

Johns Hopkins Engineering

Molecular Biology

Transcription and Translation



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Outline

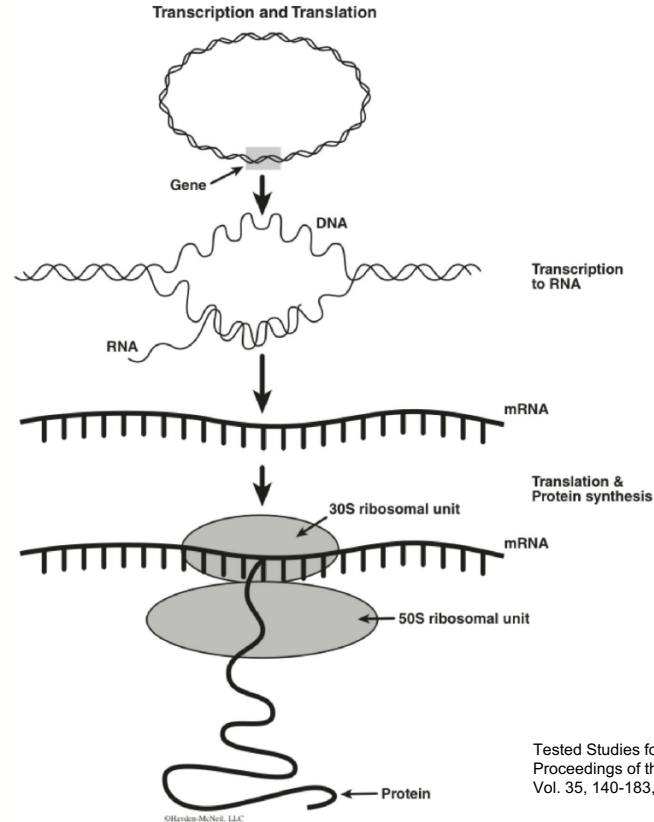
- Central Dogma
- Transcription
- RNA polymerase
- Translation

The Central Dogma of Molecular Biology

DNA → RNA → Protein

Transcription is the first step in gene expression. It involves copying a gene's DNA sequence to make an RNA molecule and is carried out by RNA polymerases.

Translation converts information in mRNAs into a chain of amino acids linked by peptide bonds.



Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 35, 140-183, 2014

The Central Dogma

- **Transcription** refers to RNA synthesis using DNA as a template
- **Translation** is the synthesis of protein using the information in the RNA
- **Messenger RNA, mRNA**, is translated into protein

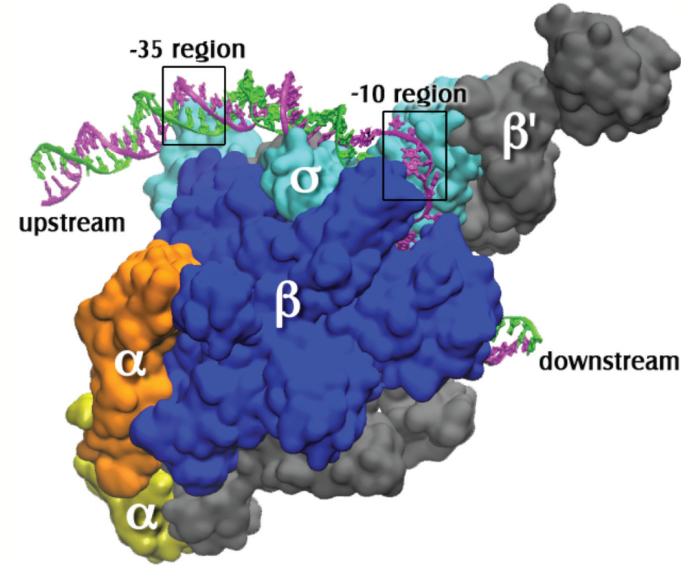
- There are exceptions to the central dogma
 - Some RNA viruses carry out *reverse transcription*, using RNA as a template for DNA synthesis
 - Other viruses produce RNAs from an RNA template

Transcription in Eukaryotes and Prokaryotes

- The fundamental principles of transcription were first determined in bacteria, where mechanisms are relatively simple
- Eukaryotic transcription involves the same four stages as prokaryotic but there are several important differences:
 - Each of three different RNA polymerases transcribes one or more different classes of RNA
 - Eukaryotic promoters are more varied than bacterial ones

