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Regulation of protein synthesis and the role of eIF3 in cancer

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Regulation of protein synthesis and the role of eIF3 in cancer

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Abstract

Maintenance of cell homeostasis and regulation of cell proliferation depend importantly on regulating the process of protein synthesis. Many disease states arise when dysregulation of protein synthesis occurs. This review focuses on mechanisms of translational control and how dysregulation results in cell malignancy. Most translational controls occur during the initiation phase of protein synthesis, with the initiation factors being the major target of regulation through their phosphorylation. In particular, the recruitment of mRNAs through the m⁷G-cap structure and the binding of the initiator methionyl-tRNA_i are frequent targets. However, translation, especially of specific mRNAs, may also be regulated by sequestration into processing bodies or stress granules, by *trans*-acting proteins or by microRNAs. When the process of protein synthesis is hyper-activated, weak mRNAs are translated relatively more efficiently, leading to an imbalance of cellular proteins that promotes cell proliferation and malignant transformation. This occurs, for example, when the cap-binding protein, eIF4E, is overexpressed, or when the methionyl-tRNA_i-binding factor, eIF2, is too active. In addition, enhanced activity of eIF3 contributes to oncogenesis. The importance of the translation initiation factors as regulators of protein synthesis and cell proliferation makes them potential therapeutic targets for the treatment of cancer.

Key words: Translational control; eIF3; Malignant transformation; Cancer

Introduction

Protein synthesis is an important step in gene expression, as it plays a pivotal role in establishing cellular protein levels and in defining the differentiated characteristics of cells. Protein synthesis is closely integrated with other metabolic pathways, influencing transcription, protein turnover, early development and neurological function, *inter alia*. It also employs a massive apparatus and is a major consumer of energy. In order to maintain cell homeostasis, it is essential to regulate the overall rate of protein synthesis, with special attention to the need for new proteins and to the availability of amino acids and energy. Such regulation, called translational control, is the topic of this review. Dysregulation may cause developmental or neurological problems or various disease states, such as diabetes and cancer, with emphasis given in this review to cancer.

Many disease states are caused by the failure to synthesize a specific active protein. Such diseases usually involve a mutation of the gene encoding the protein, leading to an altered protein level or activity. Some well-documented cases include mutations in or around initiation codons that

directly contribute to human disease (1). In such cases, the overall process of protein synthesis is normal, with only the mutated gene product being affected. There are very few instances where a disease state is due to a defect in the translational apparatus itself. Why is this so? Protein synthesis is essential for the health of cells and also is especially important in the process of early development. Substantial defects in general protein synthesis would affect the balance of proteins made, and thus would be embryonic lethal and not be seen in postnatal humans. However, subtle defects in the translational apparatus may occur and present themselves in adults. For example, a modest change in one of the initiation factors, eIF2B, leads to a neurological disease called leukoencephalopathy with vanishing white matter (2). A major challenge in studies of protein synthesis is to understand the process of protein synthesis in sufficient detail and precision in order to be able to detect the subtle changes that are responsible for certain disease states.

In addition to being caused by a defect in the apparatus

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itself, diseases may arise through aberrations in the regulatory mechanisms that affect protein synthesis. The dominant way to control translation is through phosphorylation of the protein factors that promote the various phases of protein synthesis. As most mRNAs are regulated at the initiation phase, the initiation factors are frequently regulated this way. Their phosphorylation levels are established both by protein kinases, often themselves regulated by signal transduction pathways, and by phosphoprotein phosphatases. Besides phosphorylation of the general machinery, specific proteins may regulate one or a whole class of mRNAs, leading to narrower mechanisms of translational control. Another mode of regulation involves micro-RNAs that affect either protein synthesis rates or mRNA degradation. Finally, changes in the levels of specific proteins involved in the protein synthesis machinery may affect translation rates and the balance of proteins produced. Thus, subtle changes in such regulatory mechanisms may lead to disease states that involve protein synthesis.

How to measure translational control

A number of methods are available to determine if the rate of protein synthesis has changed. A change in a protein's level may reflect a change in translation rate since its level is proportional to its rate of synthesis and inversely proportional to its rate of degradation. It is essential to examine protein degradation rates before a change in the rate of protein synthesis can be claimed to cause the altered protein level. If the protein's mRNA level also has not changed, one may conclude that translational control has occurred.

A second method is to add radiolabeled amino acid precursors to cultured cells or tissues, then measure the rate of incorporation into total protein or specific proteins. Changes in rates of incorporation reflect translational control if mRNA levels remain the same. A caveat is that the specific radioactivity of the amino acid precursors might differ in the two compared cell groups, caused for example by a change in general protein degradation.

