Recognition of the cap structure at the 5' end of mRNA is one of the first events in initiation of eukaryotic translation. This step is mediated by the translation initiation factor 4F (eIF-4F). In mammalian cells this factor is composed of the cap-binding protein eIF-4E, eIF-4A, and a 220-kDa polypeptide. In yeast Saccharomyces cerevisiae, eIF-4E is found associated with a 150-kDa protein (p150) and a 20-kDa protein (p20). The resulting protein complex is proposed to represent yeast eIF-4F. To study the functions of p150 and p20 and their interaction with eIF-4E, we disrupted the genes encoding p150 and p20 and analyzed the effects on protein complex formation and cell viability. Yeast cells with single and double disruptions were viable, but p150 single and p150/p20 double disruptions show a slow growth phenotype. Gel chromatography and immunoadsorption experiments with a monoclonal anti-eIF-4E antibody coupled to protein G-Sepharose show that both p150 and p20 bind independently of each other to eIF-4E.

Eukaryotic cellular mRNAs carry at their 5' end the cap structure m'GpppX (X means any ribonucleotide) (for a review, see Shatkin, 1976). The cap structure protects mRNAs against exonucleases (Furuichi et al., 1977) and facilitates initiation of translation (for a review, see Shatkin, 1985). During initiation the cap structure is recognized by eukaryotic initiation factor 4F (eIF-4F). In mammalian cells this factor is composed of three subunits: the cap-binding protein eIF-4E (24 kDa), eIF-4A (46 kDa), and a 220-kDa polypeptide (p220) (for reviews, see Sonenberg, 1988; Rhoads, 1991). Both, eIF-4A and eIF-4E are also found as free polypeptides not associated with eIF-4F. While eIF-4E and p220 are stably associated, eIF-4A can be separated from the complex by phosphocellulose chromatography, suggesting that its interaction with the complex is weaker (Ray et al., 1985). It is believed that binding of eIF-4E to the cap structure is the first step in translation initiation (for a review, see Thach, 1992). Binding of eIF-4F or formation of this complex at the cap structure are thought to stimulate the binding of free eIF-4A and of eIF-4B (Thach, 1992). Together, these factors have RNA helicase activity and are involved in the melting of RNA secondary structure in the leader region of mRNA (Rozen et al., 1990). Cap recognition and melting of RNA secondary structure are essential for the subsequent binding of the 40 S ribosomal subunit to most mRNAs (Rhoads, 1991).

Factor eIF-4E and eIF-4F are present in limiting amounts in mammalian cells (Duncan and Hershey, 1987; Hiremath et al., 1985), and eIF-4F was shown to have translational discriminatory activity in vitro translation systems (Ray et al., 1983). For these reasons binding of eIF-4F to or assembly of eIF-4F at the cap structure is thought to be rate limiting for translation initiation and therefore to represent a key target for translational regulation (Jagus et al., 1981; Thach, 1992). Indeed, cap binding activity in uninfected and virus-infected mammalian cells was shown to be regulated by phosphorylation of eIF-4E and p220 (Lazaris-Karatzas et al., 1990; Huang and Schneider, 1991, for reviews, see Huembelin and Thomas, 1991; Thach, 1992) or by degradation of p220 (Etchison et al., 1982, reviewed in Sonenberg, 1987).

The availability of powerful genetic techniques has made the yeast Saccharomyces cerevisiae an attractive system for studies of eukaryotic translation (for reviews, see Mueller and Trachsel, 1990; Linder and Prat, 1990). The cap-dependent translation initiation pathway appears to be highly conserved between yeast and mammals. This is demonstrated most impressively by the finding that mouse eIF-4E can substitute for its yeast homologue in vitro (Altmann et al., 1989a). Further elucidation of the functions of individual eIF-4F subunits in translation initiation may be easier to achieve in yeast than in higher eukaryotes. Yeast eIF-4E has been cloned and sequenced (Altmann et al., 1987). Coimation of eIF-4E and a 150-kDa protein (p150) in sucrose density gradients and during DEAE-cellulose chromatography (Goyer et al., 1989) and coelution of eIF-4E with p150 and a 20-kDa protein (p20) from 7-methylguanosine-diphosphate (m'GDP)-Sepharose columns (Altmann and Trachsel, 1989) suggest that these polypeptides form a protein complex. Furthermore, p150 and p20 could be shown to cross-link specifically to capped mRNA (Goyer et al., 1989). Based on these findings, we consider it to be likely that the proteins eIF-4E, p150, and p20 represent subunits of the yeast homologue of mammalian eIF-4F. In contrast to mammalian eIF-4F, yeast eIF-4F does not contain eIF-4A (Goyer et al., 1989).

