Unit 7: Translation (8 lectures)

Ribosome structure and assembly, mRNA; charging of tRNA, aminoacyl tRNA synthetases; various steps in protein synthesis, proteins involved in initiation, elongation and termination of polypeptides; fidelity of translation; inhibitors of protein synthesis; post-translational modifications of proteins.

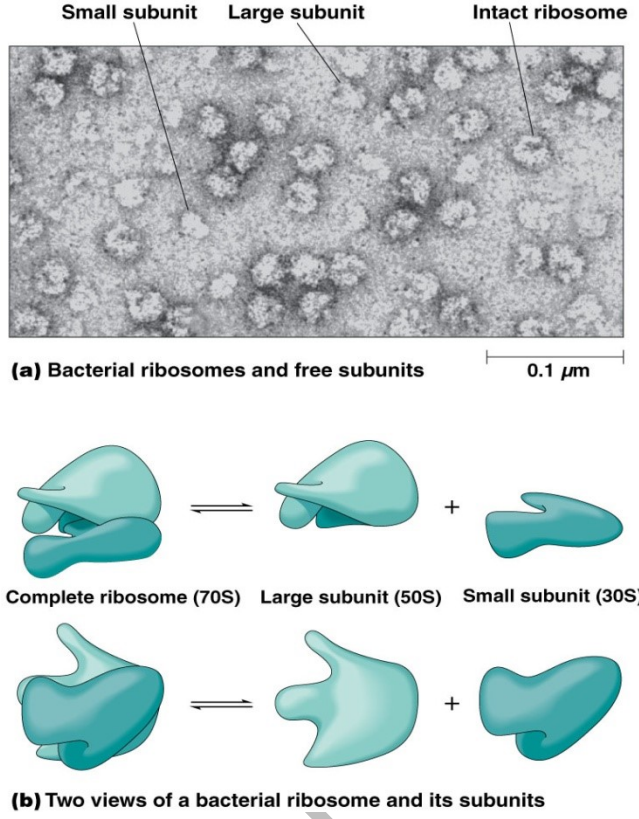
Protein Synthesis

Translation is the first & most important part of protein synthesis.

Translation is a well-conserved process among prokaryotes and eukaryotes.

Additional processing and assembly is often required to modify the proteins.

Ribosomes catalyze the joining of the amino acid monomers directed by the mRNA sequence.



(a) Bacterial ribosomes and free subunits

(b) Two views of a bacterial ribosome and its subunits

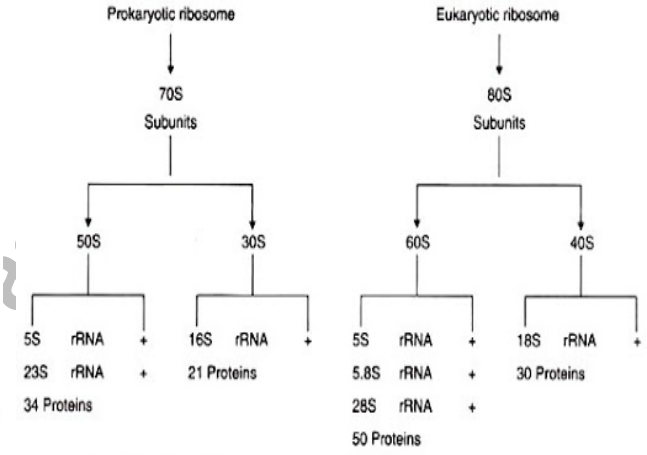


Fig. 15.18 Comparison between prokaryotic and eukaryotic ribosomes.

Amino-acyl tRNA synthetases attach amino acids to the appropriate tRNAs.

The amino-acyl tRNA act as adaptors in the translation of the nucleic acid sequence of the mRNA into the amino acid sequence of the protein.

Translation occurs in three stages...

1) In initiation, the components of the translational apparatus come together with the mRNA molecule (A tRNA carrying the first amino acid binds to the start codon).

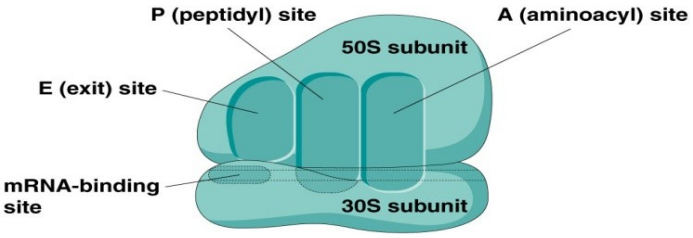
2) In elongation, amino acids are brought to the mRNA as amino-acyl tRNAs and are added one at a time to a growing polypeptide chain.

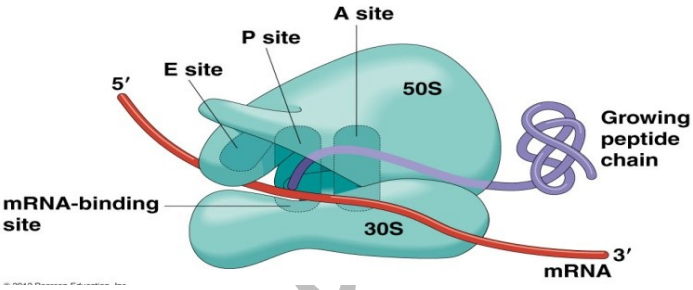
3) In termination, a stop codon in the mRNA is recognized by a protein release factor and the translational apparatus comes apart to release a completed polypeptide.

The Tools of Translation

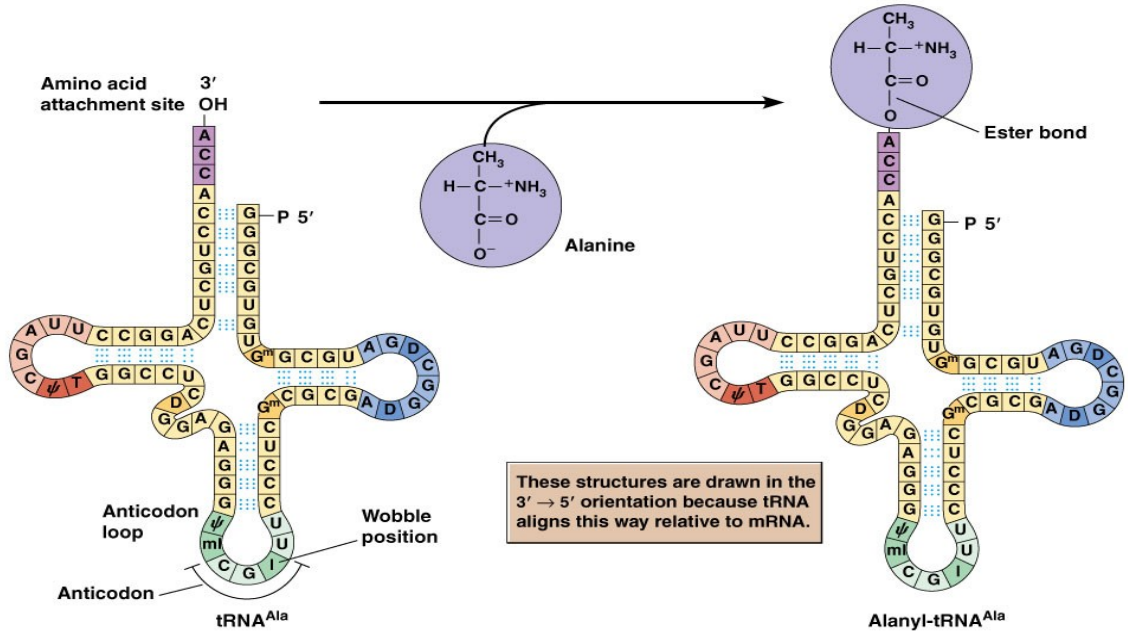
1.RIBOSOME

- The model of ribosome structure shows the A (aminoacyl) and P (peptidyl) sites as cavities on the ribosome where charged tRNA (carrying an amino acid) molecules bind during polypeptide synthesis. The recently postulated E (exit) site is the site from which discharged tRNAs leave the ribosome.
- The mRNA-binding site binds a sequence near the 5 prime end of the mRNA, placing the mRNA in the proper position for the translation of its first codon .
- The binding sites are all located at or near the interface between the large and small subunits.

