Principles of

MOLECULAR BIOLOGY

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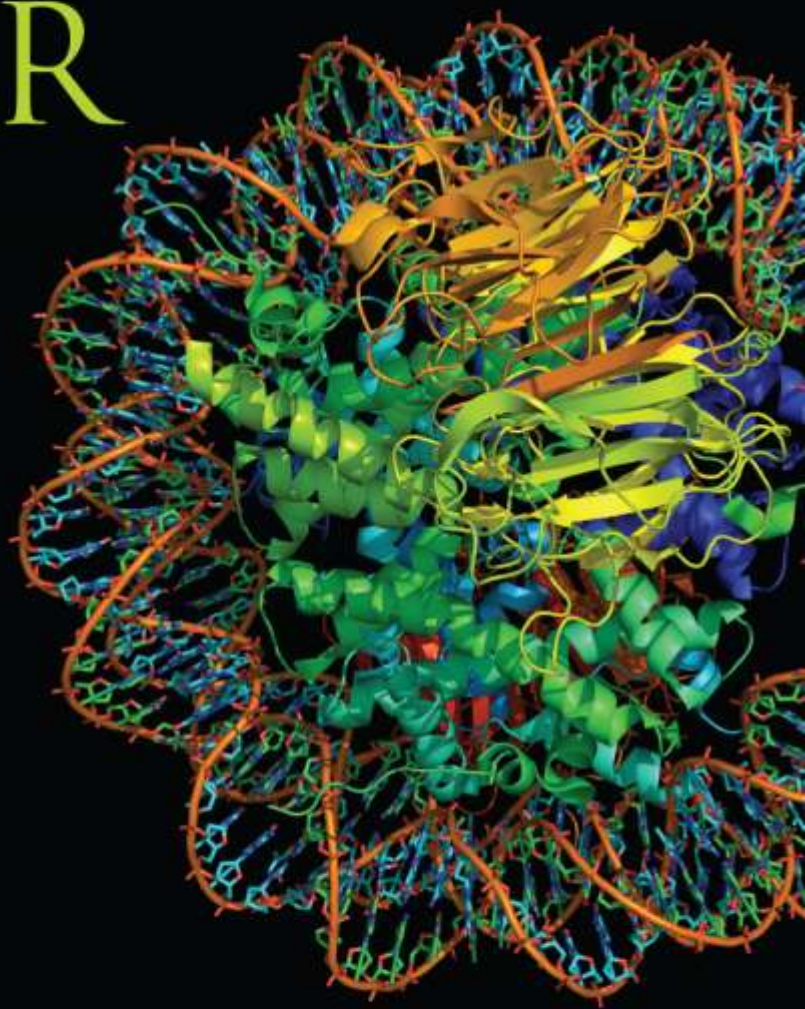


Chapter 16
Protein Synthesis

mRNA and the Genetic Code

- Three adjacent bases in mRNA that specify an amino acid are called a **codon**
- A mutation that adds or deletes a base pair shifts the reading frame – a **frameshift mutation**

mRNA and the Genetic Code

- mRNA is read in a 5’ to 3’ direction
- Because both transcription and translation are located in the cytosol bacteria can begin to translate an mRNA before its transcription is complete
  - Eukaryotes cannot begin translation before transcription is complete because transcription and translation occur in two separate biological compartments (the nucleus and the cytoplasm)

mRNA and the Genetic Code

- Three codons, UAA, UAG and UGA are polypeptide chain termination signals (stop codons)
- Mutations that change a sense codon into a stop codon are called **nonsense mutations**
  - Three different types of nonsense mutations were shown to exist and given the names:
    - Amber (UAG)
    - Ochre (UAA)
    - Opal (UGA)

mRNA and the Genetic Code

Five general statements can be made about the genetic code

1. The genetic code is nonoverlapping: each base is part of one codon
2. The genetic code is commaless: there are no intervening bases between adjacent codons
3. The genetic code is almost universal: mRNA from one species can be correctly translated by another
4. The genetic code is highly degenerate: most amino acids are specified by two or more codons
5. The genetic code is unambiguous: each codon specifies only one amino acid

mRNA and the Genetic Code

Some aminoacyl-tRNA molecules bind to more than one codon because there is some play or wobble in the third base of a codon – the **wobble hypothesis**

