Schizosaccharomyces pombe Has a Novel Eukaryotic Initiation Factor 4F Complex Containing a Cap-binding Protein with the Human eIF4E C-terminal Motif KSGST*

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Genetic and biochemical analyses were performed on the cytoplasmic cap-binding complex (eukaryotic initiation factor (eIF) 4F) of Schizosaccharomyces pombe. Genomic and cDNA sequencing of the S. pombe gene (tif1) encoding the cap-binding component eIF4E revealed the presence of two introns in a reading frame of 219 codons. The encoded sequence of 218 amino acids shows a greater degree of identity to the mammalian eIF4E sequence than does its counterpart from Saccharomyces cerevisiae. In particular, unlike its S. cerevisiae counterpart, S. pombe eIF4E has a C-terminal Ser209 within the motif KS GST that is a site of phosphorylation in hamster and rabbit eIF4E. Of relevance to its potential regulatory role, eIF4E was found to be encoded by an mRNA with six-nucleotide leader and to be of low abundance in vivo. Cross-linking experiments identified S. pombe eIF4E as the major cap-binding protein while a further protein, p36, also showed cap-dependent binding. eIF4A was not associated with the cap-binding complex. While S. pombe eIF4E was shown capable of binding S. cerevisiae p20, an equivalent protein was absent from the eIF4F complex isolated from S. pombe cells. S. pombe 4F therefore shows a remarkable combination of structural and functional properties, some of which it shares with its higher and its lower eukaryotic counterparts.

Recognition of the m7 Gppp 5’ cap by the cap-binding complex eIF4F3 (eukaryotic initiation factor 4F) is thought to be a prerequisite of efficient translational initiation on the majority of eukaryotic mRNAs (1). The “core” protein of this complex is eIF4E, which not only binds the cap structure but also interacts with the other eIF4F components (2, 3). The overall identifiable structure of eIF4F always contains eIF4E but otherwise varies somewhat from organism to organism (4). The human complex, at least as defined biochemically, comprises eIF4E (25 kDa) together with the DEAD motif protein eIF4A (46 kDa) and eIF4G (154 kDa) and is thought to require eIF4B (69 kDa) to mediate binding of the 43 S preinitiation complex to mRNA (5). One model proposes that eIF4A and eIF4E jointly “unwrap” intramolecular structures in the 5’-untranslated region of the mRNA, thus smoothing the path of the scanning ribosome toward initiation (6). Moreover, it has been suggested that eIF4G functions as a docking station for eIF4E, eIF4A, and eIF3, thus coordinating their respective activities at the 5’ end of the mRNA (7, 8). eIF4F is also a substrate for viral proteases; picornaviral protease 2A cleaves it into an N-terminal eIF4E-binding domain and a C-terminal eIF4A/eIF3-binding domain (7). The eIF4F complexes of plants (9), Drosophila (10), and Saccharomyces cerevisiae (11) have considerably different structures from that of eIF4F in mammalian cells (4). None of these complexes contain eIF4A, and they show diversity with respect to the other components. In S. cerevisiae, eIF4E can associate with either of two forms of eIF4G (Ref. 12; p150, 107 kDa; p130, 104 kDa) and a third component of unknown function, p20 (18 kDa; 13, 14). Neither p20 nor eIF4B (49 kDa) are essential in S. cerevisiae (13, 15), although disruption of the latter results in a slow growth phenotype (15).

The least variable of the eIF4F components is eIF4E. This essential protein is present in all cases examined so far and generally retains certain conserved features, most notably eight individual tryptophan residues distributed over a central region of the amino acid sequence. Moreover, eIF4E species from different organisms can be at least partly functionally exchangeable. For example, mouse eIF4E can substitute for its S. cerevisiae homologue in vivo (16). The primary evidence that eIF4E is required for translation derives from in vitro experiments, but in fact its exact role in vivo is still unknown. Indeed, there is some suspicion that this factor is involved in processes other than translational initiation. This is suggested by the observation that a fraction of the cellular population of eIF4E in COS cells (17) and S. cerevisiae (18) is located in the nucleus. Moreover, the S. cerevisiae gene encoding eIF4E has been identified as the locus of a cell cycle mutation (cdc33) that arrests the mitotic cycle at the “start” stage (19).

