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Chapter 10

Protein Synthesis and Regulation in Eukaryotes

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INTRODUCTION

The genetic information encoded in DNA becomes functionally meaningful only when it is accurately transcribed and translated into RNA and protein. Two types of RNA, transfer (t) RNA and ribosomal (r) RNA, are themselves functional

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molecules. However, the genetic information transcribed into the third RNA, messenger (m) RNA, has to be translated from a language of nucleic acids into a language of amino acids in order to produce proteins which are functional gene products. It is generally estimated that in a human cell, there are about 100,000 genes, of which between 3,000 and 10,000 are expressed and translated into a spectrum of proteins specific for every differentiated cell type.

Proteins are the most versatile macromolecules necessary for the organization of internal cellular structures, for the formation of the energy-creating and metabolic-utilizing systems in the cell, for the transport of ions and larger molecules across cell membranes and for maintaining intracellular and intercellular communication pathways. Furthermore, proteins interact with all other macromolecules, including DNA, RNA, carbohydrates, and lipids, and are required for maintenance and repair at all levels of biological organization.

Protein synthesis is thus crucial for the survival of a living system, and any disturbance at this level can cause large imbalances and deficiencies. For example, inhibition of protein synthesis is followed rapidly by cell death. If the rate of protein synthesis slows down, it affects not only the basic metabolic enzymatic processes but also the energy-supporting system, the membrane-mediated signaling pathways, and the processes for the removal of damaged and abnormal molecules. Similarly, if the process of protein synthesis becomes less accurate, that is, if unfaithful translation of the genetically specified sequence of amino acids occurs, it can result in the loss of activity and specificity of proteins with far reaching and damaging consequences for the cell and the organism. Therefore, the process of protein synthesis and its regulation during different stages of growth, division, differentiation, development, and aging is one of the most crucial aspects of a living system.

MECHANISMS OF PROTEIN SYNTHESIS

Since protein synthesis in eukaryotes occurs both in the cytoplasm and in certain cellular organelles such as mitochondria (and chloroplasts in the case of plants), the mechanisms of cytoplasmic protein synthesis are described first, followed by a discussion of the similarities and differences in protein synthesis in the organelles, particularly the mitochondria.

The primary structure of a protein is its sequence of amino acids, translated from the sequence of three-letter nucleotide codons in mRNA that itself is copied (transcribed) from DNA. The transcription of RNA, its posttranscriptional editing, processing, and transport from the nucleus to the site of translation is a huge subject, beyond the scope of this article. Interested readers are advised to consult other review articles, for example Bag (1991), Kozak (1991), and Ross (1989).

Protein synthesis is one of the most complex processes in the cell. In order to translate one mRNA molecule, almost 200 small and large components are required to function effectively and accurately while using large quantities of cellular energy

Table 1. Major Components of the Protein Synthetic Machinery Required for Translation

Component	*Subcomponents*	*Function*
1. Translational particle		
Ribosome	40S and 60S subunits 4 rRNAs and about 80 ribosomal proteins	Recognizing and translating the genetic codons in mRNA
2. Charging system		
Amino acids	At least 20	Building blocks for proteins
tRNAs	About 60	Matching codons with respective amino acids
tRNA synthetases	At least 20	Adding amino acids to tRNA
3. Translational factors		
Initiation factors	more than 24 proteins	Initiating protein synthesis
Elongation factors	4 proteins	Addition of amino acids to growing peptide chain
Release factor	1 protein	Terminating protein synthesis

in the form of GTP. There are three major components of the translational apparatus: (1) the translational particle, the ribosome; (2) the amino acid-transfer system or charging system; and (3) the translational factors. Table 1 gives an overview of the components and the subcomponents involved in eukaryotic protein synthesis, along with their major functional characteristics.

Protein synthesis can be envisaged as proceeding in three steps—initiation, elongation, and termination, followed by posttranslational modifications, including folding, which give the protein a functional tertiary structure. Of these steps, the initiation and termination steps occur only once for each polypeptide chain, whereas the elongation step is repeated as many times as the number of amino acids in a complete chain. Although there are no minimum and maximum limits on the number of amino acids required to make a functional protein, the lengths of the shortest and longest mRNA molecules in eukaryotes are not yet known.

Initiation

The translation of an mRNA begins with the formation of a so-called initiation complex between the ribosome and the initiator codon, AUG. Figure 1 is a simplified schematic representation of the major steps in the generation of an initiation complex. It is an intricate process, which consumes energy and involves at least seven-initiation factors (eIFs; e stands for eukaryotic) consisting of 24-different subunits, two subunits of ribosomes, and an initiating tRNA called Met-$tRNA_i$.

