Mutants of Eukaryotic Initiation Factor eIF-4E with Altered mRNA Cap Binding Specificity Reprogram mRNA Selection by Ribosomes in Saccharomyces cerevisiae

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The eukaryotic initiation factor eIF-4E1 (or eIF-4e1; see Ref. 1) is an essential component of the eukaryotic translation apparatus. eIF-4E constitutes part of the so-called cap-binding complex eIF-4F (eIF-4), which, in higher eukaryotes, also contains eIF-4A and p220 (eIF-4g) (2–4). This complex, together with eIF-4B, is thought to mediate binding of the 43 S preinitiation complex to mRNA (5). In the yeast Saccharomyces cerevisiae, eIF-4E has been shown to form a complex with two proteins, p150 (thought to be the homologue of mammalian p220) and p20. The functions of p20 and p150 are as yet unknown (6–9). However, it is generally assumed that yeast eIF-4E fulfills the same function(s) as its mammalian counterparts.

The sequencing of eIF-4E genes from several species has revealed strong homology within the group of known mammalian polypeptide sequences and less extensive but clearly evident homology between the yeast eIF-4E sequence and the sequences of the counterpart proteins of mammals and wheat. The mammalian and yeast proteins are immunologically distinct (10). Nevertheless, mouse eIF-4E can substitute for its homologue in vivo (11). Thus, there is also at least partial functional homology between eIF-4E from diverse species. At the same time, the functional role(s) of eIF-4E is not yet clear.

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Much attention has been paid to the fact that eIF-4E can be phosphorylated in the cell. Many apparent correlations between the level of eIF-4E phosphorylation and the rate of cellular protein synthesis have been reported (17). Clearly, any global regulation of protein synthesis mediated via eIF-4E phosphorylation would have to be based on a specific and precisely controlled mechanism, yet this remains an unresolved issue in mammalian cells. In S. cerevisiae, eIF-4E phosphorylation is unlikely to be regulated by the mechanisms proposed for its mammalian counterparts (18).

A common property of all known eIF-4E proteins is recognition of the mRNA cap structure (19). Interestingly, the estimated binding affinity is at least 4 orders of magnitude lower than that of the most tightly binding RNA-binding proteins for their targets (20), yet the significance of this binding activity for the cytoplasmic (and possibly nuclear) functions of eIF-4E remains unclear. A key question here is how the affinity of eIF-4E for the cap relates to the ability of ribosomes to bind and translate mRNA efficiently. This relationship is central to the potential function of eIF-4E as a regulatory factor, whose activity might be modulated by phosphorylation or via its interaction with other proteins. Furthermore, the availability and/or activity of eIF-4E might not influence the translation of all mRNAs equally, yet it remains uncertain whether specific mRNA populations can be differentially activated via this factor. It has been suggested that eIF-4E is “rate-limiting” for translation initiation on at least some eukaryotic mRNAs because of its low abundance in the cell relative to other initiation factors.
Yeast cells were harvested in mid-exponential phase in Proteins—end (B3) or close to the start codon (X3). Luciferase of this plasmid, B3 and X3 (33). The latter two plasmids encode CEYX1, which has a relatively unstructured 5'-UTR, and the derivatives of this plasmid, B3 and X3 (33). The latter two plasmids encode LUC mRNAs whose leaders bear a stem-loop structure positioned either close to the 5'-end (B3) or close to the start codon (X3). Luciferase assays and Northern blots were performed as described previously (33).

Preparation of Yeast Cell-Free Extracts and Purification of Cap-Binding Proteins—Yeast cells were harvested in mid-exponential phase (A600 = 0.8), washed twice with cold water, and resuspended in buffer A (20 mM Hepes (pH 7.4), 150 mM KCl, 1 mM EDTA, 2 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride). Cells were disrupted by vortexing in the presence of glass beads (0.45-0.5 mm diameter). Cellular debris was removed by centrifugation at 16,000 × g for 20 min. The extracts were either used directly for Western blots or passed through a 7-methyl-GDP-Sepharose column (Pharmacia Biotech Inc.) in order to isolate the cap-binding proteins. The latter were eluted using buffer B containing 0.1 mM 7-methyl-GTP (see below) steps using buffer A alone and buffer B containing 0.1 mM GDP, respectively.

