Protein Synthesis

Objectives:
I. Background
    A. Identify the roles of DNA, rRNA, mRNA, and tRNA in the synthesis of proteins.
    B. Explain the overall process of translation.
II. The Genetic Code
    A. Describe the essential elements of the genetic code
        1. Develop a feel for its simplicity and elegance.
    B. Define the terms:
        1. Codon
            a) Explain the role of codons in protein synthesis.
        2. Degenerate
        3. Punctuationless
        4. Non Overlapping
        5. Universal
        6. Synonymous Codons
        7. Termination or Nonsense Codons
        8. Initiation Codon
        9. Reading Frames
        10. Silent mutation
        11. Missense mutation
        12. Nonsense mutation
        13. Frameshift mutation
    C. Be able to read and translate the genetic code from an appropriate table.
III. Structure and Function of tRNA
    A. Describe the 2 dimensional and 3 dimensional shape(s) of the molecule.
    B. Describe the characteristic features of each of the arms.
    C. Define the anticodon and explain the role of the anticodon in the translation process.
        1. What is Conformational Flexibility or Wobble in the anticodon?
        2. What bases in the anticodon strictly base pair with the codon?
        3. What bases in the anticodon wobble with the codon?
    D. What is tRNA Charging?
        1. What enzymes catalyze the charging reactions?
        2. Describe the differences and similarities between the two classes of enzymes.
    E. What is the Initiating tRNA?
        1. Describe the differences and similarities between bacteria and eukaryotes with respect to the initiating tRNA.
IV. Ribosomes
    A. Describe the structure of ribosomes.
    B. Describe the component parts of the small subunit and large subunit of the bacterial ribosome.
    C. Compare and contrast the structure of the eukaryotic ribosome with the bacterial ribosome.
    D. Define the:
        1. Peptidyl Site or P Site
        2. Aminoacyl Site or A Site

3. Exit Site or E Site

V. The Translation Process in Bacteria
   A. Initiation
      1. Describe the component parts necessary for initiation of translation.
      2. Describe the process of initiation.
   B. Elongation
      1. Describe the three repeating steps of elongation.
      2. What protein factors are required for elongation and what are their functions?
      3. What is Peptidyl Transferase?
      4. What is the function of Peptidyl Transferase?
   C. Termination
      1. What protein factors are required for termination and what is their function?
      2. Describe the termination process.

VI. The Translation Process in Eukaryotes
   A. Initiation
      1. Describe the component parts necessary for initiation of translation.
      2. Describe the process of initiation.
      3. Compare and contrast the bacterial system with the eukaryotic system.
         a) Complexity of eukaryotic initiation?
   B. Elongation
      1. Describe the three repeating steps of elongation.
      2. What protein factors are required for elongation and what are their functions?
      3. What is Peptidyl Transferase?
      4. What is the function of Peptidyl Transferase?
      5. Compare and contrast the bacterial system with the eukaryotic system.
   C. Termination
      1. What protein factors are required for termination and what is their function?
      2. Describe the termination process.
      3. Compare and contrast the bacterial system with the eukaryotic system.

VII. Control of Translation in Eukaryotes
   A. Explain why eukaryotes control gene expression at the level of translation as well as transcription.
   B. Describe how translation is controlled:
      1. by phosphorylation of certain proteins.
      2. by inhibitory proteins binding to the mRNA.
      3. by proteins binding to selected initiation factors.
   C. Control of translation by small temporal RNA (stRNA) [also known as Interference RNA (RNAi)]
      1. Describe how translation is controlled by these RNA molecules
      2. What is the function of Dicer?

VIII. Protein Modification
   A. Explain the difference between Cotranslational Modifications and Post-translational Modifications.
   B. Describe some of the possible modifications.
   C. Describe the entry of proteins into the Smooth Endoplasmic Reticulum.

D. Describe the function of:
1. Signal Peptide.
2. Signal Recognition Particle (SRP).
3. Translocon Complex.
4. Docking Protein (SRP Receptor).
5. Sec61 (Ribophorin).
6. Signal Peptidase.
IX. Glycoprotein Synthesis
A. N-linked Polysaccharides
1. Describe the synthesis of the Core structure of N-linked polysaccharides on Dolichol phosphate.
2. What are the activated forms of the monosaccharides used for the synthesis of the core structure?
3. What is the function of the "Translocase"?
4. What is the function of the Oligosaccharide Transferase Complex?
5. What is the function of the Asialoglycoprotein receptor?
B. O-Linked Polysaccharides
1. Describe the synthesis of O-linked polysaccharides.

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Translation - Component Parts
The Genetic Code

Translation is the mRNA directed synthesis of the primary structure of a polypeptide. Protein synthesis is the most complex of biosynthetic processes, and deciphering/understanding it has been one of the greatest challenges in the history of biochemistry. In eukaryotic cells, protein synthesis requires the participation of 80 different ribosomal proteins and 4 ribosomal RNA's (rRNA); 40 or more different kinds of transfer RNA's (tRNA); 20 or more enzymes to activate the amino acids by attaching them to their specific tRNA; 15 or more auxiliary enzymes and other protein factors for the initiation, elongation, and termination of polypeptides; and perhaps 100 additional enzymes for the final processing of proteins. Thus almost 400 different macromolecules must cooperate to synthesize polypeptides. Many of these macromolecules are organized into the complex, highly organized three-dimensional structure of the ribosome. Ribosomes carry out the step wise translation and translocation of the mRNA as the polypeptide is assembled.

The information necessary for the synthesis of the primary (1°) structure of a protein is stored as a specific sequence of bases on DNA and is transcribed into a nearly identical (U instead of T) sequence of bases on mRNA. The information for the 1° structure of a protein exists as a code on the DNA and the mRNA molecule. This code, this specific sequence of bases on mRNA, is called the GENETIC CODE. If the information for the 1° structure of a protein is written in code, a molecule or molecules must exist in the cell to read the code and translate it into the 1° structure of a protein. These code reading molecules are TRANSFER RNA, tRNA, molecules.

As a prelude to describing the process of translation, the genetic code, the structure and function of tRNA, and finally the structure of the ribosome will be examined.

DNA and RNA contain a four letter "alphabet", A, T(U), G, and C. Protein primary structure is composed of a 20 letter "alphabet", the twenty naturally occurring amino acids. Since the amino acid "alphabet" is significantly larger than the DNA/RNA "alphabet", some unique combination of nucleotide bases must be used to code for an individual amino acid. From a mathematical point of view: four bases taken two at a time would give 4^2 or 16 unique "words", not enough for the 20 amino acids. However, four bases taken three at a time would result in 4^3 or 64 unique "words", more than enough for the 20 amino acids.

The first experiments designed to unravel the genetic code hypothesized the code contained 3 letter "words". Over the course of five years experiments performed by Sydney Brenner, Marshall Nirenberg, J. Heinrich Matthaei, H. Gobind Khorana, Francis Crick, and others unraveled the genetic code.

The genetic code is a triplet of bases on mRNA called CODONS. A table of codons, the genetic code, is given below. In this table the codons are written in the 5´ → 3´ direction. Messenger RNA when read and translated into protein is read in the 5´ → 3´ direction. There are several important facts that need to be noted about the genetic code.

First Position (5´ end)	Second Position				Third Position (3´ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

1. The genetic code is DEGENERATE. Several codons specify, code for, the same amino acid. The degeneracy of the genetic code minimizes the effects of mutations since a change of a single base

often results in a codon that specifies the same amino acid. Different codons that specify the same amino acid are known as SYNONYMOUS CODONS.

2. The first two nucleotides of a codon are often sufficient to specify a given amino acid. The degeneracy of the code usually resides in the 3´ base of the codon. A base change at the 3´ end of the codon has no to minimal effect on the structure of the protein.

3. Codons with similar sequences often code for amino acids with similar properties. For example, the codons for Ser and Thr differ by the base in the 5´ position and the codons for Glu and Asp all start with GA.

4. 61 of the 64 codons specify amino acids. The three that do not code for an amino acid are called NONSENSE CODONS, TERMINATION CODONS, or STOP CODONS. These codons mark the end of the coding sequence, the end of the information for the synthesis of the 1° structure of a protein. The three termination codons are UAA (Ochre), UGA (Opal), and UAG (Amber).

5. The genetic code is NON OVERLAPPING. In an overlapping code, letters can be parts of more than one word. In a non overlapping code a particular letter is part of one and only one word. In a non overlapping code, changing one letter only changes one word.

A C G U C A G C U A G U   non overlapping   A C G U C A G C U A G U   overlapping

6. READING FRAMES - The three base per codon genetic code allows for three possible reading frames depending upon where along the mRNA strand translation is initiated. The reading frame is set at the beginning of translation. There is a specific INITIATION CODON. The first AUG codon (the codon for Met) encountered at the 5´ end of the mRNA molecule sets the reading frame. Since AUG is the only codon for Methionine, all of the downstream AUG codons code for the insertion of Methionine into the protein.

5´ A C A U G C A U G C G U C C A G G G   First Reading Frame

5´ A C A U G C A U G C G U C C A G G G   Second Reading Frame

5´ A C A U G C A U G C G U C C A G G G   Third Reading Frame

7. The genetic code is PUNCTUATIONLESS. It is read as one continuous "sentence", there are no "commas", i.e., bases inserted between codons to offset the codons.

8. The genetic code is nearly UNIVERSAL. The same set of codons is used by nearly all organisms. Minor changes exist in the genetic code used by ARCHAEA, chloroplasts, and mitochondria.

TRANSFER RNA molecules are the genetic code readers. tRNA molecules read the genetic code. They read the codons on mRNA and translate them into the primary structure of a protein. tRNA's are from 73 to 93 nucleotides in length. The primary structure, the sequence of bases in the tRNA's are all different, but all tRNA's share common features. Eight or more of the nucleotides are modified, the 5′ end is usually a G residue, the 3′ end is always the sequence CCA, and there are 18 invariant residues (grayed residues in figure below).

All of the tRNAs have similar secondary structures. Looking at the tRNA molecule in two dimensions, they fold into cloverleaf like structures, stabilized by hydrogen bonds between complementary bases and by base stacking interactions. Hydrogen bonded regions form short, stacked, right handed helical segments, similar to those in DNA. The 3′ end of the tRNA molecule is called the ACCEPTOR STEM or ACCEPTOR ARM.
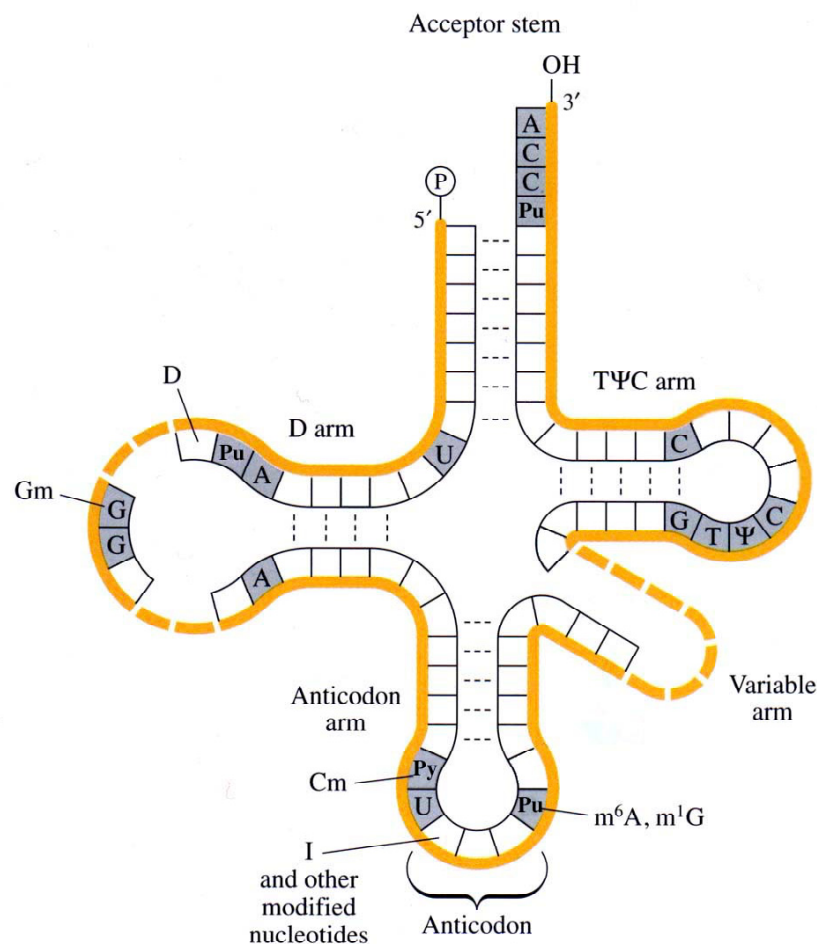
The loop of tRNA opposite from the acceptor stem is the ANTICODON LOOP or ANTICODON ARM. This loop contains the ANTICODON, a sequence of three bases complementary to the codons of mRNA. Two or three DIHYDROURIDINE (D) bases are always found in the D ARM and the TψC ARM always contains the sequence RIBOTHYMIDINE (T), PSEUDOURIDINE (ψ), CYTIDINE (C).