The process of translational initiation begins with the dissociation of inactive 80S (S stands for Svedberg) ribosomes, called monosomes, to generate free 60S

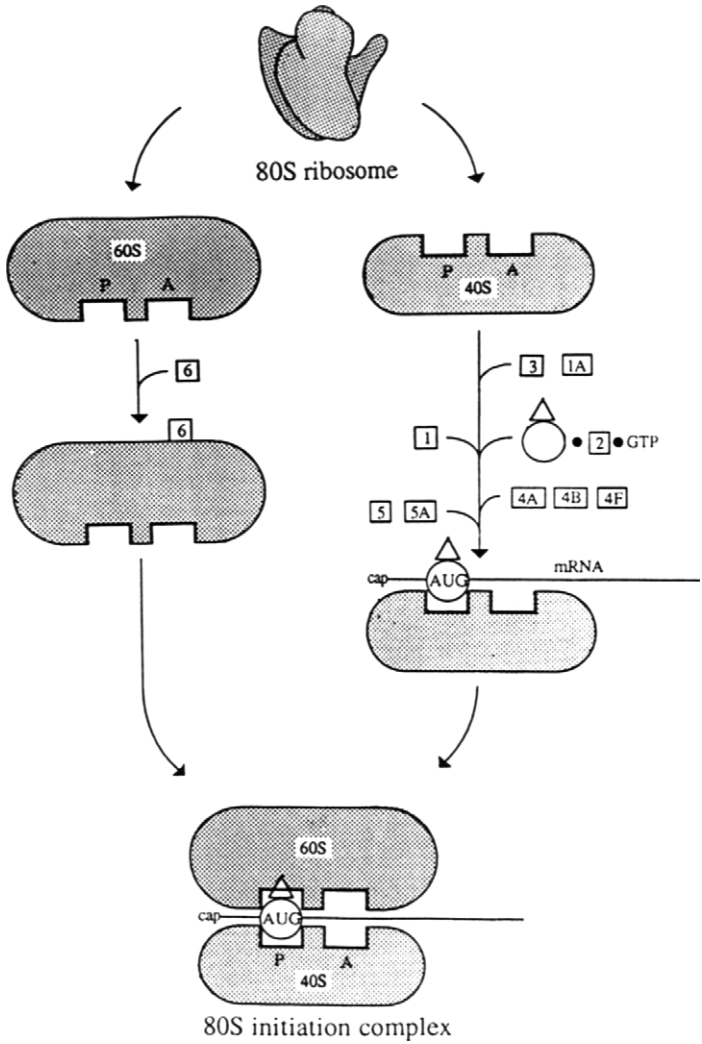

Figure 1. Schematic diagram showing the major steps of the initiation of protein synthesis in eukaryotic cells. The formation of an 80S initiation complex involves the joining of the 40S subunit bound to mRNA and met-tRNA$_i$, and the 60S subunit. Numbers in □ represent various initiation factors, ○ represents tRNA, and △ represents the amino acid. (From Johansen and Rattan, 1993).

and 40S subunits. Although far less is known about eukaryotic ribosomes than prokaryotic ribosomes, significant progress is being made in understanding the structure of eukaryotic ribosomes, particularly from yeast and from rat (Wool, 1991). Ribosomes are cellular organelles that comprise at least four types of rRNA and about 80 proteins, and are pivotal in the translation of the language of nucleic acids into a language of amino acids.

The nucleotide sequences of the four species (5S, 5.8S, 18S, and 28S) of rRNA have been determined, and these show remarkable homology among the three kingdoms (archaebacteria, eubacteria, and eukaryotes). In the case of ribosomal proteins, the amino acid sequences of about 35 proteins out of a total of about 80 have been determined. However, the exact roles of various rRNAs and proteins and their interactions in determining the activity, efficiency, and accuracy of the ribosomes is not very well understood at present. The coordinated synthesis of rRNAs and proteins, and the assembly of ribosomes is a process whose complexity is only beginning to be unravelled (Wool, 1991).

The dissociation of the inactive ribosome into its subunits is dependent on the activity of two initiation factors, eIF-3 and eIF-6, which keep subunits apart by binding to the 40S and the 60S subunits, respectively (Hershey, 1991; Rhoads, 1991; Merrick, 1992). eIF-3 is the largest of the initiation factors (molecular mass 600–650 kDa) comprising 8–10 different polypeptides. In comparison, eIF-6 is a single polypeptide of about 25 kDa.