Polysomal Gradients—In a procedure adapted from Saggiocco et al. (35), 100-ml yeast cultures (A660 = 0.5) were harvested for the preparation of polysomes. The extracts were loaded onto 12-ml 15-38% sucrose gradients prepared in 50 mM Tris-acetate (pH 7.0), 50 mM NH4Cl, 12 mM MgCl2, and 1 mM dithiothreitol. High salt conditions were achieved by addition of 2M KCl to a final concentration of 0.7 M. The extracts loaded onto the high salt gradients were adjusted to 0.8 M NaCl, thus dissociating nontranslating 80 S ribosomes. Gradients were centrifuged for 14 h at 4 °C and 60,000 × g. Fractions were collected from these gradients and treated as described previously (36) before being analyzed by Western blotting.

Electrophoration and Expression of In Vitro Synthesized mRNA—mRNA encoding luciferase was synthesized using the in vitro transcription vector LUCEX as described previously (37). The resulting run-off transcripts had A+T tails and were synthesized either capped, by adding the cap analogue m7GpppG, or uncapped. Electroporation of spheroplasts was performed essentially as described previously (38), whereby pulses were applied at 800 V, 400 ohms, and 25 microfarads using a Bio-Rad Gene Pulser. The strains used were haploid derivatives of strain 4-2 (11) that were generated by plasmid shuffling (see above). They had a disrupted chromosomal CDC33 gene and were dependent upon expression of either the wild-type CDC33 gene or the J196 mutant on a single-copy plasmid (see above). Extracts for luciferase assays were prepared after a 5-h recovery period.

RESULTS

Construction and Expression of eIF-4E Deletion Mutants—To initiate structure-function studies of yeast eIF-4E, we constructed a series of N- and C-terminal deletion mutants. In vitro mutagenesis of the gene inserted into pCYTEXP1 was used to create NdeI (CATAG) sites at various positions downstream of the authentic start codon (7, 19, 30) (Fig. 1). The DNA between the wild-type start codon and the newly created start codon could in each case be eliminated by cleavage with NdeI and religation. A newly created TgaI-GFP fusion was transcribed on initiation codons in E. coli and S. cerevisiae. To shorten the gene at the C terminus, in vitro mutagenesis was used to introduce two adjacent TAG codons at different positions 5' of the authentic termination codon (Fig. 1). The above strategy was used to generate prematurely terminated eIF-4E proteins with stops at positions 183, 200, and 206 in the amino acid sequence. The deletion constructs have been named (using the prefix Δ) according to the positions of the engineered start and stop codons, respectively. Three N-terminal deletion derivatives were combined with Δ206, yielding Δ17/206, Δ19/206, and Δ30/206. The construction of ΔJ196 followed a different strategy. EcorI cuts both in the eIF-4E gene (at position 582 of the reading frame) and in the expression vector downstream of the wild-type reading frame. Religation yielded a gene encoding the wild-type eIF-4E amino acid sequence up to position 196, extended by two codons arising from the vector sequence.