Bacterial Transcription

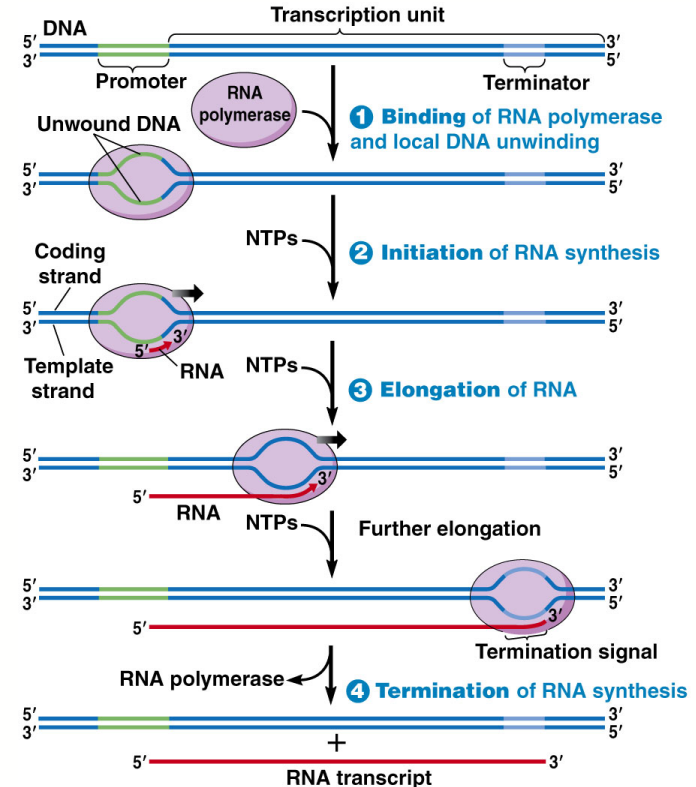
- Transcription is carried out by the enzyme RNA polymerase, which synthesizes RNA using DNA as a template
- Bacteria have a single kind of RNA polymerase to synthesize all three classes of RNA (mRNA, tRNA, and rRNA)
- The RNA polymerase of *E. coli* has two α , two β subunits, and a dissociable **sigma** (σ) **factor**



https://www.mdpi.com/journal/biomolecules/special_issues/bacterial-RNA-polymerase

Transcription Involves Four Stages: Binding, Initiation, Elongation, and Termination

- The DNA that gives rise to one RNA molecule is called the **transcription unit**
- Transcription begins when RNA polymerase binds to a promoter sequence (1) triggering local unwinding of the DNA
- RNA polymerase initiates synthesis of RNA using one DNA strand as a template (2)
- After initiation the RNA polymerase moves along the DNA template, unwinding the helix and elongating the RNA (3)
- Eventually the enzyme transcribes a termination signal which stops RNA synthesis and causes release of the RNA and dissociation of the polymerase (4)



Eukaryotic transcription

- Eukaryotic transcription differs from that of prokaryotes:
 - Transcription takes place in the nucleus
 - RNA polymerases in eukaryotes require *transcription factors*
 - RNA cleavage is more important than termination of transcription in determining the 3' end of the transcript
 - Newly forming RNA molecules undergo RNA processing, chemical modification during and after transcription
 - mRNAs can then be translated into protein in the cytoplasm

Gene Expression: Protein Synthesis & Sorting

- Questions:
 - How are messenger RNAs (mRNAs) translated into polypeptides?
 - How do the polypeptides become functional proteins?
 - How do these proteins reach the destinations where they carry out their functions?
- mRNAs encode instructions for translation, the process of assembling amino acids into a polypeptide

Translation: Key Players

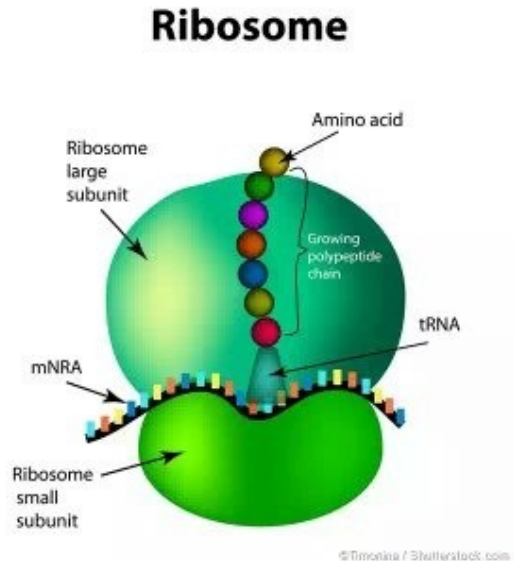
- **Ribosomes** carry out the process of polypeptide synthesis
- **tRNA** molecules align the amino acids in the correct order
- **Aminoacyl-tRNA synthetases** attach amino acids to their appropriate tRNA molecules
- **mRNA** molecules encode the amino acid sequence information (A,C,G,&U)
- **Protein factors** facilitate some of the steps of translation

The Ribosome Carries Out Polypeptide Synthesis

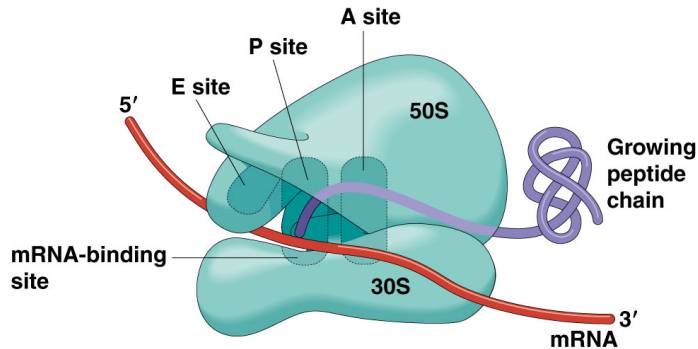
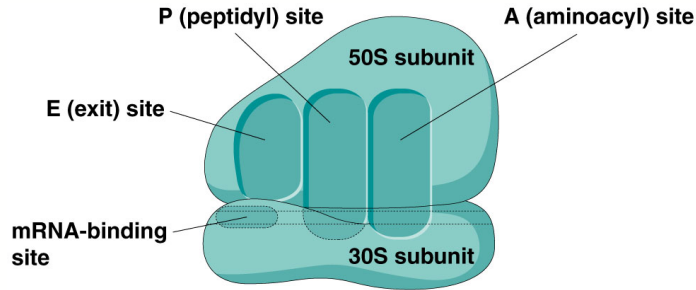
- Ribosomes orient the mRNA and amino acid-carrying tRNAs so the genetic code can be read accurately; They also catalyze peptide bonds so that amino acids are linked into polypeptides
- Ribosomes are particles made of rRNA and protein; They are “riboneucleoproteins”
- In eukaryotes, they are found free in the cytoplasm, and bound to ER and the outer nuclear envelope (80S)
- In prokaryotes, the ribosomes are smaller (70S)

