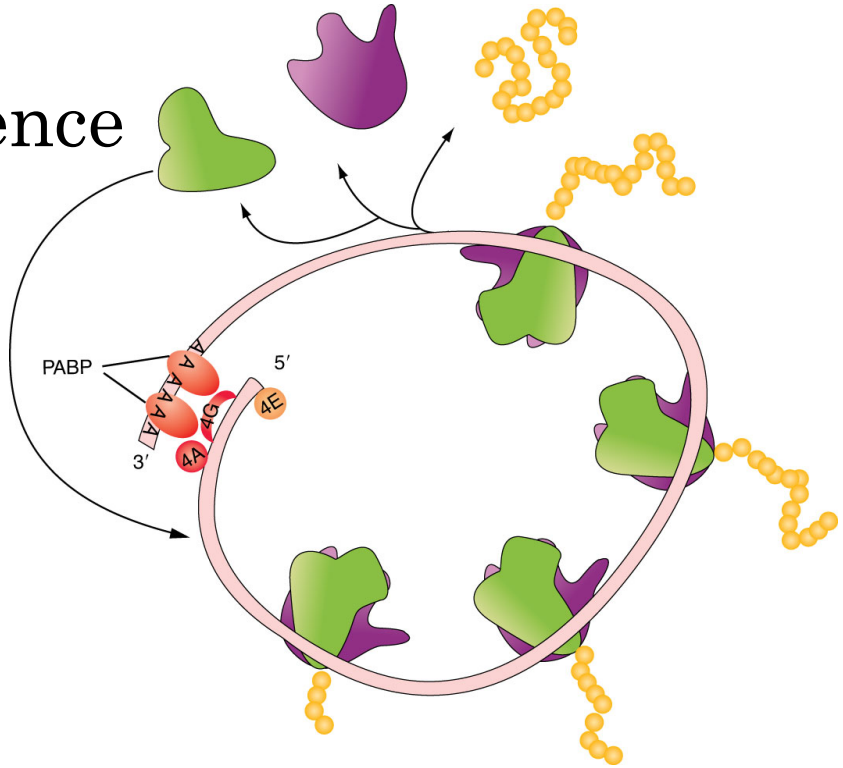
# Chapter 19

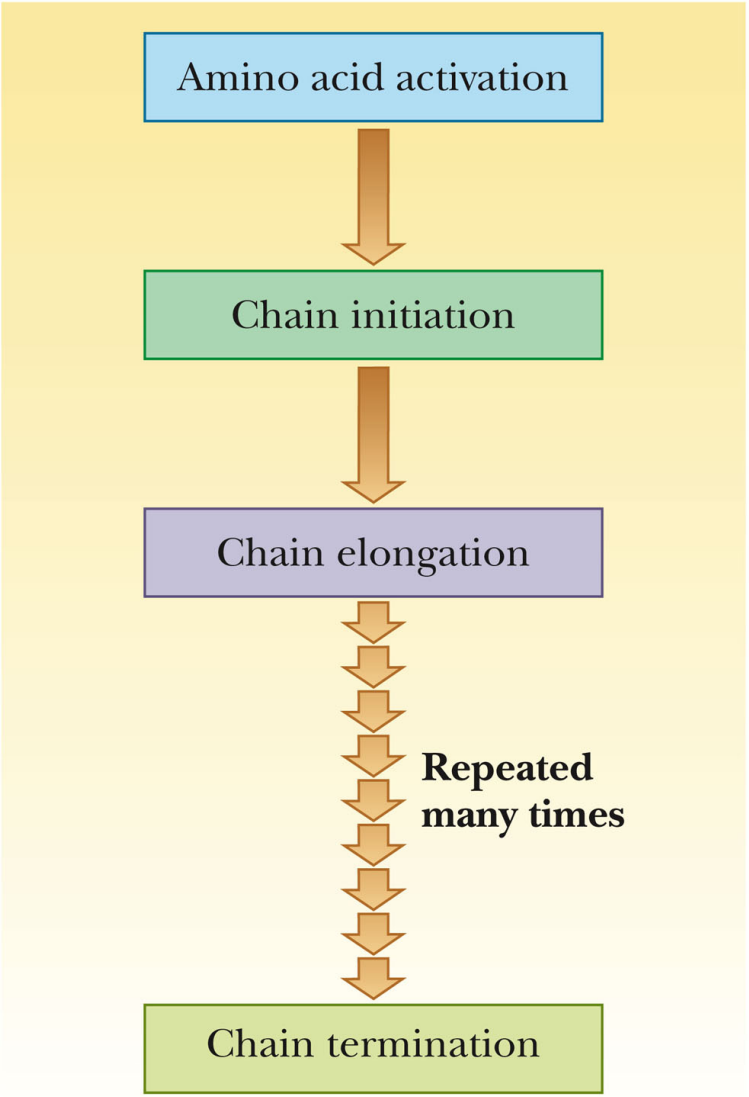
## *Overview*

- **Protein Synthesis** – genetic info encoded in nucleic acids translated into standard amino acids
- **Genetic code** – dictionary defining meaning for base sequence
- **Codon** – tri-nucleotide sequence for amino acid



# *Protein Synthesis*

- **Requires** ribosomes, mRNA, tRNA, and protein factors
  - **Formation of aminoacyl-tRNA**
    - **Aminoacyl-tRNA synthetases** – amino acid activation
  - **Formation of polypeptide chain**
    - **Chain initation** – binding of 1st aminoacyl-tRNA at start site
    - **Chain elongation** – formation of peptide bond
    - **Chain termination** – release of protein



# Section 19.1: The Genetic Code

- **Translation** – conversion of nucleic acid sequence to a amino acid sequence
  - **Genetic code** is the dictionary that specifies a meaning for each base sequence (codon)
  - **tRNA** - adaptor molecules must mediate translation process
    - Three base sequence with four different bases ($4^3 = 64$) can code for 20 amino acids
  - **Codons** - Nirenberg, Matthaei, and Khorana show that it was a triplet code
    - 64 possible trinucleotide sequences, 61 code for amino acids; four codons serve as punctuation
      - UAA, UGA, and UAG - stop codons; AUG codes for methionine - start codon

## Properties of Genetic Code:

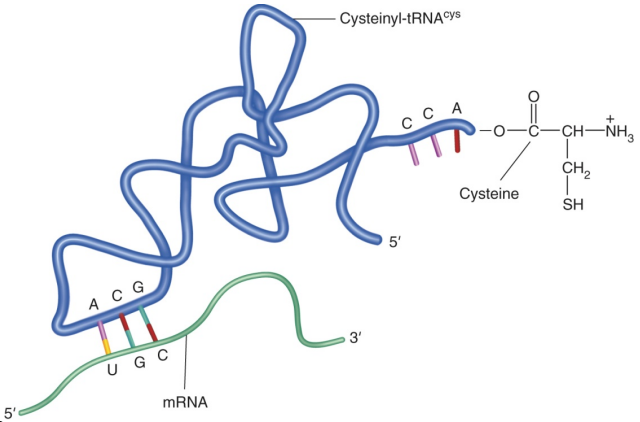
- **Triplet:** continuous sequence of three bases (a codon) specifies one amino acid
- **Non-overlapping:** bases are NOT shared between consecutive codons
- **Commaless:** no intervening bases between codons
- **Degenerate:** more than one triplet can code for the same amino acid
  - Leu, Ser, and Arg - each coded for by six triplets
- **Universal:** same in viruses, prokaryotes, and eukaryotes

**TABLE 19.1** The Genetic Code

| First position (5′ end) | Second Position: U | | | Second Position: C | | | Second Position: A | | | Second Position: G | | | Third position (3′ end) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| U | UUU | Phe | | UCU | Ser | | UAU | Tyr | | UGU | Cys | | U |
| | UUC | | | UCC | | | UAC | | | UGC | | | C |
| | UUA | Leu | | UCA | | | UAA | STOP | | UGA* | STOP | | A |
| | UUG | | | UCG | | | UAG* | | | UGG | Trp | | G |
| C | CUU | Leu | | CCU | Pro | | CAU | His | | CGU | Arg | | U |
| | CUC | | | CCC | | | CAC | | | CGC | | | C |
| | CUA | | | CCA | | | CAA | Gln | | CGA | | | A |
| | CUG | | | CCG | | | CAG | | | CGG | | | G |
| A | AUU | Ile | | ACU | Thr | | AAU | Asn | | AGU | Ser | | U |
| | AUC | | | ACC | | | AAC | | | AGC | | | C |
| | AUA | | | ACA | | | AAA | Lys | | AGA | Arg | | A |
| | AUG | Met | | ACG | | | AAG | | | AGG | | | G |
| G | GUU | Val | | GCU | Ala | | GAU | Asp | | GGU | Gly | | U |
| | GUC | | | GCC | | | GAC | | | GGC | | | C |
| | GUA | | | GCA | | | GAA | Glu | | GGA | | | A |
| | GUG | | | GCG | | | GAG | | | GGG | | | G |

*The stop codons UGA and UAG are also used by some organisms and under specific conditions (described later in the Biochemistry in Perspective box entitled Context Dependent Coding Reassignment) to insert selenocysteine or pyrrolysine, respectively into a polypeptide sequence.