The next step is the formation of the 43S ternary complex of met-tRNA$_i$, GTP, and eIF-2. This binds to the 40S subunit in the absence of mRNA. eIF-2 is one of the most widely studied initiation factors and is known to contain three polypeptides, α, β, and γ, with molecular masses of 35, 38, and 52 kDa, respectively. The primary function of eIF-2 is to bind met-tRNA$_i$ in a process that is dependent on GTP. Thereafter, through a process that is still largely a mystery, the binding of the 43S complex with mRNA occurs at its methyl-7-guanine (m^7G) cap at the 5′ end (Nygård and Nilsson, 1990). Three initiation factors, eIF-4A, 4B, and 4F, are required for optimal binding of mRNA while using one molecule of ATP. eIF-4A is a highly conserved single-polypeptide chain of about 45 kDa and is characterized as an ATP-dependent RNA helicase involved in the unwinding of RNA. A particular feature of all putative RNA helicases is the presence of the D-E-A-D (Asp-Glu-Ala-Asp) amino acid sequence motif, thought to be associated with the processing of RNA. In comparison, eIF-4B is a dimer of identical subunits of about 80 kDa and is necessary for the cross-linking of eIF-4A to mRNA. eIF-4F also contains two subunits of 26 kDa and 220 kDa, both of which interact with the m^7G cap of the mRNA.

The binding of the 43S complex to the capped 5′-end of the mRNA is followed by its migration and scanning until the first AUG codon is encountered (Kozak, 1989). Although there are some exceptions, more than 90% of cellular mRNAs appear to have "GCC GCC A/GCC **AUG** GG" as the consensus sequence for the

initiation of protein synthesis. However, it is not known what recognizes the consensus sequence and brings about a transient stopping of the complex at the AUG start codon to effect the correct positioning of mRNA for translation.

Once correct positioning occurs, and the match is made between the anticodon of the met-tRNA$_i$ and the start codon, the GTP molecule bound to eIF-2 is hydrolyzed in a reaction promoted by eIF-5. The physical nature of this reaction remains controversial. There are thought to be two forms of eIF-5 with molecular masses of 125 kDa and 60 kDa without, however, any differences in their biological properties (Hershey, 1991; Merrick, 1992). The hydrolysis of GTP causes the release of the initiation factors from the surface of the 40S ribosomal subunit, and allows attachment of the 60S subunit by triggering the release of eIF-6 from it. The formation of the 80S initiation complex culminates in the formation of the first peptide bond at the ribosomal P site. The initiation factor eIF-4D is required for the formation of the first peptide bond. eIF-4D is a small protein (about 16 kDa), and has a unique posttranslational modification of its lysine-50 residue by the action of a polyamine, spermidine, to form a hypusine residue essential for its activity (Hershey, 1991; Merrick, 1992). Furthermore, in order to allow efficient and catalytic use of eIF-2 after GTP hydrolysis and its release from the complex, another factor, eIF-2B, facilitates the exchange of eIF-2 bound GDP for GTP.

The whole process of the formation of the 80S initiation complex takes about 2–3 s in cell-free assays and is thought to occur much faster *in vivo*. Each mRNA can participate in multiple rounds of initiation, thus giving rise to a string of ribosomes called polysomes, engaged at different stages of translation. Polysomes can be observed both attached to the endoplasmic reticulum and in the cytoplasm. It is estimated that an efficiently translated mRNA at 37° C initiates protein synthesis once every 5–6 s (Hershey, 1991). How many times an mRNA can be translated depends on several aspects of its structure, including the context surrounding the AUG codon, and its lifespan expressed in terms of the rate of degradation (Ross, 1989; Kozak, 1991). What is important in a biological context is that the initiation step is considered the main target for the regulation of protein synthesis during cell cycle, growth, development, hormonal response, and under stress conditions including heat shock, irradiation, and starvation. How this regulation is brought about will be discussed in a separate section.

Elongation

The formation of the 80S initiation complex is followed by the repetitive cyclic event of peptide-chain elongation. How many times the elongation cycle will be repeated is determined by the total number of amino acids to be incorporated into the polypeptide chain, as specified by the number of codons in the mRNA. The transfer of amino acids from the cytoplasm to the ribosome is carried out by tRNAs followed by their joining in a chain, a series of reactions catalyzed by elongation

Table 2. Categories and Names of the Most Common Amino Acids, Their Abbreviations, and Respective Translatable Codons in mRNA

