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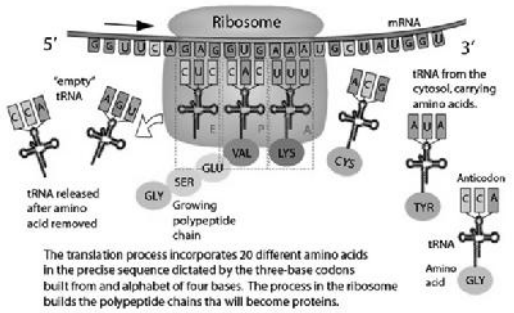
Lecture 13: PROTEIN SYNTHESIS II- TRANSLATION



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LEARNING OUTCOMES

Students, after mastering materials of the present lecture, should be able

1. to explain translation process of protein synthesis
2. to explain the site of protein synthesis
3. to explain in general the process of translation
4. to explain in detail the process of translation including initiation of translation, regulation of translation initiation, elongation of peptide chain, and termination of translation.

LECTURE OUTLINE

I. INRODUCTION

1. Definition
2. Site of Protein synthesis
3. General Mechanism

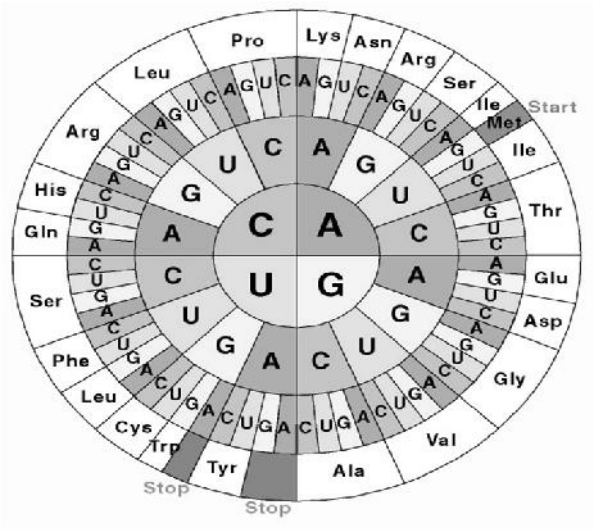
II. TRANSLATION

1. Ribosomes
2. Initiation of Translation
3. Regulation of Translation Initiation
4. Elongation of Peptide Chain
5. Termination of Translation

1. INTRODUCTION

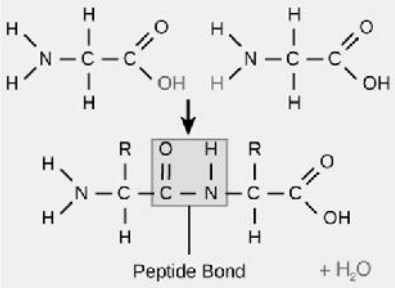
1. Definition

- **Translation** in the protein synthesis is the process of decoding the genetic information found in messenger RNA (mRNA), following transcription of DNA to RNA, that results in polypeptides or proteins.
- The information contained in the nucleotide sequence of the mRNA is made up of three (3) bases, called **codon**, from four (4) bases found in DNA resulting in $\mathbf{4^3 = 64}$ codes. As only 21 amino acids are used for the synthesis protein, so one amino acids may be encoded by more than one codes.
- The genetic codes (codons) are decoded by three-base sequence on tRNA (transport RNA), called anticodon.



Codons that encode amino acids. Anticodons are based on base pairs: A-U & G-C

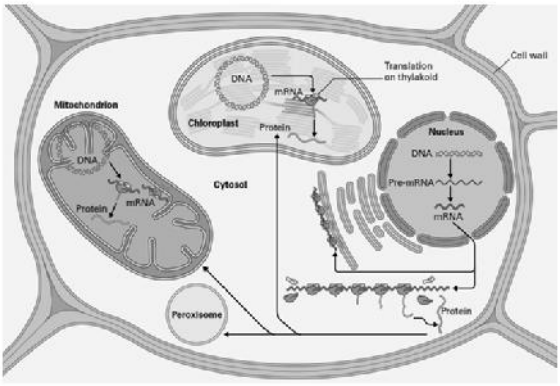
- Protein synthesis is the formation of amino acid chain formed by peptide bonds A **peptide bond** is a chemical **bond** formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H_2O). This is a dehydration synthesis reaction (also known as a condensation reaction), and usually occurs between amino acids.



