Eukaryotic translation initiation factor eIF-5A (formerly eIF-4D) is thought to function in protein synthesis by promoting synthesis of the first peptide bond because it stimulates methionyl-puromycin formation in vitro. eIF-5A is encoded by two genes (TIF51A and TIF51B) in Saccharomyces cerevisiae; the protein and its hypusine modification are essential for cell viability. To analyze the factor's function in vivo, we expressed from the repressible GAL promoter a functional but unstable eIF-5A fusion protein (R-eIF-5A) with an NH2-terminal arginine which is subject to rapid turnover through the NH2-terminal end rule proteolytic pathway. When the conditional mutant strain is shifted from galactose to glucose medium, the rapid disappearance of R-eIF-5A protein occurs within one generation, causing an immediate inhibition of cell growth. However, eIF-5A-depleted cells synthesize protein at about 70% of the wild type rate and exhibit only a slight change in polysome profiles reflecting a subtle defect in a late step of translation initiation. These results suggest that the activity of eIF-5A may not be absolutely essential for general protein synthesis. Rather, eIF-5A may be selectively required for translation of certain mRNAs and/or may be involved in some other aspect of cell metabolism.

Eukaryotic translation initiation factor eIF-5A, formerly called eIF-4D, is a small (16-18 kDa) abundant protein that is highly conserved in eukaryotes (1, 2). eIF-5A originally was isolated from the ribosomal high salt wash fraction of rabbit reticulocytes based on its activity in stimulating the synthesis of methionyl-puromycin (3, 4). This in vitro assay involves prior formation of an 80 S initiation complex containing methionyl-tRNA, and subsequently the reaction with puromycin which mimics synthesis of the first peptide bond (4, 5). Since eIF-5A is not required for 80 S initiation complex formation, it appears to be involved solely in the step where the first peptide bond is formed. Unfortunately, the methionyl-puromycin assay is the only means available for measuring the activity of eIF-5A in vitro. Furthermore, rather high concentrations of eIF-5A are required, well in excess of the amounts of methionyl-puromycin formed. As a result, there is concern whether or not eIF-5A truly functions in the initiation phase of protein synthesis. Clearly, the precise role of eIF-5A in translation in vivo has not yet been defined.

eIF-5A is the only known cellular protein that undergoes an unusual post-translational modification on a specific lysine residue to form hypusine (N²-(4-amino-2-hydroxybutyl)lysine) (6). The unique hypusine modification in mammalian cells occurs by a two-step pathway that involves the attachment of an aminobutyl group from spermidine to the ε-amino group of lysine 50, followed by hydroxylation on the number 2 carbon of the butyl group to form hypusine (7). The lysine residue is modified immediately after the synthesis of the protein, and hypusine is not altered or removed until the protein is degraded (8, 9). Hypusine in eIF-5A is found in all eukaryotic species investigated (2), suggesting an important role for the hypusinated protein in cellular metabolism. Furthermore, the hypusine modification is essential for eIF-5A activity in stimulating methionyl-puromycin synthesis in the in vitro reaction (10, 11).

To better study the structure and function of eIF-5A in eukaryotic cells, we have cloned two yeast genes encoding eIF-5A, TIF51A and TIF51B, using the mammalian eIF-5A cDNA as a probe (12). The two yeast genes encode proteins which share 90% amino acid sequence identity and are about 63% identical to human eIF-5A. Expression of at least one of the two yeast eIF-5A genes is required for cell viability, and the hypusine modification of the protein is vital for cell growth in Saccharomyces cerevisiae. Purified yeast eIF-5A protein stimulates methionyl-puromycin formation in the mammalian in vitro assay system (12), and the mammalian cDNA substitutes for the TIF51 genes in yeast, demonstrating that human and yeast eIF-5A are not only conserved at the sequence level but are functionally interchangeable in vivo (13). Interestingly, the two yeast genes are members of a duplicated gene cluster (14), where sets of homologous genes, such as the TIF51 genes and the CYC genes, are regulated by oxygen at the transcriptional level (15). Yeast cells grown aerobically express only mRNA transcribed from TIF51A, whereas cells grown anaerobically express only the TIF51B gene (16). However, growth analyses of strains constructed to express only TIF51A or TIF51B under the GAL10 promoter showed that either gene product supports cell growth identically under either aerobic or anaerobic conditions, demonstrating that the two gene products are functionally indistinguishable (13). Besides hypusination, yeast eIF-5A undergoes another post-translational modification, partial phosphorylation on a serine residue, which generates two hypusinated isolectric variants, eIF-5Aa and eIF-5Ab (17). Both isolectric forms were purified separately and shown to possess identical activity in the methionyl-puromycin assay. Thus, hypusination is an essential modification for the activity of eIF-5A both in vivo and in vitro, but phosphorylation does not have an observable effect, at least in the in vitro assay (17). The physiological significance and site(s) of phosphorylation in eIF-5A are not yet known.

