University of Anbar Department of Biotechnology

College of Science

Dr. Huda Musleh Biotechnology 1



2nd

RNA:Translation

How do we go from the language of nucleic acids to the language of amino acids?

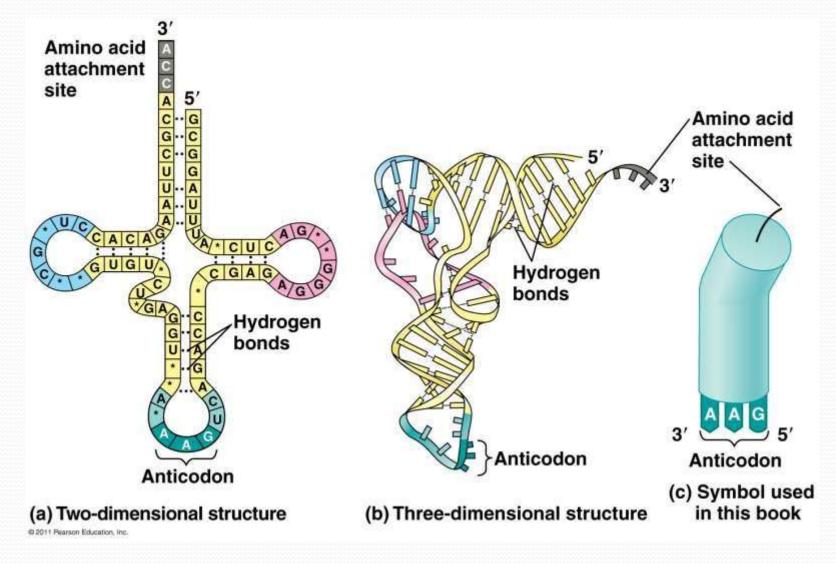
- A cell translates an mRNA message into protein with the help of **transfer RNA (tRNA)**
- tRNA transfer amino acids to the growing polypeptide in a ribosome
 - An adaptor molecule composed of RNA
 - Base-pairs with itself to form a ribozyme-like molecule

Amino acids are the monomers of proteins

Translation: The players

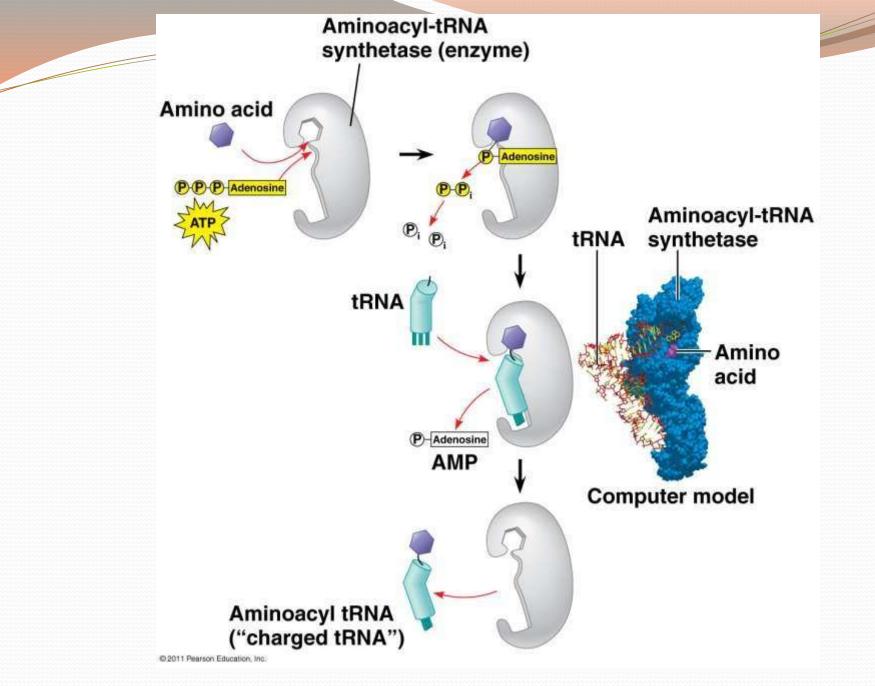
- tRNA
- Aminoacyl-tRNA synthetase
- Ribosomes
- Amino acids
- Chaperone proteins

