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TIF4631 and *TIF4632*: Two Yeast Genes Encoding the High-Molecular-Weight Subunits of the Cap-Binding Protein Complex (Eukaryotic Initiation Factor 4F) Contain an RNA Recognition Motif-Like Sequence and Carry Out an Essential Function

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The 5′ ends of eukaryotic mRNAs are blocked by a cap structure, m^{7}GpppX (where X is any nucleotide). The interaction of the cap structure with a cap-binding protein complex is required for efficient ribosome binding to the mRNA. In *Saccharomyces cerevisiae*, the cap-binding protein complex is a heterodimer composed of two subunits with molecular masses of 24 (eIF-4E, CDC33) and 150 (p150) kDa. p150 is presumed to be the yeast homolog of the p220 component of mammalian eIF-4F. In this report, we describe the isolation of yeast gene *TIF4631*, which encodes p150, and a closely related gene, *TIF4632*. TIF4631 and TIF4632 are 53% identical overall and 80% identical over a 320-amino-acid stretch in their carboxy-terminal halves. Both proteins contain sequences resembling the RNA recognition motif and auxiliary domains that are characteristic of a large family of RNA-binding proteins. *tif4631*-disrupted strains exhibited a slow-growth, cold-sensitive phenotype, while disruption of *TIF4632* failed to show any phenotype under the conditions assayed. Double gene disruption engendered lethality, suggesting that the two genes are functionally homologous and demonstrating that at least one of them is essential for viability. These data are consistent with a critical role for the high-molecular-weight subunit of putative yeast eIF-4F in translation. Sequence comparison of TIF4631, TIF4632, and the human eIF-4F p220 subunit revealed significant stretches of homology. We have thus cloned two yeast homologs of mammalian p220.

The 5′-terminal cap structure m^{7}GpppX (where X is any nucleotide) is required for efficient mRNA translation and plays a prominent role in translational control. This ubiquitous feature of eukaryotic mRNAs is also important for nuclear events. Precursor mRNA splicing (23, 46) and 3′-end processing (26, 34) are enhanced by the presence of a cap structure. In addition, the cap structure protects the mRNA against 5′ exonucleolytic degradation in both the nucleus and the cytoplasm (25, 30) and is implicated in nucleocytoplasmic transport (33). The best-characterized role of the cap structure is its stimulatory effect on ribosome binding (for reviews, see references 66 and 82).

Binding of the ribosomes to the mRNA is thought to be the rate-limiting (42) and discriminatory step (64, 76) in translation. The function of the cap structure in ribosome binding is mediated by a multisubunit complex, termed the cap-binding protein (CBP) complex (eIF-4F) (for a review, see reference 22). eIF-4F, via an RNA-dependent helicase subunit (eIF-4A), is thought to stimulate ribosome binding by unwinding the secondary structure in the 5′ untranslated region (UTR) of mRNAs (for reviews, see references 37, 58, and 82). eIF-4B, an RNA-binding protein (55), functions in combination with eIF-4F and eIF-4A to promote unwinding. eIF-4F, in all of the species studied, is composed of at least two subunits: a 24-kDa polypeptide (eIF-4E) and a larger one ranging in size from 150 to 220 kDa. eIF-4E is the bona fide cap binding subunit, as it contains the cap-binding site (83). It is complexed to a 150-kDa subunit in *Saccharomyces cerevisiae* (28), a 180-kDa polypeptide in drosophila (52), and a 220-kDa polypeptide in both mammals (20, 31, 84) and wheat germ (14, 48). An isoform of eIF-4F composed of two subunits, 28 and 80 kDa, has also been identified in plants (15, 48). It was recently shown that yeast eIF-4E can associate independently with at least two polypeptides of 18 and 150 kDa to form CBP complexes (47).

The critical role of eIF-4F in the regulation of gene expression is illustrated by the findings that the eIF-4E subunit displays characteristic properties of a proto-oncogene (49) and is encoded by the essential *CDC33* gene in *S. cerevisiae* (4, 11). Significantly, mammalian eIF-4E can substitute for its yeast homolog in vivo (6), which implies that eIF-4F is evolutionarily conserved. eIF-4A is present in mammalian eIF-4F but absent from yeast or plant eIF-4F, suggesting a weak association between this component and the complex (28, 48, 78). eIF-4F lacking eIF-4A can also be purified from mammalian cells (50, 65).

The available data derived from translational studies with poliovirus-infected cells indicate that p220 is important for cap-dependent translation. Shutoff of host protein synthesis in poliovirus-infected cells has been linked to virus-induced cleavage of p220 (9, 81). Consistent with this, addition of intact eIF-4F to cell extracts prepared from poliovirus-infected cells rescues cap-dependent translation (21, 84).

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TABLE 1. Relevant *S. cerevisiae* strains and plasmids

Strain or plasmid	Description	Source or reference
Strains		
DC66	*MATα leu2-3 leu2-112 lys2 ade6 cry1*	69
S1502B	*MATa leu2-3 leu2-112 his3Δ1 trp1-289 ura3-52*	69
S288C	*MATα mal gal2*	69
EJ101	*MATα trp1 pro1-126 prb1-112 pep4-3 prc1-126*	51
TA405-1	*MATa leu2 his3 can1*	M. Whiteway
FW1318	*MATa/MATα leu2-3 leu2-112 ura3-52 his4-917δ* (isogenic)	F. Winston
YPH149	*MATa ura3-52 lys2-801 ade2-101 his7 trp1Δ1* CFVII (*RAD2*.p.YPH149) [CFVII (*RAD2*.d.YPH146.*TRP1*)]	27
YCG127	*MATa/MATα* (DC66 × S1502B)	This study
YCG161	YCG127 *tif4631::LEU2*	This study
YCG165	*MATa leu2-3 leu2-112 trp1-289 ura3-52 his3Δ1 ade6 tif4631::LEU2*	This study
YCG209	YCG165(YCp50)	This study
YCG212	YCG165(pYCG206)	This study
YCG297	FW1318 *tif4631::LEU2*	This study
YCG312	YCG297 *tif4632::URA3*	This study
Plasmids		
pYCG2	7.0-kb fragment in YEp13; original *TIF4631* clone from YEp13 library (12)	This study
pYCG3	9.0-kb fragment in YEp13; original *TIF4631* clone from YEp13 library (12)	This study
pYCG4	7.0-kb fragment in YEp13; original *TIF4631* clone from YEp13 library (12)	This study
pYCG6	7.0-kb fragment in YEp13; original *TIF4631* clone from YEp13 library (12)	This study
pYCG206	6.7-kb *Eco*RI fragment from pYCG3 cloned into *Eco*RI site of YCp50	This study
pYCG11	15.0-kb fragment in YCp50. Original *TIF4632* clone from YCp50 library (68)	This study
Relevant subclones used in *TIF4631* or *TIF4632* characterization		
pCG200	Original 200-bp cDNA cloned into *Eco*RI site of Bluescript	This study
pCG8.4	Original 3.266-kb cDNA cloned into *Eco*RI site of Bluescript	This study
pCG61	2.0-kb *Eco*RV fragment of pYCG4 cloned into *Eco*RI site of Bluescript	This study
pCG206	6.7-kb *Eco*RI fragment of pYCG3 cloned into *Eco*RI site of Bluescript	This study
pGLD	1.4-kb *Hin*dIII fragment of pYCG11 cloned into *Hin*dIII site of Bluescript	This study
Plasmids used for gene replacement		
pCG8.4::LEU	Replacement of 2.17-kb *Bgl*II fragment of pCG8.4 by 3.0-kb *Bgl*II fragment containing *LEU2*	This study
pCG206::HIS	Replacement of 3.66-kb *Bgl*II fragment of pCG206 by 1.8-kb *Bam*HI fragment containing *HIS3*	This study
pGLD::URA	Replacement of 1.1-kb *Hpa*I fragment of pGLD by 1.5-kb *Nru*I-*Sma*I fragment containing *URA3*	This study

The precise function of the high-molecular-weight subunit of eIF-4F has yet to be defined. Genetic studies of yeast p150 should contribute to our understanding of the function of this subunit in ribosome binding and translational control.

MATERIALS AND METHODS

Materials. Restriction endonucleases, exonuclease III, mung bean nuclease, a T7 polymerase sequencing kit, and oligo(dT)-cellulose type 7 were from P-L Biochemicals. Calf intestinal alkaline phosphatase, calf liver tRNA, and T4 DNA ligase were obtained from Boehringer Mannheim. DNase I, Moloney murine leukemia virus reverse transcriptase, bovine serum albumin, T3 RNA polymerase, and vaccinia virus guanylyltransferase were from Bethesda Research Laboratories. [α-^{32}P]dATP (>3,000 Ci/mmol), [α-^{35}S]dATP (>1,000 Ci/mmol), and [^{35}S]methionine (>1,000 Ci/mmol) were from Du Pont-New England Nuclear. Autoradiographic analysis was done with Kodak XAR-5 film. Deoxyoligonucleotides were synthesized by the Sheldon Biotechnology Institute at McGill University, Montréal, Québec, Canada.

Yeast strains and plasmids. The relevant yeast strains and plasmids employed in this study are shown in Table 1. General yeast techniques were done as described by Rose et al. (69).