We tested the viability of strains dependent on these eIF-4E mutants for translation. After transformation of the diploid strain GEYX1, tetrad analysis was used to assess the viability of haploid cells lacking the wild-type gene but carrying each deletion mutant (Fig. 2). This analysis revealed that all of the deletion mutants except Δ183 allowed growth (see Tables I and II). Tetrad analysis with Δ196 gave very poor spor survival rates when expressed using the TRP1 promoter (see legend to Fig. 2), but since plasmid shuffling was possible (see below), this is not an indicator that Δ196 normally cannot support growth. In a parallel strategy, the mutant eIF-4E expression...
plasmids were transformed into strain 4-2, and the resulting transformants were cured of pMDA101. This produced haploid strains that each contained only a mutant form of \textit{cdc33}. Each of the mutant genes was expressed at low levels using the \textit{TRP1} promoter and at high levels using the \textit{GPF} promoter. 

\[ \text{D}^{30/206} \text{ allowed growth only when expressed from the GPF promoter. Complementation and growth were poor with D}^{196}. \]

Western blotting was used to estimate the relative amounts of mutant eIF-4E proteins synthesized using the two promoters (Fig. 3A). Quantitative analysis revealed that expression from \textit{FIG. 1. Expression constructs used in this work.} The wild-type or mutant eIF-4E genes were expressed using a CEN4-ARS1 plasmid (A). The promoter was either the GPF1 fusion promoter (33) or the TRP1 promoter (18). The start codon of the eIF-4E gene was part of an NdeI site engineered at the beginning of the reading frame (16). Transcription was terminated by the PKG1 terminator. The cleavage sites of the following restriction enzymes are indicated: HindIII (H), BamHI (B), and NdeI (N). B/G indicates the site of a BamHI/BglII fusion. The eIF-4E reading frame was shortened at the N and C termini (B). New start codons were engineered by introducing NdeI sites at various positions downstream of the authentic initiation codon. Premature stops were in most cases introduced as double termination codons (TAG,TAG) upstream of the wild-type stop codon. In D196, this was achieved by cleavage with EcoRI followed by religation, which in this vector results in extension of the reading frame at position 196 by two extra codons (+2). Shown in C is an amino acid sequence comparison of the N- and C-terminal ends of eIF-4E from man and the yeast \textit{S. cerevisiae}, showing the regions removed in the deletion mutants listed in B.
the GPF promoter yielded a steady-state level of eIF-4E 16-fold greater than that supported by the TRP1 promoter (Fig. 3B). Moreover, the steady-state level of eIF-4E was 2-fold lower using the TRP1 promoter plasmid than was observed in a haploid strain (YPM150A195-4C) bearing the chromosomal eIF-4E gene expressed from its own promoter (Fig. 3A). In further experiments, we compared the abundance of eIF-4E in extracts from the strains YPM150A4-2 [pMDA101], and 4-2 [YCpSuperTRP1 CDC33] (i.e. the wild-type plasmid described in Fig. 1) (data not shown). The plasmid-borne CDC33 genes supported a steady-state level of eIF-4E that was 50% less than that of the strain with a chromosomal CDC33 gene and also apparently less than that of strain 4-2. This is consistent with an earlier comparison between strains T93C and 4-2 (13).

Cap Binding Behavior of Mutant eIF-4E Proteins—Cell-free extracts were prepared from the yeast mutant strains and used for the isolation of cap complex proteins. The cap binding affinity of eIF-4E was progressively reduced by deletions of increasing length (Fig. 4A), so that this dropped below a detectable level with C-terminal deletions equal to or greater than Δ196 (Table I). These results indicated that even extreme reductions in cap analogue binding affinity did not prevent eIF-4E from functioning in vivo (Table I). In experiments where eIF-4E is isolated from yeast cells, it remains associated with the other cap complex proteins (p20 and p150) during purification using the cap analogue column. To examine the effects of the deletions on the binding of eIF-4E in the absence of these other proteins, we overexpressed some of the mutants in E. coli and recovered (and renatured) the recombinant initiation factor from the resulting cellular inclusion bodies. We found that the binding behavior of the recombinant mutant proteins was indistinguishable from that of the equivalent mutant proteins from yeast (Fig. 4, A and B). Thus, the data presented in Table I apply to the behavior of mutant eIF-4E proteins whether they are present in the yeast cap-binding complex or in a free form.