In three dimensions, the tRNA molecule folds into an L shape. The ANTICODON ARM is at the end of the short arm of the L and the ACCEPTOR ARM is at the opposite end, at the long arm of the L.
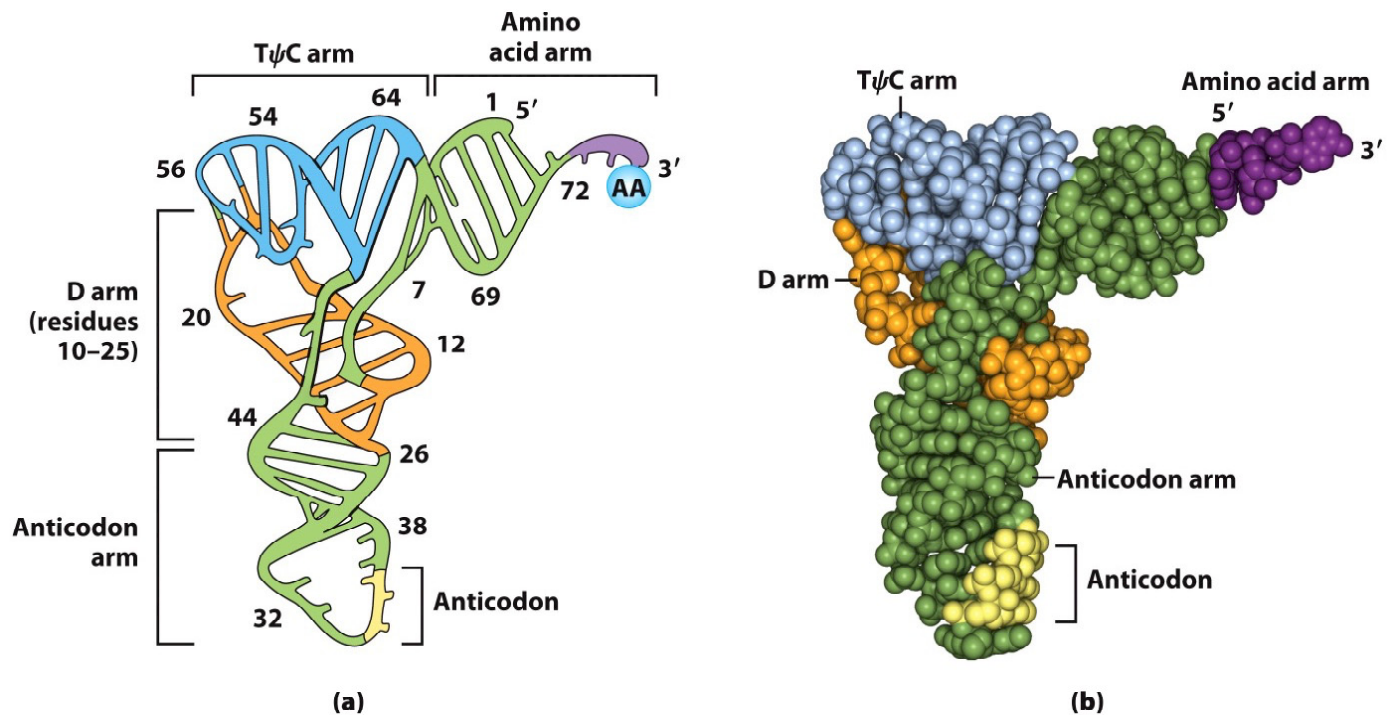
(a)

(b)

tRNA and mRNA interact with each other. Anticodons bind to codons by complementary base pairing interactions in an antiparallel manner. The 5´ end of the codon interacts with the 3´ end of the anticodon. One would expect 61 tRNA molecules to be present in a cell; one for each codon. In actuality, cells contain between 32 and 45 different tRNAs; more than enough for each of the 20 amino acids but less than the 61 readable codons. *How does the cell function with less than the optimal number of tRNA's?* The two bases at the 5´ end of the codon and the two bases at the 3´ end of the anticodon strictly follow Watson & Crick base pairing rules (A=U & G≡C). Base pairing between the last base (3´ end) of the codon and the first base (5´ end) of the anticodon shows a great deal of variability. The 5´ position of the ANTICODON shows CONFORMATIONAL FLEXIBILITY or WOBBLE. The 5´ position of the anticodon is called the WOBBLE POSITION or WOBBLE BASE. Bases in the WOBBLE POSITION and the allowed base pairing are as follows:

5´ Position of Anticodon "Wobble Position"	3´ Position of Codon
C	G
A	U
U	A or G
G	U or C
I	A or U or C

Wobble in the 5´ position of the anticodon accounts for the fact that the cells contains at most 45 different tRNA molecules and that these tRNAs can interact with and read all 61 codons on mRNA. Wobble in the 5´ position of the anticodon allows certain of the tRNA molecules to base pair with more than one codon on mRNA. If the genetic code is reexamined, it will be noted that Synonymous Codons, codons that code for the same amino acid vary at the 3´ end. Wobble at the 5´ position of the anticodon allows the same tRNA molecule to base pair with several or all of the synonymous codons of a particular amino acid. If the wobble was use to its maximum extent a total of 32 tRNA's is all that would be needed to translate the entire genetic code.

tRNA Activation - tRNA Charging

Before a tRNA can take part in translation it must be covalently linked with the correct amino acid. CHARGING a tRNA molecule is the covalent attachment of the correct amino acid to its corresponding tRNA molecule. The product is an aminoacyl-tRNA.

A bit of nomenclature
- a tRNA molecule with an anticodon that specifies, for example alanine, is designated tRNA^{Ala}.
- when this tRNA molecule (tRNA^{Ala}) is charged with Ala it is designated Ala-tRNA^{Ala}.

The enzymes that catalyze the charging of tRNA molecules are the Aminoacyl-tRNA Synthetases. There are two different classes of Aminoacyl-tRNA Synthetases.

Class I enzymes:
1. are monomeric or homodimeric enzymes
2. bind to the tRNA acceptor stem helix from the minor groove side
3. active site is formed by a five-stranded parallel β-sheet termed a Rossman fold. A structural motif common to many ATP binding enzymes.
4. recognize their cognate tRNA by the anticodon sequence, three dimensional tRNA structure, modified bases in the tRNA, and/or base sequences along the acceptor stem
5. initially attach the amino acid to the 2´ hydroxyl group of the 3´ terminal adenosine residue
6. charges Arg, Cys, Gln, Glu, Ile, Leu, Met, Trp, Tyr, & Val

Class II enzymes
1. are polymeric enzymes composed of two identical subunits or two pairs of dissimilar subunits
2. bind to the tRNA acceptor stem helix from the major groove side
3. active site contains a seven-stranded β-structure with three α-helices
4. recognize their cognate tRNA by the three dimensional tRNA structure, modified bases in the tRNA, and/or base sequences along the acceptor stem; never by the anticodon sequence
5. attach the amino acid to the 3´ hydroxyl group of the 3´ terminal adenosine residue
6. charges Ala, Asn, Asp, Gly, His, Lys, Phe, Pro, Ser, & Thr

Mechanistically, the Class I and Class II enzymes direct the carboxyl group of the amino acid to attack the anhydride bond between the α and β phosphates of ATP. Pyrophosphate is released from the ATP and the amino acid is attached to AMP by a mixed anhydride bond. Class I enzymes then pass the amino acid from the AMP moiety to the 2´ hydroxyl group on the ribose of the adenosine at the 3´ end of the tRNA. Class II

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enzymes pass the amino acid from the AMP moiety to the 3′ hydroxyl group on the ribose of the adenosine at the 3′ end of the tRNA. In both cases the mixed anhydride bond is cleaved and an ester bond is formed.

Only tRNA's with the amino acid esterified to the 3′ hydroxyl group of the 3′ terminal adenosine residue are utilized by the translation apparatus. The tRNA's charged by the Class I aminoacyl tRNA synthetases must be modified, the amino acid must be moved from the 2′ hydroxyl to the 3′ hydroxyl group. How this movement, this transesterification reaction, occurs is unknown. Some investigators suggest that the movement from the 2′ to 3′ hydroxyl group is catalyzed by the Class I enzymes. Others suggest that it is a spontaneous uncatalyzed transesterification reaction, where the 3′ hydroxyl group attacks the ester bond between the amino acid and the 2′ hydroxyl group. The amino acid is moved, forms a new ester bond with the 3′ hydroxyl group. This reaction could occur before the charged tRNA leaves the active site of the Class I enzyme or it could occur while the charged tRNA is free in the cytoplasm. If the reaction is spontaneous, the equilibrium position must very strongly favor the formation of the ester bond at the 3′ hydroxyl group since only very small amounts of the charged tRNA's with the amino acid esterified to the 2′ hydroxyl group is found in cells actively translating.
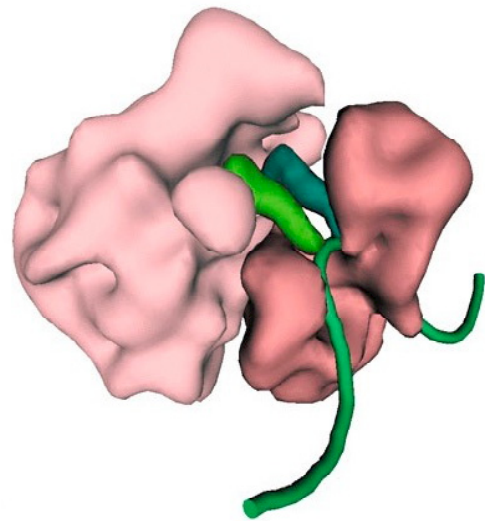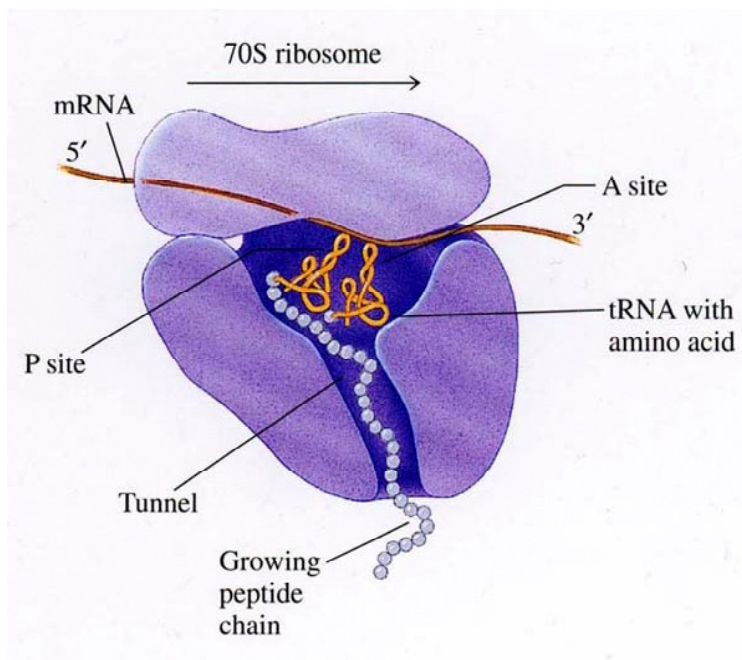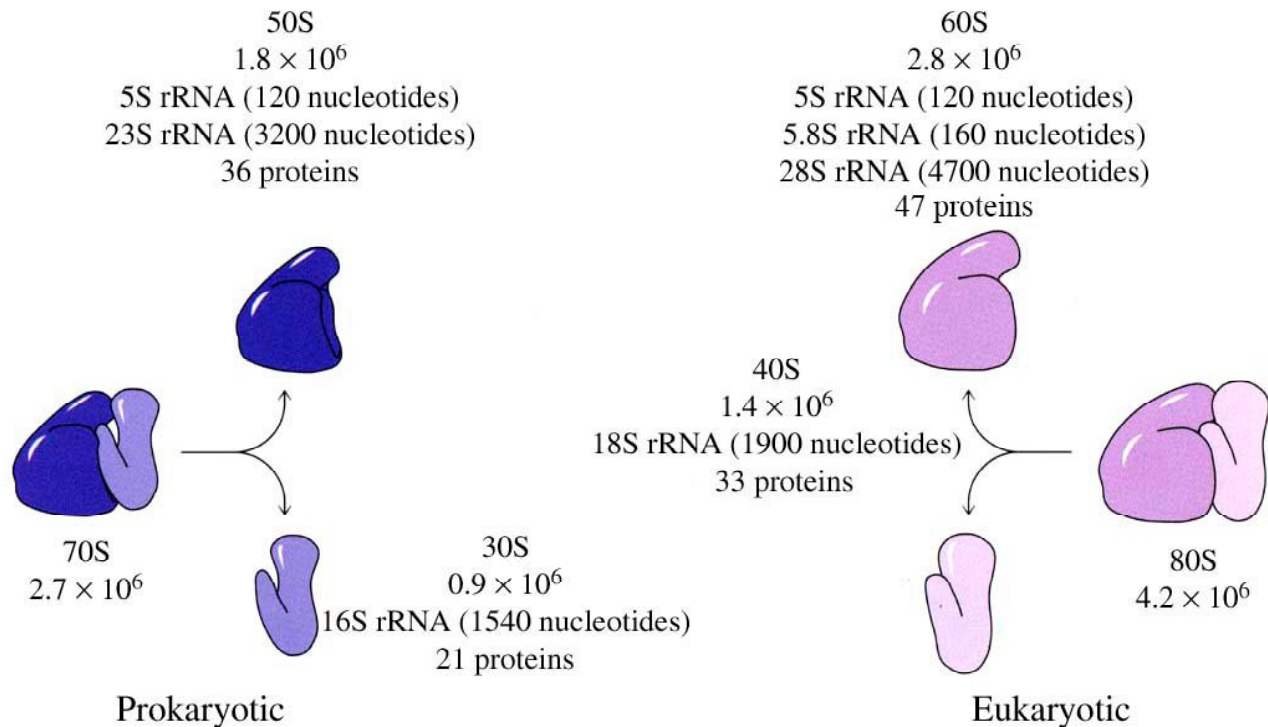
There are 20 Aminoacyl-tRNA Synthetases, one for each amino acid. Each Aminoacyl-tRNA Synthetase recognizes a specific the amino acid and all of the tRNA's that are specific for that amino acid; that should be charged with that amino acid. The amino acid is recognized on the basis of its side chain structure and chemistry. The correct tRNA is recognized as described above. Some of the Aminoacyl-tRNA Synthetases have proof reading capabilities. After the amino acid has been attached, some of these enzymes double check to make sure the correct amino acid has been covalently attached to the tRNA molecule.