Name	*Abbreviations*		*Translatable Codons*
	Three letter	*One letter*	
Aliphatic amino acids			
1. Glycine	Gly	G	GGU/GGC/GGA/GGG
2. Alanine	Ala	A	GCU/GCC/GCA/GCG
3. Valine	Val	V	GUU/GUC/GUA/GUG
4. Leucine	Leu	L	CUU/CUC/CUA/CUG
5. Isoleucine	Ile	I	AUU/AUC/AUA
Hydroxy amino acids			
6. Serine	Ser	S	UCU/UCC/UCA/UCG/AGU/AGC
7. Threonine	Thr	T	ACU/ACC/ACA/ACG
Dicarboxylic amino acids			
8. Aspartic acid	Asp	D	GAU/GAC
9. Asparagine	Asn	N	AAU/AAC
10. Glutamic acid	Glu	E	GAA/GAG
11. Glutamine	Gln	Q	CAA/CAG
Basic amino acids			
12. Lysine	Lys	K	AAA/AAG
13. Histidine	His	H	CAU/CAC
14. Arginine	Arg	R	CGU/CGC/CGA/CGG/AGA/AGG
Aromatic amino acids			
15. Phenylalanine	Phe	F	UUU/UUC
16. Tyrosine	Tyr	Y	UAU/UAC
17. Tryptophan	Trp	W	UGG
Sulfur-containing amino acids			
18. Methionine	Met	M	AUG
19. Cysteine	Cys	C	UGU/UGC
Imino acids			
20. Proline	Pro	P	CCU/CCC/CCA/CCG

factors (EFs; also abbreviated as eEFs). In this section, we shall first describe various components, particularly amino acids, tRNAs, aminoacyl–tRNA synthetases, and elongation factors involved in the elongation phase of protein synthesis and shall then describe the elongation cycle.

Amino Acids

Most commonly, there are 20-amino acids used in protein synthesis. For reasons not well understood, all the amino acids that occur in proteins are in the L form of the two enantiomers, D and L. The sequence of amino acids in a polypeptide chain gives rise to its primary structure that is, in turn, the basis of the secondary and tertiary forms of a protein. Table 2 gives a list of amino acids, their three-letter and single-letter abbreviations, and the codons on the mRNA that can be translated for each of them.

Although not every protein contains all the amino acids, lack of a single amino acid in the cytoplasmic pool can result in the inhibition of total protein synthesis.

In addition to the twenty "standard" amino acids, many nonstandard amino acids are also found in almost all proteins. Generally, these amino acids arise as a consequence of various chemical modifications after they have been incorporated into protein. Posttranslational modification of amino acids is one basis of the regulation of protein activity, specificity, and stability.

tRNAs and Aminoacyl–tRNA Synthetases

RNA molecules that physically bring the amino acids onto the template codons of mRNAs bound to ribosomes are tRNAs. There is at least one tRNA for each of the 61 codons translated into amino acids, but there are no tRNAs for the stop codons UAA, UAG, and UGA. Therefore, for several of the amino acids, for example G, A, V, L, S, and R there are 4 to 6 isoacceptor tRNA species, and their relative abundance is correlated with the codon usage in the mRNAs being translated (Lapointe and Giegé, 1991). More than a thousand sequences of tRNAs and their genes have been determined. All tRNAs are between 70 and 95 nucleotides long and can be folded into a cloverleaf secondary structure. A unique characteristic of tRNA is the presence of several modified nucleotides near and around the anticodon loop. More than 50 modified nucleotides have been discovered in eukaryotic tRNAs and these include dihydrouridine (D), inosine (I), N^6-isopentenyladenosine (i^6A), queusine (Q), and wyosine (Y). Although the exact mechanisms are not known, these modified nucleotides in tRNA are considered to be involved in codon recognition.

The function of a tRNA in transferring the amino acid to the ribosome–mRNA complex is dependent upon a specific enzyme that catalyzes the ligation of the appropriate amino acid to its acceptor arm at the 3′ end. This process of amino-acylation of an amino acid to a tRNA is also known as "charging", and the enzymes involved in this process are called aminoacyl–tRNA synthetases (aaRSs) or, more accurately, aminoacyl–tRNA ligases. A group of isoaccepting tRNAs are charged only by the single aaRS specific for their amino acid. Thus, there are only about 20 aaRSs for all tRNA species.

Functionally, it is the anticodon on tRNA that determines the specificity for an amino acid as recognized by aaRSs. Charging of tRNA is an energy-dependent process that consumes two molecules of ATP for every event of amino-acylation. The correct charging of a tRNA molecule requires specificity of tRNA recognition by aaRSs along with proofreading and editing of incorrect amino acids. Through a process of what has been called "double-sieve editing", the error frequency of tRNA charging is usually maintained at less than 1 in 10^3 amino acid additions (Fersht, 1986). Furthermore, levels of tRNAs and aaRSs are considered to be rate limiting for protein synthesis.

