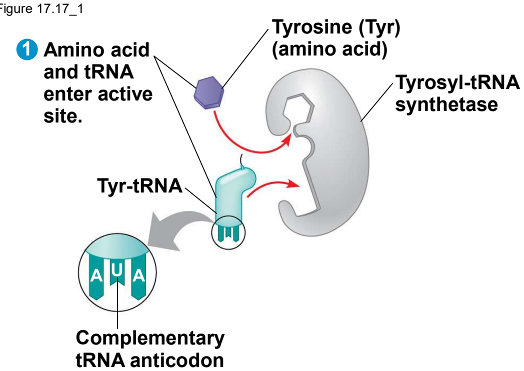


Figure 17.17_2

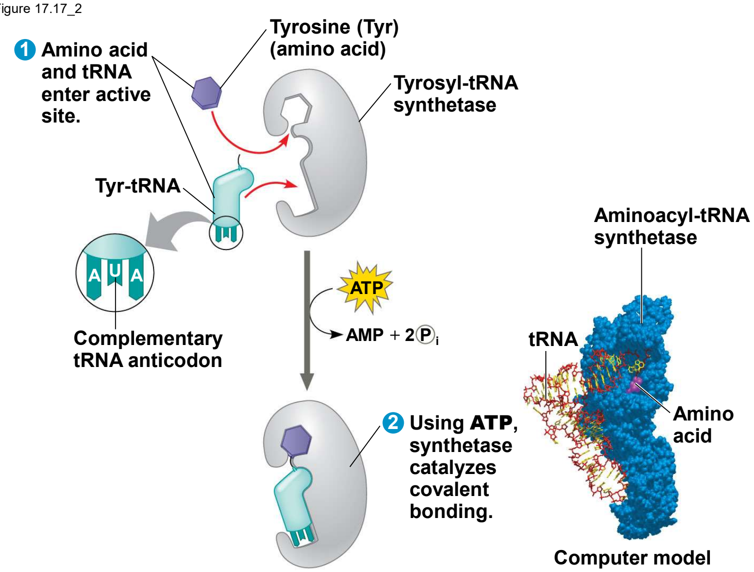


Figure 17.17_3

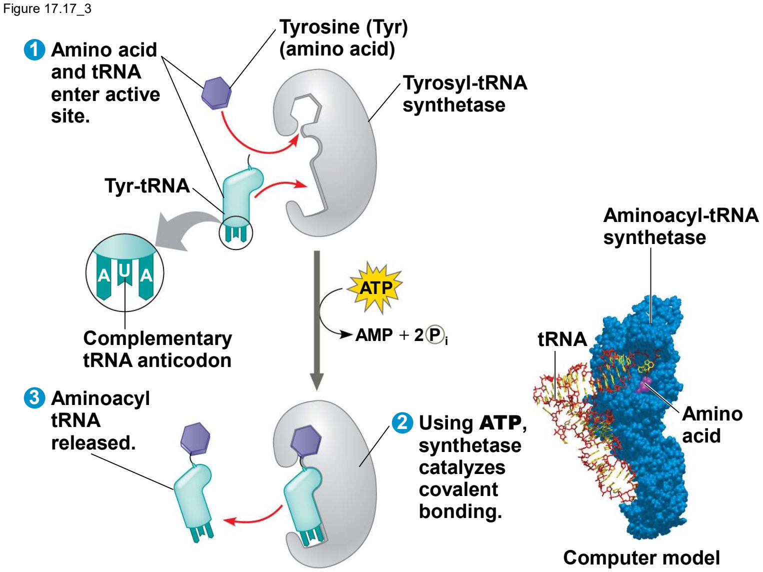
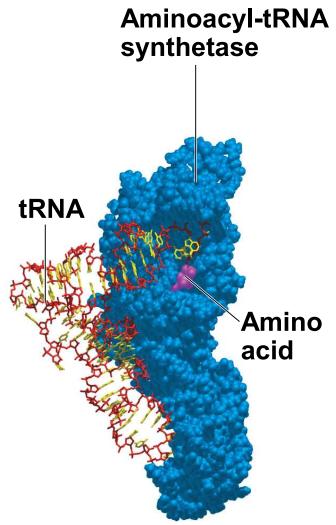


Figure 17.17a



Computer model

The Structure and Function of 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 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

