

PROTEIN SYNTHESIS

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INTRODUCTION

The regulation of protein synthesis is an important part of the regulation of gene expression. Regulation of mRNA translation controls the levels of particular proteins that are synthesized upon demand, such as synthesis of the different chains of globin in hemoglobin, or the production of insulin from stored insulin mRNAs in response to blood glucose levels, to name a few. The control of the cell cycle and cell proliferation also involves regulation of protein synthesis, and malignant transformation of cells involves loss of certain translational regulatory controls. In fact, several translation initiation factors are over-expressed in certain cancers and play key roles in tumor development and progression. The process of protein synthesis and important examples of its regulation are now understood at the molecular level. We will discuss the mechanism and regulation of protein synthesis, elucidating this complex area of gene regulation with specific examples.

Many viruses compete with their infected host cell and often dominate the protein synthetic machinery to maintain viral production and thwart innate (intracellular) anti-viral responses. For many viruses, the inhibition of host cell protein synthesis is an important component of their ability to propagate and destroy the infected cell. The infected cell, in turn, responds by enacting antiviral activities that include the production of potent biological molecules such as α -interferon that function, in part, to inhibit protein synthesis. Finally, a large proportion of antibiotics currently in use or under development inhibit protein synthesis in bacteria but not animal cells by exploiting differences in the structure of prokaryotic and eukaryotic ribosomes.

THE BASICS

Genetic Code

Since the genetic code is read in triplets (codons) comprising three of the four bases, there are 4^3 or 64 possible triplets encoding the 20 amino acids. All but 3 of these 64 codons specify amino acids. Since there are 61 codons specifying only 20 amino acids, the same amino acid may be encoded by more than one codon. The genetic code is therefore degenerate. The code is read by transfer RNAs (tRNAs) which are adapter molecules that decode the base sequence of an mRNA into the amino acid sequence of a protein. For each amino acid there is at least one corresponding tRNA which transports that amino acid to the ribosome and recognizes the particular codon(s) in the mRNA.

Code facts

1. Genetic code is read in triplets (codons) = 64 possible codons.
2. Codons are read by tRNAs which carry the amino acid to the mRNA.
3. Because 20 amino acids are specified by 61 codons, the genetic code is said to be degenerate. This means that for many, but not all amino acids, there are several related codons that can specify the same amino acid. Each related codon specifying the same amino acid corresponds to a different tRNA which transports it to the ribosome. For example, there are 4 related codons that specify the amino acid leucine. The first 2 nucleotides of the leucine codon are invariant, whereas the 3rd position can vary or wobble.
4. AUG specifies methionine, which almost always initiates polypeptide synthesis.
5. UAA, UAG, UGA specify translation termination. There are no corresponding tRNAs for termination. Rather, termination is carried out by protein factors during translation.

Wobble pairing refers to relaxed rules for basepairing that occur between the anticodon of the tRNA and the codon within families of tRNAs, such as the 4 different leucine tRNAs.

1. Wobble pairing indicates that the 3rd codon position recognizes multiple pairing partners

leucine: 4 related codons (5') - **1** - **2** - **3** - (3')

C U U
C U C
C U A
C U G

2. Most 3rd positions of codons wobble, and can therefore bind to 2 or 3 different nucleotides in the anticodon, with the following rules for pairing.

<u>codon 3rd position</u>	<u>anticodon 1st position</u>
C	G, I
G	C, U
A	U, I
U	A, G, I

3. Wobble pairing provides for multiple ways to specify a single amino acid in the genetic code.

tRNAs

1. Short molecules, 70-90 nts long.
2. All terminate with **CCA_{OH}-3'** to which an amino acid can be covalently attached.
3. Contain unusual nucleotides, which are modifications of the purine/pyrimidine bases or ribose sugar, such as methylations, reductions, altered site of sugar linking to base

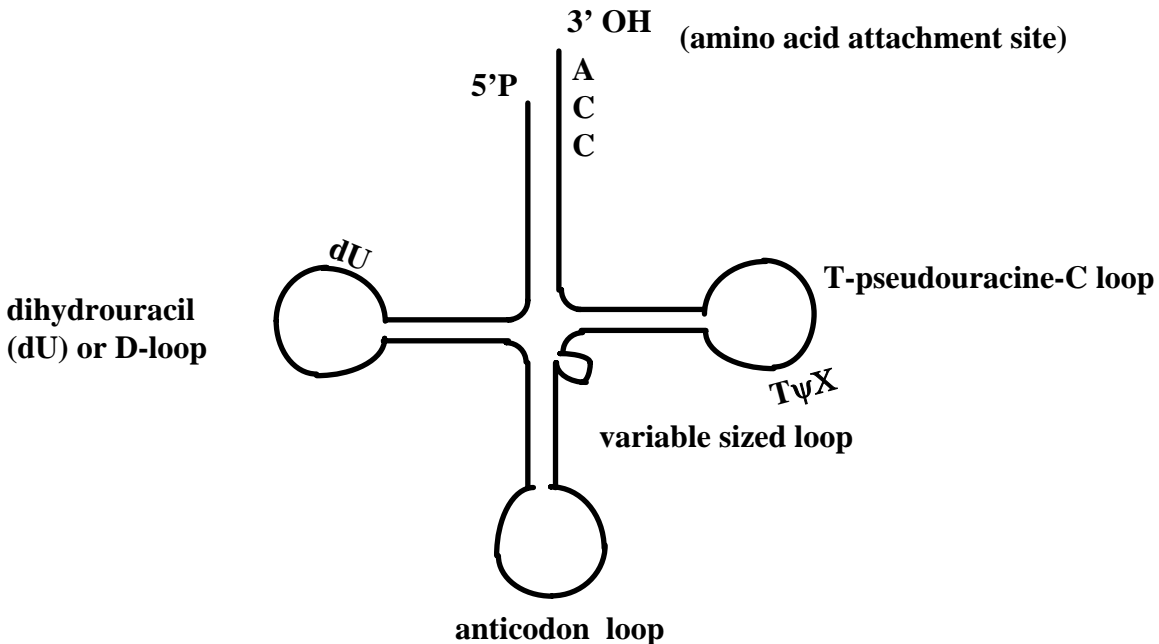
examples include:

- thymidine (uridine with C5 methyl)
- methylated guanosines, methylated adenosines
- inosine and methylinosine (modified purines)
- pseudouridine (ribose sugar attached to uridine in the C-5 rather than C-1 position)
- dihydrouridine (uridine reduced at the C5-C6 double bond).