Despite intensive investigation, the question how translational initiation can be regulated via eIF4F remains unclear. One potentially important regulatory mechanism relates to the phosphorylation status of eIF4E. Correlations between the level of eIF4E phosphorylation and the rate of cellular protein synthesis have suggested that phosphorylation of mammalian eIF4E has a positive effect on this factor’s activity (20). Moreover, it has been reported that the phosphorylated form has a 3-fold enhanced affinity for the cap (21). After some confusion about the true major site of phosphorylation in the mammalian eIF4E amino acid sequence, there is now agreement that this is
Ser\textsuperscript{209} (22, 23). This residue is found in the motif KSGST in the eIF4E sequences of human, rabbit, and mouse. However, this C-terminal motif is missing in \textit{S. cerevisiae} eIF4E, which is relatively poorly phosphorylated at two N-terminal sites (Ser\textsuperscript{4} and Ser\textsuperscript{2}; 14). A further question relevant to the potential regulatory role of eIF4E that remains unresolved is whether the activity of this factor constitutes a key point of strong kinetic control for the overall process of translational initiation. In particular, there has been uncertainty on the issue whether eIF4E is “limiting” in terms of its abundance in the cell (18, 24–26).

Looking at the known eIF4F complexes as a whole, it seems likely that they constitute variants on a common theme. However, apart from eIF4E and possibly eIF4G, the truly essential components of the minimal eIF4F complex that is functional in cap-dependent translation remain unknown. Moreover, it is unclear to what extent the different overall structures described to-date represent individual responses to specific functional requirements in the context of translation. For example, the particular features of \textit{S. cerevisiae} eIF4F raise challenging questions, including whether the observed type of structure is a general feature in lower eukaryotes, and whether eIF4E phosphorylation in yeast is not related to functional regulation via the proposed mammalian type pathway. Given the potential of work in yeast to provide important insight into the structure, function, and regulation of eIF4F, there is a pressing need to understand the significance of these apparent deviations from the mammalian type of system. In the present paper, we describe the analogous complex in \textit{Schizosaccharomyces pombe}, a fission yeast with a number of characteristics more typical of higher than lower eukaryotic cells. We report a remarkable combination of features; \textit{S. pombe} eIF4F has a novel overall structure but retains the mammalian C-terminal KSGST motif in its eIF4E component as well as sharing other properties with its counterpart complex in \textit{S. cerevisiae}.

**MATERIALS AND METHODS**

**Strains and Media**—The haploid \textit{S. cerevisiae} strain used for expression analysis was 4-2 (a eIF4E::LEU2 ura3 trpl leu2 \textit{pmDA101cdc33 \textit{[E73K, G179D]}}) (16). The diploid strain GEX1(ura3;cd33::LEU2 ura3 trpl) used for tetrad analysis was described previously (25). Yeast cells were grown in rich media (2% peptone, 1% yeast extract) containing \textit{S. pombe} (Promega). \textit{S}1 nuclease mapping was performed according to Sambrook et al. (18, 24–26).

**Nucleotide Sequence Accession Number**—The sequence of \textit{S. pombe} eIF4E was submitted to the EMBL Nucleotide Sequence Database in Cambridge, UK, and assigned the accession number X99444.

**Photochemical Cross-linking of Capped mRNA to Yeast Protein Fractions**—mRNA was labeled either cotranscriptionally via incorporation of [\textsuperscript{\textit{\alpha}-\textit{32P}]CTP or posttranscriptionally using guanylyl transferase, S-adenosyl-L-methionine, and [\textsuperscript{\textit{\alpha}-\textit{32P}]GTP (37). The UV-induced cross-linking reactions were performed essentially as described previously, with the following minor modifications. Reaction mixtures contained 32\textit{P}-labeled RNA (3–6 × 10\textsuperscript{4} cpmp; 100–200 ng) in 20 mM HEPES (pH 7,5), 0.5 mM magnesium acetate, 3% glycerol, 1 mM ATP, 100 mM potassium acetate, protein fractions (as described in the figure), and 0.65 mM GTP (where indicated) in a total volume of 30 µl. The samples were UV-irradiated, digested with RNase A, and subjected to 12.5% SDS-polyacrylamide gel electrophoresis (38) followed by autoradiography. The autoradiograms were scanned using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Immunoblotting Analysis**—Following SDS-polyacrylamide gel electrophoresis, the transfer of the proteins to Immobilon membranes was carried out according to the directions of the supplier (1986, Millipore Corp.) using a trans-blot semi-dry transfer cell (Bio-Rad) at 15 V for 30 min. The membrane was incubated in TBS (20 mM Tris-HCl (pH 8,0), 150 mM NaCl containing 2% bovine serum albumin) for 1 h, incubated with mouse antisera diluted 1:2000 in TBS for 4 h at 4°C, and further incubated with goat anti-mouse alkaline phosphatase antibody (Promega Corp.) diluted 1:200,000 in TBS for 2 h. Washes of the membrane were done by incubating it in TBS containing 0.1% bovine serum albumin. All the incubations were carried out at room temperature. Color development was performed by following the instructions of the supplier (Promega Corp.). The polyclonal antibodies were raised in mice against recombinant eIF4E from \textit{S. cerevisiae} (18) and \textit{S. pombe} (this work). In the eIF4E quantitation using either the Sequenase 2.0 kit (Amersham Corp.) or the ABI PRISM dye terminator cycle sequencing kit (Perkin-Elmer) and the ABI 373 DNA Sequencer.

