

# **Biochemistry**

**Lec:9**

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# RNA

## Structure of RNA

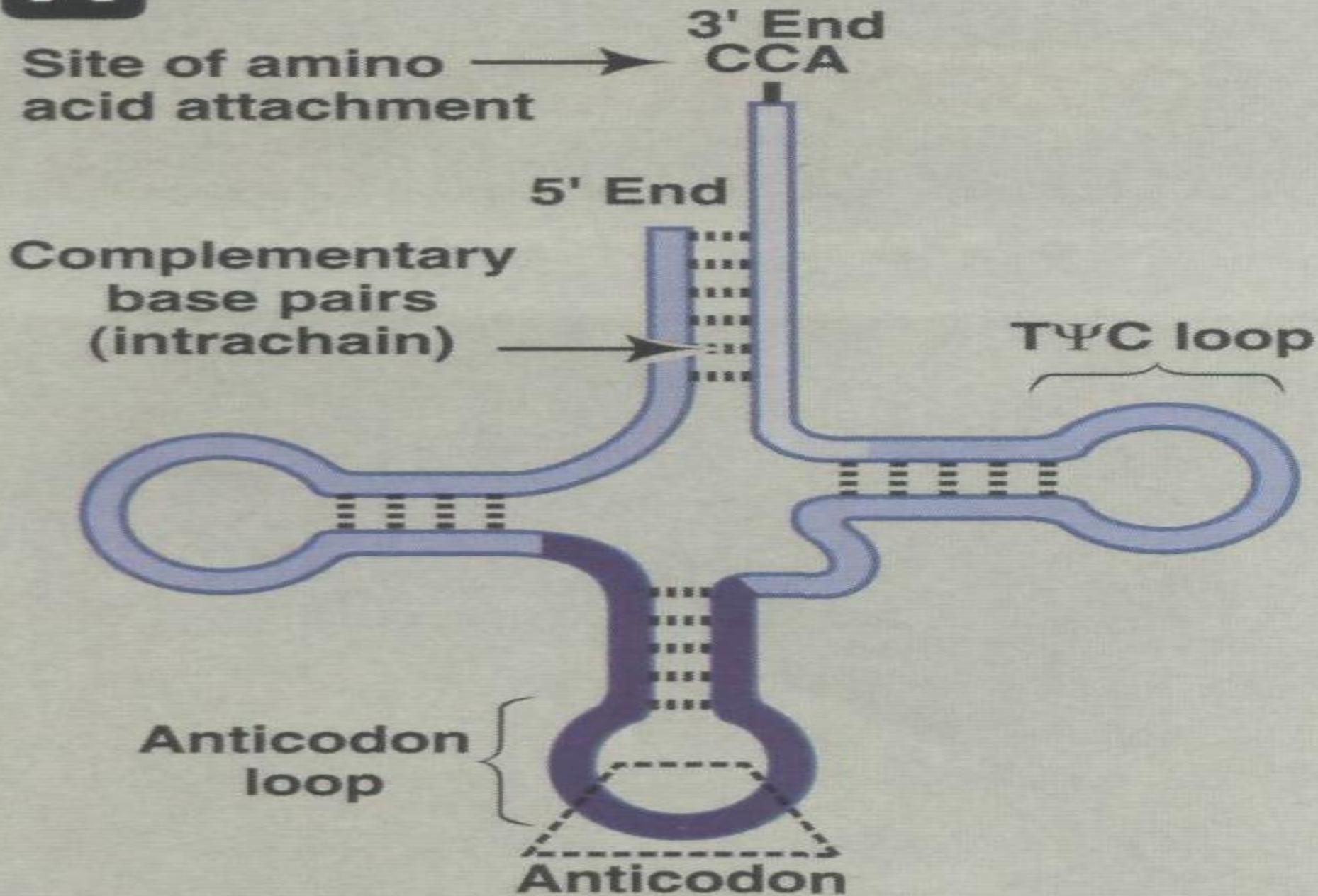
There are three major types of RNA that participate in the process of protein synthesis: **ribosomal RNA (rRNA)**, **transfer RNA (tRNA)**, and **messenger RNA (mRN)** Like DNA, However, they differ as a group from DNA in several ways, for example, they are considerably smaller than DNA, and they contain **ribose** instead of deoxyribose and uracil instead of thymine. Unlike DNA, most RNAs exist as single strands that are capable of folding into complex structures. The three major types of RNA also differ from each other in size, function, and special structural modifications.