4. Function of modifications

-control specific folding of the tRNAs. Some modifications are universal, and are therefore found in all tRNAs. These modifications contribute to the secondary structure (cloverleaf) shape of tRNAs, and the tertiary (L-shape) structure as well. Other modifications are specific to members of a family of tRNAs, and define them as such to the molecular machinery that covalently attaches a specific amino acid. Family specific modifications often serve as recognition signals for aminoacyl tRNA synthetases.

All tRNAs possess a common secondary and tertiary stem-loop structure that is critical for their function. A typical tRNA has the following secondary structure: a T-pseudouridine-C-G loop (TΨCG loop), a dihydrouracil or D-loop, and an anticodon loop. The anticodon loop contains the three complementary nucleotides that basepair with a specific codon in the mRNA. A given tRNA interacts with different codons that specify a given amino acid due to nonstandard or wobble basepairing in the 3rd position of the codon with the 1st position of the anticodon.



Anticodon facts

1. 1st anticodon position wobbles as does codon 3rd position, but with fewer choices for pairing.

<u>1st position anticodon</u>	<u>3rd position codon pair</u>
C	G
A	U
G	C, U
U	A, G
I	C, A, U

2. Inosine found in anticodon
3. Genetic code is almost universal:
 - same for prokaryotes and eukaryotes
 - in mitochondria, the codon AUA encodes methionine rather than isoleucine, and AGA/G signals stop rather than arginine.

AMINOACYL-tRNA SYNTHETASES COUPLE AMINO ACIDS TO tRNAs

Synthetase facts

1. Aminoacyl tRNA synthetases are enzymes that covalently attach a specific amino acid to a specific tRNA.
2. There are 20 different tRNA synthetases that recognize the 20 different amino acids. For example: synthetase for Ala attaches it to all 4 Ala tRNAs, in a reaction that utilizes ATP.
3. Attachment of the amino acid is to the 3'OH of the A residue ribose sugar in the conserved CCA sequence on tRNA.
4. Energy in this bond utilized later for polypeptide synthesis.
5. Synthetases recognize different characteristics of tRNAs: unusual bases and anticodon, tertiary structure.

PROKARYOTIC AND EUKARYOTIC RIBOSOMES

Ribosomes are complicated structures consisting of ribosomal RNAs and proteins that associate into a precise structure with multiple enzymatic activities. The ribosomes of prokaryotes, eukaryotes and organelles (such as mitochondria) all perform the same function and are structurally quite similar. In evolution, ribosomes from prokaryotes and eukaryotes are unrelated at the protein level, but are highly related at the rRNA level.

General Features in Common Between Eukaryotic and Prokaryotic Ribosomes

- 2 ribosome subunits, a small and large subunit.
- Consist of protein and RNA only.
- Ribosomal RNAs (rRNAs) are highly related between prokaryotes and eukaryotes, whereas ribosomal proteins (r-proteins) are not.
- Enzymatic functions of ribosomes involved in peptide synthesis are associated with rRNAs rather than r-proteins. r-proteins are thought to fine tune and enhance function of rRNAs under physiological conditions.

Bacterial ribosomes

30S and 50S subunits

- 30S:21 proteins & 16S rRNA
- 50S:32 proteins & 2 rRNAs
-23S & 5S rRNA

Eukaryotic ribosomes

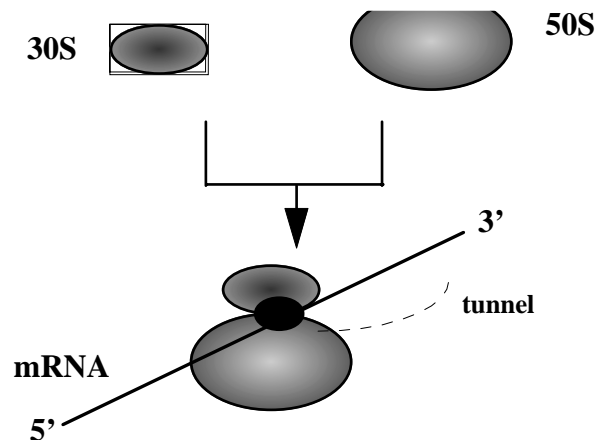
40S and 60S subunits

- 40S:30 proteins & 18S rRNA
- 60S:40 proteins & 3 rRNAs
- 28S, 5S and 5.8S rRNA

The functions of ribosomes in translation are primarily associated with rRNAs rather than r-proteins. The rRNAs:

- function to bring ribosome subunits together.
- interact with, and position mRNAs (in prokaryotes),
- bind most translation factors and create enzymatic centers
- catalyze peptide bond formation.

Ribosome structure



MECHANISM OF PROTEIN SYNTHESIS- OVERVIEW

Protein synthesis can be divided into 6 stages:

1. Amino acid activation: tRNA is charged by covalently linking it to its cognate amino acid.
2. Formation of initiation complexes: association of mRNA, ribosomal subunits and initiation factors.