Three plasmids containing deletions of *TIF4631* or *TIF4632* were constructed. A 2.17-kb *Bgl*II fragment (nucleotide positions 476 to 2644, shown in brackets in Fig. 2) was deleted from pCG8.4 and replaced by a 3.0-kb *Bgl*II frag-

ment from YEp13 harboring *LEU2* (pCG8.4::LEU2). The *LEU2*-disrupted *TIF4631* gene was isolated as a 4.1-kb *Hin*dIII-*Pst*I DNA fragment and used to transform diploid strain YCG127 by the lithium acetate method (69) to LEU^+ to generate strain YCG161. For the *tif4632*::*URA3* disruption, a 1.1-kb *Hpa*I fragment was deleted from pGLD (nucleotide positions 627 to 1730, shown in brackets in Fig. 5) and replaced by a 1.5-kb *Nru*I-*Sma*I fragment from YCp50 harboring *URA3*. The *tif4632*::*URA3* gene was isolated from pGLD::URA as a 1.8-kb *Hin*dIII fragment and used to transform diploid strain YCG297 to URA^+ to yield YCG312.

DNA methods. Preparation of plasmid DNA, DNA restrictions, agarose gel electrophoresis, transfer to nylon, and randomly primed probe synthesis were performed by standard methods (74). For high-stringency hybridization conditions, the blots were incubated at 65°C and washed in accordance with the instructions for Hybond-N (Amersham Corp.).

Yeast chromosomes were isolated from YPH149 as described by Rose et al. (69) and run alongside yeast DNA pulsed-field gel electrophoresis markers (P-L Biochemicals). The electrophoresis, transfer, and hybridization conditions used have been described previously (29).

RNA methods. mRNA isolation, electrophoresis, and Northern (RNA) blotting analysis were described previously (29). The high-stringency hybridization conditions used were in accordance with the instructions for Gene Screen Plus (Du Pont-New England Nuclear), and the dextran sulfate method was used.

The 5′ end of *TIF4631* mRNA was mapped by primer extension. A 5′-end ^{32}P-labeled 17-mer (10 ng of oligonucleotide CG8 that is complementary to nucleotide positions 81 to 97; see Fig. 2) was mixed with poly(A)$^+$ RNA from strain S288C (2 μg) and hybridized at 37°C as described by Beauchemin et al. (8). Hybridizations at 42, 50, and 55°C gave similar results (data not shown). The primer was extended in a reaction mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM deoxynucleoside triphosphates, 800 U of RNasin (Promega) per ml, 0.1 mg of bovine serum albumin per ml, and Moloney murine leukemia virus reverse transcriptase [200 U/mg of poly(A)$^+$] in a total volume of 100 μl at 37°C for 1 h. The cDNA was then phenol extracted, precipitated in ethanol, and analyzed on an 8 M urea–5% polyacrylamide sequencing gel. A control experiment with 15 μg of calf liver tRNA was performed in parallel. To localize the end of the cDNA, a sequencing reaction was done with pCG206 double-stranded DNA and oligonucleotide CG8.

To synthesize *TIF4631* mRNA, pCG8.4 was linearized at the unique *Bam*HI site in the Bluescript polylinker. In vitro transcription with T3 RNA polymerase was done in accordance with the manufacturer's specifications. Preparation of cap-labeled mRNA for UV cross-linking was done as described by Goyer et al. (28).

Protein methods. In vitro translation was performed in a nuclease-treated rabbit reticulocyte lysate in accordance with the manufacturer's (Promega) specifications. The final concentration of mRNA was 1.4 μg/ml, and 80 mM KCl was added to the reaction mixture.

Yeast extract preparation and affinity purification of yeast cap-binding proteins were done as described by Goyer et al. (28), except that all solutions starting with the breaking buffer (buffer A in reference 49) were supplemented with 0.1 mM ATP and the protease inhibitors aprotinin (50 μg/ml), benzamidine (1 mM), leupeptin (20 μg/ml), pepstatin A (10 μg/ml), phenanthroline (1 mM), phenylmethylsulfonyl fluoride (1 mM), and soybean trypsin inhibitor (50 μg/ml). Photochemical cross-linking of yeast proteins to cap-labeled RNA, immunoblotting, and immunoprecipitation of CBPs with the anti-yeast CBP polyclonal antibody were previously described (28).

Cloning of *TIF4631* and *TIF4632*. Construction of the yeast cDNA library and its immunological screening were described elsewhere (4). The *TIF4631* gene was isolated by colony hybridization (32) by screening a yeast genomic library in YEp13 (12) with the 3,266-bp cDNA as the probe (pCG8.4; see below). Six overlapping clones were isolated, and four were further characterized (pYCG2 to pYCG6; Table 1). To isolate the *TIF4632* gene, a randomly primed probe derived from the 987-bp *Eco*RV-*Acc*I fragment of pCG61 (nucleotide positions 1320 to 2307; see Fig. 2) and corresponding to the cysteine-histidine-rich region of TIF4631 was used. A YCp50 yeast genomic library (68) was screened by colony hybridization (32) under low-stringency conditions (39). One of the genomic clones obtained, pYCG11, was shown not to encode *TIF4631* and was studied further.

DNA sequence analysis. Sequencing reactions were done by the method of Sanger et al. (75). To sequence *TIF4631*, genomic DNAs from the YEp13 clones were subcloned in Bluescript (Stratagene). Initial sequences were generated by using the M13 universal or reverse primers on these subclones. Subsequent sequences were obtained by using synthetic (17-mer) oligonucleotides. The entire *TIF4631* sequence (see Fig. 2) was determined for both strands by double-stranded sequencing. pCG200 (Table 1) was sequenced in both directions; partial sequencing data were also obtained from pCG8.4.

To sequence *TIF4632*, genomic DNA from YCp50 clone pYCG11 was subcloned in Bluescript. Plasmids pGLD (Table 1), pGLC (harboring a 2.0-kb *Hin*dIII fragment 3′ to the 1.4-kb fragment contained in pGLD), and pGLB7Δ (harboring a 2.5-kb *Eco*RI-*Hin*dIII fragment 5′ to the 1.4-kb fragment contained in pGLD) were subjected to the exonuclease III-mung bean nuclease deletion protocol (Stratagene) from both directions. Double-stranded DNAs from the subclones obtained were sequenced with the M13 universal or reverse primers. The entire sequence was determined on both strands, and the contiguities between the inserts of pGLB7Δ and pGLD and between those of pGLD and pGLC were verified by sequencing with synthetic oligonucleotides.

Fractionation and characterization of ribosomal subunits and polyribosomes. Extracts were prepared and analyzed by sucrose velocity gradient centrifugation as described by Deshmukh et al. (19).

Nucleotide sequence accession number. The nucleotide sequences in Fig. 2 and 5 have been submitted to the GenBank data base and assigned accession no. L16923 and L16924, respectively.

RESULTS

Isolation of the *TIF4631* gene. A screen of a λgt11 yeast cDNA library with an anti-CBP polyclonal antibody (3) yielded, apart from eIF-4E cDNAs (4), clones that encode several different polypeptides (1a, 5). One of these λgt11 recombinant phages, harboring a 200-bp insert, was further characterized by using the Olmsted method (60). Briefly, lysates of *Escherichia coli* infected with recombinant phage were fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose, and probed with the anti-CBP polyclonal anti-

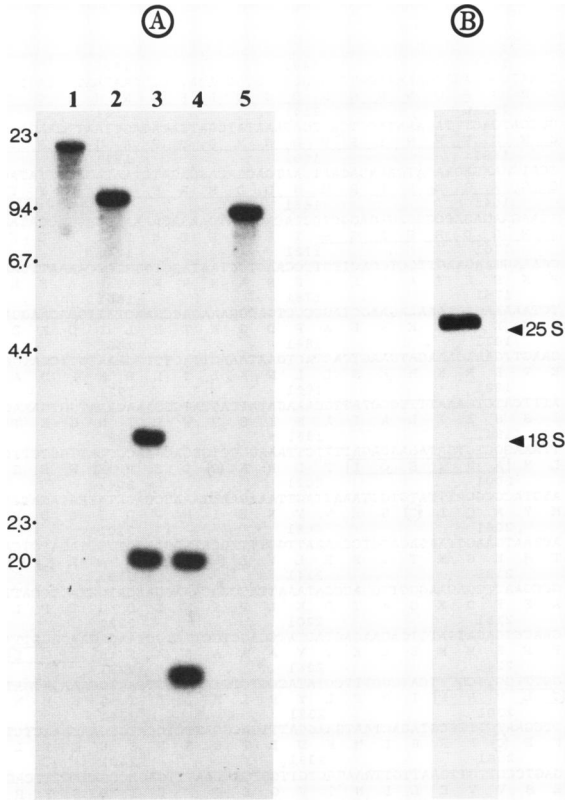

FIG. 1. p150 is encoded by a single-copy gene that gives rise to a 3,800-nt transcript. (A) Southern analysis. High-stringency Southern blotting analysis was performed as described in Materials and Methods. Genomic DNA (2 μg) isolated from strain S288C was cut with restriction enzymes, fractionated on a 1.2% agarose gel, and transferred to nylon membrane. The blot was hybridized at high stringency to the ^{32}P-labeled 3.266-kb cDNA and processed as described in Materials and Methods. The following restriction enzymes were used: *Eco*RI, lane 1; *Pst*I, lane 2; *Eco*RV, lane 3; *Eco*RV-*Hin*dIII, lane 4; *Bam*HI, lane 5. DNA standards are depicted on the left, and their sizes are expressed in kilobases. (B) Northern analysis. Northern analysis was carried out as described in Materials and Methods. Poly(A)$^+$ RNA (2 μg) isolated from strain S288C was hybridized to the ^{32}P-labeled 3.266-kb cDNA. The positions of yeast rRNAs are indicated by arrowheads on the right.