Ribosome structure

- Ribosomes are built from dissociable subunits, the *large* and *small subunits*
- Bacterial ribosomes are sensitive to different inhibitors of protein synthesis and are composed of fewer proteins and smaller and fewer RNA molecules



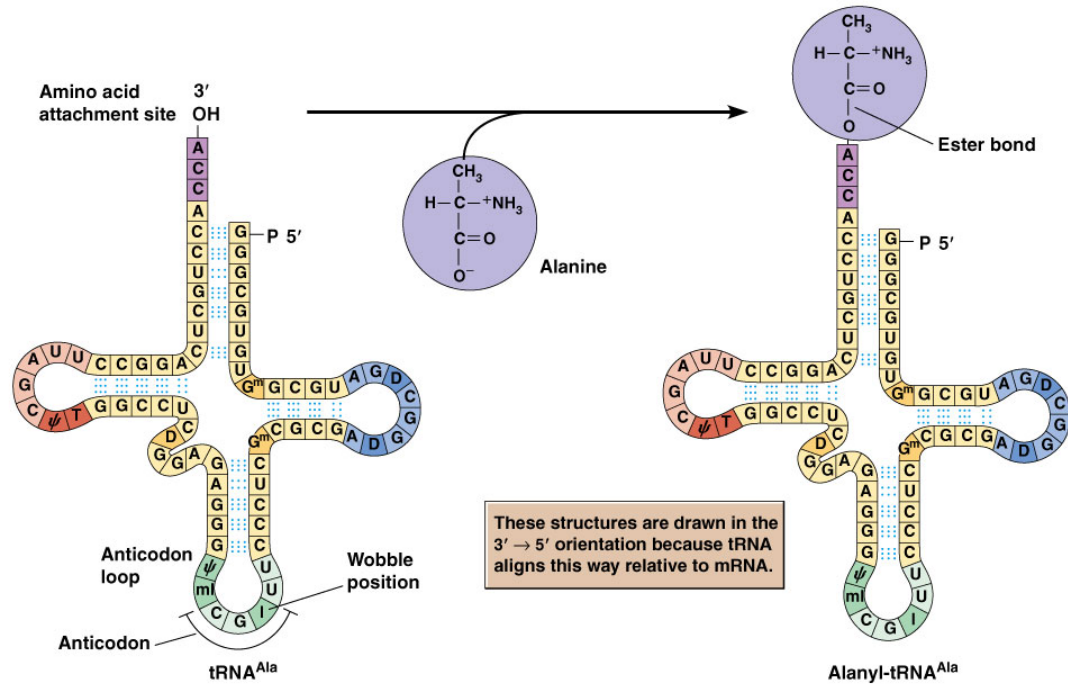
Ribosomes: Antibiotic Targets



- Nature Reviews Microbiology 12, 35–48 (2014)
 - “Ribosome-targeting antibiotics and mechanisms of bacterial resistance”
 - http://www.nature.com/nrmicro/journal/v12/n1/fig_tab/nrmicro3155_F1.html
- “selective toxicity”

Transfer RNA Molecules Bring Amino Acids to the Ribosome

- A **tRNA molecule** is an adaptor that has two specific binding sites, one for an amino acid and one for the mRNA sequence that specifies the amino acid
- Each tRNA is linked to its amino acid by an ester bond
- tRNAs are named for the amino acids attached to them, e.g., tRNA^{Ala} for alanine



(a) Secondary structure of tRNA, before and after amino acid attachment

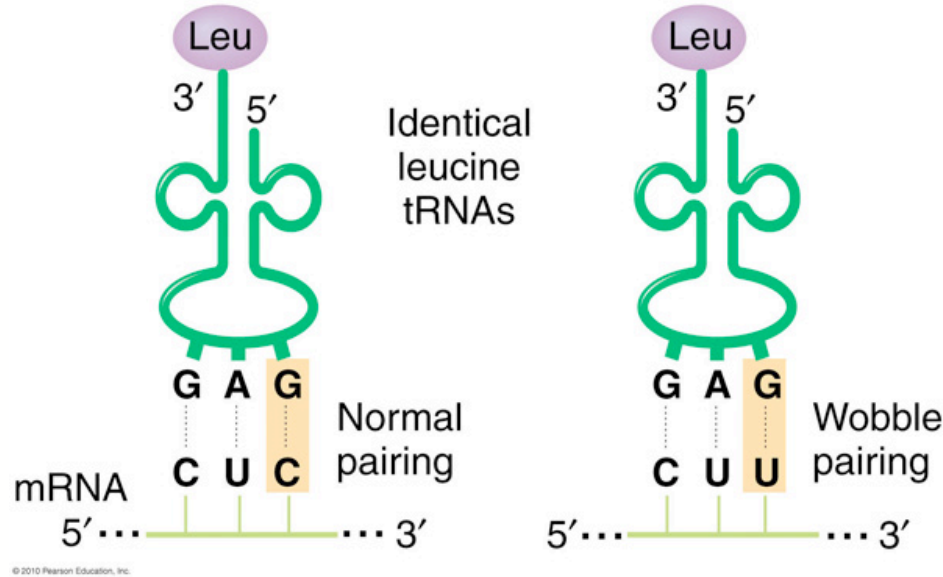
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3 major loops:

- Four base-paired regions,
- An anticodon triplet,
- A 3' terminal sequence of CCA where the appropriate amino acid is bound via an ester bond

tRNAs

- tRNAs attached to an amino acid are said to be **aminoacyl tRNAs**
- Each tRNA recognizes codons in mRNA due to their complementarity to the **anticodon** in the tRNA
- Some tRNA molecules recognize more than one codon
- mRNA and tRNA line up on the ribosome in a way that permits flexibility or wobble in the pairing between the third base of the codon and the corresponding base of the anticodon
- This is the **wobble hypothesis**, which allows for some unexpected base pairing



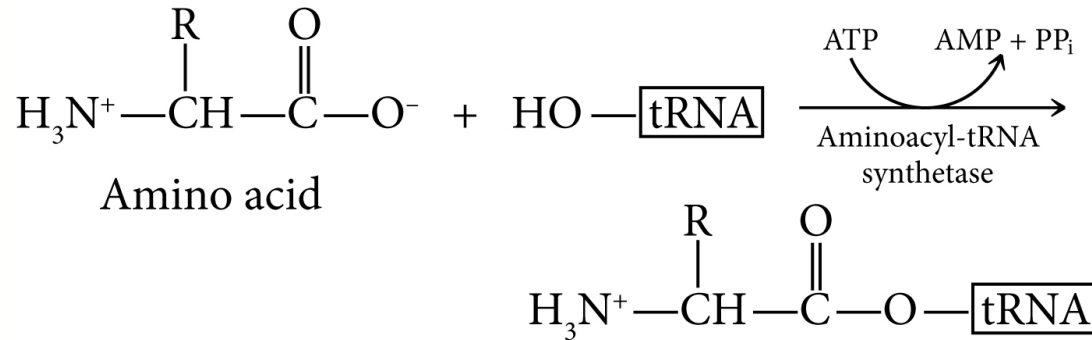
- There may be several different tRNAs capable of pairing with a given codon
- “wobble” in the third position allows flexibility, and still results in production of the same amino acid

Aminoacyl-tRNA Synthetases Link Amino Acids to the Correct Transfer RNAs

- Before the tRNA can bring its amino acid to the ribosome, the amino acid must be covalently attached to the tRNA by enzymes
- These enzymes are called **aminoacyl-tRNA synthetases**
- There is one aminoacyl-tRNA synthetase for each amino acid (20)
- This process is called “amino acid activation”

Aminoacyl-tRNA synthesis

- Aminoacyl-tRNA synthetases catalyze the attachment of amino acids to the tRNAs via an ester bond, using ATP hydrolysis



Aminoacyl-tRNA synthesis

- Both the anticodon and the 3' end of the tRNA are needed to specify the correct amino acid
- After addition of an amino acid the synthetases proofread the final product to ensure the correct amino acid was added
- It is the tRNA that then recognizes the appropriate codon in mRNA

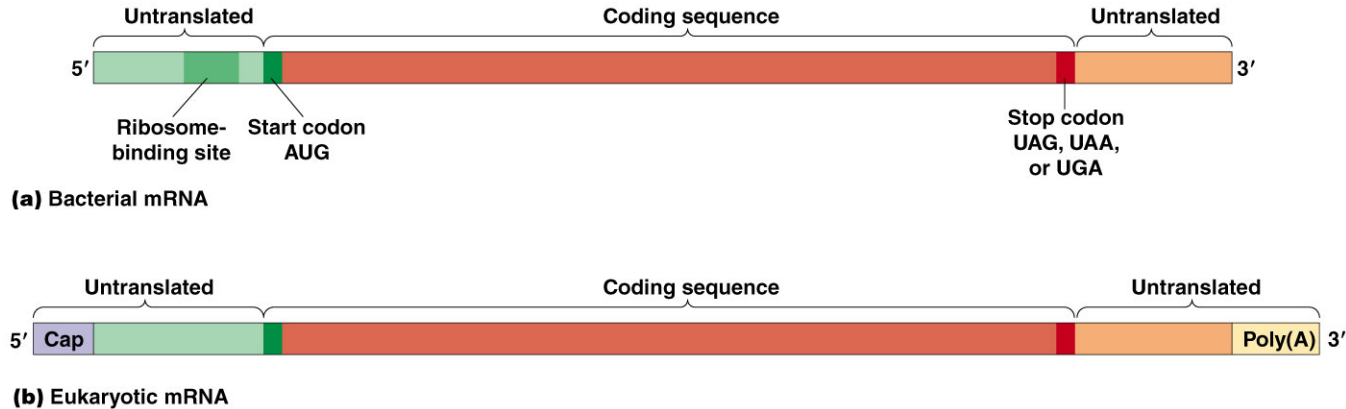
Messenger RNA Brings Polypeptide Coding Information to the Ribosome