3. Initiation of translation: assembly of stable ribosome complex at the initiation codon.
4. Chain elongation: polypeptide synthesis by repetitive addition of amino acids to the nascent (growing) chain.
5. Chain termination: release of nascent polypeptide.
6. Ribosome dissociation: subunits separate before initiating new round of translation.

INITIATION COMPLEX FORMATION

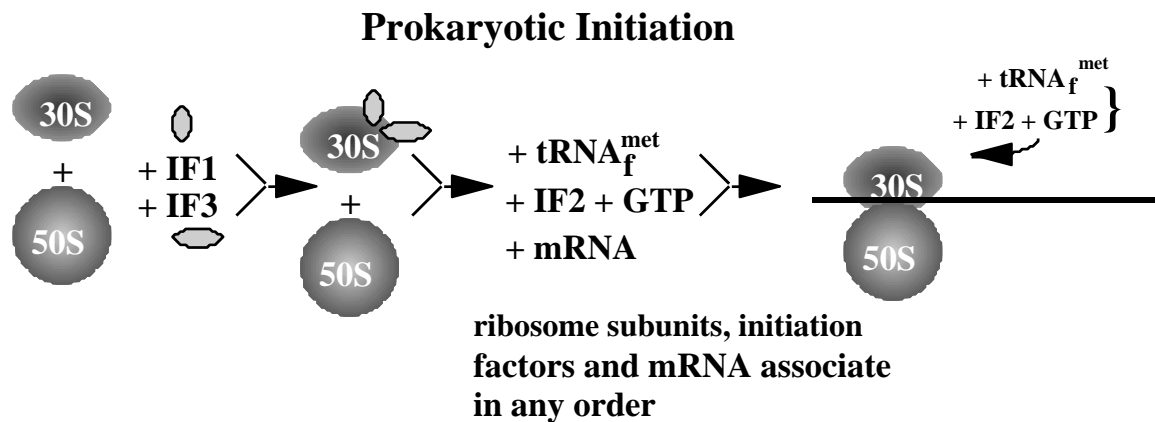
Initiating tRNA

Facts

1. Translation generally initiates with a Met encoded by AUG (prokaryotes & eukaryotes).
2. Special initiating tRNA carries Met to AUG codon.
 - In bacteria the initiating Met is modified, while attached to the tRNA, to contain an N-formyl group. It is referred to as N-formylMet ($\text{tRNA}_f^{\text{met}}$).
 - The formyl group blocks acceptance of a growing peptide chain.
 - Elongating met-tRNA is distinct (tRNA^{met}), and the Met is not modified. The formyl group is always removed from bacterial proteins.
 - In eukaryotes the initiating Met is not modified ($\text{tRNA}_i^{\text{met}}$).
 - The initiating Met is removed from roughly half of bacterial proteins, and from some eukaryotic proteins.

Initiation Complex Formation in Prokaryotes

Anti-association factors IF1 and IF3 bind the 30S subunit and prevent 50S subunit association. Eukaryotic initiation factors eIF1 and eIF3 are similar and they have the same functions. 30S subunit associates with $\text{tRNA}_f^{\text{met}}$, GTP and IF2 to form a ternary complex. Association of ternary complex components, 30S ribosome and mRNA in prokaryotes takes place in any order. In eukaryotes it is highly ordered (as described later).



Initiation of Translation

- Joining of small and large ribosomal subunits with the mRNA creates a 70S ribosome initiation complex.
- Initiation is guided by nucleotide pairing between a sequence in the mRNA and the 3' end of 16S rRNA, in a process called the Shine-Dalgarno (S/D) interaction.
mRNA 5' ----- UAAGGAGG- (5-10 nts) - AUG-----
16S rRNA 3'(OH)-----AUUCCUCC-----
- The level of translation of the mRNA is controlled by the S/D interaction, which is the extent of complementarity between mRNA:rRNA, and the most optimal AUG position (5-10 nts downstream of the S/D element). This explains why prokaryotic ribosomes can initiate protein synthesis internally. As a consequence, prokaryotic mRNAs are generally multicistronic, encoding more than one polypeptide. This also explains why the ternary complex can form on ribosomes after the subunits associate in prokaryotes, because there is no need for tRNA^{fmet} to provide anticodon identification of the initiating AUG.
- In eukaryotes there is no such sequence or S/D interaction (at least routinely). In fact, the Shine Dalgarno sequence is specifically missing from the 3' end of eukaryotic 18S rRNA.
As covered later, eukaryotes initiate translation quite differently.
- The joining of the two ribosome subunits on the mRNA creates two enzymatic regions which direct protein synthesis. This is similar in both eukaryotes and prokaryotes.
 - (i) aminoacyl (A) site: contains IF2-GTP but will contain the incoming tRNA.
 - (ii) peptidyl (P) site: contains tRNA^{fmet} but will contain the growing nascent chain.

Specific segments of 16S & 23S rRNAs have been identified that correspond to the A and P sites. Many antibiotics act by binding or blocking rRNA activity within these enzymatic sites.

Thiostrepton- binds 23S rRNA (residue A1067) and prevents 50S subunit association. Methylation of A1067 provides resistance.

Puromycin (aminoacyl-tRNA analogue)- blocks domain V of 23S rRNA responsible for peptidyl transferase activity; blocks peptide bond formation.

Tetracycline- probably binds 16S rRNA at A892, same site that tRNA binds in the A-site.

Streptomycin- probably binds and blocks activity of 16S rRNA near nt 900. Activity is similar to tetracycline.