Cells contain two different tRNAs for methionine, one is used strictly for initiation of translation and the other is used for incorporation of methionine at other, internal locations in the protein. In bacteria the methionine residue attached to the initiating tRNA (tRNA^{fMet}) is formylated by the action of a Transformylase. This enzyme transfers a formyl group from N^{10}-formyltetrahydrofolate to the amino group of the methionine covalently linked to the initiating tRNA (Met-tRNA^{fMet}) to form N-formylmethionine-tRNA^{fMet} (fMet-tRNA^{fMet}). Hence, in bacteria the initial amino terminus of all proteins is N-formylmethionine. In eukaryotes, the methionine attached to the initiating tRNA is not modified. The initiating tRNA in eukaryotes is termed tRNA$_i{}^{Met}$.
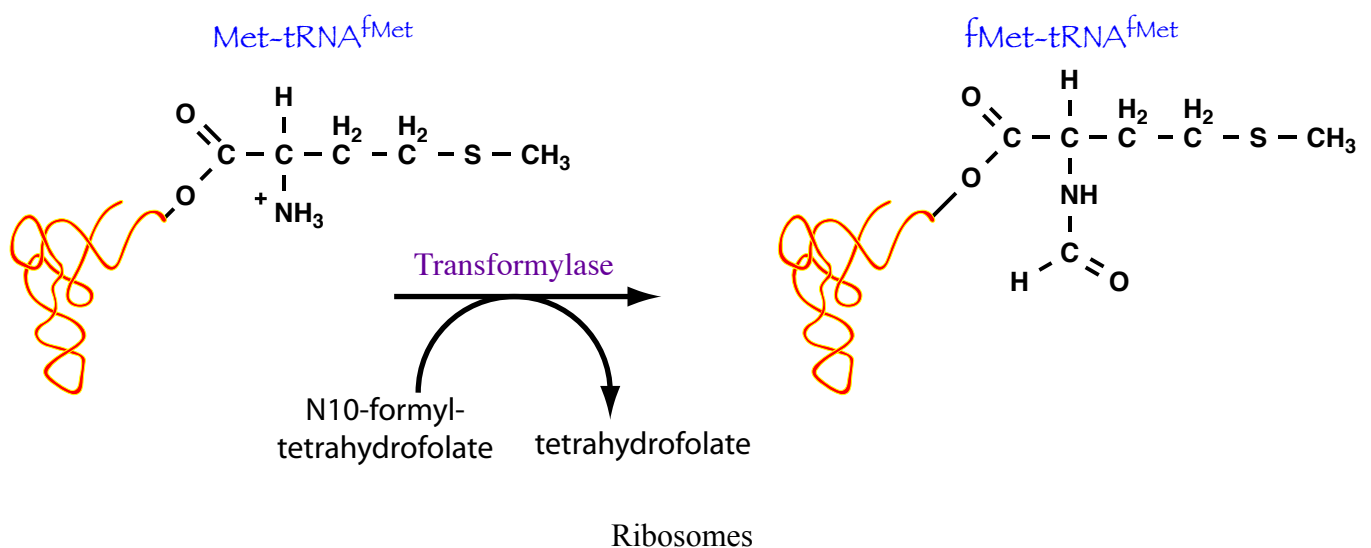
Ribosomes

RIBOSOMES are a major player in the process of translation. The translation complex is assembled upon the ribosome and the ribosome contributes several enzyme activities necessary for protein synthesis.

Ribosomes are large complexes of ribosomal RNA (rRNA) and protein. About two thirds of the mass of a ribosome is rRNA.

50S
1.8×10^6
5S rRNA (120 nucleotides)
23S rRNA (3200 nucleotides)
36 proteins

60S
2.8×10^6
5S rRNA (120 nucleotides)
5.8S rRNA (160 nucleotides)
28S rRNA (4700 nucleotides)
47 proteins

40S
1.4×10^6
18S rRNA (1900 nucleotides)
33 proteins

70S
2.7×10^6

30S
0.9×10^6
16S rRNA (1540 nucleotides)
21 proteins

80S
4.2×10^6

Prokaryotic

Eukaryotic

70S ribosome

mRNA
5′

A site

3′

tRNA with amino acid

P site

Tunnel

Growing peptide chain

Both bacterial and eukaryotic ribosomes are composed of two dissimilar subunits. In bacteria the smaller subunit has a sedimentation coefficient of 30S, a mass of 930,000 Daltons, it contains the 16S piece of rRNA (1540 bases), and 21 proteins. The larger subunit has a sedimentation coefficient of 50S and a mass of 1,800,000 Daltons. It contains the 23S rRNA (3200 bases), the 5S piece of rRNA (120 bases), and 36 different proteins. When the two subunits are joined to form the functional ribosome the particle has a

sedimentation coefficient of 70S and a mass of 2,730,000 Daltons.

The eukaryotic ribosome is similar, but almost twice as large. The functional eukaryotic ribosome contains two dissimilar subunits with a sedimentation coefficient of 80S and a total mass of 4,200,000 Daltons. The small subunit sediments at 40S, has a mass of 1,400,000 Daltons, and is composed of the 18S piece of rRNA (1900 bases) and 33 proteins. The large subunit has a sedimentation coefficient of 60S and a mass of 2,800,000 Daltons. It contains the 5S rRNA (120 bases), the 5.8S rRNA (160 bases), the 28S rRNA (4700 bases) and 47 proteins. The large ribosomal subunit from both cell types contains a tunnel through which the assembled protein emerges.

When the translation apparatus is assembled the ribosome holds the mRNA, the growing polypeptide chain, two charged tRNA molecules, and several additional protein factors. The two charged tRNA molecules are aligned so that their anticodons interact with the corresponding codons on the mRNA. The two subunits when assembled form a groove through which the mRNA moves. Interactions between the ribosome, mRNA, and the two tRNAs position the charged ends of the tRNA molecules at the active site of the enzyme that catalyzes peptide bond formation. One of the two tRNA molecules is bound to the ribosome at the PEPTIDYL SITE or P SITE. The growing polypeptide chain is covalently attached to the tRNA in this site. The second tRNA is bound at the AMINOACYL SITE or A SITE. The tRNA in this site is charged with the next amino acid to be incorporated into the growing polypeptide. Bacterial ribosomes also contain an EXIT SITE or E SITE. Empty, uncharged tRNAs reside briefly in the E Site before they dissociate from the translation apparatus.

Translation

PROTEIN SYNTHESIS or TRANSLATION is the final step in the flow of biological information.

The three cellular processes (Replication, Transcription, and Translation) involved in the flow of information are variations on a theme:

- All are polymerization reactions.
- All three can be divided into initiation, elongation, and termination steps.
- All three are carried out by large protein complexes with numerous accessory proteins.

Protein synthesis begins with tRNA charging, a process already discussed, and ends with protein folding and post translational modification of the protein. In between these two events the actual translation of information on mRNA into a polymer of amino acids occurs. In general, the initiation of translation involves the assembly of the translation complex on the initiation codon of an mRNA molecule. During elongation the translation complex moves down the mRNA molecule in a $5´ \rightarrow 3´$ direction and the protein is synthesized from the amino (N) terminus to the carboxyl (C) terminus. Finally, during termination the completed protein is released from the translation complex and the translation complex dissociates.

Initiation of Translation in Bacteria

Initiation in bacteria requires:

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1. the 30 S (small) ribosomal subunit
2. mRNA
3. the "Initiating tRNA" charged with N-FormylMethionine.
4. three INITIATION FACTORS; **IF-1**, **IF-2**, & **IF-3**.
5. GTP
6. the 50 S (large) ribosomal subunit

During the initiation process in bacteria:

1. **IF1** binds to the 30S ribosomal subunit at what will become the A site. It plays a role in separating the ribosome into its subunits for initiation and it facilitates the actions of the other two initiation factors.
2. **IF3** binds to the 30S subunit and prevents the premature association of the 50S subunit. It keeps the subunits separated until all of the necessary components have assembled.
3. The mRNA binds to the 30S subunit. A sequence near the 5′ end of the mRNA base pairs with a complementary sequence on the 16S piece of rRNA. This sequence on the mRNA is called the SHINE-DALGARNO sequence. The SHINE-DALGARNO sequence is purine rich and the complementary sequence on the 16S rRNA is pyrimidine rich. The interaction between the Shine-Dalgarno

sequence and the 16S rRNA positions the start codon correctly on the 30S subunit in what will become the P site when the subunits are joined.

4. **IF2**, a small monomeric G protein, binds a molecule of GTP and the charged fMet-tRNA^{fMet} (Initiator tRNA). This complex binds to the 30S subunit on the start codon in the P site. **IF1** facilitates the formation of this complex.

5. After the charged Initiator tRNA has base paired to the start codon, the 50S subunit binds, the GTP on **IF2** is hydrolyzed to GDP and PO_4^{-3}, and the energy released induces a conformational change in the ribosome locking the complex in place and causing the initiation factors to dissociate.

<div align="center">Elongation in Bacteria</div>

Elongation requires:

1. the initiation complex
2. the aminoacyl-tRNA specified by the next codon
3. cytosolic ELONGATION FACTORS; **EF-Tu**, **EF-Ts**, & **EF-G**
4. GTP

During elongation each amino acid is added to the growing polypeptide chain in a three step process. The three repeating steps of elongation are:

1. Positioning the correct aminoacyl-tRNA in the A site of the ribosome.
2. Peptide bond formation
3. Translocation - moving the mRNA one codon in the 3´ direction.

Elongation in bacteria occurs as follows:

1. In the inactive state, ELONGATION FACTOR-Tu (**EF-Tu**) has GDP bound to it. It is activated when ELONGATION FACTOR-Ts (**EF-Ts**) binds to the **EF-Tu**•GDP complex. **EF-Ts** facilitates the dissociation of GDP from **EF-Tu** and the binding of GTP to **EF-Tu**. **EF-Ts** is a GUANOSINE NUCLEOTIDE-EXCHANGE FACTOR; **EF-Tu** is another of the small monomeric G proteins.

2. The **EF-Tu**•GTP complex binds a charged tRNA molecule. In a bacterial cell performing translation, most/all of the charged tRNA molecules are bound by **EF-Tu**•GTP complexes since E. coli contains about 70,000 copies of **EF-Tu**.

3. The **EF-Tu**•GTP•charged tRNA complex binds to the open codon in the A site. This is a random fitting event. These complexes diffuse into the A site until the anticodon on the tRNA correctly base pairs with the codon in the A site.

4. Once the correct charged tRNA has been found and fitted, the GTP on **EF-Tu** is hydrolyzed to GDP and phosphate, the **EF-Tu**•GDP complex proof reads to make sure that the correct tRNA is in place, the **EF-Tu**•GDP dissociates from the ribosome, and the tRNA is locked in place at the A site.

