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Is there a role for eIF5A in translation?

Review Article

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Summary. The putative translation factor eIF5A is essential for cell viability and is highly conserved from archaebacteria to mammals. This factor is the only cellular protein that undergoes an essential posttranslational modification dependent on the polyamine spermidine, called hypusination. This review focuses on the functional characterization of eIF5A. Although this protein was originally identified as a translation initiation factor, subsequent studies did not support a role for eIF5A in general translation initiation. eIF5A has also been implicated in nuclear export of HIV-1 Rev and mRNA decay, but these findings are controversial in the literature and may reflect secondary effects of eIF-5A function. Next, the involvement of eIF5A and hypusination in the control of the cell cycle and proliferation in various organisms is reviewed. Finally, recent evidence in favor of reconsidering the role of eIF5A as a translation factor is discussed. Future studies may reveal the specific mechanism by which eIF5A affects protein synthesis.

Keywords: eIF5A – Hypusine – Ribosome – Translation – Protein synthesis

Abbreviations: EF-P, elongation factor P protein; eIF5A, eukaryotic translation initiation factor 5A; GC7, N1-guanyl-1,7-diaminoheptane; HIV, human immunodeficiency virus; Met-tRNA$_i$, methyonyl initiator transfer RNA; RNAi, RNA interference; SELEX, systematic evolution of ligands by exponential enrichment

1. eIF5A – an introduction

The eukaryotic translation initiation factor 5A (eIF5A) is a small (∼17 kDa) acidic protein, highly conserved and essential in all organisms from archaebacteria to mammals, but not in eubacteria (Schnier et al., 1991; Chen and Liu, 1997; Park et al., 1997). Despite being highly conserved and essential, the critical cellular role of eIF5A is not known.

eIF5A is the only protein known to contain the unusual amino acid residue hypusine (Cooper et al., 1983). Hypusine (hydroxyputrescine-lysine) is essential for eIF5A function and is formed in a posttranslational reaction, dependent on the polyamine spermidine, in which a specific residue of lysine in eIF5A is modified. This modification, called hypusination, occurs in two steps: first, the enzyme deoxyhypusine synthase transfers a 4-aminobutyl moiety from spermidine to the specific lysine residue in eIF5A, which is then hydroxylated by the enzyme deoxyhypusine hydroxylase (Park, 2006). Inhibition of either deoxyhypusine synthesis or deoxyhypusine hydroxylation affects cell growth in mammalian cells (Park et al., 1993) and the conservative mutation K51R in eIF5A, which blocks hypusination, cannot complement yeast eIF5A knockout cells (Schnier et al., 1991), demonstrating that hypusine formation is essential for eIF5A function. Curiously, although both steps of hypusination are essential in mammals, only deoxyhypusine synthesis is essential in the budding yeast *Saccharomyces cerevisiae*, while in the fission yeast *Schizosaccharomyces pombe* deoxyhypusine hydroxylation appears to be essential only at elevated temperatures (Park, 2006). For more information on this essential eIF5A modification, see the accompanying review article in this issue.

Multiple alignment of amino acid sequences of eIF5A demonstrates a high degree of conservation among different species, specially in the residues surrounding the lysine that is specifically modified to hypusine (Fig. 1). Analysing eIF5A sequences from different organisms, the N-terminal is shorter and more conserved among archaebacteria than in eukaryotes, while the C-terminal sequences are the least conserved among eIF5A proteins in general (Fig. 1). Also, eIF5A proteins from yeast and human are