1. The codon and anticodon form antiparallel base pairs
2. The first two bases in the codon form standard base pairs with the last two base pairs in the anticodon
3. There is a certain amount of play or wobble in base pairing between the first base of the anticodon and the third base of the codon that permits non standard base pairing

Wobble base pairing

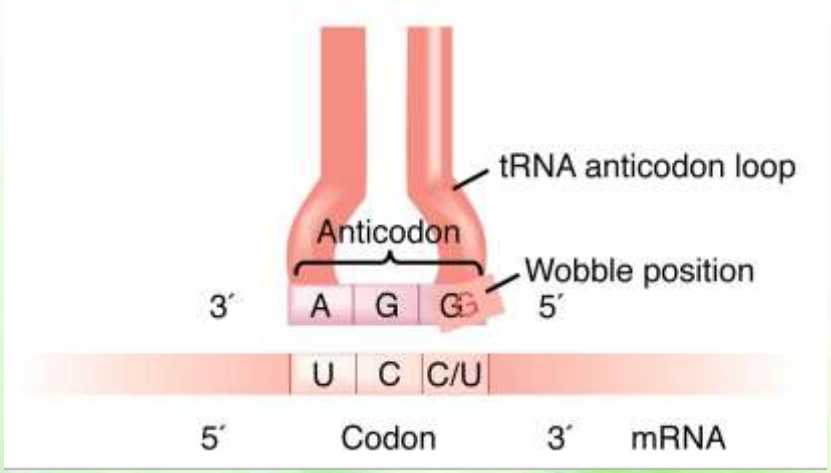
There is flexible pairing at the third base of a codon to the anticodon allowing some tRNAs to bind to more than one codon.

It is called **wobble** base pairing.

The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base,

The degeneracy of the genetic codons

It acts as a buffer against deleterious mutations.

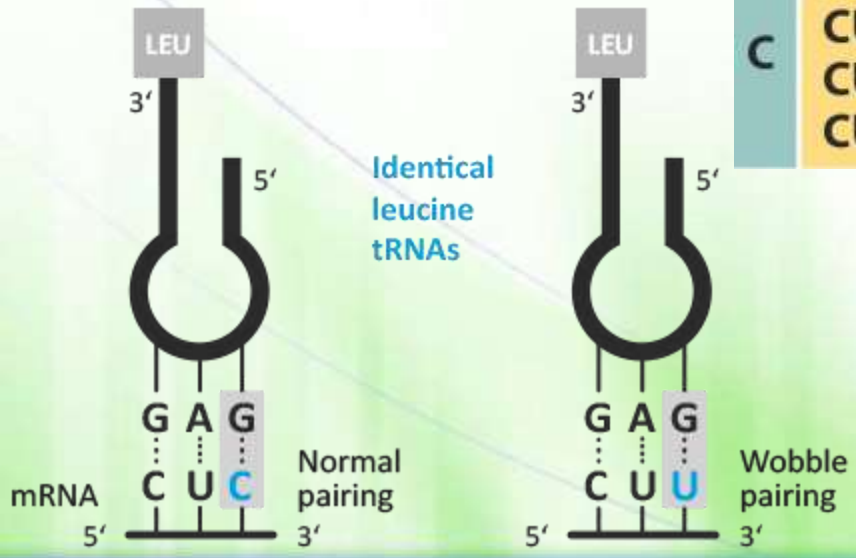


Examples of wobble base pairing

- Relaxed base pairing results from the formation of G-U base pairs.