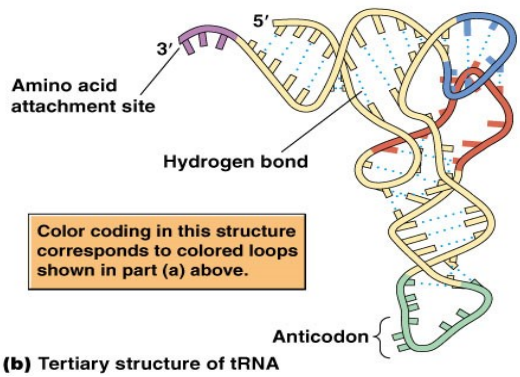




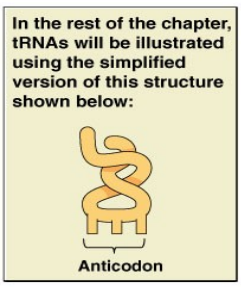
2.tRNA

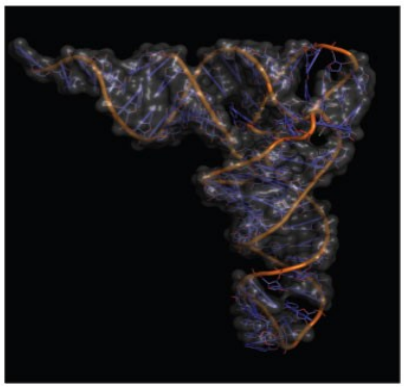


(a) Secondary structure of tRNA, before and after amino acid attachment

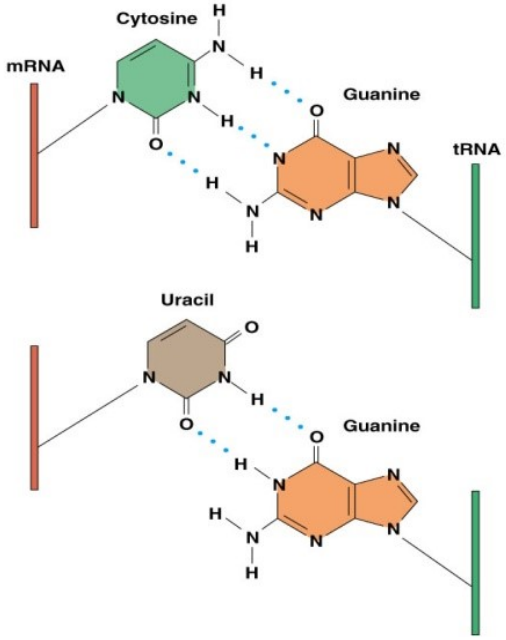


(b) Tertiary structure of tRNA



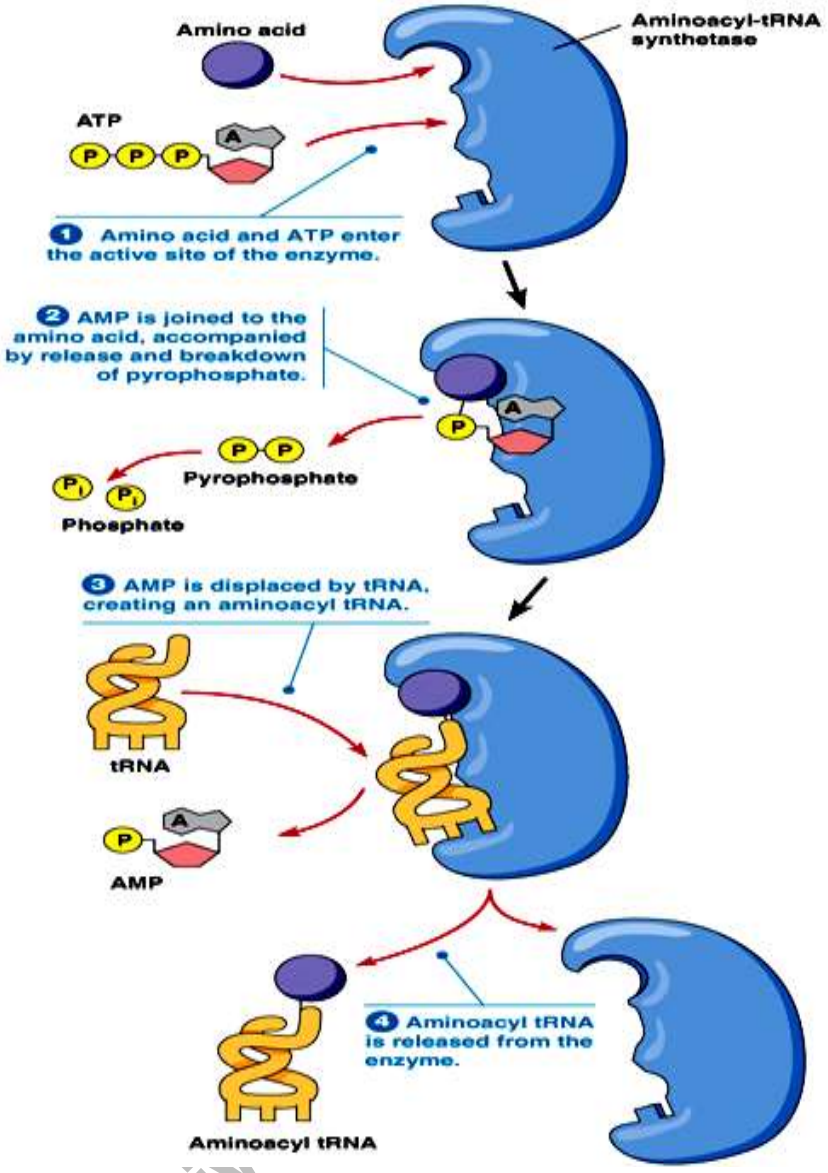


tRNA molecules contain four arms, consisting of four base-paired stem regions and three major loops.
1)The anticodon arm containing a triplet anticodon which binds the mRNA
2)An accepter arm containing a 3 prime terminal sequence of CCA (where the appropriate amino acid can be attached by an ester bond).
3) A pseudouridine/T (ψ)C arm.
4)A dihydrouridine (D) arm and a variable region.
During maturation of the tRNA molecule a number of nucleotides are modified in tRNA specific ways.
The modified nucleotides in the tRNA structure are inosine (I), methylinosine (mI), dihydrouridine (D), ribothymidine (T), pseudouridine (ψ) and methylguanosine (G^m).
The 3D structure of tRNA molecules is similar to a hockey stick (the L shaped tertiary structure of tRNA) which has the amino acid attachment site is at one end (tip of the handle) and the anticodon at the other (blade of the stick).
Twenty different aminoacyl-tRNA synthetases link amino acids to the correct tRNAs.
Some recognize only one tRNA, some recognize a few because of the redundancy in the genetic code.
Although there are 61 possible codons, there are far fewer tRNAs.
A number of codons that encode the same amino acid differ only in the third position of the codon.
A slight shift or "wobble" in the position of the base guanine in a tRNA anticodon would permit it to pair with uracil instead of its normal complementary base (cytosine).
The base pairs permitted at the third position of a codon by the wobble hypothesis are as follows:



Bases Recognized in Codon (third position only)	Base in Anticodon
U	A
G	C
A or G	U
C or U	G
U, C, or A	I (Inosine)

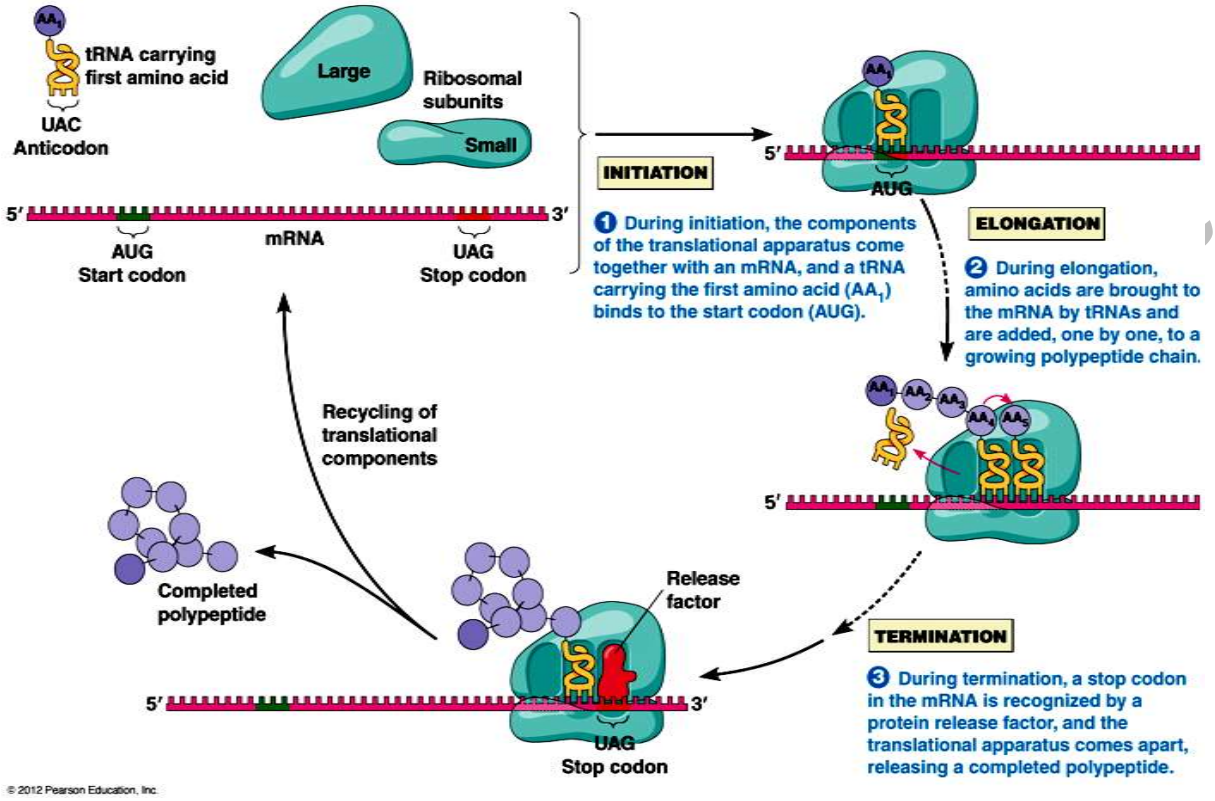
tRNA charging:



Before an amino acid can be incorporated into a growing polypeptide, it must first be attached to a molecule called transfer RNA, or tRNA, in a process known as tRNA charging. The charged tRNA will then carry the activated amino acid to the ribosome.