2. Site of Protein Synthesis

- In plants, translation (protein synthesis) occurs in three subcellular compartments (Fig. 10.1): the cytosol, plastids, and mitochondria each contain distinct protein synthetic machinery.
  - About 75% of cell proteins are made in the cytosol, where mRNAs transcribed from the nuclear genome are translated.
  - About 20% of the proteins in a photosynthetically active cell (e.g., a young leaf cell) are synthesized in the chloroplast using mRNA templates transcribed from the chloroplast genome, and a small amount of protein synthesis (2–5% of total protein) occurs in mitochondria.
  - In the cytosol, more than 25,000 different proteins may be synthesized, whereas in chloroplasts, about 40 proteins are synthesized, though plastid genomes can encode over 200 proteins.

Fig. 10.1 Sites for protein synthesis in a plant cell. A typical plant cell synthesizes proteins in three distinct compartments:the cytosol, plastids, and mitochondria.

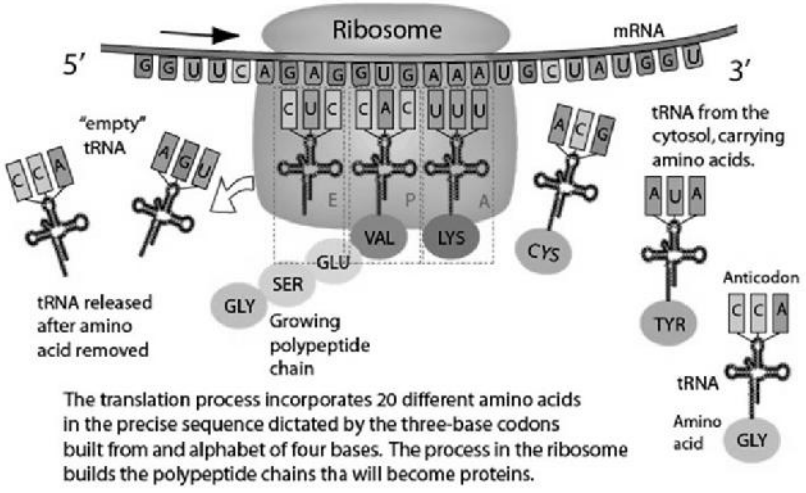


Translation of mRNAs transcribed in the nucleus occurs in the cytosol, either on soluble or membrane-bound ribosomes. In contrast, both transcription and translation of plastid and mitochondrial mRNA take place within those organelles. Similarly, ribosomes can be membrane-localized in the organelles. Proteins synthesized in the cytosol can be imported into one or more organelles— the plastid, mitochondria, or microbody. Shown here is the leaf-type microbody, the peroxisome (see Chapter 4). Organellar genomes are shown in circular form, but can exist in other conformations.

- The number of proteins synthesized in mitochondria varies widely among species; ± 20–40 proteins in the liverwort *Marchantia polymorpha*, and far fewer proteins in most plants.

3. General Mechanisms

- The overall mechanisms responsible for protein synthesis in the cytosol, plastids, and mitochondria are similar, but are clearly distinct from each other.
  - Accordingly, plant cells contain three different types of ribosomes, three groups of transfer RNA (tRNA), and three sets of translation factors for protein synthesis.
- In many cases, plant plastid and mitochondrial genomes encode tRNAs that are used specifically for protein synthesis within the organelle; there are cases, however, in which tRNAs encoded within the nuclear genome are imported into mitochondria for use in mitochondrial protein biosynthesis.



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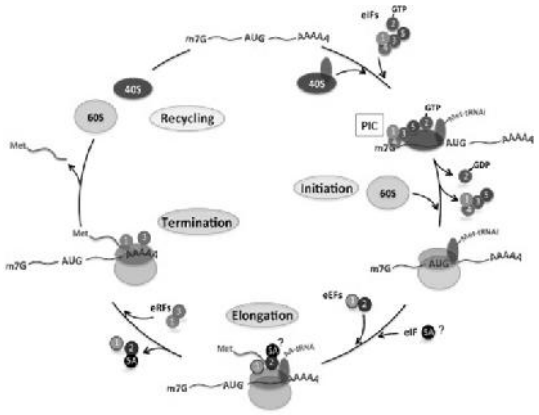

Figure 1: RNA translation in mammalian cells. The figure shows the general scheme of mRNA translation for capped [5′-methylguanylate (m7G)] mRNAs with poly-A tails (AAAA) and a start codon (AUG). Four general phases of translation are shown: Initiation, elongation, termination and recycling. The figure shows individual ribosomal components (40S and 60S) and specific initiation factors (eIFs), elongation factors (eEFs) and release factors (eRFs) that participate in the mRNA translation process. PIC, pre-initiation complex.