A third method is to employ sucrose gradient centrifugation of cell lysates to monitor the size and abundance of polysomes. Polysomes are mRNAs with multiple ribosomes attached, and represent the actively translated mRNAs. Following centrifugation, the gradient is pumped through a UV-recording cell to monitor absorbance due to ribosomes; heavy polysomes sediment more rapidly than lighter polysomes and 80S ribosomes, and thus are separated in the profile. A polysome size (number of ribosomes per mRNA) is proportional to the coding length of the mRNA and to the rate of initiation, and is inversely proportional to the rate of elongation/termination. A change in polysome size could be due to a change in the rate of initiation, so long as no change in elongation/termination rate and mRNA coding length has occurred. Specific mRNAs can be assessed by this method, as their distribution in the polysome profile

can be determined with DNA probes or by kinetic RT-PCR. Recently, the polysome distributions of essentially all of the translating mRNAs in a cell have been elucidated by high throughput sequencing of mRNA fragments protected by the translating ribosomes (3). The power of this revolutionary approach to measuring translational control, called "ribosome profiling", is impressive and is certain to alter future studies of translational control.

Brief review of the pathway and mechanism of initiation

Because most translational controls affect the initiation phase of protein synthesis, I shall concentrate on this part of the translation pathway. Initiation involves the selection of the mRNA to be translated, followed by the formation of an 80S initiation complex involving the initiator methionyl-tRNA_i (Met-tRNA_i) bound to the initiation codon in the P binding site of the ribosome. This complicated process can be separated into distinct steps: preparation of the mRNA, formation of a complex of Met-tRNA_i and the 40S ribosomal subunit, recruitment of this 40S preinitiation complex to the 5'-terminal region of the mRNA, scanning of the 40S preinitiation complex downstream on the mRNA, recognition of the initiation codon, and junction with the 60S ribosomal subunit to form the 80S initiation complex. The whole process is promoted by at least 12 protein factors called eukaryotic initiation factors (eIFs). We shall only briefly review each of these steps in the dominant pathway, called "scanning", as a number of comprehensive reviews have appeared recently (4-6). Figure 1 describes the scanning pathway in a simplified form, showing where the initiation factors function or enter the pathway. Not discussed here are less frequently utilized initiation pathways that involve either binding of the ribosome to an internal region, called the internal ribosome entry site (IRES) (7), or the primary round of initiation that occurs on mRNAs immediately following transcription and splicing (8).

mRNA selection and preparation

An mRNA is first recognized by binding of eIF4E to the m⁷G-cap at the 5'-terminus of the mRNA. The mRNA may be either one not yet involved in protein synthesis, called a free messenger ribonucleoprotein (mRNP), or one already being translated, called a polysomal mRNA. A higher order cap binding complex then forms through interaction of eIF4E with eIF4G, which in turn complexes with eIF4A (these three proteins together are called eIF4F) and the polyA binding protein (PABP). The actual order of binding of these components to one another and to the m⁷G-cap is not known, however. The tethering of the cap and polyA tail through the protein-protein interactions of eIF4E-eIF4G-PABP enhances selection of intact mRNAs possessing both the cap and polyA tail and in effect circularizes the mRNA. Since mRNAs appear to be in excess over available ribosomes in many

