Characterization of Yeast Translation Initiation Factor 1A and Cloning of Its Essential Gene*

(Received for publication, April 21, 1995, and in revised form, July 18, 1995)

Chia-Lin Wei‡, Mami Kainuma, and John W. B. Hershey§
From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616

The initiation phase of protein synthesis in eukaryotic cells is promoted by a large number of proteins called initiation factors (eIF) (1) (for reviews, see Refs. 1 and 2). One of these, eIF1A (formerly eIF-4C), has been purified from both mammalian (3-5) and plant cells (6) and is essential for maximal in vitro protein synthesis. eIF1A is a small protein (17-22 kDa) that appears to undergo no post-translational modification reactions (7). The initiation factor is implicated in 80 S ribosome dissociation, stabilizes initiator Met-tRNA\(^{i}\) binding to 40 S subunits, and facilitates mRNA binding to the 40 S preinitiation complex (2, 8). Thus, eIF1A has pleiotropic effects at different steps of the initiation pathway. Purified eIF1A from wheat germ and rabbit reticulocytes functions interchangeably in vitro (6), suggesting that the functional domains are highly conserved. Nevertheless, a dear understanding of how eIF1A promotes the initiation phase of protein synthesis is lacking.

The process of translation initiation appears to be very conserved between eukaryotes as distantly related as mammals and the yeast, Saccharomyces cerevisiae. This conclusion is based on similarities of mRNA structure and the basic mechanism of protein synthesis (9). Particularly striking are the structural similarities between yeast and mammalian initiation factors, which share amino acid sequence identities ranging from 26 to 71%. This strong conservation has made it possible to recognize several yeast genes as encoding specific initiation factors based on their sequence homology to mammalian initiation factors (10). The failure to stimulate translation rates by overexpression of the cDNA in transiently transfected mammalian cells suggests that eIF1A is not limiting for protein synthesis (22). However, because of the intrinsic complexity of the mammalian system and the limited ability to manipulate specific gene expression, we decided to study eIF1A function in yeast. We report here the purification and biochemical characterization of yeast eIF1A, the cloning of its gene, Tim11 (for translation initiation factor 1A), and in vitro and in vivo characterization of the factor.

MATERIALS AND METHODS

Strains and Genetic Manipulations—The genotypes and sources of S. cerevisiae strains used or constructed in this work are described in Table I. The diploid strain W303D was made by mating W303-1A and W303-1B (23). Construction of the strains carrying a disrupted eIF1A gene is described below. Yeast cells were grown in YP or synthetic minimal medium (5) supplemented with the relevant amino acids and 2% glucose (D) or 2% galactose (G) as described (24). Cultures were grown at 30 °C and were monitored by measuring optical density at 600 nm in a Beckman spectrophotometer. For sporulation (24), cells were grown on YPD plates for 24 h (containing 6% glucose) and then were sporulated at room temperature on Spc plates (0.3% potassium acetate, 0.02% raffinose, 10 \(\mu\)g/ml of each amino acid) specialized for strain W303D. Tetrad dissections and DNA transformations were carried out by standard procedures (25).

Fractionation of Yeast Cell Lysates—Cells from strain W303-1A were grown in YPD medium to \(A_{600}\) = 0.5 - 1 and lysed in 20 mM HEPES-KOH, 100 mM potassium acetate, 2 mM magnesium acetate, 2 mM dithiothreitol, and 0.5 mM phenylmethanesulfonyl fluoride, pH 7.4, by vortexing with glass beads. The S30 lysate was centrifuged at 100,000 \(\times\) g for 22 min to generate an S100 supernatant and ribosomal pellet. The pellet fraction was suspended in 20 mM HEPES-KOH, 500 mM KCl, 6 mM magnesium acetate, 2 mM dithiothreitol, and 0.5 mM phenylmethanesulfonyl fluoride, pH 7.4, followed by centrifugation through a cushion...
ion of the same buffer containing 20% glycerol. The supernatant (called HSW) and washed ribosome pellet (called Rb) were collected separately and stored frozen at −70 °C.