2. TRANSLATION

1. Ribosomes

- Protein biosynthesis occurs on ribosomes, which are large complexes of proteins and ribosomal RNA (rRNA) that hold tRNA and mRNA in position, and catalyze the formation of peptide bonds between amino acid residues.
- Ribosomes can be isolated intact or as two subunits: one large and one small (Table 10.1, Fig. 10.2) that reversibly associate and dissociate during protein synthesis.
- Surprisingly, it is not the protein component, but the largest RNA species of the large subunit that catalyzes peptide bond formation.

- Plant cytosolic ribosomes contain orthologs of mammalian ribosomal proteins (RPs) and have one additional plant-specific subunit (the RPP3, a member of the acidic ribosomal protein family).
- Plant ribosomes contain an estimated 80 proteins, each typically encoded by a small gene family. For example, in Arabidopsis the number of family members ranges from 2 to 11.

TABLE 10.1
Summary of the composition and properties of various plant ribosome types.

Ribosome (unit*)	Subunits (unit*)	rRNA (unit*)	Number of proteins
Plant cytosolic, 80S	40 60	18 28, 5.8, 5	32 48
Plant plastids, 70S	30 50	16 23, 5, 4.5	≈25 ≈33
Plant mitochondria, 70S	30 50	18 26, 5	>25 >30
Bacterial, 70S	30 50	16 23, 5	21 31

*S (sedimentation) value

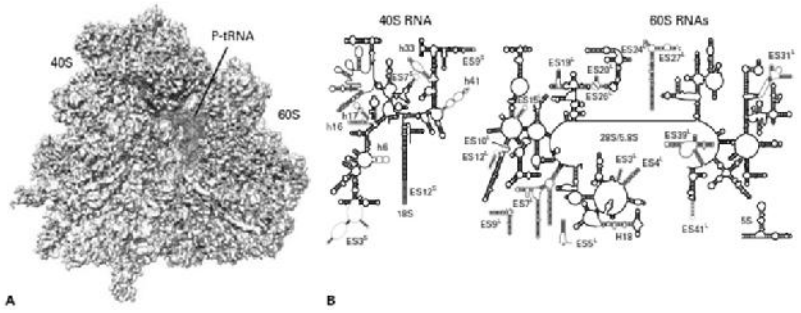

Fig. 10.2 (A) CryoEM structure of translating wheat (*Triticum aestivum*) **ribosome** at 5.5 Å resolution, with the small (40S) and large (60S) subunits colored yellow and gray, respectively, and the peptidyl-tRNA, in green, in the P site. (B) Secondary structures of the rRNAs in the 40S and 60S wheat subunits, left and right, respectively. Colored regions represent expansion segments (ES), which are additional rRNA sequences found in eukaryotic ribosomes but absent from prokaryotic ribosomes. Most ES sequences are located at the ribosome surface and are of variable lengths among eukaryotes. Source: Armache *et al*. (2010) PNAS 107: 19748–19753.

- Most ribosomal proteins are basic in nature, which facilitates their interaction with the negatively charged rRNA; however, ribosomes also contain a small family of acidic proteins, called P-proteins.
- In addition to the conserved RPP0, RPP1, and RPP2 proteins found in all eukaryotes, plant ribosomes also contain a unique member, RPP3.
- RPP3 interacts with RPP1 and RPP2 to form a mobile acidic stalk complex on the large subunit, which interacts with an elongation factor (eEF2) to promote GTP hydrolysis tRNA translocation within the ribosome following peptide bond formation.
- Many ribosomal proteins undergo post-translational modifications.

2. Initiation of Translation

- Initiation of protein synthesis establishes the reading frame on the mRNA and positions the first amino acid for incorporation.
- Initiation of protein synthesis is a complex series of events that is largely conserved in eukaryotes. The initiation of translation is facilitated by a group of auxiliary protein factors referred to as **eukaryotic initiation factors** (eIFs), which are classified on the basis of the general reaction they promote.
- Each may consist of a single or multiple polypeptides (Table 10.2), and many mechanisms that regulate cytosolic protein synthesis in eukaryotes affect the activities of one or more of these factors.

TABLE 10.2 Eukaryotic initiation factors (eIFs) and their roles in initiating translation.

