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Minireview

Regulation of protein synthesis at the elongation stage

New insights into the control of gene expression in eukaryotes

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There are many reports which demonstrate that the rate of protein biosynthesis at the elongation stage is actively regulated in eukaryotic cells. Possible physiological roles for this type of regulation are: the coordination of translation of mRNA with different initiation rate constants; regulation of transition between different physiological states of a cell, such as transition between stages of the cell cycle; and in general, any situation where the maintenance of a particular physiological state is dependent on continuous protein synthesis. A number of covalent modifications of elongation factors offer potential mechanisms for such regulation. Among the various modifications of elongation factors, phosphorylation of eEF-2 by the specific Ca^{2+}/calmodulin-dependent eEF-2 kinase is the best studied and perhaps the most important mechanism for regulation of elongation rate. Since this phosphorylation is strictly Ca^{2+}-dependent, and makes eEF-2 inactive in translation, this mechanism could explain how changes in the intracellular free Ca^{2+} concentration may regulate elongation rate. We also discuss some recent findings concerning elongation factors, such as the discovery of developmental stage-specific elongation factors and the regulated binding of eEF-1α to cytoskeletal elements. Together, these observations underline the importance of the elongation stage of translation in the regulation of the cellular processes essential for normal cell life.

Regulation of translation; Elongation factor; Protein phosphorylation; Ca^{2+}/calmodulin-dependent protein kinase

1. INTRODUCTION

Translation consists of three principal stages: initiation, elongation and termination. Most studies concerning the regulation of protein synthesis in eukaryotes are about regulation at the initiation stage. However, there is a significant amount of experimental data offering clear evidence for the existence of regulatory mechanisms at the elongation stage. Moreover, recent reports demonstrate the importance of this regulation in the control of various physiological processes, such as those governing cell proliferation and differentiation. In this review we summarize these results and discuss their possible implications.

2. EVIDENCE FOR THE REGULATION OF THE ELONGATION RATE

The rate of polypeptide chain elongation on total mRNA or on specific cellular mRNA was reported to vary depending on the stimulus, e.g. hormone treatment, viral infection, egg fertilization. In the following paragraphs we describe several examples where such changes in elongation rate were clearly demonstrated.

Addition of serum to HeLa cells resulted in a two- to three-fold increase in the overall elongation rate [1]. A significant change in the overall elongation rate was reported for human placental explants during normal placental development and in diabetic pregnancies [2]. The injection of estradiol into cockerels induced inhibition of the overall elongation rate in liver cells from an average value of seven amino acids per second per ribosome, to five amino acids per second after one day, three amino acids per second after two days and two amino acids per second after three days [3]. Intraperitoneal glucagon administration produced temporary inhibition of the elongation rate in rat liver [4]. The infection of mouse L cells with encephalomyocarditis virus led to a drastic slowing of polypeptide elongation on host mRNA, from 9–10 amino acids per second per ribosome down to two amino acids per second within five hours after infection [5]. The fertilization of sea urchin eggs was accompanied by a two-fold increase of the overall elongation rate [6,7]. During yeast-to-hyphae morphogenesis in the fungus *Mucor*

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racemosus a four-fold rise of the elongation rate was observed [8].

There are also examples where the elongation rate on a specific mRNA is significantly changed, while a relatively small effect is observed in the overall elongation rate. In the cockerel liver, one day after estradiol injection, the elongation rate for vitellogenin was reported to be about nine amino acids per second per ribosome. That for serum albumin was just two amino acids per second, and thus, reduced compared with the elongation rate of average cellular polypeptides which was about five amino acids per second [9]. An exquisitely selective effect was observed in cultured hepatoma cells: their exposure to dibutyryl cyclic AMP resulted in no change in the elongation rate of the total protein (approximately two amino acids per second per ribosome) whereas the elongation rate for tyrosine aminotransferase was increased to ten amino acids per second [10]. In rat liver, the elongation rate for ornithine aminotransferase was shown to be stimulated by the administration of a diet high in protein, in contrast to that for total protein which was somewhat reduced [11].

The examples mentioned above, demonstrate that changes in the elongation rate are indeed observed under different physiological situations. However, the identity of second messenger systems involved in the transduction of the external signal to the translational machinery, as well as which components of the translation apparatus are affected, remained undefined. These problems, and the potential physiological role for the observed changes in the elongation rate, are addressed in the following sections.