body. The bound antibodies were eluted from the nitrocellulose and used to probe a Western blot (immunoblot) of yeast extract. They reacted exclusively with a 150-kDa polypeptide (data not shown). Inasmuch as the yeast CBP polyclonal antibody recognizes the p150 subunit of yeast eIF-4F (28), these results suggest that the 200-bp insert encodes antigenic determinants of this high-molecular-weight subunit. Screening of the λgt11 cDNA library with the 200-bp insert yielded a 3.266-kb cDNA clone. High-stringency Southern blotting analysis of yeast genomic DNA suggested the existence of a single gene that encodes p150 in haploid cells (Fig. 1A). Two bands hybridized to *Eco*RV-restricted DNA (lanes 3 and 4) because of the presence of this site in the gene. Northern blot analysis identified a single mRNA band of approximately 3,800 nucleotides (nt) (Fig. 1B). To isolate the genomic copy of p150, a YEp13 yeast genomic library was screened by using the original 3.266-kb cDNA fragment as the probe, and six overlapping clones were obtained. Nucleotide sequencing revealed an open reading frame (ORF) of 952 amino acids (Fig. 2). The AUG at position 1 is most probably the initiator, since it is preceded by two termination codons in the same reading frame (positions −45 and −18). Surprisingly, the molecular weight predicted from this ORF is 107,000, considerably lower than the estimated 150,000 based on migration on SDS-polyacrylamide gels. The gene was designated *TIF4631* for translation initiation factor 4 (4 designates RNA-binding factors [73]); 6 (F, the sixth letter); 3 (γ subunit [73]); 1 (the first of two homologous genes [see below]).

***tif4631* gene disruption.** Gene disruption of one copy of *TIF4631* in diploid strain YCG127 was performed by replacing 76% of the ORF (shown in brackets in Fig. 2 at nucleotides 476 and 2644) with *LEU2*. Additionally, *HIS3* was used to replace all but 70 amino acids at the carboxy end of the single copy of *TIF4631* (starting at nucleotide 2645 in Fig. 2) in haploid strain TA405-1. The fidelity of the integration was confirmed by Southern analysis (data not shown). The *LEU2* gene disruption resulted in four viable meiotic products upon sporulation, indicating that the gene is not essential. Leu or His prototrophy, however, cosegregated with a slow-growth, cold-sensitive phenotype as the growth defect was accentuated at 18°C (see below; 47).

The growth defect of the *tif4631*::*LEU2* haploid cell (YCG165), derived from strain YCG127, could be complemented by introduction of a YCp50-based plasmid, pYCG206, harboring *TIF4631* (strain YCG212; generation time, 2 h) but not by YCp50 (strain YCG209; generation time, 4 h).

Characterization of the *TIF4631*-encoded protein. To provide evidence that *TIF4631* encodes p150, TIF4631 (with a predicted molecular weight of 107,000) was synthesized in vitro. Programming of a reticulocyte translation extract with in vitro-transcribed *TIF4631* RNA yielded a high-molecular-weight polypeptide (Fig. 3; compare lanes 1 and 2) that comigrates with cross-linked p150 (compare lanes 2 and 5). The specificity of the cross-linking to the cap structure is demonstrated by the finding that the cap analog m^{7}GDP inhibits the cross-linking of the 150-kDa polypeptide (lane 6). Additionally, in vitro-synthesized p150 could be specifically immunoprecipitated with the anti-CBP polyclonal antibody (lane 4), since no signal was observed with preimmune serum (lane 3). Finally, exonuclease III digestion of the 3′-terminal region of the 3.266-kb cDNA, followed by in vitro transcription-translation, was performed. The protein products obtained from in vitro translation of an mRNA deleted from position 2897 (39 nt downstream of the presumed termination codon) to position 3251 (the cDNA 3′ boundary), and of the wild-type *TIF4631* mRNA, comigrated on SDS-PAGE (data not shown; Fig. 2). This result shows that there is no readthrough of the predicted termination codon at position 2857 (Fig. 2) and that p150 migrates anomalously on SDS-polyacrylamide gels.

The *tif4631*-disrupted strain was used to further substantiate the conclusion that *TIF4631* encodes p150. Extracts were prepared from strains YCG209 and YCG212 and purified on an m^{7}GDP-agarose column. Immunoblotting analysis demonstrated that p150 was present in YCG212 but absent from YCG209 (Fig. 4A; compare lanes 1 and 3 with lanes 2 and 4). Consistent with this, while eIF-4F (p150 and eIF-4E) was purified by an m^{7}GDP-agarose column from extracts of YCG212 (Fig. 4B, lane 1), only free eIF-4E could be purified from YCG209 (lane 2). The photochemical cross-linking profile of these extracts obtained by using a ribosomal salt wash is shown in Fig. 4C (experiments with S-100 gave similar results). A ribosomal salt wash prepared from

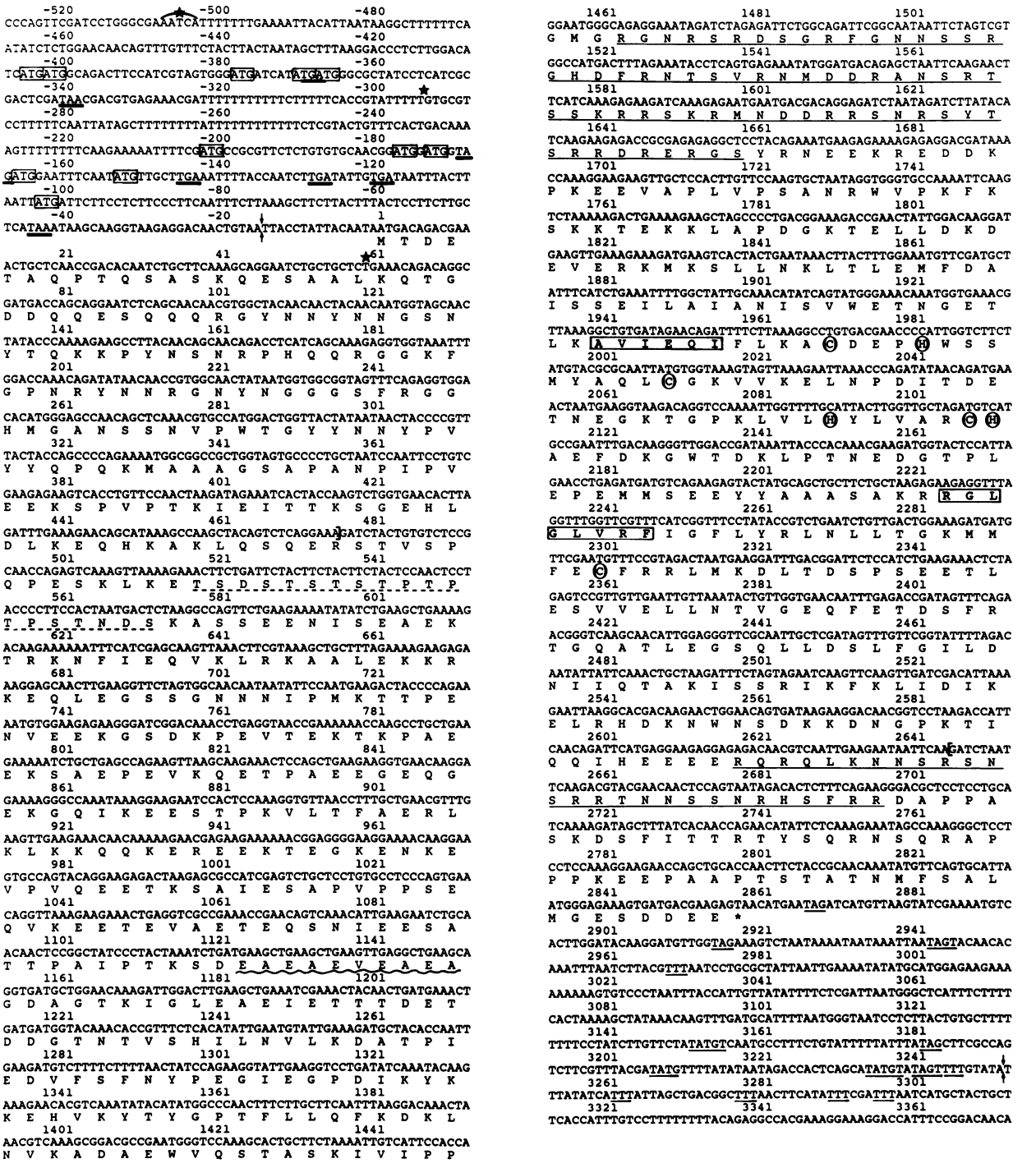

FIG. 2. Nucleotide and deduced amino acid sequences of the *TIF4631* gene. A 3.9-kb DNA region encompassing the *TIF4631* ORF is shown. Numbering starts at the predicted initiator AUG. Upstream AUGs are boxed, and termination codons are underlined. The stars indicate potential transcription start sites as determined by primer extension analysis (see below). The double arrows at positions −15 and 3,251 indicate the 5′ and 3′ ends of the 3.266-kb cDNA, respectively. In the 3′ UTR, sequences similar to the tripartite transcription termination signal described by Zaret and Sherman (88) are underlined. The brackets in the coding region (nt 476 and 2644) depict the boundaries of the *LEU2* gene disruption. Inside the coding region, a number of regions are highlighted as follows: – – – – – –, serine-threonine-proline-rich region reminiscent of the cdc2/CDC28 kinase phosphorylation consensus site; ∿∿, aspartate-alanine-rich region; underlining, arginine-serine-rich regions; boxes, RNP-1- and RNP-2-like domains; Ⓒ and Ⓗ, cysteine and histidine residues of the cysteine-histidine-rich region.