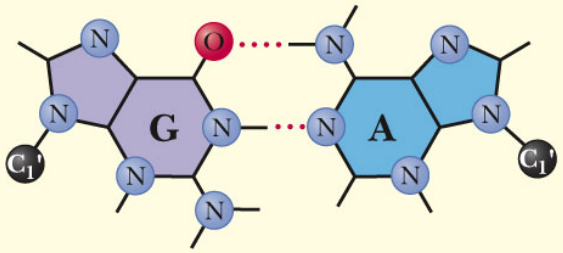
**Figure 19.1 Codon-Anticodon Base Pairing of Cysteinyl-tRNA$^{cys}$**



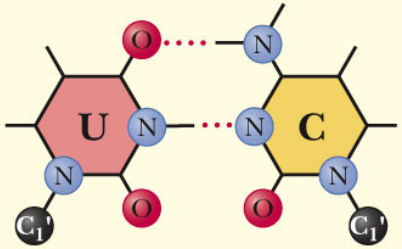

- **Codon-Anticodon Interactions**
  - tRNA carries specific amino acid and possesses an anticodon
    - Codon-anticodon pairing is antiparallel
    - 1st base anticodon (5' end) pairs with 3rd base mRNA codon (3'end)
  - **Wobble hypothesis:**
    1. First two base pairings confer most of the specificity
    2. Interaction of the third codon position and anticodon nucleotide is less stringent
       - G in the wobble position can interact with C or U
       - U in the wobble position can interact with A or G
       - Nontraditional bases can be found (e.g., inosinate)
         - I can interact with U, A, or C
    3. Minimum 32 tRNAs translate all 61 codons.

# Wobble Base Pairing Alternatives

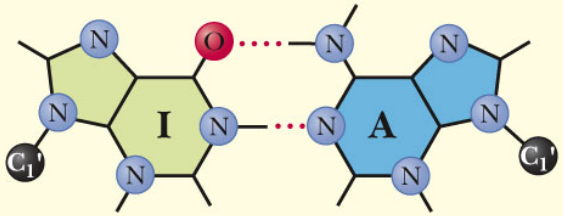
The guanine–adenine base pair



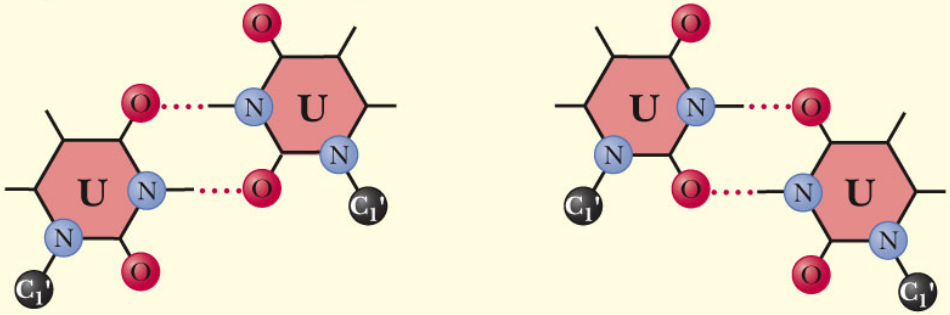

The uracil–cytosine base pair



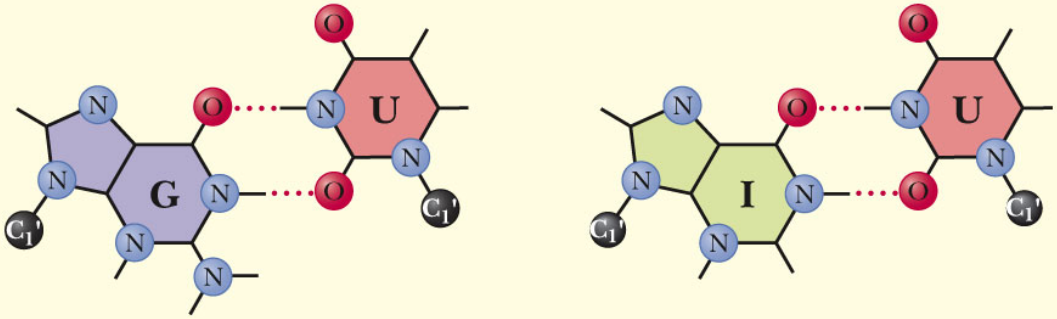

The inosine–adenine base pair




The two possible uracil–uracil base pairs




The guanine–uracil and inosine–uracil base pairs are similar



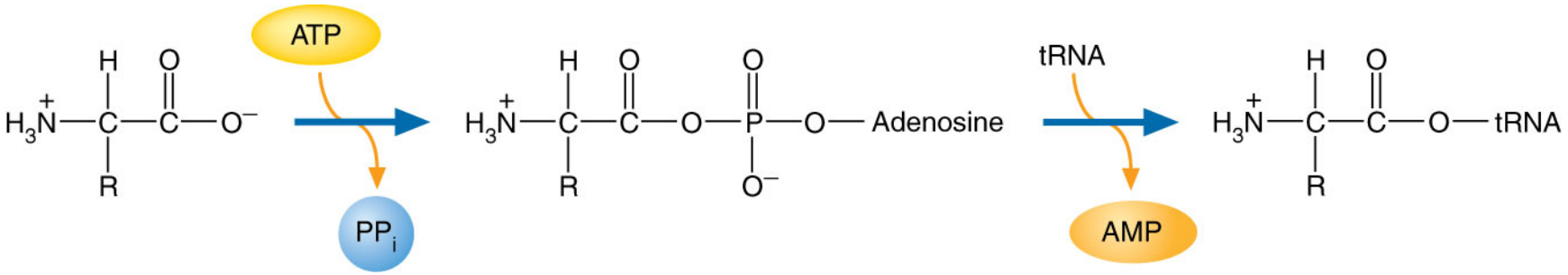



**Figure 19.3 Formation of Aminoacyl-tRNA**

## Aminoacyl-tRNA Synthetase

- **Increases accuracy** of translation (1 error/$10^4$ aa)
  - 1 aminoacyl-tRNA for each amino acid
  - Specific for both amino acid & tRNA, requires $Mg^{2+}$

## Reaction:

1. **Activation** - synthetase catalyzes formation of aminoacyl-AMP (activates amino acid)
   - ATP provides energy for bond formation
2. **tRNA linkage** - specific tRNA bound in the active site becomes covalently bound to the aminoacyl site
   - Class I – ester linkage between amino acid & 2'-OH on ribose
   - ClassII – ester linkage between amino caid &3'-OH on ribose

# Section 19.2: Protein Synthesis

1. **Initiation —** N-terminal (5') mRNA → C-terminal (3')
   - Requires $RNA^{met}$ – N-terminal AA of all proteins
     - Initiation tRNA: Eukaryotes – $tRNA^{met}$; Prokaryotes – $tRNA^{fmet}$
   - Formation of **initiation complex** - binding of small ribosomal subunit to mRNA, subsequent binding of initiator tRNA
     - Initiator tRNA binds to AUG codon on the mRNA
     - Ends with binding of large ribosomal subunit
     - P (peptidyl) site occupied by initiator tRNA
     - A (aminoacyl) site binds 2nd aminoacyl-tRNA
     - **Polysome** - multiple ribosomes can read an mRNA simultaneously