- The sequence of codons in mRNA directs the order of amino acids in the polypeptide
- mRNA is exported from the nucleus to the cytoplasm via binding to proteins that contain *nuclear export signals (NES)*; these proteins target the mRNA through nuclear pores
- An untranslated sequence at the 5' end of the message precedes the **start codon**, the first to be translated (usually AUG)

Coding information

- There is also an untranslated region at the 3' end of the mRNA that follows the **stop codon**, which signals the end of translation
- The stop codon may be UAG, UAA, or UGA
- 5' and 3' untranslated regions vary in length and are essential for mRNA function
- mRNAs have a 5' cap and 3' poly(A) tail within the untranslated region
 - The 5' cap is important for initiating translation in eukaryotes

Figure 22-6



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- a) Bacterial mRNA encodes a single peptide
- b) Eukaryotic mRNA also has a 5' cap (where ribosomes bind) and a poly(A) tail

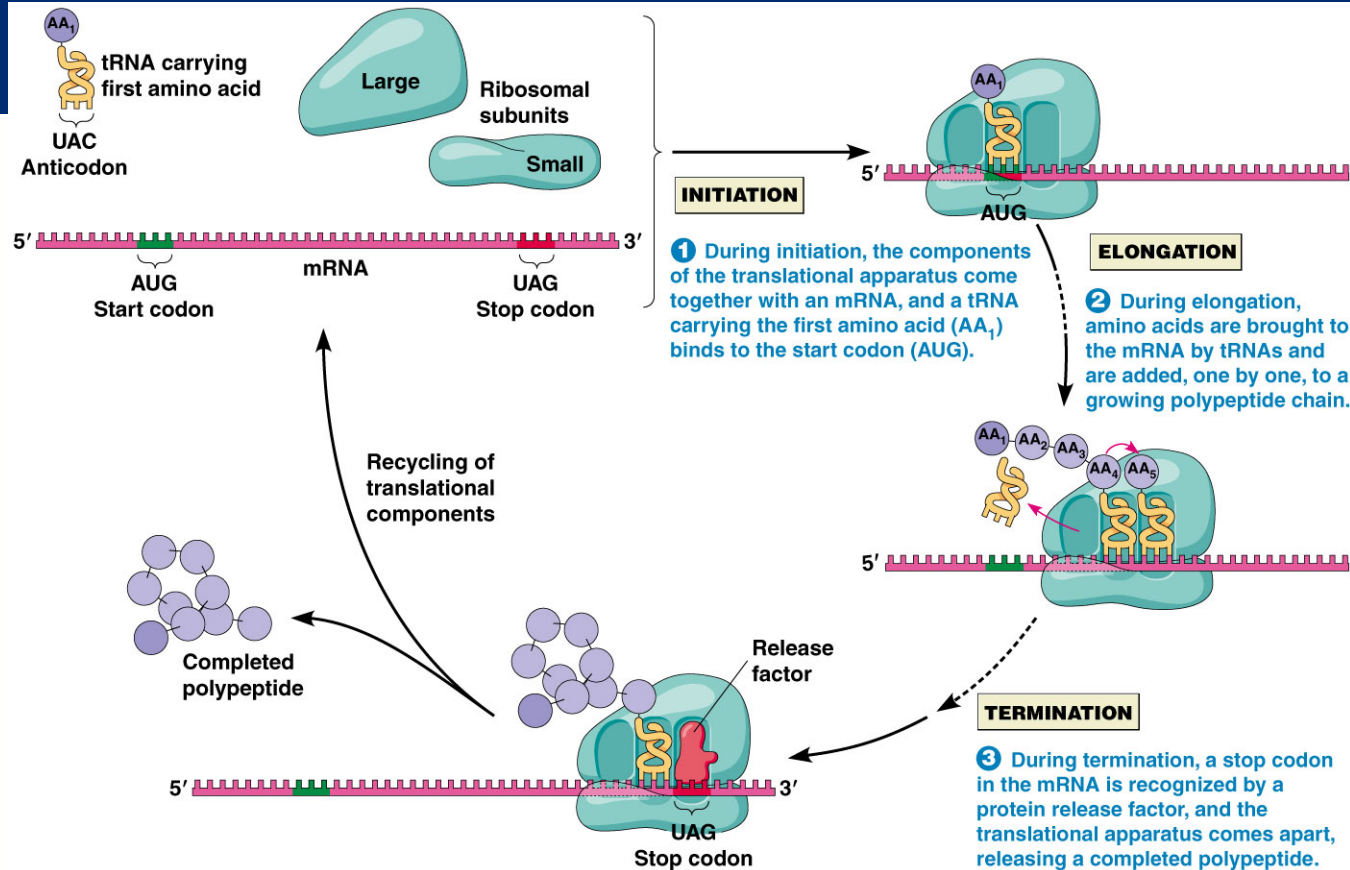
Eukaryotic mRNAs are monocistronic

- Most (but not all!) mRNAs in eukaryotes are *monocistronic*, meaning they encode just one polypeptide
- In bacteria and archaea, some are *polycistronic*, encoding several polypeptides, usually with related functions
- These polycistronic transcription units are called *operons*

