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INVITED REVIEW

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Not just for housekeeping: protein initiation and elongation factors in cell growth and tumorigenesis

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Abstract Proteins provide the structural framework of a cell and perform the enzymatic activities sustaining DNA replication and energy production. The hormones and growth factors that facilitate organ-to-organ communication are proteins as are the receptors and signaling intermediaries that integrate extracellular stimuli to intracellular action. As such, eukaryotic cells devote tremendous effort and energy to protein synthesis. The enzymes involved in protein synthesis have traditionally been described as cellular housekeepers. This was meant to imply that while they were necessary for cell viability, they were not thought to have a causal role in activating cell differentiation or neoplastic development the way that a transcription factor or hormone receptor might. However, two protein translation factors, protein initiation factor eIF4E and protein elongation factor eEF1A2, have been identified as important human oncogenes. This review summarizes recent work showing that protein initiation and elongation factors have important regulatory roles in cell growth, apoptosis, and tumorigenesis.

Keywords Cancer · Protein translation · Protein elongation · Apoptosis · Oncogene

Abbreviations *eEF:* Eukaryotic elongation factor · *eIF1:* Eukaryotic initiation factor · *eRF:* Eukaryotic release factor · *PKC:* Protein kinase C · *PMA:* Phorbol 12-myristate 13-acetate · *PTI:* Prostate tumor inducing · *UTR:* Untranslated region

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Introduction

The use of gene expression microarrays to analyze cell function has become commonplace in recent years [1]. Coupled with sophisticated computational techniques [2, 3], microarray data have exponentially increased our understanding of normal [4, 5] and malignant development [6, 7]. However, biological and phenotypic complexity ultimately derives from changes in protein concentration and localization, posttranslational protein modification and the combinatorial diversity of protein-protein interactions. Since overall protein abundance is at least partly regulated by the translational efficiency of mRNA, a priori it is predictable that the protein synthesis machinery exerts important effects on cell physiology.

Translational regulation

The central dogma of molecular biology states that the information content of DNA is transferred to mRNA by the transcription machinery and from mRNA to protein by the translation machinery. Steady-state protein abundance is therefore the net result of independent regulatory networks controlling gene transcription, mRNA

stability, translational efficiency, and protein degradation.

If protein abundance were primarily the consequence of gene transcription, one would expect a strict linear relationship between the abundance of an mRNA and the intracellular molarity of the protein that it encodes. Similarly, it would be predicted that the respective proteins of two different mRNA species of the same abundance would be present at similar levels. This is not the case. A recent study of 98 genes in 76 primary human lung adenocarcinoma samples revealed a correlation between mRNA and protein levels in less than one-fourth of the genes studied (21/98) [8]. Similarly, the ratio of mRNA to protein of glutathione *S*-transferases varies 100 times among 60 mammalian tumor cell lines studied [9]. A similar situation exists in the yeast *Saccharomyces cerevisiae*, where the variability between mRNA and protein concentration among several hundred genes is between 10- to 100-fold [10, 11]. The poor correlation between mRNA and protein levels in a single cell and among different cell lines implies that posttranscriptional processes are the prime regulators of protein abundance. The steady-state abundance of a protein is the net difference between its translation and its degradation. While it is well known that ubiquitin-mediated proteolytic destruction controls multiple cell functions [12], the rate of protein translation is also critical to cell physiology and the development of cancer [13].

Cancer, housekeeping genes, and translation factors

Cancer develops when normal cells acquire genetic mutations allowing them to circumvent the homeostatic regulators that inhibit proliferation. Cancerous cells not only have the capacity for rapid and autonomous replication but also have the ability to invade surrounding tissue and metastasize to physically distant sites. The idea that a protein translation factor could actively be promoting cancer is highly unusual and is apparently at odds with commonly held views as to the root mechanistic causes of cancer.

A commonly accepted paradigm of oncogenesis is that the primary driving force of tumor development is activating mutations in cell surface receptor kinases and/or their signaling intermediaries [14]. This model of oncogenesis can be described as "kinase-driven neoplasia." Under this model constitutive hyperactivation of kinase-dependent signaling activates proliferation by enhancing the activity of transcription factors [15]. When these changes are coupled with the inactivation of tumor suppressor genes, malignancy occurs [16]. An avalanche of experimental data supports this idea, but there are underappreciated aspects of oncogenesis that need to be further explored.

Implicit in this kinase-driven model for neoplasia is a conceptual division of the cellular machinery into two distinct classes: those proteins that act as regulators and those that act as housekeepers. The regulatory proteins are thought of as the most important class and for the most part are tyrosine and serine/threonine kinases, signaling adapters, and transcription factors. Their abundance and activity are highly dynamic and respond quickly to changes in intra- and extracellular stimuli, usually through phosphorylation. The second class of cellular proteins are the so-called housekeeping proteins, which maintain basal transcription, translate proteins, and provide the necessary enzymatic processes of a cell. Changes in the enzymatic activity of these proteins are generally thought to be a response to stimulation by regulatory proteins. Findings that housekeeping proteins are upregulated, activated, or mutated in cancer are generally dismissed as nonconsequential since that observation is not consistent with kinase-driven oncogenesis. To ascribe oncogenic properties to housekeeping proteins was, and perhaps still is, viewed with suspicion. However, it is becoming increasingly evident that far from being inactive players in the control of cell growth and replication, so-called housekeeping genes may have an important causal role in neoplasia.