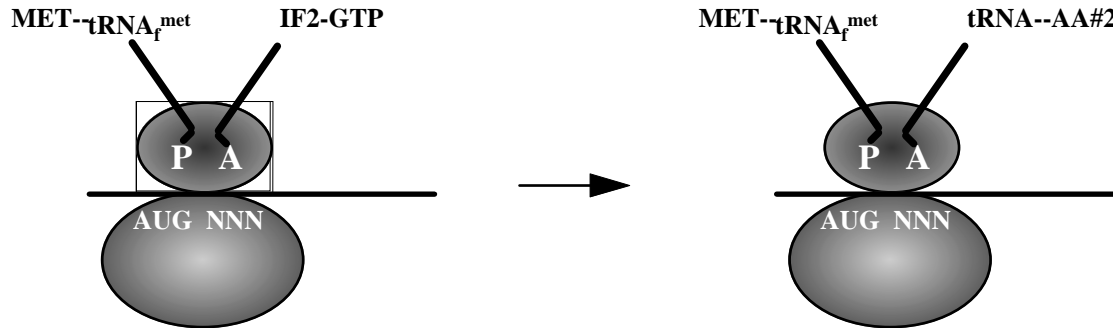
Aminoglycosides (neomycin, gentamicin, kanamycin, hygromycin)- bind specific sites in the A-site contributed by 16S rRNA, prevents translocation of the ribosome along the mRNA. Resistance is associated with mutation of sites in this region.

Edeine-binds P-site within 16S rRNA, prevents tRNA association with 30S subunit.

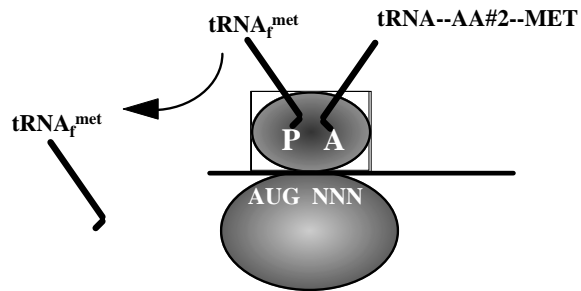
Chloramphenicol & carbomycin- bind domain V loop in 23S rRNA, inhibit peptidyl transferase activity. Resistance is associated with mutation in this region.

Mechanism of Initiation and Elongation

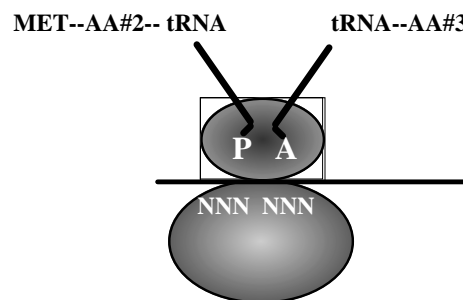
In the initiating ribosome, IF2-GTP occupies the A-site. In the elongating ribosome, the incoming tRNA will always occupy the A site. The tRNA^{f^{met}} is in the P-site. The second aminoacyl-tRNA will occupy the A-site concomitant with GTP hydrolysis and IF2 dissociation. Both the A and P sites are occupied. The peptide bond is synthesized as shown below by a peptidyl transferase activity.



The bond between f^{met} and its tRNA is cleaved and a peptide bond is formed between the f^{met} and amino acid #2 (which is attached to its tRNA in the A-site).



In the next step, the tRNA^{f^{met}} dissociates from the P-site.



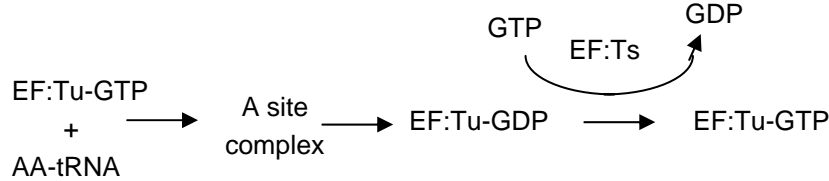
Translocation of the peptidyl-tRNA takes place from the A-site to the P site, which requires translation elongation factor EF-G and GTP.

Elongation

Elongation is a repetition of these events to form additional peptide bonds while charged tRNAs “read” the codons. Elongation utilizes a charged tRNA and 3 elongation factors, known as EF-Tu, EF-Ts and EF-G.

- The charged tRNA to the A-site as a complex with EF-Tu-GTP.
- GTP hydrolysis releases EF-Tu-GDP, and deposits the charged tRNA at the ribosome

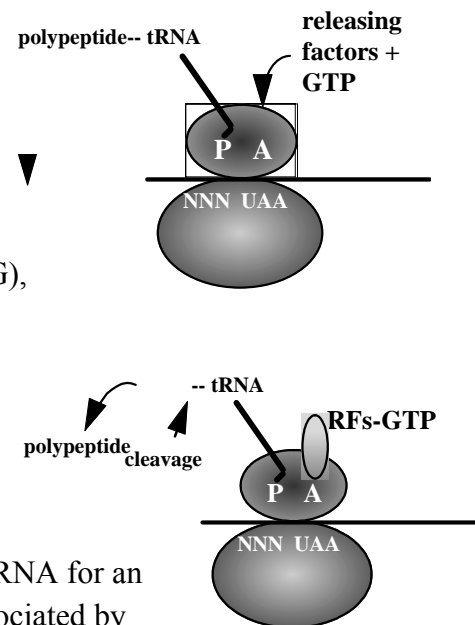
- EF-TS then recycles EF-Tu-GDP to EF-Tu-GTP.



- A similar GTP recycling scheme appears in eukaryotes for recharging initiation factor eIF2. In eukaryotes, this is the most highly regulated step in protein synthesis and it is a primary site of antiviral action of interferon- α . This is covered below.

Termination and Ribosome Dissociation

- Termination is specified by protein factors, not tRNAs.
- The growing nascent polypeptide is in the P-site.
- The termination codon is in the A-site.
- Several protein releasing factors bind to A-site in the presence of the stop codon (UAA, UGA or UAG), then activate a peptidyl-tRNA hydrolase.
- This activity cleaves the amino acid from the tRNA and releases the polypeptide chain.