Figure 17.18

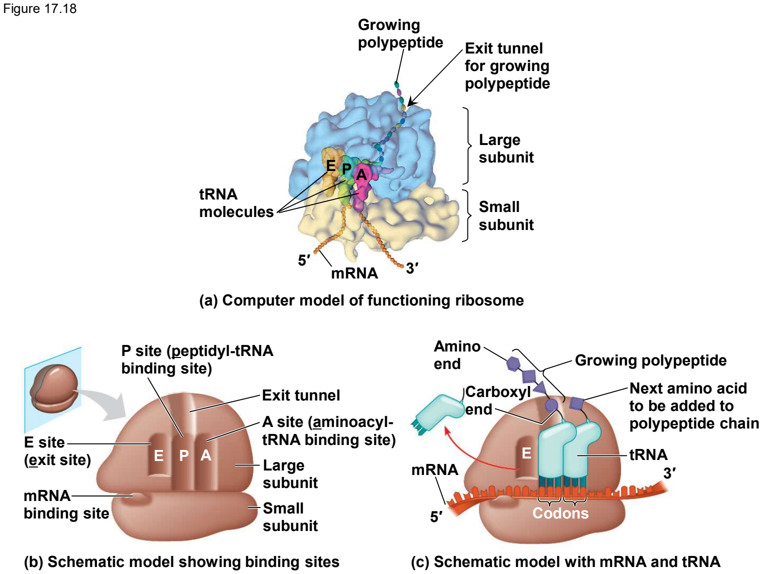
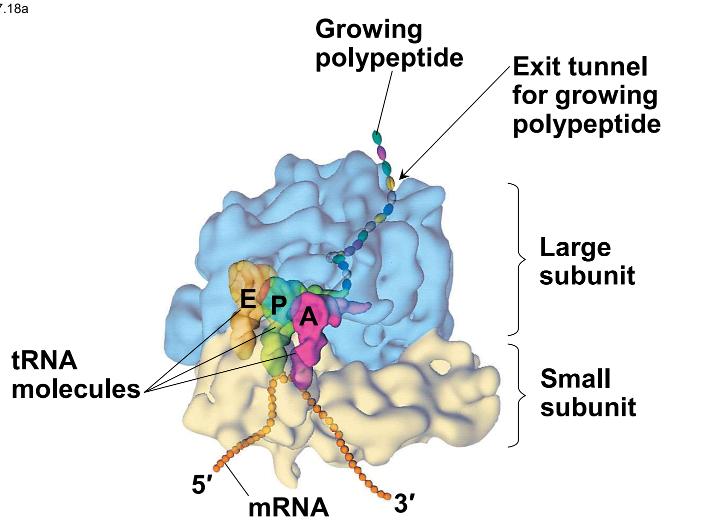
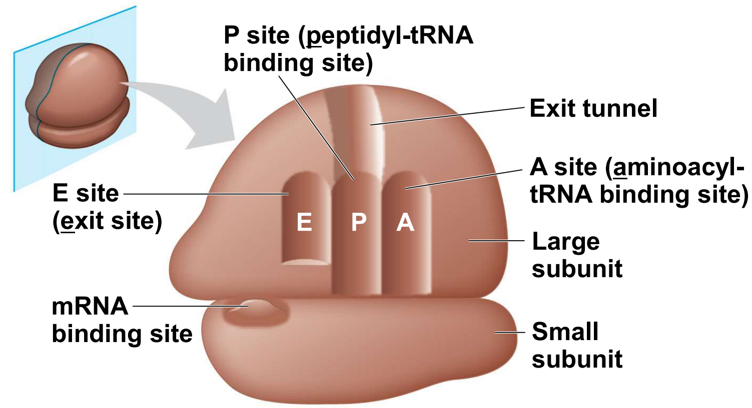