Ribosomes, for example, are archetypal housekeeping proteins. While cell viability is absolutely dependent on protein translation, ribosomal function seems plain and unexciting. Ribosomal subunits are a heterogeneous mixture of proteins (r-proteins) and ribosomal RNA (rRNA) assembled in a complex pathway [17]. Cells invest enormous amounts of energy in rRNA production [18]. rRNA accounts for 10% of the *S. cerevisiae* genome and almost 50% of its RNA polymerase II transcription [18]. But could ribosomal components have a causal role in cancer? Several r-proteins are overexpressed in human colorectal cancer [19] and leukemic blast [20] cells relative to normal controls. In addition, rRNA genes map to loci frequently amplified in human cancer [21]. Furthermore, ectopic expression of rRNA S3a leads to the acquisition of a neoplastic phenotype in NIH 3T3 fibroblast cells [22]. While no causal relationship between S3a and human cancer has been definitely established, these findings do make a case that a so-called "housekeeping gene" could have an important role in cancer [23]. Other housekeepers are equally likely to be important. One recent study in colorectal cancer found that nearly one- half of the approx. 100 genes specifically upregulated in colon cancer are ribosomal components and protein synthesis factors [24]. The reminder of this review focuses on evidence suggesting that one set of housekeeping proteins, protein translation factors, are important oncogenes.

The nomenclature of protein synthesis

A human's dry body weight is 44% protein [25], and 30–40% of the volume of a yeast cell is occupied by the ribosomal subunits on which polypeptides are made [18]. Protein synthesis is a highly organized, multicomponent pathway whose major features have been largely con-

Table 1 Eukaryotic elongation factor nomenclature and function

Current name	Previous name	Gene (human)	Functions
eEF1A1	eEF-1α1	*EEF1A1*	Recruits tRNA to ribosomal A site; GTP hydrolysis
eEF1A2	eEF-1α2	*EEF1A2*	Recruits tRNA to ribosomal A site; GTP hydrolysis
eEF1Bα	eEF-1β	*EEF1B*	GDP:GTP exchange on eEF1A
eEF1Bβ	eEF-1δ	*EEF1D*	GDP:GTP exchange on eEF1A
eEF1Bγ	eEF-1γ	*EEF1G*	Links eEF1Bα and eEF1Bβ during GDP:GTP exchange
EF2	eEF2	*EEF2*	Ribosomal translocation on mRNA; GTP hydrolysis

served between prokaryotes and eukaryotes. There are three essential stages of protein translation: initiation, elongation, and termination. In eukaryotes these independent steps are controlled by multisubunit enzyme complexes generically identified as eukaryotic initiation factors (eIF), eukaryotic elongation factors (eEF), and eukaryotic release factors (eRF).

The initiation, elongation, and termination stages of protein synthesis each involves several distinct factors that are identified by a numerical suffix. For example, eIF4 and eEF1 identify initiation factor 4 and elongation factor 1, respectively. Individual factors can themselves consist of other "subfactors" which may or may not themselves be multicomponent complexes. An uppercase letter, for example, eIF2B, identifies these subfactors. In one of the quirks of this nomenclature system, there is no identified "A" subform of eIF2, but there is eIF2 and eIF2B. Individual protein components of a particular subfactor are identified by a Greek letter, for example, eIF2Bε and eEF1Bα. In some cases a protein synthesis polypeptide has several isoforms, and these are identified numerically. For example, eEF1A1 and eEF1A2 refer to two isoforms of eEF1A.

There are two different nomenclature systems for the proteins that are part of eEF1. The eEF1 holoenzyme recruits amino-acylated tRNA to the ribosome (see below). In the current naming system, eEF1 consists of two different subfactors: eEF1A and eEF1B. eEF1A (formerly eEF1α), is a single polypeptide while eEF1B is a multimer of eEF1Bα (formerly eEF1β), eEF1Bβ (formerly eEF1δ) and eEF1Bγ (formerly eEF1γ) polypeptides. For clarity, the old and new names are summarized in Table 1. Because of the disparity between the old and modern elongation factor names, the modern name for each of the eEF1 proteins does not correspond to the gene nomenclature. For example, the protein product of the human *EEF1D* gene is eEF1Bβ. Similarly, the protein product of *EEF1G* is eEF1Bγ and the protein product of *EEF1B* is eEF1Bα. This is confusing even for investigators in the field (that is, the authors). For the remainder of this review the current elongation factor naming system is used. The eEF1A protein isoforms are referred to as eEF1A1 and eEF1A2, and they are encoded by the respective *EEF1A1* and *EEF1A2* genes.

The mechanics of protein translation

The central feature of protein initiation is the recruitment of an initiator $tRNA^{met}$ to the AUG initiation codon in conjunction with the formation of an 80S ribosomal complex, as shown in Fig. 1. The primary feature of protein initiation is the assembly of the 60S and 40S ribosomal subunits and a methionine charged tRNA at the AUG initiation codon of an mRNA. This assembly begins with the binding of eIF3, eIF1, eIF1A, eIF2, and a $tRNA^{met}$ to the 40S ribosomal subunit (part 1, Fig. 1). This complex is identified as the 43S preinitiation complex. The 43S complex is brought to the mRNA under the control of eIF4F. eIF4F is a multimer of eIF4E, eIF4G, eIF4A, and eIF4B that binds to the 7′methyl cap upstream of the AUG initiator codon (part 2, Fig. 1). eIF4A is a helicase, eIF4B and eIF4E bind the RNA, and eIF4G is a scaffolding protein that links the three proteins together. eIF4E binds the 7-methyl guanosine residues at the 5′ mRNA end. 7-Methyl guanosine is a structure present exclusively in eukaryotic mRNA and is commonly described as the mRNA "cap." Secondary structure in the 5′untranslated region (UTR) of the mRNA, often from stretches of G/C residues, sterically hinder eIF4E binding. Because of this the eIF4E-cap association may be the limiting factor in initiation of many mRNA [26]. The importance of eIF4E and the 5′ UTR secondary structure is discussed below. Binding of eIF4E to the mRNA cap is also regulated by three proteins: 4EBP1 4EBP2, and 4EBP3, which bind eIF4E and inhibit its association with mRNA. Following eIF4F binding, the 43S complex is recruited to the mRNA (part 3, Fig. 1). Next, the eIFs dissociate from the ribosome leaving the $tRNA^{met}$ bound to the initiator AUG codon and the 40S ribosome (part 4, Fig. 1). This allows for the binding of the 60S ribosomal subunit to the 40S subunit. Comprehensive reviews of eukaryotic translational initiation are available elsewhere [26].