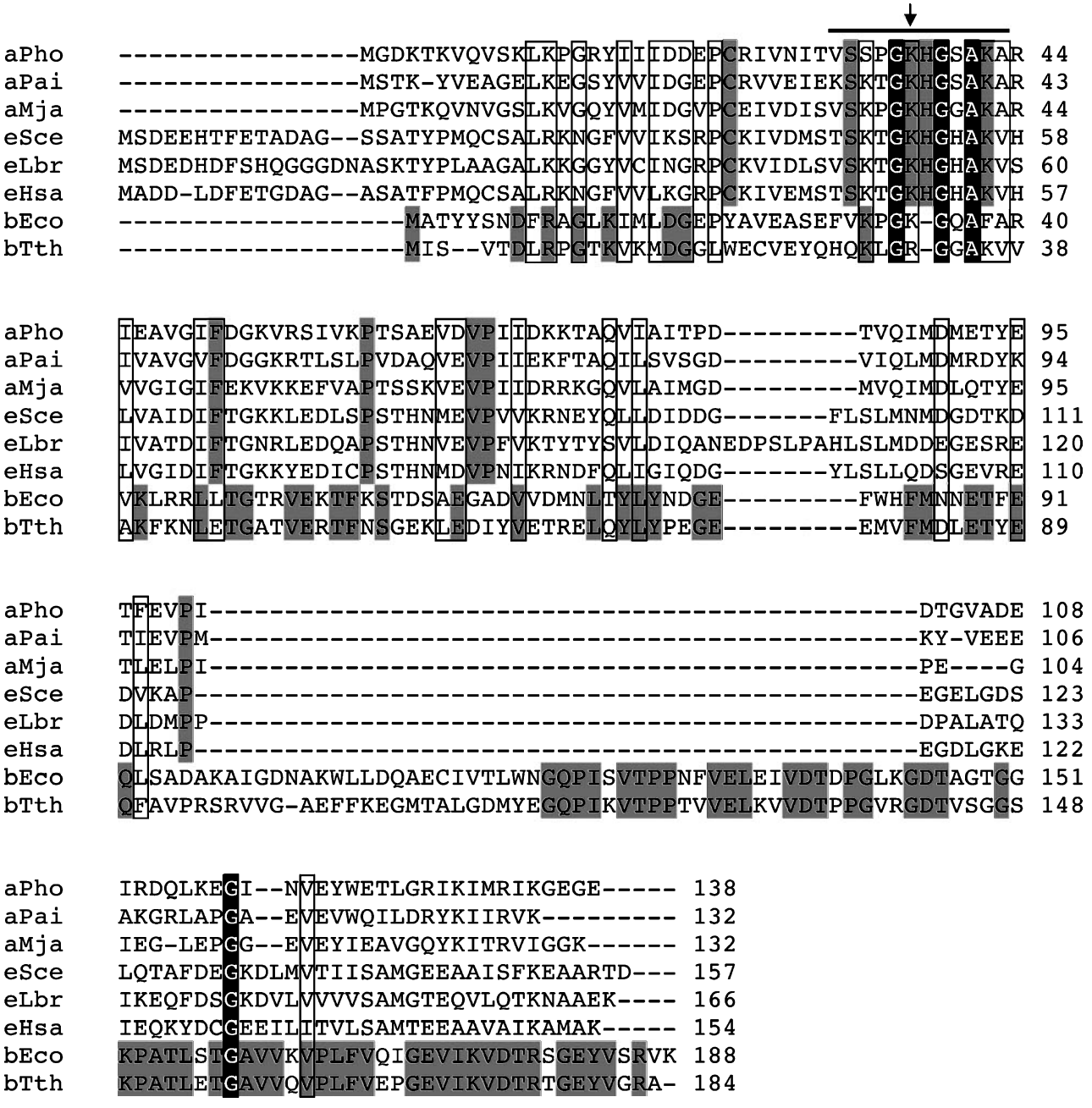

Fig. 1. Multiple alignment of the amino acid sequences of eIF5A and EF-P. aPho, aPai, aMja, eSce, eLbr and eHsa are the eIF5A proteins from *Pyrococcus horikoshii*, *Pyrobaculum aerophilum*, *Methanococcus jannaschii*, *Saccharomyces cerevisiae*, *Leishmania braziliensis* and *Homo sapiens*, respectively. bEco and bTth are the bacterial EF-P proteins from *Escherichia coli* and *Thermus thermophilus*, respectively. The amino acid residues conserved only among eIF5A or EF-P proteins are highlighted in grey. The amino acid residues completely conserved throughout eIF5A and EF-P are shown with white letters highlighted in black, and those well conserved in eIF5A and EF-P are shown in empty boxes. The hypusinated lysine residue in eIF5A proteins is marked with an arrow