Protein Factors Are Required for the Initiation, Elongation, & Termination of Polypeptide Chains

- Each part of translation requires certain *protein factors* to
 - *Initiate translation*
 - *Elongate the polypeptide chain*
 - *Terminate polypeptide synthesis*
- Translation is an ordered, stepwise process that begins at the *N-terminus* of the polypeptide and adds amino acids to the growing chain until the *C-terminus* is reached
- The mRNA is read in the 5' to 3' direction

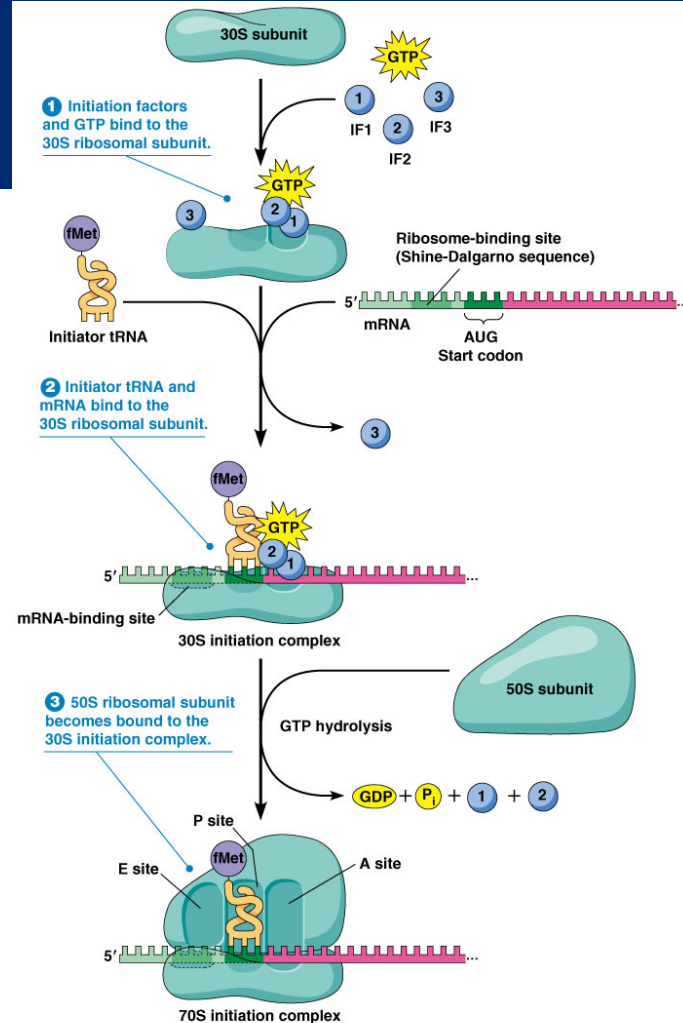
Figure 22-7



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Initiation of Translation in Prokaryotes

- Initiation of translation in bacteria can be divided into three steps:
- Three **initiation factors** (*IF1*, *IF2*, and *IF3*) bind to the small ribosomal subunit, with GTP bound to *IF2* (Step 1)
- mRNA and the tRNA carrying the first amino acid bind to the small subunit (Step 2)
- Once the *IF3* has been released, the 30S complex can bind a free 50S subunit, generating the 70S initiation complex (Step 3)



Eukaryotic Initiation

- The initiation factors bind the tRNA (these are called *eIFs*; there are about a dozen of these) and the tRNA then binds the small ribosomal subunit
 - The resulting complex then binds to the 5' end of the mRNA, recognizing the **5' cap**
- After binding the mRNA, the small ribosomal subunit (including the initiator tRNA) scans along the transcript and begins translation at the first AUG (**start codon**)
- After the initiator tRNA is base-paired with the start codon the large subunit joins the complex, facilitated by GTP hydrolysis

Chain Elongation Involves Sequential Cycles of Aminoacyl tRNA Binding, Peptide Bond Formation, and Translocation

- Once initiation has been completed a polypeptide chain is synthesized
- Amino acids are added in sequence to the growing chain (*elongation*)
- Elongation involves a repetitive cycle of three steps...