To investigate whether or not eIF-5A is truly involved in protein synthesis in vivo, we set out to deprive cells of the protein and characterize the effects on protein synthesis and on

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1 The abbreviations used are: eIF, eukaryotic initiation factor; kb, kilobase pair(s); Ub, ubiquitin; PAGE, polyacrylamide gel electrophoresis.
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intermediates in the initiation pathway. We constructed a TIF51A conditional expression system using a genetic cassette containing the repressible GAL promoter system in combination with protein destabilizing elements which has been developed as an effective way to rapidly deplete a protein of interest (18). When expression of the altered TIF51A gene fused to the protein destabilizing elements is turned off by shifting the culture from galactose to glucose medium, the pre-existing destabilized eIF-5A fusion protein is eliminated from the cells within a single generation. Thus the effect of rapid depletion of eIF-5A on cell proliferation and protein synthesis could be analyzed.

MATERIALS AND METHODS

Strains and Growth Conditions—Escherichia coli strain MC1066 (F− ΔlacX74 hsdR pslR galU galK trpC963 leuB6 pyrF− Thr−) (19) was used for the host plasmid amplification and was grown at 37 °C in LB medium (0.5% yeast extract, 1% bacto-tryptone, 1% sodium chloride) supplemented with 100 µg/ml ampicillin to propagate plasmids, if required. S. cerevisiae strain W303-1A (MATα leu2−3, 112 his3−11, 15 ade2−1 ura3−1 trp1−1 can1−100) and HYH13 (MATα leu2−3, 112 his3−11, 15 ade2−1 ura3−1 trp1−1 can1−100) (pBSTM-TIF51A) is a derivative of strain HYH13 transformed with the plasmid pSTM-TIF51A, which contains the TIF51A gene, and then streaking the Tran3 transformants on 5-flouro-orotic acid plates to select cells that have lost the URA3 plasmid, pBM-TIF51A. The plasmid pHSTC was constructed by subcloning a 1.45-kb EcoRI fragment from plasmid TRP7 containing the yeast ARS1/1TFIp sequence (20) into the EcoRI site of the bacterial vector pGEM-1 (Promega), followed by inserting a 1.6-kb blunt-ended SalI fragment from plasmid p10-2-3 containing the CEN11 sequence (obtained from M. Holland, University of California, Davis) into the pGEM-E2 site of the pGEM-1 derivative. Then a 1.3-kb BamHI-SphI fragment containing the TIF51A gene, which was generated from pSTM (12) was subcloned into BamHI and SphI sites of pHSTC to generate pHSTC-TIF51A. The strains UBHY-M (MATα leu2−3, 112 his3−11, 15 ade2−1 ura3−1 trp1−1 can1−100 (pBSTM-TIF51A)) are derivatives of strain HHY132 and express eIF-5A fusion proteins with protein destabilizing elements (see below). Yeast cells were grown at 30 °C and monitored by measuring the optical density at 600 nm in a Beckman spectrophotometer.