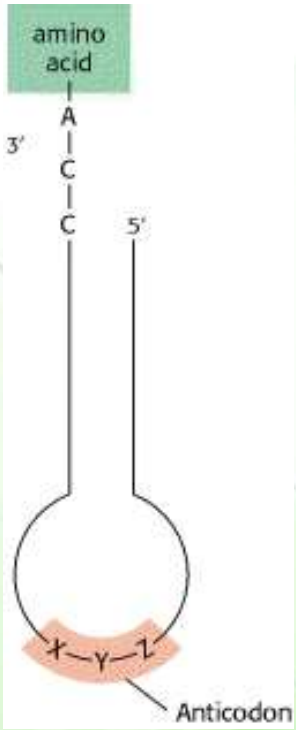
Both are hydrophobic amino acids

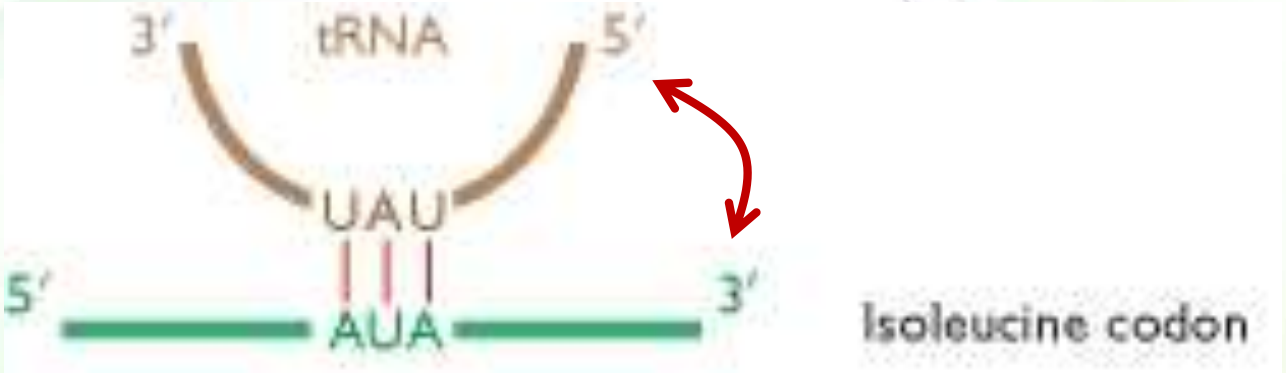
	U	C	A	G	
U	UUU, UUC } Phe	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr	UGU, UGC } Cys	U
	UUA, UUG } Leu		UAA Stop, UAG Stop	UGA Stop, UGG Trp	C, A, G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His; CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U, C, A, G



Codon vs. anticodon

- tRNAs contain a three-nucleotide sequence known as “anticodon” that pairs with the “codon’ or “triplet” mRNA molecules (note the anti-parallel alignment of mRNA-tRNA complex)





Ribosome Structure

Bacterial 30S (small) subunits and 50S (large subunits each have unique structures and functions

- The ribosome is a complicated, dynamic machine with many moving parts
- Translates 10 to 20 codons per second with an error rate usually less than 0.03%
  - The small subunit performs the ribosome decoding function (discriminating between proper and improper codon/anticodon base pairing)
  - The large subunit contains the **peptidyl transferase** activity

Ribosome Structure

- Crystal structure analysis of 70S ribosome confirms the existence of three sites
  - **A-site** (aminoacyl-tRNA binding site)
  - **P-site** (peptidyl-tRNA binding site)
  - **E-site** (exit site)

Four Stages of Protein Synthesis

Protein synthesis can be divided into four stages

1. The initiation stage: The reading frame is set by binding initiator tRNAs to ribosomes at start codons with the assistance of **translation initiation factors (IFs)**
2. The elongation stage: Amino acids are added to the growing polypeptide chain as codons on the mRNA are matched with anticodons on the tRNA with the assistance of **translation elongation factors (EFs)**

Four Stages of Protein Synthesis

3. The chain termination stage: Termination codons are recognized by **release factors** and completed polypeptide chains are released from the ribosome
4. The recycling stage: Ribosomal subunits dissociate from each other under the influence of a **ribosomal recycling factor**

Initiation Stage

Each Bacterial mRNA open reading frame has its own start codon

- Translation in bacteria can begin shortly after the 5’-end of the mRNA emerges from the RNA polymerase
- Polycistronic mRNAs have an initiation codon at the start of each open reading frame
- The translation machinery must be able to initiate polypeptide chain synthesis at multiple sites along the mRNA

Initiation Stage

Bacteria have an initiator methionine tRNA and an elongator methionine tRNA

- Bacteria use a methionine derivative: **N-formylmethionine (fMet)** to begin polypeptide synthesis
  - The formyl group prevents fMet from being incorporated into a polypeptide chain at any position other than the amino terminus
  - Has its own tRNA ($tRNA^{fMet}$) for initiation
  - $tRNA^{Met}$ is used for elongation
  - **Peptide deformylase** removes the formyl group the N-terminus

Initiation Stage

The 30S subunit is an obligatory intermediate in polypeptide chain initiation

- fMet-tRNA^{fMet} and mRNA bind to the 30S ribosomal subunit to form the **30S initiation complex**
- The 50S subunit then combines to form the **70S initiation complex**