During protein elongation amino acids are sequentially added to the growing polypeptide. Comprehensive reviews of protein elongation are available elsewhere [18]. As shown in Fig. 2, protein elongation can be divided into two stages: (a) the recruitment of amino-acylated tRNA to an existing ribosome/mRNA complex (parts 1–5, Fig. 2) and, (b) the translocation of the ribosome along the mRNA as amino acids are added to the protein (parts 5–8, Fig. 2). eEF1A and eEF1B regulate the recruitment phase and eEF2 controls translocation. eEF1A

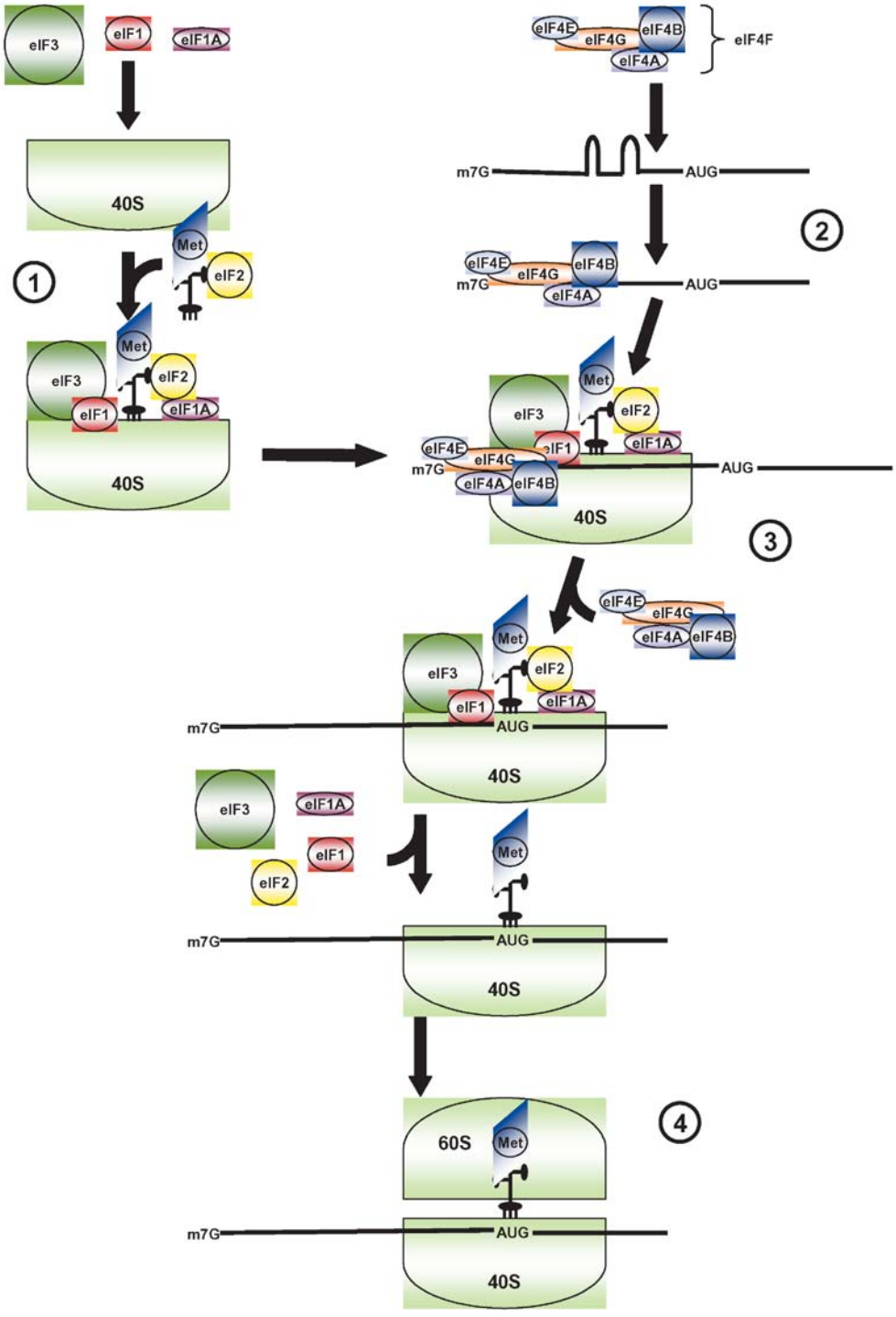

Fig. 1 The initiation phase of translation. *1* A complex of eIF3, eIF1, eIF1A and eIF2 and $tRNA^{met}$ binds to the 40S ribosome. *2* The eIF4F multimer (eIF4E, eIF4G, eIF4A, and eIF4B) binds to the 7′ methyl cap upstream of the AUG initiator codon. *3* The 40S ribosome is recruited to the mRNA molecule. *4* The eIFs dissociate from the ribosome leaving the $tRNA^{met}$ bound to the initiator AUG codon and allowing for the association of the 60S ribosomal subunit with the 40S subunit. This is the 80S ribosome

is a GTP binding protein, and it is eEF1A-GTP that physically binds amino-acylated tRNA and brings it to the A site of the ribosome (part 1, Fig. 2). Proper codon/anticodon recognition between the tRNA and the mRNA in the ribosomal A site leads to GTP hydrolysis in eEF1A and the subsequent dissociation of the eEF1A-GDP from the ribosome (part 2, Fig. 2). The tRNA is left in the ribosome. Once eEF1A-GDP leaves the ribosome, it associates with eEF1B, a complex of eEF1Bα, eEF1Bβ, and eEF1Bγ proteins (part 3 Fig. 2). The complex of eEF1A and the eEF1B proteins is referred to as eEF1H, for eEF1-heavy. It is believed that one eEF1B complex can associate with two eEF1A proteins. The composition of eEF1B is, however, variable. eEF1B consists of one molecule of eEF1Bγ bound to either: (a) one molecule each of eEF1Bα and eEF1Bβ, (b) two molecules of eEF1Bα, or (c) two molecules of eEF1Bβ. eEF1B, in any of its forms, functions to exchange GTP