Construction of Plasmids for Expressing Variant Forms of the TIF51A Gene—The plasmid pGEM-flu, which contains the BamHI-XbaI fragment encoding a lacI-flu segment (18), was used to make an in-frame fusion of the TIF51A coding region to the lacI-flu segment. An XbaI-Pml fragment containing the entire TIF51A coding sequence was generated by digesting the original DNA fragment which was amplified from the plasmid pJA2 (12) by the polymerase chain reaction by using the 5′-GGGGAATCTAGAATGTTCTGA CGAAG-3′ and 3′-GGGGAATCTAGAATGTTCTGA CGAAG-3′ primers, ligated into the XbaI and Pml sites in the multiple cloning site of pGEM-flu to generate an in-frame fusion of the lacI-flu segment to the TIF51A coding region. The resulting plasmid pGEM-flu-TIF51A was digested with HindIII and then treated with Klenow fragment to make blunt ends. The linearized blunt-ended plasmid was digested with BamHI and blunt-ended fragment containing the lacI-flu-TIF51A fragment. To make an in-frame fusion with the UBI4 gene under the control of the GAL promoter, the lacI-flu-TIF51A fragment was ligated with the BamHI/HindII fragment of pUB23 which contains: Amp′-2 micron origin-URA3-upstream activation sequence of the URA3 (13) and 5′-GGGGAATCTAGAATGTTCTGA CGAAG-3′ (22). The resulting plasmids were named pUB-Xaa-5A, where Xaa represents either methionine or arginine. To change the pHUB-Xaa-5A plasmid to a centromeric plasmid, a 2.9-kb EcoRI/FspI fragment containing the URA3 gene and the Ub-lacI-flu-TIF51A fusion gene under the GAL promoter were obtained by plasmid pUB-Xaa-5A digested with a 5′-GGGGAATCTAGAATGTTCTGA CGAAG-3′ fragment and transformed into yeast by electroporation

The transformants of HYH132 having the two plasmids, pHSTM·TIF51A and YCPUB-Xaa-5A, were grown for 20 generations in galactose containing complete minimal media but lacking uracil (SG-Ura) to deplete the pHSTC-TIF51A plasmid. Then the cells were plated on SG-Ura plates and replica-plated into 5-Gal-trypophan and SG-Ura (lacking uracil) plates to screen for colonies that cannot grow on 5-Gal due to the loss of the pHSTM-TIF51A plasmid but can grow on SG-Ura due to the presence of the YCPUB-Xaa-5A plasmid. The strain expressing the M-eIF-5A fusion protein containing methionine residues in the mature protein) was designated as UBHY-M, and the strain expressing the R-eIF-5A protein (arginine NH2 terminus) was designated as UBHY-R (Fig. 1).

Measurement of Protein Stability by Pulse-Chase Labeling and Immunoprecipitation—The W303-1A and UBHY-R strains were grown in methionine-labeling medium containing 2% galactose, 0.57% yeast nitrogen base without amino acids, uracil, adenine, and amino acids, except methionine, until the culture reached mid-exponential growth phase (OD600 = 0.25). Cultures (25 ml) were harvested, and cells were resuspended in 300 µl of the same medium. [35S]Methionine (100 µCi; 1000 Ci/mmol) was added, and the cells were incubated at 30 °C for 5 min. The cells were pelleted and resuspended in 0.6 ml of the labeling medium containing 0.5 µg/ml of cycloheximide and 0.2 mg/ml nonradioactive methionine. The cultures were incubated at 30 °C, and 100-µl aliquots were removed at various times. The aliquots were added to 700 µl of buffer A (1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 50 mM Na-HEPES, pH 7.5, 0.5 mg/ml bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml each of aprotinin, leupeptin, and pepstatin, 1 mM dithiothreitol). After adding 0.5 ml of glass beads, cells were disrupted by vortexing, and the lysate was centrifuged at 15,000 rpm for 15 min at 12,000 x g at 4 °C. The amount of [35S]methionine incorporated into protein was determined by hot trichloroacetic acid precipitation. Equal amounts of radioactivity were used for immunoprecipitations with the monoclonal antibody against yeast eIF-5A as described elsewhere (17). Immune precipitates were subjected to 15% SDS-PAGE and radioactivity in eIF-5A bands was assessed by autoradiography and densitometric scanning.