Figure 17.18a



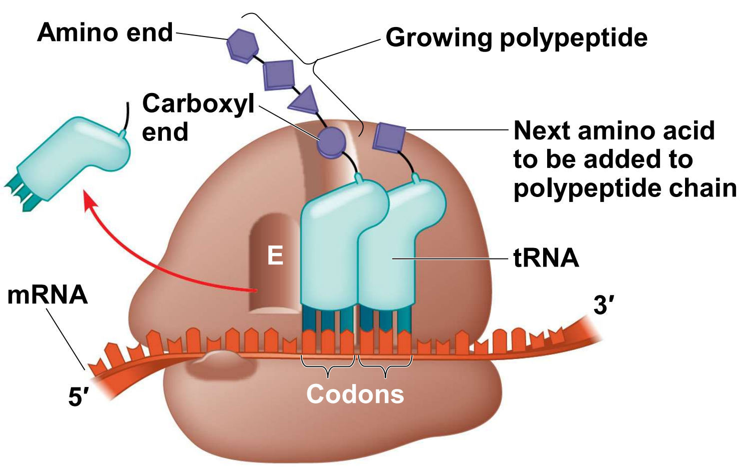
(a) Computer model of functioning ribosome

Figure 17.18b



(b) Schematic model showing binding sites

Figure 17.18c



(c) Schematic model with mRNA and tRNA

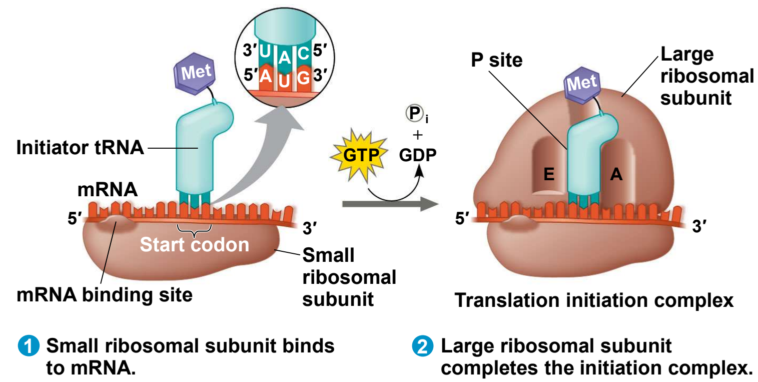
Building a Polypeptide

- The three stages of translation:
  - Initiation
  - Elongation
  - Termination
- All three stages require protein “factors” that aid in the translation process
- Energy is required for some steps, too

Ribosome Association and Initiation of Translation

- The start codon (AUG) signals the start of translation
- 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
- Proteins called initiation factors bring in the large subunit that completes the translation initiation complex

Figure 17.19



Elongation of the Polypeptide Chain

- During elongation, amino acids are added one by one to the C-terminus of the growing chain
- Each addition involves proteins called elongation factors
- Elongation occurs in three steps: codon recognition, peptide bond formation, and translocation
- Energy expenditure occurs in the first and third steps

- Translation proceeds along the mRNA in a 5′ → 3′ direction
- The ribosome and mRNA move relative to each other, codon by codon
- The elongation cycles takes less than a tenth of a second in bacteria