Initiation Stage

Initiation factors participate in the formation of 30S and 70S initiation complexes

- **IF3** binds to the 30S subunit after 70S ribosome dissociation and shifts the equilibrium to favor the dissociated subunits
- **IF1** binds at the A-site of the 30S subunit and promotes binding of IF2 and IF3
- **IF2** binds and hydrolyzes GTP at its middle domain and a C-terminal domain interacts with fMet-tRNA^{fMet}

- The 50S subunit joins the complex, GTP is hydrolyzed and the IF factors dissociate

Initiation Stage

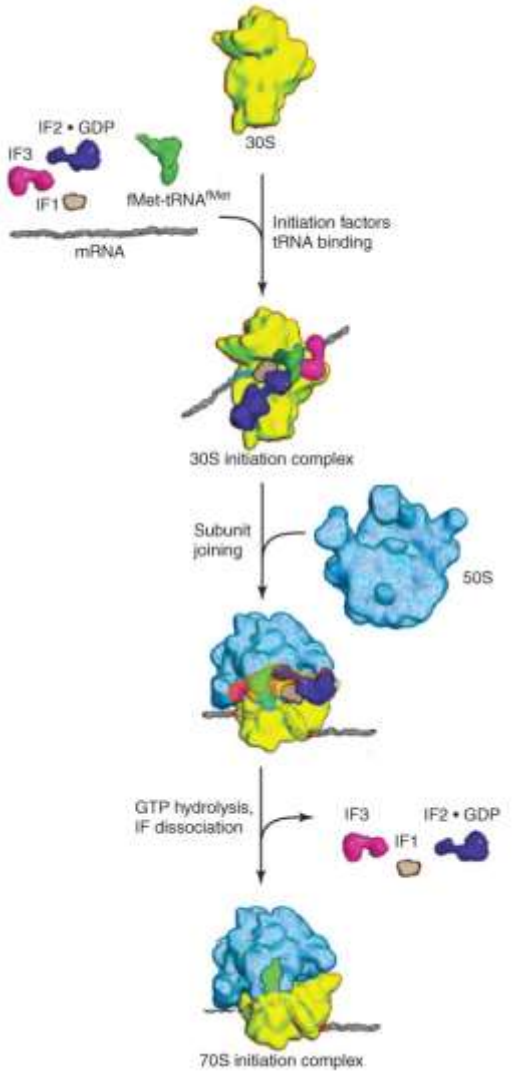


Figure 16.40: Translation initiation pathway in bacteria. Components are positioned on the ribosome according to currently available experimental information.

(Adapted from Schmeing, T. M., and Ramakrishnan, V. 2009. Nature 461:1234–1242.)

Initiation Stage

The Shine-Dalgarno sequence in mRNA interacts with the anti-Shine-Dalgarno sequence in the 16S rRNA

- The bacterial ribosome must be able to distinguish the initiator AUG from other AUGs that code for internal methionines
- The **Shine-Dalgarno** sequence is a purine-rich consensus sequence located 5-7 nucleotides upstream from the AUG
- The 16S rRNA has a complementary sequence near its 3’-end (the **anti-Shine-Dalgarno** sequence)