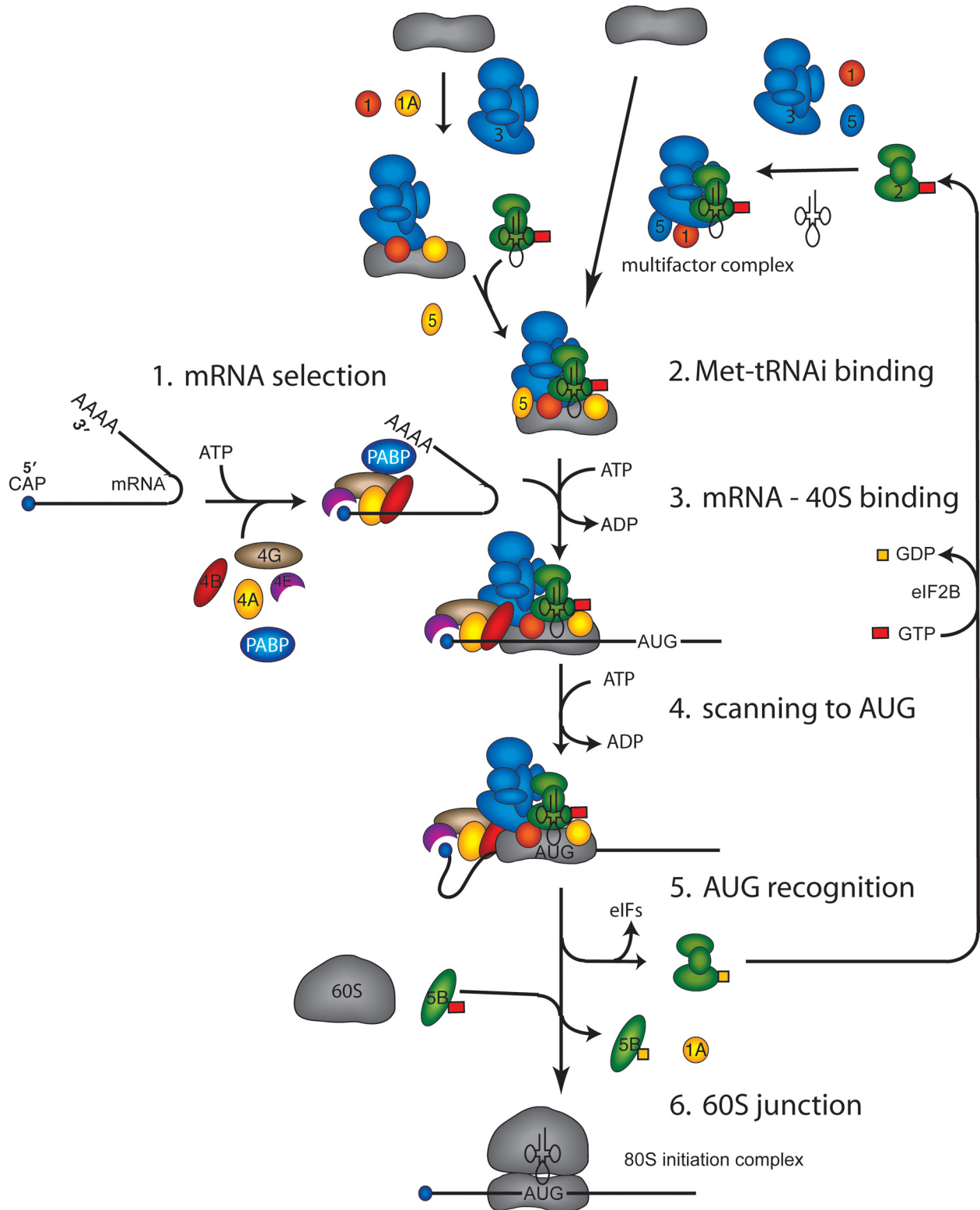


Figure 1. The translation initiation pathway initiation factors are shown as circles or complexes, each identified by its number embedded therein.

cells, the mRNAs selected are those with more accessible cap structures, whereas mRNAs with caps more occluded by secondary or higher order structures are recognized less efficiently. The process of mRNA binding is frequently regulated at the level of eIF4F, as eIF4E forms a complex with the 4E-BP family of proteins that prevents its binding to eIF4G (reviewed in Ref. 9). Following selection of the mRNA, the RNA helicase activity of eIF4A removes RNA secondary structures (see below) near to the 5'-m⁷G-cap so that the ribosome is able to bind there.

Formation of the Met-tRNA_i-40S preinitiation complex

The binding of Met-tRNA_i to the 40S ribosomal subunit appears to occur in the absence of an interaction with mRNA, but requires eIF2 and is further promoted by eIF1A and eIF3. It is generally thought that Met-tRNA_i first forms a ternary complex with eIF2 and guanosine triphosphate (GTP) prior to ribosome binding. However, an even larger complex, called the multifactor complex (MFC) comprising eIF1, eIF2, eIF3 and eIF5 may be involved. Knowledge of the actual order of interaction events leading up to formation of the 40S-Met-tRNA_i complex will require careful kinetic studies. It is likely that the 40S-Met-tRNA_i complex forms prior to mRNA binding to the ribosome, as native 40S ribosomal complexes containing Met-tRNA_i but lacking mRNA have been detected in mammalian cell lysates (10).

Recruitment of mRNA to the 40S ribosomal subunit

The mRNA complex containing eIF4E, eIF4G, eIF4A and PABP and lacking secondary structure next to the m⁷G-cap is brought to the 40S preinitiation complex bound with Met-tRNA_i, eIF1, eIF1A, eIF2, eIF3 and eIF5 through an interaction between eIF4G and eIF3. Details of this important interaction have not yet been elucidated, although one report implicates the e-subunit of eIF3 (11). Because eIF4G is tethered near the mRNA cap structure through eIF4E, the 40S ribosomal subunit is bound near the 5'-terminus of the mRNA. Although eIF3 plays a key role in bringing the mRNA to the ribosome, one of its subunits, eIF3j, plays an antagonistic role, preventing the mRNA's premature entry into the mRNA binding cleft of the 40S ribosomal subunit if Met-tRNA_i and eIF2 are not yet present (12). In contrast, eIF1 and eIF1A promote an "open" conformation of the 40S ribosomal subunit, enabling the mRNA to bind in the cleft (13). It is not known if the eIF4G-eIF3 interaction or mRNA recruitment step is regulated by phosphorylation or by other mechanisms.

Scanning and recognition of the initiation codon

In the next step the 40S preinitiation complex moves along the mRNA toward the 3'-terminus until an initiation codon is recognized. Such movement requires that there be no impeding secondary structure in the mRNA. To remove such secondary structure, the RNA helicase, eIF4A, functions to unwind the secondary structure in a reaction involving ATP hydrolysis. eIF4A has weak ATP-dependent

RNA binding ability and is tethered to eIF4G. Both eIF4B and eIF4G promote the helicase activity of eIF4A, possibly by affecting its processivity. However, the molecular mechanisms and detailed kinetics of the helicase reaction are yet to be elucidated. It is even possible that other RNA helicases, such as DHX29, may function here as well (14). Interestingly, a structure model places eIF4G near the exit side of the mRNA-binding channel (15), implying that the eIF4A helicase action "pulls" the mRNA along the 40S channel, presumably by preventing back-sliding. Molecular details, such as the rate of scanning and degree of processivity, have not yet been determined.