Purification of Yeast eIF-1A—Strain W303-1A was grown in 0.8 liters of YPD medium to an A650 of 1.5. The cells were harvested (15.2 g, wet weight) and lysed as described above except that additional protease inhibitors were added to the lysis buffer: aprotinin (2 μg/ml), leupeptin (0.5 μg/ml), and pepstatin (0.7 μg/ml). The crude S30 extract was adjusted to 500 mM KCl and centrifuged for 2 h at 100,000 × g (4 °C). The supernatant was dialyzed at 4 °C against 5 liters of buffer H containing 20% glycerol. The underlines regions are PstI and NcoI sites; P1 corresponds to the region surrounding the initiation codon (−5 to +20, where +1 is the A of the initiation codon AUG). Primer 2 (P2) is 5′-CCCATGCAGCGCCCATGGTCCCTAAAGATAAAAGG-3′. The resulting recombinant plasmid pSP72-HIF11 was digested with HindIII and EcoRI and blunted-ended with Klenow DNA polymerase; the resulting 470-bp fragment was subcloned into the blunt-ended BamHI site of pHSX3 to yield pW-h1A. inserts with the correct orientation were identified by restriction enzyme cleavage patterns (results not shown). E. coli strain HB101 was used to propagate the plasmids.

Disruption of the Chromosomal TIF11 Gene—To remove the Hpl restriction site in pSP72-TIF11, the plasmid was altered by digestion with BglII and BamHI, which flank the Hpal site, and the ends were filled in with Klenow DNA polymerase followed by blunt-ended ligation. The resulting recombinant plasmid pSP72a-TIF11 was digested with Spel and Hpal to remove a 414-bp fragment carrying 84% of the TIF11 coding region plus 27 nucleotides upstream of the AUG. The remaining 4.5-kb fragment was gel purified, the ends were filled in with Klenow DNA polymerase, and a blunt-ended DNA fragment containing the H513 gene was inserted by ligation to generate pSP72a-tif11::H513. To prepare the H513 insert, a 1.75-kb BamHI fragment carrying the entire yeast H513 gene was isolated from plasmid pHY1 (23), and the ends were filled in with Klenow DNA polymerase. pSP72a-tif11::H513 was digested with EcoRI to generate a 3.8-kb fragment carrying tif11::H513. The DNA fragment contains 1.4 and 0.6 kb of flanking DNA 5′ and 3′ to the TIF11 gene, respectively. The fragment was transformed into the diploid yeast strain W303D to create a one-step gene deletion/disruption (29). Stable His+ transformants were selected, and the disruption of one of the TIF11 genes was confirmed by Southern blot analyses (results not shown).

RESULTS

Detection and Purification of the Yeast Homologue of eIF1A—Given the fact that translational factors are very conserved from yeast to humans, it is reasonable to employ an immunoblot analysis using anti-human eIF1A antibodies to detect the corresponding yeast eIF1A protein. As shown in Fig. 1A, a single polypeptide migrating at approximately 22 kDa was detected by SDS-PAGE in a wild type W303-1A yeast cell lysate. After fractionating the yeast lysate into a low salt post-
A 3-fold stimulation of methionyl-puromycin synthesis was obtained, which compares favorably with stimulations seen with the purified recombinant human eIF1A (Fig. 2). Both proteins stimulate the assay to approximately the same extent and require the same amount of protein for maximal effect. The moles of eIF1A required to saturate the assay approximate the moles of ribosomes present, suggesting that eIF1A functions bound to ribosomes. The moles of methionyl-puromycin formed are quite low compared to the moles of initiation factors added; such results are routinely obtained in this assay system both for eIF1A and for other initiation factors (5, 28) and may reflect more the activity of the ribosomes than that of the initiation factor being assayed. These results clearly demonstrate that the purified yeast protein possesses eIF1A activity when tested in vitro.

The Cloning of a cDNA and GenomicDNAs Encoding Yeast eIF1A—Having demonstrated an antibody-cross-reacting yeast protein with eIF1A activity, we set about to clone its gene. Immunoscreening of a $\lambda gt11$ yeast cDNA expressionlibrary with an affinity-purified rabbit anti-human eIF1A polyclonal antibody yielded one positive clone from approximately $6.5 \times 10^9$ recombinant phage plaques as described under “Materials and Methods.” The phage was plaque-purified, and PCR analysis revealed the presence of a 600-bp insert, which is sufficient in length to encode eIF1A. The insert was subcloned into M13 mp18 and mp19 for sequencing of both strands (see “Materials and Methods”), and the region encoding a 153-amino acid protein was identified (the cDNA sequence is not shown, but see Fig. 3). The 0.6-kb cDNA insert was $^{32}$P labeled and used as a probe to screen a yeast genomic library as described under “Materials and Methods.” Four positive clones from $6 \times 10^5$ plaques were plaque purified for further study. Characterization of the four clones by restriction enzyme mapping and partial sequencing (data not shown) indicated that they carry portions of the same DNA. A 2.4-kb EcoRI DNA insert was then excised from one of the recombinant $\lambda$ phages, and DNA encoding eIF1A was localized on a 1.1-kb HindII -EcoRI fragment by Southern blot analysis. Sequence analysis (Fig. 3) confirmed that the genomic 1.1-kb clone contains an open reading frame encoding the same 153-residue protein as found in the cDNA clone. The first in-frame AUG codon is located at residues 92-94 and possesses the sequence $5'-AUCAUGG-3'$, which is compatible with the yeast consensus context, A(A/U)AuG (30). We have named the gene $TIF11$ (translation initiation factor 1A) based on the similarity of its encoded protein to human eIF1A (see below).
human proteins are very similar throughout the entire structure (Fig. 4). They share 65% sequence identity and 76% similarity, which reinforces the view that the derived amino acid sequence for yeast eIF1A is aligned below.