64% identical and this level of identity is maintained among eukaryotes, whereas yeast eIF5A shares much less (not more than 35% identity) of its sequence with archaebacteria. This sequence identity is reflected in protein function, since human eIF5A can substitute for yeast eIF5A, but archaebacterial eIF5A cannot (Magdolen et al., 1994).

Two eIF5A genes have been identified in *S. cerevisiae* (*TIF51A* and *TIF51B*) and in *Homo sapiens* (*EIF5A1* and *EIF5A2*), and a search for eIF5A-related proteins in different databases revealed that other eukaryotic organisms have more than one eIF5A gene (Schnier et al., 1991; Jenkins et al., 2001). In yeast, *TIF51A* and *TIF51B* encode

90% identical proteins that are differentially transcribed in response to the presence of oxygen, in that *TIF51B* is expressed only in anaerobic conditions. However, these yeast genes, once transcribed, can functionally replace each other, suggesting a functional similarity between them (Schwelberger et al., 1993). Human *EIF5A1* and *EIF5A2* genes are 84% identical and differentially transcribed among tissues and some tumor cell lines (Park, 2006). However, whether there is any functional difference between these human isoforms remains to be elucidated. Interestingly, in the case of the nematode *Caenorhabditis elegans*, the eIF5A proteins encoded by the genes iff-1 and iff-2 share 85% identity, are differentially expressed and the phenotypes observed in the mutants of each of the eIF5A genes correlate functionally to spatial expression, suggesting functional divergence of these isoforms (Hanazawa et al., 2004).

The three-dimensional structure of eIF5A homologous proteins from three archaebacteria (Kim et al., 1998; Peat et al., 1998; Yao et al., 2003) and two protozoans (Protein Database) have been determined and reveal very similar features. These studies show that eIF5A is composed of two predominantly β-sheet domains. Interestingly, the most conserved residues are those flanking the hypusinated lysine residue in the extended loop in the N-terminal domain of eIF5A and this region shows a greater concentration of positive charges, which is enhanced by the hypusine modification, whereas the overall content of acidic residues is relatively high in eIF5A protein and concentrated in the C-terminal domain (Kim et al., 1998). The comparison of eIF5A domains in protein structure databases demonstrated that the N-terminal domain shows a fold related to the SH3-like barrel found in other proteins related to translation, while the C-terminal is similar to the OB-fold present in nucleic acid-binding proteins (www.rcsb.org/pdb/).

In order to determine the capacity of eIF5A to bind mRNA, the systematic evolution of ligands by exponential enrichment (SELEX) methodology was used to identify a preferential mRNA nucleotide sequence to which eIF5A binds (Xu and Chen, 2001) and subsequent work identified some possible cellular target mRNAs (Xu et al., 2004). However, the biological significance of this eIF5A mRNA-binding capacity has not yet been clarified.

2. eIF5A identification as a translation initiation factor

eIF5A, formerly designated IF-M2Bα or eIF-4D (Safer, 1989), was originally purified from ribosomes of reticulocytes and shown to stimulate the methionyl-puromycin synthesis, indicating a role in the formation of the first peptide bond (Kemper et al., 1976; Benne and Hershey, 1978). However, although eIF5A clearly stimulates methionyl-puromycin synthesis, using either AUG triplets or globin mRNA as templates, the absence of eIF5A from the in vitro protein synthesis reaction did not affect negatively globin synthesis (Kemper et al., 1976; Benne and Hershey, 1978). The latter result was in complete contrast to that obtained for the other translation initiation factors tested: eIF1, eIF1A, eIF3, eIF5B and eIF5 (Benne and Hershey, 1978).