1. Binding of Aminoacyl tRNA

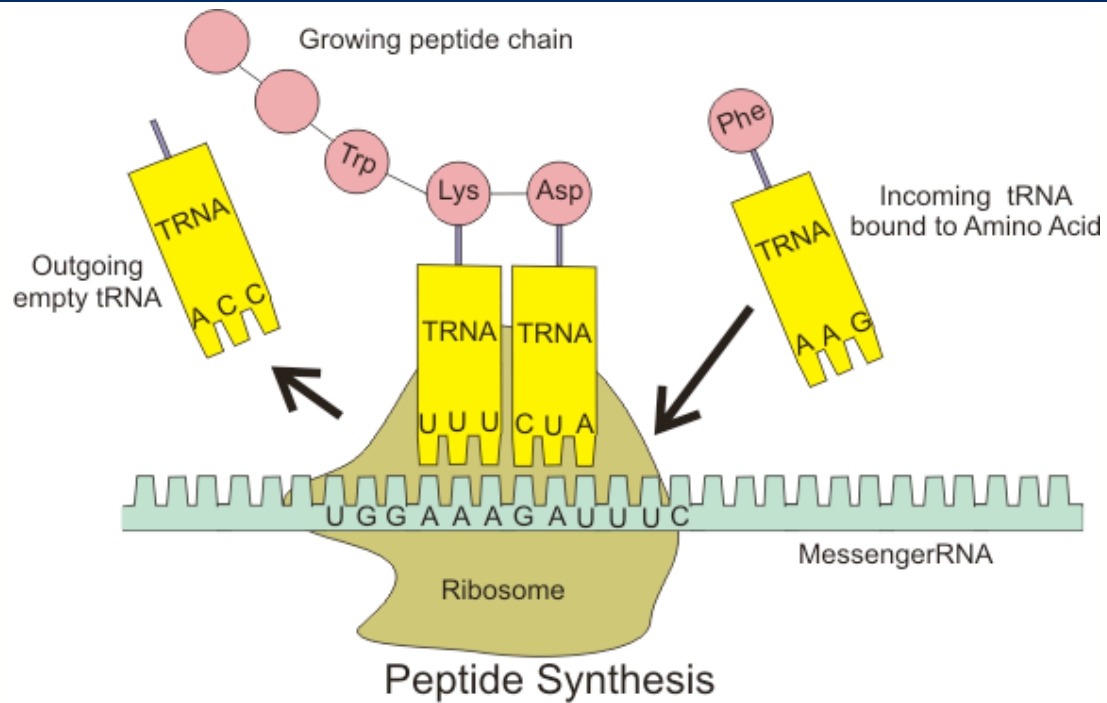
- Elongation begins as a tRNA with an anticodon complementary to the second codon binds the A site (1)
- Elongation factors don't recognize particular anticodons, so all types (except initiator tRNAs) are brought to the A site
- Only those with an anticodon complementary to the codon stay at the A site long enough for GTP hydrolysis to take place
- **Mechanisms for selecting against incorrect aminoacyl tRNA synthetases + proofreading result in a final error rate in translation of at most 1/10,000**

2. Peptide Bond Formation

- Once the aminoacyl tRNA is bound to the A site, a peptide bond forms between the amino group of the amino acid at the A site and the carboxyl group of the amino acid at the P site
- The growing peptide chain is transferred to the tRNA at the A site (2)
- No ATP or GTP hydrolysis is required for this step
- This step is catalyzed by rRNA

3. Translocation

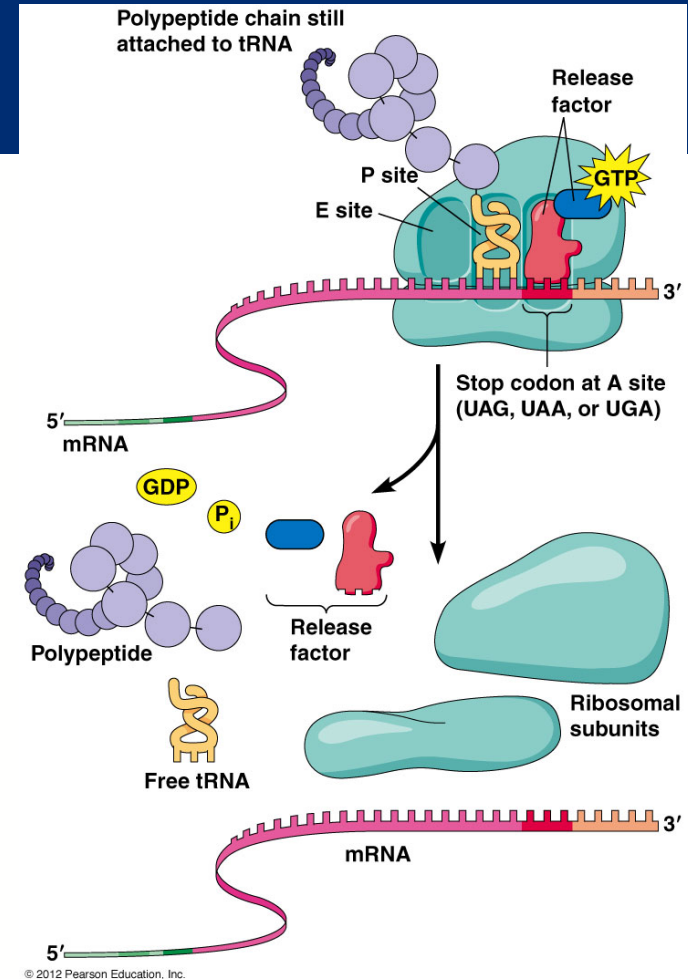
- After the peptide bond forms, the mRNA advances to bring the next codon into the proper position
- During this **translocation**, the peptidyl tRNA moves from the A to the P site, and the empty tRNA moves to the E site
- Once the next mRNA codon reaches the A site, the ribosome is now set to receive the next aminoacyl tRNA
- The elongation cycle repeats and the amino terminal of the growing polypeptide passes out of the ribosome through an *exit tunnel* in the 50S subunit
- Here molecular chaperones assist its folding



<https://courses.lumenlearning.com/boundless-biology/chapter/ribosomes-and-protein-synthesis/>