Structure of tRNA



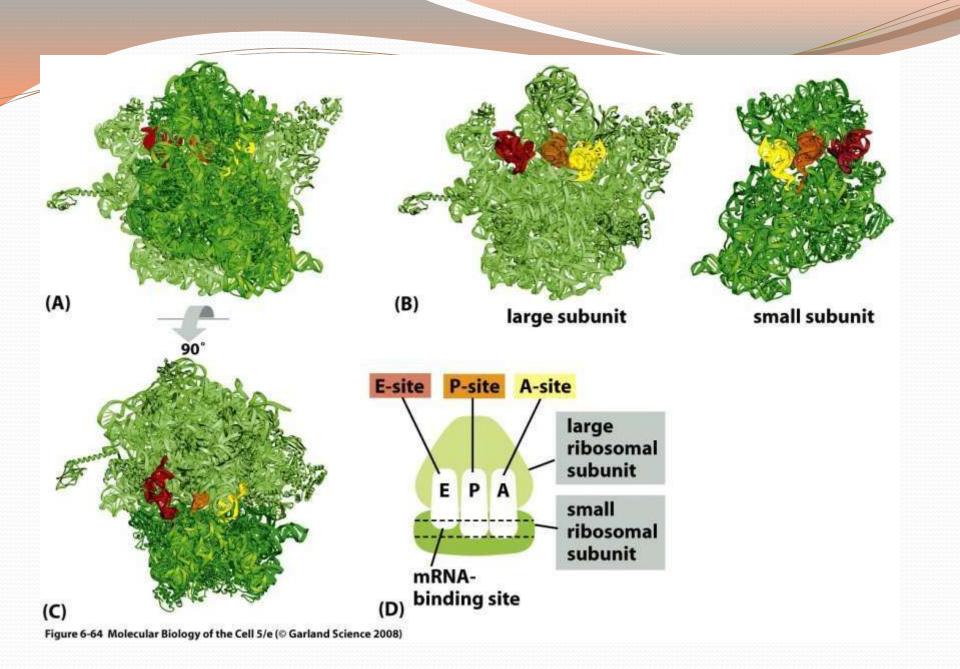
More about tRNA

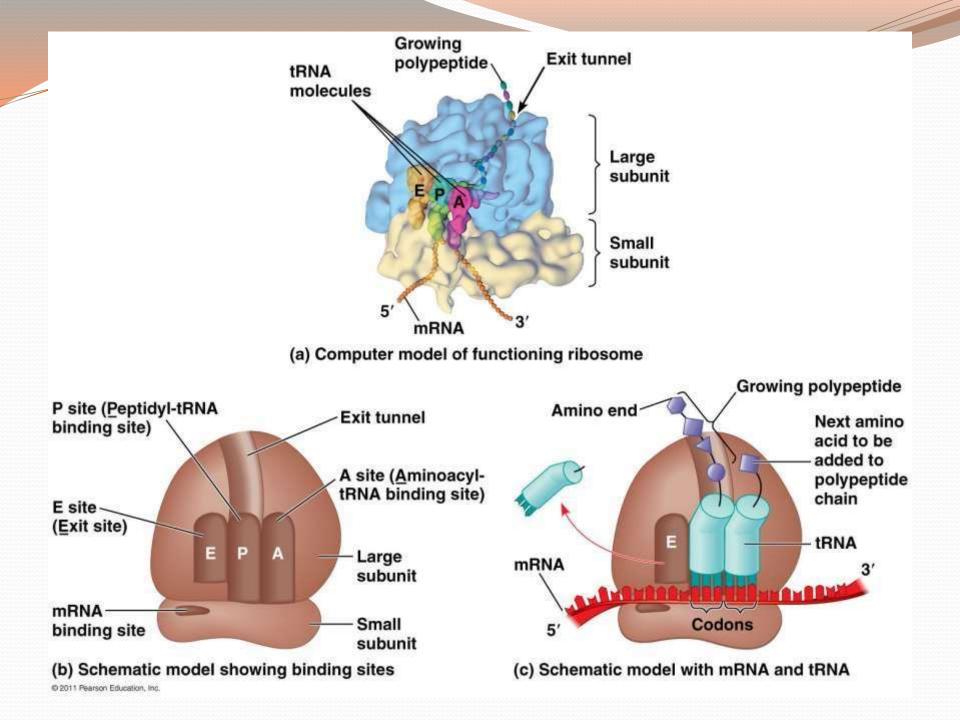
- Specific tRNAs will attach to specific amino acids
- How does it 'know' which amino acid to attach to?
 - The anticodon on the end dictates which amino acid the tRNA will attach to
 - tRNAs don't naturally come attached to their amino acids
 - An enzyme called **aminoacyl-tRNA synthetase** catalyzes the linking of an amino acid to a tRNA



Ribosomes

- Roughly the same for both eukaryotes and prokaryotes
- Ribosomes provide a site for tRNA and mRNA to come together
- Composed of rRNA and proteins
 - Large subunit and small subunit
 - Three catalytic sites: E, P, and A
 - "Aminoacyl-tRNA binding site"
 - "Peptidyl-tRNA binding site"





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

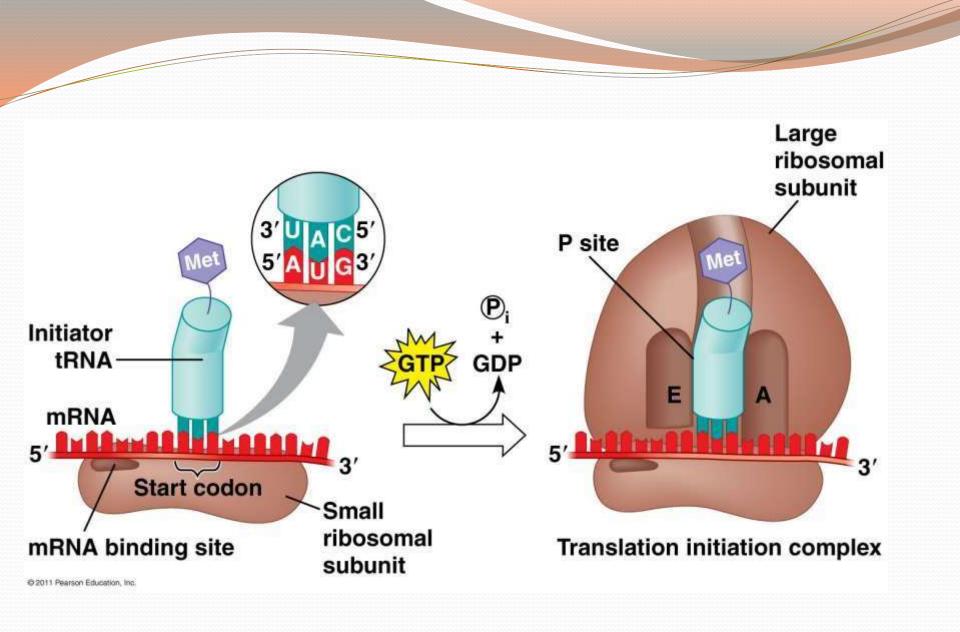
- Two populations of ribosomes in cells: free ribsomes (in the cytosol) and bound ribosomes (attached to the ER)
 - Free ribosomes mostly synthesize proteins that function in the cytosol
 - Bound ribosomes make proteins of the endomembrane system and proteins that are secreted from the cell
- Ribosomes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

Translation

- Three stages:
 - Initiation
 - Elongation
 - Termination

Translation: Initiation

- mRNA binds to the small ribosomal subunit
- An special initiator tRNA binds to the mRNA+small ribosomal subunit
 - Initiator tRNA uses its anticodon to complementary base-pair with mRNA
 - Initiator tRNA will always be methionine,AUG
- Small ribosomal subunit "scans" along the mRNA until it finds the start signal
- Large ribosomal subunit binds through GTP hydrolysis and begins elongation