A role for eIF5A during translation elongation was also considered, but no stimulation was observed on adding eIF5A to a purified protein synthesis system used to measure polyphenylalanine polymerization, containing poly-(U) RNA, 80S ribosomes and the elongation factors eEF1 and eEF2. It was therefore proposed that eIF5A might have a function specifically in the formation of the first peptide bond, but not in the elongation step of protein synthesis in general (Benne and Hershey, 1978). Interestingly, although it was not possible to detect any direct interaction between eIF5A and Met-tRNA$_i$, another experiment showed that uncharged, formylated Met-tRNA$_i$ is less dependent on eIF5A function in Met-puromycin assays, reinforcing a function for eIF5A in the formation of the first peptide bond, probably stabilizing charged Met-tRNA$_i$ binding to the peptidyl transferase center of ribosomes (Hershey et al., 1990).

However, it was shown later that depletion of eIF5A in yeast causes only a slight decrease in the overall protein synthesis rate, arguing against a role for eIF5A as a general translation initiation factor (Kang and Hershey, 1994). Similarly, a yeast eIF5A temperature-sensitive mutant, which causes instability of the eIF5A protein at the non-permissive temperature, confirmed the small effect of eIF5A function in protein synthesis (Zuk and Jacobson, 1998). Therefore, although eIF5A depletion had some effect upon translation, the extent of protein synthesis inhibition was limited (30%) compared to the effect of depletion of other translation initiation factors and did not correlate with the rapid inhibition of cell proliferation observed (Kang and Hershey, 1994). On the other hand, depletion of eIF5A in yeast caused an increase in G1-arrested cells (Kang and Hershey, 1994) and this phenotype is similar to that observed after blocking hypusination in mammalian cells, which leads to inhibition of cell proliferation by arresting the cell cycle at the G1/S boundary (Hanauske-Abel et al., 1994). Based on these results, it was hypothesized that eIF5A may be important for translating mRNAs encoding specific proteins required

for cell-cycle progression, probably a subset of mRNAs involved in S-phase onset (Kang and Hershey, 1994; Hanauske-Abel et al., 1995).

3. Involvement of eIF5A in HIV-1 Rev nucleocytoplasmic export

Shortly before the exclusion of eIF5A from the general translation scenario (Kang and Hershey, 1994), this factor was identified as the cellular partner of the HIV-1 Rev protein in UV-crosslinking experiments (Ruhl et al., 1993). Rev is a nucleocytoplasmic shuttling protein expressed early after HIV-1 infection and essential for mRNA export of full-length and partially-spliced retroviral transcripts, necessary for the HIV-1 productive cycle (Cullen, 2003). It has been demonstrated that a portion of about 10% of total cellular eIF5A is present in the nucleus, the steady-state compartment of Rev, and that eIF5A function is necessary for Rev shuttling in a *Xenopus* oocyte model (Bevec and Hauber, 1997). Subsequent studies showed that eIF5A mutants impair Rev function and that eIF5A interacts directly and physically with Rev and exportin 1/CRM1 to promote the export of Rev-mRNA complex from of the nucleus (Bevec et al., 1996; Rosorius et al., 1999). The anti-retroviral activity of deoxyhypusine hydroxylase inhibitor was also reported (Andrus et al., 1998). More recently, it was reported that blocking hypusination through deoxyhypusine synthase, using either a chemical inhibitor or RNAi, caused potent antiretroviral activity against various HIV-1 strains (Hauber et al., 2005).