Class	Members	General role
eIF1	eIF1, eIF1A	Multiple effects in enhancing initiation of complex formation and AUG selection
eIF2	eIF2, eIF2B*	GTP-dependent recognition of Met-tRNA and nucleotide exchange
eIF3	eIF3†	Ribosomal subunit dissociation; promotion of Met-tRNA and RNA binding to 40S subunits
eIF4	eIF4A, eIF4B, eIF4F‡, eIF(iso)4F‡, eIF4H	Recognition of 5′ cap on mRNA, binding 40S subunit to mRNA, and unwinding mRNA secondary structure
eIF5	eIF5, eIF5B*	Promotion of eIF2 GTPase activity, AUG selection, and release of factors from ribosome; joining of the 60S subunit
eIF6	eIF6	Binds to 60S subunits and may function in regulation and assembly of ribosomes
PABP	Poly(A)-binding protein	Binds to poly(A) at 3′ end of mRNA and interacts with eIF4F at the 5' end, facilitating circulation

* The presence of eIF2B, eIF5B, and eIF4H in plants is based only on the presence of gene sequences similar to other eukaryotes. eIF2B and eIF4H have not been purified or characterized from plants. eIF3 is composed of 13 nonidentical subunits (designated a–m) ranging from 180 to 28 kDa.

- Initiation begins when eIF2 interacts with an initiator Met-tRNA in the presence of GTP to generate a tRNA–protein complex called the **ternary complex** (Fig. 10.3). eIF2 is a heterotrimer of α, β and γ subunits.

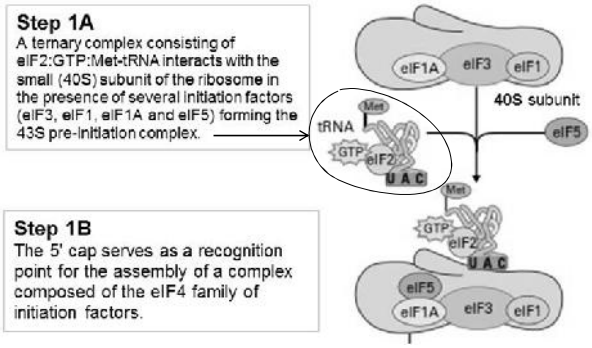

Fig 10.3 Overview of the mechanism of polypeptide chain initiation in the plant cell cytosol (Step 1).

- The eIF4s facilitate recognition of the cap structure and interaction between the 40S subunit with other associated eIFs and the mRNA.
- Once formed, the ternary complex binds to a free 40S ribosomal subunit, a process facilitated by several other eIFs (Fig. 10.3). The small subunit of the ribosome, bound to the Met-tRNA and several eIFs, then interacts with the mRNA.
- This step requires the eIF4 family of initiation factors, which includes eIF4A (DEAD box helicase), eIF4B (RNA-binding protein), eIF4G (protein interactions), and eIF4E (cap-binding protein).
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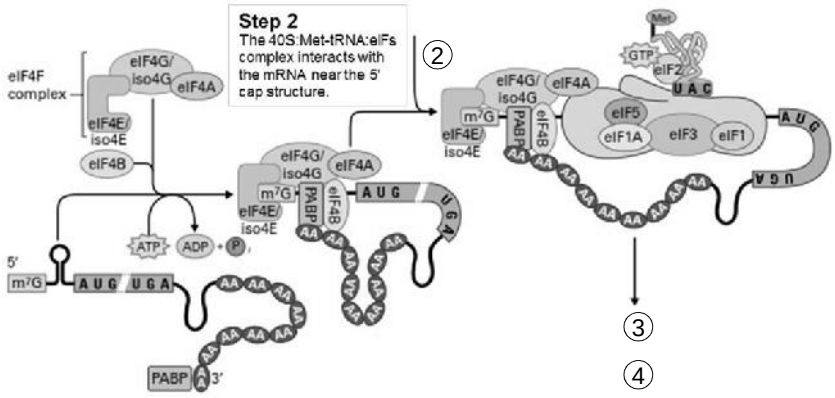


Fig 10.3 Overview of the mechanism of polypeptide chain initiation in the plant cell cytosol (Step 2).

