Translation Initiation Factor eIF-5A Expressed from Either of Two Yeast Genes or from Human cDNA

FUNCTIONAL IDENTITY UNDER AEROBIC AND ANAEROBIC CONDITIONS*

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(Received for publication, February 9, 1993)

Translation initiation factor eIF-5A (previously named eIF-4D) is an essential and highly conserved protein in eukaryotic cells that promotes formation of the first peptide bond. One of its lysine residues is post-translationally modified by spermidine to form hypusine, a unique residue required for eIF-5A activity. In Saccharomyces cerevisiae eIF-5A is encoded by two highly homologous genes, TIF51A and TIF51B. The two genes are regulated reciprocally by oxygen, where under aerobic conditions TIF51A is expressed and TIF51B is repressed, and under anaerobic conditions the opposite occurs. In order to study the products of the two genes individually, yeast strains were constructed that express either TIF51A or TIF51B under control of a galactose promoter. Each gene gives rise to two isolectric variants, eIF-5Aa (more acidic) and eIF-5Ab (more basic), both of which carry the hypusine modification. Expression of either TIF51A or TIF51B promotes growth under both aerobic and anaerobic conditions, indicating that the two gene products function indistinguishably. The human cDNA encoding eIF-5A also was expressed in yeast, and the plasmid shuffle technique was used to demonstrate that the human protein can substitute for the homologous yeast protein in vivo. These results indicate that human and yeast eIF-5A are not only conserved at the sequence level but are functionally interchangeable in vivo.

Eukaryotic translation initiation factor eIF-5A (formerly called eIF-4D) is a small protein that is highly conserved in eukaryotes (1). eIF-5A is distinguished by possession of the unique residue, hypusine [N^2-(4-amino-2-hydroxybutyl)-lysine], formed post-translationally by transfer of an aminobutyl group from spermidine to a specific lysine residue followed by a hydroxylation reaction (2, 3). The mammalian initiation factor appears to act in the final stage of the initiation phase of protein synthesis by promoting formation of the first peptide bond (4). This activity is usually studied in vivo by a model reaction, the synthesis of methionyl-phenylalanyl-arginine-poly(U)

Recently we reported the cloning and sequencing of a human cDNA encoding eIF-5A (7). The mammalian cDNA was used as a probe to clone the corresponding yeast genes TIF51A and TIF51B (8). The two yeast genes encode proteins which share 90% amino acid sequence identity and are about 65% identical to human eIF-5A. We demonstrated that eIF-5A and its hypusine modification are essential for cell viability in Saccharomyces cerevisiae (8). TIF51B is essentially identical to ANBI, differing at only 2 base pairs and encoding an identical protein (9, 8). ANBI had earlier been identified as an anaerobic gene that is repressed by heme and by oxygen and maps adjacent to the 5'-end of CYC1 on chromosome X (10, 11). TIF51A encodes the transcript denoted tr2 which cross-reacts with ANBI probes (10). We mapped TIF51A to the ARC region of chromosome V, near to the CYC1 homologue, CYC7, and showed that TIF51A and TIF51B are members of a duplicated gene cluster (12). The expression of TIF51A and TIF51B is regulated reciprocally by oxygen and heme. Yeast cells grown aerobically express only a mRNA transcribed from TIF51A, whereas a TIF51B-derived transcript is not detectable. Under anaerobic growth conditions, only TIF51B is expressed. The functional significance of the reciprocal regulation of the TIF51 genes by oxygen and heme is unclear.

By employing high resolution two-dimensional IEF/SDS-PAGE, we identified two hypusinized isolectric variants of eIF-5A in yeast and a third hypusinated form of lower molecular mass also derived from the TIF51 genes (8). It was not clear, however, how the different protein forms were related to the two genes TIF51A and TIF51B and what were the structural differences. Therefore, the major goal of this investigation was to compare the structures and functions of the two TIF51 gene products. In order to do so we constructed yeast strains that are able to express only one of the TIF51 genes but not the other, independent of regulation by oxygen and heme, and analyzed the resulting proteins. We also asked if the gene products of either TIF51A and TIF51B can support growth under aerobic and anaerobic conditions. Finally we expressed the mammalian cDNA encoding eIF-5A in yeast cells to determine whether or not the human protein can function in place of the homologous yeast initiation factor in vivo.