However, data obtained by other groups failed to confirm a functional interaction between eIF5A and HIV-1 Rev (Henderson and Percipalle, 1997; Shi et al., 1997; Lipowsky et al., 2000; Jao and Yu Chen, 2002; Valentini et al., 2002). It was demonstrated in mammalian cells that eIF5A has a cytoplasmic localization, with a perinuclear concentration, and additional analysis confirmed its association with endoplasmic reticulum (Shi et al., 1996). This cytoplasmic localization is not changed either after HIV-1 Rev expression or following treatment of cells with RNA synthesis inhibitors, which significantly affect Rev distribution (Shi et al., 1996, 1997). In contrast, only eIF5A subcellular distribution is altered when cells are treated with protein synthesis inhibitors (Shi et al., 1997). Besides that, other studies could not identify any nuclear localization sequence in eIF5A or active nuclear import and it was proposed that the small amount of eIF5A found in the nucleus may be due to passive diffusion (Lipowsky et al., 2000; Jao and Yu Chen, 2002). Also, none of a series of treatments, including blocking of CRM1-dependent nuclear export, modified the whole-cell distribution pattern of eIF5A (Jao and Yu Chen, 2002). Importantly, different assays using recombinant or endogenous eIF5A failed to detect any physical interaction with Rev or exportin 1/CRM1 proteins (Henderson and Percipalle, 1997; Lipowsky et al., 2000).

Moreover, using the yeast model, in which Rev has been shown to function in the same way as in mammalian cells (Neville et al., 1997), Rev does not seem to be exported from the nucleus in an eIF5A-dependent pathway, but clearly depends on yeast Crm1 protein (Valentini et al., 2002). Also, eIF5A shows a cytoplasmic localization with a perinuclear concentration in yeast (Valentini et al., 2002), similar to that observed in various mammalian cells (Shi et al., 1996). This localization pattern of eIF5A, differently from what happens with Rev, is not affected when Crm1-dependent nuclear export pathway is blocked by shifting a yeast temperature-sensitive *CRM1* mutant to the restrictive temperature (Valentini et al., 2002).

Finally, although the sequence conservation of eIF5A, from archaebacteria to mammals (Fig. 1), suggests a conserved eIF5A function, no essential nucleocytoplasmic shuttling function can be proposed in archaebacteria, which argues against such a function for eIF5A as its primary and conserved cellular role.

4. Implication of eIF5A in mRNA decay

eIF5A was also isolated in a screen for yeast mutants defective in mRNA degradation (Zuk and Jacobson, 1998). In that study, it was shown that the eIF5A temperature-sensitive mutant *ts1159* stabilizes various mRNAs tested. The *ts1159* mutant produces an eIF5A protein that is unstable at the non-permissive temperature. As blocking of mRNA decay could be a result of inhibition of protein synthesis, it was demontrated that the mRNA decay defect was independent of the *ts1159* mutant translation defect. Next, confirming that the mRNA decay defect was not a secondary effect, it was shown that the accumulated transcripts in this mutant did not have a 5' cap, which supported a direct function of eIF5A in the mRNA degradation process (Zuk and Jacobson, 1998).

However, although other eIF5A temperature-sensitive mutants, which also produce unstable eIF5A at the non-permissive temperature, show an mRNA accumulation phenotype as well (Valentini et al., 2002; Schrader et al., 2006), the time of growth arrest of the mutants and eIF5A depletion do not correlate with the mRNA degradation defect, strongly suggesting that the effect of eIF5A on

mRNA decay is actually secondary (Valentini et al., 2002). As discussed later in this review, recent studies have led to reconsideration of a role for eIF5A in translation and, therefore, new efforts will be necessary to address the question of exactly how eIF5A impacts mRNA decay.

5. eIF5A and cell-cycle progression

The initial observation that hypusine synthesis is correlated with mammalian cell proliferation suggested an important role for this unusual amino acid residue in the control of the cell cycle (Park, 2006). Several authors have shown that blocking hypusination can lead to inhibition of cell proliferation in various mammalian cell types (Park et al., 1997). For instance, inhibitors of deoxyhypusine hydroxylation also cause inhibition of the G1-S transition in the cell cycle (Hanauske-Abel et al., 1994) and the deoxyhypusine synthase inhibitor GC7 blocks cell growth of both nontransformed and transformed cultured cells (Park et al., 1997).