**Plasmid Construction**—The complete \textit{S. pombe} eIF4E cDNA sequence was reassembled in the \textit{S. cerevisiae} expression vector YCP33Supex2 (32) in three steps. First, the 193-bp HindIII-NcoI fragment from the genomic \textit{S. pombe} clone (containing the 5’ end of the gene) and the 800-bp NcoI-BamHI fragment from the sp22127 cDNA (a plasmid containing an \textit{S. pombe} cDNA bank containing the C-terminal coding region) were both ligated into BluescriptSK + (Stratagene). Second, an \textit{NdeI} site was engineered at the AUG start codon using polymerase chain reaction amplification. Third, the resulting \textit{NdeI}-BamHI 860-bp fragment containing the complete cDNA sequence of the eIF4E gene was cloned into YCP3Supex2 between the \textit{NdeI} and \textit{BglII} sites, yielding YCP3::SP4E. The same fragment was also cloned into pCYTExP3 (33), generating the construct pCYTExP3-SP4E. For the two-hybrid system (34), either human, \textit{S. cerevisiae}, or \textit{S. pombe} eIF4E encoding sequences were inserted between the \textit{SmaI} and \textit{BamHI} sites of the polyclin of the pGBT9 vector (Clontech) containing the DNA-binding domain of the GAL4 transcriptional activator. The gene sequence encoding the p20 protein from \textit{S. cerevisiae} was cloned as a SacI-BamHI fragment into the \textit{p20} vector (Clontech) bearing the GAL4 activation domain. \textit{pS3101} (35) was used for the cloning of chromosomal fragments.

**Protein Methods**—eIF4E was purified from \textit{S. pombe} cells grown in YPD (27) medium using a 100-liter fermentor. Cells were harvested in mid-exponential phase (\textit{A}\textsubscript{600} = 0.8), washed with cold water, and resuspended in buffer H (20 mM Tris-HCl (pH 7,4), 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride). Disruption of the cells was achieved using a Braun shaker (B. Braun type 85302/0, Germany) in the presence of glass beads (0.45–0.5-mm diameter). Cellular debris was removed by centrifugation at 30,000 × g for 20 min. The extract was passed through a column packed with a 7-methyl-GDP Sepharose resin (Pharmacia Biotech Inc.). The column was washed with buffer H, and with buffer H containing 0.1 mM GDP. Cap-binding protein was eluted with buffer H in the presence of 0.1 mM 7-methyl-GDP. Internal peptides were generated via cyanogen bromide cleavage and trypsin digestion (36). The tryptic peptides were resolved using reverse-phase high performance liquid chromatography. Microsequencing was performed using an ABI Prise 494 protein sequencer. Reconstituted \textit{S. pombe} eIF4E was purified from \textit{E. coli} cells grown in TB medium at 30°C and harvested in stationary phase (\textit{A}\textsubscript{600} = 4.5–5.0). The cells were disrupted by sonication. The cell debris was removed by centrifugation at 30,000 × g for 30 min, and affinity purification was performed according to a standard protocol (37).