MATERIALS AND METHODS

Strains and Growth Conditions—Escherichia coli strain MC1066 (F^- lacX74 hsdR recA1 galU galK trpC1608 leuB6 pyrF-6055) (13) was the host for plasmid amplification and was grown at 37 °C in LB medium (0.5% yeast extract, 1% bacto-tryptone, 1% sodium chloride)

1 The abbreviations used are: IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis; kb, kilobase pair(s); bp, base pair(s); 5-FOA, 5-fluoro-orotic acid.
supplemented with 100 mg/liter ampicillin to propagate plasmids, if required. S. cerevisiae strains W303-1A (MATa leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trpl-1 can1-100) (13) and W303D (MATa/MATa leu2-3, 112/leu2-3, 112 his3-11, 15/15 his3-11, 15 ade2-1/ade2-1 ura3-1/ ura3-1 trpl-1/trpl-1 can1-100 can1-100) (14) were used as parental strains for all the other strains constructed in this work. Construction of the pink-conferent eIF-5A gene or cDNAs is described below. YP medium and minimal media supplemented with the relevant amino acids, bases, and 2% glucose or 2% galactose were prepared as described previously (15). Yeast cells were grown at 30 °C. For sporulation, cells were streaked on 1% potassium acetate and incubated for 3 days. Growth of yeast cells was monitored by measuring the optical density at 600 nm in a Beckman spectrophotometer. For anaerobic growth, cells were streaked on YP plates supplemented with 2% galactose and 20 μg/ml ergosterol and incubated in a gas-tight chamber with the BBL Gas Pack Anaerobic System (Becton Dickinson Microbiology Systems). For anaerobic liquid cultures, cells in early exponential phase (aerobic) were inoculated into 5 ml of YP-galactose liquid media in 5 ml glass ampules, and tubes were sealed by melting their tops. A series of identical tubes was incubated at 30 °C with shaking, and growth curves were determined by opening individual tubes at various times and measuring optical density.

Migrated with a 5′-fluoro-orotic-acid select for the loss of the URA3 plasmid pBM-TIF51A (24). From this selection strains HYH131 (containing pHSHX-TIF51A), HYH141 (containing pHSHX-TIF51B), HYH151 (containing pHSHX-5AcDNA1), and HYH152 (containing pHSHX-5AcDNA2) were recovered. For all strains constructs corresponding sites of phlscript (Strategene), plasmid p4DN was digested with 5′-fluoro-orotic-acid to select for the loss of the URA3 plasmid pBM-TIF51A. For anaerobic liquid cultures, cells in early exponential phase (aerobic) were inoculated into 5 ml of YP-galactose liquid media in 5 ml glass ampules, and tubes were sealed by melting their tops. A series of identical tubes was incubated at 30 °C with shaking, and growth curves were determined by opening individual tubes at various times and measuring optical density.

Expression of Different eIF-5A Proteins—The host strain for the controlled expression of different eIF-5A genes and cDNAs, HYH13, was constructed in several steps. One chromosomal copy of TIF51A was disrupted in the diploid W303D by replacement of a 0.4-kb SalI-HpaI fragment from the coding region with a 2-kb SalI-HpaI fragment carrying the LEU2 gene as described by Schmitter et al. (18), resulting in strain HYH131. Insertion of the blunt-ended BamHI fragment containing HIS3 at the SaI site in the coding region of one of the TIF51B genes (8) of HYH21, which then has one intact and one disrupted copy of both TIF51A and TIF51B. Strain HYH23 was transformed with plasmid pBM-TIF51A, which has a blunt-ended 723-bp HindIII-FspI fragment containing the entire coding region of TIF51A cloned into the blunt-ended unique BamHI site of the centromeric expression vector pBM272 (obtained from R. W. Davis, Stanford University). The vector pBM272 is a derivative of pBM150 and contains elements for amplification and selection in E. coli (OrI, Ap') and the yeast mating type marker (Trpl), as well as the URA3, CEN4, and TRP1 markers (21) that controls expression of DNA fragments cloned into the unique BamHI site. One of the resulting diploid transformants was named HYH23[pBM-TIF51A] and was then sporulated. Tetrads were dissected and Leu+ His+ Ura+ spores were selected. One of the selected haploids was named HYH13 (MATa leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trpl-1 can1-100 trpl-tf51A:LEU2 tf51B1:His3 [pBM-TIF51A]) and is shown schematically in Fig. 1C.