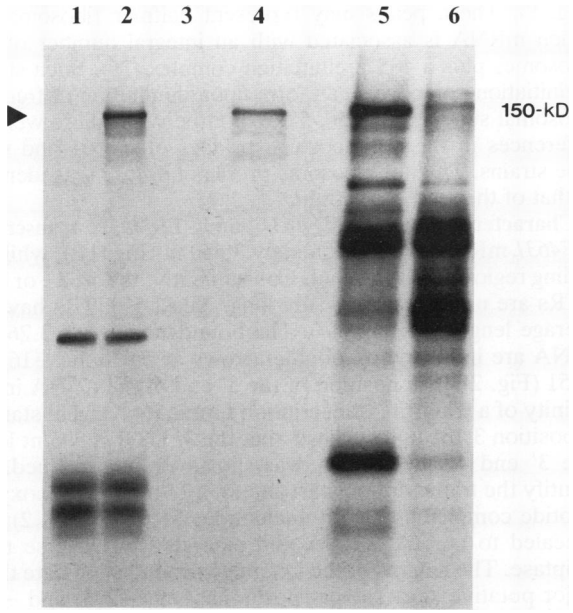

FIG. 3. The 107-kDa polypeptide encoded by *TIF4631* migrates aberrantly on SDS-PAGE and is specifically immunoprecipitated by the anti-CBP antibody. *TIF4631* RNA was transcribed in vitro as described in Materials and Methods and used to program a reticulocyte translation extract. Translation was done for 60 min at 30°C. Aliquots (5 μl) were subjected to SDS–12.5% PAGE. Gels were processed for autoradiography, dried, and exposed against X-ray film for 3 days. Lanes: 1, negative control RNA; 2, *TIF4631* RNA. The extract from lane 2 was immunoprecipitated with preimmune sera (lane 3) or an anti-CBP polyclonal antibody (lane 4) as described by Goyer et al. (28). UV-induced cross-linking of yeast extract to α-^{32}P-cap-labeled RNA in the absence (lane 5) or in the presence (lane 6) of the cap analog m^{7}GDP was performed as described by Goyer et al. (28).

YCG209 failed to show p150 cross-linking (lanes 1 to 3), while CBPs of 18 (5, 47), 24 (eIF-4E) (3, 28), 93 (10, 28), and 64 (possibly eIF-4B; 29a) kDa were specifically labeled by the radioactive cap structure (compare lanes 1 and 3 with lane 2). However, cross-linking of p150 was evident in extracts from YCG212 (lane 4) and was inhibited by m^{7}GDP (lane 5) but not by GDP (lane 6). It is noteworthy, however, that cap-specific polypeptides migrating slower and faster than p150 (indicated by dots) were observed in extracts prepared from both strains YCG209 and YCG212. The possible identity of these CBPs is described below. These results demonstrate that *TIF4631* encodes p150 and that although it is not essential for growth under laboratory conditions, it is required for normal cell growth.

Isolation and characterization of the *TIF4632* gene. The viability of *tif4631* strains and the observation of high-molecular-weight proteins cross-linked to the cap structure in extracts prepared from these strains raised the possibility that a functionally homologous gene was present. We reasoned that the clustering of all four cysteines, and a number of histidine residues in a small region of p150 (circled in Fig. 2), could be functionally important. A DNA fragment from this region was therefore used to probe genomic DNA under low-stringency hybridization conditions (39). The Southern blot suggested the presence of a related gene (data not shown). Consequently, a YCp50 genomic library was screened under the same conditions and a clone encoding a *TIF4631*-related gene was isolated and termed *TIF4632*. The sequence of *TIF4632* is shown in Fig. 5. The AUG at position 1 is presumed to be the initiator, as it is preceded by an in-frame termination codon (position −48). In addition, the predicted amino-terminal sequence of TIF4632 is homologous to that of TIF4631 (MTDE/MTDQ).

An alignment of TIF4631 and TIF4632 sequences is shown in Fig. 6. The 914-amino-acid protein encoded by *TIF4632* (with a predicted molecular weight of 104,000) is 53% identical to the product of *TIF4631*, with the most conserved region in the carboxy-terminal half. In the region encompassing the cysteine-histidine-rich domain (amino acids 565 to 800 of TIF4631; the cysteine and histidine residues are boxed, and the ribonucleoprotein consensus-like sequences, RNP-1 and RNP-2 [see below], are boxed and shaded), the homology is 90%.

A *tif4631 tif4632* double mutant is inviable. Gene disruption was carried out to investigate the importance of *TIF4632* for cell growth. By using *URA3* as a selectable marker, we replaced 44% of the ORF of *TIF4632* (shown in brackets in Fig. 5 at nucleotides 627 and 1730), transformed *tif4631*::*LEU2* diploid strain YCG297, and selected Ura$^+$ transformants. Replacement at the *TIF4632* locus was confirmed by Southern blot analysis (data not shown). The results of tetrad analysis of the double gene disruption strain (YCG312, containing the disrupted genes *tif4631*::*LEU2* and *tif4632*::*URA3*) are shown in Fig. 7. *tif4632* gene-disrupted cells displayed no detectable phenotype (Fig. 7A and B; *TIF4632*::*URA3* cells are indicated by the letter U [for Ura$^+$]). Plating of *tif4632* cells on synthetic or rich media with different carbon sources and at various temperatures failed to show any obvious growth defect (data not shown). As mentioned above, cells harboring the *tif4631* null allele showed a slow-growth phenotype (Fig. 7A, cells indicated by the letter L [for Leu$^+$] in panel B). However, disruption of both genes caused lethality, as the segregation pattern of the Leu and Ura markers shows that every dead spore carried the *LEU2* and *URA3* genes. If *TIF4631* and *TIF4632* are unlinked genes, the expected ratio of parental ditypes to nonparental ditypes is one. The data in Table 2 confirm that *TIF4631* and *TIF4632* are unlinked, since the difference between the numbers of parental and nonparental ditypes (six versus nine) is not significant (79). Were the double gene disruption viable, 25% of the germinated spores would be expected to be Leu$^+$ Ura$^+$. However, no germinated spore showing a Leu$^+$ Ura$^+$ phenotype was observed in a total of 36 tetrads (Table 2 and Fig. 7B), while 39 (27%) of 144 germinated spores were Leu$^-$ Ura$^-$ (Table 2 and Fig. 7A and B). These data demonstrate that *TIF4631* and *TIF4632* are homologous and that the presence of one of them is essential for viability.

The *tif4631* mutant is deficient in 60S ribosomal subunits. To determine whether the absence of TIF4631 or TIF4632 affects protein synthesis or the protein synthetic machinery, we assayed the levels of 40S and 60S ribosomal subunits, 80S monoribosomes, and polyribosomes in extracts of wild-type, *tif4631*, and *tif4632* strains. *tif4631* cells accumulated fewer free 60S ribosomal subunits relative to 40S ribosomal subunits than did wild-type cells, but *tif4632* cells contained wild-type ratios of ribosomal subunits (Fig. 8). A further decrease in the ratio of 60S to 40S subunits was observed in cold-sensitive strain *tif4631* shifted from 30 to 15°C for 2 h, while a lesser effect was observed in both the wild type and strain *tif4632* shifted to 15°C (data not shown). The polyribosome profile of *tif4631* cells grown at 30°C differed from that of wild-type cells in that additional discrete peaks were observed sedimenting slightly faster than polyribosomes containing two or three ribosomes