In the experiments described below, we investigated the interactions of the putative yeast eIF-4F subunits p150 and p20 with eIF-4E by disrupting the genes encoding p150 and p20 and analyzing the effects on cell viability and protein complex formation.

**MATERIALS AND METHODS**

**Stains and Gene Disruptions**—The stains used in this work are listed in Table I. If not stated otherwise, restriction enzymes and DNA manipulating enzymes were purchased from Boehringer Mann-
et vector pUC8 (Sigma). This plasmid was digested with BglII, which cut BglII fragment carrying the URA3 gene was inserted, thus destroying the gene. Transformants were selected for Ura+ prototrophy.

Transformants were selected for growth on minimal medium lacking uracil. The gene TIF4631 encoding protein p150 has been cloned and sequenced. A 3.2 kb EcoRI cDNA fragment containing the complete TIF4631 open reading frame (ORF) was inserted into the EcoRI-cut vector pUC19 (Sigmod). This plasmid was digested with BglII, which cuts out a 2.2 kb fragment of the 2.9 kb TIF4631 ORF, and a 1.2 kb BglII fragment carrying the URA3 gene was inserted, thus destroying the reading frame of TIF4631. A 3.5 kb EcoRI fragment of this construct containing the deleted/disrupted TIF4631 was used to transform Ura- haploid (T105D) and diploid (T210A) strains, and transformants were selected for growth on minimal medium lacking uracil.

To construct CAF20 TIF4631 double disruptions, we first selected for a spontaneous mutant carrying tif4631::ura3 (i.e. a strain which had its genomic TIF4631 gene disrupted by an inactive URA3 gene). The CAF20 allele was obtained by growing strains indicating an inactive CAF20 allele, which were selected for growth on minimal medium lacking uracil. The gene TIF4631-encoding protein p150 has been cloned and sequenced.

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TABLE I

Genotype of strains used in this study

| Strain   | Name                          | Genotype                  | Source  |
|----------|-------------------------------|---------------------------|---------|
| T105D    | Wild-type                     | MATa, ura3-52, trp1, leu2, ade2-101, his3 | This study |
| T138B    | tif4631::URA3                 | MATa, tif4631::URA3, leu2, ura3-52 | This study |
| T143C    | caf20::URA3, tif4631::ura3    | MATa, caf20::URA3, tif4631::ura3, trp1, leu2, ade2, his3 | This study |
| T145A    | caf20::URA3, tif4631::ura3    | MATa, caf20::URA3, tif4631::ura3, trp1, leu2, ade2, his3 | This study |
| T149B    | caf20::URA3                   | MATa, caf20::URA3, his3, leu2, trp1 | This study |
| T149D    | Wild-type                     | MATa, ura3-52, his3        | This study |
| T210A    | Wild-type                     | MATa/a, ura3-52/ura3-52, trp1/trp1, leu2/leu2, ade2-101/ade2-101, his3/his3 | This study |

TABLE II

Generation times of wild-type and mutant strains

| Strain   | Name                          | Generation time (min) | Relative to wild type |
|----------|-------------------------------|-----------------------|-----------------------|
| T149D    | Wild-type                     | 110                   | 1                     |
| T138B    | tif4631::URA3                 | 150                   | 1.36                  |
| T149B    | caf20::URA3                   | 100                   | 0.91                  |
| T145A    | caf20::URA3, tif4631::ura3    | 240                   | 2.18                  |