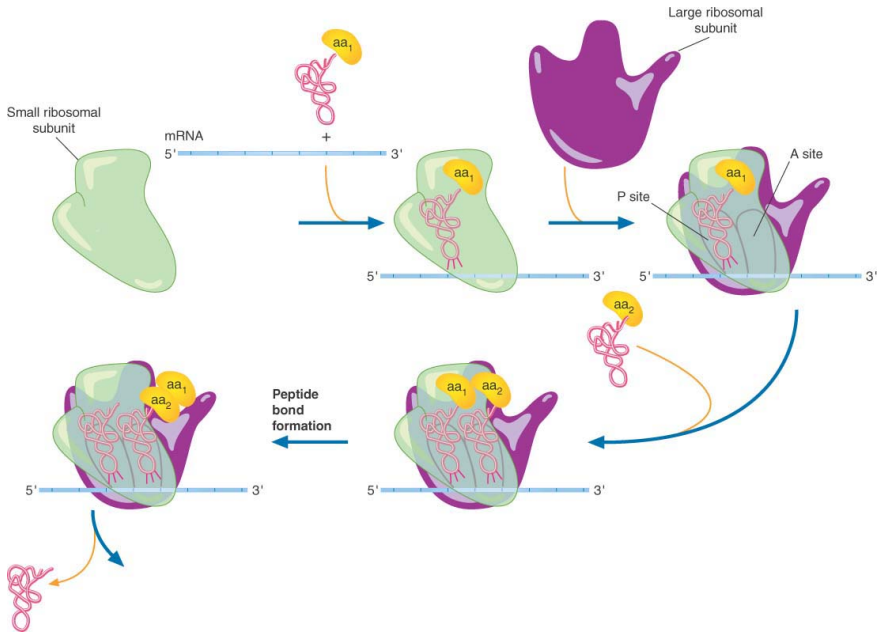



**Figure 19.4a Protein Synthesis**

# Section 19.2: Protein Synthesis

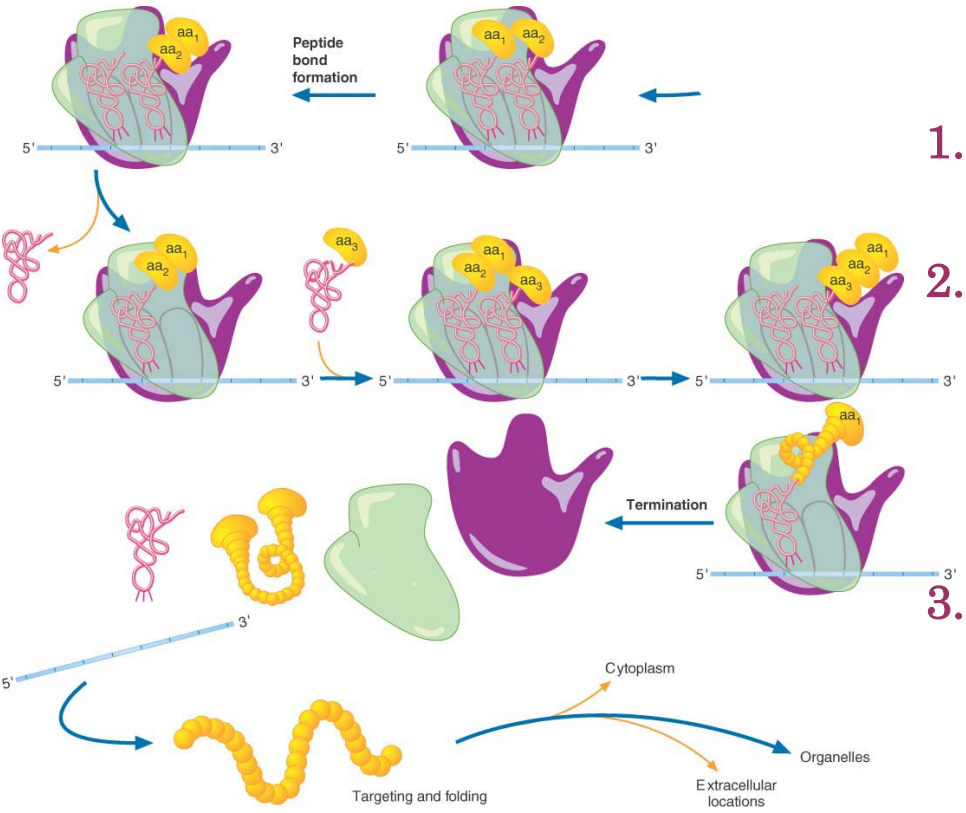



**Figure 19.4b Protein Synthesis**

**2. Elongation** - mRNA is read in 5′→3′ direction protein is assembled from N-terminus to the C-terminus

1. **Addition** of 2nd amino acid in A site
   - ✓ Specified by mRNA in A-site
2. **Peptidyl transferase** catalyzes peptide bond formation between aas in P-site & A-site
   - ✓ Dipeptidyl-tRNA in A-site
   - ✓ Uncharged tRNA at P-site
3. **Translocation** - transfer of peptidyl-tRNA to the P site
   - ✓ GTP provides energy
   - ✓ A-site peptide chain shifted to P-site
   - ✓ Uncharged tRNA in P-site released

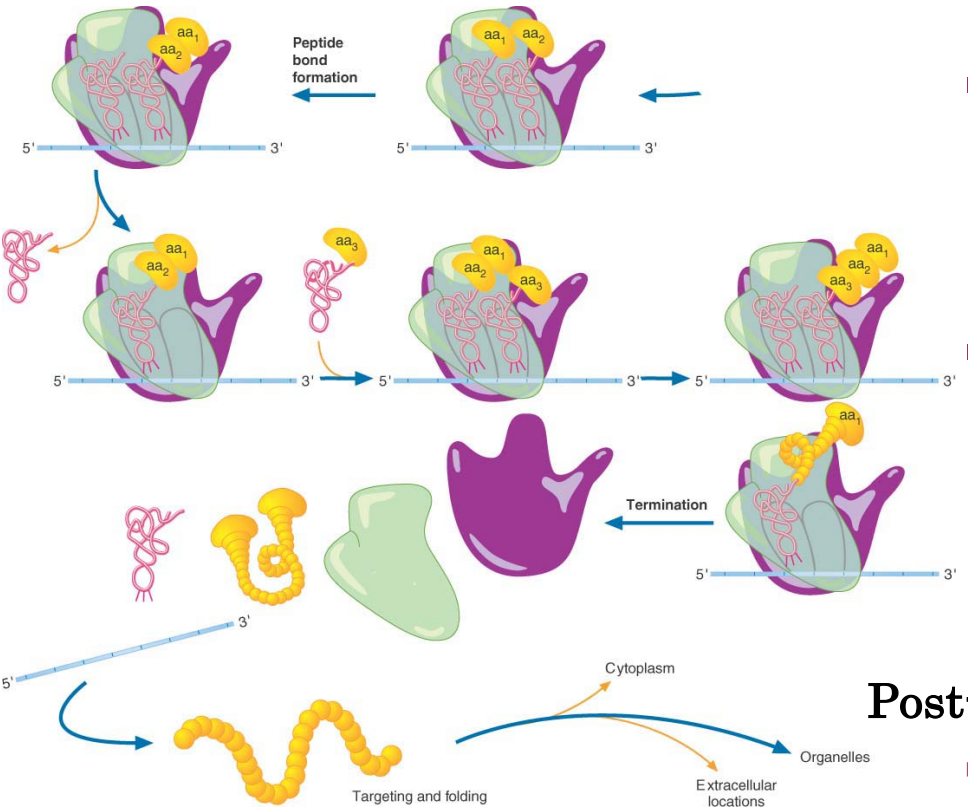



Figure 19.4b Protein Synthesis

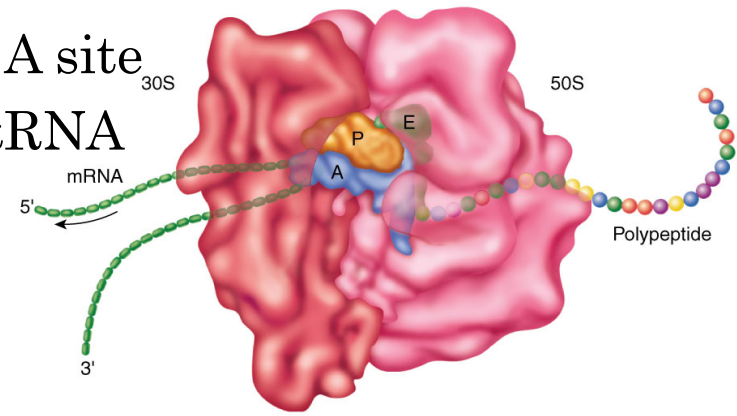
3. **Termination** – no aminoacyl-tRNA to bind with stop codon
   - **Protein-releasing factor** binds to A site
     - ✓ Cleaves bond between protein and last tRNA
   - Ribosome releases mRNA
     - ✓ Dissociates large & small subunits
     - ✓ GTP required
     - ✓ Wide variety of protein factors