5. A transfer RNA with the growing peptide chain covalently linked to its 3´ end is present in the P site and a charged tRNA has just bound to the A site. Binding of the tRNAs to the ribosome properly positions the groups for catalysis. The enzyme Peptidyl Transferase catalyzes the formation of the peptide bond. The amino group of the amino acid in the A site attacks the carboxyl carbon of the ester bond between the nascent polypeptide chain and the 3´ hydroxyl group on the ribose of the

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peptidyl-tRNA. Aminoacyl group transfer results in the formation of the peptide bond.
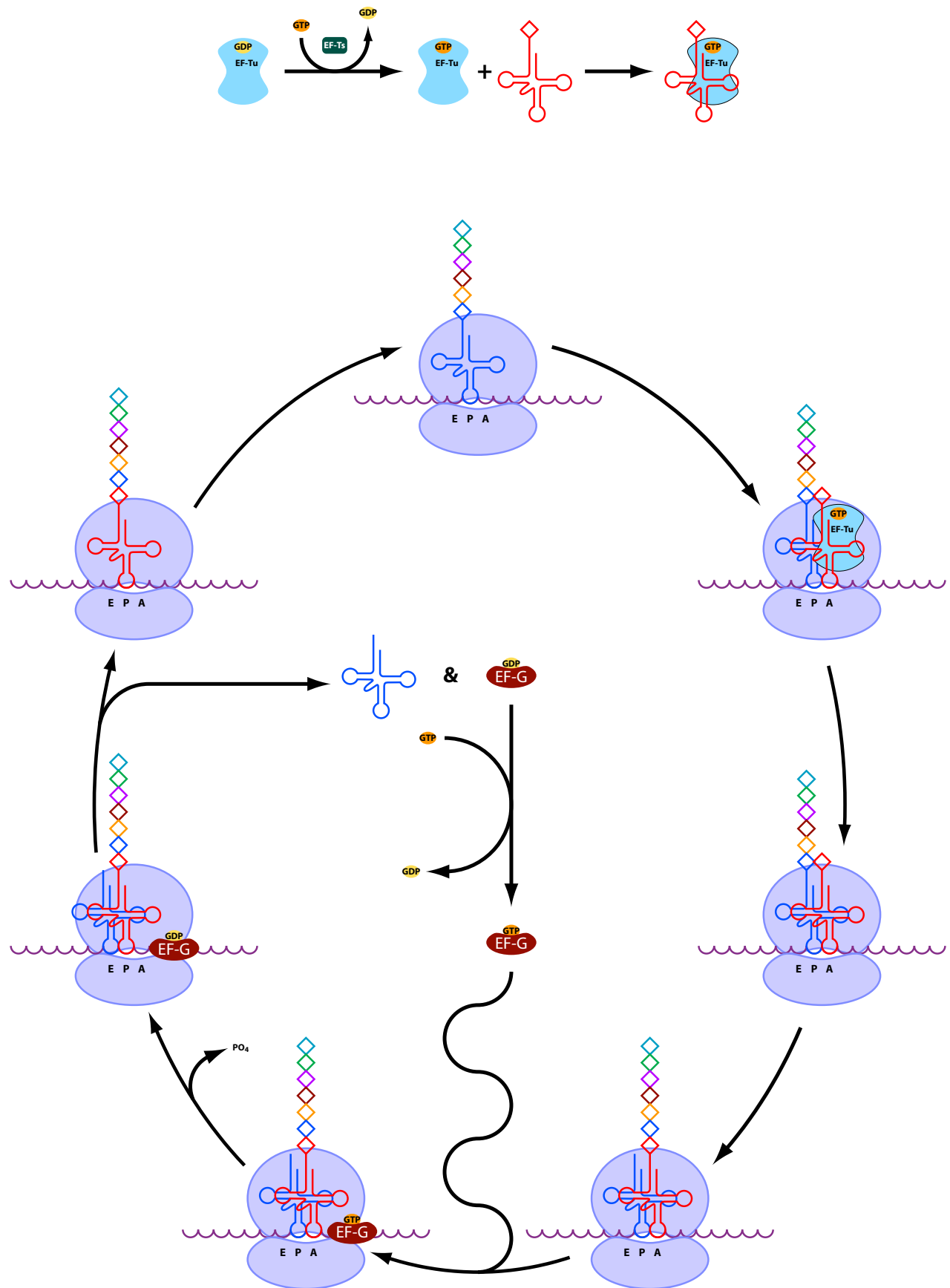
During this reaction the peptide chain is increased in length by one amino acid and the growing polypeptide is transferred from the tRNA in the P site to the tRNA in the A site. The enzyme Peptidyl Transferase is contained within the large ribosomal subunit. The 23S rRNA and proteins of the large subunit form the substrate site. The catalytic activity resides in a specific adenosine residue that is part of the 23S piece of rRNA. The enzyme is RNA; it is a RIBOZYME. An accessory protein, **EF-P**, has been implicated as a necessary factor for the formation of the first peptide bond; the peptide bond between N-formlymethionine and the second amino acid. However, not everyone agrees that **EF-P** is necessary.

6. After the peptide bond is formed the ribosome undergoes TRANSLOCATION. ELONGATION FACTOR-G (**EF-G**), a small monomeric G protein, binds GTP and the **EF-G**•GTP complex binds to the ribosome. **EF-G**•GTP facilitates translocation. During translocation the ribosome moves one codon down the mRNA in the 3′ direction. This movement shifts the empty tRNA from the P site to the E site, the empty tRNA dissociates from the ribosome. The tRNA with the growing polypeptide chain moves, is moved from the A site to the P site. The energy released by hydrolysis of the bound GTP to GDP and phosphate is used to drive the translocation process.

7. This cycle of tRNA binding to the A site, peptide bond formation, and translocation continues until a stop codon is encountered in the A site.

Once elongation has moved the translation complex 80 to 100 nucleotides from the initiation site, a new initiation complex often forms. mRNA with numerous translation complexes attached are called POLYSOMES. Endonucleases that specifically cleave the 5′ ends of single stranded RNA (mRNA) are also hydrolyzing the mRNA while it is being transcribed. As long as the Shine-Dalgarno sequence is intact, translation will be initiated. Once the Shine-Dalgarno sequence is damaged or destroyed, the RNA molecule is no longer recognized by the ribosome as mRNA. The number of times a piece of mRNA is translated by bacteria is dependent upon the number of bases in the mRNA molecule upstream from the Shine-Dalgarno sequence.
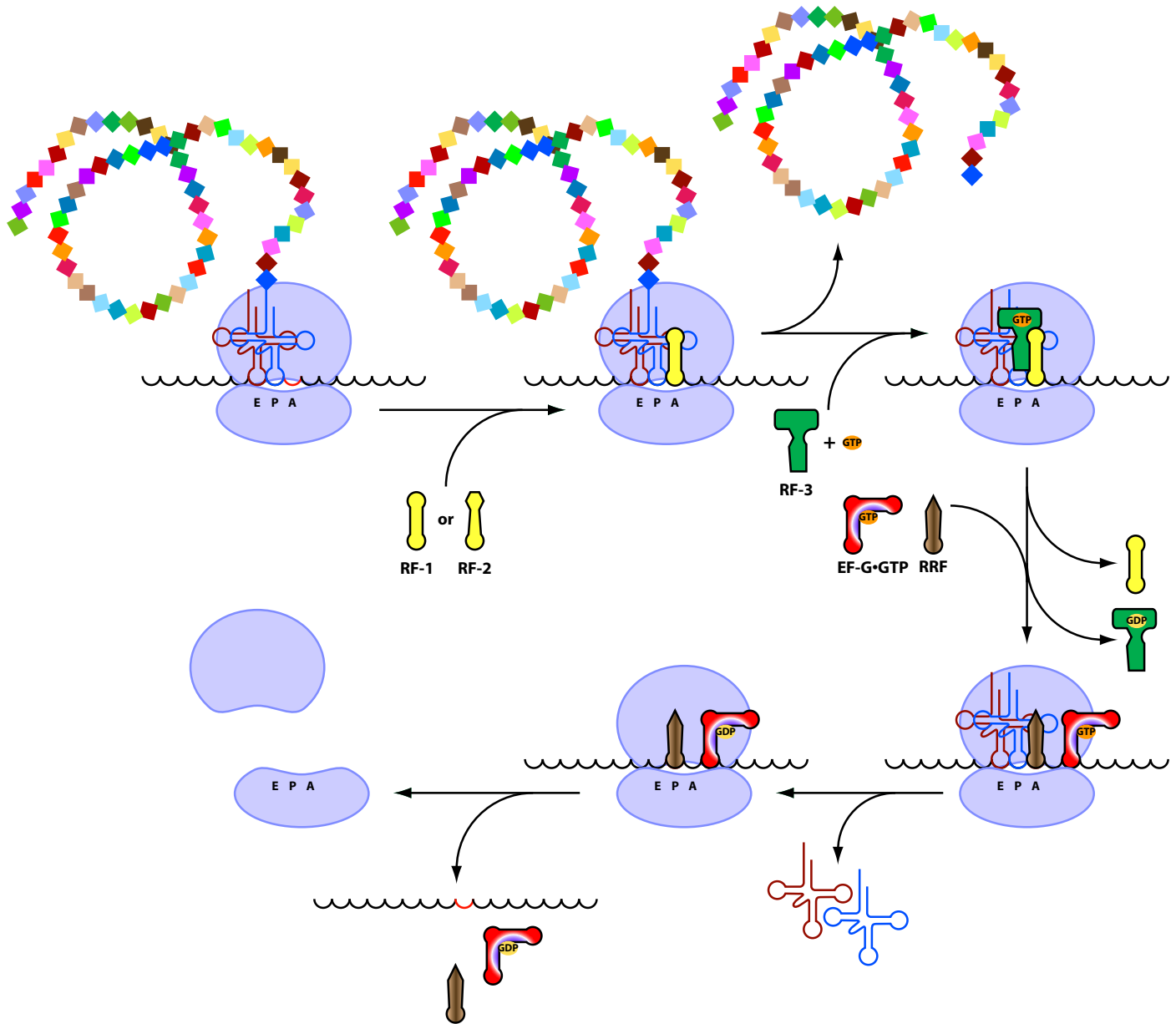
Termination in Bacteria

In bacteria, two out of three RELEASE FACTORS, **RF1** or **RF2** and **RF3**, are required for the termination of protein synthesis.

1. When a stop codon is present in the A site of the ribosome either **RF1** or **RF2** binds to this codon. **RF1** recognizes and binds to UAG or UAA; **RF2** binds to UAA or UGA.

2. **RF1** or **RF2** when bound to the A site of the ribosome changes the specificity of Peptidyl Transferase. Peptidyl Transferase transfers the completed polypeptide to a water molecule rather than to the amino group of an amino acid on a charged tRNA. It catalyzes the hydrolysis of the ester bond between the completed polypeptide and the 3′ end of the last tRNA in the P site. This "hydrolysis" releases the completed polypeptide from the ribosome.

3. **RF3**, a small monomeric G protein, binds GTP and then binds to the ribosomal complex. The GTP is hydrolyzed to GDP and phosphate The energy liberated causes the dissociation of **RF1** or **RF2** (which ever one was bound).

4. **RIBOSOMAL RECYCLING FACTOR** (**RRF**) binds to the A site followed by the **EF-G**•GTP complex and **RF3**•GDP complex dissociates. The GTP is hydrolyzed to GDP and phosphate, the energy released translocates **RRF** from the A site to the P site, and the tRNA's in the P and E site dissociate.

IF-3 binds, RRF is released from the ribosome followed by the EF-G•GDP complex and the mRNA. IF-3 separates the ribosome into small and large subunits ready for the next round of initiation.


Initiation of Translation Eukaryotes

Initiation of translation in eukaryotes differs dramatically from initiation in bacteria. In eukaryotes the ribosomal subunits are larger, 40S & 60S, and at least twelve initiation factors are required. Many of these initiation factors are multisubunit proteins (quaternary structure). New / additional initiation factors and/or accessory proteins are still being identified and characterized. The EUKARYOTIC INITIATION FACTORS are abbreviated as eIF. The Initiator tRNA is a special tRNA^{Met}, designated tRNA$_i^{Met}$. This tRNA must be charged with methionine (Met-tRNA$_i^{Met}$) and is recognized by eukaryotic INITIATION FACTOR 2 (eIF2).