The Proteins p150 and p20 Bind to eIF-4E — The results of previous experiments (Altmann and Trachsel, 1989) suggested that p150 and p20 bound through eIF-4E to the cap affinity column. To verify that p150 and p20 bind to eIF-4E, and to address the question of whether complex maintenance requires the presence of a cap structure or cap analog, we fractionated RSW preparations from wild-type strains and strains carrying caf20::URA3 or tif4631::URA3 disruptions by molecular sieve chromatography (AcA44) and analyzed individual fractions by SDS-polyacrylamide gel electrophoresis.
and Western blotting. Fig. 2 shows a Western blot of the different fractions obtained after separation of RSW fraction from the wild-type strain T149D. Polypeptide p150 is only found in fractions eluting early from the column (fractions 3–5, high molecular weight fractions). In contrast, p20 is found in high molecular weight fractions (fractions 4–10) and low molecular weight fractions (fractions 18–21). The 70-kDa heat shock protein is distributed between fractions 4 and 11. Initiation factor eIF-4E appears in low molecular weight fractions and broadly distributed between fractions 3 and 14, indicating that it may form several different high molecular weight complexes.

To test whether p150 and p20 interact directly with eIF-4E, we performed immunoadsorption experiments, in which eIF-4E was reacted with a monoclonal anti-yeast eIF-4E antibody and antibody-antigen complexes adsorbed to protein G-Sepharose. In this way, polypeptides complexed to eIF-4E can be identified by their (indirect) adsorption to protein G-Sepharose. To assay the association of p150 and p20 with eIF-4E, AcA44 column fraction 3 containing all three proteins (Fig. 2, lane 2) was analyzed. Antigen-antibody complexes bound to protein G-Sepharose were eluted with SDS sample buffer, fractionated by SDS-PAGE, blotted onto nitrocellulose, and reacted with the polyclonal anti-eIF-4F antibody. Fig. 3 shows that p150 and p20 in this fraction (lane 2) were bound to eIF-4E and could be adsorbed with the monoclonal antibody to protein G-Sepharose (lane 3). Only minor amounts were not bound and remained in the supernatant (lane 4). The 70-kDa protein did only bind partially to eIF-4E and appeared mainly in the supernatant (lane 4, the distortion of the 70-kDa band in the supernatant is due to albumin in the monoclonal antibody solution). We conclude from these data that eIF-4E can bind both p150 and p20 and that the complex is stable in the absence of a cap structure.

The Proteins p150 and p20 Interact Independently of Each Other with eIF-4E—To investigate whether p150 and p20 can bind independently of each other to eIF-4E, RSW preparations of strains disrupted in the gene encoding p150 (tif4631::URA3) or p20 (caf20::URA3) were size fractionated on AcA44 columns and individual fractions analyzed for complex formation. In the RSW preparation from a caf20::URA3 disrupted strain (Fig. 4), both eIF-4E and p150 were found in lower molecular weight fractions (fractions 5–15) than in wild-type RSW preparations (Fig. 2). The 70-kDa heat shock protein fractionated very similarly in the two preparations and served as an internal molecular weight standard. Cofractionation of p150 and eIF-4E was seen in fractions 5–10. When AcA44 fraction 5 (Fig. 2, lane 5, and Fig. 3, lane 6) was analyzed for eIF-4E-p150 complex formation, polypeptide p150 appeared exclusively in the protein G-Sepharose-bound fraction (Fig. 3, lane 7). No p150 was detected in the supernatant (lane 8). About 20% of eIF-4E was not bound to protein G-Sepharose and appeared in the supernatant (lane 8). We conclude from these results that complex formation between eIF-4E and p150 is independent of p20.

Fig. 5 shows the molecular sieve separation of a RSW preparation from a tif4631::URA3-disrupted strain. Both, eIF-4E and p20 in the high molecular weight fractions (fractions 8–10) eluted later from the column than in RSW preparations from wild-type cells (Fig. 2). Fraction 8, which contains eIF-4E and p20 (Fig. 5, lane 8, and Fig. 3, lane 9) was assayed for eIF-4E-p20 complex formation. The results show that p20 was bound to protein G-Sepharose (Fig. 3, lane 10) and no p20 was detected in the supernatant (lane 11). These results demonstrate that p20 can form a complex with eIF-4E in the absence of p150.