The DNA sequence of the HindII--EcoRI fragment, which contains the coding region of TIF11 and its flanking sequences, was determined as described under "Materials and Methods." The derived amino acid sequence for IF1A is aligned below. Residue numbers for nucleotides and amino acids are shown on the right. The reported sequences as well as another 1.3 kb of DNA sequence upstream are available from GenBank under accession number U11585.

human proteins are very similar throughout the entire structure (Fig. 4). They share 65% sequence identity and 76% similarity, which reinforces the view that TIF11 encodes yeast eIF1A. The strong conservation of structure also is seen in the hydrophobicity profiles (results not shown). Both proteins contain basic N-terminal domains (29 mol % Lys + Arg in the first 42 amino acids for human eIF-1A, 30 mol % Lys + Arg in the first 40 amino acids for yeast eIF1A) and acidic C-terminal domains (54 mol % Asp + Glu in the C-terminal 28 amino acids for human eIF1A, 54 mol % Asp + Glu in the C-terminal 37 amino acids for yeast eIF1A). The highly charged terminal domains may be responsible for the apparent slow mobility of yeast eIF1A upon SDS-PAGE (apparent mass of 22 kDa versus a calculated mass of 17.4 kDa). The major difference between the yeast and mammalian proteins is the "insertion" of 8 amino acids in the yeast protein between residues 131 and 132 in human eIF1A. Comparison of the predicted amino acid sequence of yeast eIF1A with protein sequences in the GenBank and EMBL data banks indicate no significant amino acid sequence homology to other known proteins except human eIF1A. However, another gene is located upstream from TIF11 in the original 2.4-kb cloned genomic fragment. By searching the data bases, we determined that this gene encodes a homolog of the CIF1 gene (GenBank accession no. M88172). CIF1 encodes trehalose-6-phosphate synthetase in yeast and is required for cells to grow on glucose.

The number of TIF11 genes was investigated by Southern blot analyses of genomic DNA. The generation of a single 32P-labeled band in every one of the nine different restriction digests (results not shown) is consistent with a single copy gene. However, the analyses do not rule out the possibility of other genes encoding eIF1A that have diverged sufficiently to evade detection under the conditions used here.

eIF1A is Essential for Cell Viability—Although eIF1A stimulates assays for initiation in vitro, it was not known if it is essential for protein synthesis and/or cell viability in intact eukaryotic cells. We therefore generated a null mutant strain in which TIF11 is substantially deleted and is disrupted by the His3 gene. The plasmid pSP72a-tif11::HIS3 was constructed and was used to disrupt TIF11 in the diploid strain W303D as described under "Materials and Methods." About 84% of the coding region of TIF11 is deleted, and the 3.8-kb tif11::HIS3 EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene). The EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene). The EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene). The EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene). The EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene). The EcoRI-linearized fragment contains 1.4 and 0.6 kb of TIF11 flanking sequences separated by the open reading frame (but lacking the TIF11 gene).

![Fig. 3. Sequence of TIF11. The DNA sequence of the HindII--EcoRI fragment, which contains the coding region of TIF11 and its flanking sequences, was determined as described under "Materials and Methods." The derived amino acid sequence for eIF1A is aligned below. Residue numbers for nucleotides and amino acids are shown on the right. The reported sequences as well as another 1.3 kb of DNA sequence upstream are available from GenBank under accession number U11585.](http://www.jbc.org/content/279/15/22791.full.html)
haploid strain (Fig. 6B, upper row). This fact rules out the possibility that the 2:2 segregation pattern seen when CMD1 was sporulated (Fig. 5B) could be due to down-regulation of the adjacent CIF homolog gene, since pW-y1A does not carry the CIF homolog gene. A haploid cell colony containing the tif11::HIS3 allele and plasmid pW-y1A was selected and named CM1.