During the initiation process in eukaryotes:

1. **eIF2B** binds to the **eIF2**•GDP complex and "recharges" the bound GDP to GTP. It appears that after **eIF2B** binds to the **eIF2**•GDP complex, it catalyzes the transfer of a high energy phosphate from ATP to the GDP on **eIF2** forming ADP and the "recharged" **eIF2**•GTP complex.
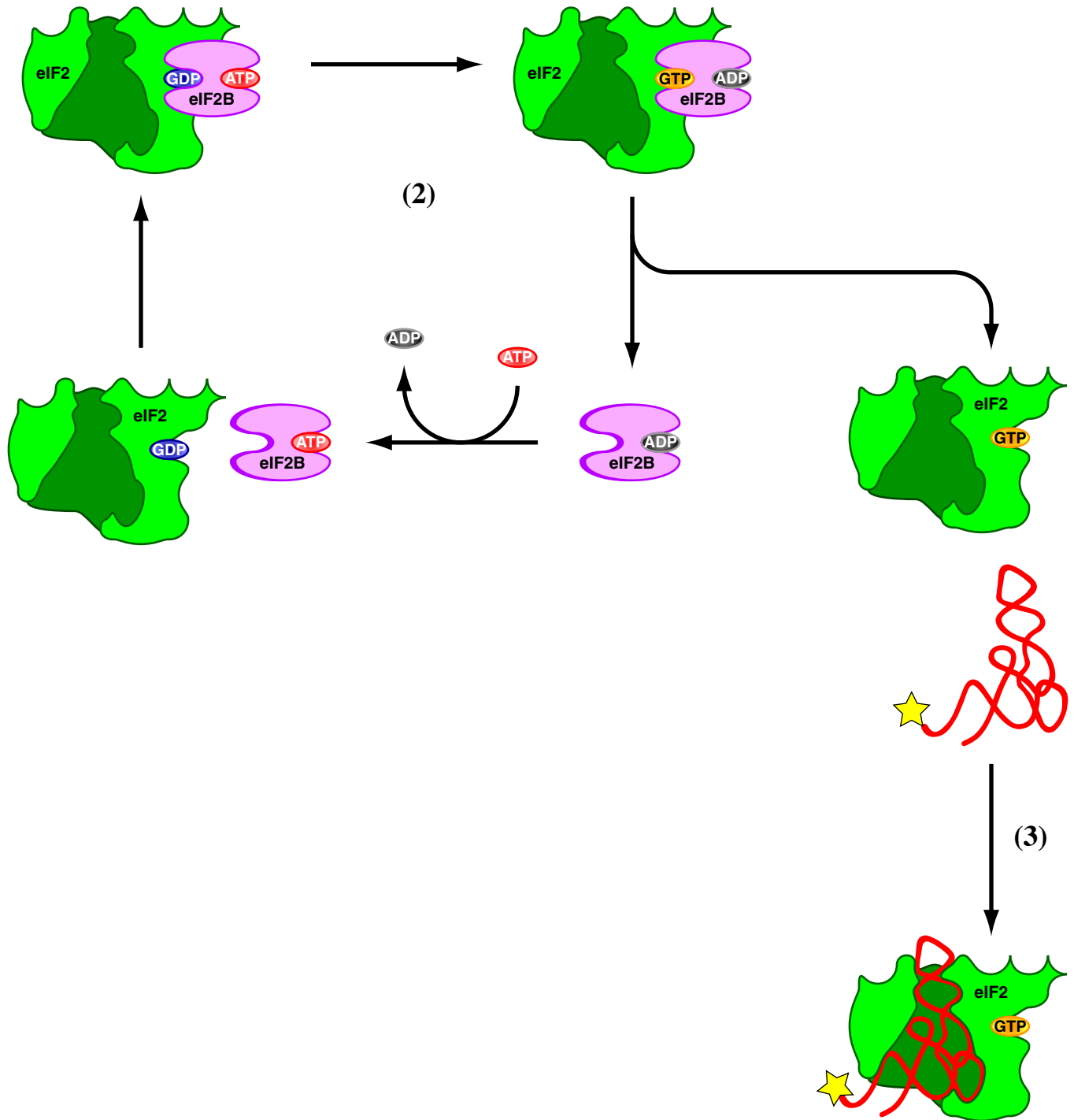

2. The **eIF2**•GTP complex, binds to the Met-tRNA$_i^{Met}$ to form an **eIF2**•GTP-Met-tRNA$_i^{Met}$ complex.

3. **eIF6** binds to the ribosome and dissociates inactive ribosomes into small and large subunits. **eIF6**

prevents the premature (re)binding of the large subunit to the small subunit or to the partially assembled initiation complex.
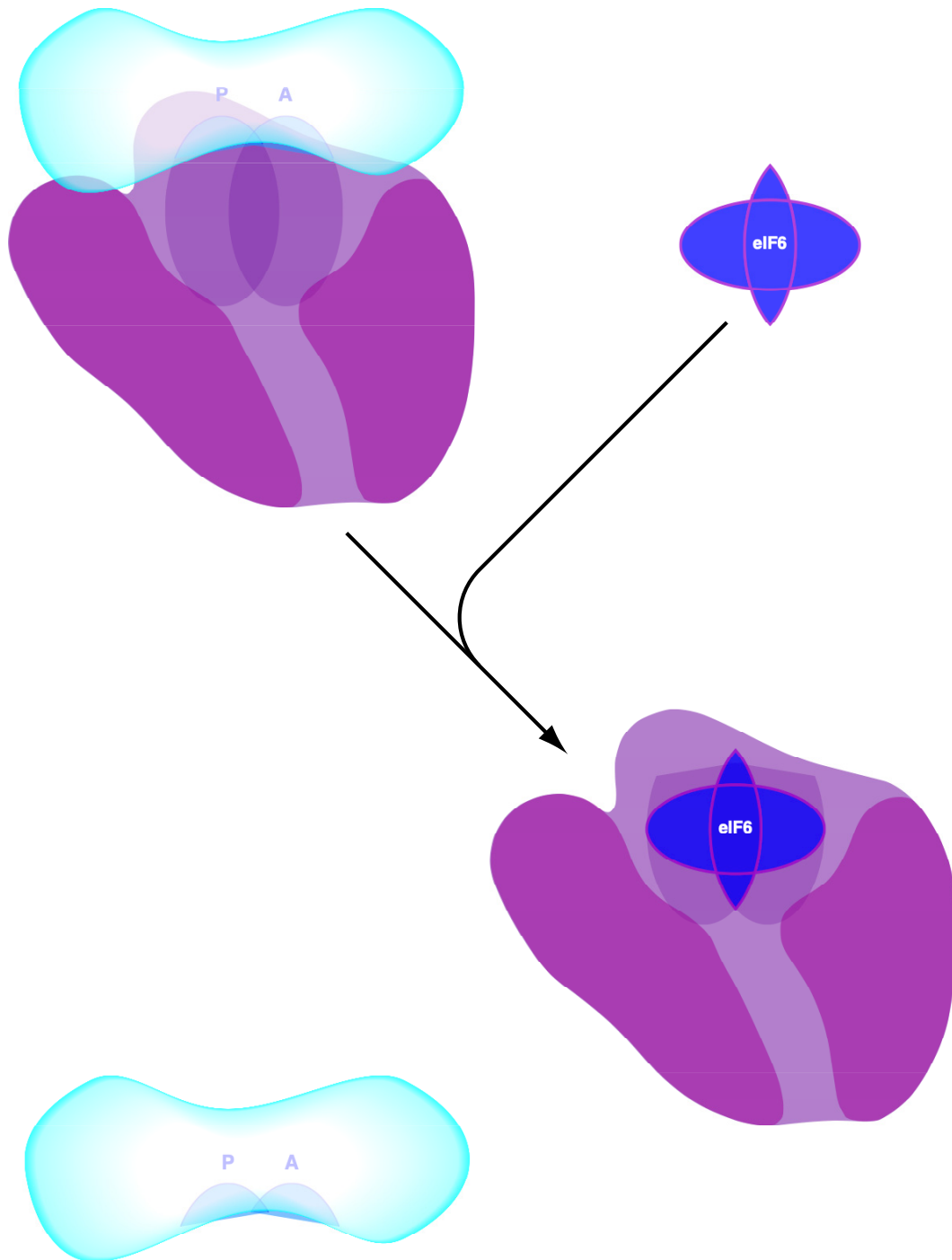

4. **eIF1**, **eIF1A**, and **eIF3** bind to the 40S subunit. **eIF1** binds at or near the P SITE and **eIF1A** binds at or near the A SITE. All three of these initiation factor prevents ribosome re-association in the absence of an initiation complex. **eIF3** acts as a scaffold for the assembly of subsequent initiation factors.
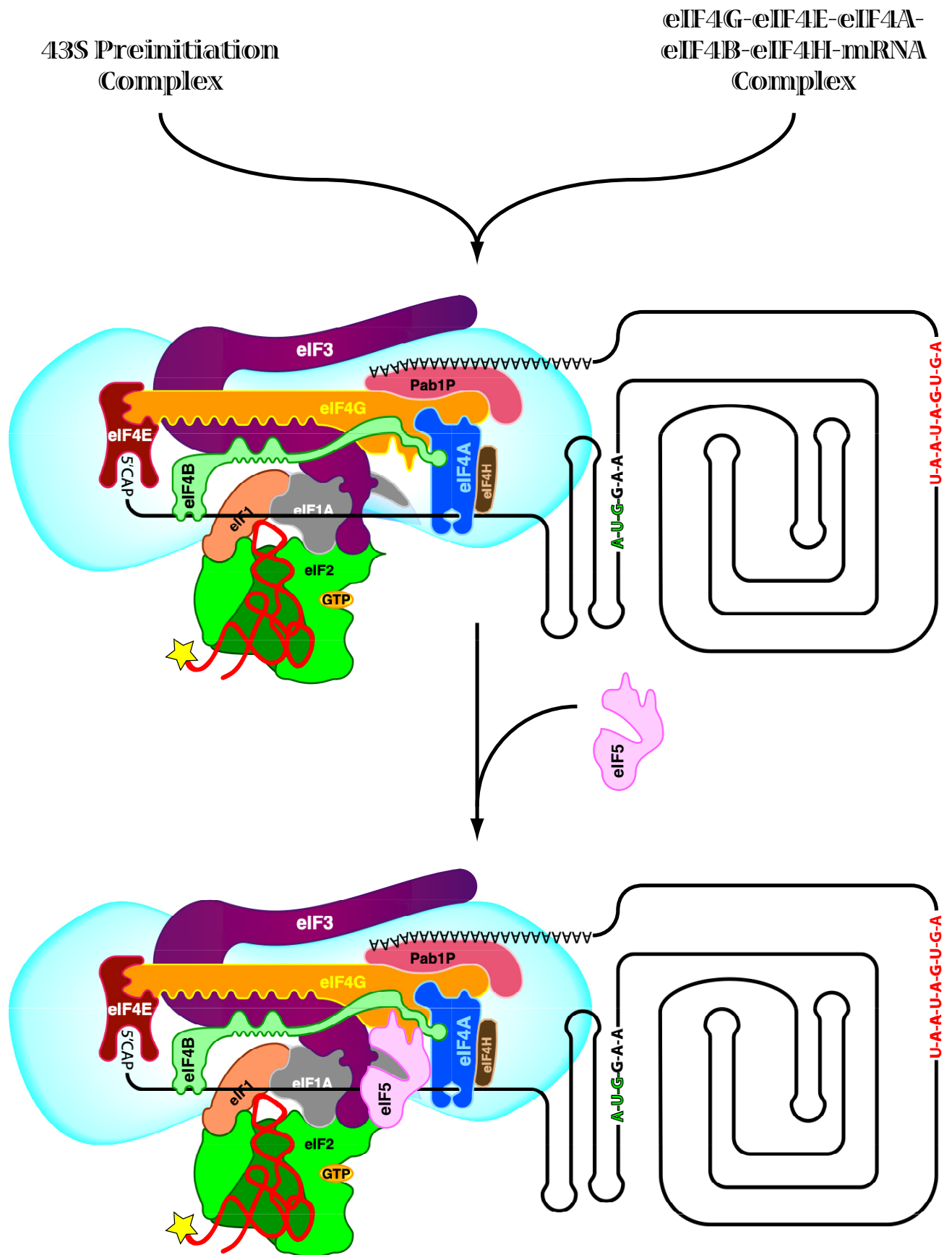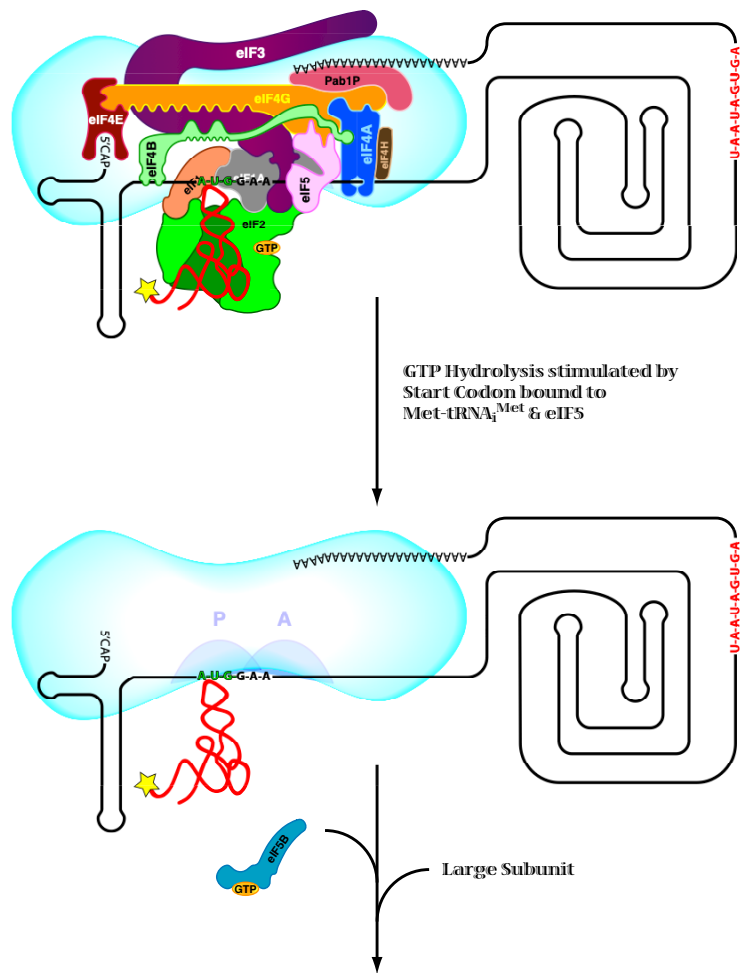
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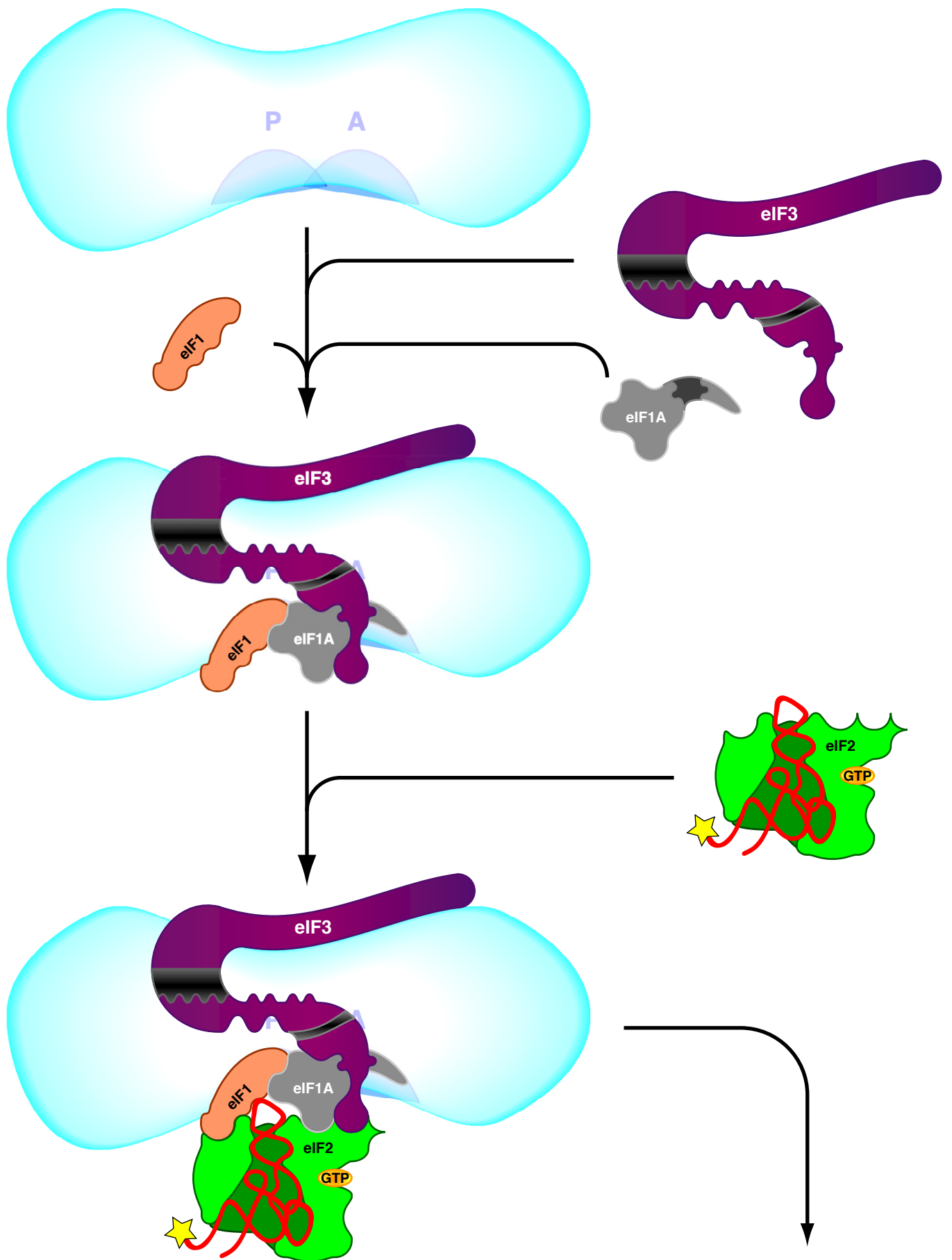
5. **eIF1A** facilitates/mediates the binding of **eIF2**•GTP-Met-tRNA$_i^{Met}$ to the 40S ribosomal subunit. The resulting complex is termed the 43S preinitiation complex. 43S refers to its sedimentary velocity.