Functional Characterization of eIF-4E Mutants in Vivo—Measurements of the growth rates of the various strains revealed extended doubling times for the mutant genes expressed from the relatively weak TRP1 promoter (P<sub>Trp</sub>-eIF-4E; in the strain YPM150A) and that maintained by the TRP1 promoter. In an additional approach to analysis of the eIF-4E mutants, we used electroporation to introduce in vitro transcribed mRNA into yeast spheroplasts (Fig. 5). This method allowed us to compare the translation of capped and uncapped mRNAs in vivo. The results clearly demonstrate that the deletion mutant Δ196, when expressed at a level comparable to that of eIF-4E in wild-type yeast cells, confers reduced selectivity upon the translational apparatus with respect to capped mRNA. The uncapped mRNA is able to compete more effectively as a translational template in the strain containing the mutated eIF-4E.

The luciferase activity encoded by a capped mRNA in a strain containing wild-type eIF-4E was up to 5 times greater than that encoded by an uncapped mRNA, whereby the ratio averaged over the four different amounts of mRNA used (Fig. 5A) in

### Table I

| eIF-4E derivative | in vitro complementation | complementation in vivo<sup>a</sup> |
|------------------|-------------------------|-------------------------------|
| wt               | +                       | +                             |
| Δ200             | −                       | −                             |
| Δ196             | −                       | −                             |
| Δ183             | −                       | −                             |
| Δ7/206           | ≈                       | ≈                             |
| Δ19/206          | ≈                       | ≈                             |
| Δ30/206          | ≈                       | ≈                             |

<sup>a</sup>The amount of protein bound to the cap analogue column relative to that obtained with the wild-type protein. The experiments were performed using recombinant proteins from E. coli (●) or both the E. coli-derived proteins and extracts from the appropriate yeast strains (○). Four symbols (e.g. ☑️) – the wild-type binding affinity (100%); three symbols (e.g. ☑️) = 75%; two symbols = 50%; one symbol = 25%; − = not detectable.

### Table II

| Strain          | Generation time<sup>b</sup> min | TRP1 promoter | GPF promoter |
|-----------------|---------------------------------|--------------|-------------|
| Wild-type       |                                 | 156          | 156         |
| Δ200            |                                 | 186          | 156         |
| Δ196            |                                 | 240          | 190         |
| Δ7/206          |                                 | 168          | 156         |
| Δ19/206         |                                 | 186          | 156         |
| Δ30/206         |                                 | 186          | 156         |

<sup>b</sup>Doubling times of the respective strains grown in lactate/galactose medium. Each value represents the average of duplicated measurements made with at least three independent cultures.

<sup>c</sup>NV, not viable.
wild-type.

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tion on 7-methyl-GDP-Sepharose. The combination of the N-terminal dele-

stained gels show fractions from all stages of affinity chromatography

eIF-4E genes from pCYTEXP1 (4).

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B (Fig. 5 ).

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Distinction between capped and uncapped mRNAs is also

basis of the modification status of its 5'-end. However, the

leaders bearing stem-loops more effectively than translation of

mRNA with a relatively unstructured control

contains stable inhibitory secondary structure. We compared

the translational efficiencies of two luciferase mRNAs bearing

leader. The measurements were made in strains carrying ei-

scribed deletions. Δ196 (not shown here) was not detectably bound in

this assay. Similar elution behavior was seen using inclusion body

preparations from E. coli strains expressing the respective mutant

eIF-4E genes from pCYTEXP1 (B). The latter two Coomassie Blue-

stained gels show fractions from all stages of affinity chromatography

on 7-methyl-GDP-Sepharose. The combination of the N-terminal dele-

tion Δ7 with the C-terminal deletion Δ206 resulted in a further reduc-

tion in cap binding affinity over either of the deletions alone, wt, wild-type.