Termination of Polypeptide Synthesis Is Triggered by Release Factors That Recognize Stop Codons

- Codons are read on the mRNA one after the other, until a stop codon arrives at the A site
- Stop codons are recognized by protein **release factors**, rather than tRNAs
- Once release factors bind to the stop codons, translation is terminated through release of the completed polypeptide



Polypeptide Folding Is Facilitated by Molecular Chaperones

- Proteins must fold into their correct three-dimensional shapes before they can function
- Protein folding is usually facilitated by proteins called **molecular chaperones**; often several are required, acting in sequence
- Chaperones bind polypeptide chains during the early stages of folding

Molecular chaperones

- If folding goes awry, chaperones can sometimes rescue the proteins and fold them properly; Alternatively, improperly folded proteins may be destroyed
- Some kinds of incorrectly folded proteins bind to each other and form insoluble aggregates within and between cells (e.g. resulting in diseases like Alzheimer's disease; mad cow disease)
- Two of the most widely occurring chaperone families are *Hsp70* and *Hsp60*
- Members of each family function differently but both involve ATP-dependent cycles of binding and releasing their protein substrates
- Chaperones also perform other functions, such as assembling polypeptides into multisubunit proteins

A Summary of Translation

- Translation converts information in mRNAs into a chain of amino acids linked by peptide bonds
- Most messages are read by many ribosomes simultaneously
- RNA molecules play important roles in translation: mRNA, tRNA, rRNA
- <http://www.dnalc.org/resources/3d/16-translation-advanced.html>

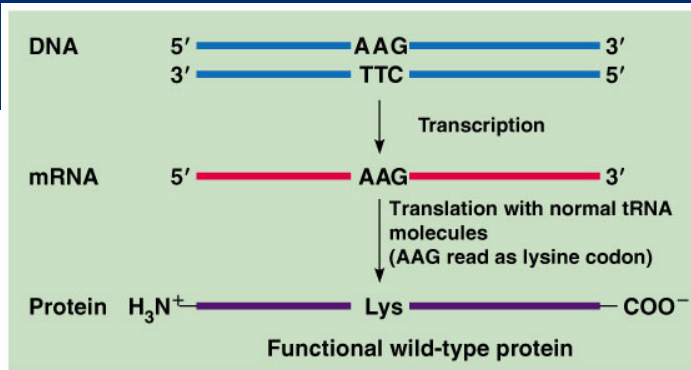
Mutations and Translation

- mRNAs may contain mutant codons that cause errors in the polypeptide chain synthesized
- Most codon mutations alter a single amino acid and some (in the third base of a codon) don't alter the amino acid at all
- Mutations that add or remove **stop codons** or **alter the reading frame** can severely disrupt translation

Suppressor tRNA Overcomes the Effects of Some Mutations

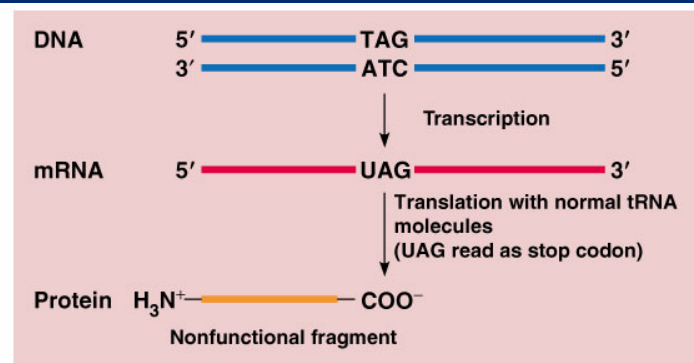
- Mutations that convert amino acid-coding codons into stop codons, called **nonsense mutations**, typically lead to incomplete, nonfunctional polypeptides
 - e.g. cystic fibrosis
- These mutations are often lethal, but can sometimes be overcome by an independent mutation affecting a tRNA gene
- A tRNA molecule that negates the effect of a mutation is called a **suppressor tRNA**
- Suppressor tRNAs recognize stop codons and insert amino acids, suppressing nonsense mutations

Figure 22-12A, B



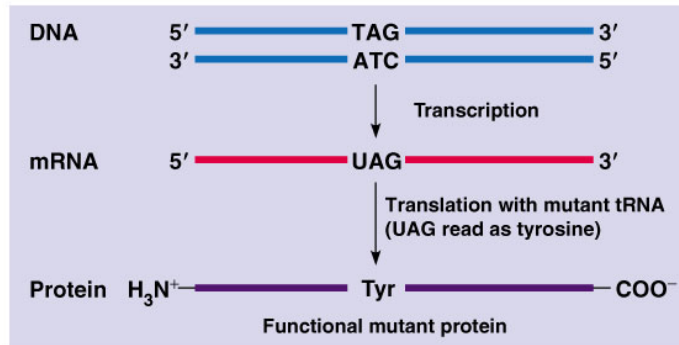
(a) Normal gene, normal tRNA molecules

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(b) Mutant gene, normal tRNA molecules

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(c) Mutant gene, mutant (suppressor) tRNA molecule

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A suppressor
tRNA protects
against
mutation

Summary

- Central Dogma
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 - Prokaryotic and eukaryotic
- RNA polymerase
- Translation
 - Ribosomes and peptide synthesis



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