6. **eIF4E** and **eIF4G** bind to each other and then **eIF4A** binds. The complex of **eIF4E-eIF4G-eIF4A** is called **eIF4F**. **eIF4F** (**eIF4E-eIF4G-eIF4A**) is the CAP BINDING PROTEIN (CBP). **eIF4E** binds the 7-methylguanosine cap structure at the 5′ end of the message. **eIF4A** is an ATP dependent helicase that "melts" any secondary structures in the mRNA that would prevent mRNA binding to the initiation complex. Both **eIF4E** and **eIF4G** cooperate with **eIF4A** for mRNA "melting".
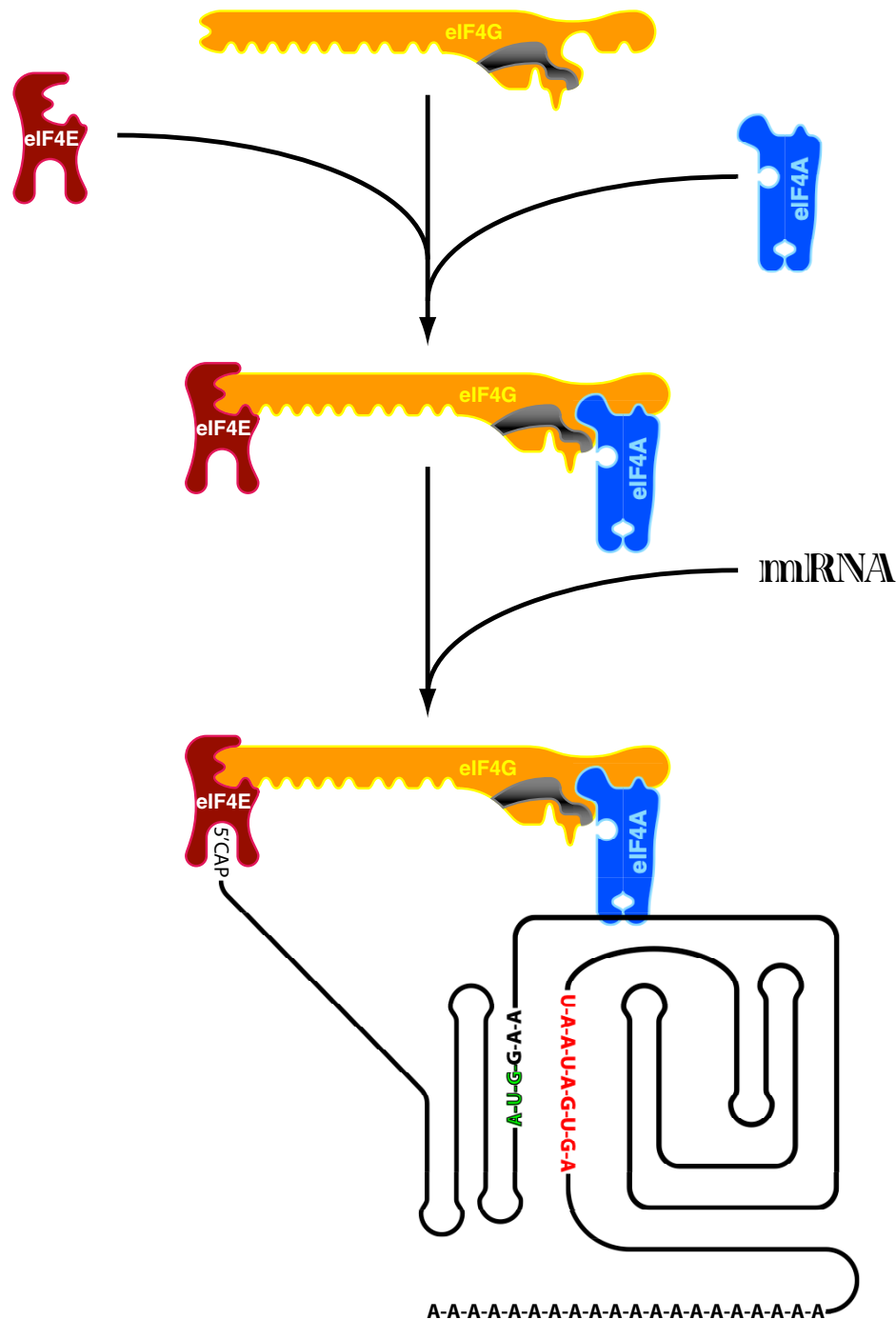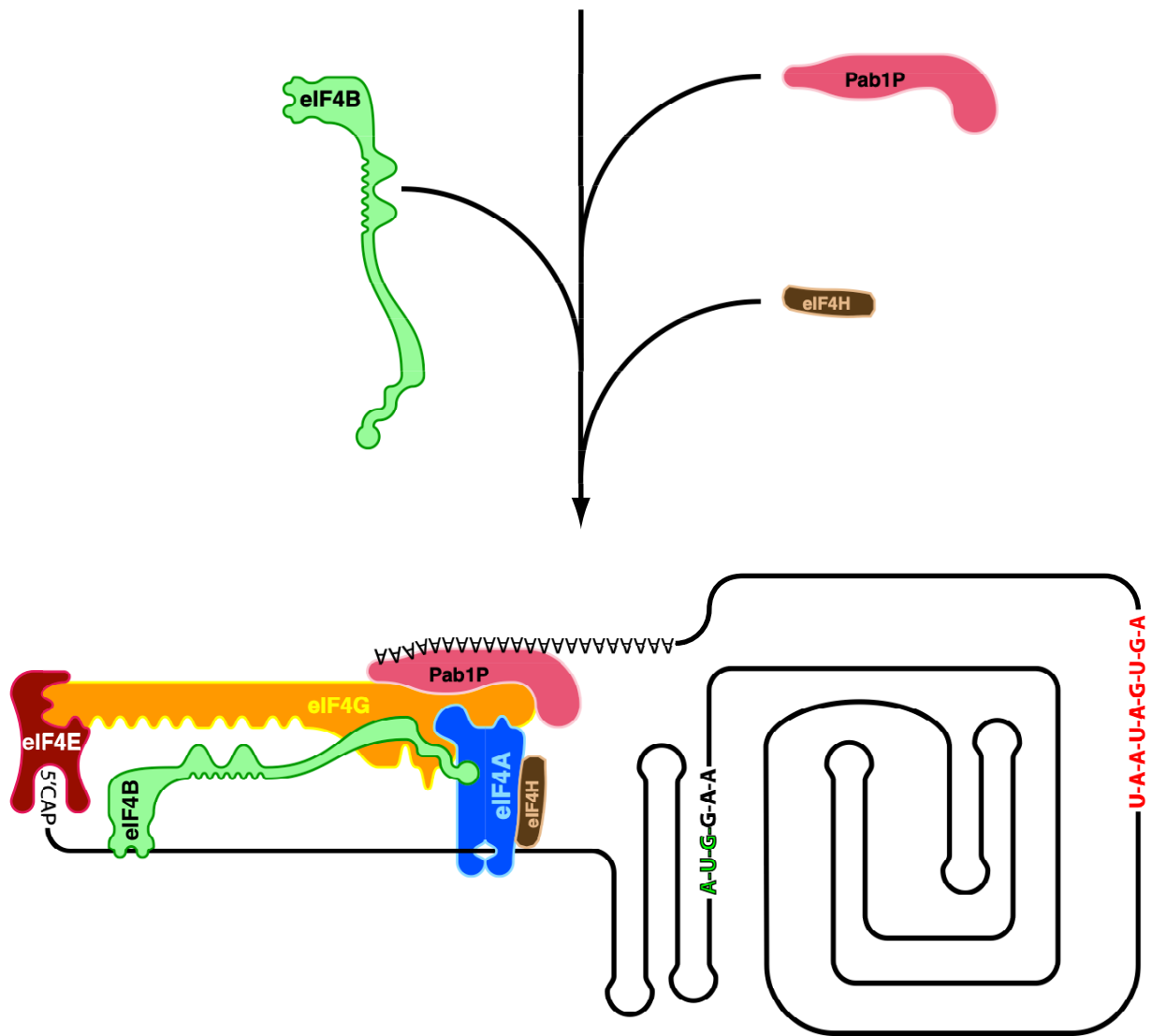
7. **POLY A BINDING PROTEIN** (**Pab1P**) binds to the poly A tail of mRNA and to **eIF4G**. The 7-methyl-GTP cap and the poly A tail act synergistically to increase translational efficiency. **eIF4G** serves as a bridge between **eIF4E** bound to the cap and Pab1P bound to the poly A tail.

8. **eIF4B** and **eIF4H** binds to the **eIF4E-eIF4G-eIF4A** complex after it has bound the cap and poly A tail.

9. **eIF4B** delivers the **eIF4E-eIF4G-eIF4A-eIF4B-eIF4H**-mRNA complex to the 43S preinitiation complex and facilitates **eIF4G** binding to the **eIF3** already associated with the 40S subunit. **eIF4G** serves as a bridge between **eIF4E** bound to the cap, the poly A tail, and **eIF3** bound to the 40S subunit.

10. **eIF5** joins the growing initiation complex.

11. The 40S subunit now scans down the mRNA in search of the AUG start codon. Scanning is an ATP requiring process. **eIF4A-eIF4G-eIF4H** complex utilizes the ATP to melt secondary structures in the mRNA and facilitates the scanning process.