- The post-translational ribosome can proceed on the mRNA for an unknown distance, but is thought to ultimately be dissociated by factors IF1/IF3.

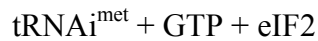
EUKARYOTIC INITIATION OF TRANSLATION

There are major differences between prokaryotic and eukaryotic initiation, which leads to different mechanisms for the regulation of protein synthesis. One difference is that in eukaryotes, there is no "Shine-Dalgarno" interaction typically between the mRNA and 18S rRNA to select the proper protein coding region. Consequently:

- Prokaryotic mRNAs are usually multicistronic. Ribosomes bind prokaryotic mRNAs internally, specified solely by Shine-Dalgarno interactions.
- Eukaryotic mRNAs are generally monocistronic. Eukaryotic ribosomes usually (but not always) initiate translation through a precisely regulated process by scanning from the 5' end of mRNA, surveying the mRNA for the initiating AUG codon in a nucleotide by nucleotide manner which is highly ordered.

In prokaryotes the components comprising the initiation complex can be individually assembled on the small ribosomal subunit (30S subunit) in any order. In eukaryotes this process follows a precise ordering and is highly regulated.

1) A ternary complex is formed prior to 40S ribosome subunit binding to mRNA:



2) The ternary complex binds to the 40S ribosome subunit.

3) Ribosomal 40S subunit binds to a complex of proteins at the 5' end of capped mRNA.

-Almost all eukaryotic mRNAs are capped, i.e. contain an inverted ⁷MethylGTP (m⁷GTP) attached to the first nucleotide.

-The cap is a signal to ribosomes that an mRNA is to be translated.

-Most uncapped mRNAs are poorly translated, if at all.

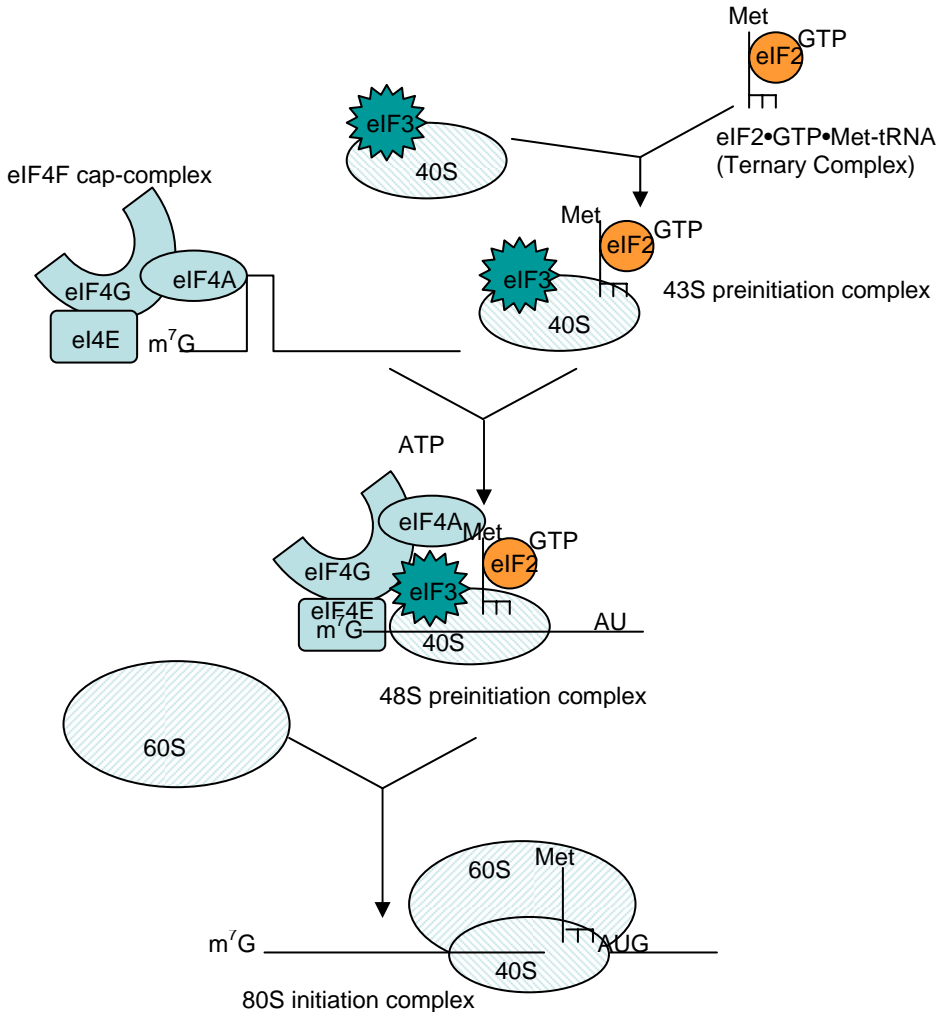
A group of initiation factor proteins binds the capped end of the mRNA and directs 40S ribosome-mRNA interaction in eukaryotes. They are collectively known as the cap-initiation complex or factor eIF4F.

40S ribosome cannot recognize mRNA without these proteins.