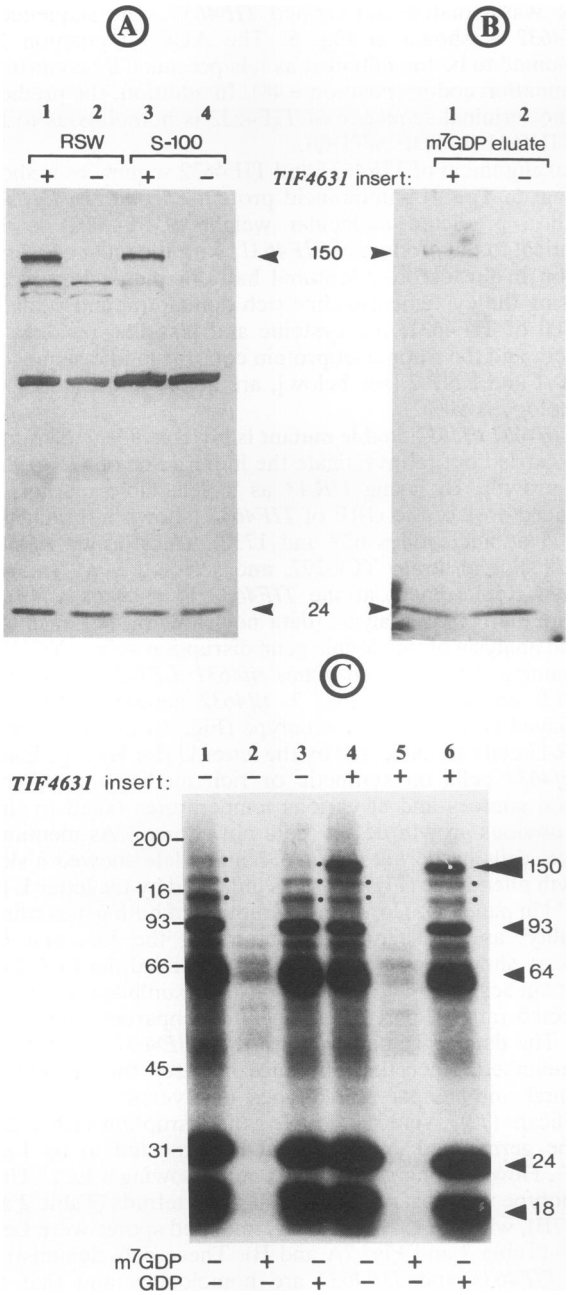

FIG. 4. *TIF4631* encodes p150. (A) Immunoblotting of yeast extracts. Yeast extracts (ribosomal salt wash or S-100, lanes 1 to 4) derived from strains YCG209 and YCG212 were immunoblotted with the anti-CBP polyclonal antibody as described in Materials and Methods. Samples of protein were loaded (70 and 125 μg in lanes 1 and 2 and 3 and 4, respectively). Strain YCG212 (*tif4631*::*LEU2* [YCp50 with a *TIF4631* insert; pYCG206]), odd-numbered lanes. Strain YCG209 (*tif4631*::*LEU2* [YCp50 minus insert]), even-numbered lanes. (B) m^7GDP-agarose chromatography of p150. Purification of CBPs by m^7GDP affinity chromatography with S-100 was done as described in Materials and Methods, and the gel was Coomassie blue stained. Equal amounts of proteins were loaded in lanes 1 and 2 (lane 1, YCG212; lane 2, YCG209). The 150- and 24-kDa (eIF-4E) CBPs that constitute yeast eIF-4F are indicated by arrowheads between panels A and B. (C) Photochemical cross-linking. UV cross-linking of ribosomal salt washes prepared from YCG209 (lanes 1 to 3) and YCG212 (lanes 4 to 6) and processing of samples for SDS-PAGE and autoradiography were performed as described in reference 28. Where indicated, the cross-linking mixture included cap analogs GDP and m^7GDP at 0.65 mM each. The different yeast CBPs are indicated by arrowheads on the right. Cap-specific cross-linking polypeptides running below or at the level of the 150-kDa CBP are highlighted by dots (lanes 1, 3, 4, and 6). Molecular mass standards (sizes are expressed in kilodaltons) are indicated on the left.

(Fig. 8). These peaks may represent halfmer ribosomes, in which mRNA is associated with an integral number of 80S ribosomes plus a 48S preinitiation complex (36). Such stalled preinitiation complexes can form upon diminution of free 60S ribosomal subunits (19, 56, 57, 67). Otherwise, there were no differences in the polyribosome profiles of *tif4631* and wild-type strains. The polyribosome profile of *tif4632* was identical to that of the wild-type strain.

Characterization of *TIF4631* and *TIF4632* transcripts. *TIF4631* mRNA is approximately 3,800 nt (Fig. 1B), while its coding region spans 2,859 nt. Consequently, the 5′, 3′, or both UTRs are uncharacteristically long. Yeast 5′ UTRs have an average length of 52 nt (17). The boundaries of the 3.266-kb cDNA are indicated by double arrows at positions −16 and 3,251 (Fig. 2). The position of the 3′ end of the cDNA in the vicinity of a tripartite transcription termination signal starting at position 3,181 (88) suggests that the 3′ UTR is 393 nt long. The 3′ end of the mRNA was, however, not defined. To identify the transcription start site(s), a 17-mer oligodeoxynucleotide complementary to nucleotides 81 to 97 (Fig. 2) was annealed to poly(A)$^+$ RNA and extended by reverse transcriptase. The lengths of the extended products indicate three major putative start sites at positions +59, −295, and −508 (Fig. 9; indicated by stars in Fig. 2). The two putative transcription start sites at −295 and −508 are consistent with the results of a Northern blot analysis with a −422 to −74 probe (Fig. 2) which detected the *TIF4631* 3,800-nt transcript (data not shown) and indicate that the stop of reverse transcription inside the coding region (position 59) is artifactual. However, the distinction between the start sites at −295 and −508 will have to be determined by S1 analysis. Nonetheless, it is evident that the *TIF4631* 5′ UTR is uncharacteristically long and contains numerous upstream AUGs (boxed in Fig. 2). Northern blot analysis showed a *TIF4632* transcript of approximately 3,600 nt (data not shown). As with *TIF4631*, the *TIF4632* transcript showed an uncharacteristically long UTR(s) since its ORF is 2,745 nt long (Fig. 5). Sequences similar to the tripartite transcription termination signal described by Zaret and Sherman (88) were observed downstream of the *TIF4632* termination codon (underlined in Fig. 5).

***TIF4631* and *TIF4632* are localized to chromosome VII.** Chromosome localization of the two homologous genes was determined by probing a chromoblot. Use of strain YPH149 (27) permitted unambiguous mapping of both genes to the RAD2-proximal arm of chromosome VII (Fig. 10, lanes 2 and 4). Blotting of an ordered array of lambda clones harboring yeast DNAs that have been mapped in the yeast genome (66a) refined the map position of *TIF4631* to the *RSR1* region and that of *TIF4632* to a region between *TRP5* and *RAD6*. Genetic mapping showed that *tif4631* is tightly linked to *kre11*, since all 14 tetrads of a *kre11-tif4631* cross were parental ditypes (12a). *kre11* has been genetically mapped to the *RSR1* region, 5 centimorgans centromere proximal to *ade3* on the right arm of chromosome VII (13).

***TIF4631* and *TIF4632* encode yeast homologs of mammalian p220.** Protein sequence comparison of TIF4631 with human

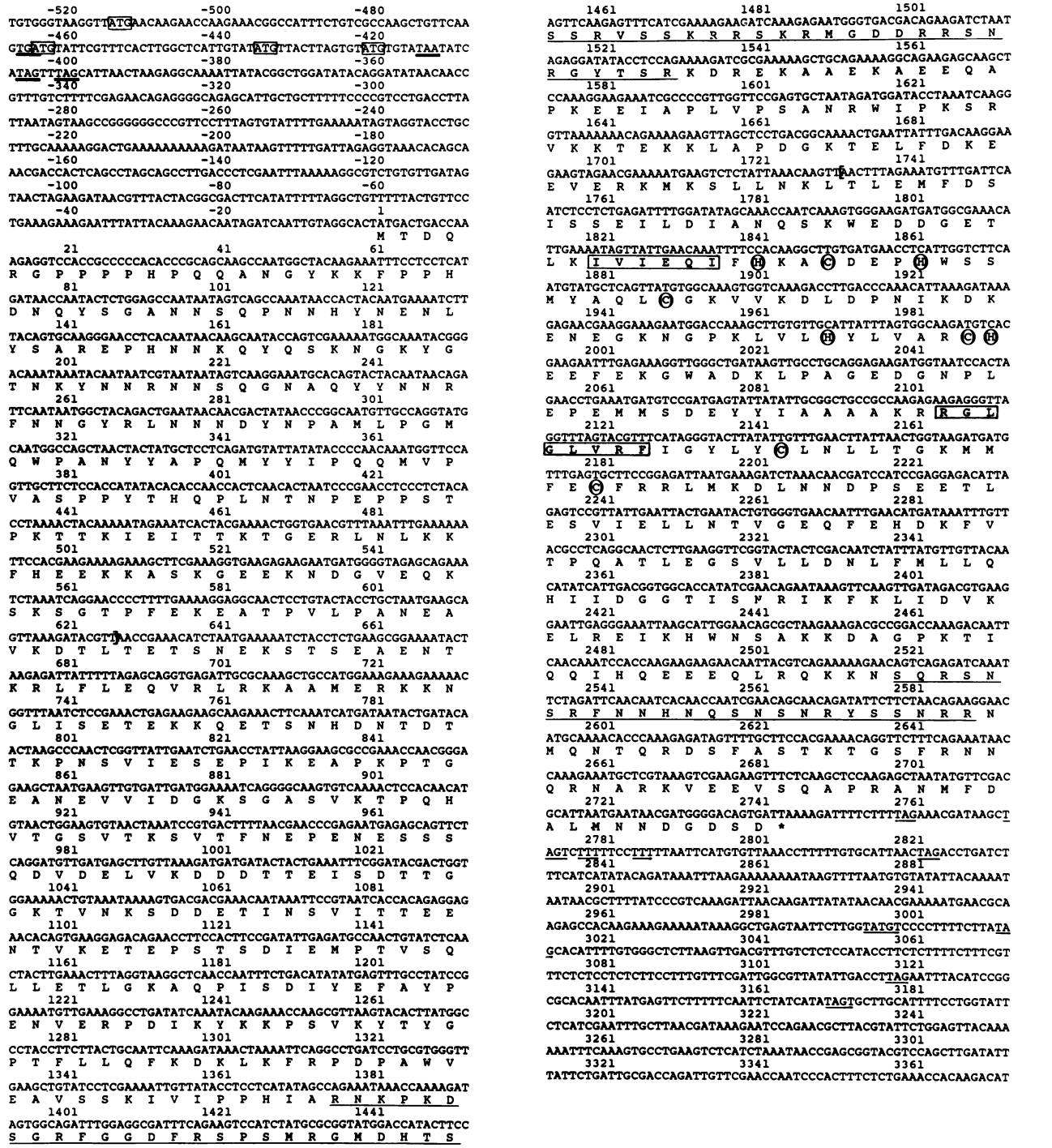

FIG. 5. Nucleotide and deduced amino acid sequences of the region containing the *TIF4632* gene. A 3.9-kb DNA region encompassing the *TIF4632* ORF is shown. Numbering starts at the predicted initiator AUG. Upstream AUGs are boxed, and the termination codons are underlined. In the 3′ UTR, sequences similar to the tripartite transcription termination signal are underlined. The brackets in the *TIF4632* coding region depict the boundaries of the *URA3* gene disruption (nt 627 and 1730). Inside the coding region, a number of regions are highlighted. Two arginine-serine-rich regions are underlined. The RNP-1- and RNP-2-like domains are indicated by boxes. The cysteine and histidine residues of the cysteine-histidine-rich region are circled.

p220 (85) and the high-molecular-weight polypeptide of plant iso-4F (p82; 1) by using the GAP program (Genetics Computer Group, Inc., Madison, Wis.) showed overall similarities of 43 and 39%, respectively. Values of 40 and 39% were obtained by using the TIF4632 sequence. Alignment of the most conserved region among TIF4631, TIF4632, human p220, and plant p82 is shown in Fig. 11. This region corresponds to the RNA recognition motif (RRM)-like sequence of TIF4631 and TIF4632.