Figure 17.20_1

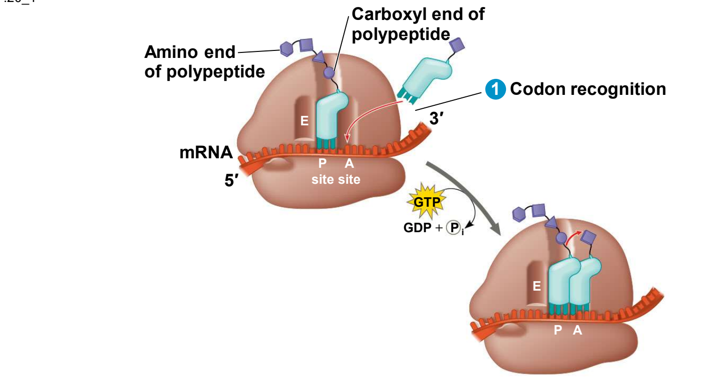


Figure 17.20_2

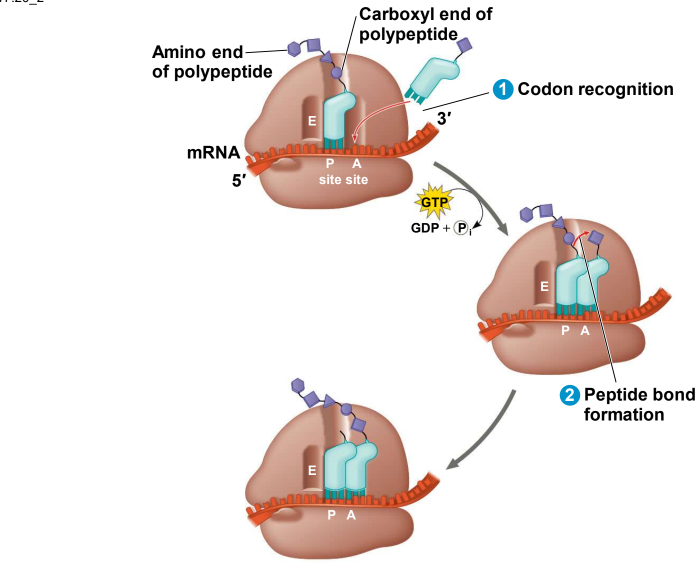
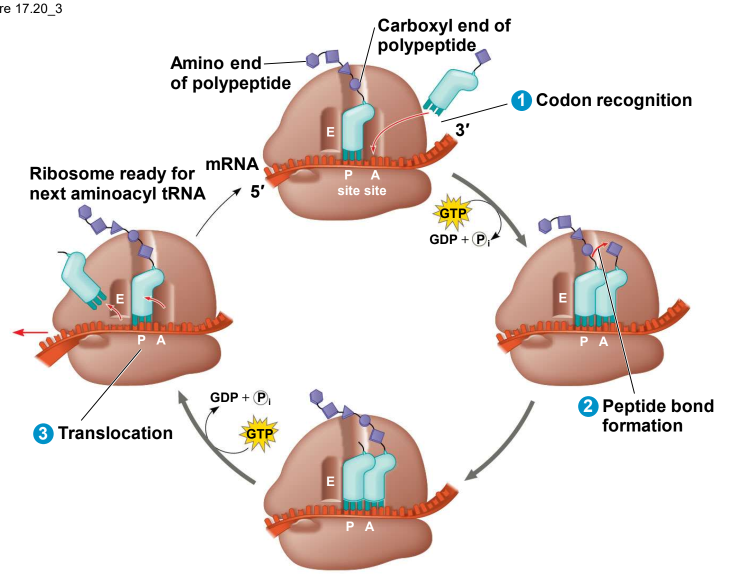


Figure 17.20_3



Termination of Translation

- Elongation continues until 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 comes apart

Figure 17.21_1

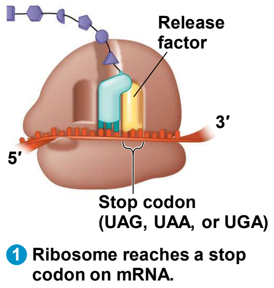


Figure 17.21_2

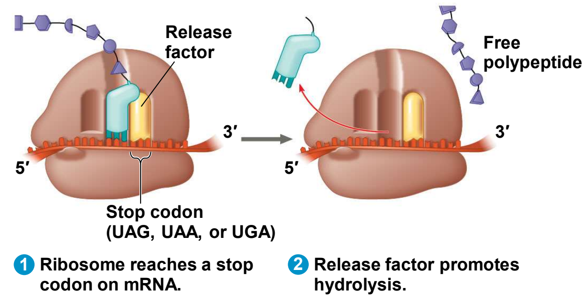
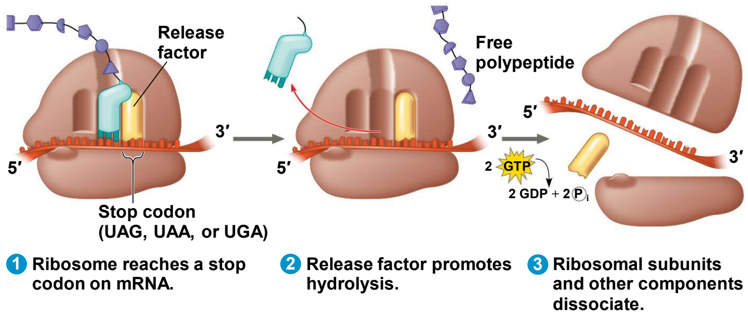


Figure 17.21_3



Completing and Targeting the Functional Protein

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

Protein Folding and Post-Translational Modifications

- During its synthesis, a polypeptide chain begins to coil and fold spontaneously into a specific shape—a three-dimensional molecule with secondary and tertiary structure
- A gene determines primary structure, and primary structure in turn determines shape
- Post-translational modifications may be required before the protein can begin doing its particular job in the cell