The expression vector pHSHX3 shown in Fig. 1A was constructed as follows. The bacterial vector pGEM-1 (Promega) was opened at the HindIII site, and the ends were filled in with Klenow fragment and ligated to 950-bp blunt-ended fragment from pHS8101 (14) containing the termination sequences of the FBA1 gene. The resulting plasmid pH81 was then cut with EcoRI and SalI, and the larger fragment was ligated with a 960-bp EcoRI-SalI fragment from plasmid pBM272 containing the GALI-GAL10 promoter (21), giving plasmid pH82. Next, a 1453-bp EcoRI fragment from the plasmid YRp7 containing the yeast AR51/TRP1 sequences (22) was cloned into the EcoRI site of pH82, resulting in the high-copy-number expression vector pHSX1. Finally, a 1.6-kb blunt-ended SalI fragment from plasmid p16-2-3 containing the CEN1 sequence (obtained from M. MacKay, University of California, Davis) was inserted in the PvuII site of pHSX1 to give the centromeric expression vector pHSX3.

For the expression of TIF51A, plasmid pHSHX-TIF51A was constructed by cloning a blunt-ended 723-bp HindIII-FspI fragment from plasmid pBM-TIF51A containing the centromeric expression vector pHSX3 into the blunt-ended BamHI site of pHSX3. For the expression of TIF51B, plasmid pHSHX-TIF51B was constructed by cloning a blunt-ended 566-bp Macl fragment from plasmid pJSB1 (8) containing the TIF51B coding region into the blunt-ended BamHI site of pHSX3. For the expression of the human eIF-5A-cDNA, two sources of the eIF-5A-cDNA were used: plasmid p4DN contains a 561-bp EcoRI-PstI fragment with the wild-type cDNA sequence (7) cloned into the corresponding sites of phLScript (Strategene), plasmid p4DN has the same size EcoRI-PstI fragment of the eIF-5A-cDNA inserted into pBlueScript, but the two A's immediately 5' to the translational start codon in the cDNA had been changed to G's, thus creating a NcoI site (23). Plasmids pHSHX-5AcDNA1 and pHSHX-5AcDNA3 contain a blunt-ended 579-bp EcoHl-BamHI fragment of p4DN and p4D, respectively, in the blunt-ended BamHI site of pHSX3. Plasmids pHSH-5AcDNA2 and pHSHX-5AcDNA4 contain a blunt-ended 554-bp HindIII-BamHI fragment of p4DNS and p4D, respectively, in the blunt-ended BamHI site of pHSX3. For all constructs the respective eIF-5A gene or cDNA was inserted into pHSX3 in both orientations relative to the GALI transcription initiation site and the constructs with the insert in the wrong orientation were denoted by the suffix -R.

The expression constructs were transformed individually into strain HYH13 and Trp+ Ura+ transformants were selected. Thirty transformants from each transformation were then streaked onto plates containing 5-fluoro-orotic-acid for selection for the loss of the URA3 plasmid pBM-TIF51A (24). From this selection strains HYH131 (containing pHSHX-TIF51A), HYH141 (containing pHSHX-TIF51B), HYH151 (containing pHSHX-5AcDNA1), and HYH152 (containing pHSHX-5AcDNA2) were recovered. For all strains constructs corresponding sites of phlscript (Strategene), plasmid p4DN was digested with 5′-fluoro-orotic-acid to select for the loss of the URA3 plasmid pBM-TIF51A.