Initiation Stage

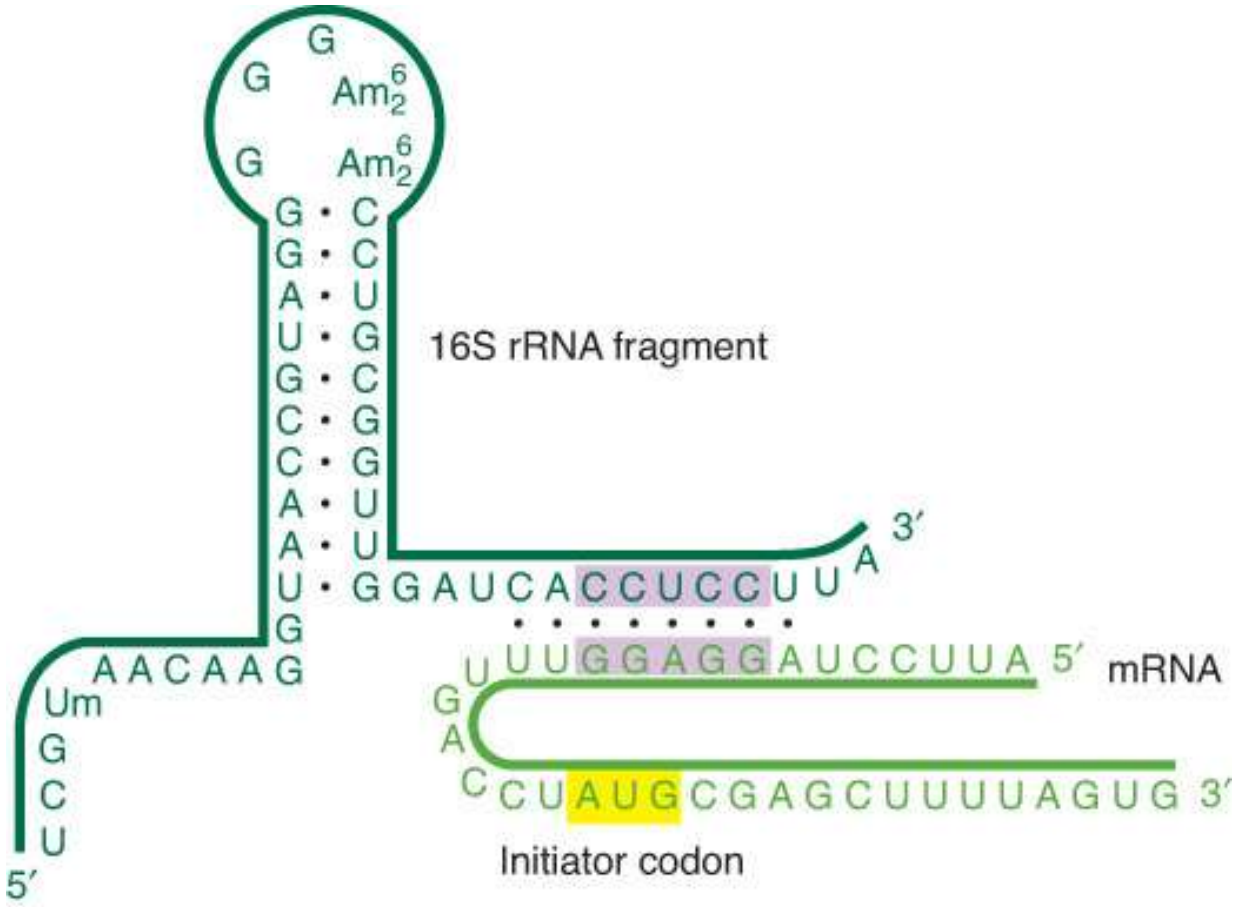


Figure 16.42: Binding of mRNA to a complementary sequence in 16S rRNA.

Initiation Stage

- Eukaryotic initiator tRNA is charged with a methionine that is *not* formylated
- However, like bacteria, eukaryotes have an initiator Met-$tRNA_i$ and an elongator tRNA ($tRNA_m^{Met}$)
- There are a variety of unique sequence features present in initiator tRNA but not in elongator tRNA