**Post-translation modifications**

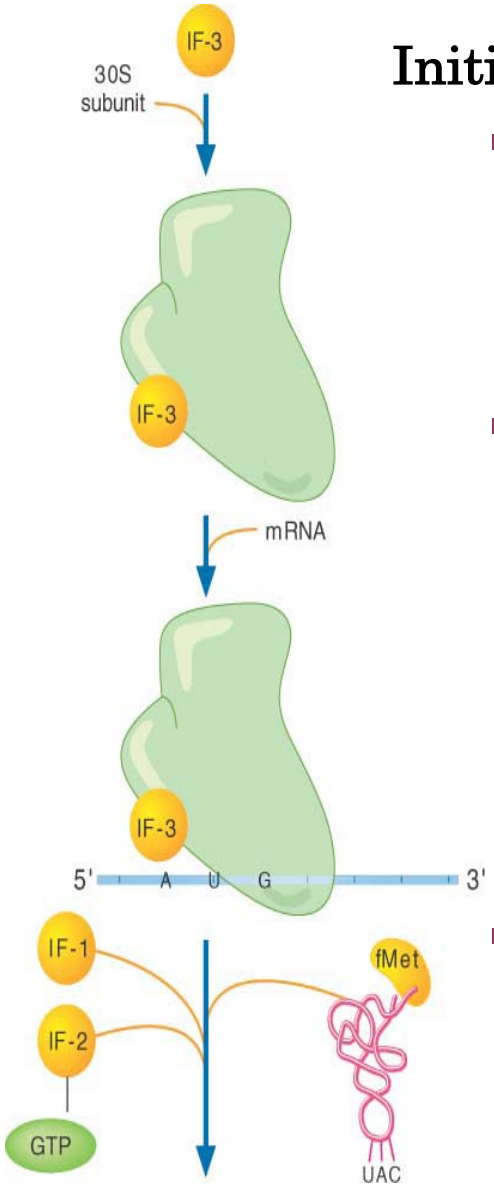
- Amino acid removal
- Side chain modifications
- Combining with other polypeptides

## **Prokaryotic Protein Synthesis** – rate of 20 aa/sec

- 70S Ribosome composed of a 50S/30S subunit
  - **peptidyl transferase center** (PTC) binds 3′ ends of aminoacyl- and peptidyl-tRNAs for peptide bond formation
    - ✓ Located on 50S in 23S rRNA subunit
  - **GTPase associated region** (GAR) is a set of overlapping binding sites of 23S structural elements
    - ✓ Drives GTP hydrolysis (acts as GAP) causing conformational change
  - **decoding center** (DC) in 30S located in A site
    - ✓ mRNA codon is matched with a tRNA anticodon

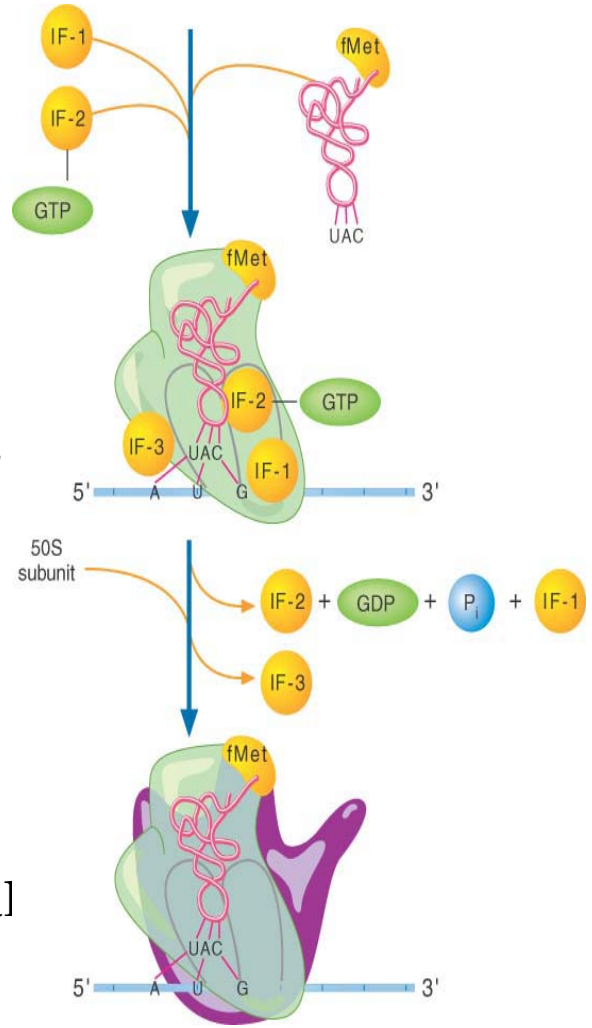


# Section 19.2: Protein Synthesis




**Initiation complex** formation

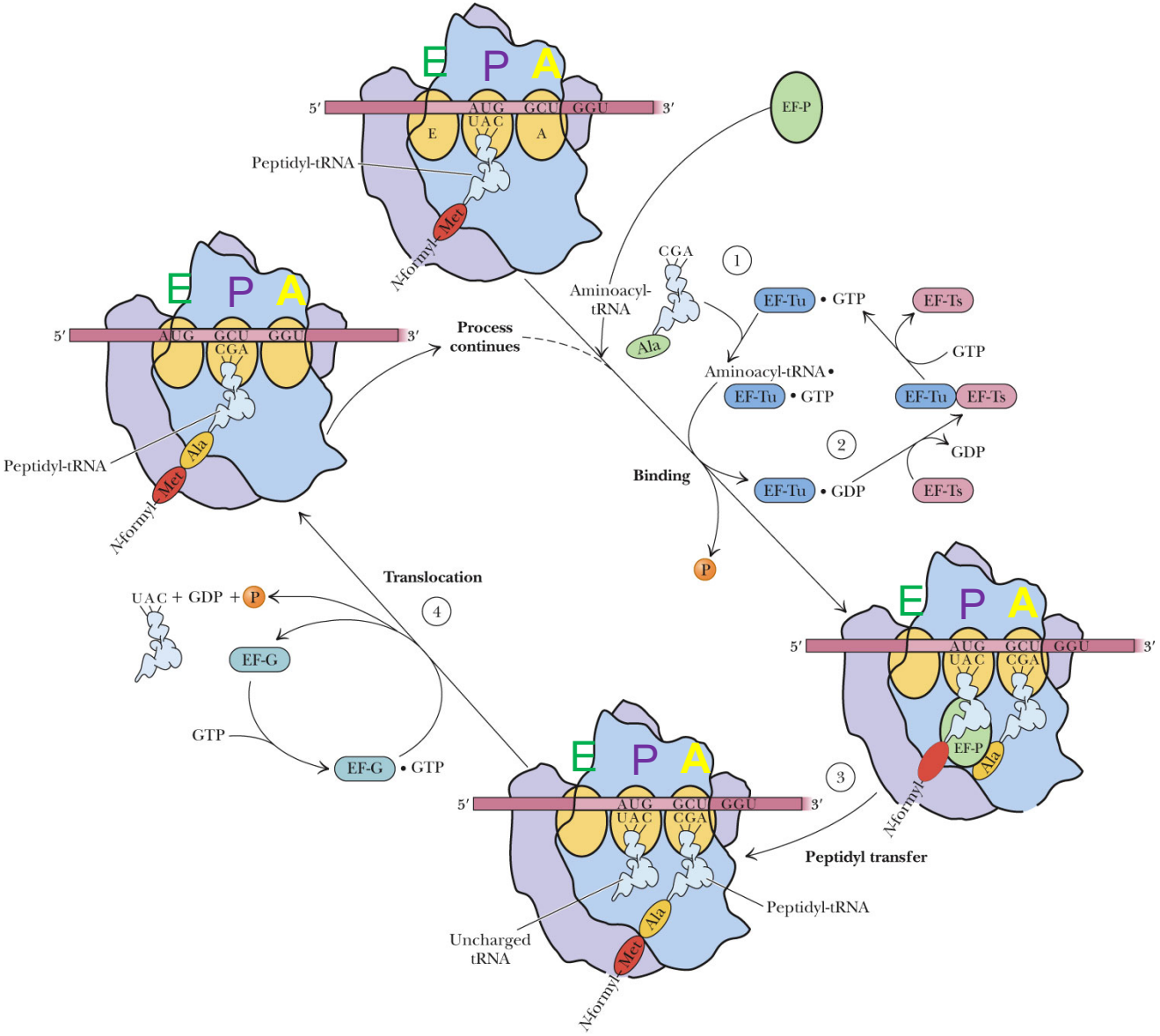
- **IF-3** binds to 30S subunit
  - ✓ Prevents premature binding to 50S subunit
  - ✓ Promotes mRNA binding
- **IF-1** binds to A site blocking tRNA binding
  - ✓ **Shine-Dalgarno sequence** guides binding of 30S subunit to AUG
    - ✓ Located on 16S region
    - ✓ Upstream from AUG
    - ✓ Unique to each mRNA
- **IF-2** GTPasebinds to initiator fmet-tRNA$^{fmet}$, P-site entry
  - ✓ **GTP hydrolysis** conformational change
  - ✓ 50S subunit binds