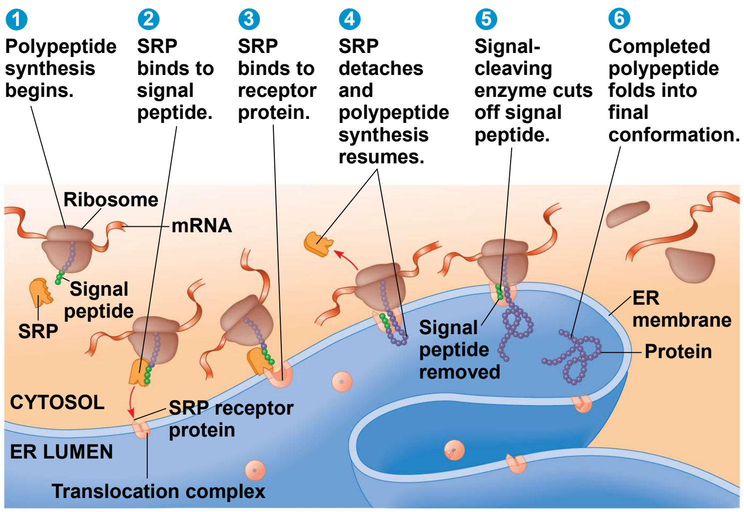
Targeting Polypeptides to Specific Locations

- 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 escorts the ribosome to a receptor protein built into the ER membrane

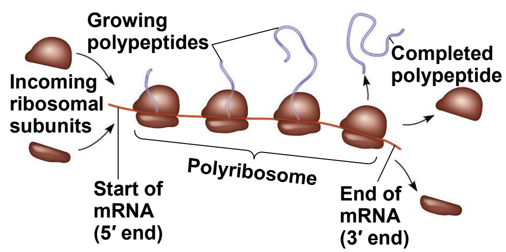
Figure 17.22



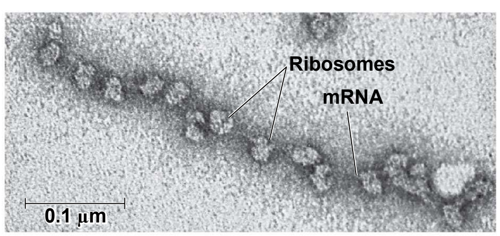
Making Multiple Polypeptides in Bacteria and Eukaryotes

- Multiple 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

Figure 17.23

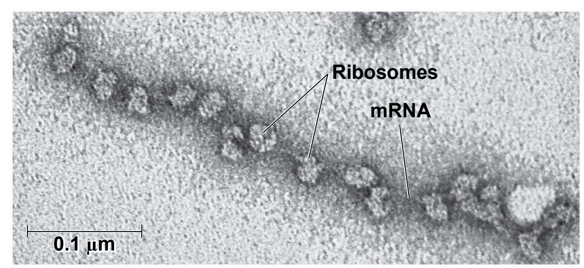


(a) Several ribosomes simultaneously translating one mRNA molecule



(b) A large polyribosome in a bacterial cell (TEM)

Figure 17.23a



(b) A large polyribosome in a bacterial cell (TEM)

- A bacterial cell ensures a streamlined process by coupling transcription and translation
- In this case the newly made protein can quickly diffuse to its site of function

Figure 17.24

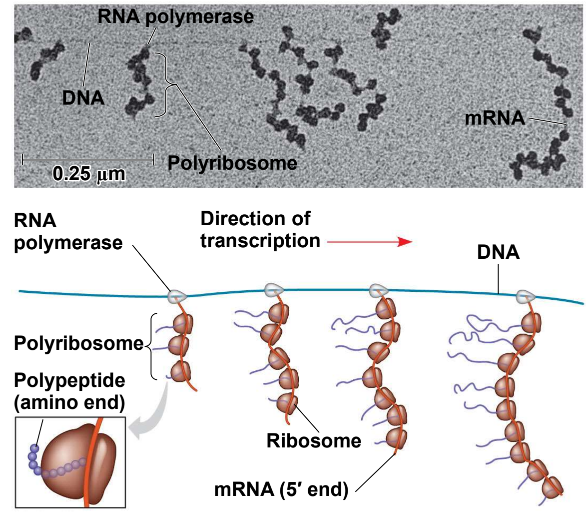
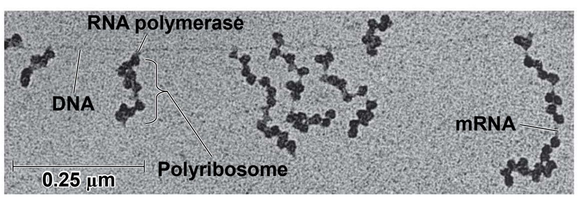