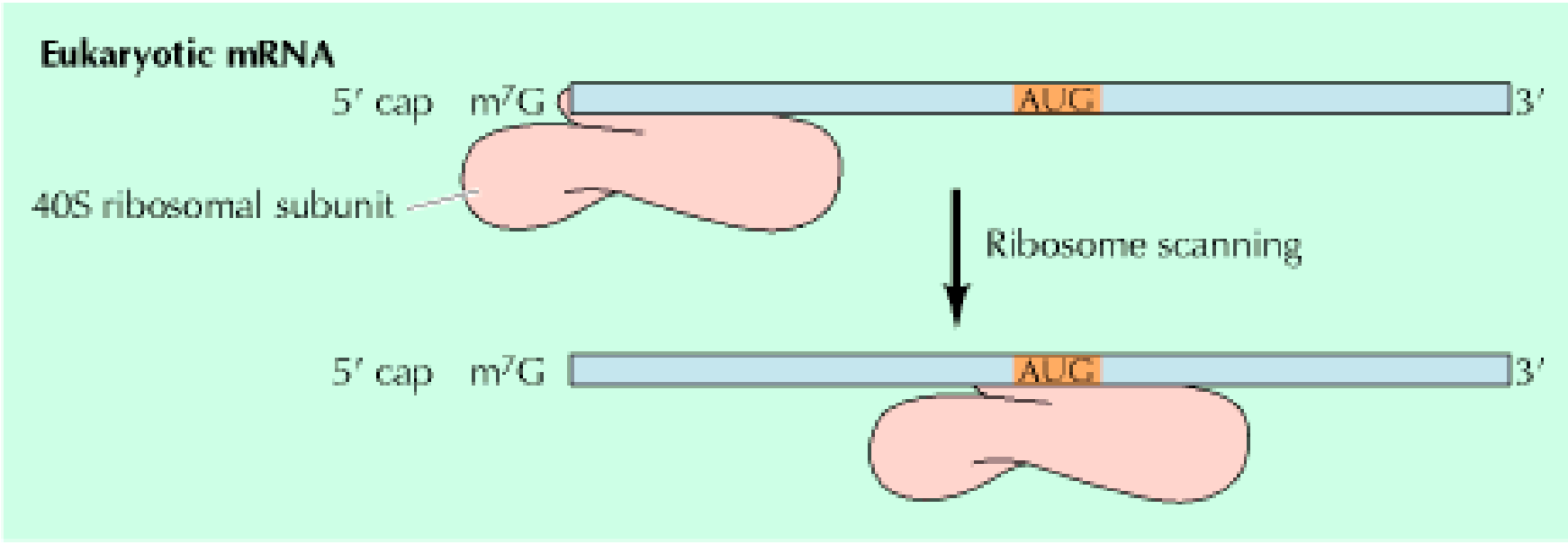
Initiation Stage

Eukaryotic translation initiation proceeds through a scanning mechanism

- Eukaryotic mRNA does not have a Shine-Dalgarno sequence
- Instead, the AUG is embedded within a sequence
    - ACC**AUG**G
- Marilyn Kozak in 1978 proposed the **scanning model**
  - The pre-initiation complex binds to the mRNA 5’-cap and scans 5’→3’ until it detects the initiation codon

But in eukaryotes…

- Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5´ terminus.



Initiation Stage

Key stages

1. Ternary complex formation: eIF2 binds GTP to form the eIF2•GTP•Met-tRNA$_i$ ternary complex
2. Ternary complex binding to 40S (small) subunit: Assisted by initiation factors
3. mRNA activation: Secondary structures and proteins are removed
4. mRNA entry into the complex formed in stage 2
5. 5’→3’ scanning to detect the initiation codon: complex from stage 2 moves along the mRNA until the AUG codon is aligned with the anticodon on the initiator tRNA
6. 80S initiation complex formation