RESULTS

Either the TIF51A or the TIF51B Gene Product Supports Growth under Aerobic and Anaerobic Conditions—Since TIF51A is expressed under aerobic conditions, and TIF51B is expressed anaerobically, it is possible that each of the gene products plays a unique essential role in their respective conditions. To test this idea, we asked if TIF51B alone can support growth aerobically and if TIF51A alone can function under anaerobic conditions. In previous work (8), we had reported that a TIF51A-disrupted strain grows slowly, implying that the intact TIF51B product functions aerobically, but the level of expression of TIF51B was not assayed. In order to study the function of the TIF51A and TIF51B gene products individually, we constructed the plasmids pBM-TIF51A and pBM-TIF51B. As a host we used the haploid strain HYH13 which was constructed as outlined under "Materials and Methods" and is depicted in Fig. 1C. Strain HYH13 carries null mutations in the chromosomal copies of TIF51A and TIF51B and harbors the centromeric URA3 plasmid pBM-TIF51A containing the TIF51A gene under control of the GALI promoter. For the expression of different eIF-5A proteins we constructed the centromeric vector pHSX3 which contains a TRP1 marker and a GALI promoter with a unique cloning site followed by termination sequences as shown in Fig. 1A. The TIF51A coding region was cloned into pHSHX3 (see "Materials and Methods"), resulting in plasmids pHSHX-TIF51A (correct orientation with respect to the GALI promoter) and pHSHX-TIF51A-R (wrong orientation). The TIF51B coding region was inserted into pHSX3, resulting in plasmids pHSHX-TIF51B (correct orientation) and pHSX-TIF51B-R (wrong orientation). Since these constructs (Fig. 1B) have very short 5′-untranslated regions derived from TIF51A (97 bp) and TIF51B (44 bp), the gene-specific transcription signals are eliminated, and expression is controlled only by the GALI promoter. The vector and the plasmids were transformed individually into strain HYH13 and Trp+ Ura+ transformants were selected. Thirty transformants from each transformation were then streaked onto plates containing 5′-fluoro-orotic-acid (5-FOA) to select for the loss of the URA3 plasmid, pBM-TIF51A (24). All cells transformed with
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A

B

C

pHSX3 (7.2 kb)

TRP1

ARS1

GAL1-P

FBA1-T

AmpR

Ori

CEN1

TTIF51A

TTIF51B

elf-5A-cDNA

100 bp

LEU2

TIF51A

chromosome V

TIF51A

chromosome X

A, linear map and relevant restriction sites of the expression vector pHSX3 which was constructed as described under "Materials and Methods." Transcription of DNA fragments cloned into the unique BamHI site is controlled by the GAL1 promoter (GAL1-P) and transcripts terminate at the FBA1 termination sequence (FBA1-T). B, DNA fragments inserted into the BamHI site of pHSX3 for the expression of TIF51A (pHSX-TIF51A), TIF51B (pHSX-TIF51B), and the HeLa elf-5A-cDNA (pHSX-5AcDNA1 and pHSX-5AcDNA2), respectively. Thick lines indicate the respective coding regions (N terminus on the left) and thin lines the 5' and 3'untranslated regions. After transformation of strain HHY13 with each of the recombinant plasmids, cells were streaked onto plates containing 5-fluoro-orotic acid to select for the loss of the URA3 plasmid, pBM-TIF51A, and the names of the various strains recovered from this selection are given in parentheses. C, the haploid host strain HHY13 which was used for the controlled expression of the different elf-5A genes or cDNAs. HHY13 carries null mutations in the chromosomal copies of TIF51A and TIF51B and depends for growth on TIF51A carried by the centromeric plasmid, pBM-TIF51A. The restriction sites are A = HpaI, B = BamHI, E = EcoRI, F = FspI, H = HindIII, K = KpnI, M = MaeI, N = HinfI, P = PstI, S = Sal.

The growth rates of strains HHY131 and HHY141 were determined in liquid medium containing galactose by measuring the optical density under aerobic growth conditions (Fig. 3A). The doubling times calculated from these curves for HHY131 and HHY141 are exactly the same and are about 1.3-fold greater than that of the wild-type strain W303-1A (Fig. 3C). This shows that the TIF51B gene product, although normally repressed in the presence of oxygen in wild-type cells (10), can function under aerobic growth conditions as well as the TIF51A gene product. The growth rates of strains HHY131 and HHY141 also were determined in liquid medium under anaerobic conditions (Fig. 3B). Both strains showed an early burst of growth due to remaining oxygen in the sealed tubes, then grew identically in a slower exponential phase with doubling times of about 4.2 h (Fig. 3C). Strains HHY131 and HHY141 also were streaked onto plates containing galactose and ergosterol and incubated under anaerobic conditions. When inspected visually every 24 h for a period of 10 days, the sizes of the colonies of strains HHY131 and HHY141 were comparable (results not shown). Therefore the TIF51A gene product, whose expression is barely detectable under
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Fig. 2. Growth analysis of yeast strains expressing different eIF-5A proteins. The haploid yeast strain HHY13 was transformed with the different recombinant plasmids identified on the right side of the figure which carry either the yeast TIF51A or TIF51B coding sequences or the human eIF-5A cDNA (see Fig. 1); the designation R denotes constructs that contain the respective eIF-5A gene or cDNA in the reverse orientation relative to the GAL1 promoter. Transformants were selected on minimal media plates containing galactose as a carbon source. Seven individual transformants from each transformation were suspended in water, and the cell suspensions were spotted in parallel on minimal media plates containing galactose (left panel, labeled -Trp -Ura Gal) and minimal media plates containing galactose supplemented with uracil and 5-fluoro-orotic acid (right panel, labeled 5-FOA Gal). The plates were incubated at 30 °C for 2 and 5 days, respectively. The figure shows a photograph of the plates.

anaerobic conditions in wild-type cells (10), supports growth in the absence of the TIF51B gene product under anaerobic growth conditions.