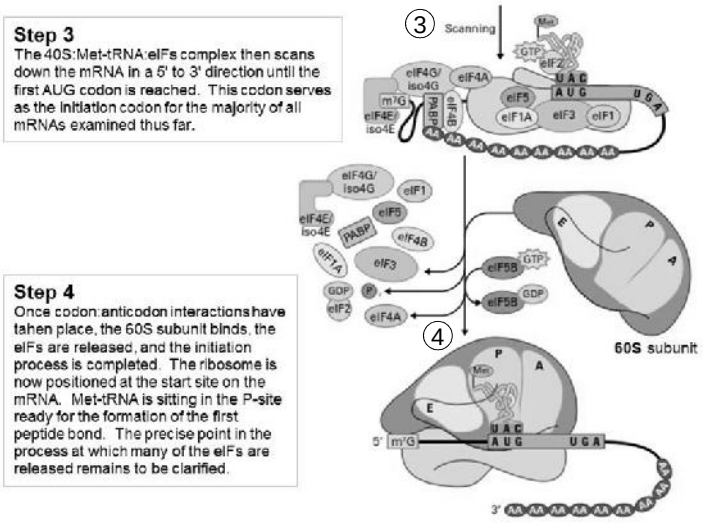
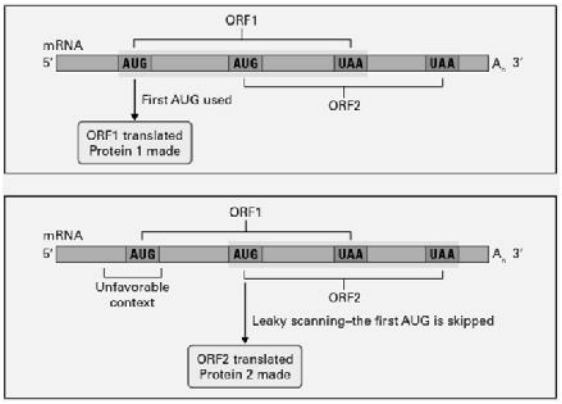


Fig 10.3 Overview of the mechanism of polypeptide chain initiation in the plant cell cytosol (Step 3 & 4).

- The 40S subunit must then identify the correct AUG codon to begin reading the mRNA.
  - Typically, the 40S subunit carrying the bound Met-tRNA migrates away from the cap along the 5′ untranslated region (UTR) of the mRNA in a 5′ to 3′ direction, a process referred to as **scanning**. Based on model plants, the 5′ UTRs range from <10 to >200 nucleotides (nt), but on average are ≈125 nt.
- For initiation, the ribosome generally selects the first AUG codon it encounters; however, there are significant exceptions (Box 10.2; viral templates).
  - AUG selection is facilitated by codon:anticodon hydrogen bonding between the AUG codon and the Met-tRNA bound to the ternary complex on the 40S subunit with the associated factors eIF1, eIF1A, and eIF5B.
- This pairing fixes the start site on the mRNA and establishes the correct reading frame.

Box 10.2. Most eukaryotic mRNAs are monocistronic, initiating protein synthesis from the AUG codon closest to the 5' end.



If the first AUG is in an unfavorable context, some ribosomes may bypass it, and initiate translation at the second AUG codon, in a process referred to as **leaky scanning**. Leaky scanning allows plant viruses to translate multiple polypeptides from a single mRNA. ORF: open reading frame

- Ribosomal selection of the initiation AUG codon is not strongly dependent on the nucleotide sequence surrounding the codon.
  - A consensus sequence surrounding the AUG translation initiation codon for Arabidopsis and rice (Oryza sativa) was identified through in silico analysis of predicted translation initiation sites.
  - The consensus sequence is highly degenerate: aa(A/G)(A/C)aAUGGcg and c(g/c)(A/G)(A/C) (G/C)AUGGCg, where lower-case letters are variable and upper-case letters are conserved nucleotides
- Other properties of the 5′ UTR that can affect translation of its downstream open reading frame (ORF) are the presence of potential secondary structures, such as a loop of self- complementarity, or short ORFs called upstream ORFs (uORFs).

- Next, the large ribosomal subunit binds to the small sub unit, with the mRNA and Met-tRNA remaining in the correct position (Fig. 10.3).
  - During this stage, eIF5 acts as a GTPase-activating protein and stimulates the hydrolysis of Met GTP by eIF2 present in the ternary complex; eIF2-GDP is then released. The final step of joining of the 60S subunit requires eIF5B, another GTPase that interacts with both the large and small subunits.
- This mechanism for the initiation of protein biosynthesis in the cytosol of plants appears to be very similar to that in other eukaryotes.
  - One difference, however, is the presence of two forms of eIF4F, the protein complex involved in recognizing the 5′ mRNA cap before the interaction between the mRNA and 40S ribosomal subunit. Plants have two forms of this initiation factor, eIF4F and eIF(iso)4F, each comprised of two subunits, eIF4G/eIF4E or eIFiso4G/eIFiso4E.