Measurement of Protein Synthesis Rates—Preculture of strains W303-1A or UBHY-R were inoculated into methionine-labeling medium (see above) containing 2% galactose and grown to early log phase. Cultures were harvested, and cells were resuspended in the same labeling medium except that it contained 2% glucose instead of galactose in order to deplete eIF-5A protein in the UBHY-R strain. At various times after the shift to glucose-containing medium, cultures were harvested, and cells (corresponding to OD600 = 1) were resuspended in 300 µl of 2% glucose-containing labeling medium. [35S]Methionine was added, and cells were incubated at 30 °C for 5 min. To stop incorporation of [35S]methionine, 1 ml of labeling medium containing cycloheximide (0.5 µM) and unlabeled methionine (1.2 mg/ml) was added to the culture, followed by quick-freezing in liquid nitrogen. Cells were lysed in Na-EDTA, β-mercaptoethanol, and proteins were precipitated with hot trichloroacetic acid (24). The pellet was washed with acetone, dissolved in 200 µl of 1% SDS solution, and heated immediately at 95 °C for 5 min. The incorporation of [35S]methionine into total protein was determined by trichloroacetic acid precipitation. The labeled proteins in the SDS extract was analyzed by the micro-BCA protein assay reagent kit (Pierce Chemical Co.) as described by the manufacturer. The rate of protein synthesis is expressed as counts/min of [35S]methionine incorporated into 1 µg of trichloroacetic acid-precipitable protein/min.

Immunoprecipitation Analysis—Cultures grown to mid-exponential phase in YP medium containing 2% galactose were shifted to YP medium containing 2% glucose. At various times after the shift to glucose medium, 100 µg/ml cycloheximide was added to the culture, followed by quick-cooling by swirling the culture on ice. The cells were collected by centrifugation and washed with buffer B (10 mM Tris-HCl, pH 7.4, 100 mM KCl, 10 mM MgCl2, 1 mM dithiothreitol) and resuspended with 100 µg/ml cycloheximide. The cell pellets were broken by vortexing with glass beads, and cell lysates were clarified by centrifugation at 10,000 x g for 5 min at 4 °C. The extracts corresponding to OD600 = 10 were layered onto 12.5-mL sucrose density gradients (7–47% in Buffer A) prepared in a Gradient Master 105 (Biocomp) apparatus. The gradients were centrifuged in a Beckman SW40 rotor at 40,000 rpm for 150 min at 4 °C and fractionated in an ISCO density gradient fractionator.
Effect of eIF-5A Depletion on Protein Synthesis

In order to deplete eIF-5A protein more rapidly, we constructed the haploid strain HY13 which has the chromosomal copies of both TIF51A and TIF51B disrupted, but harbors a centromeric plasmid containing the TIF51A coding region under the GAL promoter (13). The steady state concentration of TIF51A mRNA in the wild type strain W303-1A and that of the hybrid TIF51A mRNA from the GAL promoter in strain HY13 are very similar in the exponential growth phase. Likewise, HY13 expresses almost the same level of eIF-5A protein as do wild type cells when analyzed by Western immunoblotting (data not shown). When a culture of strain HY13 is shifted from galactose to glucose medium, transcription of TIF51A from its own promoter. This indicates that the lack of growth on glucose-containing medium is caused by depletion of eIF-5A.