**Elongation** – addition of amino acids to growing protein

1. **Positioning an aminoacyl-tRNA** in the A site
   - EF-Tu-GTP binds to aminoacyl-tRNA then guides to A site
   - Positions the aminoacyl-tRNA in the A site
     - ✓ Protects aminoacyl linkage from hydrolysis
   - After binding, GTP hydrolysis occurs; EF-Tu is released
2. **Peptide bond formation** - catalyzed by **peptidyl transferase**
   - A Site – dipeptidyl-tRNA; P Site – uncharged tRNA
     - ✓ Catalyzes nucleophilic attack of A-site a-amino group on carbonyl carbon of p-site amino acid
     - ✓ Release of polypeptides from ribosome
3. **Translocation** - movement of mRNA by ribosome
   - Uncharge tRNA moves from P site to E site; released
   - Peptidyl-tRNA translocates from A site to P site
     - ✓ EF-G required - another GTP-binding protein
   - **A site** occupied by next aminoacyl-tRNA

# Section 19.2: Protein Synthesis

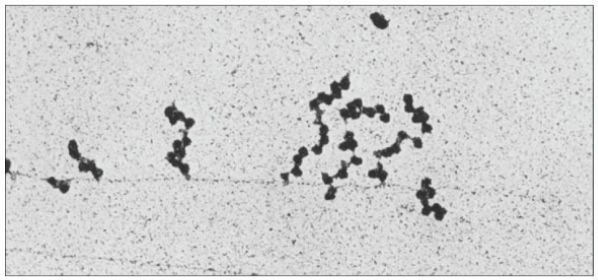


- **Termination** - termination codon enters A site
  - ✓ UAA, UAG, UGA
  - Three release factors (RF1, RF2, and RF3) are involved
    - Both RF1 and RF2 resemble tRNAs in shape and size
    - RF1 recognizes UAA and UAG
    - RF2 recognizes UAA and UGA
    - RF3 - GTPase necessary for RF1 and RF-2 binding to ribosome
  - **Complex dissociates** – freeing release factors, tRNA, mRNA, 30S/50S subunits

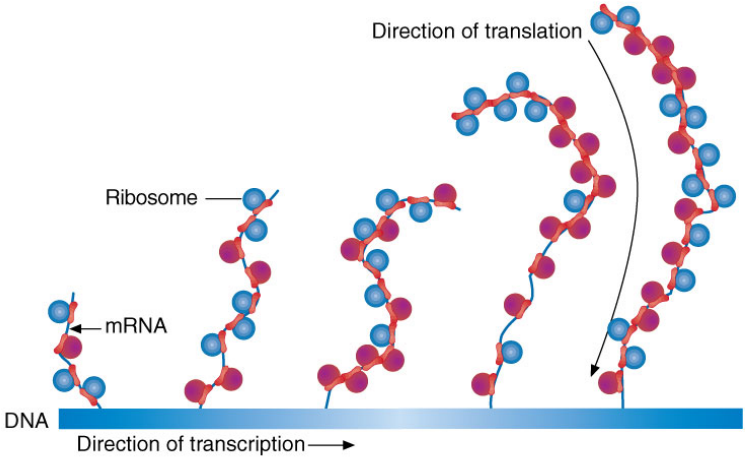
## Posttranslational Modifications

- ***Trigger factor*** **(TF)** - molecular chaperone helps begin folding process as each nascent polypeptide emerges from exit tunnel
  - Covalent alteration –removal of signal peptides; formylmethionine residue
  - Chemical modifications - methylation, phosphorylation, carboxylation, & covalent linkage to lipid molecules

**Figure 19.10 Transcription and Translation in *E. coli***



(a)




(b)

## Translational Control

- Prokaryotes occurs at transcription initiation
  - Transcription and translation are coupled
  - Prokaryotic mRNA has a short half-life (1-3 minutes)
  - Rates of translation also vary
    - Differences in Shine-Dalgarno sequences

- Functional and structural differences between prokaryotic and eukaryotic protein synthesis are the basis of the therapeutic and research uses of antibiotics

**TABLE 19.2** Selected Antibiotic Inhibitors of Protein Synthesis

| Antibiotic | Action |
|---|---|
| Chloramphenicol | Blocks prokaryotic A site |
| Cycloheximide | Inhibits eukaryotic peptidyl transferase |
| Erythromycin | Blocks prokaryotic exit site |
| Lincosamide | Binding to 23S rRNA of the 50S subunit |
| Streptomycin | Blocks binding of fmet-tRNA$_i$ to 30S P site |
| Streptogramins | Premature release of polypeptide chain |
| Tigecycline | Binding to 16S rRNA prevents entry of aa-tRNA to A site |