Elongation Factors

The addition of an amino acid to the growing polypeptide chain is facilitated by at least two elongation factors, EF-1 and EF-2. A third factor, EF-3, is reported only in some fungi and yeast. EF-1 is composed of three distinct parts: a G-binding protein, EF-1α, and a nucleotide-exchange protein complex, EF-1βγ. EF-1 usually occurs in multiple molecular forms, composed of varying amounts of EF-1α and EF-1βγ. Sequences of EF-1α genes, cDNAs and protein from more than 15 different species have been determined and found to be highly conserved during evolution. For example, in comparison with human EF-1α, the most distant eukaryotic sequence is that of tomato (78% homology), the closest nonmammal is *Xenopus laevis* (95% homology), whereas almost 100% homology exists among mammals (Riis et al., 1990a).

Some other interesting features of EF-1α include its high abundance (between 3% and 10% of the soluble protein) and multiple copies of the gene that in some cases undergo cell type and/or developmental stage-specific expression as reported in yeast, fungi, brine shrimp, fruit fly, toad, and human cells (Merrick, 1992). Furthermore, EF-1α appears to have several other functions in addition to its requirement in protein synthesis. For example, EF-1α has been reported to bind to cytoskeletal elements, particularly actin. It is associated with the endoplasmic reticulum, and is part of the valyl–tRNA synthetase complex; it is also associated with the mitotic apparatus, and is involved in protein degradation and ribosome association (Merrick, 1992). Similarly, the role of an extra copy of the EF-1α gene in life prolongation, (e.g., transgenic *Drosophila* at high temperature, Shepherd et al., 1989), and in an increase in the accuracy of protein synthesis in yeast cells (Song et al., 1989) indicate the pluripotent nature of this factor.

The other elongation factors, EF-1βγ and EF-2, are involved, respectively, in the posthydrolytic exchange of GDP with GTP and in the translocation of peptidyl–tRNA on the ribosome. Of these, EF-2 (95 kDa) has a unique characteristic in the form of a histidine residue at position 715 modified into diphthamide, as a result of which it can be ADP-ribosylated either endogenously or by bacterial toxins such as diphtheria toxin (Riis et al., 1990b). EF-3, which is present in some yeast and fungal species, is a 125 kDa polypetide chain. A unique property of EF-3 is its ability to function with any of the three purine nucleosides (A, G, and I), perhaps to facilitate the interaction of EF-1α, GTP, and aminoacyl–tRNA. The requirement for EF-3, even *in vitro*, is an exclusive property of yeast ribosomes. When yeast factors are tested with mammalian ribosomes, EF-3 has no effect (Riis et al., 1990).

The elongation cycle proceeds in three steps (Figure 2). Once the initiator tRNA is bound in the ribosomal P site thereby forming the 80S initiation complex, the codon-directed repetitive cycle of peptide-chain elongation sets in. In the first step, the binding of an aminoacyl–tRNA carrying the appropriate amino acid is directed by EF-1α bound to GTP. After the correct match has been made and a process of

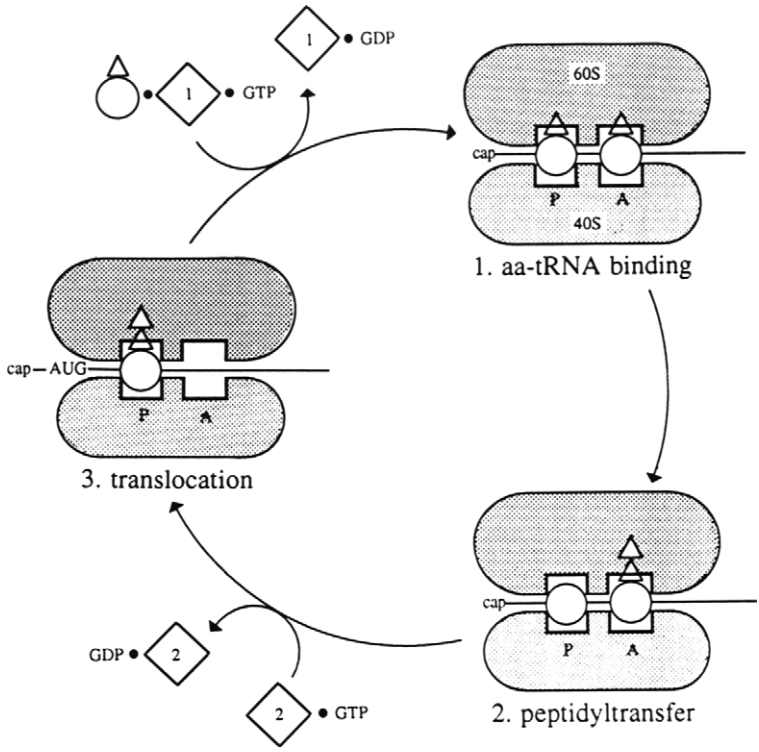

Figure 2. Schematic representation of the cyclic and repetitive event of polypeptide chain elongation. Numbers in ◇ represent various elongation factors, ○ represents aminoacyl–tRNA, and △ represents the amino acid. (From Johansen and Rattan, 1993).

proofreading completed, the hydrolysis of GTP is initiated by some as yet unidentified signal, followed by the placement of the aminoacyl–tRNA in the A site and the release of the EF-1α·GDP complex. The GDP on EF-1α is exchanged for GTP through a process that involves EF-1βγ, thereby regenerating an active EF-1α.