Once an initiation codon (nearly always AUG) is encountered, a tight codon-anticodon interaction is formed between the mRNA and the Met-tRNA_i. This tight interaction generates a "closed" 40S conformation that prevents further scanning. Recognition of the AUG is influenced by the so-called Kozak consensus sequence surrounding the AUG (16) and by the initiation factors, especially eIF1. AUGs surrounded by a poor consensus sequence may be passed over, a phenomenon called "leaky scanning". Following formation of a correct codon-anticodon interaction, the GTP bound to the eIF2 is hydrolyzed in a reaction promoted by eIF5, and eIF1 and inorganic phosphate are released (17). Then, eIF2-GDP (guanosine diphosphate) leaves to complete formation of the 40S initiation complex and is recycled by eIF2B to the active eIF2-GTP complex. The affinity of eIF3 for the 40S initiation complex also is reduced following GTP hydrolysis, but evidence suggests that this factor, and possibly others such as eIF4G, do not dissociate immediately and remain bound during 80S initiation complex formation (18). Although many of the initiation factors present during scanning and AUG recognition are phosphorylated, it is not known if this or other post-translational modifications influence the rate or fidelity of initiation codon recognition.

Junction of the 60S ribosomal subunit with the 40S initiation complex

The 60S ribosomal subunit joins the 40S initiation complex in a reaction requiring eIF1A, eIF5B and GTP (19). This step also involves the release of other initiation factors, although as mentioned above, there is evidence that eIF3 and possibly eIF4G remain bound for some time, but with lower affinity. eIF1A and eIF5B are homologs of bacterial IF1 and IF2, and presumably serve to direct the Met-tRNA_i into its correct position in the P site of the 60S subunit. Following 60S junction, the GTP bound to eIF5B is hydrolyzed, releasing eIF1A and eIF5B, and the 80S initiation complex is formed with Met-tRNA_i bound to the P-site. This complex is now ready to enter the elongation phase of protein synthesis.

Overview

Initiation by the scanning mechanism occurs rather

rapidly, up to one initiation event every 6 s on a polysomal mRNA (20), whereas the rate of elongation is even faster, up to 5 residues inserted per second. It is a highly complex pathway, involving at least 12 initiation factors, which comprise more than 30 different proteins. In contrast with bacterial protein synthesis, where initiation involves mostly RNA-RNA interactions and only three initiation factors, the process in mammalian cells is dominated by protein-protein interactions amongst the initiation factors. This additional complexity likely is due to a greater need for sophisticated translational control mechanisms.

Regulation of general protein synthesis by phosphorylation

The most prevalent mechanism for controlling the overall rate of protein synthesis involves the phosphorylation of the soluble factors involved in initiation and elongation. Nearly all of these protein factors are phosphoproteins, although whether or not their activities are in fact regulated by such phosphorylation is yet to be established for many of them. We shall first consider the mechanisms that regulate Met-tRNA_i and mRNA binding to the 40S ribosomal subunit, as these appear to be most important. Other reactions in the translation pathway are less well studied and are treated only briefly.

Met-tRNA_i binding

Following completion of a round of protein synthesis, eIF2 leaves the ribosome as a complex with GDP. In order to bind another Met-tRNA_i and form a ternary complex, eIF2 must exchange the GDP for GTP. The exchange reaction is catalyzed by eIF2B, which comprises 5 non-identical subunits and functions as a guanine nucleotide exchange factor for eIF2. When the alpha subunit of the heterotrimeric eIF2 is phosphorylated at serine-51, it acts as a competitive inhibitor of eIF2B, by binding and sequestering eIF5B (reviewed in Ref. 21). Since the cellular level of eIF2B is thought to be lower than that of eIF2, only partial phosphorylation of eIF2 may be sufficient to inhibit all of the eIF2B, resulting in a failure of eIF2 to exchange GDP for GTP. This in turn leads to a decrease in the level of the ternary complex and an inhibition of protein synthesis. eIF2 is phosphorylated by at least four different protein kinases, each of which is activated by a form of cell stress: PKR, by double-stranded RNA and viral infection; HRI, by heme deficiency; GCN2, by amino acid starvation, and PERK, by the unfolded protein response and endoplasmic reticulum stress. Phospho-eIF2 α in turn is dephosphorylated by the phosphoprotein phosphatase, PP1A. eIF2B also is phosphorylated by a number of different protein kinases, causing either activation or inhibition, but the importance of eIF2B regulation by its phosphorylation is unclear. A strong phosphorylation of eIF2 severely inhibits the translation of essentially all mRNAs. However, weaker phosphorylation may only par-

tially inhibit the rate of protein synthesis, allowing some mRNAs to be translated. Under these circumstances, the balance of protein synthesis may be altered, with some classes of mRNAs more severely inhibited than others. Regulation of eIF2 activity by phosphorylation plays a role in the regulation of cell proliferation and cancer, as described in a later section of this review.