# Ribosomal RNA

Ribosomal RNAs (rRNAs) are found in association with several proteins as components of the ribosome the complex structures that serve as the sites for protein synthesis . There are three distinct size species of rRNA (23S, 16S, and 5S) in prokaryotic cells . In the eukaryotic cytosol, there are four rRNA size species(28S,18S,5.8S) and 5S). [Note: "S" is the **Svedberg** unit, which is related to molecular weight and shape of the compound.] Together, rRNAs make up eighty percent of the total RNA in the cell.

# Transfer RNA

Transfer RNAs (tRNAs), the smallest of the three major species of RNA molecules (4S), have between 74 and 95 nucleotide residues. There is at least one specific type of tRNA molecule for each of the twenty amino acids commonly found in proteins. Together, tRNAs make up about fifteen percent of the total RNA in the cell. The tRNA molecules contain **unusual bases** (for example, pseudouracil) and have **extensive intrachain base-pairing** . Each tRNA serves as an "adaptor" molecule that carries its specific amino acid covalently attached to 3`end to its the site of protein synthesis. There it recognizes the genetic code word on an mRNA which specifies the addition of its amino acid to the growing peptide chain .

**A**

## **Messenger RNA**

Messenger RNA (mRNA) comprises only about five percent of the RNA in the cell, yet is by far the most heterogeneous type of RNA in size (500 to 6000 nucleotides) and base sequence. The mRNA carries genetic information from the nuclear DNA to the cytosol, where it is used as the template for protein synthesis. Special structural characteristics of eukaryotic mRNA (but not prokaryotic) include a long sequence of adenine nucleotides (a poly A tail") on the 3'-end of the RNA chain, plus a “**cap**” on the 5'-end consisting of a molecule of 7-methylguanosine.

# **Transcription of prokaryotic genes**

The structure of RNA polymerase, the signals that control transcription, and the varieties of modification that RNA transcripts can undergo differ among organisms, and particularly from prokaryotes to Eukaryote.

## **Properties of prokaryotic RNA polymerase**

In bacteria, one species of RNA polymerase synthesizes all of the RNA except for the short RNA primers needed for DNA replication (RNA primers are synthesized by a specialized enzyme primase).

# Steps in RNA synthesis

The process of transcription of a typical gene of E. Coli can be divided into three phases: initiation, elongation, and termination. [Note: Within the DNA molecule, regions of both strands can serve as templates for specific RNA molecules. However, only one of the two DNA strands serves as a template within a specific stretch of double helix.]

**1. Initiation:** Initiation of transcription involves the binding of the RNA polymerase holoenzyme to a region on the DNA that determines the specificity of transcription of that particular gene. That DNA sequence is known as the **promoter region** .

Characteristic "consensus" nucleotide sequences of the prokaryotic promoter region are highly conserved, that is, many different promoters contain some very similar or identical sequences.