We also analyzed the expression of the different TIF51 genes in strains W303-1A, HHY131, and HHY141 by Northern blot hybridization of total RNAs prepared from mid-log phase cells grown aerobically in galactose-containing medium (Fig. 4). When probed with TIF51A DNA a strong 0.9-kb band is generated in the case of the wild-type strain W303-1A (lane 1), whereas no signal is detected in W303-1A with a TIF51B-specific probe (lane 6). Strain HHY131 also generates a strong band when probed with TIF51A DNA (lane 2). The size of this RNA is slightly larger than that of wild-type TIF51A mRNA due to the short 5' leader derived from the GAL1 sequence. A faint band of ~1.0 kb is seen in HHY131 with a TIF51B probe (lane 7). This band is detected with the TIF51B DNA probe in all strains carrying the tifs1B-HIS3 allele (lanes 7–10) and is probably a transcript made from the disrupted chromosomal TIF51B gene containing HIS3, although this has not been proven. In HHY141 no TIF51A-specific signal is detected (lane 3), whereas there is a strong band of ~0.85 kb when the blot is probed with TIF51B DNA (lane 8). This RNA also is slightly larger than wild-type TIF51B mRNA due to the 5'-leader derived from the GAL1 promoter. Since the same amount of total yeast RNA was loaded onto each lane and probes of comparable length and specific activity were used, the strength of the signal may be an approximate measure of the mRNA concentration. Comparing the band intensities in Fig. 4, we estimate that the steady state concentrations of TIF51A mRNA in W303-1A (lane 1), of hybrid TIF51A mRNA in HHY131 (lane 2), and of hybrid TIF51B mRNA in HHY141 (lane 8) are very similar in the exponential growth phase.

The Protein Product of TIF51A Is Smaller than That of TIF51B, but Each Gene Product Comprises Two Isoelectric Variants—Employing high resolution two-dimensional IEF/SDS-PAGE, we were previously able to identify three yeast proteins of ~18–20 kDa that are labeled with radioactive spermidine and that are barely or not at all detectable in a haploid strain carrying a disrupted TIF51A gene (8). In order to find out how these proteins are related to the TIF51 genes, cells of strains W303-1A, HHY131, and HHY141 were labeled either with [35S]methionine or [3H]spermidine, and total proteins of the labeled cells were analyzed by IEF/SDS-PAGE (Fig. 5). The identities of the two spots in the [35S]methionine-labeled protein pattern were made on the basis of the spermidine labeling and on previous analyses (8). In the wild-type strain W303-1A, two proteins of 20 kDa and slightly different isoelectric points are labeled by [3H]spermidine and are also strongly labeled by [35S]methionine. These two proteins correspond to the proteins of ~20 kDa previously identified in other yeast strains as being eIF-5A and which were then called spot a (more acidic) and spot b (more basic) (8). A protein previously called spot c (8), exhibiting a mass of ~18 kDa and a more basic pI, was not detected in strain W303-1A; it is very likely that this protein is a proteolytic product of proteins a and/or b. The intensity of protein b is much greater than that of protein a in the [35S]methionine-labeled lysate compared with the [3H]spermidine-labeled lysate (compare the left and right panels of W303-1A in Fig. 5). This is due to the shorter [35S]methionine labeling time (the precursor-product relationship of the b and a forms will be addressed in a separate publication).