# Eukaryotic Protein Synthesis

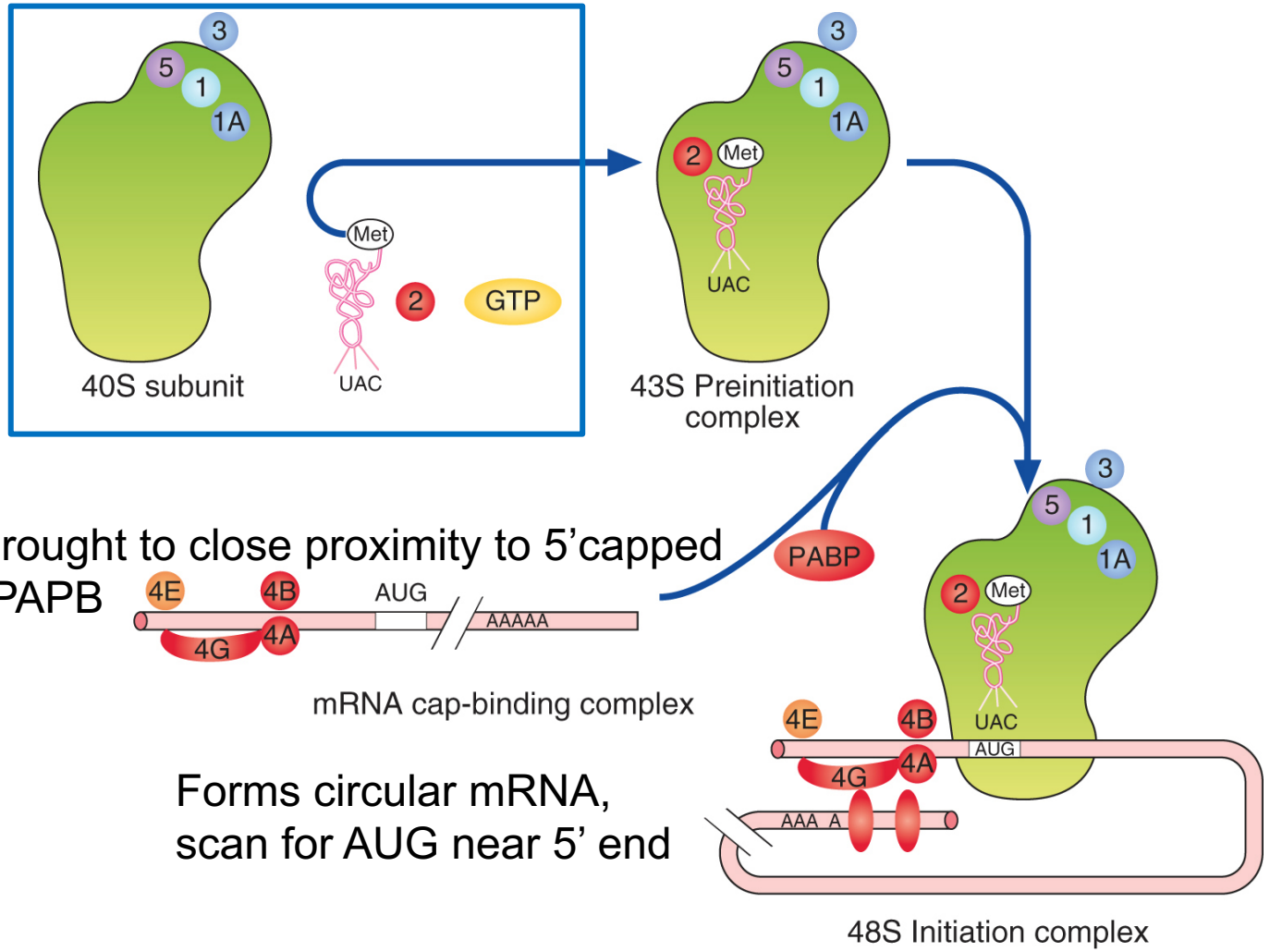
## Chain Initiation

- **Extra processing mRNA secondary structure –** methylguanosine cap, poly(A) tail, removal of introns
  - ✓ Associates with ribosome after leaving nucleus; complexed with several proteins
- **Scan for TSS –** no Shine-Dalgarno sequence, ribosome migrates in 5' -> 3' direction

- **Initiation** – begins with assembly of pre-initiation complex (PIC)
  - **Pre-initiation complex (**PIC) - binding of 40S subunit to eIF1 A, eIF2 (GTP-binding protein), GTP, and methionyl-$tRNA^{met}$
  - **eIF2-GTP** mediates the binding of the initiator tRNA to the 40S subunit
  - **eIF3** (bound to the 40S subunit) prevents association with large subunit (60S)
  - **43S preinitiation complex** - binds to 5'-cap, has **cap-binding complex** (CBC or eIF4F) associated

# Section 19.2: Protein Synthesis

Binding 40S to elf1 A, eIF2, GTP, $tRNA^{met}$ to AUG



- **80S complex** - initiation complex binds the 60S subunit
  - Hydrolysis of GTP bound to eIF2
  - eIF5-acts as a guanine nucleotide activating protein
  - Initiation factors are released from the ribosome

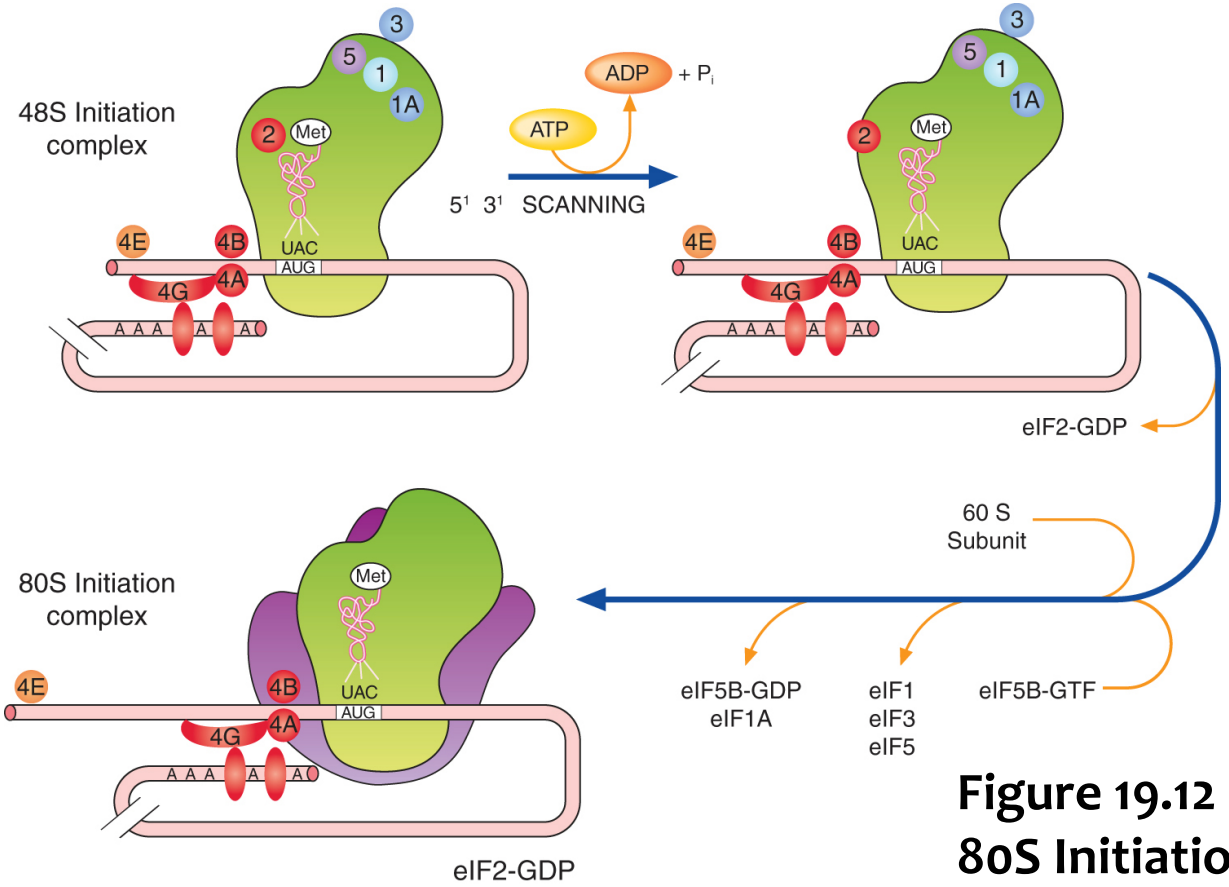
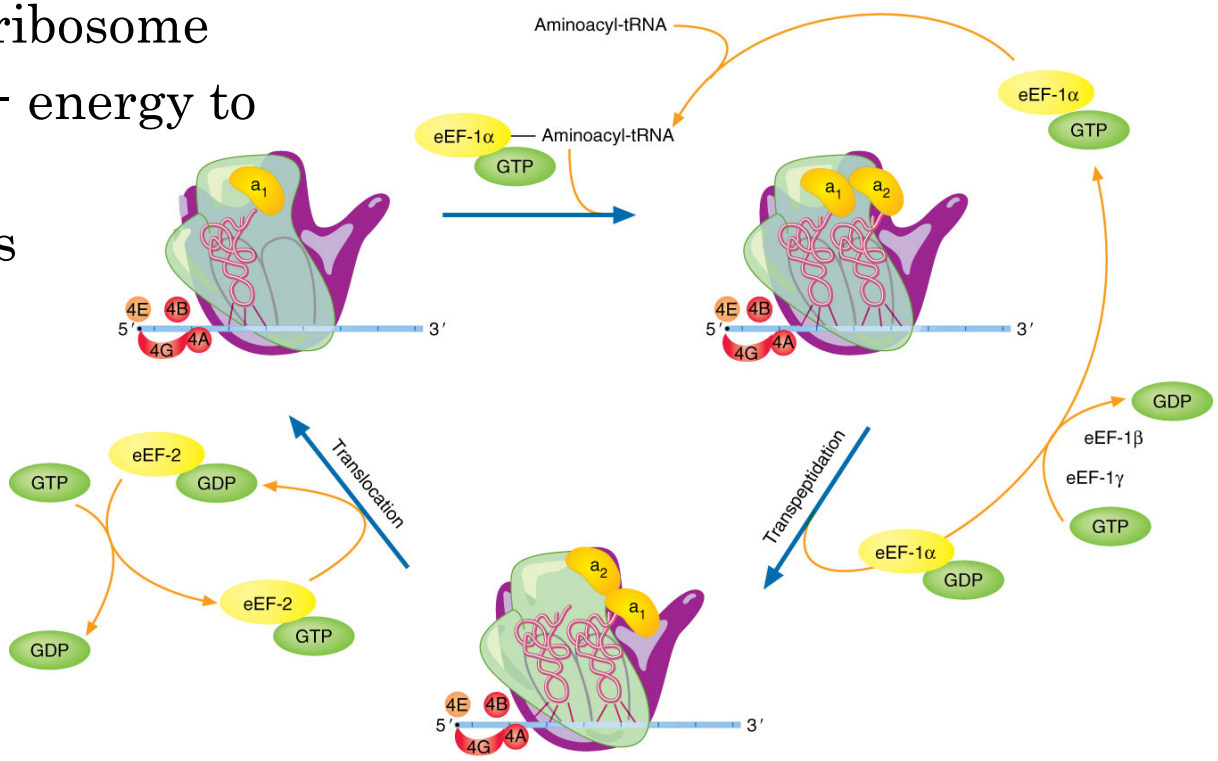
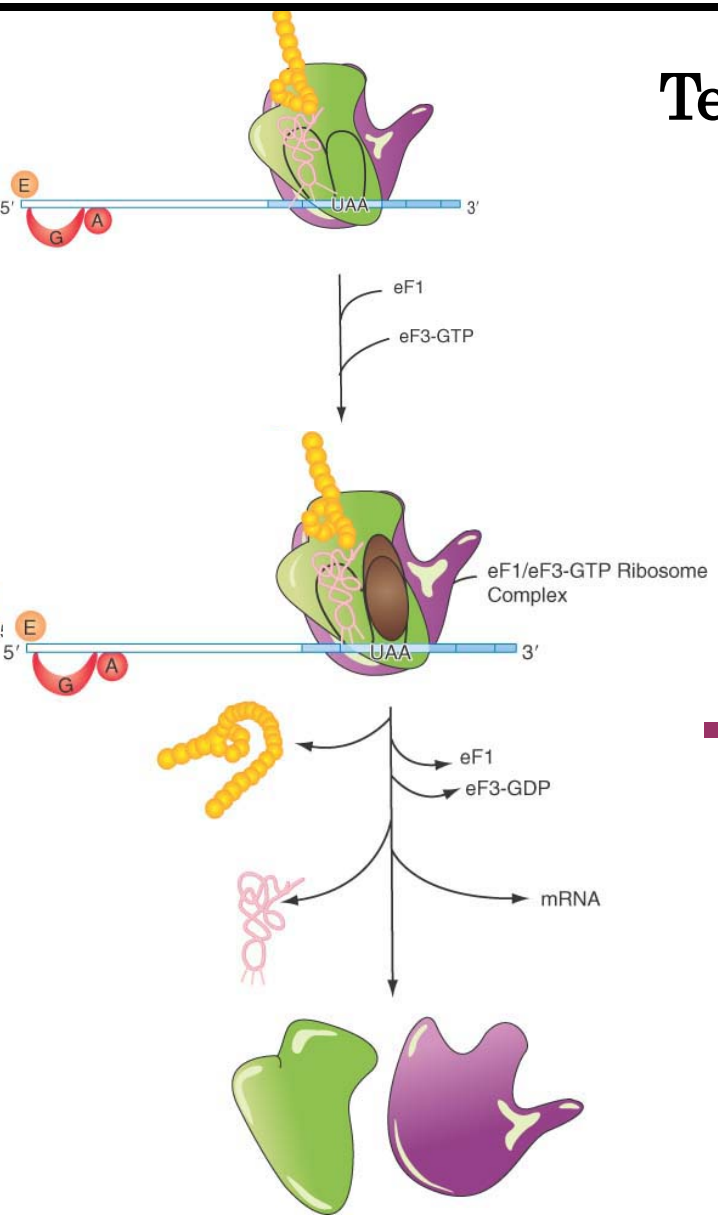