Figure 17.24a



- In eukaryotes, the nuclear envelope separates the processes of transcription and translation
- RNA undergoes processing before leaving the nucleus

Figure 17.25

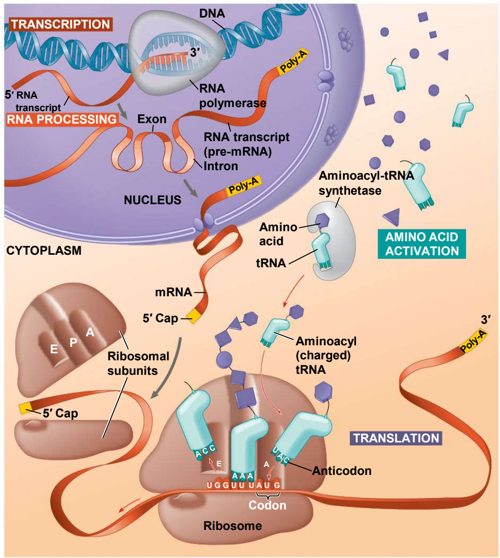


Figure 17.25a

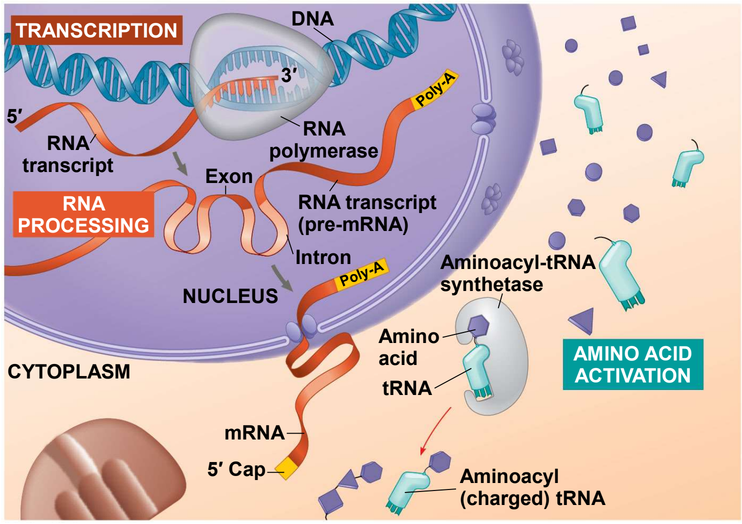
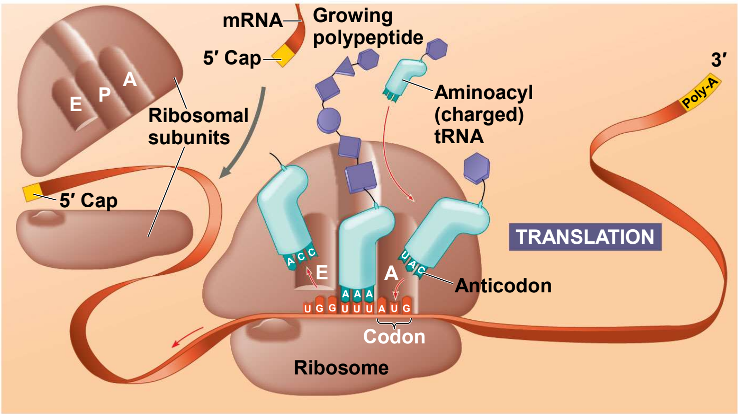