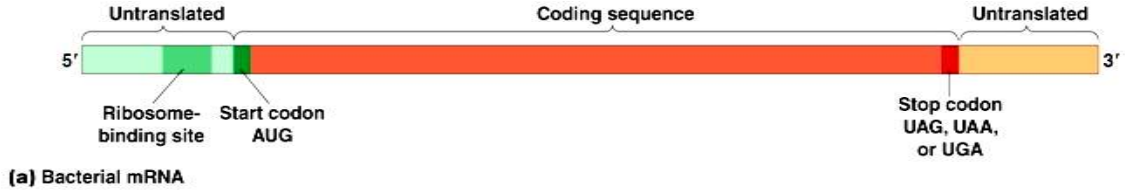
In two chemical steps, aminoacyl-tRNA synthetases catalyzes the formation of an ester bond between the carboxyl group of an amino acid and the 3 prime hydroxyl (OH) group of the appropriate tRNA.
They are as follows:
Step 1) The amino acid and a molecule of ATP enter the active site of the enzyme.
The ATP loses pyrophosphate and the resulting AMP bonds covalently to the amino acid.
The pyrophosphate is hydrolyzed into two phosphate groups.
Step 2) The tRNA covalently bonds to the amino acid to displace the AMP and the aminoacyl tRNA is then released from the enzyme.

The Process of Translation



Messenger RNA brings polypeptide-coding information to the ribosome.

A prokaryotic mRNA molecule encoding a single polypeptide has 5 prime non-coding leader sequence.



(a) Bacterial mRNA

(b) Eukaryotic mRNA

The leader contains a ribosome binding site (or Shine-Dalgarno sequence.

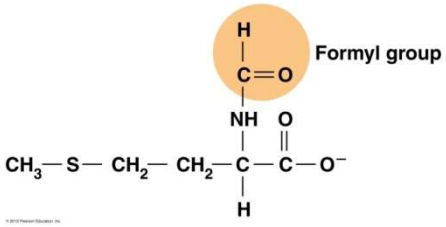
The rest of the mRNA contains a coding sequence that starts with an AUG start codon and ends with a stop codon (UAA, UAG or UGA) and a 3 prime non-coding trailer sequence.

A polycistronic prokaryotic mRNA would generally have a set of these features for each gene.

A eukaryotic mRNA molecule has, in addition to the above, a 5 prime cap and a 3 prime poly(A) tail.

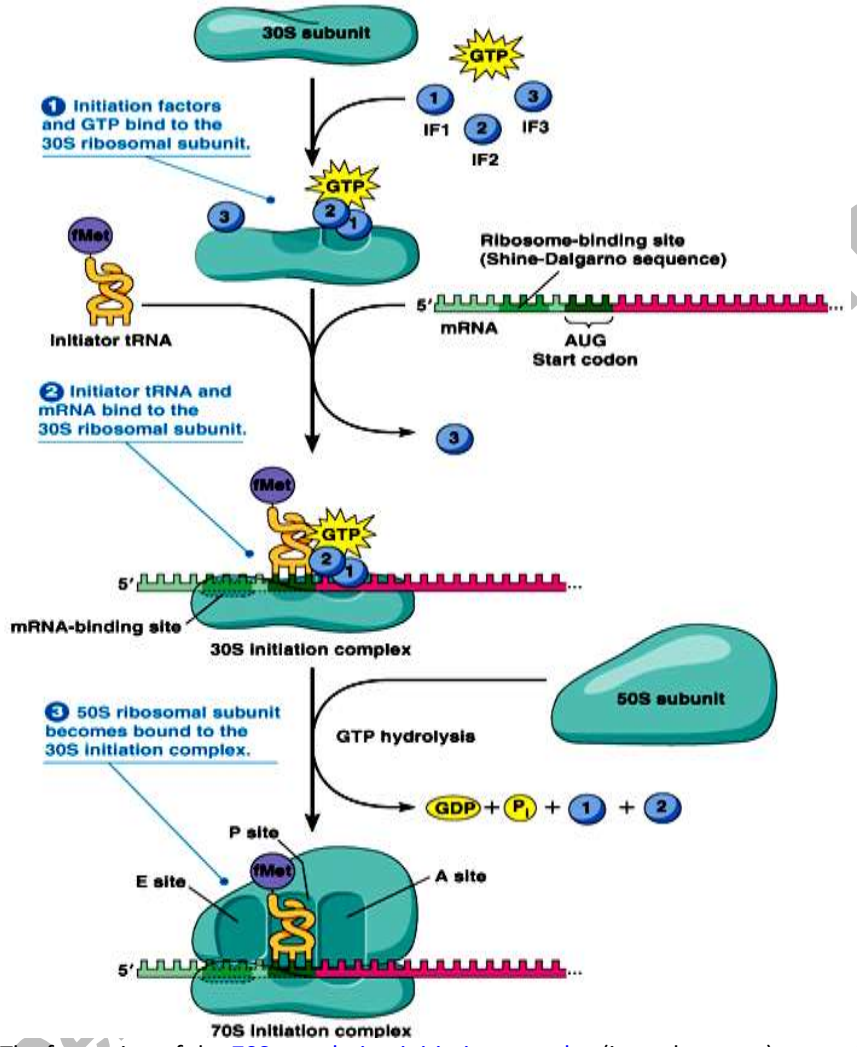
One important difference is that eukaryotic mRNAs lack a ribosome binding site (SD site).

A number of protein factors are required for the initiation, elongation and termination of translation.



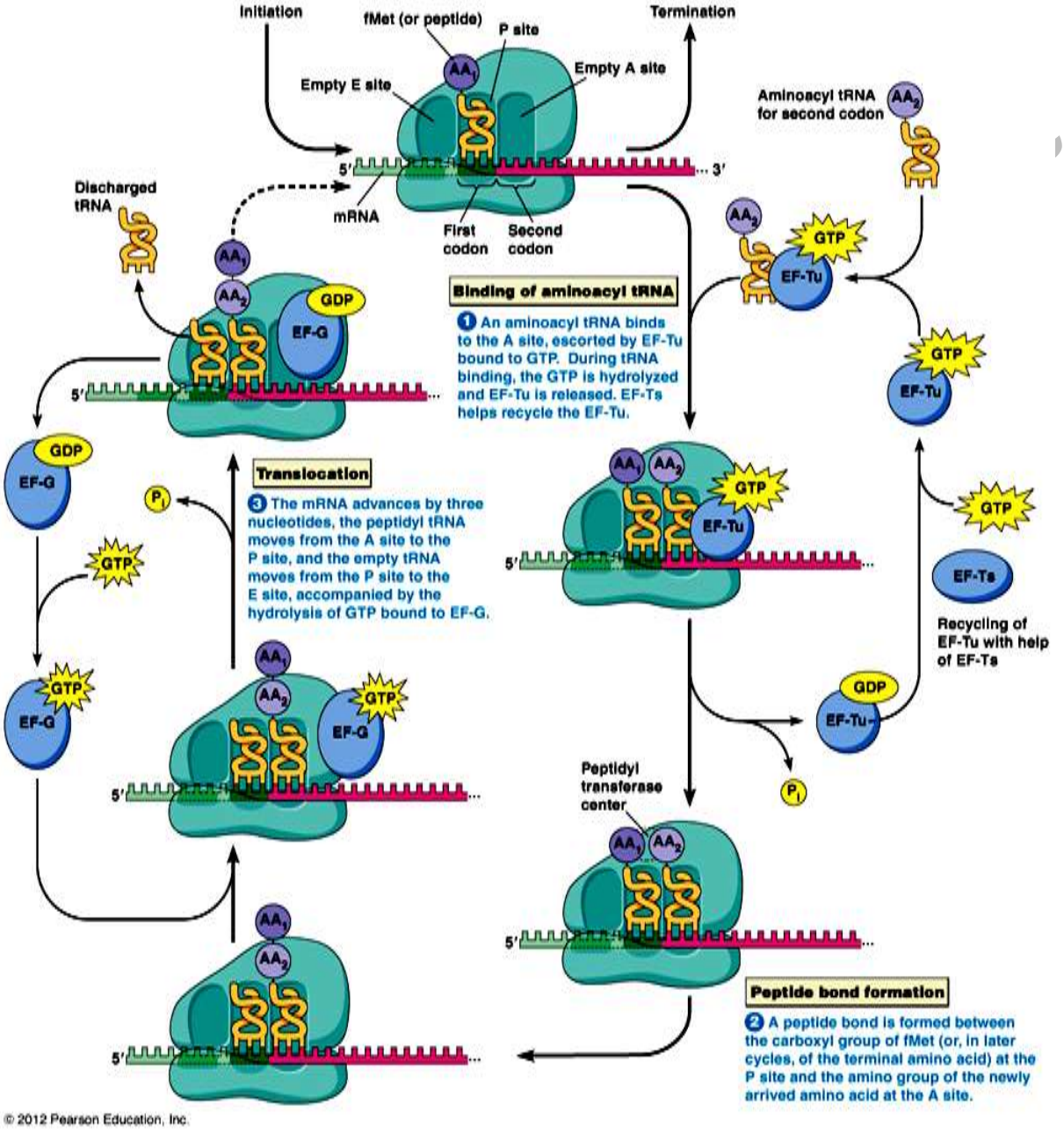
N-formylmethionine (fMet) is the modified amino acid with which every polypeptide is initiated in prokaryotes.