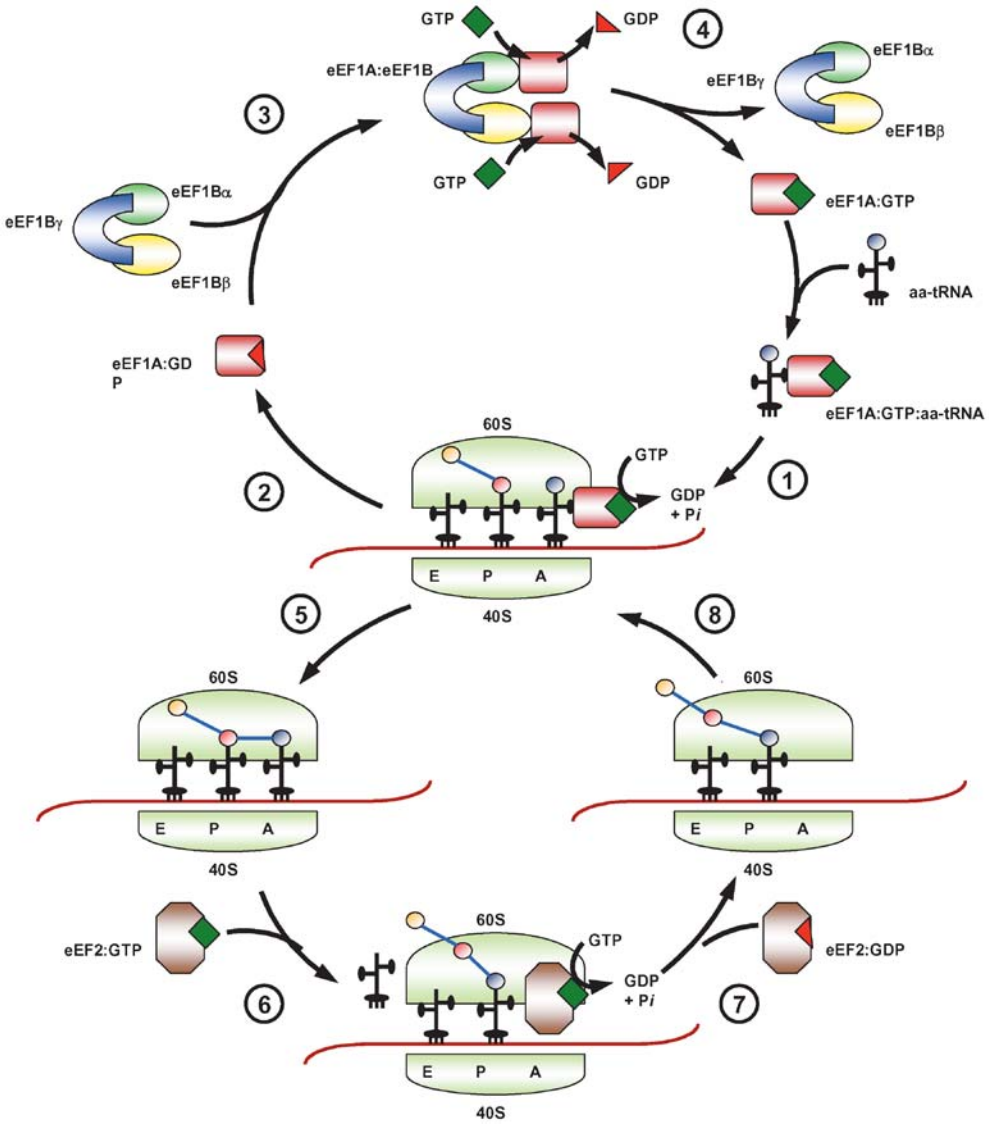

Fig. 2 The elongation phase of translation. *1* eEF1A recruits an amino-acylated tRNA to the A site of the ribosome. *2* GTP hydrolysis allows eEF1A to dissociate from the ribosome and bind eEF1B. *3* eEF1B exchanges GDP for GTP on eEF1A. *4* eEF1A is free to recruit a new amino-acylated tRNA to the ribosomal A site. *5* A peptide bond forms between the amino acids in the A and P sites. *6* eEF2 transiently binds near the ribosomal A site. *7* GTP hydrolysis by eEF2 causes the ribosome to translocate 3 amino acids down the mRNA and leads to dissociation of eEF2 from the ribosome. *8* The A site is left free for eEF1A to bring in a new amino-acylated tRNA

for GDP on eEF1A, priming it for another round of tRNA binding and recruitment (part 4, Fig. 2). Both eEF1Bα and eEF1Bβ have GTP exchange activity for eEF1A. eEF1Bγ has no known enzymatic activity but is believed to facilitate the physical association of eEF1Bα or eEF1Bβ with each other or with eEF1A [27].

The translocation phase of protein synthesis begins with the formation of a peptide bond between amino acids in the P and A ribosomal pockets (part 5, Fig. 2). After bond formation the monomeric eEF2-GTP protein binds to the ribosome (part 6, Fig. 2). eEF2 then physically forces the peptide formerly in the A site to the P site. The tRNA and peptide formerly in the P site moves to the E site and the tRNA from the E site is released. During this translocation step eEF2 moves the ribosome exactly 3 nucleotides 3′ down the mRNA. This GTP-dependent process ends with hydrolysis of the GTP moiety on eEF2 and eEF2-GDP dissociation from the ribosome (part 7, Fig. 2). Unlike eEF1A, no eEF2 specific exchange factors have yet been identified. The cycle then resumes (part, 8 Fig. 2), with new eEF1A-GTP-tRNA recruited to the now unoccupied A site and fresh eEF2-GTP utilized for subsequent translocation.