16 independent experiments was 4.3. This ratio was never

more than 2.0 in a strain containing Δ196 instead of wild-type
eIF-4E, whereby the average for the equivalent set of exper-

ments was 1.9. These results show that eIF-4E has a major

fluence on the ability of ribosomes in vivo to select mRNA on

the basis of the modification status of its 5'-end. However, the

distinction between capped and uncapped mRNAs is also

markedly reduced when wild-type eIF-4E is overexpressed

(Fig. 5B).

The presence of a defective eIF-4E protein resulted in

changes in the profiles obtained from polysomal gradient anal-

ysis. Thus, as clearly evident in the case of Δ196, the relative

proportion of ribosomes in the 80 S peak increased markedly

(Fig. 6). Salt treatment resulted in the dissociation of most of the

80 S ribosomes into 40 S and 60 S subunits, thus indicating that

a large proportion of the 80 S ribosome population com-

prised nontranslating 80 S "couples" (compare with Ref. 36).

Examination of the influence of the promoter used on the

relative sizes of the 80 S peaks reveals that there is again an
eIF-4E dosage effect. The increased abundance of the Δ196
eIF-4E mutant synthesized from the GPF promoter compen-

sated partially for its reduced specific activity, allowing more

ribosomes to be translationally active. A further unusual as-

pect of the polysomal profiles was the enlarged 40 S/43 S peak,

which was particularly exaggerated in extracts from cells con-
taining GPF promoter constructs. One possible explanation of

this phenomenon might be the release of non-scanning 40 S

subunits that are normally involved in complexes (e.g., post-

termination complexes) with the mRNA.

Both wild-type eIF-4E and the mutant derivatives were pre-

sent in all fractions of the gradient (Fig. 6). This contrasted with

the distribution of eIF-2, which was found mainly in the 40 S/43

S fractions, with only low levels in the polysomal fractions. This

is consistent with eIF-2 dissociating upon formation of 80 S

monosomes and being present in much reduced amounts in the

polysomes. The results obtained with the eIF-4E antibody in-
dicate that this factor remains bound to cytoplasmic mRNA at

least during translation initiation and elongation. Moreover,

the greatly reduced cap binding activity of Δ196 does not pre-

vent this truncated form of eIF-4E from interacting with

mRNA in vivo.

Rate Control on Translation of mRNAs with Structured and

Unstructured Leaders—The availability of the expression con-

structs and mutants described in this paper provided us with a

unique opportunity to assess the influence of changes in eIF-4E

activity on the translational efficiency of specific mRNAs. It

has been proposed that eIF-4E is involved in the "unwinding" of

leader structure during the scanning process (2). According to

this model, variations in eIF-4E activity are expected to be

especially critical for the translation of mRNAs containing

stable structure in their 5'-UTRs (23). We therefore investi-
gated whether the eIF-4E deletion mutations preferentially

attenuate the translation of a reporter mRNA whose leader

contains stable inhibitory secondary structure. We compared

the translational efficiencies of two luciferase mRNAs bearing

a stable stem-loop (see "Materials and Methods" and Ref. 16)

with that of an mRNA with a relatively unstructured control

leader. The measurements were made in strains carrying ei-

ther a wild-type or mutant eIF-4E gene expressed at a high

amount of luciferase mRNA relative to the abundance of cellu-

lar PKG1 mRNA associated with the substitution of wild-type

eIF-4E by the truncated forms of this factor. Thus, for any one

LUC construct, the luciferase activities indicated in Fig. 7

reflect the relative translational efficiencies of the respective

mRNAs. According to these results, partial inactivation of

eIF-4E does not restrict the translation of the mRNAs with

leaders bearing stem-loops more effectively than translation of

the control mRNA.

DISCUSSION

In this work, we have generated and analyzed a series of

deletion mutants in CDC33 that encode proteins with reduced

affinity for the 5'-cap of mRNA. These mutants have proved to

be useful tools in investigations of the in vivo function of eIF-

4E, which we have studied using a combination of in vivo and

in vitro methods.