Figure 17.25b

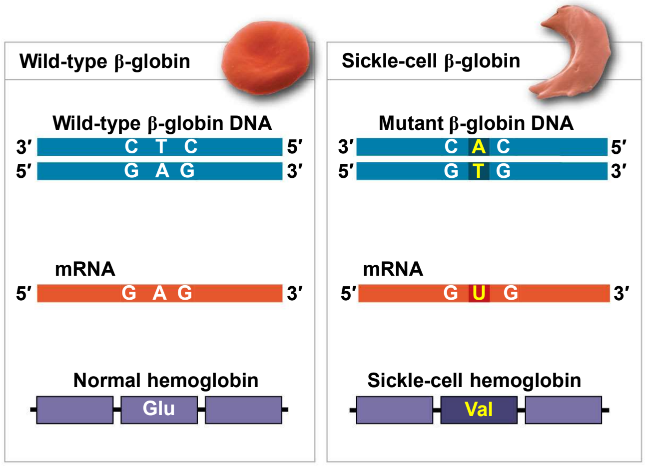


Concept 17.5: Mutations of one or a few nucleotides can affect protein structure and function

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

- If a mutation has an adverse effect on the phenotype of the organism, the condition is referred to as a **genetic disorder** or **hereditary disease**

Figure 17.26



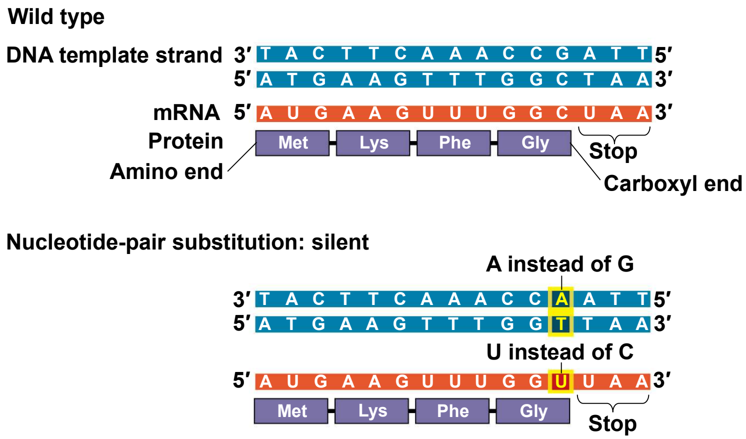
Types of Small-Scale Mutations

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

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*; most lead to a nonfunctional protein

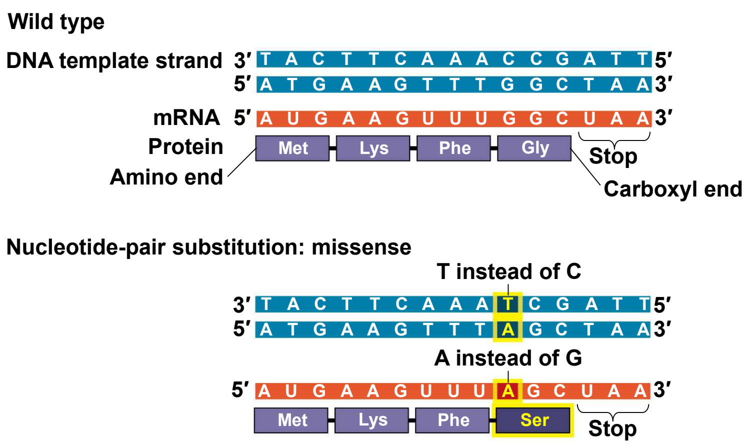
Figure 17.27a



Insertions and Deletions

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene
- These mutations 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**

Figure 17.27b



New Mutations and Mutagens

- Spontaneous mutations can occur during errors in DNA replication, recombination, or repair
- **Mutagens** are physical or chemical agents that can cause mutations
- Chemical mutagens fall into a variety of categories
- Most carcinogens (cancer-causing chemicals) are mutagens, and most mutagens are carcinogenic

What Is a Gene? *Revisiting the Question*

- The idea of the gene has evolved through the history of genetics
- We have considered a gene as
  - a discrete unit of inheritance
  - a region of specific nucleotide sequence in a chromosome
  - a DNA sequence that codes for a specific polypeptide chain

- A gene can be defined as a region of DNA that can be expressed to produce a final <u>functional product</u> that is either a polypeptide or an RNA molecule