Likewise, mature eIF5A-1 is a marker of aberrant proliferating cells in intraepithelial neoplasia of the vulva (Cracchiolo et al., 2004), while eIF5A-2 transcript is overexpressed in ovarian cancer and colorectal cancer cell lines (Park, 2006). Also, eIF5A-1 was found to be downregulated after growth inhibition of BCR-ABL-positive leukemia cells treated with Imatinib and the blocking of hypusination in these cells with either GC7 or ciclopirox showed a synergistic effect, demonstrating that mature eIF5A-1 is necessary for active proliferation of these cancer cells (Balabanov et al., 2007).

Furthermore, mutations in eIF5A genes of other eukaryotic model organisms also revealed a correlation of eIF5A function and control of cell proliferation. In *Drosophila melanogaster*, eIF5A interferes with cell-cycle control and development through a mechanism related to the cyclin B pathway (Lee et al., 2001), and in *C. elegans*, iff-1 and iff-2 mutants show slow larval growth and defects in the organization of defined organs (Hanazawa et al., 2004).

In the budding yeast, eIF5A depletion leads to accumulation of cells arrested in G1, as mentioned before (Kang and Hershey, 1994). Using the same organism, our recent findings demonstrated that the growth arrest of a temperature-sensitive mutant of eIF5A, *tif51A-1* ($eIF5A^{P83S}$), is suppressed by high-copy genes involved in cell-wall integrity and actin cytoskeleton polarization (Fig. 2). Consistently, eIF5A mutants *tif51A-1* and *tif51A-3* ($eIF5A^{C39Y,\ G118D}$) show an actin cytoskeleton defect at the non-permissive temperature, which is also partially corrected by the suppressors identified (Zanelli and Valentini, 2005). Another study, using a different eIF5A temperature-sensitive mutant (tsL102A), confirmed involvement of eIF5A in cell-cycle progression in yeast (Chatterjee et al., 2006). Interestingly, a similar defect in actin cytoskeleton organization is observed in yeast cells depleted of spermidine and spermine (Park et al., 1997). These results imply that eIF5A function is essential for polarized growth in yeast, a process necessary for the G1/S transi-

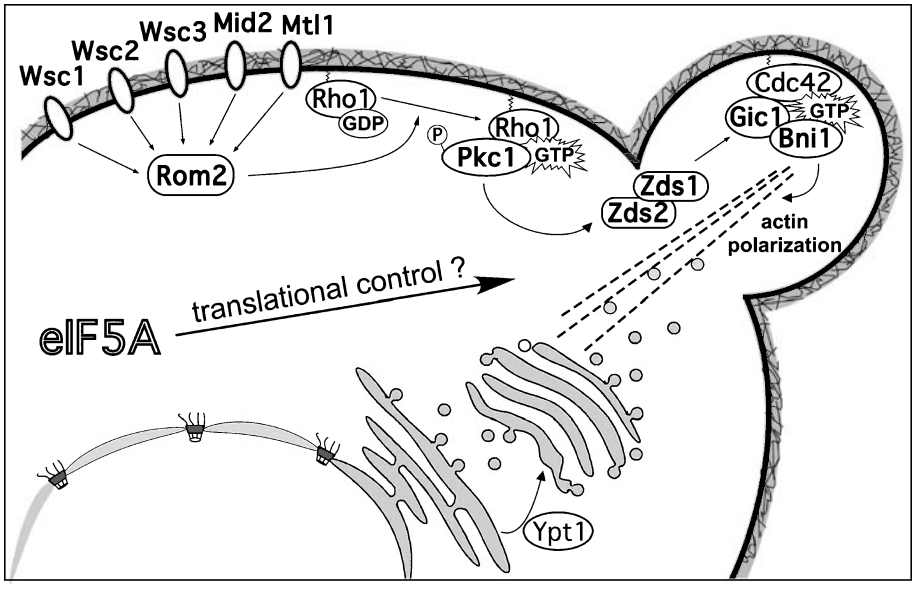

Fig. 2. Schematic of pathways comprising the eIF5A genetic interactors in yeast. High-copy suppressors of an eIF5A yeast mutant are related to cell-wall integrity and actin cytoskeleton polarization (*WSC1-3*, *MID2*, *MTL1*, *PKC1*, *ZDS1-2* and *GIC1*) and the synthetic lethal gene with eIF5A mutants is associated with vesicular trafficking and secretion (*YPT1*)

tion in this organism, and that eIF5A may mediate a large range of effects of the polyamine spermidine in the cell.