The second step in the elongation of protein synthesis is the peptidyl-transferase reaction in which a peptide bond is formed between the amino acid in the P site and the amino acid coupled to aminoacyl–tRNA in the A site. This reaction is facilitated by the intrinsic activity of the ribosome. As a result of this reaction, the growing polypeptide chain becomes elongated without moving forward. The movement or translocation of the dipeptide–tRNA from the A site to the P site is achieved by the action of EF-2, while another molecule of GTP is hydrolyzed. This process results in the relative movement of the mRNA by three nucleotides, so that a new codon becomes readable in the A site. The deacylated tRNA is pushed out of the ribosome after a transient halt at the so-called exit (E) site. At this point, all the components

involved in the elongation cycle become ready to undergo the next cycle. In terms of energy consumption, the addition of each new amino acid to a growing polypeptide chain costs 4 high-energy phosphates from 2 molecules of ATP during amino-acylation and 2 molecules of GTP during elongation. Various estimates of the elongation rates in eukaryotic cells give a value in the range of 3–6 amino acids incorporated per ribosome per second, which is several times slower than the prokaryotic elongation rate of 15–18 amino acids incorporated per second (Hershey, 1991).

Termination

The cycle of peptide-chain elongation continues until one of the three stop codons (UAA, UAG, UGA) is reached. There is no aminoacyl–tRNA complementary to these codons, and instead a termination factor or a release factor (RF) with bound GTP binds to the ribosome and induces hydrolysis of both the aminoacyl-linkage and GTP, thereby releasing the completed polypeptide chain from the ribosome. The 475 amino acid-long sequence of rabbit liver RF has been deduced from its cDNA sequence, and it shows 90% homology with mammalian tryptophanyl–tRNA synthetase (Lee et al., 1990). It has also been reported that for efficient and accurate termination, an additional fourth nucleotide (most commonly an A or a G) after the stop codon is required (Tate and Brown, 1992). The exact role of the fourth nucleotide in the termination of protein synthesis is not fully understood at present.

Organeller Protein Synthesis

Mitochondria and chloroplasts are the two main eukaryotic cellular organelles that contain their own genome and undertake the semi-independent processes of transcription and translation. Most of the constituent proteins of these organelles are imported from the surrounding cytoplasm, but each organelle also synthesizes its own proteins. Here we shall limit our discussion to mitochondrial protein synthesis, because the chloroplast is not present in animal cells.

Since nucleic acids generally cannot go in and out of mitochondria, all mitochondria appear to code for their own rRNAs and tRNAs. For the same reason, only the mRNAs that have been transcribed from the mitochondrial genome are translated in the mitochondria. A unique feature of mitochondrial mRNAs is the lack of a m^7G cap at the 5′ end (reviewed by Bag, 1991). There are only 22–25 tRNA species in the mitochondria, indicating that a single tRNA can recognize more than one codon. There are some structural and sequence differences in the mitochondrial tRNAs. Furthermore, deviations from the standard genetic code, for example, utilization of AUA as the initiation codon instead of AUG, and reading UGA as a tryptophan instead of a stop codon, are a unique feature of mitochondria (Lapointe

and Giegé, 1991). The proteins imported from the cytoplasm into mitochondria include ribosomal proteins, initiation, elongation, and termination factors, and aminoacyl–tRNA synthetases.

Although there is a 5-fold difference between the sizes of the mitochondrial genomes of yeast (84 kb) and mammals (16 kb), the number of proteins synthesized within mitochondria is similar. Proteins produced by mammalian mitochondria are those involved in electron-transport and oxidative-phosphorylation systems. These include cytochrome *b*, three subunits of cytochrome oxidase, one subunit of ATPase, and six subunits of NADH dehydrogenase. Apart from these differences, protein synthesis in mitochondria follows the same steps and mechanisms as those in the cytoplasm.