mRNA recruitment through m⁷G-cap recognition

eIF4E binds the m⁷G-cap of mRNAs and recruits eIF4G, eIF4A and PABP prior to mRNA binding to the 40S ribosomal subunit. eIF4E binding to eIF4G is prevented by a family of eIF4E binding proteins called the 4E-BPs, which mask the eIF4G binding site on eIF4E. The binding of 4E-BP to eIF4E is regulated by phosphorylation, where hyperphosphorylation of the 4E-BPs decreases their affinity for eIF4E, enabling the initiation factor to bind to eIF4G and recruit an mRNA for translation. 4E-BPs are phosphorylated by mTOR, which in turn is regulated through a complex net of signal transduction pathways (reviewed in 9,22). For example, growth factors and other cell mitogens activate the PI3K pathway and subsequently mTOR. Depending on the extent of 4E-BP phosphorylation, cap-dependent initiation can be severely or only partially inhibited. However, mRNAs possessing an IRES have the capacity to be translated without the involvement of eIF4E, and thus evade this regulatory mechanism. A second kind of regulation of eIF4E activity occurs by phosphorylation at Ser-209. While early studies gave conflicting results, recent work with cells obtained from a knock-in mouse where eIF4E is mutated to eIF4E(S209A) to prevent its phosphorylation showed that the cells are resistant to malignant transformation (Sonenberg N, personal communication). As in the case of eIF2, regulation of eIF4E and the 4E-BPs plays an important role in establishing and maintaining cell malignancy.

Other initiation reactions

Other initiation factors known to be phosphorylated in mammalian cells are eIF3, eIF4B, eIF4G, eIF5, eIF5B and PABP (reviewed in detail in Ref. 22). One of the better-characterized phosphorylations concerns eIF4B, a protein that promotes the RNA helicase activity of eIF4A during mRNA recruitment and scanning. eIF4B is phosphorylated at Ser-422 by the S6 kinases, S6K1 and S6K2 (23). Also, Ser-406 may be a target in some cells, and the kinases RSK and Akt may contribute to these phosphorylations (24). Phosphorylation of eIF4B stimulates initiation and the protein's binding to 40S initiation complexes (24,25). However, the precise function of eIF4B affected by phosphorylation has not yet been identified. Also noteworthy, another protein involved in the RNA helicase reaction, programmed cell death 4 (PDCD4), binds to and inhibits eIF4A; phosphorylation of PDCD4 leads to its degradation, resulting in cell malignancy (reviewed in Ref. 22).

Besides eIF4E and the 4E-BPs, mRNA recruitment

may be regulated by eIF4G, which is phosphorylated at a number of different sites, but how the function of the protein is affected has not been elucidated. eIF5 is phosphorylated by casein kinase 2, which promotes initiation and affects cell-cycle progression (26). eIF5B and PABP can be phosphorylated, but possible functional effects are not known. Finally, numerous phosphorylation sites have been mapped to various subunits of eIF3 (27). Phosphorylation at two sites in eIF3f by CDK11p46 during apoptosis enhances the protein's ability to inhibit protein synthesis (28), whereas phosphorylation at a single site in eIF3h promotes cell malignancy, as described below. Clearly much additional work is needed to better elucidate the roles of phosphorylation in controlling protein synthesis.

Elongation factors and ribosomes

The translocation factor, eEF2, is phosphorylated on Thr-56 by a specific Ca^{2+} /calmodulin-regulated protein kinase, eEF2K, leading to inhibition of its activity. eEF2K itself is regulated by a variety of signal transduction pathways, for example by S6 kinase or AMP kinase. Phosphorylation of eEF2 and eEF2K is implicated in a number of diseases, but how these events affect protein synthesis and cell homeostasis is not well characterized (see review in 22). eEF1A and eEF1B, involved in the binding of aminoacyl-tRNAs to the ribosome, also are known phospho-proteins, but a role in regulating their activities has not yet been established.

A number of ribosomal proteins are phosphorylated. The best characterized is S6, which is phosphorylated by the S6 kinases at a number of sites near its C-terminus. S6 phosphorylation correlates with an activation of protein synthesis in many, but not all, cases. It was first implicated in the translation regulation of terminal oligopyrimidine (TOP) mRNAs that encode ribosomal proteins and some protein factors, but more recent findings challenge this view. The lack of strong phenotypes in a knock-in mouse carrying a mutant S6 gene with all phospho-serine targets changed to alanine indicates that S6 regulation through phosphorylation is not very important (29). Other ribosomal proteins (e.g., the L7/L12-like P1 and P2 proteins) also can be phosphorylated, but no role in the regulation of protein synthesis has been reported.

Other mechanisms of translation regulation

Besides phosphorylation, other mechanisms and post-translational modifications of the translational machinery may regulate protein synthesis. The availability of mRNA to the translational machinery is affected by their dynamic sequestration into processing bodies (PBs) or stress granules (SGs). Recent work suggests that small differences between ribosomes, some due to ribosomal protein mutations or altered levels, may affect the translation of specific mRNAs (30). Methylation of lysine and arginine residues occurs frequently in ribosomal proteins and some of the

protein factors, although no compelling evidence links these changes to translational control. O-glycosylation is another type of modification that could occur, but only one instance of its regulation of protein synthesis has been reported, involving p67, a protein that prevents eIF2 kinases from phosphorylating eIF2 α (31). Finally, two different ubiquitin E3 ligases have been found to bind to eIF3, suggesting that components of the initiation pathway may be ubiquitinated (see below). A regulatory role for any of these modifications is yet to be determined.