3. COVALENT MODIFICATION OF ELONGATION FACTORS COULD BE RESPONSIBLE FOR THE REGULATION OF THE ELONGATION RATE

In theory, the elongation rate can be regulated through modification or alteration of the concentration of any component participating in the elongation cycle, such as ribosomes, aminoacyl-tRNA synthetases, tRNA, elongation factors or even GTP. In reality, there is more evidence in favor of elongation factors as primary targets for regulation of the elongation rate. There are several examples where a change in the overall rate of protein synthesis has been demonstrated to correlate with elongation factor activity. This was the case in toad-fish liver during cold acclimation [12], in cultured mammalian cells depending on the presence of serum [13,14], in rat liver after thyroidectomy [15,16], in sea urchin eggs as a result of fertilization [17], in rat spleen during the immune response [18], as well as during ageing in rat liver [19], in *Drosophila* [20], and in human cell lines [21]. The nature of the modification responsible for the alteration of elongation factor activity in these cases remains unknown. However, the existence of several covalent modifications of both elongation factors has been reported (Table I).

Elongation factor-1 (eEF-1) is composed of three subunits: α (50 kDa), β (32 kDa) and γ (48 kDa). All three subunits are subject to covalent modifications. eEF-1α can be methylated [22–26], phosphorylated [27] and modified with glycerylphosphoryl-ethanolamine [26,28,29]. The role of the latter two modifications is completely unclear. Concerning methylation of eEF-1α in the fungus *Mucor racemosus*, it was found that the observed increase of methylation correlated with the elevation in the overall elongation rate during the yeast-to-hyphae transition. The reduction of methylation correlated with the decrease in eEF-1 activity during spore germination [25]. In contrast, purified hypo- or hypermethylated eEF-1α were equally active in partial reactions of the elongation cycle in vitro [30].

Phosphorylation of eEF-1β was reported in *Artemia* [31] and wheat embryos [32] presumably due to the presence of an intrinsic protein kinase in the preparations of eEF-1. The effect of eEF-1β phosphorylation

Table I

Covalent modifications of elongation factors

Elongation factor	Type of modification	Site	References
eEF-1α	methylation	Lys-55, Lys-165 (dimethyllysine) Lys-36, Lys-79, Lys-318 (trimethyllysine)	[22–26]
	phosphorylation	?	[27]
	attachment of glyceryl phosphorylethanolamine	Glu-301, Glu-374	[26,28,29]
eEF-1β	phosphorylation	Ser-89	[31]
eEF-1γ	phosphorylation	?	[33,59]
eEF2	formation of diphtamide	His-714	[34]
	phosphorylation	Thr-53, Thr-56, Thr-58	[39,40]
		Ser? Tyr?	[72]

on eEF-1 activity is unclear: both stimulation [32] and inhibition [31] were reported in vitro.

The most intriguing modification is the phosphorylation of eEF-1γ catalysed by maturation promoting factor ($p34^{cdc2}$ kinase) [33,59]. Since $p34^{cdc2}$ protein kinase has now been shown to be a universal regulator of mitosis and cell cycle progression in eukaryotes, this finding suggests a possibility of the involvement of eEF-1 in the regulation of cell cycle traverse. It is difficult to speculate on this since the function of eEF-1γ is as yet unknown.

There are also several covalent modifications of eEF-2. The best described being the modification of His-714 into diphthamide (cf. [34] for review). Diphthamide is a trivial name for 2-[3-carboxyamido-3-(trimethyl-ammonio)propyl]histidine and at least five enzymes participate in this modification. eEF-2 can be ADP-ribosylated at the diphthamide residue by diphtheria and *Pseudomonas* toxins, thereby rendering eEF-2 inactive in translation (reviewed in [34]). The physiological role of diphthamide is unknown. The most recently described modification of eEF-2 is phosphorylation on threonine residues (reviewed in [35]) and on serine and tyrosine residues [72]. The phosphorylation of eEF-2 on threonine requires special attention because it has been clearly shown to play an important role in the regulation of protein synthesis.

4. Ca^{2+}/CALMODULIN-DEPENDENT PHOSPHORYLATION OF eEF-2: THE LINK BETWEEN THE EXTERNAL SIGNAL AND PROTEIN SYNTHESIS

Phosphorylation of eEF-2 was recently found to be the most prominent and rapid phosphorylation in mammalian cell extracts [36] (reviewed in [35]). It is catalyzed by a specific Ca^{2+}/calmodulin-dependent protein kinase called Ca^{2+}/calmodulin-dependent protein kinase III [37] or eEF-2 kinase [38]. This kinase phosphorylates threonine residues in the N-terminal part of eEF-2 [39,40].