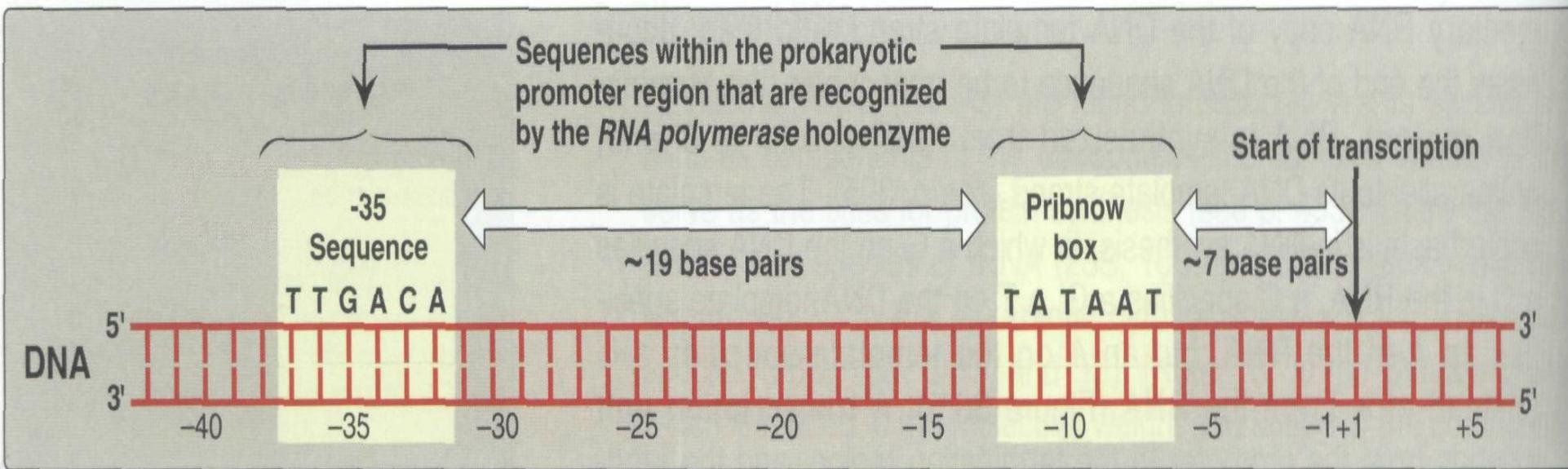
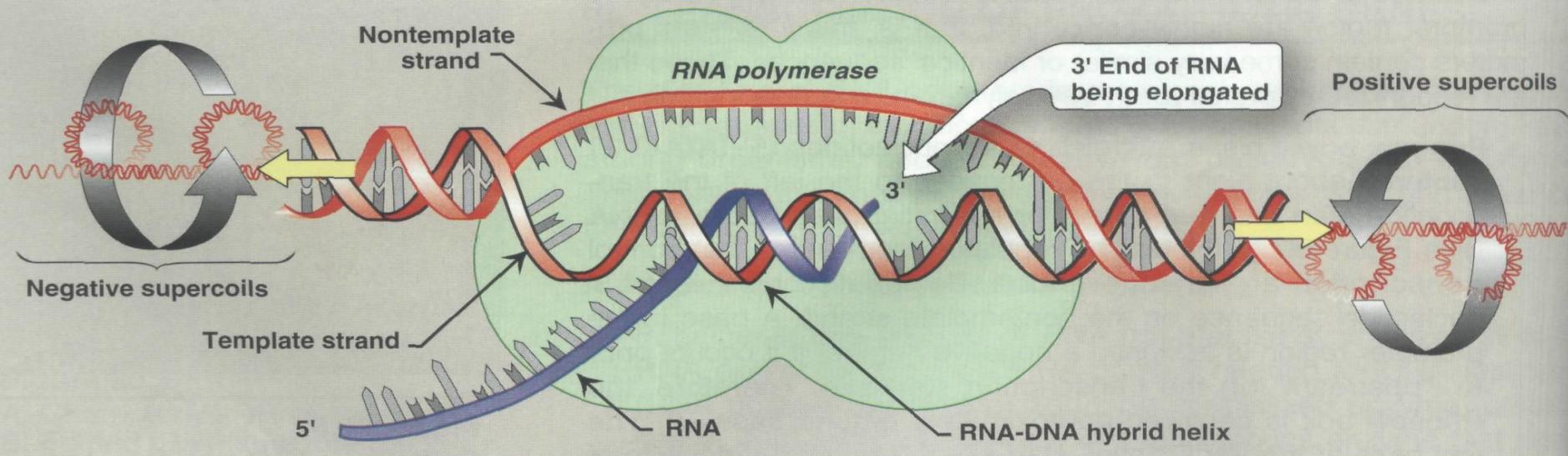


Figure 30.7

Structure of the prokaryotic promoter region.

**Elongation:** Once the promoter region has been recognized by the holoenzyme RNA polymerase begins to synthesize a transcript of the DNA sequence (usually beginning with a purine), it has no ability to repair mistakes in the RNA, as does DNA polymerase during DNA synthesis. RNA polymerase uses **ribonucleoside triphosphates**, and releases pyrophosphate each time a nucleotide is added to the growing chain. [Note: As in DNA synthesis, two high-energy bonds are thus used for the addition of each nucleotide.] The binding of the enzyme to the DNA template results in a local unwinding of the DNA helix (Note: This process can generate supercoils that can be relaxed by DNA topoisomerase).