Initiation Stage

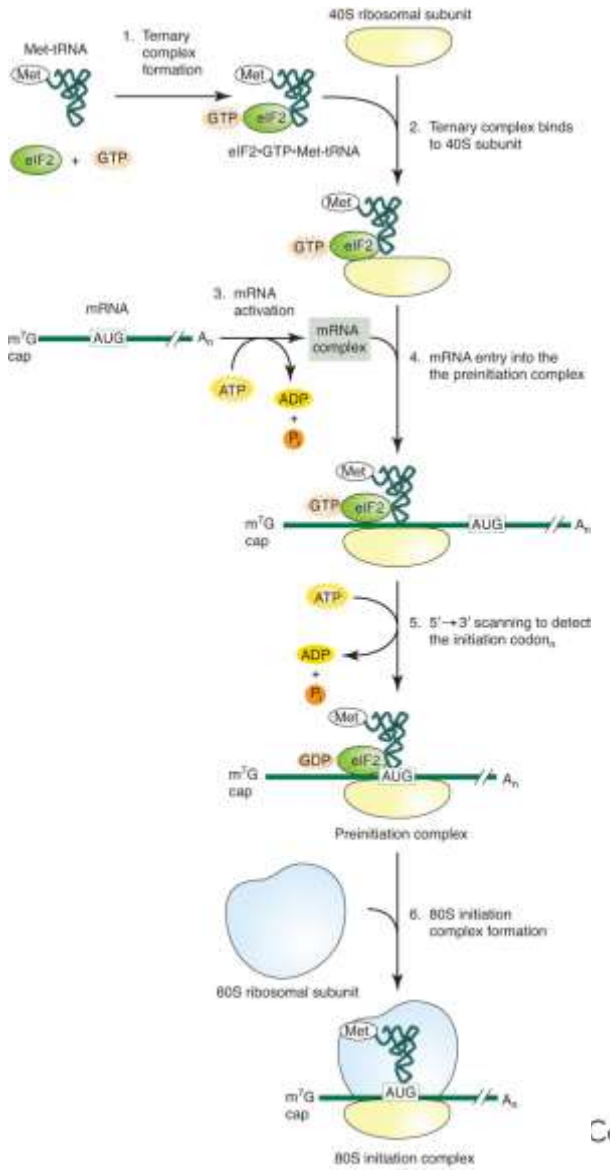


Figure 16.45: Eukaryotic translation initiation pathway.

Initiation Stage

Translation initiation factor phosphorylation regulates protein synthesis in eukaryotes

- eIF2·GDP, which is released during 80S complex formation, must be converted back to eIF2·GTP
- Phosphorylation of eIF2α (a subunit of eIF2) can block further translation by binding eIF2B (eIF2 GTP exchange factor)
- Four different protein kinases, each regulated by a different signal, can phosphorylate eIF2α

Elongation Stage

- Polypeptide chain elongation requires elongation factors
  - In bacteria
    - EF-Tu, EF-Ts and EF-G
  - In eukaryotes
    - eEF1A, eEF1B and eEF2
- The elongation factors act through a repeating cycle

Elongation Stage

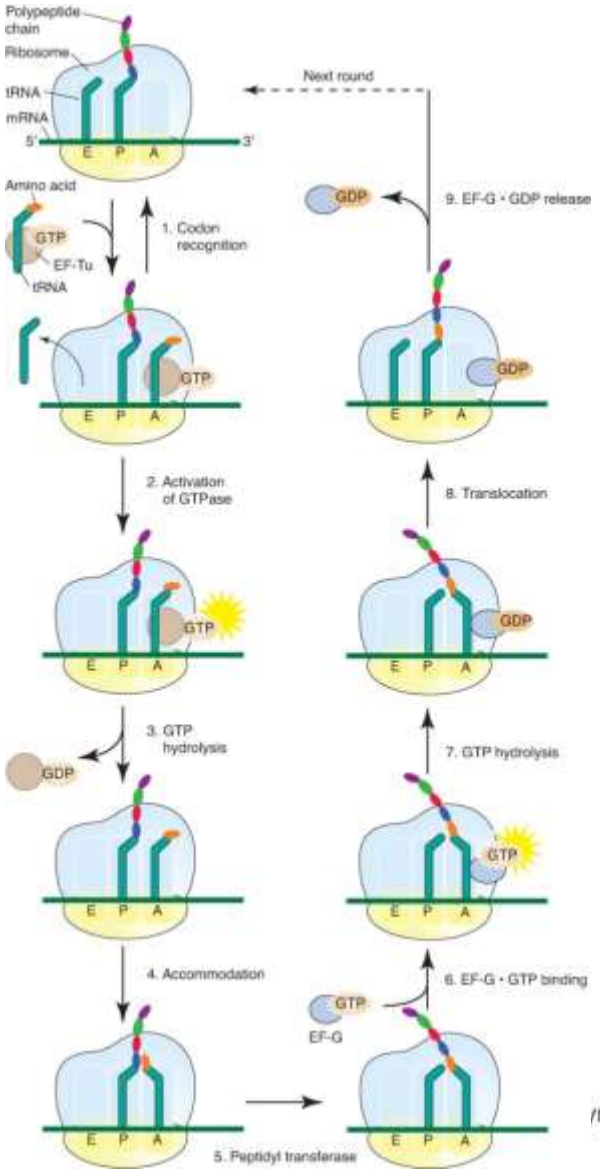
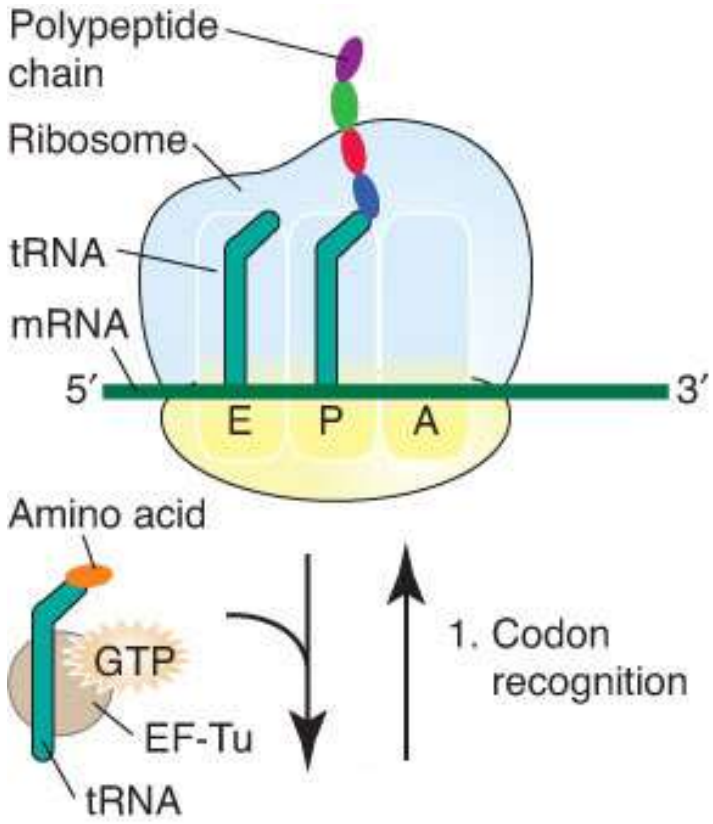