INITIATION:



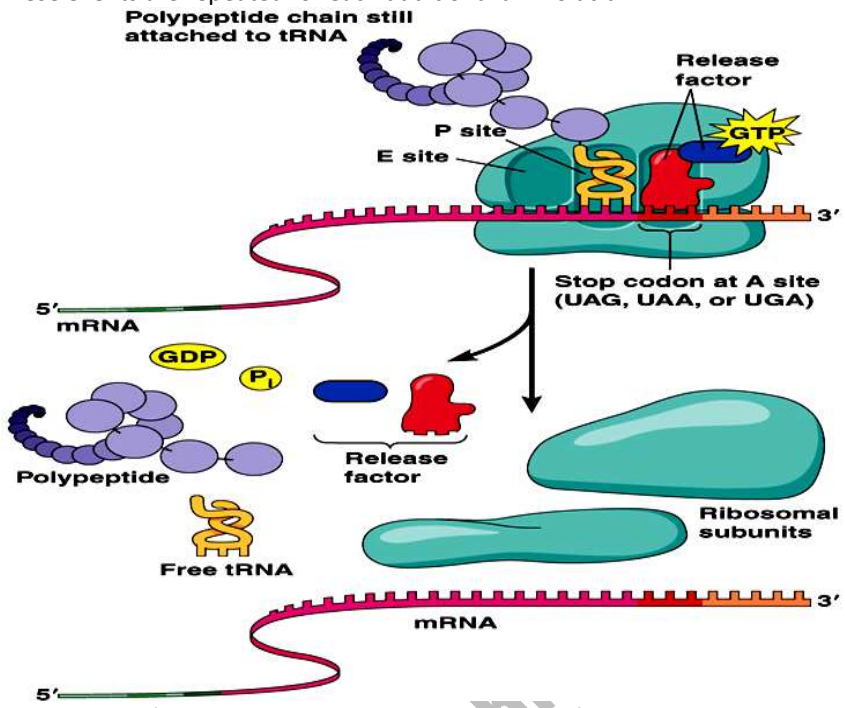
The formation of the 70S translation initiation complex (in prokaryotes) occurs in three steps.
1) Three initiation factors (IF) and GTP bind to the small ribosomal subunit.
2) The initiator aminoacyl tRNA and mRNA are attached.
The mRNA-binding site is composed, at least in part, of a portion of the 16S rRNA of the small ribosomal subunit.
The 3 prime end of the 16S rRNA bears a pyrimidine-rich stretch that base pairs with the Shine-Dalgarno sequence of the mRNA (in prokaryotes).
3) The large ribosomal subunit joins the complex.
The resulting 70S initiation complex has fMet-tRNA^{fMet} residing in the ribosome's P site.
In eukaryotes, translation includes a different set of initiation factors (eIFs), a slightly different assembly pathway and a non-formylated tRNA ^{met}.

eIF2 binds the initiator tRNA ^{met} before the small ribosomal subunit.
This complex can attach to the 5 prime cap structure of the mRNA.
As there is no Shine-Dalgarno sequence, the ribosome begins translation at a AUG that is located within the Kozak consensus sequence (often a good match to CAAA**AUG**).

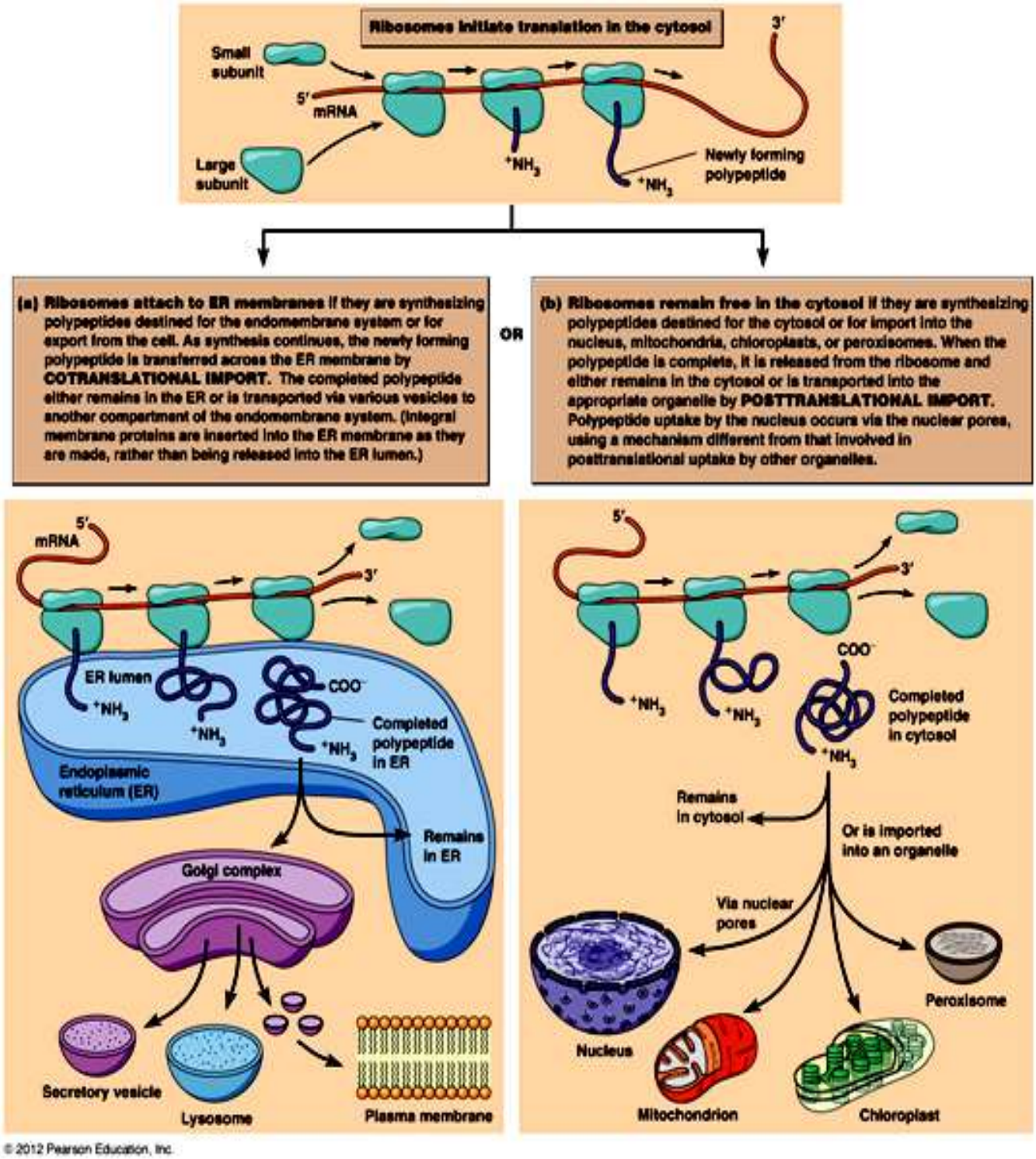


Chain elongation during protein synthesis requires the presence of a peptidyl tRNA or, in the first elongation cycle, an fMet-tRNA^{fMet} at the peptidyl (P) site.
1) Elongation begins with the binding of the second aminoacyl tRNA at the ribosomal aminoacyl (A) site.
The tRNA is escorted to the A site by the elongation factor EF-Tu, which also carries two bound GTPs.
As the tRNA binds, the GTPs are hydrolyzed and EF-Tu is released. EF-Ts helps recycle the EF-Tu.
2) A peptide bond is formed between the carboxyl group of the terminal amino acid (or fMet in the first cycle) at the P site and the amino group of the newly arrived amino acid at the A site.

This reaction is catalyzed by the peptidyl transferase activity of the 23S rRNA molecule in the large ribosomal subunit.
3) After EF-G-GTP binds to the ribosome and GTP is hydrolyzed, the tRNA carrying the elongated polypeptide translocates from the A site to the P site.
The discharged tRNA moves from the P site to the E (exit) site and leaves the ribosome.
As the peptidyl tRNA translocates, it takes the mRNA along with it.
Consequently, the next mRNA codon is moved into the A site, which is open for the next aminoacyl tRNA.
These events are repeated for each additional amino acid.



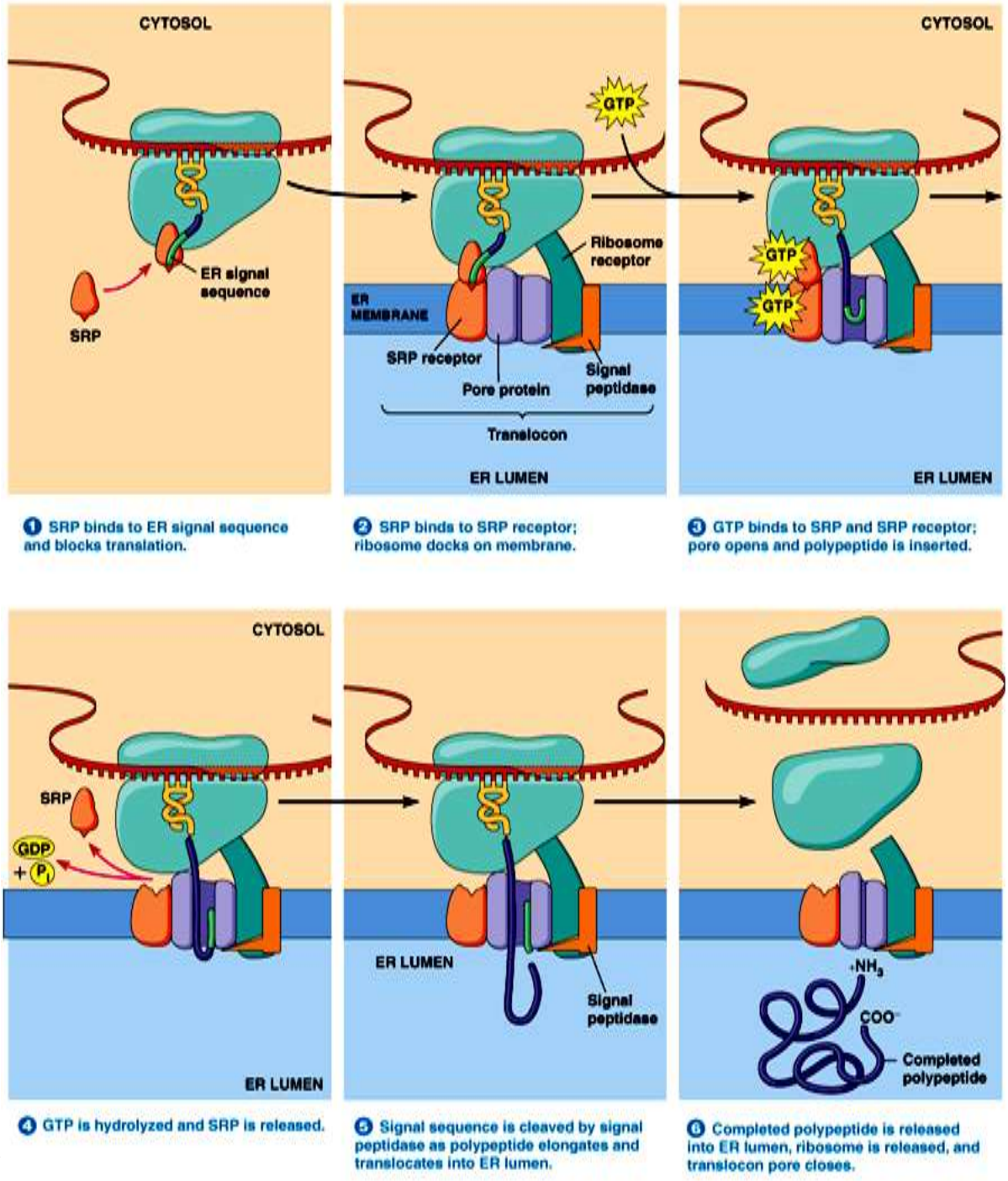
Termination of protein synthesis depends on release factors that recognize the three stop codons.
When a stop codon (UAG, UAA, or UGA) arrives at the A site, it is recognized and bound by a protein release factor.
This protein causes the polypeptide to be transferred to a molecule of water to cause its release from the tRNA and the dissociation of the other components of the elongation complex.