**DISCUSSION**

Initiation of translation of the vast majority of eukaryotic mRNAs is cap dependent, and the recognition of the cap structure is mediated by the cap-binding protein eIF-4E in...
the eIF-4F complex. In this report, we characterized the interaction of yeast eIF-4E with the proteins p150 and p20. These proteins are encoded by the genes CAF20 (p20) and TIF4631 (p150). Size fractionation of RSW preparations and immunoadsorption of fractions to a monoclonal anti-yeast eIF-4E antibody revealed that both proteins bind independently of each other to eIF-4E. This was further substantiated by the findings that (i) p20 was bound to eIF-4E in extracts from a TIF4631-disrupted strain and (ii) p150 was complexed to eIF-4E in the absence of p20 thus demonstrating the existence of eIF-4E-p20, eIF-4E-p150, and eIF-4E-p20-p150 complexes. In addition, we showed that these complexes are stable in the absence of a cap structure.

Previous studies reported the cross-linking of a 150- and a 20-kDa protein to the cap structure (Goyer et al., 1989). The 150-kDa protein was suggested to be the equivalent of p220 in mammalian eIF-4F. There is strong evidence that this 150-kDa protein and p150 described in this report are identical, since both polypeptides are recognized by the same polyclonal anti-yeast eIF-4E antibody, run identically in SDS-polyacrylamide gels, and coelute with eIF-4E from a cap affinity column. In addition, Goyer et al. (1989) showed that their 150-kDa protein copurifies with eIF-4E through a DEAE-cellulose chromatographic step and cosediments with eIF-4E in a sucrose density gradient. Taking all these observations together, we suggest that the yeast eIF-4F complex includes eIF-4E, p150, and p20. However, the existence of additional subunits cannot be excluded.

When the amounts of eIF-4E in wild-type and mutant strains were compared, it was evident that eIF-4E was less abundant in RSW preparations from CAF20- and TIF4631-disrupted strains, and very much reduced in RSW preparations from double-disrupted strains. Three explanations seem to be plausible to account for this finding: (i) in CAF20- and/or TIF4631-disrupted strains, eIF-4E may be less stably associated with the ribosome and therefore lost during purification of ribosomes. However, we have observed a similar reduction of eIF-4E in preribosomal extracts of disrupted strains (results not shown); (ii) in the eIF-4F complex individual subunits are protected from proteolytic degradation. In cells deficient in p150 or p20, eIF-4E would no longer be incorporated into an eIF-4F complex and would therefore be degraded. A similar observation was made in HeLa cells, where depletion of eIF-4E lead to a concomitant and drastic decrease in p220 levels (De Benedetti et al., 1991); (iii) disruptions of CAF20 and TIF4631 could alter eIF-4E gene expression.

Results from CAF20 and TIF4631 disruption experiments suggest that none of the two proteins is essential for survival of yeast cells. In addition, double mutants were viable as well. The CAF20-disrupted strains behave like wild-type cells in terms of growth rate, mating and sporulation, and in vivo translation (not shown) despite the reduced eIF-4E level suggesting that, at least in S. cerevisiae, the amount of this factor in the cell is not limiting under normal conditions for initiation of protein synthesis. TIF4631 and, more pronounced, the double-disrupted strains show a slow growth phenotype, but behave normally in mating and sporulation (not shown). These findings may be interpreted to mean that (i) neither of the two proteins is required for translation; (ii) they play a role in translation only under special conditions, or (iii) there exist other genes in the yeast cell encoding proteins which can take over their biological functions. As judged from high stringency Southern blotting analysis, there is no evidence for the presence of a second gene having homology to CAF20. In the case of the TIF4631 gene, however, our recent results show that a second gene exists whose encoded protein shows remarkable homology in its COOH-terminal half with the TIF4631-encoded protein p150. Preliminary results revealed that double disruptions of TIF4631 and this second gene are lethal. Thus, it is possible that the function of p150 can be fulfilled by a second gene product. Such a redundancy is known for many other genes encoding translational components. Most ribosomal protein genes are duplicated, and two initiation factors, eIF-4A and eIF-4D, are encoded by duplicate genes. Therefore, it would not be too surprising if a second gene product exists, which can functionally replace p150.

In summary, we have described complexes between eIF-4E, p150, and p20, and proposed that this complex represents yeast eIF-4F.

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