3. Regulation of Translation Initiation

- In all eukaryotes, translation is primarily regulated at the initiation. In mammals and yeast, phosphorylation of eIF2 by several different kinases prevents the exchange of GDP bound by eIF2.
  - Whether phosphorylation of eIF2 is also a major regulatory event in plant cells is still unknown and an area of active research.
  - Several lines of evidence suggest this regulatory system may function in plants. Unphosphorylated eIF2α is barely detectable under normal growth conditions, but the phosphorylated form increases in seedlings depleted for amino acids. Concomitantly, overall protein synthesis declines (Fig. 10.4).
- An Arabidopsis kinase (GCN2) that phosphorylates plant eIF2α subunit has been identified.

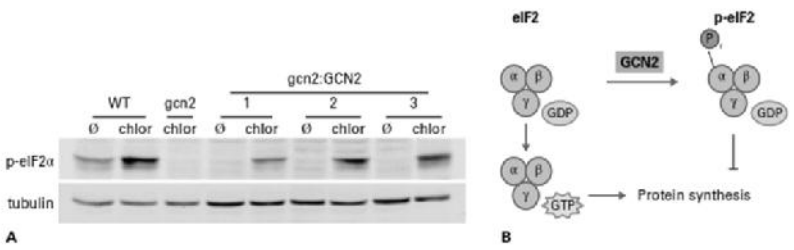


Fig. 10.4 (A) The alpha subunit of eIF2 is phosphorylated in response to amino acid deprivation (here by treatment of seedlings with the herbicide chlorosulfuron (chlor), an inhibitor of branched amino acid synthesis). Phosphorylation is detected with an antibody that specifically detects the phosphorylated form. eIF2α phosphorylation is not seen in a gcn2 mutant background, but is restored when a GCN2 expression construct is re-introduced into the mutant background. Three independent transgenic lines are shown. The tubulin immunoblot (below) indicates that equal protein was present in each sample. (B) Schematic of the proposed control of protein synthesis initiation by eIF2α phosphorylation. Phosphorylation correlates with reduced overall protein synthesis; however, the mechanism of the inhibition has not been shown to be similar to that in mammals and is currently unknown. Source: (A) Lageix et al. (2008). BMC Plant Biology 8:134.

- GCN2 is activated by amino acid deprivation, among other stresses, and eIF2 is phosphorylated in a GCN2dependent manner under the same conditions (Fig. 10.4).

- These changes correlate with an overall reduction in protein synthesis; however, a direct effect on initiation of translation by phosphorylation of eIF2α by plant GCN2 kinase has not been demonstrated.
- A second major regulatory pathway in yeast and mammals is through a small protein that competes with eIF4G for binding to eIF4E. These eIF4E-binding proteins, known as 4E-BPs in mammals, are regulated through phosphorylation by kinases in signal transduction cascades.
- This group of regulatory proteins has not been detected yet in plants, either biochemically or through bioinformatics.

4. Elongation of Polypeptide Chain

- The sequential addition of amino acids to the growing poly peptide chain involves the use of three sites (the A-, P-, and E-sites, Fig. 10.5) on the fully assembled 80S ribosome.
- The P-site (peptidyl-tRNA binding site) participates in chain initiation and donates the growing polypeptide chain to the incoming aminoacyl-tRNA (aa-tRNA) in the A-site.
- The A-site (aa-tRNA binding site or decoding site) exposes the next codon to be read on the mRNA, where the incoming charged tRNA binds.
- The P-site tRNA occupies the E-site (exit site) after it has released its growing polypeptide chain, just before it leaves the ribosome.