Physiological Characterization of the Conditional System—
To examine the conditional expression and the steady state level of eIF-5A protein, exponential cultures of strains UBHY-M and UBHY-R were shifted from galactose to glucose medium and cell lysates were prepared at various times thereafter. Western immunoblot analysis shows that the level of the M-eIF-5A fusion protein decreases steadily over a period of 6 h (Fig. 2B), whereas eIF-5A from the nonrepressed wild type gene in W303-1A remains constant (A). In contrast, strain UBHY-R shows a rapid disappearance of R-eIF-5A within 1 h after the shift to the glucose-containing medium (Fig. 2C). This result demonstrates that the R-eIF-5A protein, which has Arg as the NH2 terminus, is so unstable that repression of transcription from the GAL promoter leads to a rapid depletion of R-eIF-5A protein. To demonstrate directly that the rapid disappearance of the R-eIF-5A protein is due to protein destabilization, pulse-chase labeling followed by immunoprecipitation was carried out with strains W303-1A and UBHY-R as described under “Material and Methods.” Fig. 3 shows that unmodified eIF-5A expressed in the wild type cell is stable over a 60-min chase period (A), which is consistent with the greater than 24-h half-life of wild type eIF-5A protein reported previously (2). In contrast, the half-life of R-eIF-5A is about 10 min (Fig. 3B), which is a little longer than the 2-min half-life of Arg-β-galactosidase reported by Bachmair et al. (22). These results clearly show that R-eIF-5A, whose mature NH2 terminus is the destabilizing residue arginine, is subject to rapid turnover through the NH2-terminal end rule proteolytic pathway.

To investigate the effect of eIF-5A depletion on cell growth and viability, growth was monitored by measuring OD600 of liquid cultures and viability was analyzed by plating cells onto galactose medium throughout the growth period of strains UBHY-M and UBHY-R. The strains expressing the eIF-5A fusion proteins grow well with doubling times of 2.0 and 2.2 h, comparable with 1.9 h for the wild type strain W303-1A in
After a 5-min pulse labeling, cells were analyzed immediately for detection of eIF-5A protein, and the de-ubiquitinated eIF-5A fusion protein was detected using an antibody as described. The UBHY-M strain grows normally for up to four generations, then either directly or indirectly.

Analyses of Protein Synthesis Parameters upon Depletion of eIF-5A—Having established that the R-eIF-5A protein is rapidly depleted after transfer to glucose-containing medium, we used the UBHY-R strain to investigate the effect of the depletion of eIF-5A on protein synthesis. The rate of UBHY-R protein synthesis was measured with 5-min pulses of [35S]methionine over the first 5 h of culture in glucose medium as described under “Material and Methods.” After 1–2 h the cells “grow” for several more hours at about 40% of the rate of wild type as detected by OD600, but apparently have ceased cell division as analyzed by cell viability. We chose to analyze the early stage of the culture after the shift to glucose medium in order to avoid indirect effects of prolonged depletion of eIF-5A on protein synthesis, which may be caused by the decreased growth rate or by other factors. Surprisingly, the rate of protein synthesis is about 60–70% of that of wild type cells from 1 to 5 h (Fig. 5). Continuation of protein synthesis well after essentially complete depletion of eIF-5A suggests that eIF-5A is not required for global protein synthesis.

To investigate whether the slight decrease in the incorporation of [35S]methionine into protein comes from a small defect in the protein synthesis machinery, polysome profiles were obtained from eIF-5A-depleted and nondepleted cells to determine whether or not the initiation phase might be affected. When initiation of translation is slowed or blocked, ribosomes finish translating mRNA but do not efficiently reinitiate translation, leading to a reduction in the size of polysomes (number of ribosomes per mRNA) and the accumulation of 80 S ribosomes. Exponential cultures of strains W303-1A (control) and UBHY-R (R-eIF-5A) were shifted to glucose medium and extracts were prepared and fractionated by sucrose density gradient centrifugation as described under “Material and Methods.” The polysomes obtained from either strain grown in galactose medium show almost the same profile (Fig. 10). After 4-h growth in glucose medium, the polysome profile from the control cells is essentially unchanged (Fig. 6A, right panel), whereas that from the eIF-5A-depleted cells shows only slight differences (Fig. 6B, right panel). Substantial amounts of intact polysomes are seen in these UBHY-R cells, although a slight shift toward smaller polysomes is detected. In addition, a small increase in the 60 S peak and the presence of “half-mers” are observed for monosomes and small polysomes that are not seen in control cells. Half-mers are not detected.
Effect of eIF-5A Depletion on Protein Synthesis

Fig. 4. Effect of eIF-5A depletion on cell growth. Exponential cultures of strain W303-1A (◼), UBHY-M (○), and UBHY-R (△) grown in YP media containing galactose were diluted to an OD₆₀₀ = 0.06–0.08 in YP media containing either galactose (A) or glucose (B). Growth was monitored by measuring optical density at 600 nm. C, the yeast strains W303-1A, UBHY-M, and UBHY-R were streaked on YP plates containing either galactose (YP-Gal) or glucose (YP-Glu) and grown at 30 °C for 3 days. The figure shows a photograph of the plates; a template indicating the locations of the streaked strains is shown on the right.