12. When the complex stops at the AUG start codon, hydrolysis of the GTP that is part of the **eIF2**•GTP-Met-tRNA$_i^{Met}$ complex causes ejection of the initiation factors bound to the 40S subunit. **eIF5** promotes the GTPase activity of **eIF2** and the release of the eIFs.

43S Preinitiation Complex

eIF4G-eIF4E-eIF4A-eIF4B-eIF4H-mRNA Complex

eIF3

Pab1P

eIF4G

eIF4E

eIF4A

eIF4H

5'CAP

eIF4B

eIF1

eIF1A

eIF2

GTP

A-U-G-A-A

U-A-A-U-A-G-U-G-A

eIF5

13. **eIF5B** and GTP bind to the small subunit complex. The large subunit enters. GTP is hydrolyzed to GDP and phosphate. The energy released causes the expulsion if **eIF5B** and **eIF6** and allows the 60S subunit to associate to form the 80S initiation complex.
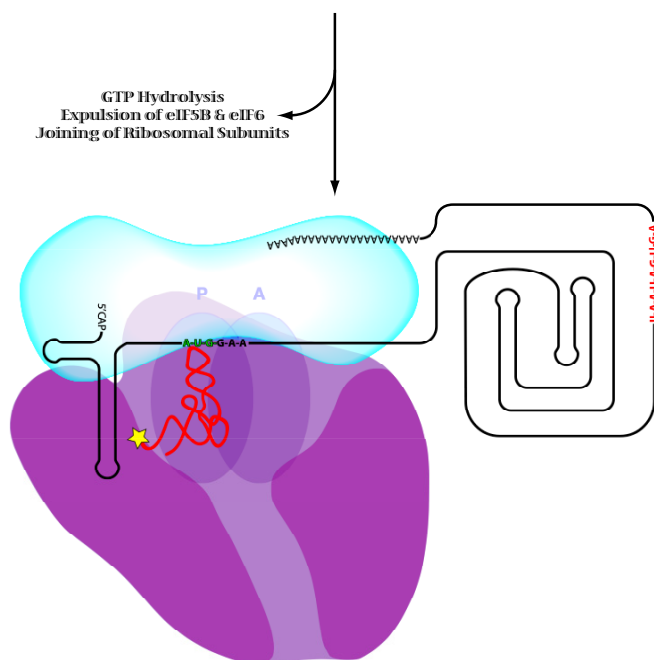
In summary there are 2 major differences in the initiation of translation in bacteria compared to eukaryotes.

1. Three initiation factors in bacteria, at least 12 in eukaryotes
2. In bacteria, the message has an affinity for the small ribosomal subunit and binds to the ribosome first. The Shine-Dalgarno sequence on the message base pairs with a sequence on the rRNA of the small subunit. This base pairing orients the start codon in the P Site of the ribosome. After the message is bound then the Initiator tRNA binds. In eukaryotes the Initiator tRNA is brought to the ribosome first. The small subunit of the ribosome has no affinity for message. Initiation factors bind to the ends of the mRNA and deliver it to the ribosome. These same initiation factors then use the bound Initiator tRNA to find the start codon on the mRNA and set the reading frame.

<center>Elongation in Eukaryotes</center>

Elongation in eukaryotes occurs by an identical sequence of steps. Eukaryotes contain only two elongation factors **eEF1** and **eEF2**. **eEF1** is a multisubunit protein composed of three subunits, **eEF1α**, **eEF1β**, and **eEF1γ**. **eEF1α** is the eukaryotic counterpart of bacterial **EF-Tu**. **eEF1βγ** serves the same function as **EF-Ts**. **eEF2** functions in translocation like its bacterial kin **EF-G**.

<center>Termination in Eukaryotes</center>

In eukaryotes the termination process is similar. However, release factor, **eRF1** takes the place of **RF1** and **RF2**, this factor recognizes all three termination codons. **eRF1** binds and changes the specificity of Peptidyl Transferase for the release of the completed protein. **eRF3**•GTP complex then binds and from this point on termination is similar to that seen with bacteria with one exception. Eukaryotes do not contain a RIBOSOMAL RECYCLING FACTOR so the mechanism for dissociation of the ribosome and release of the mRNA is unknown.

<center>Control of Translation in Eukaryotes</center>

Transcription and translation are tightly coupled in bacteria. Since the mRNA in bacteria requires no processing translation is often initiated before transcription is terminated. The control point of protein synthesis in bacteria is at the level of transcription. Eukaryotes control protein synthesis at both the transcriptional and translational level. Transcripts in the eukaryotic nucleus must be processed and transported into the cytoplasm for translation, a process that can take up to hours. When a rapid increase in protein production is needed in eukaryotes, a translationally repressed mRNA already in the cytoplasm can be activated without delay. Eukaryotes have at least four mechanisms for translational regulation.

I. Ribosomal proteins and initiation factors are subject to phosphorylation by a number of different protein kinases.
   A. Phosphorylation of 40S ribosomal protein S6 facilitates translation by shifting the ribosomal population from inactive ribosomes to actively translating polysomes.
   B. The action of **eIF4F**, the cap binding complex, is promoted by phosphorylation.
   C. Phosphorylation of **eIF2** inhibits protein chain initiation because the phosphorylated **eIF2** binds to **eIF2B** much more tightly than the dephosphorylated (unphosphorylated) form. With **eIF2B** bound, **eIF2**•GTP cannot be regenerated from the **eIF2**•GDP complex for additional

cycles of initiation.

    D. The activity of **eIF2B** and **eIF3** are also inhibited by phosphorylation.

II.   Inhibitory proteins bind directly to mRNA and act as translational repressors. Many of them bind to specific sites in the 3′ untranslated region. When bound to the mRNA, these proteins interact with other translation initiation factors (e.g., POLY A BINDING PROTEIN) and prevent translation initiation.

III. Several proteins disrupt the interaction between **eIF4E** and **eIF4G**. These proteins are called **eIF4E** BINDING PROTEINS (**4E-BP**). During periods of slow cell growth, these proteins limit translation by binding to the site on **eIF4E** that normally interacts with **eIF4G**. When cell growth resumes or increases the binding proteins are inactivated by protein kinase dependent phosphorylation.

IV. Gene Silencing occurs by RNA Interference. A small RNAi molecule binds to (base pairs with) an mRNA at a 3′ untranslated region and once bound either stimulates mRNA degradation or inhibits translation. This type of gene regulation controls developmental timing in some organisms and the RNAi is sometimes called Small Temporal RNA (stRNA). RNAi are transcribed as precursor RNA's with internal complementarity allowing for the formation of hairpin structures. The precursor RNA's are cleaved into small pieces (20 to 25 bases) by endonucleases. The best characterized endonuclease of this type is called DICER. One strand of the processed RNA is then transferred to the mRNA that will be silenced.

Energy Cost

Protein synthesis is a very energy expensive process. During initiation the one molecule of GTP on eIF2 and numerous ATP molecules to "melt" secondary structures in the mRNA are hydrolyzed. On average, four high energy phosphate bonds are required for the formation of each peptide bond in a protein. Two high energy bonds are used during tRNA charging reaction, the attachment of the amino acid to the tRNA, and two are used during each elongation cycle. GTP is also hydrolyzed during the process of termination and release. This energy expenditure accounts for up to 75% of all energy usage in eukaryotes. With this large energy expense it is surprising that proteins survive only a short time in the cell. Half lives for intracellular and extracellular proteins are on the order of minutes to weeks.

Inhibitors (Xenobiotics, Antibiotics and/or Toxins)

Many of the medicinally useful antibiotics inhibit steps in the translation process

Streptomycin inhibits initiation in bacteria and causes misreading of the mRNA.

Chloramphenicol binds to the 50S subunit and inhibits Peptidyl Transferase.

Tetracycline binds to the 30S subunit and inhibits tRNA binding to the ribosome.

Erythromycin binds to the 50S subunit and inhibits Peptidyl Transferase.

Cycloheximide inhibits translocation of peptidyl-tRNA in eukaryotes. It is used as an antivirus and a cancer chemotherapeutic agent.

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Puromycin is an aminoacyl-tRNA analog which acts as a peptidyl acceptor stopping further peptide elongation. It is toxic to bacterial and eukaryotic cells.

Diphtheria Toxin inactivates eEF2.

Ricin isolated from the castor bean (*Ricinus communis*) catalytically inactivates the 28S rRNA of eukaryotic ribosomes. A single molecule of Ricin can rapidly inactivate 50000 ribosomes and kill a eukaryotic cell.

Mutations Revisited

POLYMORPHISMS are present in a small percentage of the population and are base changes in the nucleotide sequence of a particular gene. The base change in a polymorphism results in little or no significant change in the structure and function of the resulting protein. Most polymorphisms result from a MISSENSE MUTATION where one base is substituted by another different base. In a polymorphism the base change results in no change in the protein (due to the degenerate nature of the code) or it results in the substitution of one amino acid by a different amino acid with similar physical and chemical properties. For example the substitution of Leu by Ile would result in minimal changes in protein structure and function, as would the substitution of Asp for Glu or Lys for Arg. Individuals carrying a polymorphism show no clinical signs or symptoms.

A TRUE MUTATION is likewise a base change in a particular gene present in a small percentage of the population. In a true mutation the base change results in a significant deleterious change in the structure and function of the resulting protein. The diminished function or nonfunctional protein causes some significant clinical sign or symptom. Some mutations are the result of a base change that causes the substitution of one amino acid for another. This type of mutation is also a MISSENSE MUTATION. When a mutation causes a change from a codon that specifies an amino acid to a termination (stop) codon, the mutation is called a NONSENSE MUTATION. This type of mutation results in premature termination of protein synthesis and a short nonfunctional protein. Some mutations involve the insertion or deletion of one or more nucleotides. The insertion or deletion of a nucleotide changes the reading frame downstream from the mutation. This type of mutation is called a FRAMESHIFT MUTATION and always results in a nonfunctional protein.

Protein Modification

As translocation proceeds, 25 to 40 of the amino acids most recently added to the growing peptide reside in the tunnel of the ribosome. The remaining amino acids, the amino terminal amino acids, have been extruded into the cytoplasm. These N-terminal residues begin to fold into a "native" three dimensional structure. As residues emerge from the ribosome they can be acted upon by enzymes that modify the protein into its final functional form.

Protein modifications that occur before chain elongation is complete are called CO-TRANSLATIONAL MODIFICATIONS. Those that occur after elongation is complete are called POST-TRANSLATIONAL MODIFICATIONS.

Some examples of the hundreds of possible modifications include removal of the initiating N-terminal

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methionine residue, disulfide bond formation, prosthetic group attachment, and glycosylation of integral membrane proteins and secreted proteins.

One of the most important CO-TRANSLATIONAL events is the processing and transport of proteins through membranes. Protein synthesis occurs in the cytoplasm, but many proteins are located on the outside of the cell membrane, localized in subcellular organelles such as lysosomes & mitochondria, and/or are secreted from the cell. In each of these cases the protein must be transported across a cellular membrane.
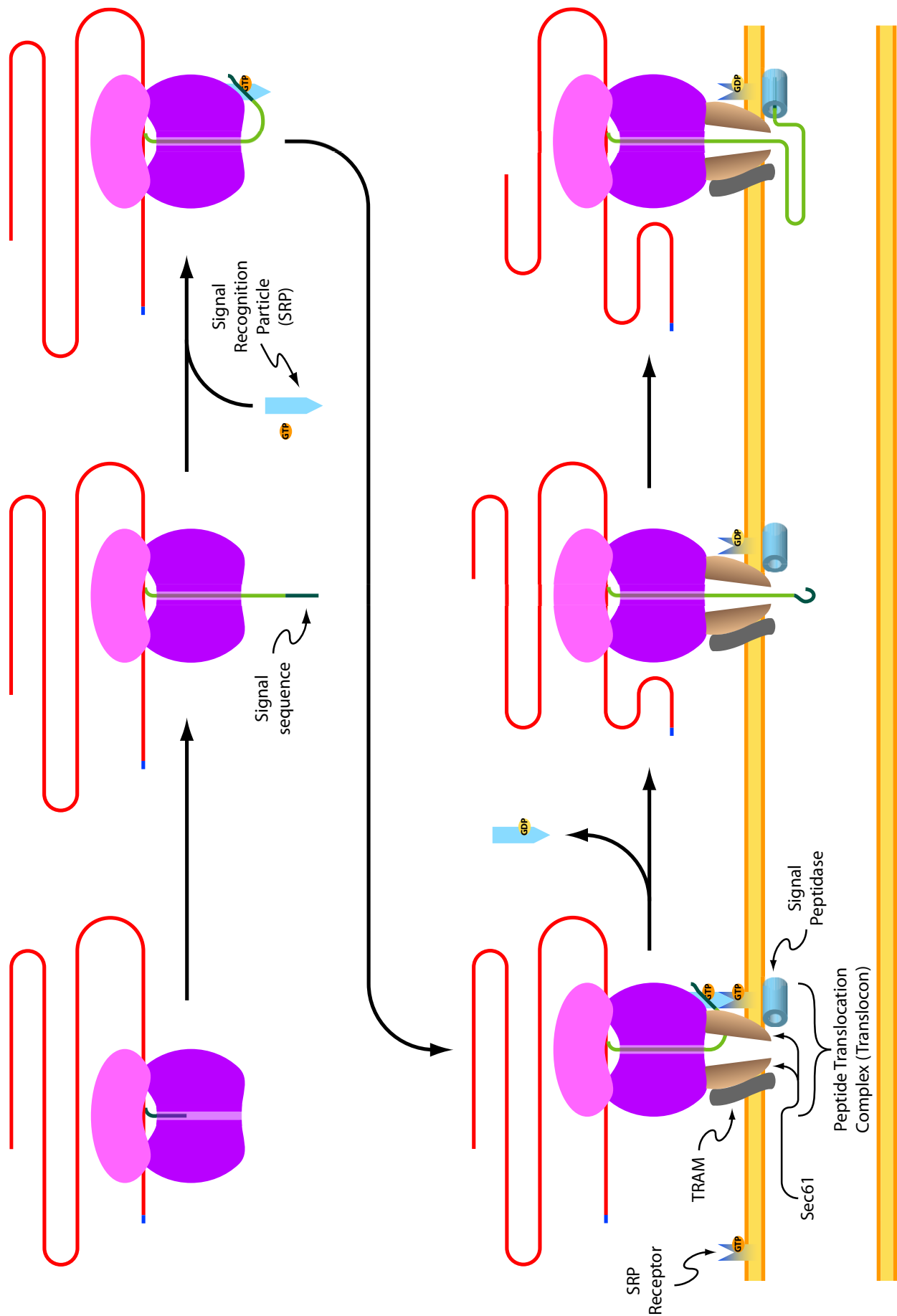
In eukaryotes, proteins destined for insertion into cellular membranes and/or secretion are transported across the membrane of the endoplasmic reticulum into its lumen. The lumen of the endoplasmic reticulum provides a network of channels through which proteins can travel on their way to the plasma membrane and/ or other cellular organelles. The newly synthesized proteins contain "marker sequences" that direct them to specific cellular locations, a phenomenon known as PROTEIN TARGETING. From the endoplasmic reticulum proteins are transported into the Golgi apparatus. In the Golgi carbohydrate is added to the newly synthesized proteins. Other post-translational modifications occur in the Golgi, but the primary function of the Golgi is glycosylation of proteins.