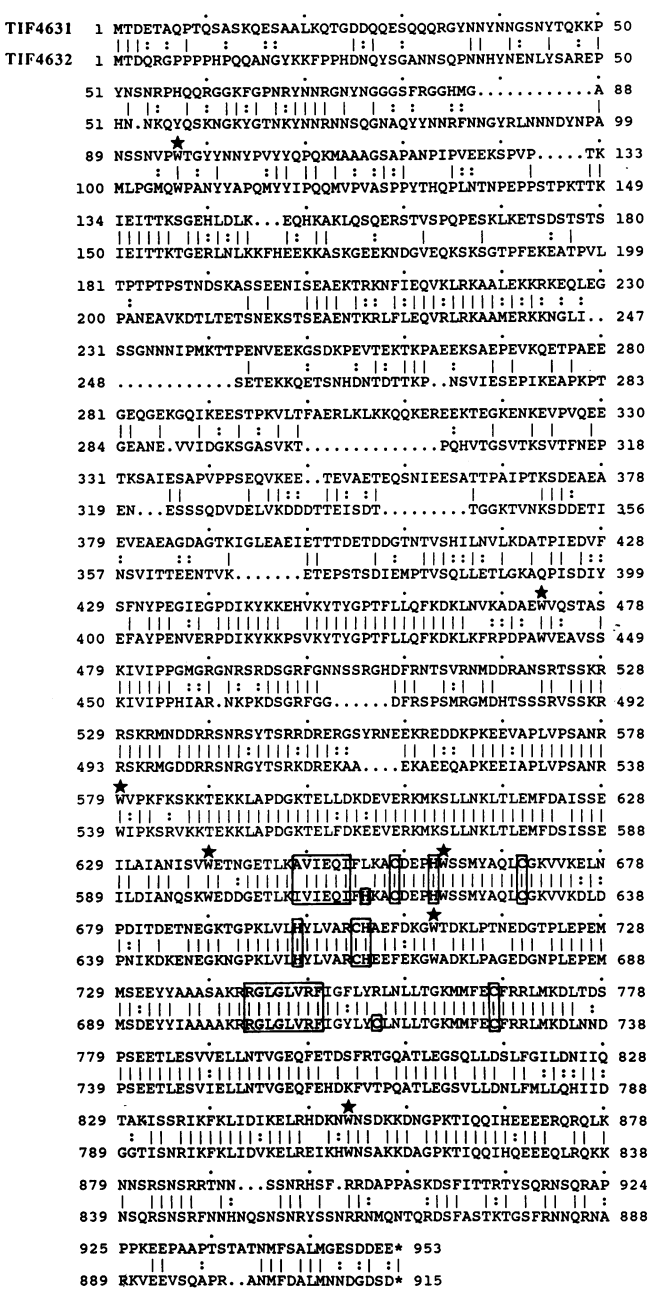

FIG. 6. Comparison of the predicted protein sequences encoded by *TIF4631* and *TIF4632*. TIF4631 appears above TIF4632. The two proteins were compared by using the GAP program of the Genetics Computer Group Sequence Analysis Software Package, version 7.0. Identical and conserved amino acids are indicated by solid bars and colons, respectively. The overall identity is 53%. Note the high degree of conservation in the carboxy-terminal halves of TIF4631 and TIF4632, especially around the two RNP domains (boxes) and the cysteine-histidine-rich region. Seven conserved tryptophan residues are indicated by stars.

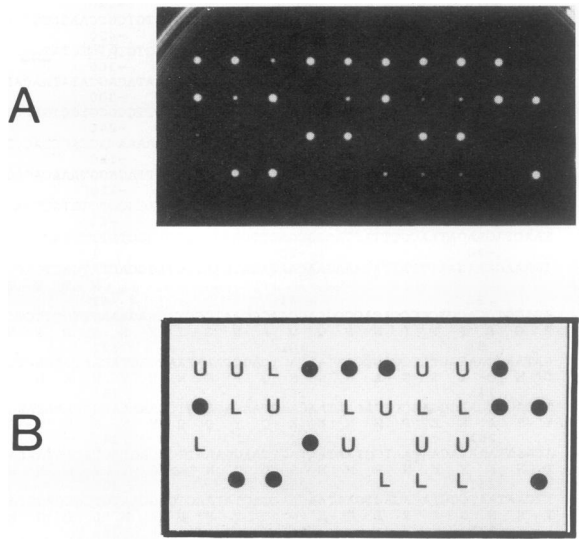

FIG. 7. *tif4631*::*LEU2 tif4632*::*URA3* double gene disruption is lethal. (A) Tetrad analysis. A diploid strain (YCG312) heterologous for *TIF4631* and *TIF4632* was sporulated, and tetrads were dissected. Representative tetrads are shown. (B) Phenotypes of the germinated spores. Schematic representation of the phenotypes of the different colonies shown in panel A. URA^+ and LEU^+ cells are represented by the letters U and L, respectively. The black circle represent a URA^- LEU^- cell.

DISCUSSION

The high-molecular-weight subunit of yeast eIF-4F is essential for growth. We have cloned two homologous genes and shown that *TIF4631* encodes the high-molecular-weight subunit of the putative yeast eIF-4F. We do not know whether TIF4632 also associates with eIF-4E to form a similar complex. However, the presence of high-molecular-weight polypeptides that cross-link to the cap structure in extracts from a *tif4631* mutant strain (Fig. 4C) is consistent with the idea that TIF4632 is a subunit of the putative yeast eIF-4F. Likewise, the inviability of the *tif4631 tif4632* double mutant strain and the degree of conservation of TIF4631 and TIF4632 strongly suggest that the two proteins have a function in common. The essential function of TIF4631-TIF4632 is in agreement with the requirement for an intact p220 subunit for efficient cap-dependent translation in mammalian cells (24). The cold-sensitive phenotype of the *tif4631* mutant strains supports the contention that the high-molecular-weight subunit of the putative eIF-4F is involved in a protein-protein interaction (16) and might reflect a weaker interaction of TIF4632 with eIF-4E or other translation initiation components. The reason for the lack of an effect of the *tif4632* gene disruption on growth is unclear. Despite our finding that the protein encoded by *TIF4631* is a component of the CBP complex, we found no obvious defect in the polyribosome profile of the *tif4631* mutant lacking TIF4631 other than a diminution of 60S ribosomal subunits and the appearance of halfmer polyribosomes. This result plus the absence of effects on growth or on the ribosome-polyribosome profile in the *tif4632* mutant indicate that the product of both genes is necessary for protein synthesis; cells lacking one or the other gene product may still contain sufficient protein to carry out translation at wild-type rates. Because the *tif4631 tif4632* double mutant is inviable, it should be possible to construct a strain conditional for synthesis of both proteins to examine whether these gene products are necessary for protein synthesis.

The phenotype of *tif4631* is similar to that of yeast mutants defective in assembly of 60S ribosomal subunits, namely, diminished amounts of 60S subunits relative to 40S subunits

TABLE 2. Meiotic segregation pattern of *tif4631::LEU2* and *tif4632::URA3* upon sporulation of strain YCG312

Ascus type	No. of viable spores	No. of asci
Parental ditype	4[a]	6
Nonparental ditype	2[b]	9
Tetratype	3[c]	21

[a] Two large (Leu$^-$ Ura$^+$) and two small (Leu$^+$ Ura$^-$).
[b] Two large (Leu$^-$ Ura$^-$).
[c] Two large (one Leu$^-$ Ura$^-$ and one Leu$^-$ Ura$^+$) and one small (Leu$^+$ Ura$^-$).