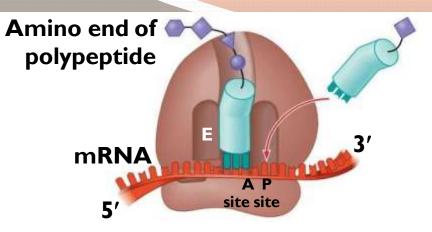


Translation: Elongation

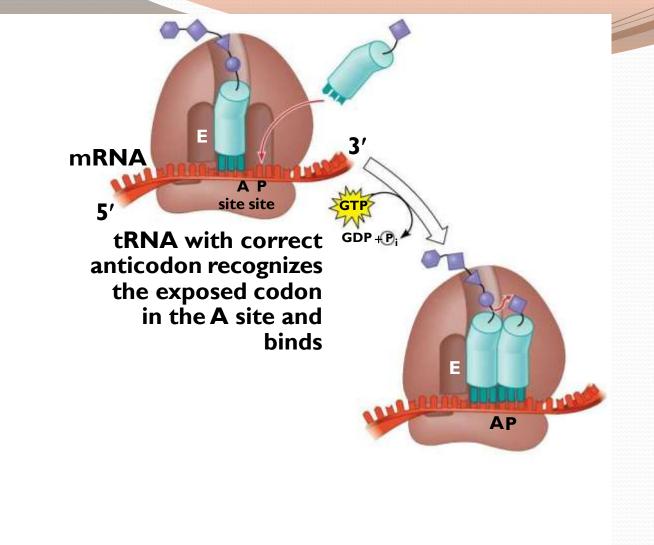
- Amino acids are added one by one to the preceding amino acid at the C-terminus of the growing chain
- Each addition involves proteins called elongation factors and occurs in three steps: codon recognition, peptide bond formation, and translocation

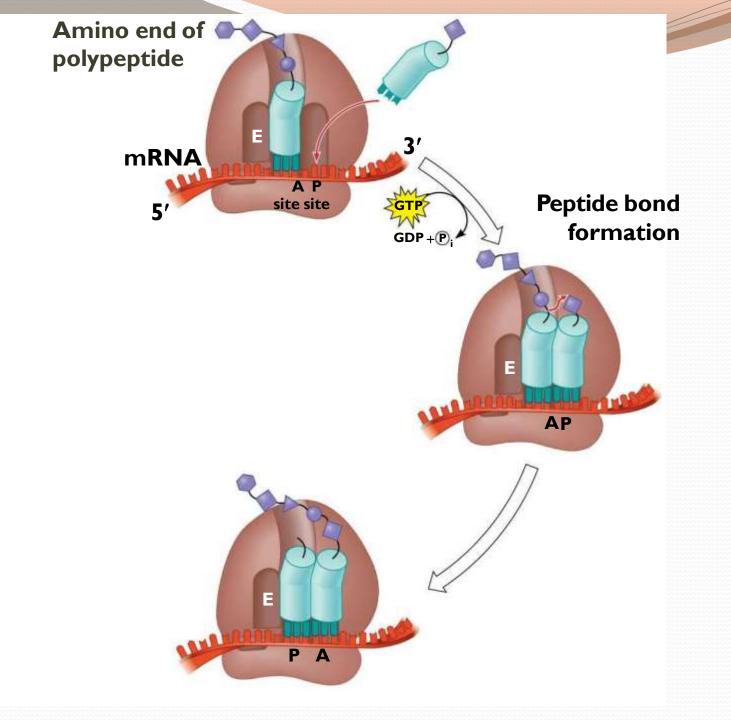
Translocation: Elongation

- Codon recognition
 - Three ribonucleotides are exposed in the "A" site
 - A tRNA with the correct anticodon will recognize the exposed codon and enter the "A" site
- Peptide bond formation
 - A peptide bond is formed between the two amino acids of adjacent tRNAs on the "A" and "P" sites
- Translocation
 - The ribosome moves one codon forward
 - The tRNA now in the "E" site exits
 - A new codon is now exposed in the "A" site

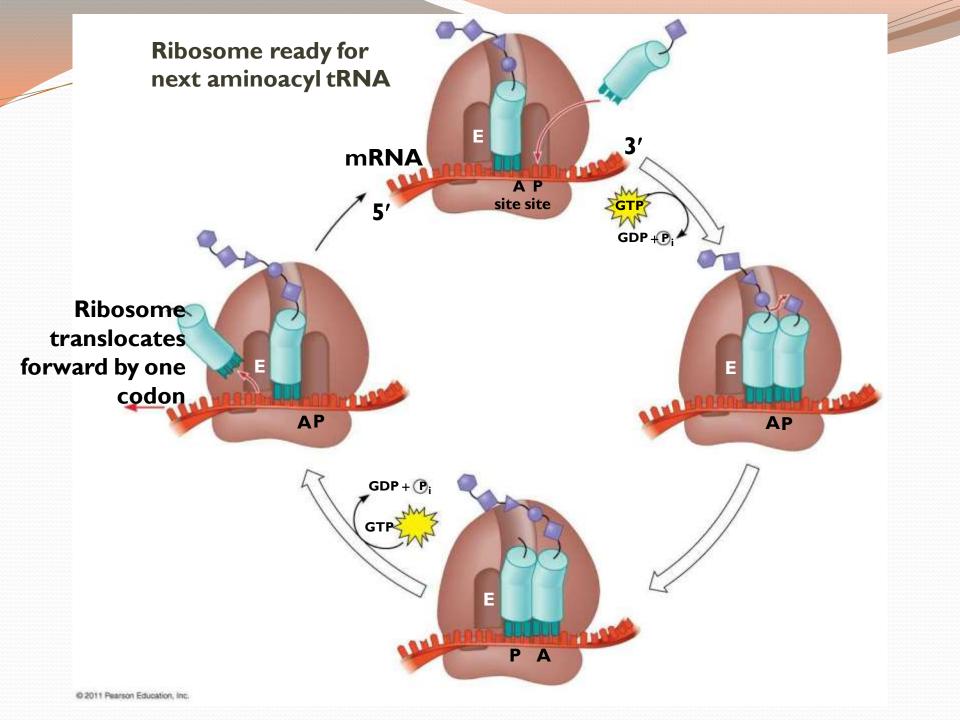


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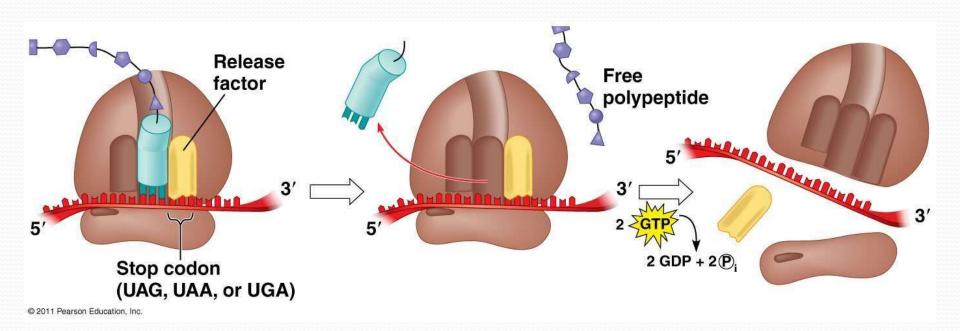
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Translation:Termination