REGULATION OF PROTEIN SYNTHESIS

The regulation of protein synthesis means two things: first, the regulation of translation of individual mRNA species, and second, the regulation of the rates of total protein synthesis in a cell. For specific proteins, the rate-limiting factor is usually the mRNA, whose levels and several secondary and tertiary features such as the presence and accessibility of the cap, the placement and context of the AUG initiator codon, and the presence and length of the poly(A) tail determine the rate of translation. Some of the most important examples of translational regulation at the level of individual mRNAs include mRNAs for ribosomal proteins, heat shock proteins, several hormonally regulated proteins, transcription activator protein GCN4 (in yeast), and proteins involved in iron metabolism (e.g., ferritin). The regulation of the rates of mRNA production, processing, transport, stability, and turnover is a complex and wide subject beyond the scope of this chapter, and other review articles (Ross, 1989; Bag, 1991; Kozak, 1991; Rhoads, 1991), should be consulted.

The regulation of total- or bulk-protein synthesis is independent of mRNA levels and the rate-limiting factors, in principle, can be any of the components of the protein synthetic machinery. Therefore, all three steps of initiation, elongation, and termination are targets in the regulation of protein synthesis.

Regulation at the Level of Initiation

Global regulation of protein synthesis occurs most commonly at the level of initiation as exemplified by studies carried out on protein-synthetic rates during the cell cycle, differentiation, embryonic development, and by altered physiological conditions such as serum starvation, amino acid starvation, glucose starvation, hypertonic conditions, ionic changes, heat shock, and other stresses.

The availability of the 43S ternary complex of met-tRNA$_i$, GTP, and eIF-2 is the rate-limiting step for translation initiation. In this context, both the levels of ribosomes and the amounts and activities of soluble factors including eIFs are

critical. However, it is generally believed that the regulation of specific activities rather than levels of translational components allows the cell to alter rates of bulk protein synthesis rapidly (Hershey, 1991). Posttranslational modification, particularly phosphorylation of eIFs, is considered the main mode of regulation of their activities.

The phosphorylation of the α subunit of eIF-2 at ser 48 and ser 51 appears to regulate its activity in terms of increasing its binding with GDP, hindering the GDP/GTP exchange reaction, and thus abolishing its recycling activity (Mathews et al., 1990; Hershey, 1991). At least two protein kinases and phosphatases are involved in the phosphorylation and dephosphorylation of eIF-2. Examples of the regulation of the ternary complex by the phosphorylation of eIF-2 include physiological conditions such as heat shock, nutrient deprivation, heme deficiency, and viral infection.

Other soluble factors whose activities are regulated by phosphorylation and which are involved in determining the rates of protein initiation include eIF-3, eIF-4A, eIF-4B, eIF-4F, and eIF-5. However, unlike eIF-2 in which phosphorylation is related to inhibition of protein synthesis, phosphorylation of all other factors is correlated with stimulation of protein synthesis at the level of initiation. Similarly, phosphorylation of S6 ribosomal protein is also considered to increase the rates of initiation by increasing the activity of 40S ribosomal subunits. In some cases (increased amounts and activities of eIFs, for example), overexpression of eIF-4E has been shown to cause malignant transformation of mammalian fibroblasts (Lazaris-Karatzas et al., 1990). In addition, the initiation factor eIF-4D, which is required for the formation of the first peptide bond, has a unique posttranslational modification, hypusine, whose absence can block the initiation of protein synthesis (Park et al., 1991).

Regulation at the Level of Elongation

The regulation of protein synthesis can also occur totally and differentially at the level of polypeptide-chain elongation. Examples of differential regulation include the rapid translation of heat shock-induced mRNAs in *Drosophila* and chick reticulocytes, translation of viral S1 mRNA in reovirus-infected cells, synthesis of vitellogenin in cockerel liver after estradiol injection, and the synthesis of tyrosine aminotransferase in cultured hepatoma cells treated with cAMP (Spirin and Ryazanov, 1991). The regulation of bulk-protein synthesis at the level of elongation has been reported for normal and transformed cells during cell cycle transition, amino acid starvation, serum stimulation, and phorbol ester treatment (Spirin and Ryazanov, 1991; Johansen and Rattan, 1993). Similarly, alterations in the rates of elongation have also been reported in full-term human placenta from diabetic mothers, in rat livers during fasting and refeeding, and during aging in various cells, tissues, and organs of *Drosophila*, rats, and mice (Rattan, 1992).

In principle, elongation rates can be regulated through changes in the concentration of aminoacyl-tRNAs, modifications of ribosomes, and changes in the amounts and activities of elongation factors. However, most of the available evidence points towards elongation factors EF-1 and EF-2 as the main regulators of protein-elongation rates. For example, changes in the total-protein-synthesis rate have been correlated with the amounts and activities of EF-1 in mammalian cells in culture, both during the cell cycle and during aging, in rat and mouse organs during regeneration and aging, in rat spleen during the immune response, in sea urchin eggs after fertilization, and in a fungus *Mucor* during spore germination (Spirin and Ryazanov, 1991). Similarly, the amount of active EF-2 has been reported to vary with the protein synthetic status of mammalian cells in different phases of the cell cycle and during aging (Riis et al., 1990b).