Translation termination begins with the recognition of a termination codon by eRF1 [28]. eRF1 is believed to be a structurally similar to an amino-acylated tRNA and occupies the ribosomal A site in place of it. The eRF3 complex then removes eRF1 from the A site in a GTP-dependent process. A combination of eIF3 and eRF4 causes dissociation of the ribosomal subunits from the mRNA, allowing both to begin a new initiation step.

Initiation factor eIF4E is an oncogene

An oncogene, a gene that promotes cancer, has two basic properties: it is hyperactivated in primary human cancers, and it is capable of activating cell growth and repli-

cation. For the purposes of this review we define cell growth as an increase in size and mass of an individual cell while cell replication refers to the division of a mother cell into two daughters. The activation may be the result of gene mutation or increased protein or mRNA expression. Nearly all known oncogenes cause morphological and functional changes in cell lines cultured in vitro. Rodent cell lines, usually NIH 3T3 or Rat1 cells, are used to test for this. NIH 3T3 cells are cultured mouse fibroblasts that are said to be "immortalized" but not "transformed." Immortalization and transformation are thought to represent the laboratory manifestation of two critical developmental steps in human malignancy [16]. Immortalization refers to the process by which cells acquire an unlimited lifespan, whereas transformation refers to the acquisition of malignant properties. The in vitro dividing potential of nonimmortalized cells is usually limited to 20–40 doublings, a potential described as the Hayflick limit [29]. Immortalized cells grow beyond the Hayflick limit but retain some characteristics of nonmalignant cells. They do not form solid tumors when xenografted into immunocompromised animals, for example. In addition, they require attachment to a solid substrate for in vitro proliferation. The requirement for a solid substrate is described as "anchorage-dependent" replication. When an immortalized cell becomes transformed, it grows as a vigorous colony in nonsolid media (usually the gel-like soft agarose), a type of replication termed "anchorage-independent." Transformed cells also form three-dimensional, dome-like outgrowths, termed foci, in tissue culture that are easily distinguished from the one-cell thick monolayer culture of nontransformed cells. Transformed cells also usually acquire the ability to grow as solid tumors in mice. The well studied oncogenes *RAS* and *MYC* easily transform the immortal NIH 3T3 cells and make them malignant.

The first evidence implicating the protein translation machinery in malignancy was the observation that eIF4E, an mRNA cap-binding factor, transforms NIH 3T3 cells and allows them to grow as tumors in mice [30]. eIF4E expression activates the Ras oncogene, and the observation that an inhibitor of Ras activity, the GTPase GAP, reverses eIF4E-mediated transformation [31] suggests that eIF4E is part of a pathway of malignancy involving Ras. Abnormally high eIF4E expression is observed in a substantial fraction of breast carcinomas [32], colorectal adenocarcinomas [33], squamous larynx cancers [34], invasive carcinomas of the head and neck [35], as well as bladder [36] and bronchioalveolar lung tumors [37]. Furthermore, *EIF4E*, the human eIF4E gene, maps to a locus amplified in the vast majority of non-small-cell lung cancers [38] and some breast tumors [39, 40]. Increased eIF4E expression and *EIF4E* gene amplification is correlated with increased malignancy, poor survival probability, and increased chance of tumor recurrence after initial therapy in breast cancer [32, 39, 40]. Elevated eIF4E levels are also associated with poor survival in bladder and larynx tumors [35, 36, 41]. Taken together, these observations make a convincing argument that eIF4E, and by implication, the process of protein initiation, has an important causal role in multiple human cancers. It is not yet known whether other initiation factors have similar roles.

Elongation factor eEF1A1 and eEF1A2 are oncogenes

Tatsuka and colleagues [42] were the first to implicate the protein elongation machinery in cell transformation. They screened a mouse expression library for genes that would enhance the rate of both spontaneous and chemically induced transformation of mouse and hamster fibroblasts. One of the genes that they identified was the mouse homologue of *EEF1A1*, which was found dramatically to increase the ability of 3-methylcholanthrene and ultraviolet light to cause transformation.

The first report linking human eEF1A1 to cancer was work from Paul Fisher's group at Columbia University. Using differential display [43], a polymerase chain reaction based technique used to clone genes expressed in one tissue but not another, they identified a gene expressed in primary prostate carcinomas but not in normal prostate [44]. They named this gene prostate tumor inducing (PTI) gene 1. Expression of PTI-1 in nontumorigenic prostate cell lines was found to make these cells tumorigenic in nude mice. Furthermore, antisense-mediated abrogation of PTI-1 expression inhibits tumorigenesis and anchorage-independent cell replication in PTI-1 expressing prostate tumor cells [45]. When fully sequenced, PTI-1 proved not to be a complete human gene but rather a fusion between a *Mycoplasma hyopheumoniae* 23S ribosomal RNA gene and human *EEF1A1* encoding amino acid residues 65–462. There are also six amino acid changes between wild-type eEF1A1 and homologous sequences in PTI-1. At first, Fisher's work raised the radical possibility that the fusion of a gene from an infectious agent (*Mycoplasma*) and a normal human gene could have created a novel prostate cancer oncogene. Alternatively, the mutation of the eEF1A1 coding sequences in PTI-1 could have rendered the wild-type eEF1A1 oncogenic. However, the unusual composition of PTI-1 and the fact that error-prone polymerase chain reaction analysis was used for its identification raised the possibility that, notwithstanding its oncogenic properties, PTI-1 is a laboratory epiphenomenon. The relevance of PTI-1 to prostate and other cancers still remains an unresolved, albeit intriguing, issue. One issue that was never addressed in subsequent studies was the possibility that wild-type eEF1A1 is itself a transforming gene, and its fusion to the *Mycoplasma* gene as well as the observed gene mutations was not directly related to the ability of PTI to promote malignant growth. None of the control experiments in Fisher's work seem to have addressed this issue, possibly because it was not appreciated that a wild-type translation factor could be a human oncogene.