In contrast to many other phosphorylations of proteins involved in translation, phosphorylation of eEF-2 has a strong effect on its activity. Phosphorylated eEF-2 is inactive in a poly(U)-directed cell-free translation system [41–43] because it is unable to catalyze ribosome translocation [44]. Phosphorylation of eEF-2 can also affect translation of natural mRNA, as shown in a cell-free system based on the rabbit reticulocyte lysate. For example, the cAMP-dependent activation of globin mRNA translation in a rabbit reticulocyte cell-free translation system was shown to correlate with dephosphorylation of eEF-2 [45]. Inhibition of globin mRNA translation by the phosphatase inhibitor okadaic acid, was found to correlate with the subsequent increase in the amount of the phosphorylated form of eEF-2 [46]. It was also shown that phosphorylated eEF-2 was unable to restore translation of globin mRNA after addition to the eEF-2-depleted rabbit reticulocyte cell-free translation system [47] whereas addition of the unphosphorylated form could.

eEF-2 becomes intensely phosphorylated in vivo in response to elevation of the cytoplasmic Ca^{2+} concentration. This was shown in the case of treatment of quiescent human fibroblasts with mitogens [48] or treatment of human umbilical vein endothelial cells with thrombin or histamine [49]. In both cases, the increase in eEF-2 phosphorylation was transient and continued for just a few minutes [48,49]. Since almost 100% of the total cellular eEF-2 was phosphorylated under these conditions [49], one would expect that this would result in a transient shut-off of protein synthesis.

Phosphorylation of eEF-2 may also be a mechanism by which protein synthesis could be inhibited during mitosis. It is well known, that protein synthesis is decreased during mitosis, but the molecular mechanism of this is unknown. During mitosis, there is an increase in Ca^{2+} concentration (reviewed in [50]). When changes in the level of phosphorylated eEF-2 were investigated throughout the cell cycle of transformed human amnion cells, a dramatic increase in the level of phosphorylated eEF-2 was found specifically in mitosis [51]. Together, these observations support the postulate that the regulation of the phosphorylation state of eEF-2, represents a mechanism via which cytoplasmic free Ca^{2+} concentration could modulate the rate of protein synthesis.

5. CHANGES IN THE ELONGATION RATE CAN AFFECT GENE EXPRESSION IN DIFFERENT WAYS

In previous sections we presented several examples of changes in the rate of polypeptide chain elongation in vivo, as well as several post-translational modifications of elongation factors which could be responsible for such changes. There are at least three possible ways via which changes in the rate of elongation can affect the steady-state level of a given protein in a cell. These possibilities are discussed below.

5.1. *Stimulation of translation of 'weak' mRNA through inhibition of the overall elongation rate*

Cellular mRNAs have various initiation rate constants and can be classified into two categories: 'strong' (with high constant for initiation) and 'weak' (with low constant for initiation). Under the most common conditions, the rate-limiting stage in translation in vivo is initiation. Under these conditions, different mRNAs are translated according to their 'strength' rather than their concentrations. Thus, 'strong' mRNAs have an advantage over 'weak' mRNA during translation. As originally pointed out by Lodish [52], the inhibition of overall elongation rate has a more pronounced effect on

the translation of 'strong' mRNA than of 'weak' mRNA. This is due to the fact that when the elongation stage becomes rate-limiting, the efficiency of translation of each mRNA is determined by the relative amount of that mRNA. Thach and his colleagues [53-58] further developed this model demonstrating both theoretically and experimentally that inhibition of overall elongation rate could actually *stimulate* translation of 'weak' mRNA. This is due to the existence of specific initiation factor(s) which can be limiting in translation and have different affinities for different mRNAs. Reduction of the elongation rate, makes elongation rate-limiting only on 'strong' mRNAs. Thus, translation of 'weak' mRNA is stimulated by mobilization of the limiting initiation factor from 'strong' mRNA. For example, when protein synthesis in mouse fibroblasts was partially inhibited by cycloheximide, the synthesis of several polypeptides was stimulated [57,58].

5.2. *Effect of elongation rate changes on mRNA stability*

There are numerous examples where mRNA stability is dependent on ongoing protein synthesis. In some cases, inhibition of elongation results in stabilization of mRNA, probably by simple physical protection of mRNA by the stalled ribosomes. In other cases, a quite opposite effect is observed: inhibition of elongation results in the stimulation of mRNA degradation. The behaviour of α-tubulin mRNA illustrates both situations: a 95% inhibition of protein synthesis in mouse fibroblasts with the elongation inhibitor cycloheximide, resulted in enhanced degradation of α-tubulin mRNA, while a 99% inhibition of protein synthesis stabilized the α-tubulin mRNA [60].

5.3. *Inhibition of the elongation rate and elimination of short-lived proteins*

Recently, it became evident that there are proteins in the cell that have extremely short half-lives [61]. For example the α2 repressor in yeast has a half-life of ~5 min [62]. The steady-state levels of such proteins could be regulated by changes in the overall elongation rate. If cells contain a repressor of transcription or translation having a half-life of several minutes, it is very easy to imagine how a transient block of elongation could result in elimination of such a repressor, thereby resulting in an apparent induction of transcription or translation of the gene or mRNA repressed by this short-lived protein.