Proteins have to be folded into the proper three dimensional conformation to work properly.
A number of diseases, including Alzheimer's disease, may be considered to be protein-folding diseases.
Sometimes the primary sequence of amino acids is sufficient to spontaneously direct the folding of proteins into their proper shape.
However, often newly-made proteins require the help of molecular chaperones to attain their final shape.
The heatshock proteins, Hsp70 and Hsp60, are molecular chaperones.

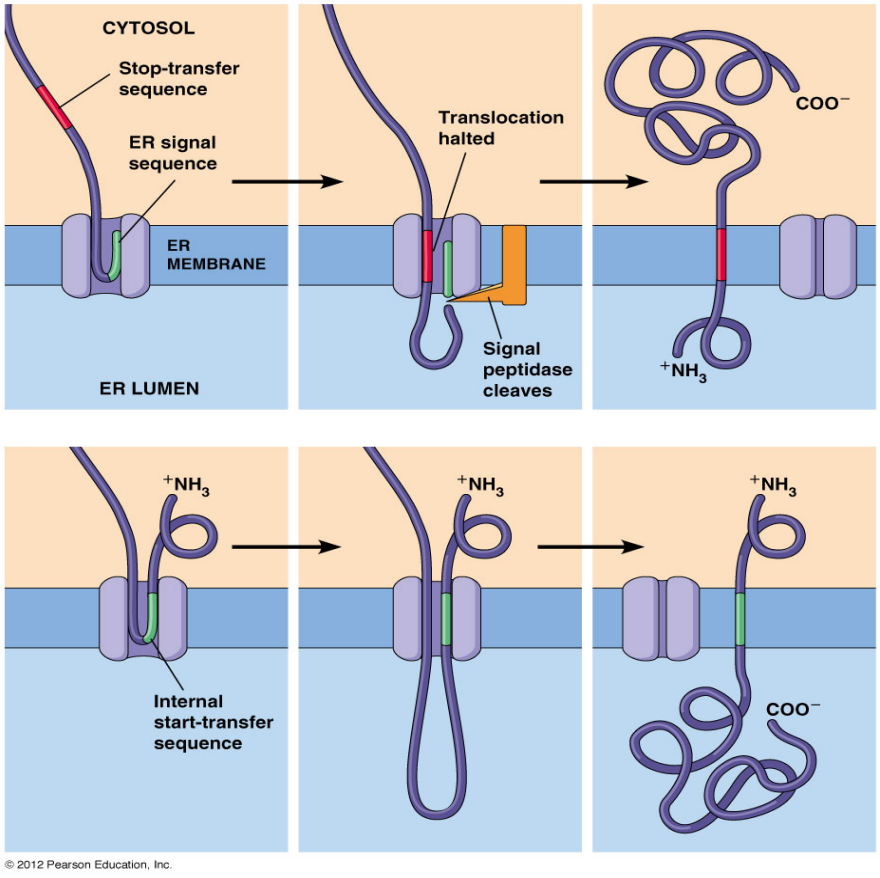
Heat-denatured proteins can be renatured through the activity of molecular chaperones and heatshock proteins are made during times of stress.
Prion diseases, such as "mad cow" disease, may "self-propagate" based upon a misfolded protein that can, in turn, misfold other versions of the same protein.
After the amino chain is made, many proteins undergo posttranslational processing (including removal of stretches of amino acids).
1) In prokaryotes, the N-formyl group is always removed in the mature protein and often the methionine and, sometimes, a number of N-terminal amino acids are cleaved away from the final protein product.
2) The protein hormone insulin provides an example of posttranslational processing.
Proinsulin is converted to the active hormone by the enzymatic removal of a long internal section of polypeptide. The two remaining chains continue to be covalently connected by disulfide
bonds connecting cysteine residues in insulin.
3) Recently discovered, the process of protein splicing (analagous to RNA splicing) removes inteins and splices the exteins together to make a mature protein.

Protein Targeting and Sorting



Synthesis of all polypeptides encoded by nuclear genes begins in the cytosol.
The large and small ribosomal subunits associate with each other and with the 5 prime end of an mRNA molecule, forming a functional ribosome that starts making the polypeptide.
When the polypeptide is about 30 amino acids long, it enters one of two alternative pathways.
1) In cotranslational import, if the newly forming polypeptide is destined for any of the compartments of the endomembrane system, it becomes associated with the ER membrane and is transferred across the membrane into the lumen (cisternal space) of the ER as synthesis continues.
The completed polypeptide then either remains in the ER or is transported via various vesicles and the Golgi

complex to another final destination.
Integral membrane proteins are inserted into the ER membrane as they are made, rather than into the lumen.
2) If the polypeptide is destined for the cytosol or for import into the nucleus, mitochondria, chloroplasts, or peroxisomes, its synthesis continues in the cytosol.
When the polypeptide is complete, it is released from the ribosome and either remains in the cytosol or is transported into the appropriate organelle by posttranslational import.
Polypeptide uptake by the nucleus occurs via the nuclear pores, using a mechanism different from that involved in posttranslational uptake by other organelles.

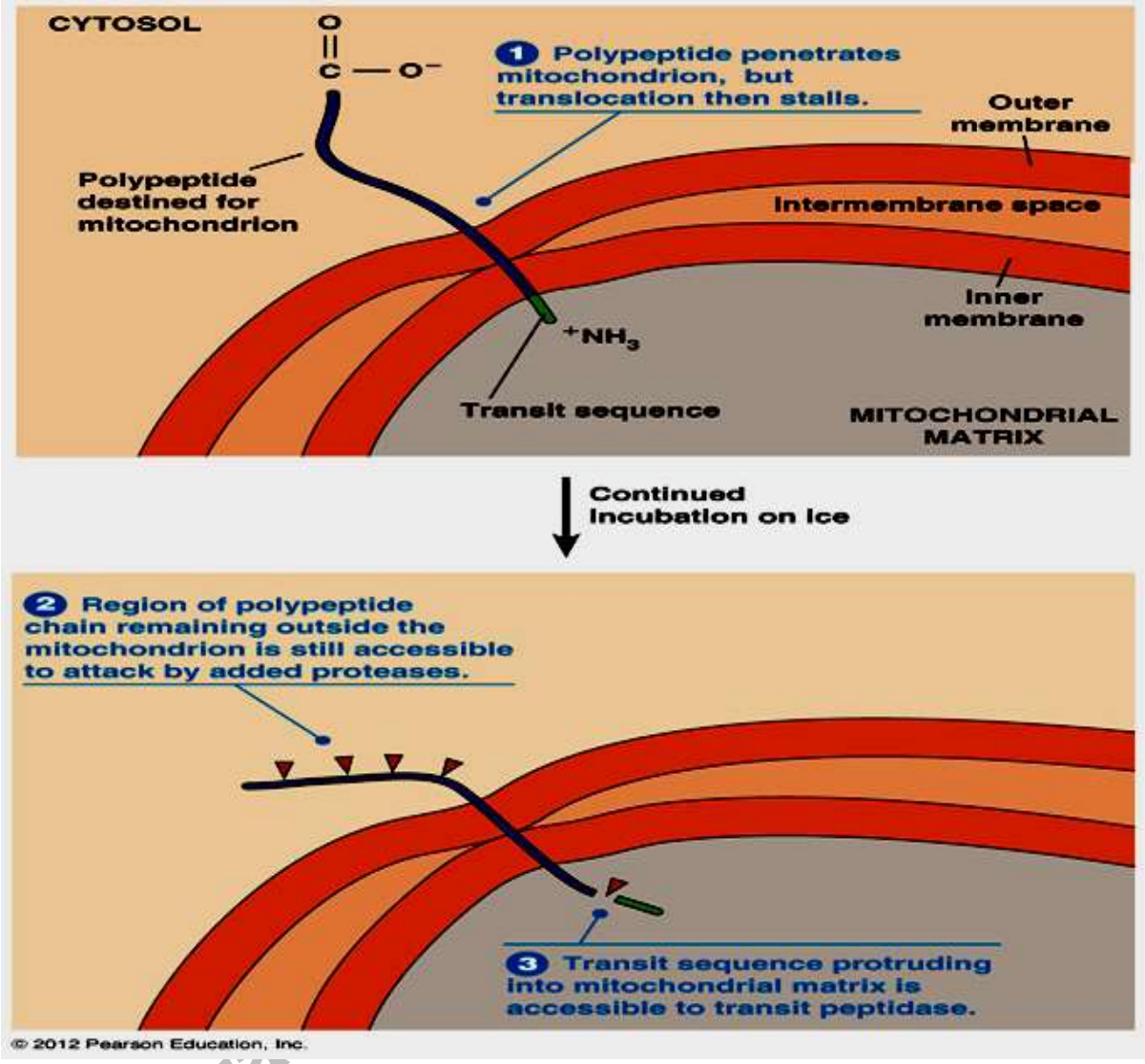


(a) Polypeptide with an internal stop-transfer sequence and a terminal ER signal sequence. The stop-transfer sequence halts the process of translocation and moves out through a side opening in the translocon to anchor the polypeptide in the membrane, creating a transmembrane protein with its N-terminus in the ER lumen and its C-terminus in the cytosol.