**Termination:** The process of elongation of the RNA chain continues until a termination signal is reached. An additional protein,  $\rho$  (rho) factor, may be required for the release of the RNA product ( $\rho$ -dependent termination). Alternatively, the tetrameric RNA polymerase can, in some instances, recognize termination regions on the DNA template ( $\rho$ -independent termination).



**Figure 30.8**  
Local unwinding of DNA caused by *RNA polymerase*.

**Action of antibiotics:** Some antibiotics prevent bacterial cell growth by inhibiting RNA synthesis. For example, rifampin inhibits the initiation of transcription by binding to the  $\beta$ -subunit of prokaryotic RNA polymerase, thus interfering with the formation of the first phosphodiester bond. Rifampin is useful in the treatment of tuberculosis. Dactinomycin (known to biochemists as Actinomycin D) was the first antibiotic to find therapeutic application in tumor chemotherapy. It binds to the DNA template and interferes with the movement of RNA polymerase along the DNA

# **Transcription of eukaryotic gens**

Transcription of eukaryotic genes is a far more complicated process than transcription in prokaryotic cells. In addition to RNA polymerase recognizing the promoter region and initiating RNA synthesis, several supplemental transcription factors bind to distinct sites on the DNA— either within the promoter region or some distance from it. The binding of different factors determines which genes are to be transcribed. For RNA polymerase and the transcription factors to recognize and bind to their specific DNA sequences, the double helix must assume a loose conformation and dissociate temporarily from the nucleosome core.

# **Nuclear RNA polymerases of eukaryotic cells**

There are three distinct classes of RNA polymerase in the nucleus of eukaryotic cells.

**1. RNA polymerase I:** This enzyme synthesizes the precursor of the large ribosomal RNAs (28S, and 5.8S) in the nucleolus. [Note: mRNA and tRNA are synthesized in the nucleoplasm.]

**2. RNA polymerase II:** This enzyme synthesizes the precursors of messenger RNAs that are subsequently translated to produce proteins. Polymerase II also synthesizes certain small nuclear RNAs (snRNA), and is used by some viruses to produce viral RNA.

**3. RNA polymerase III:** This enzyme produces the small RNAs, including tRNAs, the small 5S ribosomal RNA, and some snRNAs.