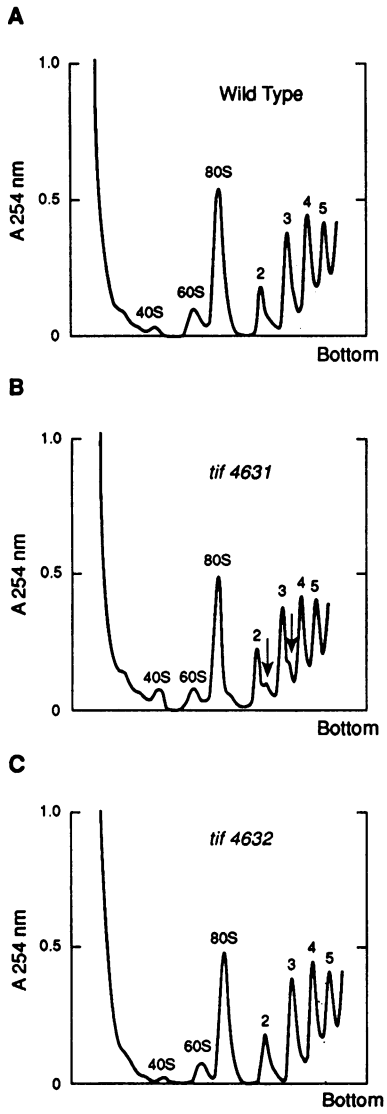

FIG. 8. The *tif4631* mutant is deficient in 60S ribosomal subunits and contains halfmer polyribosomes. Wild-type cells (A), and *tif4631* (B) and *tif4632* (C) mutants were grown at 30°C to 4 × 10^7 cells per ml, cell lysates were prepared and free ribosomal subunits, monoribosomes, and polyribosomes were resolved by centrifugation on 7 to 47% sucrose velocity gradients. Peaks representing free 40S and 60S ribosomal subunits, 80S monosomes, and polyribosomes are labeled. Halfmer polyribosomes are indicated by the arrows.

and accumulation of halfmer polyribosomes (19, 56, 57, 59, 67, 72). This result suggests the interesting possibility that TIF4631 is necessary for ribosome assembly. The lack of effect on ribosome biogenesis in the *tif4632* mutant might reflect a quantitative difference in the levels of expression of *TIF4631* versus *TIF4632* and/or a qualitative difference in the functions of the products of these two genes. Consistent with the first possibility, RNA levels of TIF4631 are severalfold higher than those for TIF4632 (27a). Nonequal contribution to growth by homologous genes was also observed for the translation factor eIF-5A encoded by yeast genes *TIF51A* and *TIF51B* (77).

Primary sequence analysis of *TIF4631*, *TIF4632*, and their encoded products. Searches of the GenBank DNA data base (release 72.0) and the SwissProt (version 22.0) and Mark Goebl (Indiana University) amino acid data bases did not identify previously characterized genes or gene products related to *TIF4631*, *TIF4632*, or the proteins they encode.

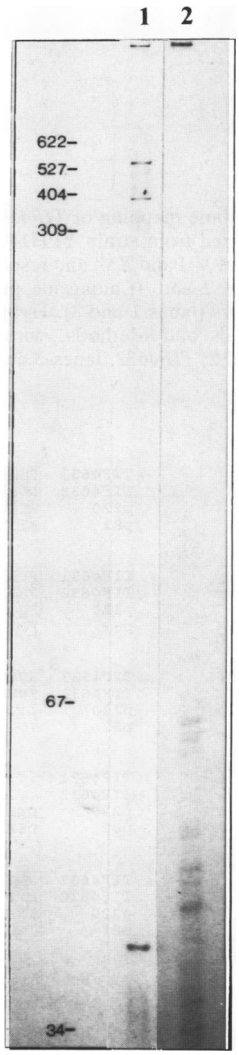

FIG. 9. Primer extension analysis of *TIF4631* transcript. A 17-mer oligodeoxynucleotide complementary to region 81 to 97 of *TIF4631* was hybridized to poly(A)$^+$ RNA (2 μg) isolated from strain S288C (lane 1) or to tRNA (15 μg, lane 2) and extended with reverse transcriptase as described in Materials and Methods. The sizes of the DNA standards on the left are in base pairs.

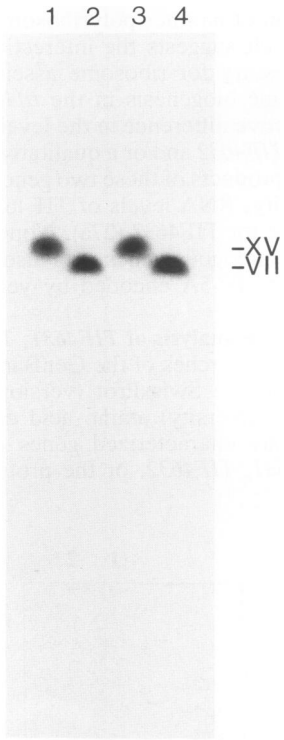

FIG. 10. Chromosome mapping of *TIF4631* and *TIF4632*. Chromosomes were prepared from strain YPH149 (which permits separation of chromosomes VII and XV) and resolved by pulsed-field gel electrophoresis (lanes 2 and 4) alongside pulsed-field gel electrophoresis DNA markers (lanes 1 and 3). Hybridization was done as described in Materials and Methods, with gene-specific probes (*TIF4631*, lanes 1 and 2; *TIF4632*, lanes 3 and 4).

Signature searches with the PROSITE program of the Genetics Computer Group Sequence Analysis Software Package (version 7.0) revealed the presence of numerous putative phosphorylation sites. Consistent with this, TIF4631 has recently been shown to be a phosphoprotein (49a). Also, three putative cdc2-CDC28 phosphorylation site sequences are observed in the serine-threonine-proline-rich region of TIF4631 (nucleotides 517 to 573 in Fig. 2) (62).

A schematic representation of the structures of TIF4631 and TIF4632 is shown in Fig. 12A. Sequences similar to the RRM (for reviews, see references 7 and 45) are present in both TIF4631 and TIF4632, in the most conserved region between the two proteins (Fig. 6 and 12B). The RRM, found in members of a large family of proteins that bind to a wide variety of RNAs, consists of a conserved sequence of approximately 80 amino acids that folds into a characteristic tertiary structure possessing four antiparallel β strands and two α helices (for reviews, see references 35 and 45). It is also composed of two conserved ribonucleoprotein (RNP) consensus sequences: RNP-1, an octamer which is part of the β3 strand, and RNP-2, a hexamer which is part of the β1 strand. Structural motifs similar to the RRM and sequences related to the RNP concensus sequences are present in TIF4631 and TIF4632 (Fig. 12B). However, they contain two leucine residues at positions 3 and 5 of RNP-1 rather than the consensus aromatic amino acids (Fig. 12B). These aromatic amino acids, however, are not found in all RRM family members. For example, in the human polypyrimidine tract-binding protein and the human heterogeneous nuclear RNP-L protein, each containing three RRMs, only once is an aromatic amino acid found at position 3 or 5 of RNP-1 (45). Leucine residues are sometimes present at these positions (45). It is also noteworthy that ribosomal proteins L7/L12 and L30 have no primary structure homology to RRM proteins, but their tertiary structure is similar to that of RRM

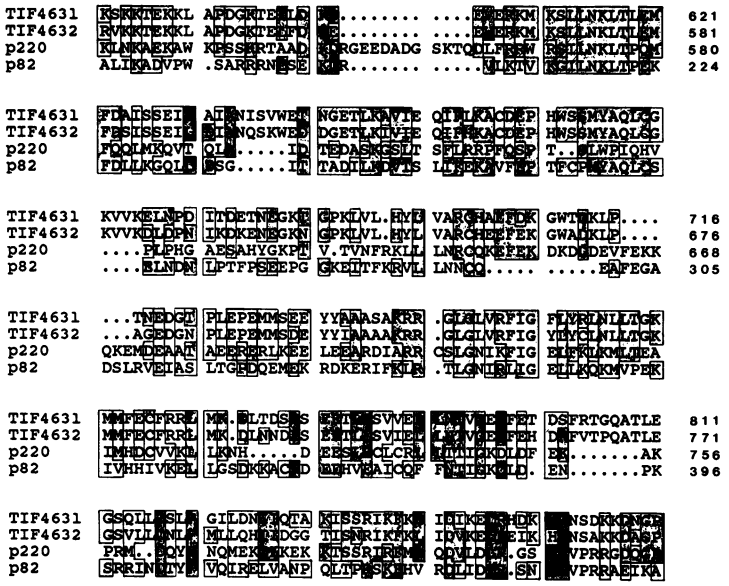

FIG. 11. Comparison of the predicted protein sequences encoded by *TIF4631* and *TIF4632*, human p220 (85), and the high-molecular-weight subunit of wheat germ iso-4F (p82; 1). The four proteins were compared by using the PileUp program of the Genetics Computer Group Sequence Analysis Software Package, version 7.0. Identical and conserved amino acids between the yeast proteins and their homologs are indicated by shaded and cross-hatched boxes, respectively. Over this 300-amino-acid region, the percentages of identity and similarity of TIF4631 versus TIF4632, p220, or p82 are 80 and 87, 33 and 59, and 29 and 55%, respectively. Over that same region, p220 and iso-4F are 35% identical and 58% similar.