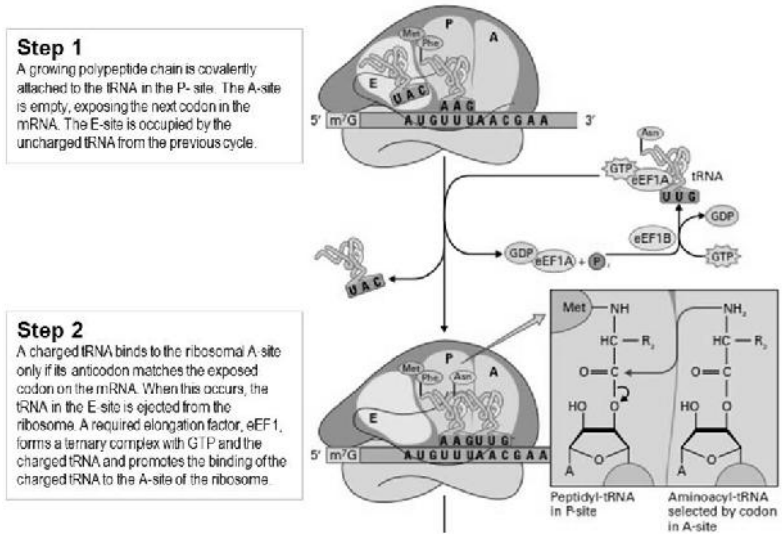
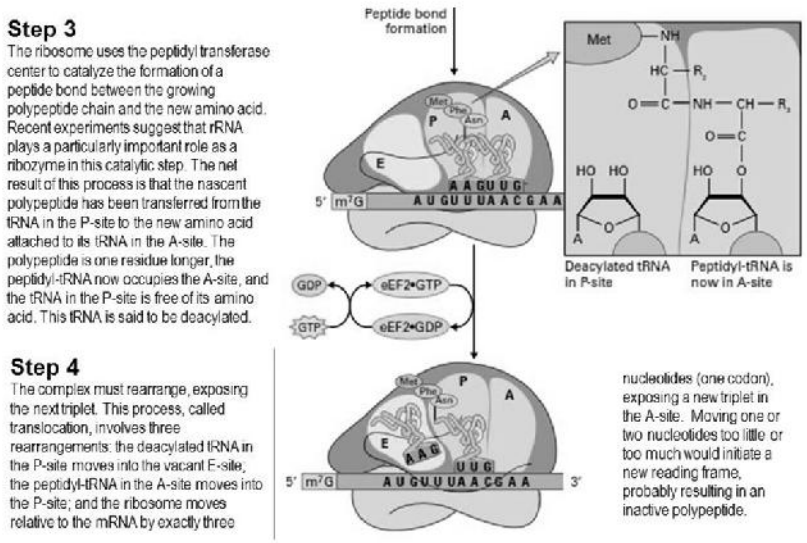


Fig. 10.5 The elongation phase of cytosolic protein synthesis in plants



- These three sites are used sequentially as the polypeptide chain is synthesized, and a complete cycle requires as little as 0.05 seconds.
- The overall chain elongation process occurs in three steps (Fig. 10.5) and requires three elongation factors: eEF1A, eEF1B, and eEF2.
  - eEF1A in the GTP-bound form binds to the aa-tRNA and delivers it to the ribosome, accompanied by GTP hydrolysis.
  - eEF1B exchanges the GDP for GTP on eEF1A to allow recycling of eEF1A.
  - eEF2 catalyzes translocation of the mRNA through GTP hydrolysis after formation of the peptide bond.
- eEF2, unlike other G proteins involved in translation, such as eIF2 and eEF1A, does not require a recycling factor to replace the GDP for GTP.

5. Termination of Translation

- Centrifuge and fractionate 40% sucrose
- Polypeptide elongation ceases when a ribosome reaches one of three stop codons on the mRNA: UAA, UAG, or UGA (Fig. 10.7).
- Termination of protein synthesis requires two proteins, known as release factors (RFs), eRF1 and eRF3, to bind to the A-site.
  - eRF1 is a structural mimic of tRNA, and it recognizes all three stop codons and binds eRF3 and GTP.
  - eRF3 has GTPase activity.
- RF interaction with the ribosome triggers a series of events, including hydrolysis of the bond between the completed protein and the final tRNA at the P-site.

- GTP hydrolysis by RF3 stimulates release of the polypeptide and the RFs from the ribosome. The tRNA and the ribosome are also released from the mRNA and become available to participate in another cycle of translation.
- Dissociation of the 80S ribosome into 40S and 60S subunits is promoted by eIF6 and ATPbinding cassette E (ABCE1) protein in an ATP-dependent process, and may not occur until 40S subunits are required for preinitiation complex formation.
- Consequently, 80S ribosomes lacking an mRNA accumulate when initiation of translation is limited, such as under conditions of stress (e.g., hypoxia, heat shock, or dehydration stress).