**Figure 19.12 mRNA Scanning and 80S Initiation Complex Formation**

**Elongation** – no E site, A & P sites only, 2 elongation factors

- **eEF1α** mediates binding of aminoacyl-tRNAs to A site
  - ✓ Correct match – eEF1α leaves site
  - ✓ Wrong – complex leaves site
- **Peptidyl transferase** of 60S subunit catalyzes peptide bond formation
- **eEF2-GTP** binds to ribosome
  - ✓ GTP hydrolysis - energy to move mRNA
- Stops at stop codons






**Termination** - release factors

1. **eRF1** – recognizes/binds to stop codons & to eRF3
2. **eRF1 binding** causes peptidyl transferase to hydrolyze the ester linkage, releasing polypeptide
3. **eRF3** triggers dissociation of eRF1 and ribosomal subunits

- **Translation efficiency** may be related to circular conformation of polysomes

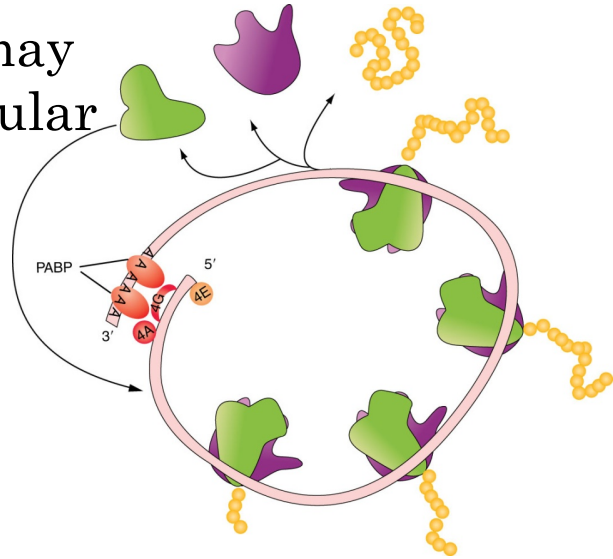
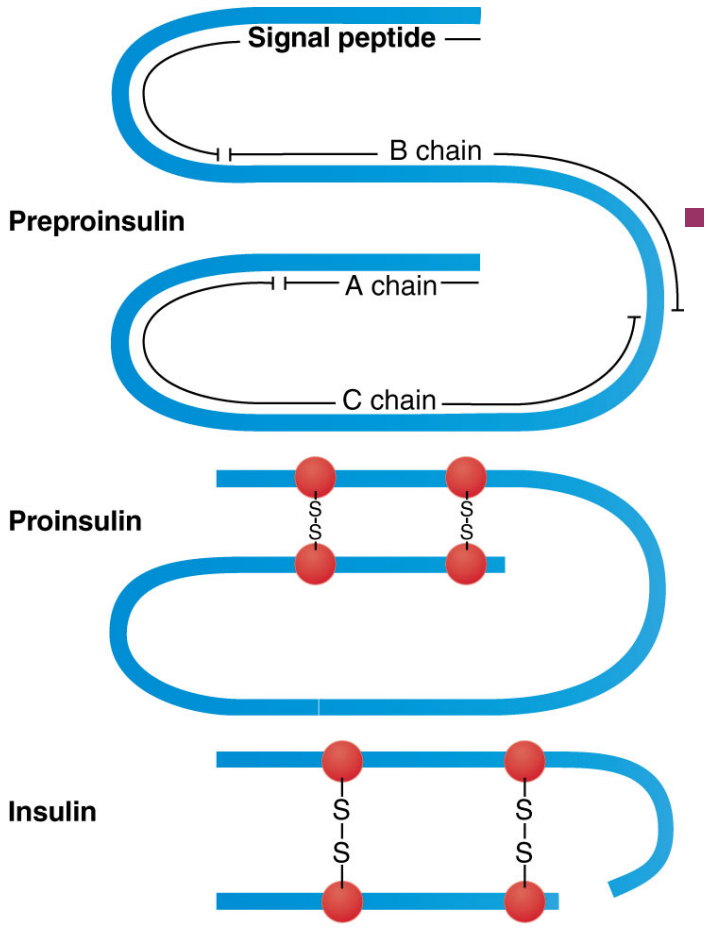