More recently, searching for synthetic lethal genes with the eIF5A mutant *tif51A-3*, a mutant of *YPT1* was identified (*ypt1*^{G80D}), and this gene encodes a Rab small GTPase, which is essential for vesicular trafficking between endoplasmic reticulum and Golgi (Frigieri et al., 2007; Fig. 2). Cytoskeletal reorganization, secretion and cell-wall deposition are all required to produce a new bud. These activities must be orchestrated, and transcriptional as well as post-transcriptional regulatory mechanisms are expected to be important for regulating the different processes involved in polarized cell growth in yeast (Pruyne and Bretscher, 2000). It would not be appropriate to propose a direct function for eIF5A in the establishment of cell polarity in *S. cerevisiae*, as eIF5A function has been conserved throughout evolution, while budding is not a mechanism ubiquitously used by eukaryotes to progress through the cell cycle. On the other hand, eIF5A was initially characterized as a translation initiation factor (Benne and Hershey, 1978) and the association of this protein with translation has recently been reconsidered, as discussed below, suggesting that eIF5A may have a role in translational control. Thus, the genetic interactions identified in yeast (Fig. 2) may reveal a connection between translation and the polarized distribution of membrane components for cell growth and division, suggesting that the proteins encoded by the genetic interactors work together in the cell to guarantee proper bud formation during G1/S transition and further studies will be necessary to clarify this hypothesis.

Summarizing, eIF5A may control the expression, at the translational level, of factors important for G1/S transition. Examples of such factors could be those involved in the polarized cytoskeleton and polarized secretion in yeast, as revealed by our genetic findings. This is in agreement with the previous hypothesis for a function of eIF5A in translation of a specific subset of mRNAs (Park et al., 1993; Kang and Hershey, 1994) and with the new evidence described in the next section, showing that eIF5A may play a role in protein synthesis. It is hoped that future studies involving additional analysis of the factors important for cell-cycle progression and the use of other strategies to identify differentially-expressed proteins in eIF5A mutants will reveal whether eIF5A affects specific gene expression.

6. New evidence to bring eIF5A back to the translation scenario

At this point, all the functions proposed for eIF5A are controversial rather than conclusive and its involvement in cell-cycle progression seems clearly to be indirect, acting most probably by regulating gene expression at the translational level.

In agreement with the hypothesis of a role for eIF5A in translation, our study in yeast, using co-purification of protein complexes with eIF5A followed by protein identification by mass spectrometry, revealed that eIF5A interacts physically with protein components of the translational machinery components. Importantly, an eIF5A mutant that does not allow hypusine formation, eIF5A^{K51R}, also does not bind efficiently to the translational machinery components identified, establishing a correlation between the essential function of eIF5A and its association with translational machinery. It is important to note that not only structural components of the ribosome were identified, but also elongation factors, suggesting that eIF5A specifically binds to actively translating ribosomes (Zanelli et al., 2006). Using a similar approach, it was also demonstrated that eIF5A binds to translating 80S ribosomes in a hypusine-dependent manner (Jao and Chen, 2006).