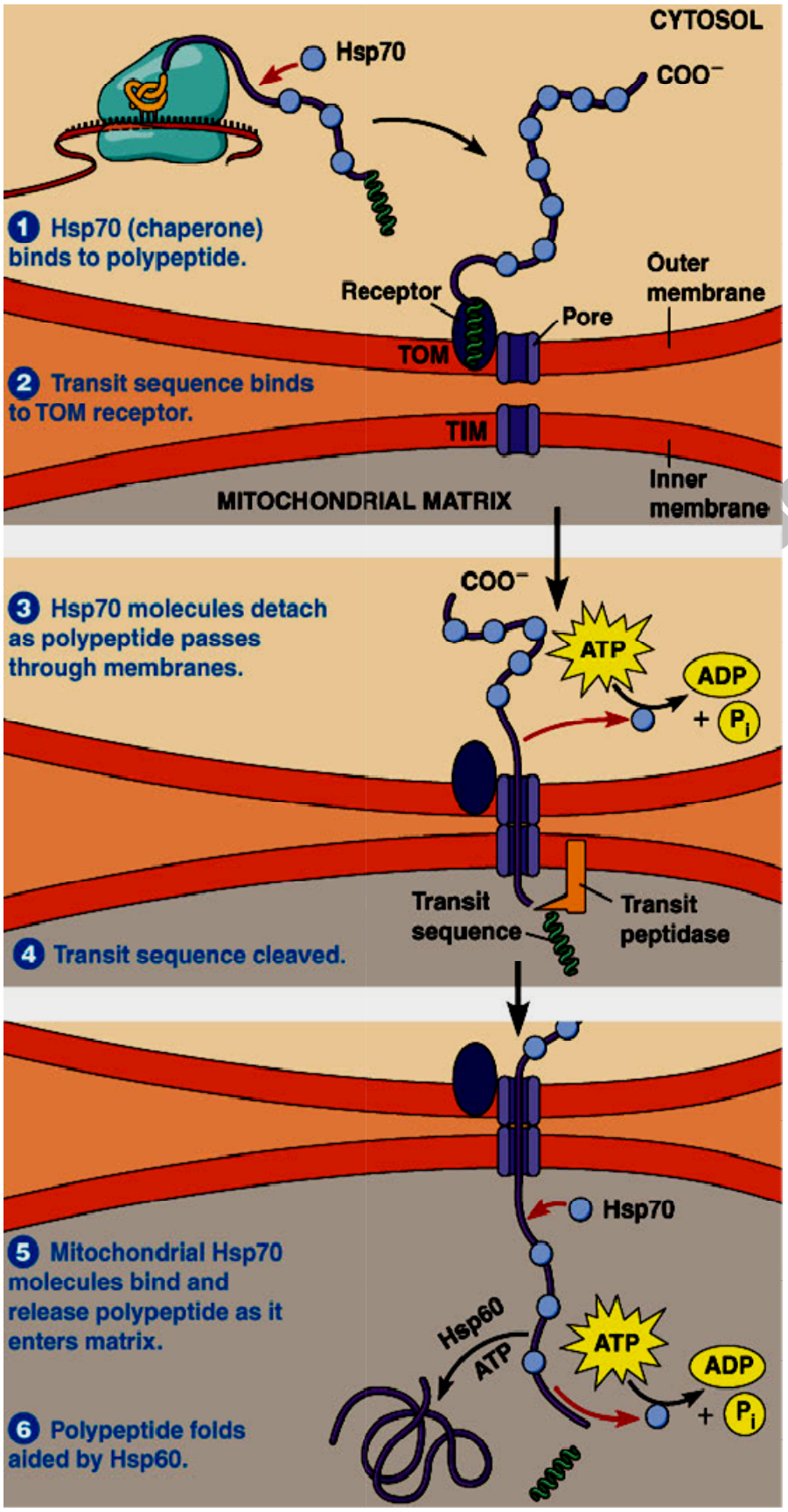
(b) Polypeptide with only a single, internal start-transfer sequence. This single start-transfer sequence starts polypeptide transfer and then moves through a side opening in the translocon to anchor itself in the membrane. (If this polypeptide also had a stop-transfer sequence that prevented complete transfer of the polypeptide through the translocon, the result would be a transmembrane protein with both its N-terminus and its C-terminus in the cytosol.)

In cotranslational import, proteins to be targeted to the endoplasmic reticulum initially have an N-terminal peptide, the ER signal sequence, translated by a cytosolic ribosome.
The ER signal sequence is bound by a signal-recognition particle (SRP), a ribonucleoprotein complex composed of 6 peptides and a 300 nucleotide RNA molecule.
The SRP binds to the SRP receptor to dock the ribosome on the ER membrane.
When the SRP receptor binds GTP, the nascent polypeptide enters the pore.
The SRP is released with hydrolysis of the GTP.
The growing polypeptide translocates through a hydrophilic pore created by one or more membrane proteins called the translocon.
The most recent evidence suggests that the ribosome fits tightly across the cytoplasmic side of the pore and that the ER-lumen side is somehow closed off until the polypeptide is about 70 amino acids long.
When the polypepide is complete, the signal peptidase cleave the signal to release the protein into the ER lumen while retaining the signal peptide, for a time, in the membrane.
Afterwards the ribosome is released and the pore closes completely.
In the endoplasmic reticulum, folding of the newly-made proteins may also require molecular chaperones and other proteins involved in protein folding.
Bip (binding protein), a member of the Hsp70 chaperone family, briefly binds to and stabilizes hydrophobic regions of proteins (especially rich in Trp, Phe, Leu) allowing proper folding instead of aggregation with other inmature

proteins.
Protein disulfide isomerase catalyses the formation and breakage of disulfide bonds between cysteine residues to produce a stable conformation.



There are two possible mechanisms for the insertion of integral membrane proteins having a single transmembrane segment.
1) Type I: Insertion of a polypeptide with both a terminal ER signal sequence and an internal stop-transfer sequence.
The terminal peptide is eventually cut off, leaving a transmembrane protein with its N-terminus in the ER lumen and its C-terminus in the cytosol.
2) Type II: Insertion of a polypeptide with only a single, internal start transfer sequence, which both starts polypeptide transfer and anchors itself permanently in the membrane.
The amino-carboxyl orientation of the completed protein depends on the orientation of the start-transfer sequence when it first inserts into the translocation apparatus.

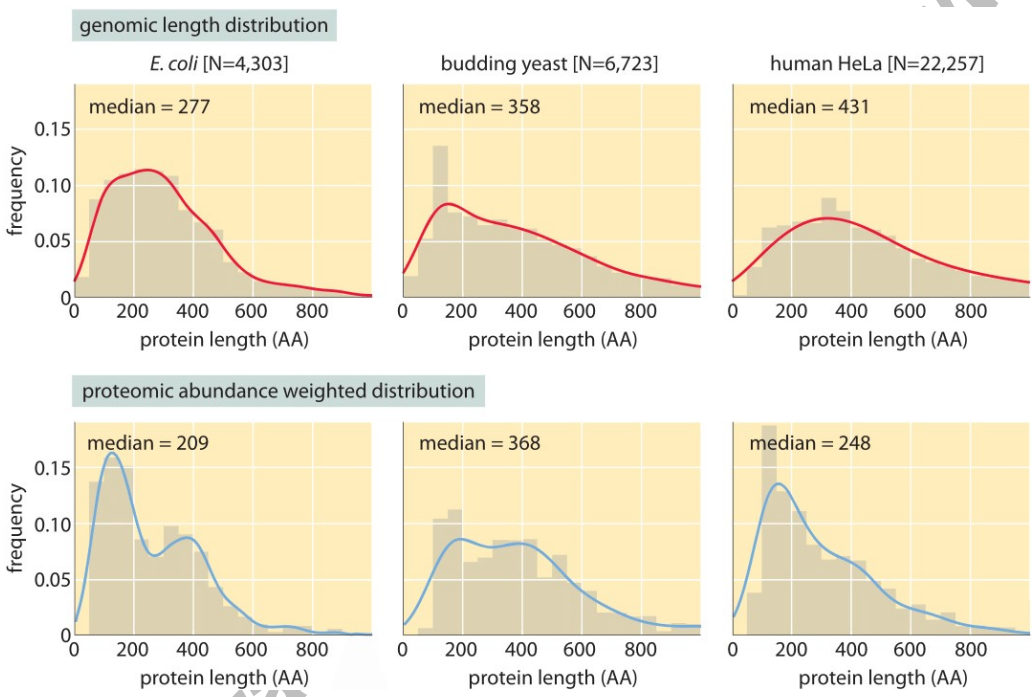

CYTOSOL
Hsp70
COO⁻
1 Hsp70 (chaperone) binds to polypeptide.
Receptor
Pore
Outer membrane
TOM
2 Transit sequence binds to TOM receptor.
TIM
Inner membrane
MITOCHONDRIAL MATRIX
COO⁻
3 Hsp70 molecules detach as polypeptide passes through membranes.
ATP
ADP
+ Pi
Transit sequence
Transit peptidase
4 Transit sequence cleaved.
Hsp70
5 Mitochondrial Hsp70 molecules bind and release polypeptide as it enters matrix.
Hsp60
ATP
ATP
ADP
+ Pi
6 Polypeptide folds aided by Hsp60.