Figure 16.46: Polypeptide chain elongation pathway in bacteria.

(Adapted from Ramakrishnan, V. 2002. Cell 108:557–572.)

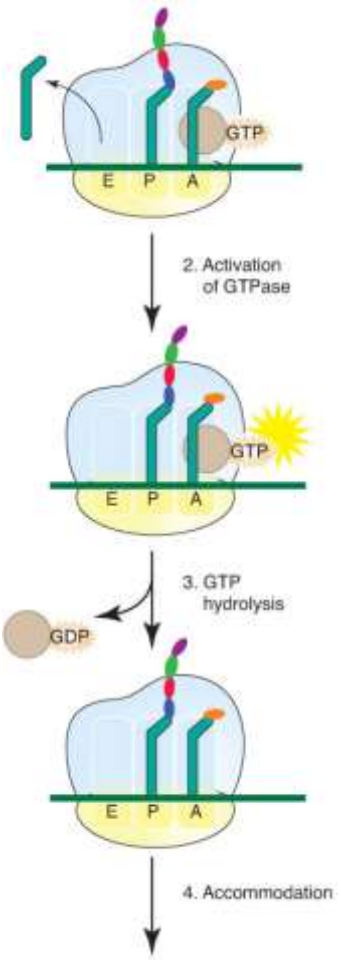
An EF-Tu•GTP•aminoacyl-tRNA ternary complex carries the amino-acyl tRNA to the ribosome

- The tRNA is in an A/T state
  - Anticodon interacting with mRNA in the A site
  - Acceptor end remains bound to the elongation factor



An EF-Tu•GTP•aminoacyl-tRNA ternary complex carries the amino-acyl tRNA to the ribosome

- If the codon/anticodon match is correct EF-Tu's GTPase activity is activated
- Free aminoacyl end of the A-site tRNA moves into the peptidyl transferase center in a process called **accommodation**



Elongation Stage

EF-Ts is a GDP-GTP exchange protein

- The EF-Tu•GDP released during the elongation cycle must exchange its GDP for GTP
  - Requires EF-Ts to carry out the exchange
  - Eukaryotic eEF1B catalyzes the same reaction

Termination Stage

Polypeptide chain termination requires release factors

- Bacteria have three protein release factors (RFs)
  - RF1 and RF2 interact with termination codons
  - RF3 stimulates the rate of peptide release
- Eukaryotes have two release factors
  - eRF1 recognizes all three termination codons
  - eRF3 acts with eRF1 to guarantee efficient termination codon recognition
- A mutant tRNA (with an anticodon that recognizes a nonsense codon) can suppress mutations that create termination codons

Recycling Stage

- At the end of the termination stage, the ribosome complex is still associated with mRNA
- A new translation factor known as the **ribosome release factor** is required for the bacterial ribosomal complex to disassemble
- EF-G•GTP and IF3 assist
  - Nothing is known about the recycling stage in eukaryotes
- After ribosome disassembly the small subunit is free to interact with IFs to start a new round of translation