Figure 17.27

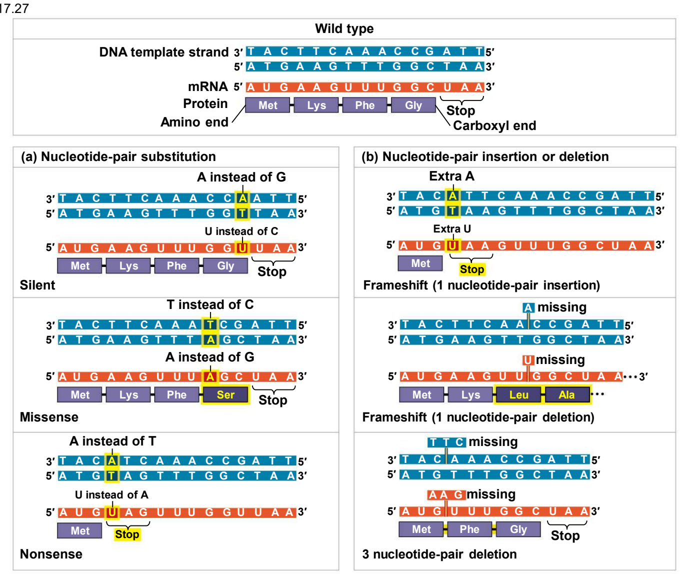


Figure 17.27c

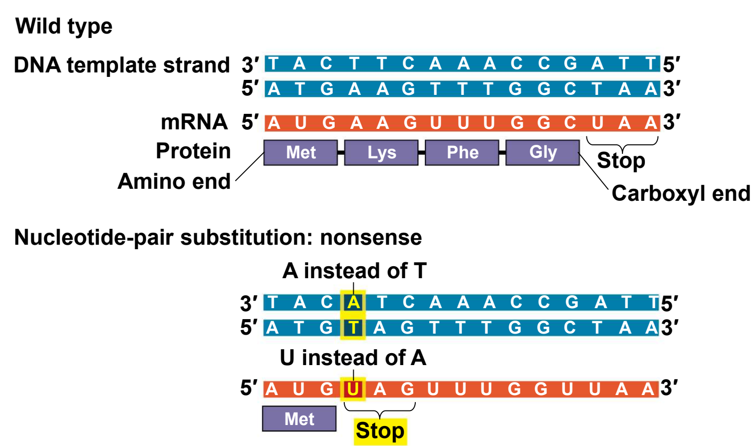


Figure 17.27d

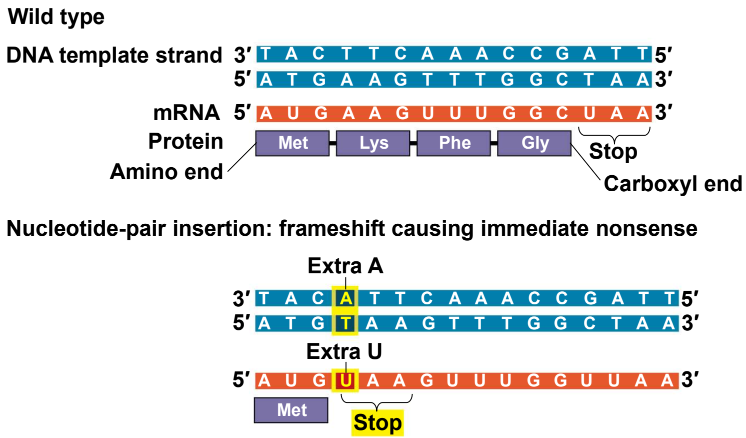


Figure 17.27e

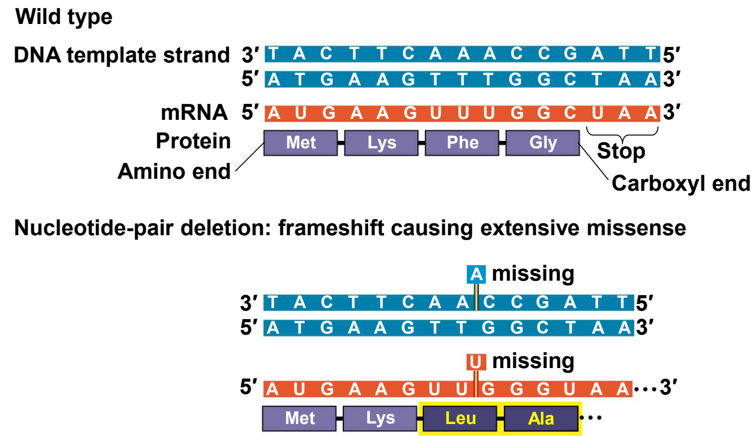


Figure 17.27f