Posttranslational import allows some polypeptides to enter organelles after protein synthesis.
Like cotranslational import into the ER, posttranslational import into a mitochondrion (and chloroplast) involves a signal sequence (called a transit sequence), a membrane receptor, pore-forming membrane proteins, and a peptidase.
Polypeptides being imported into the mitochondrion span both membranes at the same time.
This was demonstrated in a cell-free import system incubated on ice in which the polypeptides begin to penetrate the mitochondrion but then stall.
The transit sequence is cleaved by the transit peptidase present in the matrix, indicating that the N-terminus of the polypeptide is within the mitochondrion.
At the same time, most of the polypeptide molecule is can be attacked by exogenously added proteolytic enzymes on the outside of the mitochondrion.
Therefore, the polypeptide must span both membranes transiently during import at a contact site between the two membranes.
However, in the mitochondrion, the membrane receptor recognizes the signal sequence directly without the intervention of a cytosolic SRP.
Furthermore, chaperone proteins play several crucial roles in the mitochondrial process:
1) Chaperones keep the polypeptide partially unfolded after synthesis in the cytosol so that binding of the transit sequence and translocation can occur.
2) Chaperones drive the translocation itself by binding to and releasing from the polypeptide within the matrix, an ATP-requiring process and
3) Chaperones often help the polypeptide fold into its final conformation.
Polypeptides synthesized on cytosolic ribosomes but destined for either the intermembrane space or the inner membrane of the mitochondrion require two separate targeting sequences (both located at the N-terminus).
1) The polypeptide is directed to a contact (translocation) site on the mitochondrion by a positively charged or amphipathic transit sequence.
2) Cleavage of the transit sequence by a peptidase in the mitochondrial matrix uncovers a highly hydrophobic second signal sequence.
3) This second signal sequence causes the polypeptide to be inserted into the inner membrane in the same way that mitochondrially encoded polypeptides are targeted to this membrane.
4) The remainder of the polypeptide is then moved across the membrane into the intermembrane space (or into the inner membrane for integral inner membrane proteins).
5) Cleavage by a second peptidase can release the polypeptide into the intermembrane space leaving the signal sequence behind in the inner membrane.

What are the mechanisms that ensure the fidelity of translation?

During protein synthesis (translation), the overall rate of misincorporation has been estimated to be in the range of 6×10−46×10−4 to 5×10−35×10−3 per amino acid incorporated. This error rate represents a compromise between the opposing requirements to keep the production of functionally inactive proteins to a tolerably low level, versus the heavy energetic costs that would be necessary to achieve higher levels of fidelity, and the need for speed and efficiency in translation. Given the typical distribution of protein lengths in organisms, Poisson statistics informs us that allowing more than somewhere in the range of 10−310−3 errors per amino acid incorporated would result in an unacceptable rate of production of functionally inactive proteins for the larger translation products:[note 1][note 1]

Source: How big is the "average" protein?



Three strategies are employed to improved the fidelity of translation: editing, kinetic proofreading and induced fit.

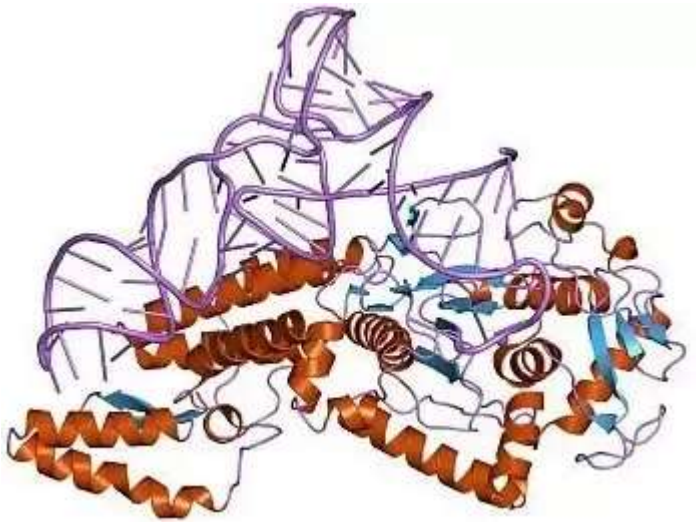
Errors are generally substitution or missense errors occuring during two key steps of the translation process

- Incorrect linking of an amino acid to its cognate tRNA during the aminoacyl-tRNA synthetase step
- Selection of an incorrect tRNA during elongation.

Other types of error, including incorrect start site selection, frameshifting and inappropriate termination, also occur during translation but have been less well studied.

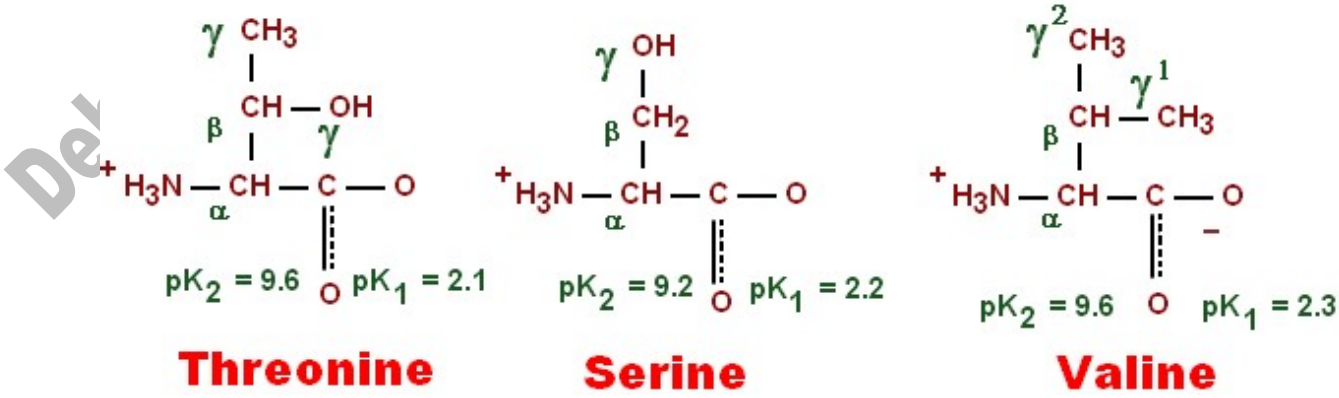
Editing in the aminoacyl-tRNA synthetase step

Aminoacylation involves the selection of two appropriate substrates, the tRNA and the amino acid, by the corresponding aminoacyl-tRNA synthetase (aaRS)



Source: Aminoacyl tRNA synthetase

1. tRNAs are relatively large and contain a large number of determinants that enable specific interactions with the aaRS.
a. Association of tRNA wih its specific aaRS is hence highly accurate
b. Above is a “cutaway” illustration of a tRNA complexed with the binding domain of its corresponding aaRS, showing its extensive tight and specific associations. Alpha helices of the binding site are brown, beta sheets are blue, and the skinny lavender threads represent random coil stretches of the binding site.
2. Amino acids are smaller and their side chains have fewer distinguishing determinants.
a. Most amino acids are easy to distinguish
b. Some pairs of amino acids are difficult to distinguish. For example, threonyl-tRNA synthetase must discriminate threonine from valine, which is “isoteric”, and from serine, which is smaller than threonine but has a γ-hydroxyl group.

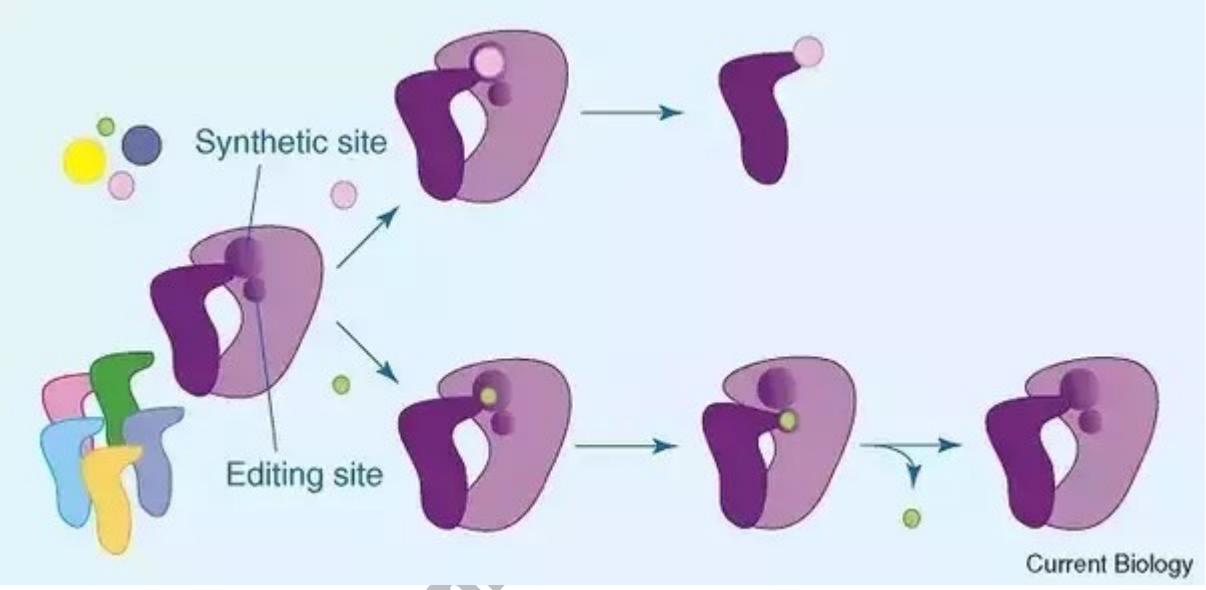


Source: Threonine Structure

The aminoacylation reaction occurs in two steps.