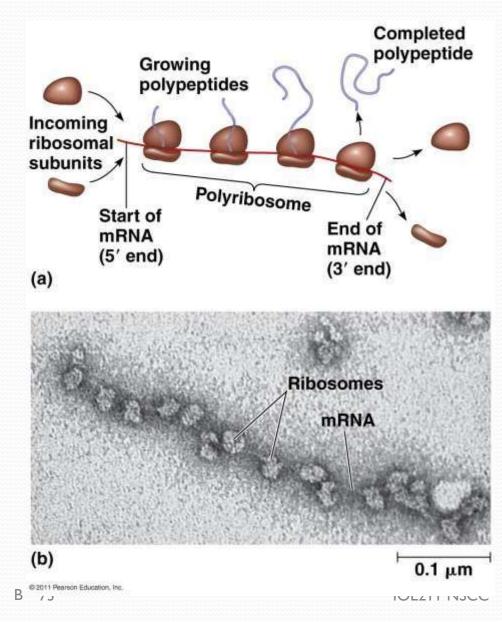
- The codons UGA, UAA, and UAG are **stop signals**
- Instead of a tRNA binding there, a protein called a release factor binds and causes the ribosome to release
 - The release factor causes the addition of a water molecule instead of an amino acid
 - This reaction releases the polypeptide, and the translation assembly then comes apart

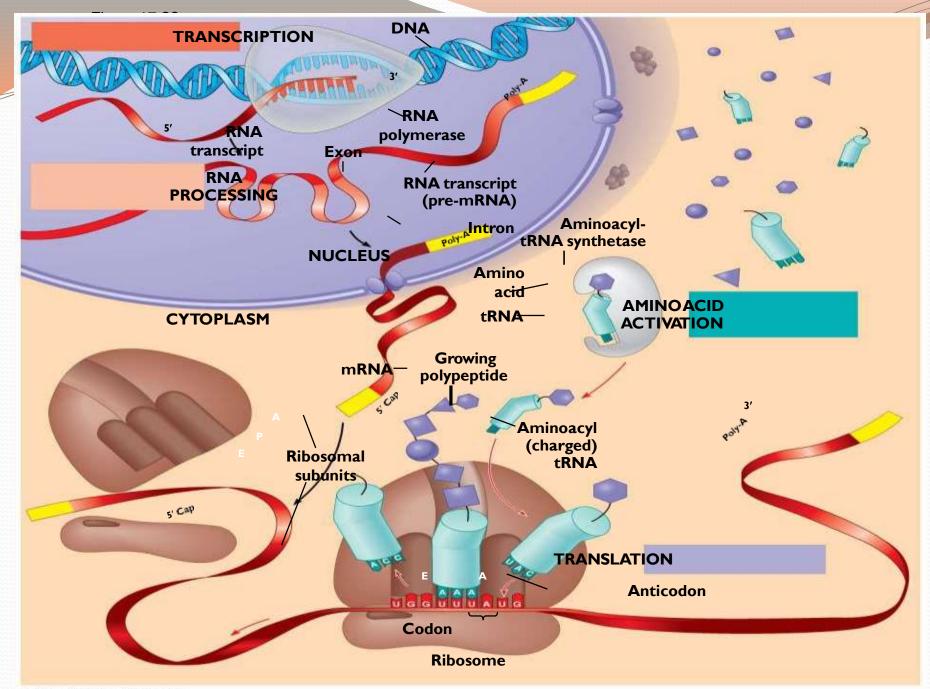
Translation:Termination



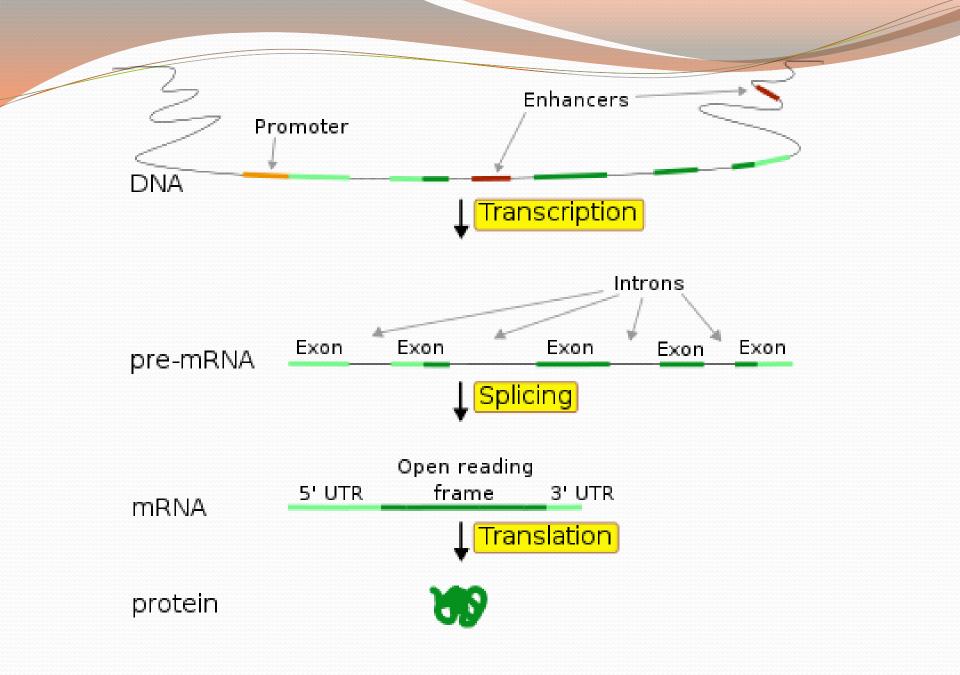
Polyribosomes

- Many ribosomes can attach to one mRNA at once
- This allows for very fast translation of multiple copies of the protein