As in the case of initiation factors, posttranslational modifications of EF-1 and EF-2 are considered important for determining their activities. Methylation of EF-1α at five-lysine positions appears to regulate its activity in a way that is correlated with changes in protein-synthetic rates (Riis et al., 1990a). Furthermore, phosphorylation and the addition of glycerol-phosphoryl-ethanolamine to EF-1α have been suggested to alter its activity. Similarly, phosphorylation of the recycling protein, EF-1$\beta\gamma\delta$, is reported to regulate its activity either positively or negatively, depending on the kinase responsible for phosphorylation (Merrick, 1992).

The site of regulation of the activity of EF-2 is considered to be his715, which is modified to diphthamide (Riis et al., 1990a). ADP-ribosylation of this diphthamide residue then results in abolition of the translocation activity of EF-2. Phosphorylation of EF-2 by a calcium/calmodulin-dependent protein kinase III (CaM PK III), also known as the EF-2 kinase (Redpath and Proud, 1993), is considered another mode of regulation of EF-2 activity. Changes in the amounts of phosphorylated EF-2 during the mammalian cell cycle have been correlated with the changes in protein-synthetic rates (Celis et al., 1990; Spirin and Ryazanov, 1991). Furthermore, the level of phosphorylated EF-2 can be regulated by specific protein phosphatases, for example the type 2A, whose activities are increased by treatment with phorbol esters (Merrick, 1992).

Regulation at the Level of Termination

It was once commonly believed that the termination step of polypeptide-chain synthesis was not a target for regulation of protein synthesis. However, in recent years significant evidence has accumulated showing that in several instances a termination codon can be translated as a sense codon (nonsense suppression), or it can be skipped by frame-shift resulting in the synthesis of elongated proteins. For example, the opal termination codon, UGA, can be translated as trp in mitochondria, and UAA and UAG termination codons can be translated as gln in ciliates (Valle and Morch, 1988). Similarly, owing to ribosomal frame-shifting (when a ribosome

shifts from one reading frame to another at a position in the mRNA before reaching a termination codon), elongated proteins can be produced, such as during the expression of retroviruses and coronavirus in eukaryotes (Valle and Morch, 1988; Tate and Brown, 1992). Furthermore, in some proteins, for example mammalian glutathione peroxidase, the incorporation of selenium-cysteine (Se-cys) is facilitated by an opal suppressor tRNA that can translate UGA as Se-cys (Valle and Morch, 1988). Thus, although the termination step of protein synthesis is not a common site of regulation, in special cases it can regulate the rate and extent of expression of specific proteins.

SOME GENERAL COMMENTS

Although faithful translation of the genetic information encoded in mRNA into a polypeptide chain is a prerequisite for accurate protein synthesis, it is not enough to guarantee efficient functioning of the protein. According to one estimate, more than 140 types of posttranslational modifications of proteins have been described that determine the activity, stability, specificity, and transportability of a protein (Alix and Hayes, 1983; Rattan et al., 1992). These modifications include covalent modification reactions involving amino acid side-chain residues (for example, phosphorylation, oxidation, methylation, acetylation, glycation, and ADP-ribosylation), deamidation, racemization, and noncovalent spontaneous changes in protein conformation and folding.

In addition to posttranslational modifications that can regulate translational rates by regulating the activities and efficiencies of various components of protein synthetic machinery, regulation of protein synthesis can also be achieved by intracellular ionic levels, energy charge, pH, nutrient availability, growth factors, heat shock, heavy metals, and other physiological conditions. Thus, the subject of protein synthesis and its regulation still awaits the filling of several gaps and the gathering of more knowledge in addition to what is already known about this most fundamental process in the cell.

SUMMARY

Protein synthesis is one of the most complex processes in the cell. Its regulation during different stages of growth, division, differentiation, development, aging, and death is a crucial aspect of a living system. In order to translate one mRNA molecule transcribed from a gene, almost 200 small and large components are required to function effectively and accurately, while using large quantities of cellular energy. Ribosomes, initiation factors, elongation factors, amino acids, tRNAs, and aminoacyl–tRNA synthetases are the major components of the protein synthetic apparatus. Protein synthesis proceeds in three steps—initiation, elongation, and termination, followed by posttranslational modifications. The rate-limiting factors for the regulation of total protein synthesis can be any of the components of the

protein synthetic machinery. The availability of mRNA and the amounts and activities of ribosomes, initiation factors, and elongation factors are the major regulators of protein synthesis. Posttranslational modifications such as phosphorylation of various protein synthetic components are involved in determining their activity and stability.

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