If eIF1A is essential for cell viability, the His-Trp spores will germinate and grow on plates with galactose medium, which induces eIF1A expression, but should not grow when transferred to plates with glucose medium. On the other hand, if eIF1A is required only for germination, the germinated spore colonies will continue to grow on glucose medium. When the spore colonies were streaked on glucose-containing SD-his or SD-trp plates, growth ceased (Fig. 6B, bottom row). The results show that TIF11 is required for cell growth and viability.

Human eIF1A cDNA Complements Yeast tif11::HIS3—The amino acid sequences of human and S. cerevisiae eIF-1A share 65% identity and 76% similarity. The fact that yeast eIF1A functions as well as human eIF1A in in vitro methionylpyruvomycin synthesis assay reconstituted with mammalian components strongly suggests that the two proteins are functionally equivalent. Since TIF11 is essential for yeast cell growth, we are able to use the no-growth phenotype to test if the human cDNA can replace the yeast gene in vivo. cDNA encoding the entire human eIF1A open reading frame was placed under control of the yeast GAL1 promoter in plasmid pHSX3 as described under “Materials and Methods.” The resulting plasmid pW-h1A was introduced into the diploid strain CMD1 to yield CMD3. The diploid strain was sporulated, and dissected spores were germinated on YPG plates, which allow the expression of the human form of eIF1A. The appearance of viable to nonviable spores in the ratio of 2:2, 3:1, and 4:0 (Fig. 7A) resembles that obtained when yeast TIF11 is expressed in vivo.

DISCUSSION

We report here the purification of a yeast initiation factor called eIF1A and the cloning of its gene, TIF11. Several lines of
may speculate that the N terminus is responsible for the RNA binding property of eIF1A (27). The yeast eIF1A sequence exhibits no significant homology to other proteins (other than human eIF1A) in the data bases. eIF1A is one of the most conserved proteins among the initiation factors. Sequence identities between the yeast and mammalian initiation factors range from 26% for eIF4B (15, 16) and 33% for eIF4A (17), representing the least conserved proteins, to 65% for eIF4A (31) and 71% for eIF2γ (32), the latter being the most conserved initiation factor known at this time. Thus, initiation factors are nearly as conserved as ribosomal proteins where the sequence identity between all cognate yeast and mammalian ribosomal proteins is 60%, with individual proteins falling in the range from 40 to 88% (33).

The similar primary structures of yeast, mammalian, and plant eIF1A are manifested in their biochemical activities. Either yeast or wheat germ eIF1A (6) functions in place of mammalian eIF1A in an in vitro assay for initiation based on mammalian components. Furthermore, the human cDNA encoding eIF1A complements a yeast strain lacking a functional TIF11 gene, indicating that the human protein functions in vivo with yeast components of the translational machinery. Many other mammalian cDNAs can relieve a growth defect of cells where the corresponding yeast gene has been disrupted. For example, the effects of disruption of SU12, SU13, TIF51A/B, and CDC33 are reversed by expressing the cDNAs for eIF2ε, eIF2δ, eIF5A, and eIF4A, respectively (16, 23). Only in the case of eIF4A does the mammalian cDNA fail to relieve disruption of TIF1 and TIF2 (31).

Most initiation factor proteins in yeast are essential for cell growth and viability. The only exceptions known to date are the α-subunit of eIF2B (GCN3) (34) and eIF4B (TIF3) (15, 16), where cells grow in the absence of the protein, albeit more slowly. The cloning of TIF1 and the demonstration that it is essential for cell growth will allow us to address the function of this protein by genetic and biochemical studies. Both approaches likely will be required to explain the pleiotropic effects of this small yet essential initiation factor.

Acknowledgments—We thank Susan MacMillan for performing the methionyl-puromycin synthesis assays, Elizabeth Shuster for advice on sporulation procedures, and Charles Moehle for helpful comments on the manuscript.