In HHY131 where only the TIF51A gene is expressed, we find exactly the same proteins labeled with [3H] and [35S] as in W303-1A. When lysates of W303-1A and HHY131 are mixed and electrophoresed, all proteins comigrate, including the eIF-5A proteins a and b (result not shown). So both proteins a and b are products of the TIF51A gene, as expected, since the TIF51A gene is the only TIF51 gene expressed in wild-type cells under aerobic growth conditions (Fig. 4) (10). In HHY141 where only TIF51B is expressed, there are also two proteins of ~20 kDa labeled with [3H] and [35S]. These TIF51B gene products migrate to the same position in the IEF dimension and therefore have the same pI values as the TIF51A gene products a and b, but they migrate slightly more slowly in the SDS-polyacrylamide gel, even though the molecular masses of the TIF51A and TIF51B proteins (17,101 and 17,120 Da, respectively) calculated from the gene sequences are similar (8). This is more obvious on gels where lysates of strains HHY131 and HHY141 are mixed and electrophoresed (not shown). In summary, both TIF51A and TIF51B individually give rise to two isoelectic forms, eIF-5Aa (more acidic) and eIF-5Ab (more basic), which have the same respective pI.

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After incubation with shaking at 30 °C for the times indicated, where oxygen in the state was assumed to be exhausted. Individual ampules were broken for determining the optical density at 600 nm. C, growth rates. Doubling times were calculated from the growth curves after five generations of growth (absorbency about 0.4), where oxygen in the ampule was assumed to be exhausted.

Human eIF-5A Can Substitute for the Corresponding Yeast Protein in Vivo—Human eIF-5A shares 63% sequence identity with the gene products of the yeast TIF51 genes (8), and both human and yeast proteins contain the unique hypusine post-translational modification. The finding that yeast eIF-5A is active in the in vitro mammalian methionyl-tRNA synthetase assay (8) strongly suggests that the TIF51A and TIF51B genes encode proteins that are functionally equivalent to eIF-5A in humans. Since eIF-5A is an essential protein in yeast (8), we asked whether or not yeast cells could grow in the presence of only human eIF-5A but no yeast eIF-5A. To answer this question we employed the plasmid shuffle system described above. A number of different restriction endonuclease fragments containing the human eIF-5A cDNA were cloned into the expression vector pHSX3 (Fig. 1A) as described under “Materials and Methods.” The resulting plasmids were transformed individually into the yeast strain HHY13 (Fig. 1C), and Trp+ Ura+ transformants were selected. The transformants were then streaked onto plates containing 5-FOA and galactose to select for the loss of the URA3 plasmid pBM-TIF51A. Cells growing on 5-FOA plates should express only human eIF-5A derived from the human eIF-5A-cDNA cloned into pHSX3 and should have no yeast eIF-5A.

When we tested plasmids pHSX-5AcDNA3 and pHSX-5AcDNA4 which contain a 579-bp EcoRI-BamHI fragment and a 554-bp HindIII-BamHI fragment, respectively, of wild-type eIF-5A cDNA (7), none of 60 Trp+ Ura+ transformants grew on the 5-FOA plates. Then we tried the same size EcoRI-BamHI and HindIII-BamHI fragments of the eIF-5A cDNA where the two A's immediately 5' to the translational start codon had been changed to C's, thus creating a NcoI restriction site (23). HHY13 was transformed with plasmids pHSX-5AcDNA1 and pHSX-5AcDNA2 which have, respectively, a 579-bp EcoRI-BamHI fragment and a 554-bp HindIII-BamHI fragment of the mutant cDNA inserted in pHSX3 (Fig. 1B). Interestingly all of 30 Trp+ Ura+ transformants from each transformation grew on the 5-FOA plates, whereas cells that had been transformed with plasmids containing the respective cDNA inserts in the wrong orientation relative to the GAL1 promoter (pHSX-5AcDNA1-R and pHSX-5AcDNA2-R) did not grow (Fig. 2). This suggests that human eIF-5A can function in place of yeast eIF-5A in vivo. We have no explanation why the NcoI mutant works in this experiment and the wild-type form does not. We can only speculate that the change of only two A's to two C's immediately 5' to the translation start codon (confirmed by DNA sequence analyses) somehow improves the expression of the cDNA at the transcriptional or translational level in S. cerevisiae.