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Template sequence (from problem):

3'-TTCAGTCGT-5'

Nontemplate sequence: 5'-A mRNA sequence: 5'-A

5'- AAGTCAGCA-3'

5'-AAGUCAGCA-3'

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Antibiotics: Ribosome inhibitors

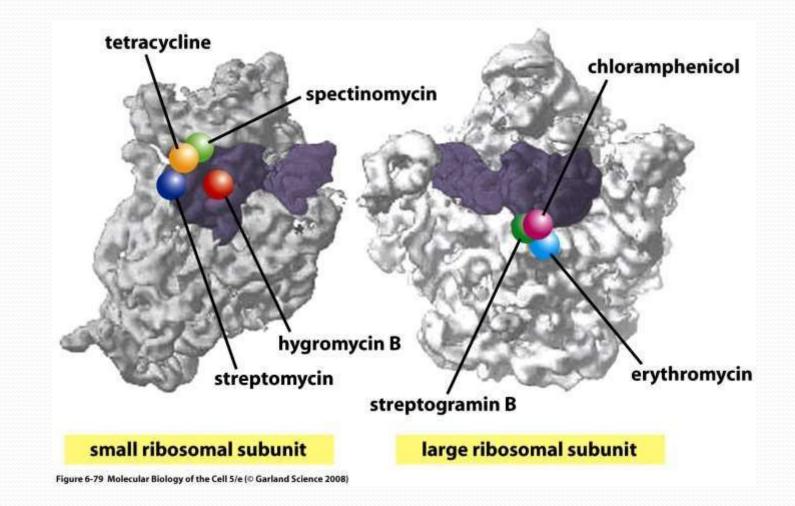


Table 6–4 Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
Erythromycin	binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

Table 6-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-translational modifications

- Once translated, proteins aren't finished! They must undergo post-translational modification
 - Help with proper folding
 - Addition of functional groups
 - Building subunits together into a protein with quaternary structure
 - Cleaving apart a protein to activate it

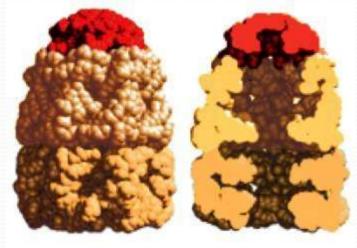
Why do modification?

- Modification affects the:
 - Lifespan
 - Cellular location
 - Activity



Protein Folding

- After translation, some proteins require additional help to fold properly
- Chaperonins are specialized proteins that help other proteins fold correctly
 - They provide a protected, neutral chamber



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Protein folding

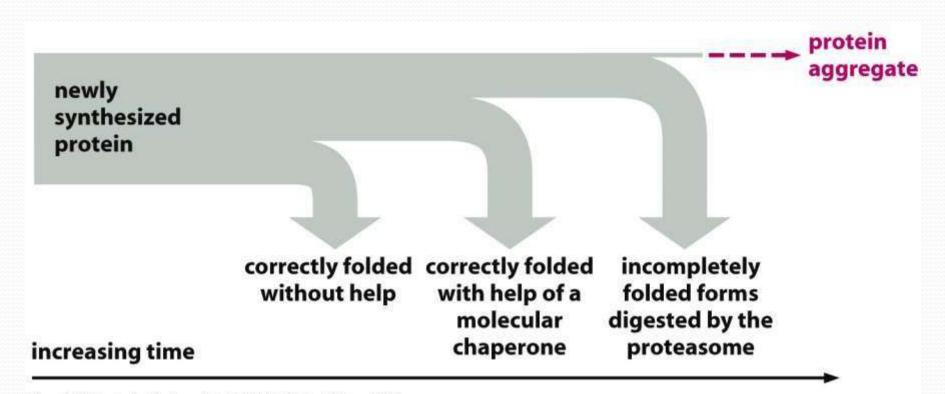
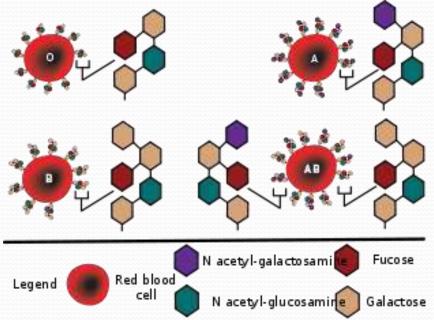
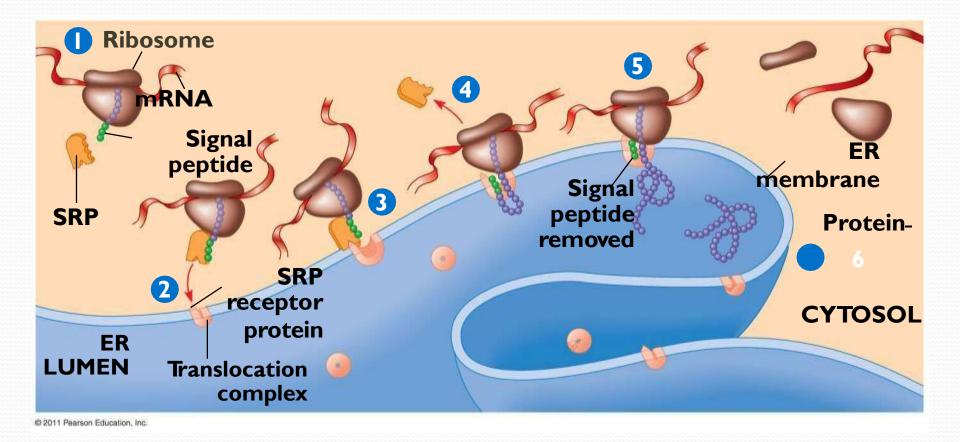


Figure 6-88 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Addition of functional groups