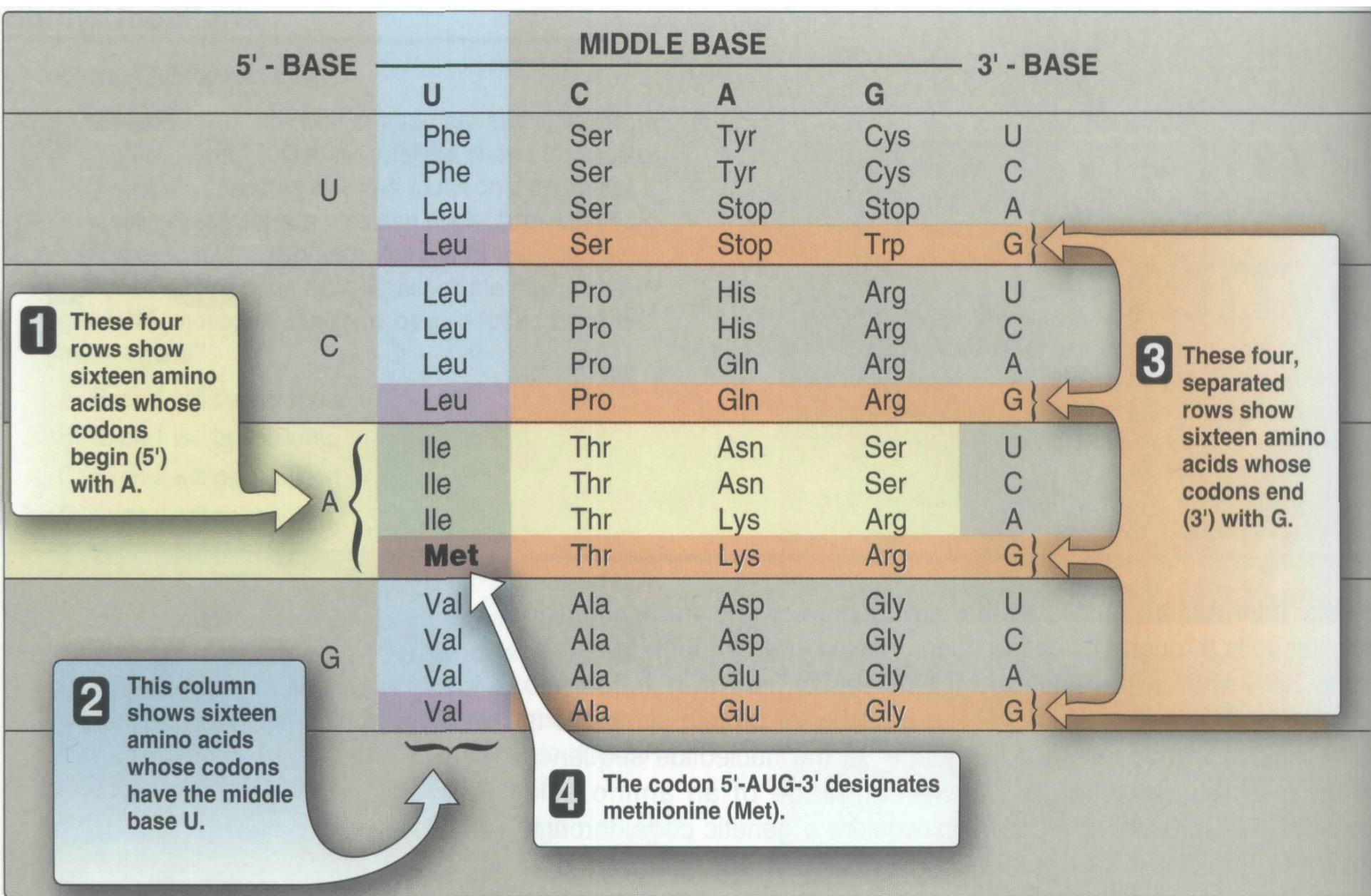
# Protein Synthesis

## The Genetic code

The genetic code is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids. Each individual word in the code is composed of three nucleotide bases. These genetic words are called codons.

### A. Codons

Codons are usually presented in the messenger RNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5'-end to the 3'-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time .



**Figure 31.2**  
Use of the genetic code table to translate the codon AUG.

**How to translate a codon:** This table (or "dictionary") can be used to translate any codon sequence and, thus, to determine which amino acids are coded for by an mRNA sequence. For example, the codon codes for methionine . Sixty-one of the 64 codons code for the twenty common amino acids.

**Termination ("stop" or "nonsense") codons:** Three of the codons, UAG, UGA, and UAA, do not code for amino acids, but rather are termination codons. When one of these codons appears in an mRNA sequence, it signals that synthesis of the peptide chain coded for by that mRNA is completed.

# Characteristics of the genetic code

- 1. Specificity:** The genetic codes specific (unambiguous), that is, a specific codon always codes for the same amino acid.
- 2. Universality:** The genetic codes virtually universal .
- 3. Redundancy:** The genetic code is redundant sometimes called **degenerate**). Although each codon corresponds to a single amino acid, a given amino acid may have more than one triplet coding for it. For example, arginine is specified by six different codons.
- 4. Nonoverlapping** The genetic code is nonoverlapping and commaless, that is, the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time.

# **Components required for translation**

## **A. Amino acids**

All the amino acids that eventually appear in the finished protein must be present at the time of protein synthesis.

## **B. Transfer RNA (tRNA)**

At least one specific type of tRNA is required per amino acid. In humans, there are at least fifty species of tRNA, whereas bacteria contain thirty to forty species. Because there are only twenty different amino acids commonly carried by tRNAs, some amino acids have more than one specific tRNA molecule. This is particularly true of those amino acids that are coded for by several codons.

### **C. Aminoacyl- tRNA synthetases**

This family of enzymes is required for attachment of amino acids to their corresponding tRNAs. Each member of this family recognizes a specific amino acid and the tRNAs that correspond to that amino acid.

### **D. Messenger RNA (mRNA)**

The specific mRNA required as a template for the synthesis of the desired polypeptide chain must be present.