From the 5-FOA plates we recovered strains HHY151 (harboring pHSX-5AcDNA1) and HHY152 (harboring pHSX-5AcDNA2) and confirmed the genotypes of the respective eIF-5A loci by Southern blot analyses (results not shown). Neither strain forms detectable colonies on plates containing YP-glucose, demonstrating that growth of the null strains is indeed dependent on expression of the human eIF5A-cDNA (Fig. 6B). We then determined the growth rates of strains HHY151 and HHY152 in liquid culture containing galactose as a carbon source under aerobic conditions (Fig. 6A). The doubling time of strain HHY151 is 4.47 h and is ~2.5-fold higher than that of wild-type strain W303-1A and is ~2.0-fold higher than those of strains HHY131 and HHY141 which express yeast eIF-5A from the GAL1 promoter (Fig. 3C). Strain HHY152 has a doubling time of 2.51 h which is only ~1.1-fold higher than the doubling times of strains HHY191 and HHY141; HHY152 grows ~1.4-fold more slowly than the wild-type strain W303-1A. We analyzed the eIF-5A-specific mRNAs by Northern blot hybridization of total RNAs prepared from aerobically growing mid-log phase cells of strains HHY151 and HHY152 (Fig. 4)., we found no TIF51A-specific signal (lanes 4 and 5) and could detect only the faint band derived from the disrupted tif51B::HIS3 allele with a TIF51B probe (lanes 9 and 10). When the blot was probed with the human eIF-5A-cDNA, we found a very strong band of ~1.1 kb in strain HHY151 (lane 14) and a slightly smaller
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**FIG. 4. Northern blot analyses.** Total yeast RNA was prepared from exponentially growing cells of W303-1A (lanes 1, 6, and 11), HHY131 (lanes 2, 7, and 12), HHY141 (lanes 3, 8, and 13), HHY151 (lanes 4, 9, and 14), and HHY152 (lanes 5, 10, and 15); 15 µg of RNA were loaded on each lane of a formaldehyde, 1.75% agarose gel, electrophoresed, and then blotted onto Hybond-N (Amersham). The blot was probed with the 791-bp HinfI-FspI fragment containing TIF51A (lanes 1–5), the 566-bp Mael fragment containing TIF51B (lanes 6–10), or the 561-bp EcoRI-PsiI fragment containing the HeLa-eIF-5A-cDNA (lanes 11–15), respectively, each labeled to a specific activity of ~10⁶ cpm/ng. The blots were exposed to Hyperfilm (Amersham) for 4 h.

**FIG. 5.** Gel electrophoretic analyses of eIF-5A forms. Total protein in lysates of W303-1A (parental strain), HHY131 (expressing only TIF51A), HHY141 (only TIF51B), and HHY152 (only the human eIF-5A-cDNA) was subjected to two-dimensional IEF/SDS-PAGE as described by O'Farrell (27). A 3:1 ratio of Servalytes, pH 4–6 and 3–10, was used in the IEF dimension and 15% SDS-polyacrylamide gels were run in the second dimension. Prior to analysis, the cells analyzed in the left panels were labeled for 0.25 generations with 75 pCi of [35S]methionine (1000 Ci/mmol; Du Pont-New England Nuclear) in 2 ml of galactose minimal medium, and the cells in the right panels were grown for three generations in 2 ml of galactose minimal medium containing 30 µCi of [3H]spermidine (24 Ci/mmol; Du Pont-New England Nuclear) during the mid-log phase. W303-1A Cells were pelleted for 5 min at 3000 x g at 4°C, washed twice with ice-cold water, and lysed in 500 µl of 10 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride by vortexing with glass beads. After centrifugation for 10 min at 30,000 x g at 4°C, proteins were precipitated from the supernatant by addition of trichloroacetic acid to a final concentration of 10% and redissolved in IEF-sample buffer (27). The figure shows the autoradiograms on Hyperfilm (Amersham) which were exposed overnight (left panels) for 2 days (right top, upper, and lower middle panels) or for 3 weeks (right bottom panel). The migration positions of the yeast eIF-5A proteins are indicated by arrows and the positions of the proteins derived from the human eIF-5A-cDNA by arrowheads.

In order to prove that the human eIF-5A protein is in fact expressed in yeast strains HHY151 and HHY152, we labeled cells of both strains with either [35S]methionine or [3H]spermidine and analyzed the cell lysates by two-dimensional IEF/SDS-PAGE. For strain HHY151 we were unable to identify...
PI values in the [3H]spermidine labeling which correspond to the translational machinery of the basic protein is usually not found in HeLa lysates and could be considered to be precursors that are seen only in yeast due to a very slow conversion in this organism or could be modifications in wild-type yeast cells in the [35S]methionine labeling experiment. However, as shown in this work, the two gene products are distinguishable by their slightly different migration rates when analyzed by SDS-PAGE. The similar primary structures suggest that the two gene products would function identically. However, TIF51A and TIF51B are regulated reciprocally in yeast, resulting in the exclusive presence of the TIF51A product aerobically and the TIF51B product anaerobically. Such regulation suggests that the TIF51A product might be required for the expression of specific aerobic genes, whereas the TIF51B product might be required for the expression of anaerobic genes. It was therefore important to determine whether or not the two proteins have different functional roles in yeast.