Most recently work in our laboratory has established that one of the human eEF1A isoforms, eEF1A2, has many of the accepted properties of an oncogene [46]. There are two known eEF1A isoforms, eEF1A1 and eEF1A2, and these share more than 95% DNA and protein identity. eEF1A1 is expressed ubiquitously whereas eEF1A2 expression is restricted to the heart, brain, and skeletal muscle in humans, rats, and rodents [47, 48, 49]. The functional significance of the tissue-specific expression pattern of eEF1A2 has yet to be determined, but eEF1A1 and eEF1A2 are believed to have the same enzymatic function in protein translation. *EEF1A2*, the gene for human eEF1A2 maps to 20q13.3 [50], a locus that is increased in copy number in a 30% fraction of breast [51, 52, 53] and ovarian cancers [54, 55, 56]. In both cancers 20q13 amplification is correlated with poor clinical prognosis and increased tumor aggressiveness [56, 57, 58]. We found that not only is *EEF1A2* amplified in about one-third of primary ovarian tumors, but that the level of its mRNA is also increased in a similar tumor fraction [46]. Importantly, ectopic expression of wild-type eEF1A2 transforms mouse and rat fibroblasts and allows them to grow as tumors when xenografted into mice. When eEF1A2 is expressed in a human ovarian cell line that does not express eEF1A2, the eEF1A2-expressing line is more tumorigenic than its parental control [46]. These results indicate that eEF1A2 can directly promote malignancy, at least in ovarian cancer. It has yet to be demonstrated whether eEF1A2 can directly promote tumorigenesis in transgenic mouse models of ovarian cancer, but this is a research avenue that our laboratory is actively pursuing. Nor is it yet known whether eEF1A2 is overexpressed or otherwise involved in cancers other than those of the ovary.

The involvement of eEF1A2 in ovarian cancer and its strong sequence similarity to eEF1A1 imply that human eEF1A1, as with eEF1A2, is also involved in cancer. The eEF1A1 gene (*EEF1A1*) maps to 6q14 [50] and one would suppose that, as in the case of *EEF1A2* and 20q13, 6q14 and *EEF1A1* are amplified in some human cancers. Gains at 6q14 have been reported in some childhood brain tumors [59], but for the most part it is losses at 6q14 rather than gains that are associated with cancer [60, 61, 62]. Given the strong transforming and tumorigenic aspects of eEF1A2 it is perhaps surprising that *EEF1A1* gene amplification is not linked to any malignancy. While there are several nonconservative amino acid differences between the eEF1A1 and eEF1A2 proteins, none would be predicted to affect protein translation capacity. Earlier results with mouse eEF1A1 suggested that it cannot transform cells on its own, but requires cooperation with other mutagens. However, work in our laboratory has found that human eEF1A1, as with eEF1A2, can also be transforming (K. Jackson, N. Anand, J. Lee, unpublished observations). Perhaps *EEF1A1* amplification is not linked to cancer because the 6q14 locus contains a potent, yet uncharacterized tumor suppressor gene and any 6q14 amplifications would decrease cell growth even in the increased presence of growth-enhancing *EEF1A1* [61]. If this were the case, an increase in eEF1A1 mRNA expression independent of changes in the 6q14 locus might be an important parameter to investigate. Overexpression of eEF1A1 occurs in melanomas and tumors of the pancreas, breast, lung, prostate and, colon [24, 63, 64, 65, 66, 67, 68]. In addition, higher expression of eEF1A1 is found in metastatic rat adenocarcinoma cells than in controls [68, 69]. The increased expression is only circumstantial evidence that eEF1A1 is causally involved in cancer however, and further work must investigate its possible causal role.

Immortality, senescence, and eEF1A

The lifespan of animals and their constituent cells is genetically defined. When normal mammalian cells stop dividing in culture at their Hayflick limit [29], they are said to be senescent. These nondividing cells do not die and continue to have active, albeit slow, metabolic functions. In this respect, senescence can be thought of as the opposite of malignancy.

Part of the aging and senescent process is an overall decrease in the rate of protein synthesis. A decrease in steady-state eEF1A mRNA levels and a decrease in its catalytic activity is associated with senescence of nonimmortal human fibroblasts [70]. When the rate of protein elongation was compared between tissues of young (3–5 months of age) and old (23–27 months of age) C57Bl/6 mice, it was found that elongation rates were 60–80% lower in the brain, liver, kidney, and muscle of old mice than those of young animals [71]. Similarly, eEF1A protein and mRNA abundance falls in step with decreases in protein synthesis during the aging of the *Drosophila melanogaster* fruit-fly [72, 73]. This has led to the idea that decreases in overall eEF1A abundance and activity is one of the factors causing aging and senescence. This idea gained great credence when *Drosophila* that were transgenic for eEF1A under the control of a highly active heat shock promoter lived substantially longer than controls [74, 75]. However, this finding is a matter of some controversy [76]. It is not clear, moreover, whether increased eEF1A1 or eEF1A2 expression has any capacity to inhibit senescence or aging in mammalian cells. Nonetheless, the possibility that changes in eEF1A levels or activity is causally involved in aging is an idea that merits further investigation.

eEF1A and cell proliferation

When cells grown in tissue culture are deprived of growth factors they cease to divide. This quiescence is reversible and when appropriate growth factors are added to the media, the cycle of DNA synthesis and mitosis resumes. As with senescence, the quiescence of human fibroblasts involves a decrease in eEF1A enzymatic activity and its protein levels [77]. Elongation rates, for example, drop about 40% in the absence of serum [77].

This is a reasonable response to growth factor deprivation since a nondividing cell has a much reduced need for protein synthesis. When growth factors are added to the medium, eEF1A levels and corresponding elongation rates rise quickly [77]. These observations suggest that eEF1A activity is coordinated with cell replication: its activity is high when cells are dividing and low when cells do not. It is tempting to speculate that the modulation of eEF1A activity may be one mechanism by which eukaryotic cells actively control proliferation. This idea is consistent with observations that one of the events rapidly activated by growth promoters is eEF1A activation.