<center>Entry of Proteins Into the Endoplasmic Reticulum</center>

The first 20 or so amino acids of the newly synthesized protein are important for protein targeting. These residues indicate that the protein is to enter the endoplasmic reticulum. Once in the ER they are removed to form the mature amino terminus of the functional protein. This N-terminal sequence of amino acids that targets a protein to the ER is called the SIGNAL PEPTIDE since it signals to the translation apparatus that the protein being synthesized is to cross a membrane.

The SIGNAL PEPTIDE is 16 to 30 amino acids in length and contains a majority of hydrophobic amino acids - Val, Leu, Ile, and Phe. The hydrophobic nature of the peptide is more important than the precise sequence of amino acids.

As the ribosome and associated factors translate the mRNA into protein, the SIGNAL PEPTIDE is the first region synthesized and extruded from the tunnel of the ribosome. The extruded SIGNAL PEPTIDE is bound by a protein-RNA complex called the SIGNAL RECOGNITION PARTICLE (SRP). After binding to the SIGNAL PEPTIDE, SRP binds GTP and further translation is blocked. The ribosome-SRP•GTP complex migrates, is directed through the cytoplasm until it encounters the PEPTIDE TRANSLOCATING COMPLEX or The TRANSLOCON. The Translocon complex consists of four proteins, SRP RECEPTOR, SEC61, TRANSLOCATING CHAIN-ASSOCIATED MEMBRANE PROTEIN (TRAM), and Signal Peptidase. SRP RECEPTOR (also known as the DOCKING PROTEIN), is present on the cytosolic side of the ER. SRP RECEPTOR binds the ribosome-SRP complex and positions the ribosome over SEC61 (also called RIBOPHORIN). The ribosome becomes anchored to the ER by binding to SEC61. Once the ribosome-SRP complex is bound to the membrane, SRP is released in a reaction coupled to GTP hydrolysis, and the inhibition of translation is released. The SIGNAL PEPTIDE passes through the membrane of the ER by a pore that is part of the SEC61 protein complex. As translation continues the growing polypeptide chain is extruded through the membrane and the SIGNAL PEPTIDE is hydrolytically removed by Signal Peptidase. When translation terminates all of the new protein is in the lumen of the ER and the ribosome dissociates from the ER.

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Signal Recognition Particle (SRP)

Signal sequence

SRP Receptor

Signal Peptidase

Peptide Translocation Complex (Translocon)

TRAM

Sec61

29

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The core polysaccharide of an N-linked polysaccharide is synthesized on a lipid molecule that is embedded in the membrane of the Endoplasmic Reticulum. The lipid molecule is the isoprenoid **Dolichol phosphate**.

Dolichol phosphate

$$n = 9 \text{ to } 22$$
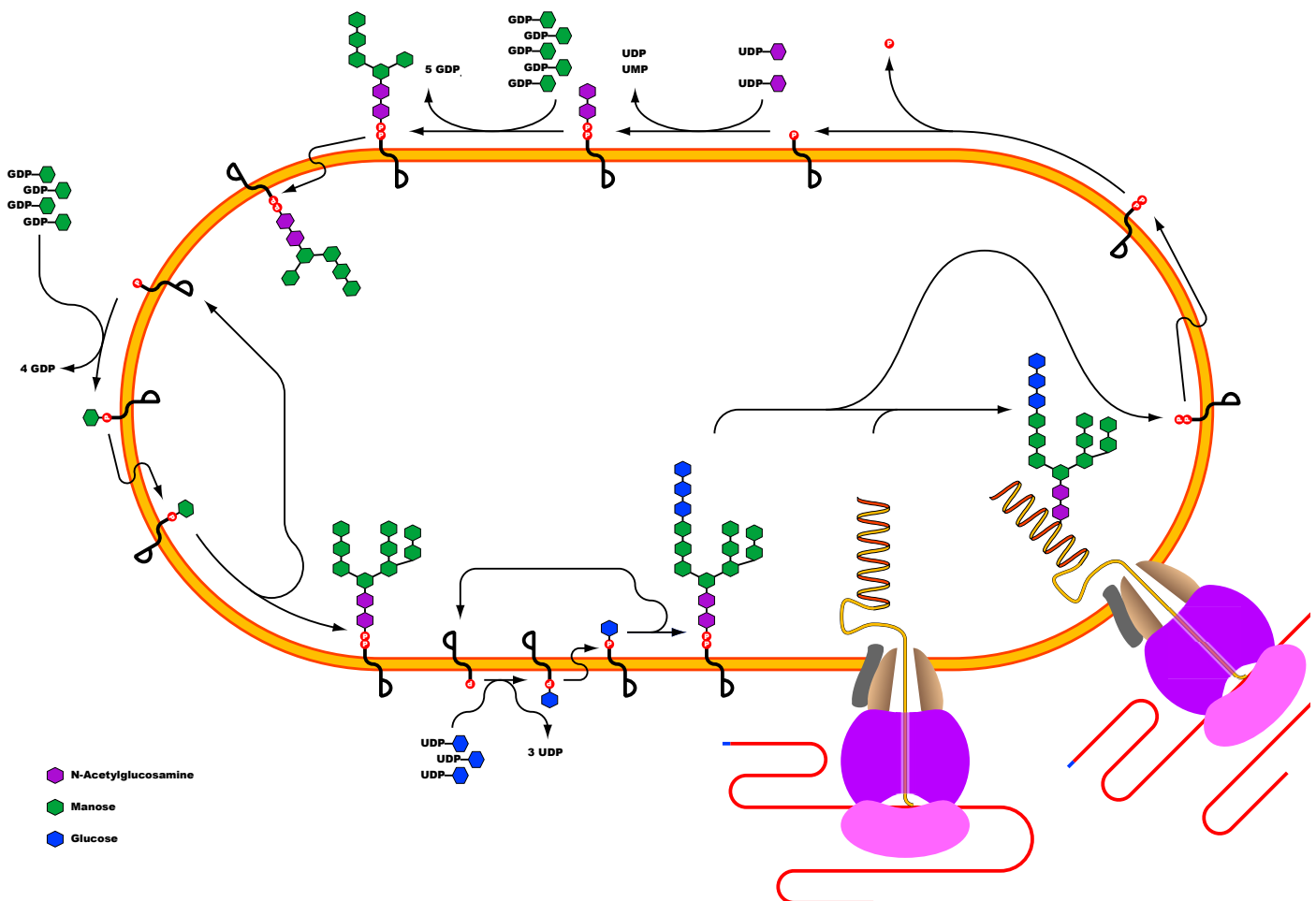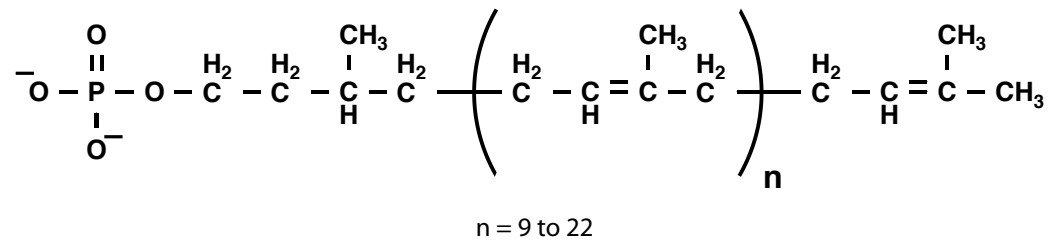
In the first step of the synthesis of the core polysaccharide N-acetyl glucosamine phosphate is transferred to the dolichol phosphate from UDP-N-acetyl glucosamine liberating UMP. A second N-acetyl glucosamine (GlcNAc) donated by UDP-N-acetyl glucosamine is added to the first. A branched structure of 5 mannose residues, donated by GDP-mannose, is then added. The first seven reactions of core polysaccharide

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synthesis occurs on the cytoplasmic face of the ER and each of the sugar residues is added by the action of a specific Glycosyltransferase. A "Translocase" has been postulated to "flip" the partially synthesized core polysaccharide from the cytoplasmic face of the ER to the lumenal face.

Once in the lumen of the ER specific Glycosyltransferases add four mannose residues and three glucose residues to complete the CORE STRUCTURE. The sugars added to the core structure in the ER are activated in the cytoplasm by coupling them to nucleotides (GTP for Mannose, UTP for Glucose) and then the activated sugars are passed to **dolichol phosphate** present on the cytoplasmic surface of the ER. The "Translocase" flips the dolichol phosphate sugars into the lumen of the ER where they are substrates for the Glycosyltransferases in the lumen of the ER. An Oligosaccharide Transferase Complex catalyzes the transfer of the core polysaccharide from dolichol pyrophosphate to (a) specific Asparagine (Asn) residue(s) on the protein. The Oligosaccharide Transferase Complex recognizes the sequences Asn-X-Ser or Asn-X-Thr as acceptor sites, X can be any amino acid except Pro. Once attached to the protein two of the three terminal glucose residues are removed forming binding site(s) for the Ca^{2+} requiring chaperones Calnexin (membrane bound) and/or Calreticulin (lumen). These proteins guide protein folding. Once properly folded, the last glucose residue is removed and the protein is transferred to the Golgi for additional processing. The resulting final polysaccharide can be of the high mannose type, the complex type, or the mixed type.

Some of the newly synthesized N-linked glycoproteins contain a three-branch oligosaccharide in which sialic acid (N-acetylneuraminic acid) residues cap galactose residues. Many/Most of the glycoproteins of this type are plasma proteins. As these glycoproteins circulate in the plasma, enzymes on the blood vessel walls randomly cleave off the sialic acid groups, exposing the galactose residues below. The number of sialic acid groups removed increases as the time in the circulation increases. When all three sialic acid groups have been removed the **asialoglycoprotein receptor**, in the liver, binds to the exposed galactose residues with very high affinity. The complex of receptor and glycoprotein is taken into the liver cell by endocytosis and the glycoprotein is degraded in cellular lysosomes.

O-Linked Polysaccharides

O-Linked polysaccharides are synthesized by a mechanism different from that used for the synthesis N-Linked polysaccharides. O-Linked polysaccharides are linked to Ser or Thr by a glycosidic bond to N-acetylgalactosamine. O-Linked polysaccharides are synthesized and N-Linked polysaccharides are modified in the Golgi apparatus. O-Linked polysaccharides are built up directly on the protein one sugar residue at a time. Nucleotide diphosphate Sugars (most sugars are coupled to UTP forming UDP-sugars; mannose is coupled to GTP forming GDP-mannose) are the source of the individual sugar moieties and the synthesis of the heteropolysaccharide is catalyzed by numerous specific Glycosyltransferases. The Golgi transports Nucleotide diphosphate Sugars from the cytoplasm, their site of synthesis, by an antiport mechanism. A nucleotide diphosphate sugar is transported in as a nucleotide diphosphate is transported back into the cytoplasm. The ER membrane does not contain this antiport system which explains why the ER uses dolichol phosphate sugars for glycoprotein synthesis in the lumen.

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