REFERENCES
1. Merrick, W. C. (1992) Microbiol. Rev. 56, 291–315
2. Hershey, J. W. B. (1991) Annu. Rev. Biochem. 60, 717–754
3. Kemper, W. M., Berry, K. W., and Merrick, W. C. (1976) J. Biol. Chem. 251, 5551–5557
4. Schröer, M. H., Erni, B., and Staehelin, T. (1977) J. Mol. Biol. 116, 727–753
5. Benne, R., Brown-Luedi, M.-L., and Hershey, J. W. B. (1978) J. Biol. Chem. 253, 3070–3077
6. Tilmers, R. T., Lax, S. R., Hughes, D. L., Merrick, W. C., Ravel, J. M., and Brown-Luedi, M.-L., and Hershey, J. W. B. (1978) J. Biol. Chem. 253, 3070–3077
7. Hershey, J. W. B. (1989) J. Biol. Chem. 264, 20823–20826
8. Thomas, A. A. M., Benne, R., and Voorma, H. O. (1981) FEBS Lett. 128, 177–185
9. Linder, P., and Prat, A. (1990) BioEssays 12, 519–526
10. Cigan, A. M., Pabich, E. K., Fang, L., and Donahue, T. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2784–2788
11. Donahue, T. F., Cigan, A. M., Pabich, E. K., and Valavidus, B. C. (1988) Cell 54, 621–632
12. Nanninga, E. M., Cigan, A. M., Freeman, B. A., and Kinzy, T. G. (1993) Mol. Cell. Biol. 13, 506–520
13. Bushman, J. L., Asu, A. I., Mats, R. L., and Kinzy, T. G. (1993) Mol. Cell. Biol. 13, 1920–1932
14. Linder, P., and Slonimski, P. P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2286–2290
15. Coppolecchia, R., Buser, P., Stotz, A., and Linder, P. (1993) EMBO J. 12, 4005–4011
16. Altmann, M., Müller, P., Wittmer, B., Ruchti, F., Lanker, S., and Trachsel, H. (1993) EMBO J. 12, 3907–4003
17. Altmann, M., Händschin, C., and Trachsel, H. (1987) Mol. Cell. Biol. 7, 998–1003
22794

Cloning of Yeast eIF1A

18. Goyer, C., Altmann, M., Lee, H. S., Blanc, A., Deshmukh, M., Woolford, J. L., Trachsel, H., and Sonenberg, N. (1993) Mol. Cell. Biol. 13, 4860–4874
19. Chakravarti, D., Maiti, T., and Maitra, U. (1993) J. Biol. Chem. 268, 5754–5762
20. Schnier, J., Schweberger, H. G., Smit-McBride, Z., Kang, H. A., and Hershey, J. W. B. (1991) Mol. Cell. Biol. 11, 3105–3114
21. Dever, T. E., Wei, C. L., Benkowski, L. A., Browning, K., Merrick, W. C., and Hershey, J. W. B. (1994) J. Biol. Chem. 269, 3212–3218
22. Wei, C.-L., MacMillan, S. C., and Hershey, J. W. B. (1995) J. Biol. Chem. 270, 5764–5771
23. Schweberger, H. G., Kang, H. A., and Hershey, J. W. B. (1989) J. Biol. Chem. 264, 14018–14025
24. Rose, M. D., Wisston, F., and Hieter, P. (1990) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
25. Struhl, K. (1985) Nucleic Acids Res. 13, 8587–8601
26. Laemmli, U. K. (1970) Nature 227, 680–685
27. Lazaris-Karatzas, A., Montine, K. S., and Sonenberg, N. (1990) Nature 345, 544–547
28. Smit-McBride, Z., Schnier, J., Kaufman, R., and Hershey, J. W. B. (1989) J. Biol. Chem. 264, 18527–18530
29. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–211
30. Cigan, A. M., and Donahue, T. F. (1987) Gene (Amst.) 59, 1–18
31. Prat, A. (1990) Biochim. Biophys. Acta 1050, 140–145
32. Gaspar, N. J., Kinzy, T. G., Scherer, B. J., Hümbelin, M., Hershey, J. W. B., and Merrick, W. C. (1994) J. Biol. Chem. 269, 3415–3422
33. Wool, I. G., Chan, Y. I., Gluck, A., and Suzuki, K. (1993) Biochimie (Paris) 75, 861–870
34. Hannig, E. M., and Hinnebusch, A. G. (1988) Mol. Cell. Biol. 8, 4808–4820
35. Hill, J. E., Meyers, A. M., Koerner, T. J., and Tzagoloff, A. (1986) Yeast 2, 163–167
Characterization of Yeast Translation Initiation Factor 1A and Cloning of Its Essential Gene
Chia-Lin Wei, Mami Kainuma and John W. B. Hershey

J. Biol. Chem. 1995, 270:22788-22794.
doi: 10.1074/jbc.270.39.22788

Access the most updated version of this article at http://www.jbc.org/content/270/39/22788

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 18 of which can be accessed free at http://www.jbc.org/content/270/39/22788.full.html#ref-list-1