Rather than modifications of the machinery, *trans*-acting elements are known to affect the translation of mRNAs. Here I will review recent progress in elucidating the regulatory functions of mRNA sequestration, *trans*-acting proteins and microRNAs. In general, these elements affect only one or a class of mRNAs, through their interactions with specific mRNAs. In this sense, they differ substantially from regulation by phosphorylation of initiation or elongation factors, which affects general protein synthesis.

mRNA sequestration into P-bodies or stress granules

Cytoplasmic mRNAs are either actively translated by ribosomes (polysomal mRNPs) or are translationally inactive (free mRNPs), with no ribosomes attached. The free and polysomal mRNPs are thought to be in rapid equilibrium, with a given mRNA transiting back and forth between the two compartments. Inactive mRNAs may assemble together to form either PBs or SGs, subcellular structures visualized by microscopy. PBs are found in normal mammalian cells, whereas SGs are seen only following cell stress (e.g., heat, hypoxia, virus infection). Inhibition of protein synthesis is required, but is not sufficient, to form PBs or SGs; additional RNA-binding proteins are needed. Some of the components of PBs and SGs are the same, yet each contains proteins specific for that type of granule. A detailed review of the assembly and functions of PBs and SGs has been published recently (32). Here, I simply point out that translational control and PB or SG assembly are closely linked, with mRNAs residing in such particles being inaccessible to the translational machinery. Nevertheless, mRNAs in PBs and SGs can be recruited into polysomes, although the specific mechanisms enabling their activation have not yet been elucidated.

Trans-acting proteins

One of the first well-characterized proteins that regulate protein synthesis is the iron regulatory element (IRE)-binding protein (IRP) that inhibits the translation of ferritin mRNA in the absence of iron (33). The IRE is a *cis*-acting hairpin structure found in the 5'-UTR of ferritin mRNA. When the IRP binds, eIF4F can still interact with the mRNA, but the 40S ribosome is prevented from binding to the 5'-terminus of the mRNA due to IRP stabilization of the IRE hairpin. However, when iron ions enter the cell, they bind to the IRP,

resulting in its reduced binding to the IRE and elimination of its inhibitory properties.

While certain characteristics of IRP action are typical of many *trans*-acting proteins, e.g., its binding to a specific site in the mRNA and its interference with a step in initiation, its binding to a *cis*-element in the 5'-UTR is unusual. Most *trans*-acting proteins bind to elements in the 3'-UTR, yet affect initiation at the 5'-UTR. A well-characterized example is the CPEB/maskin complex. CPEB binds to the cytoplasmic polyadenylation element (CPE) in the 3'-UTR to promote cytoplasmic polyadenylation of a class of mRNAs. However, CPEB also binds to maskin, a protein that contains a motif that binds to eIF4E. By the interaction of maskin with eIF4E, the eIF4E-eIF4G interaction is prevented and initiation is impaired. Another example involves the binding of bicoid to a specific element in *Drosophila* caudal mRNA. Bicoid in turn recruits the *Drosophila* 4E-homologous protein d4EHP, which can bind to m⁷G-caps but does not interact with eIF4G. d4EHP competes with eIF4E for cap recognition, thereby reducing the rate of caudal mRNA translation. There are many other examples of *trans*-acting regulatory proteins that bind to 3'-UTR *cis*-elements, especially those functioning during early development (33,34).

MicroRNAs

MicroRNAs are small non-coding RNAs containing about 21 nucleotides that have been implicated in the post-transcriptional regulation of gene expression (35). Early studies established these RNAs as promoters of mRNA degradation; however, more recent evidence indicates a role in translational control as well. There are at least 800 genes encoding microRNAs in the mammalian genome, suggesting that these molecules affect a wide range of gene expression. MicroRNAs interact with a complementary sequence in an mRNA, usually in its 3'-UTR. If the complementarity is perfect, the mRNA is degraded, sometimes through a deadenylation mechanism. However, if the complementarity is imperfect, inhibition of translation may occur instead. There are reports that either the initiation phase is inhibited through a mechanism involving eIF4E or that the elongation phase is inhibited. To further complicate the situation, there are reports that microRNAs can stimulate translation of specific mRNAs, whereas a recent paper questions whether microRNAs affect protein synthesis at all (36). Clearly, a detailed understanding of how microRNAs affect protein synthesis is not yet available. Nevertheless, it can be anticipated that future studies will rapidly elucidate such mechanisms, as microRNA regulation of gene expression appears to be important in many disease states.

Defects in the regulation of protein synthesis affect cell proliferation

Hyperactivation or the failure to down-regulate the rate of protein synthesis contributes importantly to cell

malignancy. It is hypothesized that a moderately repressed translational apparatus leads to a balance of protein synthesis that enables cells to control their rates of proliferation. However, when protein synthesis is over-activated, "weak" mRNAs, often containing considerable amounts of secondary structures in their 5'-UTRs, are translated relatively more efficiently, leading to an imbalance of proteins made. Such "weak" mRNAs encode numerous proteins involved in promoting cell growth and proliferation, such as c-myc, cyclin D1 and growth factors. It is thought that when such protein levels increase due to the dysregulation of overall protein synthesis, cells become malignant. I shall review a number of examples where this mechanism appears to cause cancer.