To address the question whether eIF5A binds specifically to actively translating ribosomes, polysome profile experiments were carried out. In these experiments, eIF5A showed co-sedimentation with translating ribosomes and this co-sedimentation was affected by translation inhibition or 80S disassembly, supporting the model in which eIF5A binds specifically to ribosomes engaged in protein synthesis. Besides, association with co-sedimenting 80S ribosomes was tested, to confirm specific interaction of eIF5A with the translational machinery. In this experiment, polysome profile fractions containing 80S ribosomes were re-sedimented on a sucrose density gradient and it was shown that eIF5A was still fractionated together with the 80S ribosomal peak, confirming its specific association. Also, in this experiment it was observed that, although most eIF5A protein remains bound to 80S ribosomes, there is a small fraction that dissociates from the ribosomes, indicating that eIF5A has only a transient interaction with ribosomes. However, the exact way in which eIF5A interacts with ribosomes remains unclear, since no direct protein ligand was found for eIF5A (Zanelli et al., 2006). Also, it is important to test whether eIF5A binds to ribosomes through protein-RNA interactions, which could explain the failure to find a protein of the translational machinery that binds directly to eIF5A.

Moreover, our study showed that yeast eIF5A temperature-sensitive mutants that lead to eIF5A protein depletion (*tif51A-1* and *tif51A-3*) have altered polysome profiles at the non-permissive temperature. However, these results were not in agreement with those observed for well-known translation initiation mutants (Asano et al., 2000), but instead greatly resembled that seen for the translation

elongation mutant *cca1-1*, which blocks tRNA maturation at the restrictive temperature (Peltz et al., 1992). Thus, these data suggest a defect in translation elongation, not in translation initiation, caused by loss-of-function eIF5A mutants.

Furthermore, eIF5A mutants are sensitive to the protein synthesis inhibitors paromomycin, sparsomycin and anysomycin (Zanelli et al., 2006), reinforcing the suggestion of a role for eIF5A in translation. Interestingly, although there is no clear homologue of eIF5A in eubacteria, the bacterial elongation factor P protein (EF-P) shows considerable sequence similarity to eIF5A, mainly in the N-terminal portion where hypusination occurs (Fig. 1; Hanawa-Suetsugu et al., 2004). In agreement with our findings, EF-P function is specifically affected by peptidyl-transferase inhibitors in vitro (Ganoza et al., 2002), analogously to the sparsomycin and anisomycin sensitivity of eIF5A mutants. Like eIF5A, EF-P is essential to cell viability, stimulates the first peptide bond formation in the methionyl-puromycin assay and binds to elongating ribosomes. Nevertheless, EF-P is essential for protein synthesis (Ganoza et al., 2002).

Interestingly, another laboratory has recently re-evaluated the impact of eIF5A on translation and several results suggested that eIF5A depletion in yeast and inhibition of hypusination in mammalian cells lead to a significant decrease in both polysomal fractions and amino acid incorporation during protein synthesis (E. Gerner, personal communication).

Finally, it is important to highlight that the eIF5A depletion experiment that ruled out a general function for eIF5A in translation initiation was recently revisited. It was realized that the defined growth medium used in the original eIF5A depletion experiment (Kang and Hershey, 1994) results in somewhat less efficient protein synthesis, as evidenced by polysome profiles and ^{35}S-methionine incorporation. When a more complex medium is used, the system is more active, and then it is possible to see a distinct shift of ribosomes from very large polysomes into small polysomes upon eIF5A depletion. Moreover, in an in vitro protein synthesis assay using eIF5A depleted lysates, a 2 to 2.5-fold stimulation of protein synthesis occurs after eIF5A addition as measured by ^{35}S-methionine incorporation (A. Henderson and J. Hershey, personal communication).

Taken together, these data do support a function for eIF5A in translation. As has been proposed before, eIF5A may act as an ''interphase'' factor whose role bridges the initiation and elongation phases of translation (Moldave, 1985). This hypothesis finds support in our observations in yeast that, although eIF5A mutants affect translation, the results are totally different from those of well-known translation initiation factor mutants, but rather resemble those observed in a tRNA maturation mutant. Future studies in diverse organisms and the development of new approaches to test in more detail the steps of translation may reveal the specific mechanism by which eIF5A affects protein synthesis.

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