First, the binding characteristics of eIF-4E have been shown
to determine the selectivity of the cellular translational appar-
atus with respect to capped and uncapped mRNAs in vivo. The

competitiveness of uncapped mRNA as a template for transla-

tion can be greatly increased as a result of mutations in eIF-4E

structure function analysis of yeast eIF-4E
that reduce this factor’s specificity for the cap. The electroporation method we have used here allows mRNAs to be introduced into the cytoplasm of yeast independently of the normal pathway of (pre-)mRNA synthesis, processing, and transport. Expression of the in vitro synthesized mRNAs reflects primarily the relative rates of their translation. Apart from clearly demonstrating the role of eIF-4E in mediating the interaction between ribosomes and mRNA in vivo, our data show how regulatory changes in eIF-4E binding activity can be expected to influence the global pattern of translation in the cell. For example, potential modulation of the binding activity of eIF-4E due to phosphorylation or via the influence of regulatory proteins would also be expected to change the pattern of mRNA recognition in an analogous fashion. A further striking aspect of these data is that overexpression of eIF-4E (via the GPF fusion promoter) reduces the difference in translation rates between capped and uncapped mRNAs. Saturation of the recognition process is achieved for uncapped mRNA only at higher intracellular activities of eIF-4E. Under normal conditions in the cell, the activity of eIF-4E is set at a level that is optimal for the translation of capped mRNA, but greatly suboptimal for the translation of uncapped mRNA.

Second, while the reductions in cap binding affinity affected the selectivity of ribosomes for capped mRNA, we observed no differential effects related to structure in the mRNA leader. This therefore extends our previous study of the influence of secondary structure in the 5′-UTR of an mRNA on the translational efficiency of the yeast translational apparatus, at least under the chosen laboratory growth conditions. Expression of the Luciferase activities were measured in extracts from spheroplasts 5 h after electroporation with 0.5, 1.0, 2.0, or 4.0 μg of in vitro synthesized LUC mRNA. Typical data are shown, each point representing the average of two parallel measurements. Capped (●) or uncapped (○) polyadenylated mRNA was introduced in various amounts into spheroplasts of a strain with wild-type CDC33 expressed from the relatively weak TRP1 promoter (A). The same transcripts were also introduced into spheroplasts of a strain with the Δ196 gene expressed from the TRP1 promoter (capped LUC mRNA (●) and uncapped LUC mRNA (○)). The second set of results (B) were obtained with spheroplasts derived from strains in which either CDC33 (capped (●) and uncapped (○)) or Δ196 (capped (●) and uncapped (○)) was expressed from the relatively strong GPF fusion promoter. The amounts of mRNA added were all below the level of saturation for the translational apparatus.

Fig. 5. Expression of electroporated mRNA in spheroplasts of S. cerevisiae. Luciferase activities were measured in extracts from spheroplasts 5 h after electroporation with 0.5, 1.0, 2.0, or 4.0 μg of in vitro synthesized LUC mRNA. Typical data are shown, each point representing the average of two parallel measurements. Capped (●) or uncapped (○) polyadenylated mRNA was introduced in various amounts into spheroplasts of a strain with wild-type CDC33 expressed from the relatively weak TRP1 promoter (A). The same transcripts were also introduced into spheroplasts of a strain with the Δ196 gene expressed from the TRP1 promoter (capped LUC mRNA (●) and uncapped LUC mRNA (○)). The second set of results (B) were obtained with spheroplasts derived from strains in which either CDC33 (capped (●) and uncapped (○)) or Δ196 (capped (●) and uncapped (○)) was expressed from the relatively strong GPF fusion promoter. The amounts of mRNA added were all below the level of saturation for the translational apparatus.
A reduction in the cellular abundance of eIF-4E by a factor of 2 does not limit the growth rate of the host cell (compare Fig. 3 and Table II). It is therefore likely that the cellular concentration of eIF-4E is normally above that which would have a measurably restrictive effect. This observation helps explain why the overproduction of eIF-4E has little effect on cell growth. Only at very high levels is a (negative) effect observed (16), possibly due to the movement of increased amounts of eIF-4E into the nucleus (16), the sequestering of proteins that bind eIF-4E, or other nonspecific effects. However, the fact that eIF-4E levels are not normally restrictive for translation does not rule out that translation can be regulated via modulation of eIF-4E activity. It simply means that greater changes in eIF-4E activity are required in order to achieve a given regulatory effect than would be the case if eIF-4E activity were strongly rate-controlling. Finally, a corollary of the above is that high cap specificity and cap binding activity per se are not necessary for eIF-4E function.