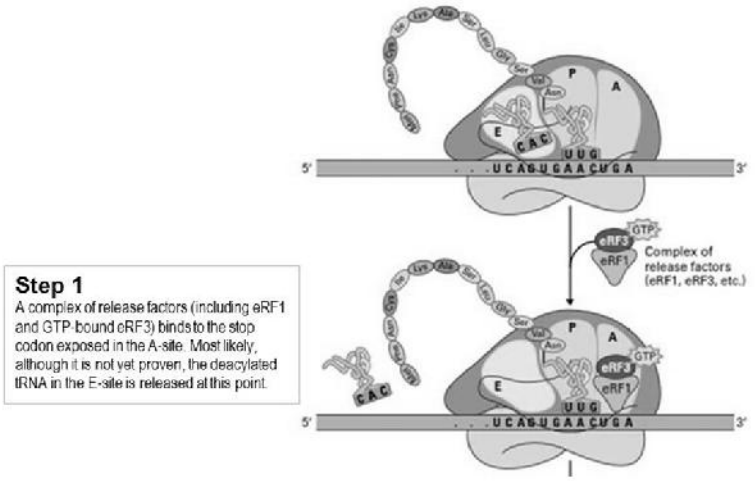
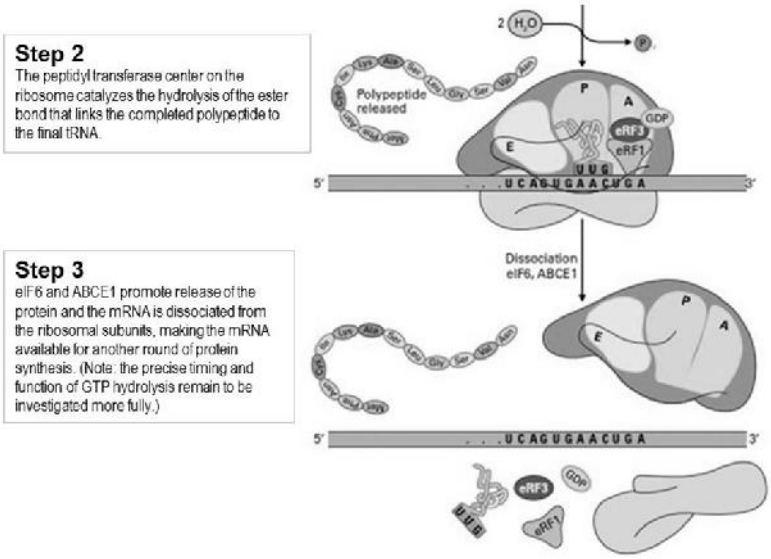


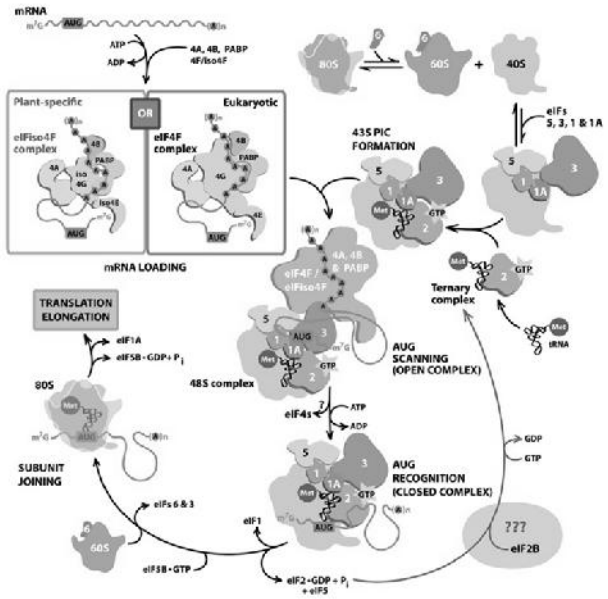
Fig. 10.7 Events during the termination of protein synthesis. Polypeptide chain termination occurs when one of three stop codons appears in the A-site of the ribosome.



QUESTIONS

1. What is translation in protein synthesis?
2. What is codon and anticodon?
3. What is peptide bond?
4. Where does protein synthesis take place in plant cells?
5. What is the function of tRNA in protein synthesis?
6. What is the actual component of ribosomes catalyzing the formation of peptide bonds?
7. What is eIFs and their function?
8. What is the role of the intiation factor of PABP?
9. What is the factor that initiates the translation of protein synthesis?
10. What proteins are that required to terminate protein synthesis?

Overview of the steps of translation initiation in the cytoplasm of plants.



Source: Browning & Bailey-Serres (2015)