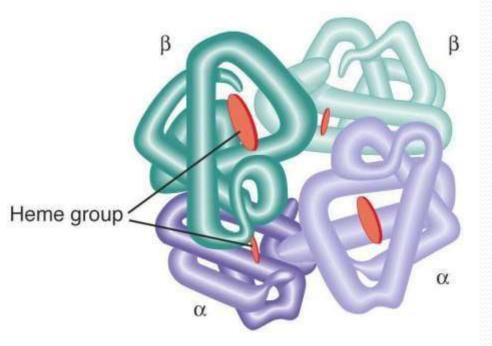
- -OH, -CH₃,-SH₂
- Major one is glycosylation, which attaches polysaccharides to proteins
 - Most proteins translated by ribosomes in the rough ER are glycosylated
 - Proteins are flagged to be sent to the ER by a special signal peptide



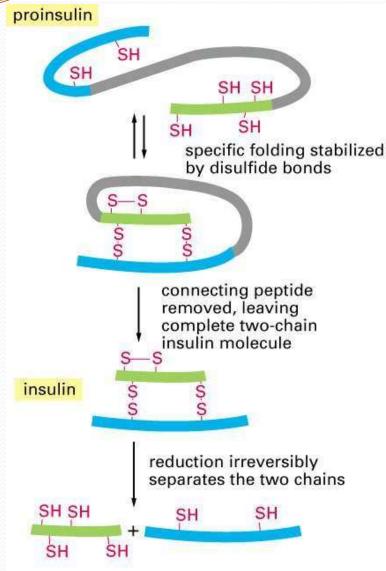


Quarternary Structure

- Each subunit of a protein with quaternary structure is transcribed and translated separately
- The subunits are brought together after translation



Cleavage



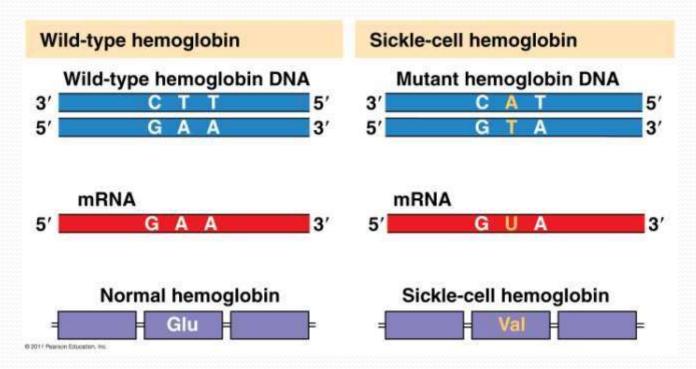
- Splitting off part of the peptide chain in order to activate the protein
 - Some cell signaling pathways signal the cleavage of other proteins

Mutations

- Large-scale, chromosome-wide
 - Translocations, inversions, deletions, insertions
- Small-scale
 - Point mutations
 - Indels
 - Frameshift

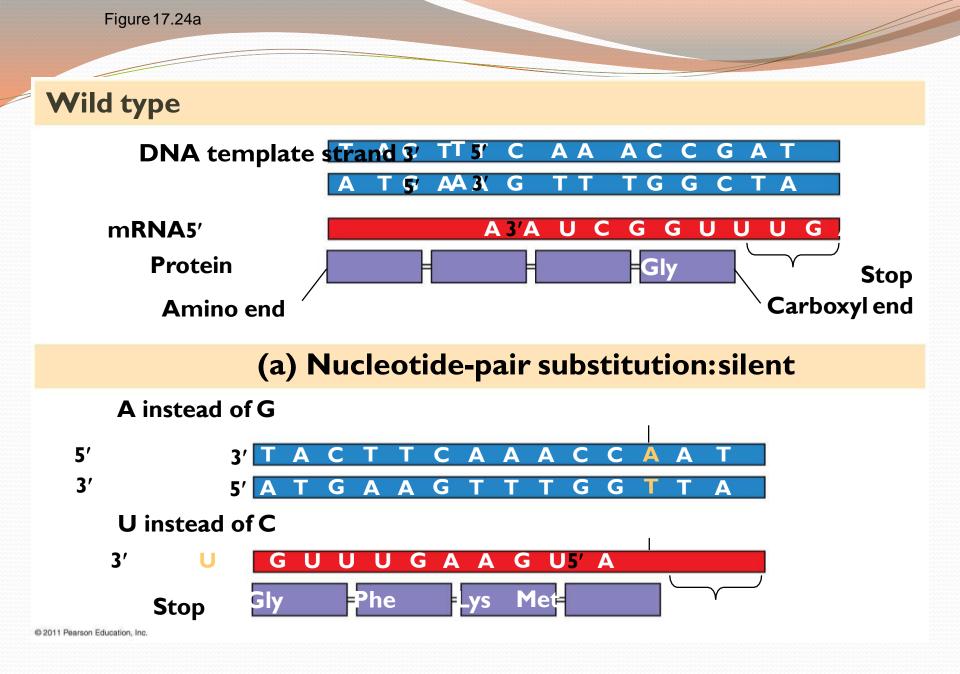
Point mutations

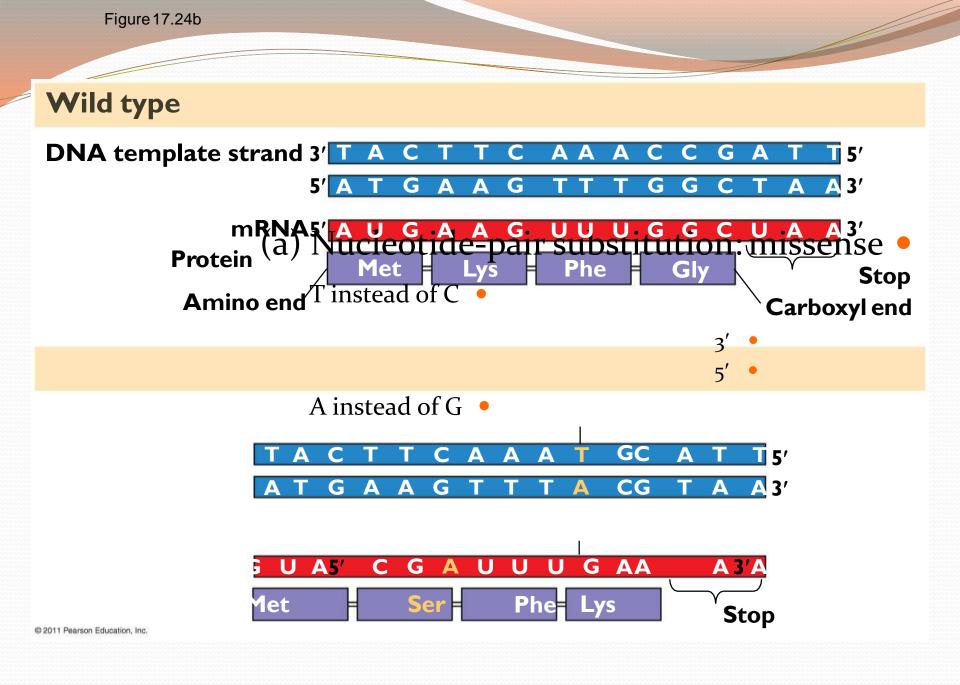
- Changing a single nucleotide in the template strand
- Point mutations are the cause of SNPs