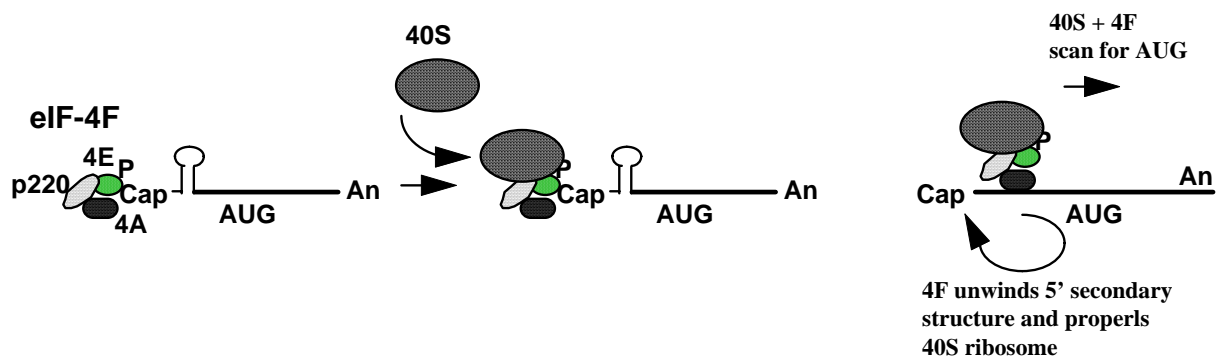
eIF4F acts as a molecular bridge, bringing the mRNA and the 40S subunit together.

eIF4F acts as a cap-dependent mRNA helicase, promoting ribosome binding while unwinding the 5' end of mRNA, permitting the 40S ribosome subunit to search for the initiating AUG codon.

The protein complex eIF4F consists of the cap binding protein eIF4E, the ATP-dependent helicase eIF4A, and the adapter protein eIF4G upon which the complex assembles. Also associated with eIF4F is a protein kinase, Mnk1, that phosphorylates eIF4E, activating initiation of translation. The large initiation factor eIF3 is also associated. eIF3 has many functions in translation, one of which is to bind the 40S ribosome and add the ternary complex. Thus, the 40S ribosome associates with the mRNA through eIF3. Also associated with the eIF4F complex are poly(A) binding protein (PABP), which coats the poly(A) tail on the mRNA, protecting the mRNA from degradation. Interaction of PABP with eIFG is also thought to stimulate initiation. The eIF4F complex, with associated polypeptides, binds the cap, unwinds the 5' end of the mRNA to promote 40S ribosome loading, and probably propels the 40S ribosome subunit on its scanning search for the initiating AUG codon. The functional implications of the interaction of PABP with the complex are not established, but it is thought to provide a mechanism by which only fully intact mRNAs, possessing a cap and polyA tail are translated. The PABP-eIF4G interaction may also facilitate ribosome reinitiation on the mRNA, possibly by tethering the initiation complex and thereby functionally circularizing mRNAs during translation.



5) 40S ribosomes normally “scan” processively from the 5’ capped end of the mRNA to the first appropriate AUG, directed by the eIF4F complex. Scanning involves a nucleotide by nucleotide search for the initiation codon (AUG), beginning at the cap of the mRNA.



In eukaryotes, the nucleotides flanking the initiation codon contribute to recognition of the start codon by the 40S ribosome and the anticodon of the initiating tRNA. This is known as the AUG context. Poor context is associated with usage of downstream AUGs in better context.

Codon context							Frequency of initiation at AUG
-3	-2	-1	+1	+2	+3	+4	
A	c	c	A	U	G	G	100%
G						G	50%
C						A/C/U	0-5%
U						A/C/U	0-5%

6) 60S large ribosome subunit joins at the AUG.

As in prokaryotes, upon 60S ribosome subunit joining, the hydrolysis of GTP and release of eIF2:GDP prepares the ribosome for an incoming aminoacylated tRNA.

Recycling of eIF2:GDP to eIF2:GTP is a highly controlled step in eukaryotic protein synthesis and a primary site of antiviral action of α -interferon. Recycling of GDP to GTP on eIF2 occurs catalytically using recycling factor (RCF) (also called guanine nucleotide exchange factor or GEF). RCF exchanges GDP for GTP on eIF2 (eIF2-GDP \rightarrow eIF2-GTP).

INITIATION OF TRANSLATION IN EUKARYOTES IS HIGHLY REGULATED

The regulation of mRNA translation has evolved in many cell types and tissues to coordinate the levels of different protein products that assemble to form biologically active molecules, and which are required in precise amounts. One of the best studied examples is the regulation of α and β - globin synthesis in red blood cells, which assemble with the molecule heme in the ratio of 2:2:4. This same mechanism of regulation has been exploited by non-red blood cells as the basis for protection against many infecting viruses by the antiviral agent α -interferon.