Fig. 5. Protein synthesis rates in cells after a shift from galactose to glucose medium. W303-1A (◼) and UBHY-R (△) cells grown to early exponential phase in galactose-containing methionine-labeling medium were inoculated into glucose-containing labeling medium. At the times indicated, cells were taken from each culture and subjected to 5-min pulse labeling and analysis as described under “Material and Methods.” The rate of [³⁵S]methionine incorporation into protein (counts/min/µg of protein/min) was calculated by dividing the counts/min of the hot trichloroacetic acid precipitate by the µg of protein in the sample and by the time of pulse labeling (5 min).

thought to be polysomes with an extra 40 S ribosomal subunit attached, presumably in the scanning mode or bound to the initiator AUG awaiting junction with the 60 S ribosomal subunit. These observations are consistent with a small effect of eIF-5A on a late step of initiation, leading to the slight decrease of the protein synthesis rate, but rule out that eIF-5A is essential for the initiation process in general protein synthesis.

DISCUSSION

eIF-5A is unique in that it is the only known cellular protein that undergoes the hypusine modification by spermidine at one of its lysine residues. Polyamines, and specifically spermidine, have been implicated in a wide variety of physiological effects, including cellular proliferation and differentiation. It has been reported that polyamines affect nucleic acid and protein synthesis in vivo and in vitro (28). It is possible that eIF-5A is the intermediate through which polyamines exert such effects. Based on its activity in the methionyl-puromycin assay, a model system that mimics the initiation phase of eukaryotic translation, eIF-5A has been thought to be involved in the initiation step of protein synthesis. Since eIF-5A stimulates methionylpuromycin synthesis after formation of the 80 S initiation complex (4), eIF-5A may be required specifically for formation of the first peptide bond. We have speculated that the positive charge on the non-acylated Met-tRNAi, may not be favorable for tight binding in the donor site of the peptidyl transferase center of the 80 S initiation complex, and thus eIF-5A may provide stabilization for the binding and positioning of Met-tRNAi, in the 80 S initiation complex (29). Demonstration of the involvement of eIF-5A in a reconstituted in vitro protein synthesis system with natural mRNA has been largely unsuccessful. It has been reported that eIF-5A slightly lowers the Mg²⁺ concentration for optimal protein synthesis, but does not affect the level of translation (30).

As a result of the facts cited above, there has been concern about the role of eIF-5A in the methionyl-puromycin assay. Even though stimulation of up to 3–6-fold has been obtained in the mammalian assay system, it is troubling that quite large amounts of protein are required to obtain such stimulation. Nearly 40–50 pmol of homogenous eIF-5A protein are added to obtain 0.2–0.3 pmol of methionyl-puromycin synthesis in the assay (12). Therefore there is doubt that eIF-5A is truly an initiation factor, as opposed to a protein that artifactually stimulates the formation of methionyl-puromycin. Thus it is important to test the possibility that while eIF-5A is an important yeast protein required for yeast growth and viability, it may not be a translation factor required for protein synthesis.