Regulation of m⁷G-cap binding by eIF4E

eIF4E levels are thought to be limiting in most cells. In addition, active eIF4E capable of binding to eIF4G is reduced through the activity of the 4E-BPs, which in turn are down-regulated by phosphorylation. Overexpression of eIF4E in NIH 3T3 and CHO immortal cell lines causes only a modest increase in the rate of overall protein synthesis, but leads to malignant transformation of these cells (37). The phenotype is reversed if 4E-BPs also are overexpressed along with eIF4E. In related studies, overexpression of eIF4G also can malignantly transform cell lines (38). Thus, when eIF4F activity is elevated by higher levels of eIF4E or eIF4G, cells lose their ability to control their proliferation. There are numerous examples of tumors where eIF4E levels are substantially higher than normal. Indeed, combined analysis of eIF4E and 4E-BP expression is a strong prognostic indicator of breast cancer (39).

Regulation of Met-tRNA_i binding

The major initiation factor involved in Met-tRNA_i binding to the 40S ribosomal subunit is eIF2. Phosphorylation of Ser-51 in the α -subunit converts eIF2 into a competitive inhibitor of eIF2B, resulting in a failure to exchange GDP for GTP in eIF2 and a subsequent inhibition of Met-tRNA_i binding. When the phosphorylation of eIF2 by PKR is reduced by overexpression of a dominant negative mutant form of PKR, immortal cells become malignant (40). Overexpression of a mutant form of eIF2 α where the phosphorylation target, Ser-51, is changed to alanine, also causes malignant transformation since the mutant eIF2 α cannot be phosphorylated (41). These results all point to eIF2 phosphorylation as an important mechanism for establishing a suitable rate of overall protein synthesis and for maintaining cell homeostasis.

Other mechanisms involving protein synthesis and cancer

Besides initiation factors affecting mRNA recognition through eIF4F and Met-tRNA_i binding through eIF2, other factors involved in protein synthesis have been implicated

in cancer. Overexpression of eIF4A occurs in primary hepatocellular carcinomas (42), and eIF4A binds to the tumor suppressor Pdc4 (43), suggesting that down-regulation of eIF4A activity may be important in the regulation of cell proliferation. The eIF5A2 gene is amplified in a number of cancers, and its down-regulation by siRNA decreases a number of malignant phenotypes in an ovarian cancer cell line (44). A number of eIF3 subunits have been implicated in cancer, as discussed in detail in the section following. Finally, elongation factor eEF1A levels are enhanced in a number of cancers and its overexpression causes malignant transformation of NIH 3T3 cells (45). Further work is needed to better elucidate the roles of these, and possibly other, factors in regulating cell proliferation.

Regulation of eIF3 is important in controlling cell proliferation

eIF3 is the largest of the initiation factors, exhibits a 5-lobed structure that binds to the back of the 40S ribosomal subunit (15), and plays a central role in the initiation pathway. Yet there has been little evidence to indicate that the factor's activity is regulated. Many of the 13 eIF3 subunits are phosphoproteins, and an increase in their phosphorylation correlates with activation of protein synthesis (27), but regulation of eIF3 activity by phosphorylation has been documented only in a few cases (see below). Alternatively, specific subunits may associate with other proteins that regulate protein synthesis or other processes. For example, eIF3e binds to P56, an interferon-induced protein that inhibits protein synthesis (46). eIF3g binds to PAIP, a PABP-binding protein that stimulates initiation (47); in addition, plant eIF3g binds to TAV, required for shunting of the 40S initiation complex during scanning/reinitiation (48). eIF3f binds to Atrogina/MAFbx, a ubiquitin E3 ligase that degrades the subunit during muscle atrophy (49). Another ubiquitin E3 ligase, TRC8, interacts with both eIF3h and eIF3f, leading to inhibition of protein synthesis, possibly through ubiquitination of eIF3 or some other translational component (50). The latter two observations suggest that regulation of protein synthesis and protein degradation may be coordinated through eIF3. The mRNA export factor hGle1 interacts with eIF3f, affecting initiation and possibly linking mRNA export and translation (51). eIF3 also provides docking sites for protein kinases such as mTOR and the S6Ks, which regulate protein synthesis (52). eIF3f binds directly to mTOR, but whether or not the mTOR-dependent insulin stimulation of eIF3-eIF4G association requires this interaction is not clear. Finally, eIF3i binds to, and is phosphorylated by, the type II TGF- β receptor (53).