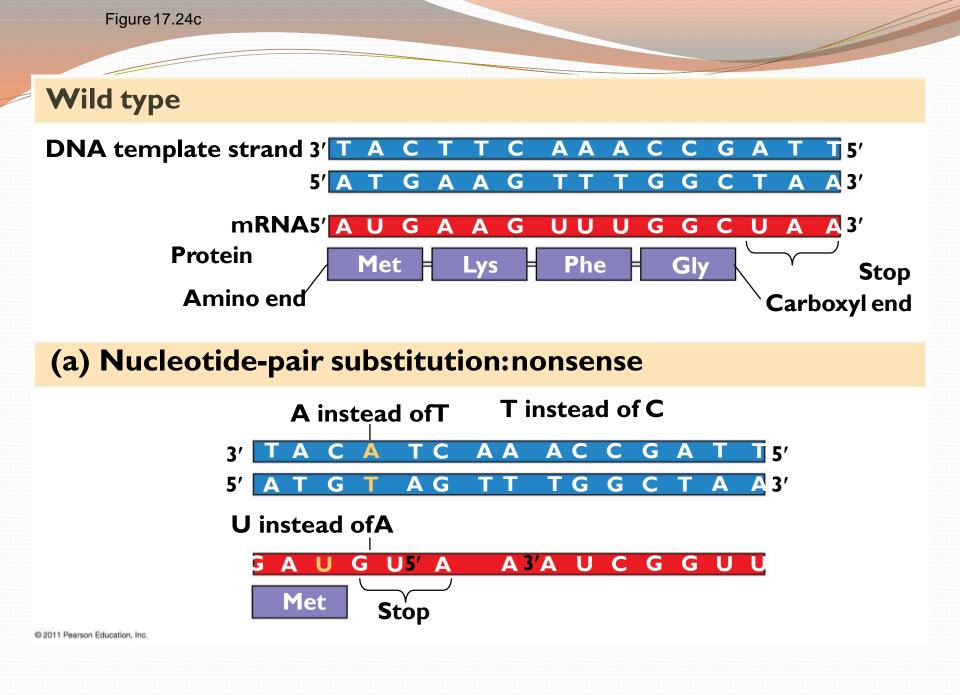


Types of point mutations

- 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

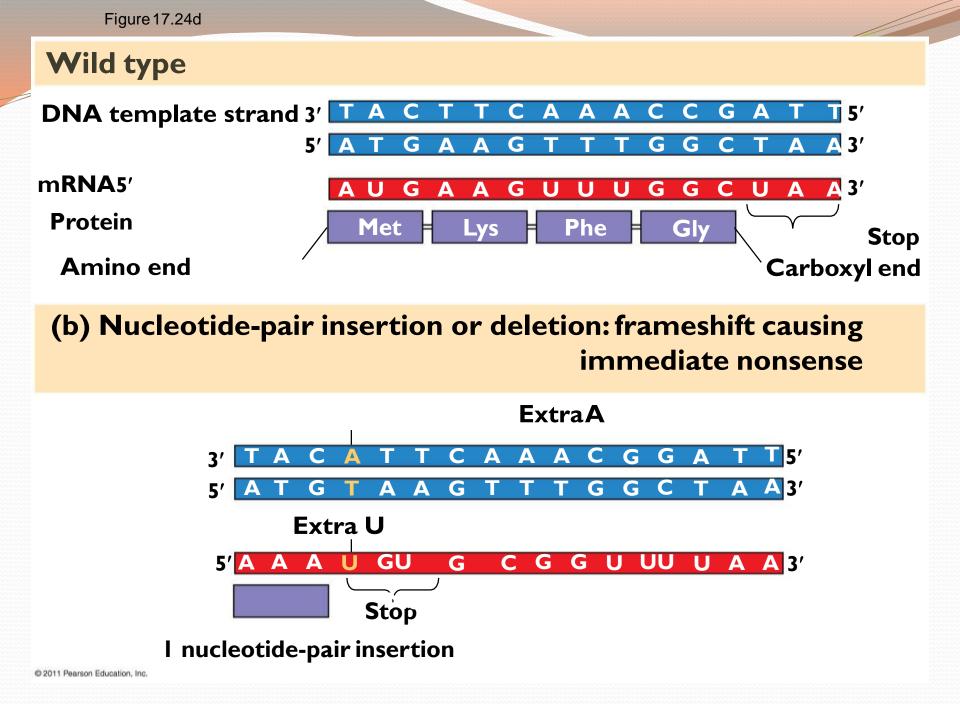


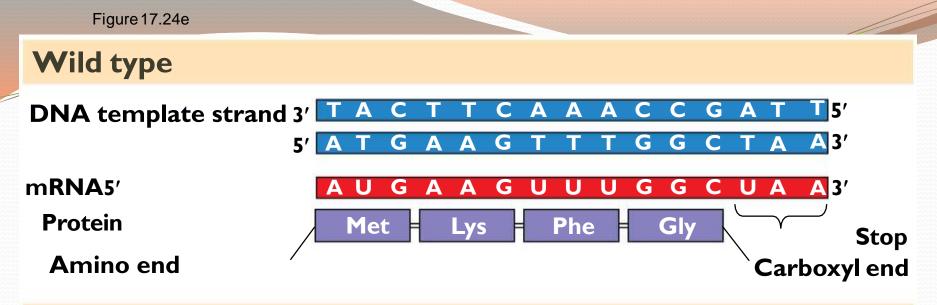




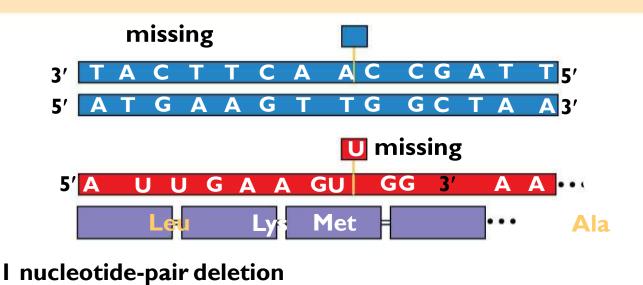
Indels

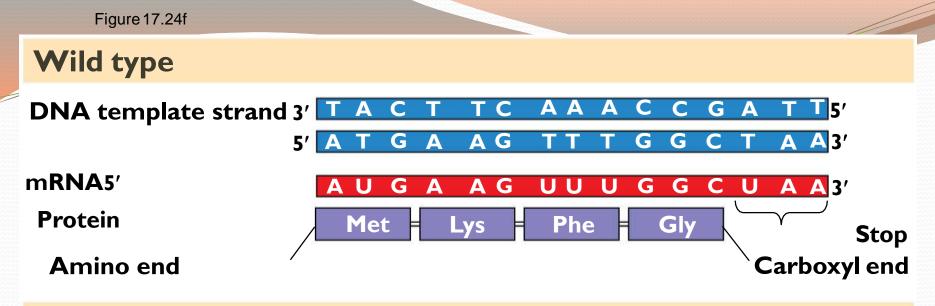
- Insertions and deletions are additions or losses of nucleotide pairs in a gene
- These mutations have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame, producing a frameshift mutation



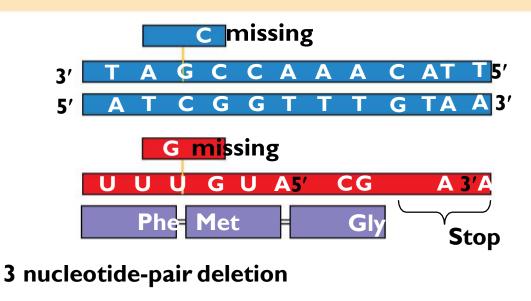


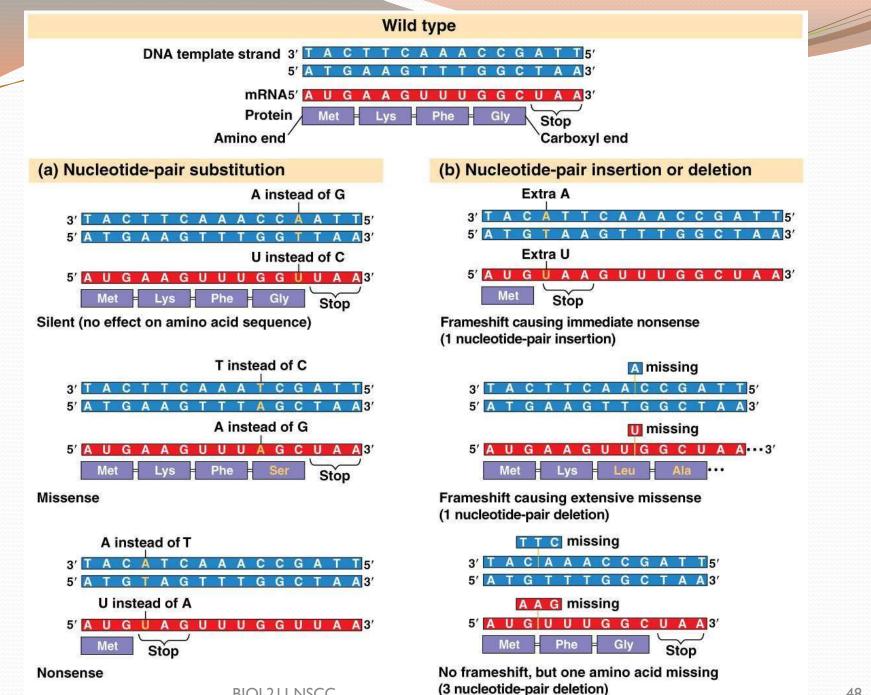
(b) Nucleotide-pair insertion or deletion: frameshift causing extensive missense





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





Mutagens

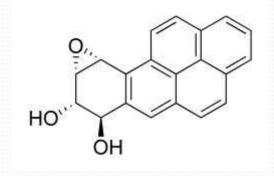
- Spontaneous mutations can occur during DNA replication, recombination, or repair
- **Mutagens** are physical or chemical agents that can cause mutations
 - Carcinogens are mutagens that can cause cancer

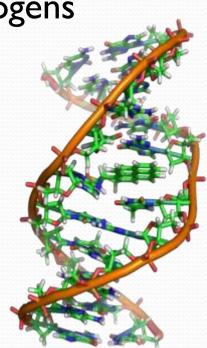


Carcinogens

- Heterocyclic amines are produced when meat is charred
- Nitrosamines, used in preserving meat, fish, and beer
- **Tobacco smoke** 19 known carcinogens

- Benzopyrene diolepoxide





OH

- Carcinogen
- Mutagen
- Point mutation
- Silent mutation
- Missense mutation
- Nonsense mutation
- Frameshift mutation
- Indel
- Post-translational modification
- Glycosylation
- Chaperonins
- Antibiotics
- Polyribosomes
- Stop signals
- Release factor
- Translation

Vocabulary

- Initiation, elongation, termination
- Methionine
- tRNA
- Anticodon
- Aminoacyl-tRNA synthetase
- Ribosome
- Small subunit,large subunit
- EPA
- Spliceosomes
- Alternative splicing
- Introns, exons
- RNA splicing
- preMRNA
- 5' cap, Poly-A tail

- Transcription
- Initiation,elongation, termination
- Promoter
- Enhancer
- Silencer
- TATA box
- -10 and -35 sequences
- Transcription factors
- Sigma factors
- RNA polymerase
- Codon bias
- Degenerate code
- Codon
- Template strand

Reference

• - Molecular biology of the cell, Garland Science , 2008.