- The amino acid is activated by adenylation (which consumes ATP)
- Then it is transferred to the tRNA (releasing AMP).

In most cases, steric exclusion of amino acids with incorrect side-chains and recognition of specific properties of each amino acid make these steps sufficiently specific so that only the correct amino acid can be activated and transferred. But where the chemical specificities of alternative selections of amino acids are not distinct enough to guarantee precise selection, the aaRS enzymes include a second active site called the editing site, which is distinct from the synthesis site.



"Double-sieve" model of fidelity. Source: Fidelity in protein synthesis

In the "double-sieve" model of fidelity, the synthetic site of the enzyme acts as the first sieve, excluding amino acids that are too large or that cannot establish specific interactions. Threonyl-tRNA synthetase, for example, excludes amino acids that have too large a side chain, as well as amino acids such as valine, which are similar in size but lacks a γ-hydroxyl group. This first sieve is not perfect, however.

The role of the second sieve is provided by the editing site. In the case of Threonyl-tRNA synthetase, threonine has too bulky a side chain to reach this second site, but small amino acids such as serine that slipped through the first selection **can** reach this second site. Editing decreases the error rate of threonyl-tRNA synthetase from 10^{-3} to the 10^{-4}–10^{-5} range.
In general, editing contributes a 5–100-fold increase in the overall selectivity. Editing may occur at the level of the activated amino acid (i.e. before the aminoacyl bond is formed) or at the aminoacyl-tRNA level (i.e. post-transfer). In general, post-transfer editing seems to predominate *in vivo*.

Kinetic proofreading and induced fit in tRNA selection

Given the low error frequency (10^{-4}–10^{-5}) of tRNA aminoacylation, the 6×10^{-4} to 5×10^{-3} error frequency seen in the overall translation process must primarily stem from errors in selection of the cognate aminoacyl-tRNA to the codon presented by the mRNA in the ribosome.
Discrimination between the different AA-tRNAs results from differences in the free energy of codon-anticodon binding between the cognate and non-cognate aminoacyl tRNAs. It is easy to discriminate when the mismatch amounts to two or three base pairs. But discriminating between codon-anticodon pairings with only a single mismatch is not easy. Furthermore, selection among the twenty-some choices of AA-tRNAs needs to be fast to

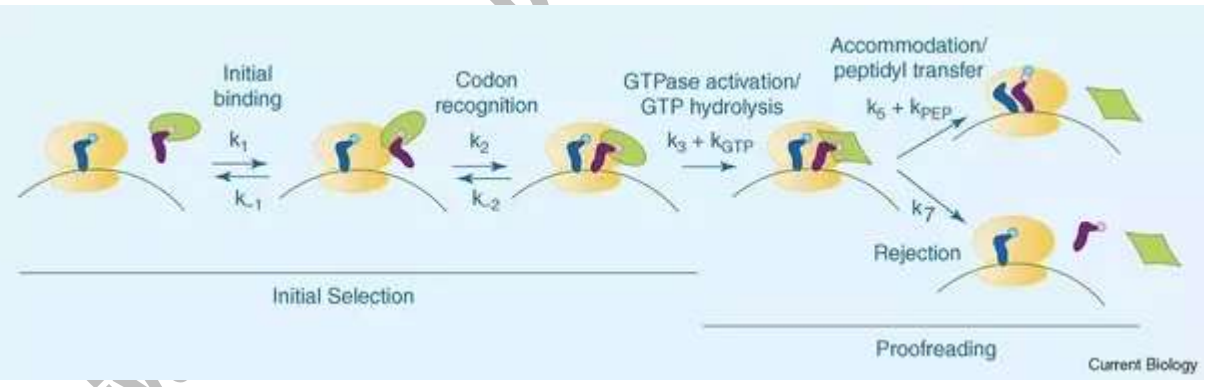
keep up with the translation rates. There is simply no time to establish an equilibrium between the various AA-tRNAs and the ribosome–mRNA complex.

organism	rate (aa/s)
E. coli	10-20
S. cerevisiae	3-10
N. crassa	5-8
M. musculus	6

Translation rates. Source: What is faster, transcription or translation?

The relatively poor selectivity possible between the twenty-some choices of AA-tRNA is improved by "kinetic proofreading".

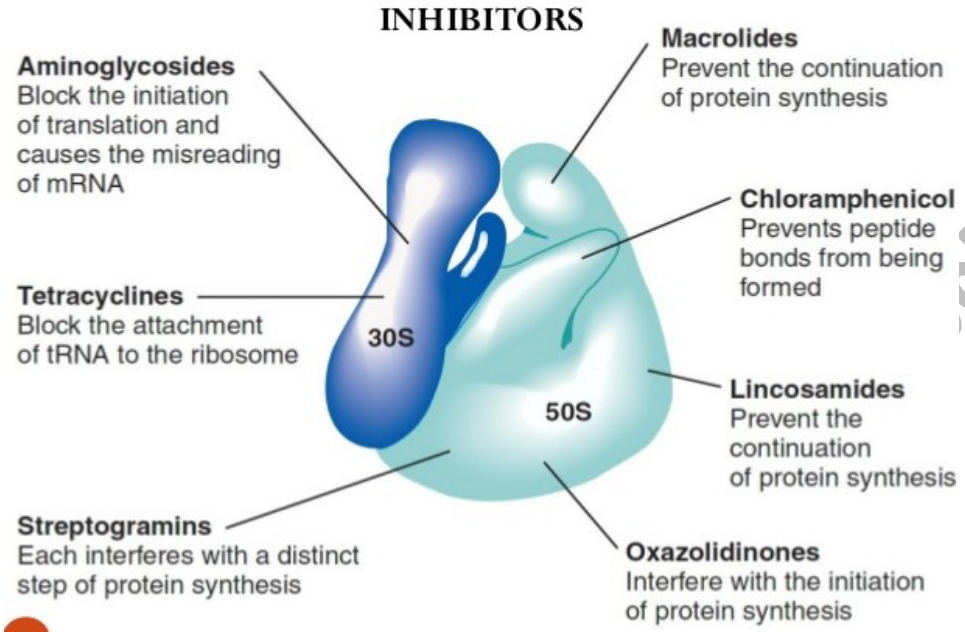
In the diagram below, note that substrate selection is separated into two distinct phases by an irreversible step (in this case GTP hydrolysis). This permits **two** opportunities to examine and discard an incorrect AA-tRNA. The first selection occurs when AA-tRNAs are delivered to the ribosome in ternary complexes with the GTPase elongation factor EF-Tu (in bacteria; EF1A in eukaryotes) and GTP. Correctly paired complexes are more likely to trigger hydrolysis than incorrectly paired complexes. After GTP hydrolysis, the proofreading step enables a second chance to test the accuracy of the codon-anticodon pairing. The cognate AA-tRNA is more likely to actually participate in peptidyl transfer than a non-cognate AA-tRNA, which is likely to be released from the ribosome.



Kinetic proofreading. Source: Fidelity in protein synthesis

Near-cognate AA-tRNAs, differing in their anticodons by only one base from the correct anticodon, pass through initial selection, triggering GTP hydrolysis, with a error frequency of approximately 3×10^{-2}. These improperly bound AA-tRNAs are usually rejected during the proofreading stage, thus increasing selectivity by ~15–45-fold.
During the initial selection step, k_3 is larger for cognate AA-tRNAs than for near-cognate AA-tRNAs. Likewise, during proofreading, k_5 is larger for cognate AA-tRNAs than for near-cognate AA-tRNAs. These differences in forward rates have been attributed to *induced fit:* The correct substrate, but not the incorrect one, can induce the conformational changes in the enzyme/substrate complex that result in catalysis.
Conflicting requirements for fidelity, speed and efficiency have dictated the accuracy of the translation process. It appears to be as accurate as necessary without incurring excessive costs in other areas.

Inhibitors of protein synthesis:



CLASSIFICATION OF PROTEIN SYNTHESIS INHIBITORS

TETRACYCLINES 1

Demeclocycline DECLOMYCIN
Doxycycline VIBRAMYCIN
Minocycline MINOCIN
Tetracycline SUMYCIN

GLYCYLCYCLINES 2

Tigecycline TYGACIL

AMINOGLYCOSIDES 3

Amikacin AMIKIN, OTHERS
Gentamicin GARAMYCIN
Neomycin NEO-FRADIN
Streptomycin STREPTOMYCIN
Tobramycin TOBREX

MACROLIDES/KETOLIDES 4

Azithromycin ZITHROMAX
Clarithromycin BIAXIN
Erythromycin E-MYCIN
Telithromycin KETEK

OTHERS 5

Chloramphenicol CHLOROMYCETIN
Clindamycin CLEOCIN
Linezolid ZYVOX
Quinupristin/Dalfopristin SYNERCID