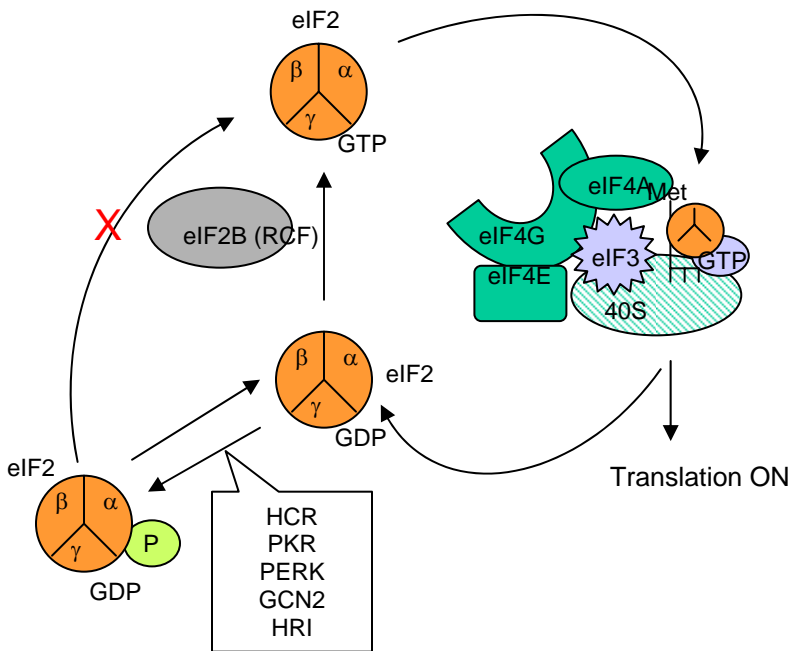
Regulation of Hemoglobin Synthesis

Red blood cells (RBCs) synthesize large amounts of globin proteins, which account for ~90% of RBC protein synthesis. When heme is in excess of globin proteins, protein synthesis in the RBC is stimulated (which corresponds largely to globin synthesis). When heme levels are low, protein synthesis is inhibited. Under conditions of inhibition, about 30% of the pool of eIF2 is phosphorylated on the alpha subunit (eIF2 contains 3 subunits, α , β , γ). The phosphorylated eIF2(α P) that accumulates is associated with GDP rather than GTP. This indicates that eIF2 is phosphorylated after it participates in protein synthesis, but before the GDP can be exchanged for GTP, which is essential for the participation of eIF2 in translation.

The phosphorylation of eIF2 is not the direct cause of inhibition. Rather, eIF2(α P) inactivates the GTP exchange factor RCF. Phosphorylated eIF2(α P)-GDP has a 10-fold higher binding

affinity for RCF than non-phosphorylated eIF2(α)-GDP. RCF is only $\sim 1/10$ - $1/20$ as abundant as eIF2 in most cells. Therefore, RCF is rapidly sequestered by eIF2(α P)-GDP into inactive complexes, blocking GTP recycling on eIF2 and preventing translation initiation. Thus, the total increase in eIF2 phosphorylation needs to rise by only 30% to shut-off all protein synthesis in the RBC.

The kinase that phosphorylates eIF2 α is called the heme controlled repressor (HCR). As heme synthesis catches up to globin levels, heme becomes abundant enough to suppress the HCR activity. eIF2(α P) is constitutively dephosphorylated by a phosphatase. The loss of eIF2(α P) liberates RCF which converts eIF2-GDP to eIF2-GTP for protein synthesis once again.



Regulation of eIF2 α activity eIF2 is part of the ternary complex, eIF2•GTP•Met-tRNA, responsible for bringing the first amino acid to the initiation codon. This process requires hydrolysis of GTP associated with the gamma subunit. GDP release and acquisition of a new molecule of GTP is catalyzed by eIF2B (also known as RCF). Phosphorylation of the alpha subunit of eIF2, under conditions of cellular stress, results in the inhibition of 2B activity, leading to a decrease in translation initiation.

Regulation by Interferon

The same mechanism used to regulate globin synthesis underlies the antiviral action of α -interferon. Non-red blood cells contain a kinase related to HCR, that targets eIF2 α as its substrate. The HCR related kinase is not regulated by heme, but is activated by double-stranded RNA (dsRNA). Hence, it is known as protein kinase double-strand RNA regulated, or PKR.

Cells infected by different viruses often synthesize and secrete α -interferon, which in turn stimulates synthesis of PKR in uninfected cells. Activation of PKR is mediated by low levels of dsRNA. Why dsRNA? Most viruses containing DNA genomes have opposing transcription units that are simultaneously active at some point in their life cycles, and therefore generate dsRNA. Other viruses use RNA as a genome, and therefore must generate dsRNA to replicate. Formation of dsRNA is therefore a signal to cells that they are infected by a virus. In uninfected cells, dsRNA is quite rare so the PKR kinase is not activated. Activation of the kinase inhibits all protein synthesis in the cell, both host and virus. Although the cell may die, it destroys the virus in the process. In other cases, the viral RNA or infecting genome may be degraded, removing the source of dsRNA and permitting recovery of the cell. The activity of PKR kinase is such an important control point in translation, and a critical marker of infection to the cell, that many viruses have evolved mechanisms to prevent its activation.

PKR activation is inhibited by adenoviruses, pox viruses (Vaccinia virus), influenza viruses, and reoviruses, in some cases utilizing similar mechanisms. A great many viruses, however, do not possess mechanisms to inhibit the activation of PKR and remain acutely sensitive to the antiviral effects of interferon α .

Inhibition of PKR by viruses

Adenovirus has a dsDNA genome with many opposing transcription units. Adenovirus synthesizes a small RNA called Virion Associated (VA) RNA I. VA RNA I is a highly structured RNA that lacks a cap, AUG, and polyA tail. VA RNA I binds to PKR and prevent its activation by dsRNA.

Vaccinia virus produces two proteins called E3L and K3L. E3L binds dsRNA, scavenging and removing the low levels of dsRNA that are present in a Vaccinia virus infected cell, preventing activation of PKR kinase. The K3L protein mimics the site of eIF2 α subunit phosphorylation, binding and sequestering PKR. Vaccinia has evolved a two-pronged strategy for inhibition of PKR activity.