The hormone insulin has long been known to stimulate both cell cycle progression and protein synthesis [78, 79]. Activation of protein synthesis activation occurs within 8 minutes of insulin stimulation and protein elongation rates typically double during this time [80, 81]. This rapidity suggests that the activation of protein elongation is not a simple consequence of insulin-stimulated cell proliferation but might rather have an important regulatory function. Insulin stimulation is associated with activation of several protein kinases, among them, multipotential S6 kinase [81]. Multipotential S6 kinase can phosphorylate eEF1A on, as yet unidentified, residues [81]. This phosphorylation increases eEF1A activity two- to threefold in in vitro assays, although the mechanism by which phosphorylation does this is unclear [81, 82].

As in the case of insulin, phorbol esters are also potent mitogens and protein elongation activators. Phorbol 12-myristate 13-acetate (PMA) induced cell proliferation is associated with doubling of protein elongation rate and activation of the protein kinase C (PKC) family [83]. PKC isoform PKCγ can phosphorylate purified eEF1A in vitro on threonine 431 [83, 84] and increases GDP for GTP exchange on eEF1A, possibly by stimulation of eEF1Bα activity [85]. PKCγ phosphorylates all the eEF1 subunits, thus stimulating elongation activity.

Why is it necessary to regulate the rate of elongation at all? It is well known that protein initiation rates are subject to multiple levels of regulation, and many investigators have postulated that protein initiation is the limiting factor in overall protein translation rates [25, 26]. However, increases in the rate of protein elongation must occur in step with any increased initiation rates to prevent a backlog of ribosomes crowding around the initiation AUG. Since increases in elongation rate have been linked with increasing translational errors [86], it would be advantageous for a cell to keep elongation rates at the lowest rate consistent with initiation. Thus a need to regulate elongation upon cellular activation is likely to exist. In addition, regulating elongation rates allows for a more rapid response to cell growth conditions, since protein levels can be controlled from preexisting ribosome/mRNA complexes rather than requiring the initiation-dependent assembly of these complexes.

Over and above the teleological argument for the advantages of regulating elongation rates, it is evident from the above work with PMA and insulin that elongation rates and the proteins involved indeed are subject to regulatory control. The key issue is whether elongation rates and elongation factors have *causal* roles in regulating the cell cycle. The rapid activation of elongation upon growth factor stimulation argues for a causal role, although this has yet to be definitely demonstrated. The activation of protein elongation and eEF1A phosphorylation following insulin and PMA stimulation, however, argues that the protein elongation machinery is a target for multiple growth promoters. One possibility is that the growth-enhancing potential of known oncogenes such as Ras and Myc somehow involve activation of the protein elongation network. It is well known that Myc is capable of inducing an increase in cell size by positively regulating protein synthesis, possibly by the upregulation of eIF4E [87]. It is not unreasonable then to speculate that a similar mechanism exists for the activation of certain elongation factors.

Other elongation factors and cancer

If eEF1A proteins truly are oncogenes, other elongation factors could behave similarly. Since eEF1A activity is dependent on its GTP loading, eEF1A exchange factors could be oncogenic through their ability to increase the amount of GTP-loaded eEF1A. The best evidence for this idea stems from a study of cadmium-mediated transformation of mouse fibroblasts. In this study, Joseph and collaborators [88] used differential display to identify genes that were present in high levels in cadmium-transformed BALB/c-3T3 fibroblasts relative to non-transformed cells. One of the genes that they identified was eEF1Bβ (eEF1δ) and transfection of eEF1Bβ was sufficient to transform 3T3 fibroblasts and make them tumorigenic in nude mice. It is not yet known whether eEF1Bα, the other eEF1A GTP exchange factor, is similarly transforming, nor is it known whether eEF1Bα or eEF1Bβ is mutated or overexpressed in any human cancer or whether, as with *EEF1A2*, their respective genes are amplified.

More information is known about eEF1Bγ in human cancer. Increased levels of eEF1Bγ mRNA, as detected by northern blotting is observed in hepatocellular carcinoma [89], almost three-fourths of gastric carcinomas [90], 15% of esophageal carcinomas [91], and over one-half of colorectal adenoma [43] compared to normal controls. While eEF1Bγ serves to couple eEF1Bα and eEF1Bβ to eEF1A and therefore could activate eEF1A by enhancing eEF1A GTP exchange, no data exist yet as to whether eEF1Bγ can transform cells. Taken together, however, the balance of evidence suggests that eEF1 components, in addition to eEF1A1 and eEF1A2, could in fact be oncogenes with important roles in human cancer.

Nontranslation functions of eEF1A proteins

eEF1A proteins, in addition to their role in translation, are thought to be involved in cytoskeletal regulation. The cytoskeleton of a eukaryotic cell is composed of long polymers of actin proteins called filaments and tubulin polymers termed microtubules. These structures provide structural integrity to a eukaryotic cell and regulate cell motility and endocytosis. eEF1A proteins from several species and genera associate with the cellular actin network and can bind actin filaments in vitro [92, 93, 94, 95, 96, 97]. The association of eEF1A with actin filaments either inhibits actin depolymerization or promotes actin monomer assembly into filament polymers at higher eEF1A concentrations [98]. This has the net effect of increasing the length of actin-containing filaments. eEF1A can also promote the bundling of actin fibers into large gel-like structures [99]. Actin also affects eEF1A function and actin-bound eEF1A interacts poorly with aminoacylated tRNA [100]. This suggests that eEF1A associated with the cytoskeleton is not competent to facilitate translation and it is possible that actin-linked eEF1A provides a readily activated pool of elongation factors whose release from the cytoskeleton rapidly increases protein translation. This intriguing idea remains unverified.