### **E. Functionally competent ribosomes**

Ribosomes are large complexes of protein and rRNA .They consist of two subunits- one large and one small-one relative sizes are generally given in terms of their sedimentation coefficients, or S (Svedberg) values.

# Steps in protein synthesis

## Initiation

Initiation of protein synthesis involves the assembly of the components of the translation system before peptide bond formation occurs. These components include the two ribosomal subunits, the mRNA to be translated, the aminoacyl-tRNA specified by the first codon in the message, GTP (which provides energy for the process), and initiation factors that facilitate the assembly of this initiation complex.

**B. Elongation** of the polypeptide chain involves the addition of amino acids to the carboxy end of the growing chain. During elongation, the ribosome moves.

## **C. Termination**

Termination occurs when one of the three termination codons moves into the A site.

## **D. Polysomes**

Translation begins at the 5'-end of the mRNA, with the ribosome proceeding along the RNA molecule. Because of the length of most mRNA more than one ribosome at a time can generally translate a message . Such a complex of one mRNA and a number of ribosomes is called a polysome or **polyribosome**.

## **E. Protein targeting**

Although most protein synthesis occurs in the cytoplasm of eukaryotic cells, many proteins are destined to perform their functions within specific cellular organelles.

## **Regulation of translation**

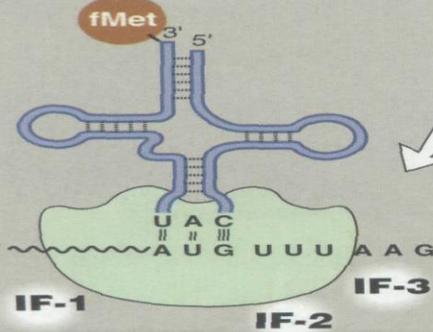
Although gene expression is most commonly regulated at the transcriptional level. For example, heme stimulates overall translation by preventing the phosphorylation of eukaryotic initiation factor eIF-2 which is only active in its unphosphorylated form. The translation of some messenger RNA molecules is regulated by the binding of regulatory proteins, which sometimes block translation (for example, of ferritin mRNA), and sometimes stabilize the mRNA to extend its lifetime (for example, of transferrin receptor mRNA). [Note: In the presence of adequate supplies of iron, these regulatory proteins dissociate from the mRNA molecules, causing the rate of ferritin synthesis to increase and the rate of transferrin receptor synthesis to decrease.]

### STREPTOMYCIN<sup>1</sup>

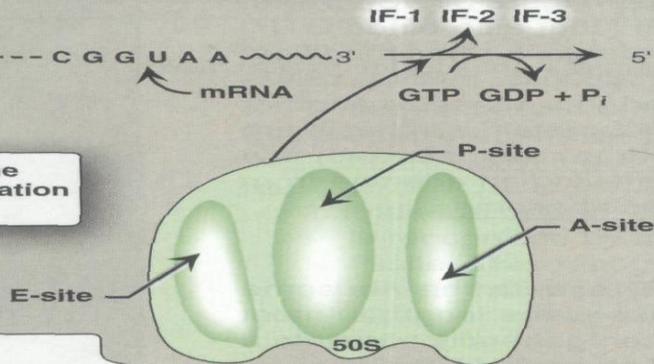
Binds to the 30S subunit and distorts its structure, interfering with the initiation of protein synthesis.

## INITIATION

**2** GTP is cleaved and initiation factors are released when the 50S subunit arrives to form the 70S initiation complex.



**1** Initiation factors aid in the formation of the 30S initiation complex.



### TETRACYCLINES<sup>2</sup>

Interact with small ribosomal subunits, blocking access of the aminoacyl-tRNA to the mRNA-ribosome complex.

**3** Elongation factors direct the binding of the appropriate tRNA to the codon in the empty A-site.

EF-Tu  
EF-Ts

## ELONGATION

**4** Peptidyltransferase, a component of the 50S ribosomal subunit, transfers the amino acid (or peptide chain) from the P-site onto the amino acid at the A-site, and catalyzes peptide bond formation.

### PUROMYCIN

Bears a structural resemblance to aminoacyl-tRNA and becomes incorporated into the growing peptide chain, thus causing inhibition of further elongation in both prokaryotes and eukaryotes.