As an approach to investigate the role of eIF-5A in protein synthesis in vivo, we tried to deplete eIF-5A in cells and analyze the effect of eIF-5A depletion on protein synthesis. Depletion of a protein of interest has the advantage that it leads to the complete loss of function of the protein, in contrast to temperature-sensitive mutants that may still retain partial activity, especially if the protein has more than one function. For example, analysis of cell-free extracts from eIF-4A-depleted cells demonstrated that eIF-4A is truly involved in translation (31). Using protein destabilizing elements and the GAL promoter, we constructed a strain that shows rapid disappearance of eIF-5A protein within one generation in glucose medium. Analyses of protein synthesis parameters such as protein synthesis rate and polysome profiles upon rapid eIF-5A depletion show that cells support protein synthesis well, even when cell division is severely inhibited. A similar observation has been also made with strain HHY151, a null strain complemented by the human eIF-5A cDNA (13). This strain expresses human eIF-5A protein from the GAL promoter at such a low level that
Fig. 6. Polysome analyses of wild type and eIF-5A-depleted cells. W303-1A(A) and UBF1-R(B) cells grown in galactose medium (YPGal, left) and those grown for 4 h after a shift to glucose medium (YPD, right) were harvested, lysed, and 10 A260 units from each extract were fractionated by centrifugation on 7-47% sucrose gradients as described under "Materials and Methods." Gradients were collected from the top by using an ISCO density gradient fractionator and were scanned at A254, with an ISCO UA-5 detector. Sedimentation is from left to right. The small arrows in B, right, identify possible half-mers as discussed in the text.

it is barely detected by radiolabeling in galactose medium, yet the strain grows at an appreciable rate. The doubling time of strain HYH151 is ~2.5-fold greater than that of the wild type strain W303-1A, but we do not see any significant differences in the former's polysome profiles except for a very slight diminution of large polysomes. The lack of a clear correlation between protein synthesis activity and growth rate in eIF-5A-depleted cells strongly argues against the idea that eIF-5A is required for general protein synthesis. It rather suggests that eIF-5A may be involved in some other aspect of cellular metabolism. But based on the observation that a slight decrease in protein synthesis occurs upon eIF-5A depletion, it still remains possible that eIF-5A is required for translation of a small set of mRNAs. We also cannot rule out that very low (undetectable) levels of eIF-5A generated by leaky transcription of the GAL promoter in glucose are sufficient to promote translation. This seems unlikely, however, since eIF-5A is an abundant protein in both yeast and mammalian cells.

Although eIF-5A was originally isolated from the ribosomal high salt wash (3), it is a very abundant protein in the cytoplasm, unlike most of the other initiation factors (1). It is largely present unbound to ribosomes, and only a small fraction of the protein is in the ribosomal high salt fraction. Such abundance of eIF-5A in the cytoplasm may imply multiple functions, like another abundant translation factor, eIF-1α (32). Recently the TIF51A gene was isolated as a multicity suppressor of a defective RNA polymerase II (RP021-mCTD51) in which a portion of the mouse COOH-terminal domain was placed at the carboxyl terminus of the largest subunit of yeast RNA polymerase II. The mutant strain shows cold sensitivity and an Ino- phenotype. In another case, TIF51A was isolated as a multicopy suppressor of a fus3 null strain, which shows defects in its ability to arrest in G1 phase in the presence of mating factor treatment. However, it is not yet known how overexpression of eIF-5A suppresses these mutations. It is enigmatic that overexpression of eIF-5A suppresses such mutant phenotypes, when it already is an abundant protein in the nonsuppressed cells. The overproduction of eIF-5A in wild type cells does not change the growth phenotype of either HrLa cells (33) or yeast, suggesting that it is not limiting.

On the other hand, the observation that eIF-5A depletion leads eventually to enlarged cells in G1 arrest is intriguing in that certain metal chelation inhibitors of deoxyhypusine hydroxylase, which is involved in one of the steps of hypusination, exhibit antiproliferative effects by arresting mammalian cells at the G1/S boundary of the cell cycle (34). Based on these observations, it was speculated that eIF-5A acts as an initiation factor selective for a subset of mRNAs encoding proteins that have a critical function in the initiation of DNA replication (35). To test the requirement of eIF-5A for a small class of mRNAs, we are in the process of evaluating the relative rates of synthesis of individual proteins in eIF-5A-depleted and nondepleted yeast cells. Work also is in progress to investigate possible other functions of eIF-5A by using the R-eIF-5A construct where eIF-5A can be depleted rapidly.

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