### Sequence Analysis

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**RESULTS AND DISCUSSION**

**Cloning and Sequencing of the Chromosomal Gene Encoding eIF4E from *S. pombe***—We initiated this study of eIF4E in *S. pombe* by cloning and sequencing the gene encoding the core component of the cap-binding complex, eIF4E. Most of the reading frame encoding *S. pombe* eIF4E was initially obtained as a cDNA sequence identified via a homology search through a cDNA data base established in the course of a genome sequencing project for *S. pombe*. We used the identified partial cDNA as a probe in subsequent analysis of *S. pombe* DNA. Southern analysis of *S. pombe* total chromosomal DNA restricted with several enzymes showed that the genome contains only one copy of the gene encoding eIF4E, located on a 2.8–3-kilobase HindIII DNA fragment (Fig. 1A). Genomic DNA fragments in this range were therefore isolated from an agarose gel and used to generate a partial *S. pombe* DNA bank in *pBluescript* II SK+. Positive clones bearing the chromosomal *S. pombe* eIF-4E-encoding gene were identified via colony filter hybridization. Sequence analysis revealed that the chromosomal HindIII restriction fragment extended on the 5′ side only to position −61 relative to the eIF4E reading frame. Southern blot analysis of *S. pombe* genomic DNA using a 193-bp HindIII-NcoI fragment (containing the 5′ end of the HindIII fragment and part of the reading frame up to position +127) showed that a 3.5-kilobase EcoRI-NcoI fragment contained the sequence contiguous with the HindIII fragment 5′ of the eIF4E reading frame. This fragment was inserted into pSL301 using the same strategy as used for the HindIII fragment.

Pulsed-field gel electrophoresis of *S. pombe* genomic DNA followed by Southern blotting revealed that the eIF4E reading frame is located on chromosome 1 of the *S. pombe* genome (data not shown).

This work generated a complete nucleotide sequence for the chromosomal gene and its flanking regions (Fig. 2). The chromosomal gene has three exons and two introns. Using the same abbreviation as that adopted for *S. cerevisiae*, we have named this first *S. pombe* gene characterized that encodes a translation initiation factor *tif1*. The donor site for the first intron conforms to the standard consensus motif (G/G)UA(A/U)GU (39), whereas the donor site of the second intron deviates from this motif at position +4. The 3′ splice site of each intron has a YAG motif on the intron side. There are putative branch sites at positions −16 to −12 (CUAAC) and at −15 to −11 (CUAUU), respectively. The longer second intron contains several poly(U) runs. Examination of the sequence 5′ of the main ORF reveals the presence of a putative TATA box, whereas in the 3′-untranslated region two potential polyadenylation signals are identifiable (compare Ref. 40). The translational start codon has an A at −3, a feature typical of many initiation sites in *S. cerevisiae* (41). However, unlike the majority of initiation sites in *S. cerevisiae* and *S. pombe* (39), the *tif1* start context is C/G-rich.

The main open reading frame (ORF) of *S. pombe* eIF4E was deduced from examination of the cDNA and genomic sequences; it encodes a polypeptide of 218 amino acids (Fig. 2). The amino acid sequence predicted on the basis of the DNA sequences was confirmed at the N terminus by direct analysis of amino acid sequencing of tryptic fragments (Fig. 2). Moreover, the cDNA sequence was recloned by means of S1 nuclease mapping analysis. Poly(A) tails were added to poly(A)-untranslated RNA. The results obtained were consistent with the existence of a 5′ end of the mRNA located 6 nucleotides upstream of the translation initiation site (Fig. 1D). We also checked this result independently by means of S1 nuclease mapping analysis. Poly(A)+ mRNA
FIG. 2. Structure and sequence of the chromosomal gene encoding \textit{S. pombe} eIF4E (\textit{tif1}). A restriction map shows the sequencing strategy. \textit{A}, each strand was sequenced using a set of primers generating a set of overlapping sequence data (see arrows). \textit{B}, The sequenced region comprising 1868 bp contained the complete reading frame (indicated in capitals) of 219 codons (including the stop codon), encoding a protein of 218 amino acids with a predicted molecular mass of 24.9 kDa. The chromosomal gene has two introns (lowercase nucleotides 228–295 and 471–605, respectively). A potential "TATA" promoter box (nucleotides 35 to 39) and two possible polyadenylation signals (nucleotides 1009–1015 and 1025–1030) are boxed and underlined, respectively. The transcription start site is indicated by an arrow, whereas the 3' end of the mature mRNA (polyadenylation site) is at the bold and underlined A at position 1044. The regions of the predicted amino acid sequence confirmed are indicated by bold capitals, underlined bold capitals (CNBr fragments), and by boxed italic capitals (tryptic peptides).
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**FIG. 3.** Synthesis of *S. pombe* eIF4E in *E. coli* and *S. cerevisiae*.