6. POSSIBLE ROLE FOR THE REGULATION OF ELONGATION RATE

Two main roles for regulation of protein synthesis at the elongation stage can be proposed. Since it is possible to affect translation of 'weak' and 'strong' mRNA differentially (see above) by decreasing the overall rate of elongation, regulation at the elongation stage could serve to coordinate translation of selected mRNA. This could be important in the case of activation of certain cells and tissues by hormones or in the case of fertilization, when a large amount of new mRNA is mobilized for translation. If these new mRNAs have very high rate constants for initiation, the sudden demand for their translation could limit the availability of initiation factors and thus favor the synthesis of new proteins at the expense of that of constitutive proteins. For example, estradiol injection into cockerels was demonstrated to cause a rapid increase in the amount of specific mRNA in the liver, together with a decrease in the overall elongation rate [3]. The most evident explanation for the role of this decrease in elongation rate is that it could minimize translational discrimination and hence, prevent interruption of synthesis of constitutive proteins when many egg-related proteins begin to be synthesized.

Another process in which regulation at the elongation stage could be important is transition of a cell from one physiological state, dependent on continuous protein synthesis, into another. For example, maintenance of the quiescent state in some types of cells is dependent on continuous protein synthesis as illustrated by the fact that transient inhibition of elongation results in transition of the cells to a proliferative state [63]. Similarly, metaphase arrest during meiotic maturation of oocytes from different vertebrates and invertebrates is dependent on continuous protein synthesis and can be overcome by inhibition of elongation rate. This was shown in the case of metaphase II arrest in mouse oocytes [64] and metaphase I arrest in oocytes of the annelid *Chaetopterus* [65], the mollusc *Patella* [66] and the mollusc *Mytilus* [67].

Usually, the fact that protein synthesis inhibition can induce transition of a cell into a new physiological state, is explained in the literature by the presence of short-lived proteins which maintain the cell in the initial state. It should be mentioned, however, that an additional explanation can be applicable in some cases. As we already discussed, inhibition of elongation could not only result in elimination of short-lived proteins, but it could also result in a change in the stability of, or induce the translation of, specific physiologically critical mRNAs.

7. RECENT FINDINGS

7.1. *Developmental stage-specific elongation factors*

Recently, it was found that in *Drosophila*, there are two genes for eEF-1α. One, called F1, is expressed in all cells at every stage of development, while another, F2, is expressed mainly during the pupal stage [68].

In *Xenopus*, three genes for eEF-1α were identified [69]. One gene is expressed in somatic cells while

another is expressed in oocytes [69,70]. The third gene encodes a form of eEF-1α called thesaurin-a. Thesaurin-a is a component of a 42 S ribonucleoprotein particle, consisting mainly of aminoacyl-tRNA, that is present at early stages of oogenesis [69,71]. The role of these forms of eEF-1α is unknown. The situation becomes even more puzzling in the light of results of a recent publication [71] where it was found, that while thesaurin-a is distributed uniformly throughout the cytoplasm of pre-vitellogenic oocytes, the oocyte-specific form of eEF-1α is concentrated in, or near, the Balbiani body which is the large mitochondria of small oocytes.

7.2. *Binding of eEF-1 to actin and other macromolecules*

Binding of eEF-1 to different cellular proteins has been reported recently. Complexes between eEF-1 and valyl-tRNA synthetase have been isolated from different mammalian tissues [73–75]. It has been found that the entire cellular complement of valyl-tRNA synthetase is present in a complex with eEF-1 [73–75]. It has also been demonstrated, that eEF-1α can bind to the components of the sea urchin mitotic apparatus and that it represents the major GTP-binding protein therein [76,77].

Another recent discovery is the observation that eEF-1α is the major actin-binding protein from *Dictyostelium* [78]. It was observed, that eEF-1α can bind both G-actin and F-actin [78]. However, while F-actin has no effect on the eEF-1α activity in a cell-free system, binding to G-actin completely inhibits eEF-1α activity [79]. This observation raises an intriguing possibility that binding of eEF-1α to actin can be a sensory mechanism which regulates the rate of protein synthesis in response to cytoskeletal rearrangements.

7.3. *Prolongation of life by eEF-1α*

The most exciting recent finding concerning elongation factors is the influence of eEF-1α mRNA concentration on the lifetime of the fruit fly. It was previously reported that, during ageing in *Drosophila*, there is a selective decrease in the amount of mRNA for eEF-1α [20]. When *Drosophila* were transformed with a P-element vector containing the eEF-1α gene, the mean lifetime of transformed flies was 20–40% longer than that of wild type flies [80].

This observation, together with the others described in this review, argues strongly in favor of a paramount role for the regulation of protein synthesis at the elongation stage in the modulation of gene expression.

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