### CHLORAMPHENICOL<sup>3</sup>

Inhibits prokaryotic peptidyltransferase. High levels may also inhibit mitochondrial protein synthesis.

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Figure 31.13

Steps in prokaryotic protein synthesis (translation). (Continued on the next page)

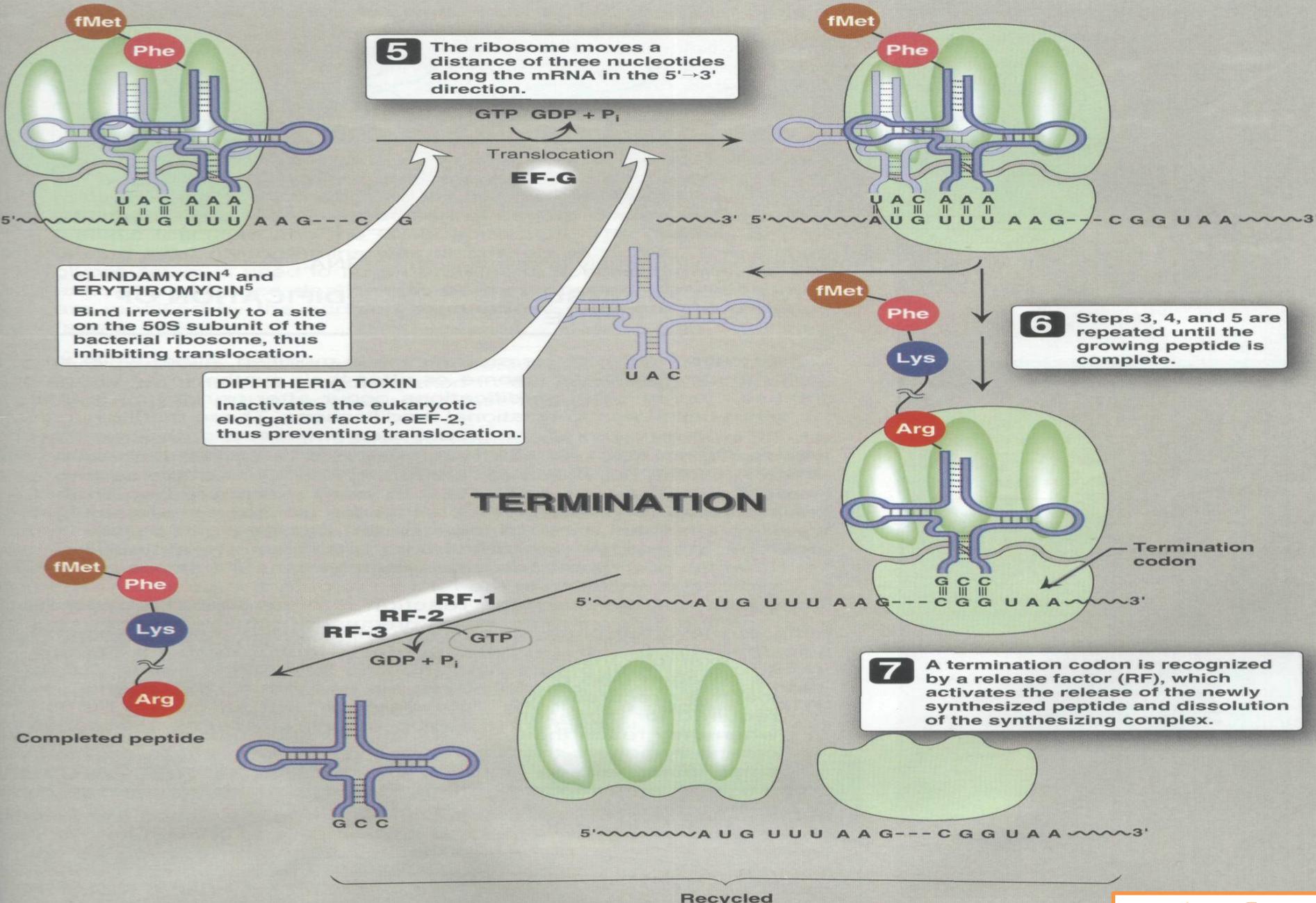


Figure 31.13 (Continued)