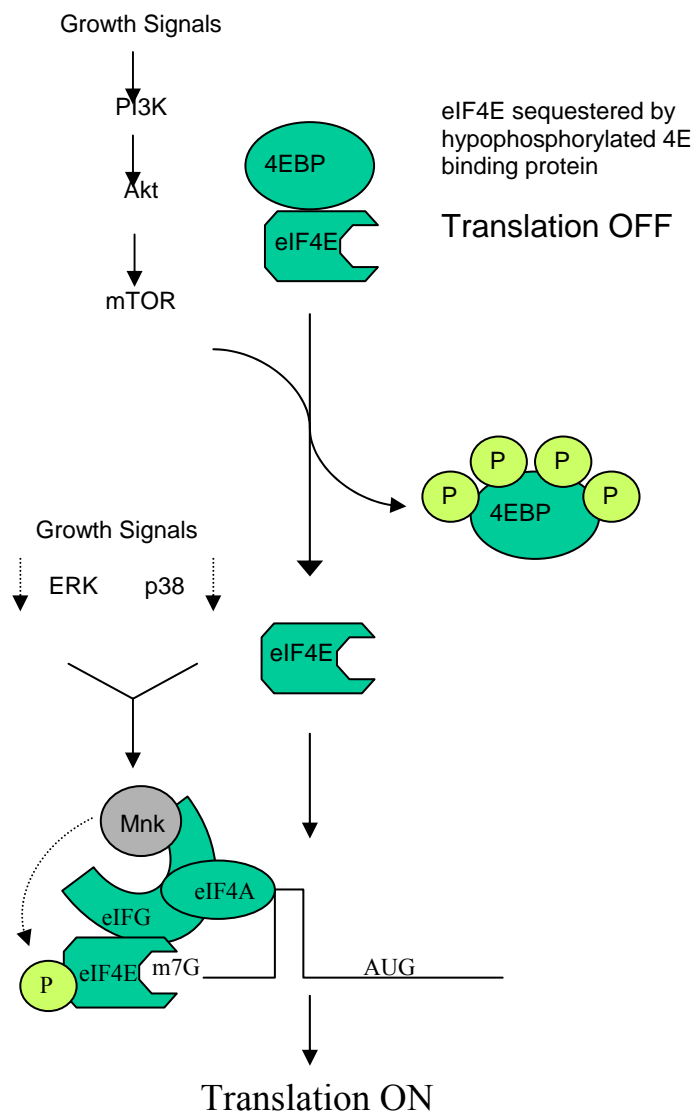
Other eIF2 Kinases

Nutrient deprivation of cells has been shown to inhibit protein synthesis by activation of a third eIF2 kinase known as GCN2. GCN2 is activated by low levels of the amino acid histidine, and in turn phosphorylates eIF2 in the α -subunit, blocking protein synthesis as described above.

Oxidative and Other Stresses can activate an eIF2 kinase that resides in the endoplasmic reticulum (ER) known as PERK. PERK detects changes in ER status, including the unfolding of proteins, and inhibits protein synthesis by phosphorylation of the eIF2 α -subunit.

Regulators of Translation Acting on Cap Binding Protein eIF4E

The activity of eIF4E is tightly regulated in cells by distinct mechanisms. The available cellular pool of eIF4E is controlled by inhibitory eIF4E-binding proteins (4EBPs). These proteins bind to the eIF4G binding site of eIF4E, removing eIF4E from eIF4G and blocking initiation of translation. The activity of the 4EBPs (i.e. the ability to bind and sequester eIF4E) is in turn regulated by phosphorylation in response to growth factors including insulin, IGF-1, and angiotensin II (signalling occurs by the PI3-kinase/Akt/mTOR signal transduction pathway). In the presence of activating growth factors, 4EBP phosphorylation increases, thereby *lowering* the amount bound to eIF4E and therefore stimulating translation.



Regulation of eIF4E activity

eIF4E is the mRNA 5' cap-binding component of the eIF4F cap-complex. Its availability is limited by sequestration via the eIF4E binding proteins (4EBPs), which prevent eIF4E interaction with the eIF4G scaffold component. The affinity of 4EBP for eIF4E is greatly diminished by multiple phosphorylation mediated by mTOR kinase via the cell growth signaling PI3K-Akt pathway. In addition the eIF4E kinase, Mnk, activated under conditions of cellular growth, interacts with the eIF4G scaffold and can phosphorylate eIF4E, which may potentiate cap-dependent initiation.

The cap-binding activity of eIF4E is also thought to be regulated, although less stringently, by phosphorylation, which occurs on Ser-209. The phosphorylation is catalized by the serine/threonine kinase, Mnk1, which is associated with eIF4G. Generally, increased eIF4E phosphorylation correlates with increased translational activity.

Cellular transformation and the control of protein synthesis.

During malignant transformation, a cell accumulates characteristics necessary for unchecked proliferation and enhanced survivability by means of up-regulation of oncogenes and down-regulation of tumor suppressor genes. One means of control of proto-oncogene and tumor suppressor gene expression is regulation of protein synthesis. Modifications in the translational apparatus of cells, particularly changes in several initiation and elongation factors, are associated with malignant transformation and the acquisition of transformed and oncogenic properties of tumor cells. Cells that are highly transformed generally show higher rates of protein synthesis as compared with non-transformed, quiescent cells. Up-regulation of protein synthesis may promote critical aspects of tumor progression, such as angiogenesis (induced growth of new blood vessels and revascularization to tumors), metabolic adaptation, and invasiveness; or there may be specific components or pathways that are selectively up-regulated to achieve these responses.

Several specific components of the protein synthesis machinery have been significantly associated with malignant transformation of cells. Elevated cellular levels of protein synthesis initiation factors, particularly eIF4E and eIF4G, have been implicated as promoters of malignancy and suppression of apoptosis (programmed cell death) which generally limit tumor invasiveness. In addition to the components of the eIF4F-cap dependent initiation complex, other key components of translational initiation have been cited for potential roles in transformation.

<u>Initiation Factor</u>	<u>Cancer</u>
eIF4E	Up-regulated in some invasive ductal breast cancers, head and neck carcinomas, lymphomas, bladder carcinomas, colon carcinomas
eIF4A	Up-regulated in some melanomas, hepatocellular carcinomas
eIF4G	Up-regulated in squamous cell lung carcinomas, breast cancers
eIF2 α	Up-regulated in lymphomas, gastro-intestinal carcinomas
eIF2B	Up-regulated in breast carcinomas

TARGETED mRNA DEGRADATION