**Figure 19.14a Eukaryotic Protein Synthesis Termination**




**Posttranslational Modifications** – prepares protein for functional role, folding into native conformation

- **Proteolytic cleavage** – common regulatory mechanism
  - Remove N-terminal methionine and signal peptides
  - Convert **proproteins** into their active forms
    - **Preproprotein** – inactive precursor with removable signal peptide
      - Preproinsulin – proinsulin - insulin

**Figure 19.16 Proteolytic Processing of Insulin**

## Posttranslational Modifications

- **Glycosylation** – catalyzed by glycosyl transferase
  - N-linked oligosaccharide is assembled in association with phosphorylated dolichol (polyisoprenoid)
  - Vital role in protecting ER from misfolded glycoproteins
    - Cannot be correctly folded are targeted for ER-associated protein degradation by ubiquitin proteasome in cytoplasm
- **Hydroxylation** - proline and lysine is required for structural integrity collagen and elastin
  - Vitamin C (ascorbate) is required to hydroxylate proline
  - Inadequate dietary intake of vitamin C can result in scurvy, which is caused by weak collagen fiber structure
- **Phosphorylation** plays critical roles in metabolic, control, signal transduction, and protein-protein interaction

## Posttranslational Modifications

- **Lipophilic Modifications** - covalent attachment of lipid moieties to proteins
  - Acylation and prenylation are the most common
- **Methylation** - marking proteins for repair or degradation or changing their cellular function
- **Carboxylation** - increases a protein's sensitivity to $Ca^{2+}$-dependent modulation
- **Disulfide Bond Formation** – conformation stabilization

**Targeting** - directing protein to proper destination

- **Transcript localization** - specific mRNA is bound to receptors creating protein gradients
  - mRNA is moved by motor proteins (e.g., dynein and kinesin) along cytoskeletal filaments
- **Signal hypothesis** – polypeptides targeted to proper location by sorting signals (**signal peptides**)
  - Facilitate insertion of polypeptide into the appropriate membrane

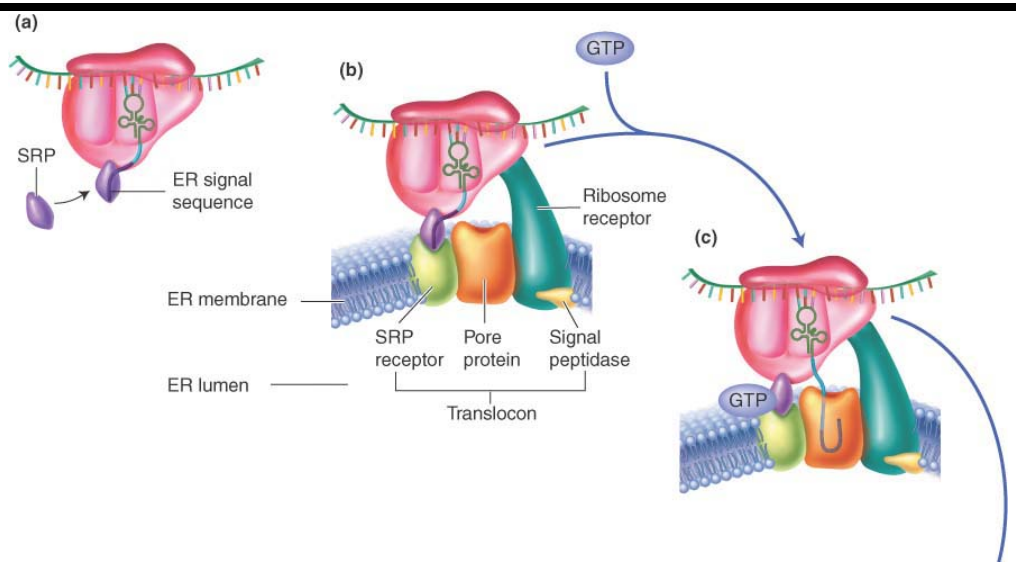


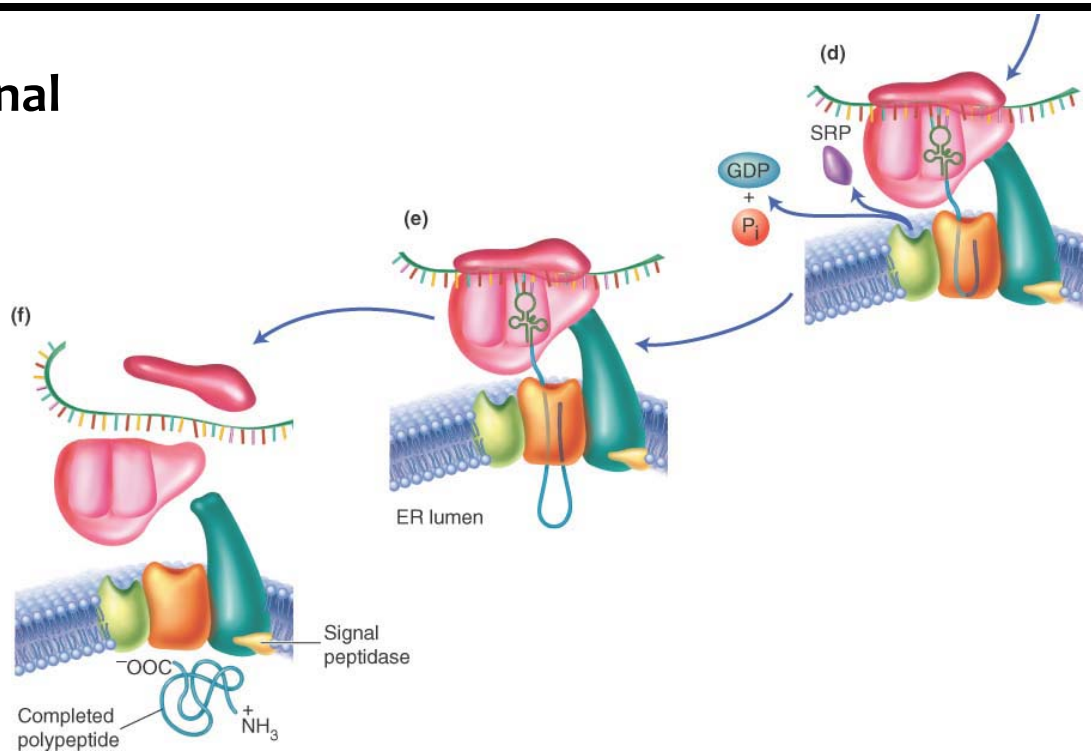

**Figure 19.19 Cotranslational Transfer across the RER Membrane**

a) Polypeptide protrudes from ribosome, SRP binds to signal sequence causing a transient cessation of translation

b) Subsequent binding of SRP to SRP receptor results in binding of ribosome to translocon complex in RER membrane

c) Translation restarts; polypeptide inserts into the membrane

**Figure 19.19 Cotranslational Transfer across the RER Membrane**




d) Dissociation of SRP from receptor

e) Continuation of elongation

f) Signal peptide removal by signal peptidase

# Section 19.3: The Proteostasis Network

- Proteotoxic stress-related protein misfolding and other types of damage are a sever threat to cell function
- Proteostasis Network monitors proteins from their synthesis, through folding, refolding, transport, and degradation
- PN processes utilize molecular chaperones, stress response transcription factors, detoxifying enzymes and degradation processes

# Section 19.3: The Proteostasis Network

- Heat shock response is the best understood stress response.
  - Works to protect an affected cell and its proteome by making rapid and global changes in gene expression that inhibit nonessential protein synthesis on ribosomes and increase the concentration of PN elements

# Section 19.3: The Proteostasis Network

- Defective protein folding is responsible for a large number of human diseases
  - Examples include cystic fibrosis and CBS
- Large number of protein folding disorders where there is chronic proteostasis dysfunction arising from adverse interactions between aggregated proteins and proteosomal components
  - Examples include Alzheimer's, Parkinson's, Huntington's, and Type II Diabetes