Fifth, large deletions in yeast eIF-4E do not inactivate this protein, and the described mutants help define the minimal functional protein structure. We have shown that up to 30 of the N-terminal amino acids, or up to 17 C-terminal amino acids, can be eliminated without giving rise to a lethal phenotype. In those mutants where amino acids were removed from both ends, deletion of a total of 37 amino acids was tolerated. This resulted in the generation of far fewer 80 S couples (compare F and D). All strains were grown with galactose as carbon source.
Evidently, more amino acids can be removed from the N terminus without inactivating the protein than from the C terminus. The deleted sequences belong to those regions of yeast eIF-4E showing no significant amino acid sequence identity to the equivalent regions of the higher eukaryotic eIF-4E proteins (Fig. 1). None of the deletions eliminated any of the eight tryptophan residues that are generally conserved in eIF-4E amino acid sequences from various sources and that are suspected to be significant for the recognition of the cap structure (40, 41). Mutation of either the first (Trp-43) or the last (Trp-166) of these to phenylalanine was found previously to abolish cap binding activity (41). Given that the mutation of especially these two respectively N-terminal and C-terminal proximal tryptophans has the most drastic effect on cap binding (41), it may be possible to delete further sections of the interior region of the eIF-4E sequence without fully eliminating activity. The limits to this type of mutation will be at least partially dictated by the necessity of individual residues for the maintenance of the active conformation and/or stability of the protein. In another study, we have shown that the N-terminal sequence also contains two phosphorylation sites of yeast eIF-4E, both of which are recognized by casein kinase II (18). An important further issue to be considered here is that cap binding is evidently not the only property of eIF-4E that is relevant to cell viability. This is illustrated by comparison of, for example, Δ30/206 and Δ196. Δ196 binds more weakly to the cap analogue column, yet allows growth at the low level of eIF-4E supported by the TRP1 promoter. In contrast, Δ30/206 shows measurable cap binding activity, yet cannot support growth when expressed from the TRP1 promoter. One possible explanation is that the N-terminal deletion affects the ability of eIF-4E to interact with the ribosome and/or p20 or p150/p130.

In conclusion, we have obtained in vivo evidence that eIF-4E normally mediates the selectivity of yeast ribosomes for capped mRNAs. However, cells can survive with a greatly reduced preference for the capped state, accompanied by an overall reduction in the ability of eIF-4E to promote functional ribosomal binding, provided the selectivity does not drop to a point where uncapped mRNA becomes significantly competitive. The absolute priority for capped mRNA is clearly not essential for cell viability. Overexpression of CDC33 mutants apparently allows the cell to partially compensate for the defective ribosome-mRNA binding pathway. The simplest rationalization of these observations is that eIF-4E directly mediates 40 S ribosomal interactions with the mRNA, whereby the factor itself provides the specificity of cap-dependent binding. However, this does not rule out that eIF-4E can act indirectly, for example, by promoting the localization of (capped) mRNAs to a compartment where ribosomes can bind, by mediating the action of other proteins, or via another step preceding or subsequent to the actual ribosome-mRNA binding interaction.
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