In earlier work (8), disruption of TIF51A in the haploid strain S173-6B (strains JSDA1 and JSDA2) resulted in a slow growth phenotype. Expression of TIF51B occurred at a low level, since its eIF-5A gene product, which migrates more slowly than the eIF-5A expressed from TIF51A, was detected by Western immunoblot analysis of cell lysates. The diminished repression of TIF51B in the TIF51A-disrupted strain under aerobic conditions suggested that eIF-5A expressed from TIF51A normally may repress TIF51B by a mechanism of intergenic dosage compensation. However, when TIF51A was disrupted in the haploid strains W303-1A and W303-1B employed in the experiments described in this paper, no viable colonies were detected under aerobic conditions. This indicates that the expression of TIF51B is tightly regulated by oxygen, but not by the TIF51A gene product. We postulate that in the TIF51A-disrupted S173-6B strain JSDA1 or JSDA2, there is an alteration in the TIF51B allele such that repression by oxygen is reduced. This loss of tight repression likely resulted from the selective pressure imposed by the aerobic conditions used to isolate JSDA1 and JSDA2. Indeed, expression of TIF51A from a plasmid transformed into JSDA1 and JSDA2 does not suppress transcription from TIF51B (results not shown).

The growth of JSDA1 or JSDA2 indicates that the TIF51B gene product can promote growth under aerobic conditions, but the slow growth phenotype could be due either to reduced levels of eIF-5A or partial loss of function. To better address the issue of whether or not the two genes are functionally interchangeable, we constructed a haploid strain (HHY151) with disruptions in the chromosomal copies of TIF51A and TIF51B and with the wild-type TIF51A gene carried on a URA3 centromeric plasmid. By using the plasmid shuffle technique, we transformed this strain with a TRP1 centromeric plasmid carrying either TIF51A or TIF51B under control of the GAL1 promoter. Following loss of the URA3 plasmid, each strain was grown in galactose medium under either aerobic or anaerobic conditions. The finding that either gene supports equally well the growth of yeast under both conditions strongly indicates that the two gene products function indistinguishably. It therefore appears that the reciprocal regulation of TIF51A and TIF51B is gratuitous, serving no essential function in yeast cells. However, the possibility that other tests might eventually reveal subtle functional differences between the TIF51A and TIF51B products cannot be excluded.

Possession of yeast strains that express either TIF51A or TIF51B allowed us to analyze and compare the two gene products. Surprisingly, the TIF51B product migrates slightly more slowly when analyzed by SDS-PAGE. The cause of the slower migration in these gels is not apparent, since both proteins have 157 amino acid residues, are hypusinated, and share 90% amino acid sequence identity. Each gene product exhibits two isoelectric forms when analyzed by isoelectric

\[ \text{H. A. Kang, unpublished results.} \]
focusing gel electrophoresis. Since both forms contain the hypusine modification based on [3H]permidine labeling, the different isoelectric points appear to be due to a second partial modification of the protein. This view is reinforced by the labeling experiments reported in Fig. 5. In these analyses, short labeling times produce a more intense basic form relative to longer labeling times, suggesting that a slow post-translational acidic modification occurs. Work is in progress to identify the nature and site of this putative modification.

The ability of human eIF-5A to substitute for yeast eIF-5A in vivo reinforces the claim that the TIF51 genes in yeast encode the functional homologs of mammalian eIF-5A. The results are consistent with the finding that yeast eIF-SA can replace HeLa eIF-5A in the in vitro mammalian assay for methionyl-puromycin synthesis (8). Mammalian eIF-4E can substitute for its yeast homologue even though the respective proteins share 65% identity. Furthermore, human eIF-2α cDNA complements the corresponding null strain. The finding that a number of initiation factors are interchangeable between the yeast and mammalian systems strongly suggests that the mechanism of initiation of protein synthesis in all eukaryotic cells is highly conserved. It is therefore likely that insights derived from genetic studies of yeast translation will be relevant to understanding initiation in mammalian cells as well.

* M. Kainuma, K. Chakraburttty, T. Donahue, and J. W. B. Hershey, unpublished results.

REFERENCES