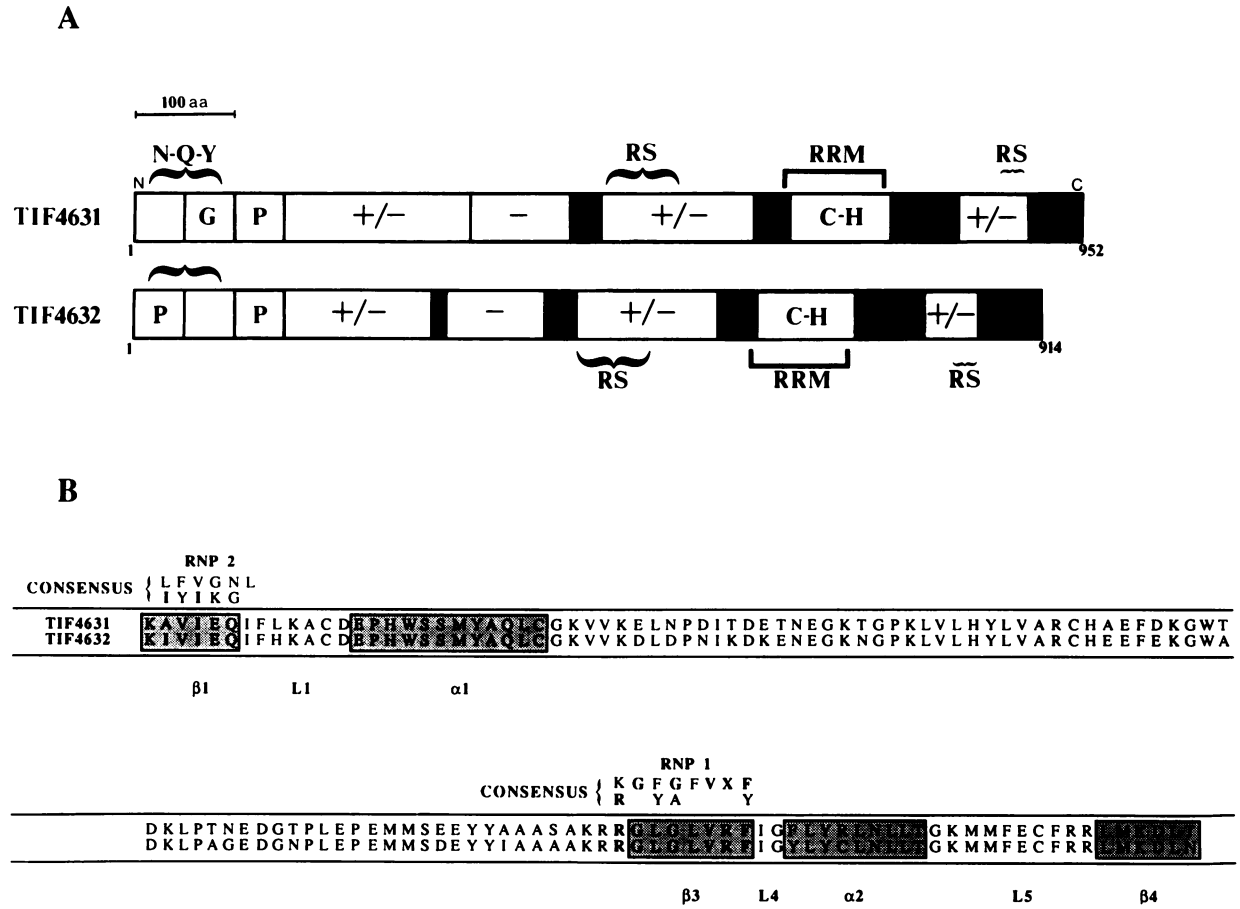

FIG. 12. Regions of TIF4631 and TIF4632 predicted to be involved in RNA binding or protein-protein interactions. (A) Schematic representation of TIF4631 and TIF4632. N-Q-Y, G, and P indicate regions rich in asparagine-glutamine-tyrosine, glycine, and proline, respectively. RS, arginine-serine-rich region; C-H, cysteine-histidine-rich region. The +/− sign depicts a highly charged region. The functional relevance of these domains is discussed in the text. (B) Primary sequences and predicted structures of the RRM-like motifs of TIF4631 and TIF4632. The consensus sequences for RNP-1 and RNP-2 are shown above the TIF4631 sequence, and the predicted α helices and β strands are indicated by shaded boxes.

proteins (40). Notably, TIF4631 and TIF4632 possess a longer spacer region between RNP-1 and RNP-2 (including loop 3) than RRM-containing proteins.

Most RRM-containing proteins contain an auxiliary domain(s) that is distinct from the RRM (reviewed in references 7, 35, and 44). Whether glycine rich, proline rich, or arginine-serine rich, these domains are believed to contribute to protein-protein and protein-nucleic acid interactions (7, 86). A glutamine-rich region, present in a number of transcription factors, is required for the transcriptional activity of SP1 and is believed to be involved in protein-protein interactions (18). Proline-rich transcription activation domains likely serve similar functions (54). Auxiliary domains are also present in TIF4631 and TIF4632. The amino-terminal regions of both proteins (the first 150 amino acids) are rich in proline and glycine (20%) (Fig. 12A). In addition, the N-terminal regions have high glutamine contents (12 of 60 [20%] and 13 of 100 [13%] in TIF4631 and TIF4632, respectively) and asparagine-tyrosine contents (26 of 72 [36%] and 37 of 81 [46%] in TIF4631 and TIF4632, respectively).

Both TIF4631 and TIF4632 display extensively charged regions (Fig. 12A, +/− or −). One of these (Fig. 6, amino acids 488 to 553 and 459 to 510 for TIF4631 and TIF4632, respectively) is similar to the arginine-serine-rich (RS) motif found in a number of splicing factors (for reviews, see references 53 and 87). The RS motif has recently been proposed to be involved in protein-protein interactions (86). In the carboxy-terminal charged region of TIF4631 and TIF4632 (Fig. 12A), a shorter RS region is present (amino acids 873 to 908 and 840 to 863 in TIF4631 and TIF4632, respectively; Fig. 6). The RS region of the 70-kDa U1 small nuclear RNP is responsible for much of its aberrant migration in SDS gels (63). In translation initiation factor eIF-4B, a similar motif is presumed to retard its migration in SDS gels (55). Thus, it is likely that the RS region, as well as the glycine- and proline-rich regions, is responsible for the anomalous migration of TIF4631 in SDS gels.

In summary, we have shown that the high-molecular-weight subunits of the putative yeast eIF-4F share homology with members of the RRM family of proteins. eIF-4B is the only other translation initiation factor that contains an RRM (55). Although the highest homology among the human eIF-4F p220 subunit (85), the wheat germ eIF-4F p82 subunit (1), and their yeast homologs is found in the region encompassing the RRM-like sequence of TIF4631 and TIF4632

(Fig. 11), no obvious RRM can be predicted by examining the primary or predicted secondary structures of p220 and p82 (44a). However, a divergent version of RNP-1 is present in both p220 and p82 (Fig. 11, positions 698 to 705 and 334 to 341, respectively). Whether p220 and p82 have a region that folds in a structure compatible with an RNA-binding function remains to be determined.

***TIF4631* mRNA 5′ UTR and implications for the regulation of its expression.** The 5′ UTR of *TIF4631* is atypical among yeast mRNAs. Northern blot analysis and primer extension data predict a 5′ UTR of at least 300 nt, which is uncharacteristically long. Strikingly, there are 11 AUGs in the 5′ UTR (Fig. 2). All of the upstream ORFs terminate upstream of position 1 and encode relatively small ORFs (from 3 to 21 amino acids; Fig. 2). Only a minority of yeast mRNAs (5%; 17) carry one or more upstream AUGs. The translation of mRNAs with long 5′ UTRs and upstream ORFs is tightly regulated (e.g., *GCN4* and *CPA1*; 38). It is therefore likely that expression of *TIF4631* is translationally regulated. The polypyrimidine stretches observed in the upstream region of *TIF4631* might be implicated in such a control, since they are essential determinants in internal binding of ribosomes on picornavirus RNAs (41). Of relevance, internal binding of yeast ribosomes to poliovirus RNA has been reported (2, 76a). Strikingly, upstream AUGs and polypyrimidine stretches are also found in the 5′ UTR of p220 (85).

Function of the high-molecular-weight subunit of eIF-4F. Several features of mammalian eIF-4F have been described: (i) eIF-4A and eIF-4B require the presence of eIF-4F to cross-link to the cap structure (20); (ii) eIF-4E cross-linking to the cap is greatly enhanced when it is part of eIF-4F (50); (iii) eIF-4F, in combination with eIF-4B, exhibits bidirectional helicase activity (70); (iv) eIF-4F displays enhanced helicase activity relative to eIF-4A alone (70); (v) eIF-4F forms a more stable complex with RNA than does eIF-4A (43). Taken together, these data suggest an RNA-binding property for the high-molecular-weight subunit of eIF-4F. This idea is supported by the presence of an RRM-like sequence in TIF4631 and TIF4632. Furthermore, three findings demonstrate that TIF4631 and, possibly, TIF4632 are RNA-binding proteins. First, TIF4631 and, most probably, TIF4632 can cross-link to RNA (Fig. 4C). Second, when purified yeast eIF-4F is cross-linked to the cap structure, TIF4631 (p150) cross-linking is not inhibited by a cap analog, suggesting that it interacts with the mRNA independently of eIF-4E (29a). Third, Northwestern analysis reveals that TIF4631 binds to RNA (29a). It is of interest that p220 also cross-links, albeit inefficiently, to the cap structure (61, 71).

One model for 40S ribosomal subunit binding to mRNA posits that eIF-4F first binds to the cap structure in an ATP-independent fashion. Subsequently, in combination with eIF-4A and eIF-4B, it unwinds, at the expense of energy derived from ATP hydrolysis, the 5′-proximal mRNA secondary structure. Once unwound, the RNA serves as a "landing pad" for the ribosome (82). Thus, the RNA-binding property of the high-molecular-weight subunit of eIF-4F might serve to stabilize the interaction of eIF-4E with the cap structure. It is also possible that this interaction stabilizes the interaction of eIF-4A with the mRNA, ensuring more efficient unwinding. It is therefore likely that the enhanced cap cross-linking and unwinding activities of mammalian eIF-4F, relative to those of its free subunits, are due to the RNA-binding properties of its high-molecular-weight component (p220). Studies of the RNA-binding properties of TIF4631 and TIF4632 should increase our understanding of the translation initiation process.

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