Besides these intriguing possibilities for eIF3 involvement in numerous cellular pathways, eIF3 has been implicated in oncogenesis and the maintenance of the cancer state. Transcriptome analyses of tumors and malignant cell lines identified a number of elevated mRNAs that encode

eIF3 subunits. Some examples are listed here (for literature citations, see Ref. 54). eIF3a mRNA is overexpressed in breast, cervical, esophageal, lung and gastric cancers; eIF3b, in breast carcinomas; eIF3c, in testicular seminomas, and eIF3h, in breast, prostate and hepatocellular carcinomas. On the other hand, eIF3e is underexpressed in breast and lung carcinomas, usually associated with loss of heterozygosity at the *int6* locus (55). The eIF3e gene also is the site of frequent insertion of the mouse mammary tumor virus genome. Another eIF3 subunit, eIF3f, also is underexpressed in prostate cancer and is discussed below. In the case of eIF3a, the translation of specific mRNAs involved in cell proliferation (e.g., the cell-cycle regulator p27) is affected by eIF3a levels (56). These observations strongly suggest that eIF3 activity is important in regulating cell proliferation and in tumorigenesis.

To examine the possibility that overexpression of eIF3 subunit proteins might contribute to the malignant phenotype, stable cell lines of immortal NIH 3T3 cells were constructed such that each overexpresses one of the eIF3 subunits. Of the 12 cell lines analyzed (all except eIF3m), individual overexpression of five of the twelve subunits results in a modest increase in protein synthesis rate, the ability for anchorage-independent growth, resistance to apoptosis and other malignant phenotypes (54): namely, the eIF3a, eIF3b, eIF3c, eIF3h and eIF3i subunits. Overexpression of the 3a, 3b or 3c subunit leads to enhanced levels of the whole eIF3 complex by a mechanism that is not understood. Nor is it clear how overexpression of the 3h or 3i subunit, which does not alter the level of eIF3, stimulates protein synthesis and cell proliferation. Further experiments with a stable cell line where eIF3h synthesis can be induced show that the malignant phenotypes emerge within about 8 h of induction, just as the level of eIF3h and the tightly coupled rate of protein synthesis increase, suggesting that protein synthesis is directly involved (57). However, no malignant transformation occurred with induced overexpression of a mutant form of eIF3h, where Ser-183 is changed to alanine to prevent phosphorylation at that site, indicating that phosphorylation of eIF3h is required for induction of malignancy. Further evidence implicating eIF3h levels in cancer comes from experiments that reduce the level of eIF3h in a prostate cell line that exhibits high eIF3h levels. Down-regulation of eIF3h causes the cells to lose their ability for anchorage-independent growth and to become more susceptible to apoptosis (57). The results indicate that elevated levels of eIF3h are required to maintain the cancerous state of these prostate-derived cells.

A number of other eIF3 subunits also have been implicated in the regulation of protein synthesis and cell proliferation. The level of eIF3f is abnormally low in pancreas, breast, ovary and vulva tumors and in a number of melanoma and pancreatic cancer cell lines (58). Overexpression of eIF3f inhibits protein synthesis and the growth of these cancer cells and induces apoptosis. Interestingly, eIF3f is the target

of CDK11p46, a protein kinase whose activity is induced during apoptosis; the kinase phosphorylates eIF3f on serine-46 and threonine-117 (59). Addition of eIF3f to a cell lysate inhibits protein synthesis *in vitro*. When the kinase is added along with wild-type eIF3f, protein synthesis is more strongly inhibited, whereas a mutant form of the subunit with alanine substitutions at the two phosphorylation sites does not inhibit (28). Overexpression of this mutant form of eIF3f in transiently transfected cells also does not inhibit protein synthesis. These results suggest that eIF3f may dampen the activity of eIF3 and that its phosphorylation enhances this down-regulatory activity. eIF3f can be considered a tumor suppressor protein whose activity is augmented by phosphorylation.

High levels of eIF3i are found in a number of tumors. There is evidence suggesting that mTOR may phosphorylate eIF3i, an event that correlates with the malignancy caused by eIF3i overexpression in stable cell lines (60). In summary, overwhelming evidence indicates a clear role for eIF3 in establishing and maintaining a malignant phenotype. It appears likely that eIF3 accomplishes this through its role in initiation of protein synthesis, although other roles cannot be rigorously excluded. The findings support the general hypothesis that regulation of cell proliferation involves establishing a proper level of translation initiation, with over-activation of the translational apparatus leading to excessive translation of mRNAs encoding oncogenic proteins.

Perspectives

A large body of evidence supports the hypothesis that hyper-activation of the translational machinery causes loss

of the control of cell proliferation and malignant transformation. Although this activation results in the overproduction of oncogenic proteins that in turn are likely responsible for the malignancy, it is difficult to show a direct causal relationship between translation and cancer. One of the problems is that the process of protein synthesis is intimately entwined with many other cellular processes that contribute to the maintenance of cell homeostasis. Another challenge is that only subtle changes in translation rates can affect the delicate balance of gene expression. Nevertheless, regulation of Met-tRNA_i and mRNA binding through initiation factors eIF2 and eIF4E/eIF4G are well understood. In contrast, explanations for how eIF3 activity also contributes to the malignant state are less obvious and will require more detailed studies of the initiation pathway. In particular, determining an atomic resolution structure for eIF3 will enable a better understanding of how this protein complex organizes other initiation factors and regulatory proteins on the surface of the ribosome. A more detailed understanding of the kinetics of the initiation process also is needed. It can be anticipated that the increasing knowledge of the initiation pathway will enable a more rational therapeutic approach to drug design and the treatment of cancer and other human diseases.

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