It has also been reported that microinjection of human eEF1A1 protein into cells depolymerizes α-tubulin microtubules [101]. However, another report states that eEF1A stabilizes microtubules [102]. Over and above the role of eEF1A in controlling microtubule length, the full physiological significance of eEF1A association with the actin and tubulin cytoskeleton is unclear. It has been suggested that eEF1A couples cytoskeletal function to localized protein translation and vice versa, but this idea has yet to be experimentally tested.

Elongation factors and apoptosis

Our laboratory first became interested in eEF1A2 because of the Wasted mouse (*wst*/*wst*), first described by Shultz and collaborators [103] at the Jackson laboratories in 1982. Wasted is a spontaneous recessive mutation in HRS/J mice that leads to immunodeficiency, neural abnormalities, and progressive muscular wasting [103, 104]. Cells derived from Wasted mice also show higher sensitivity to DNA damage-induced cell death and apoptosis than their control littermates [105, 106, 107]. These observations initially led to the idea that Wasted was a model for the human disease ataxia telangiectasia (AT) because it shares with the disease the phenotypes of DNA-damage sensitivity and neurological defects [103]. However, ataxia telangiectasia is known to be the result of a mutation of the ATM kinase [108] while the Wasted phenotype results from a deletion of the promoter and first exon of the unrelated mouse *Eef1A2* gene, the human *EEF1A2* homologue [109].

Before the Wasted defect was cloned, we observed that Wasted mice have increased apoptosis in their thymic and lymphoid organs [107]. This elevated apoptosis in the absence of eEF1A2 suggested that eEF1A2 functions to inhibit apoptosis. Consistent with this idea, a recent report from Talapatra and colleagues [110] identified eEF1A1 in a screen for genes that would protect against the apoptosis caused by interleukin 3 withdrawal. Another report found that rat eEF1A2 expression protects muscle cells from caspase-3 induced apoptosis [111]. Taken together, these findings strongly imply that eEF1A1 and eEF1A2 both inhibit apoptosis. Interestingly, a recent report suggests that the acquisition of resistance to the anticancer agent cisplatin in a cell line derived from a human head and neck cancer is associated with increased eEF1A1 expression [67]. Given the high expression of eEF1A1 and eEF1A2 in some human cancers it is tempting to speculate that increased eEF1A expression contributes to resistance to anticancer therapy and reduces survival probability. It is also intriguing to think that the ability of both eEF1A1 and eEF1A2 to enhance cell growth and tumorigenesis could be related to their ability to inhibit apoptosis.

How does it work?

How might the ectopic expression of an elongation factor enhance cell growth and inhibit apoptosis? Presumably it is the ability of eEF1A to activate translation that is responsible for its ability to regulate cell growth and not some unknown cryptic function. Thus there are at least two possible mechanisms: either eEF1A specifically upregulates the production of proteins that activate cell growth, or increased eEF1A expression causes an overall increase in protein translation, and it is this bulk protein production, without a specific target, that causes an increase in cell proliferation.

eIF4E, the oncogenic protein initiation factor, is believed to promote cell growth by specifically upregulating the initiation of growth- and replication-promoting proteins [112]. Overexpression of eIF4E contributes to an increase in the translation of specific proteins due to the presence of extensive secondary structure in the 5′ UTR of their respective mRNA. An analysis of 5′ UTR sequences revealed that many known oncogenes such as Ras and the nuclear transcription factor κB have more 5′ UTR secondary structure than mRNA with no known growth- and division-promoting activity such as globins, albumins, and histones [113]. Leader sequences contained within these Ras and nuclear transcription factor κB mRNA molecules are GC-rich and are predicted to contain more secondary structure than mRNA without the GC sequences. This increased amount of secondary structure would be predicted to keep the initiation rate of these oncogenes low when there are small amounts of eIF4E present. When eIF4E protein levels increase, as might occur in malignancy, there is a specific increase in the initiation and translation of oncogene-derived mRNA because eIF4E binding is the limiting factor in their initiation. mRNA that do not have secondary structure re-

strictions are subsequently less affected by eIF4E levels because eIF4E and initiation rates are not limiting their translation.

It is hard to understand, however, how similar specificity for growth and replication promoters can be accomplished by a protein elongation factor. The coding region of an mRNA is the only place where an elongation factor interacts with a functional, initiated ribosome/mRNA complex. A protein's mRNA coding region presumably lacks any information other than that for the polypeptide's primary amino acid sequence. Thus there is no elongation factor analogue to the 5′ UTR secondary structure sequences for initiation factors. It therefore seems unlikely that those proteins with oncogenic potential could carry some kind of specific information or secondary structure in their coding region that would make them specific candidates for elongation activation in the presence of high amounts of eEF1A. The answer to this issue will be the identification of those proteins whose abundance increases upon eEF1A overexpression. Recent advances in proteomic sampling of individual cells could provide some insight to this idea.

If specific protein targets are not the explanation for eEF1A's ability to enhance proliferation, it is possible that eEF1A overexpression promotes cell growth and replication by contributing to an overall increase in protein translation. An increase in overall protein synthesis may enhance cell replication because cell division requires sufficient protein production to fulfill the metabolic and size requirements of two new daughter cells [114]. Increasing the abundance of an elongation factor may decrease the total time required to translate the total mass of proteins necessary for cell division. If this is the case, it would be expected that anything that increases protein translation rates is oncogenic. The reverse is certainly true, and inhibitors of protein translation are universally and highly toxic to cells and organisms.

The future

Protein translation factors and other so-called housekeepers have important roles in controlling cell growth, apoptosis, and cancer. The important role for translation in human cancer implies that members of the protein translation network, specifically the initiation and elongation complexes, are likely to be targets for anticancer therapy. Indeed clinical trails of rapamycin, an inhibitor of eIF4E-dependent initiation are now beginning [115]. It might be argued that because all cells require protein synthesis, inhibitors of translation would have substantial cytotoxic effects on normal tissues. However, it is possible that rapidly growing tumor tissue is more sensitive to small decreases in protein synthesis than normal tissue. A similar relationship has been exploited in the use of DNA-damaging agents such as cancer chemotherapeutics.

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