A 870-bp fragment containing the *S. pombe* *tif1* ORF was inserted between the *NdeI* and *BamHI* restriction sites of the pCYTEXP3 expression vector polylinker (Schnepp et al. (33)) and expressed in *E. coli* TG2. A SDS-polyacrylamide gel electrophoresis was performed with total cell extracts from *E. coli* TG2 containing the recombinant *tif1* plasmid (lane 3) and containing the starting vector pCYTEXP3 (lane 2). Purified *S. pombe* eIF4E is shown for comparison in lane 4. The relative molecular masses (in kDa) of standards (lane 1) are indicated on the left side of the gel. *S. pombe* *tif1* (inserted into the inducible expression vector YCpSupex1 (32)) was also expressed in the *S. cerevisiae* strain 4-2 and recovered from this host using a cap-analogue affinity column. B, either the *S. cerevisiae* gene (CDC33; *S. cerevisiae* eIF4E in 4-2) or the *S. pombe* gene (*tif1*; *S. pombe* eIF4E in 4-2) was expressed in the haploid *S. cerevisiae* host 4-2. Both types of eIF4E complemented the CDC33 disruption of 4-2 (see also Fig. 5). Two controls are presented for comparison: the *S. cerevisiae* haploid strain 1773 (42), which has a wild-type chromosomal copy of CDC33, and the wild-type strain *S. pombe* DSM 7057 (*S. pombe* eIF4E from *E. coli*). In each experiment, the total protein extract derived from the strain indicated was subjected to cap-analogue affinity purification, thus isolating eIF4E and associated proteins. The amounts of extract loaded were adjusted to yield comparable staining between the lanes of the gel.

**FIG. 4.** Comparison of the predicted eIF4E amino acid sequence encoded by the *S. pombe* cDNA with the counterpart sequences of man, rabbit, mouse, *Drosophila*, wheat, and *S. cerevisiae*. Gaps resulting from alignments are represented by dots. Conserved residues present in all sequences are indicated by bold letters. The positions of serines (potentially) linked to eIF4E phosphorylation are indicated by asterisks: Ser2 and Ser15 of *S. cerevisiae* (14); Ser325, which is present in both the human sequence (22, 23) and in the *S. pombe* sequence; Ser47, which is missing in the *S. pombe* sequence and now thought not to be a major site of phosphorylation.

Finally, the growth rate of the derivative of *S. cerevisiae* 4-2 containing only *S. pombe* eIF4E was slower than that of the 4-2 strain when this contained the wild-type *S. cerevisiae* CDC33 gene.

**Cap and Protein Binding Functions of *S. pombe* eIF4E—**We analyzed the interactions of *S. pombe* eIF4E in the *in vivo* environment of *S. cerevisiae* (Fig. 3). A cap-analogue affinity column was used for the preparation of cap-binding proteins from extracts derived from the derivatives of *S. cerevisiae* strain 4-2. The *S. pombe* eIF4E isolated in this way from *S. cerevisiae* cells was found bound to a protein showing similar electrophoretic mobility to *S. cerevisiae* p20 (Fig. 3B). In contrast, no protein of equivalent electrophoretic mobility was evident in cap-analogue-binding fractions isolated from *S. pombe* (Fig. 3B). This suggested that, despite the absence of the p20 type protein in the *S. pombe* cap-analogue-binding fractions, *S. pombe* eIF4E may be capable of binding *S. cerevisiae* p20. We therefore turned to two-hybrid analysis to provide an independent means of testing this potential property of *S. pombe* eIF4E. The *S. cerevisiae* strain HF7c was transformed with two types of plasmid, pGBT9 expressing the eIF4E protein from either *S. cerevisiae*, *S. pombe*, or *Homo sapiens*, and pGAD424, containing the p20-encoding sequence from *S. cerevisiae*. β-Galactosidase activity was measured using either...
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Fig. 5. Viability of a S. cerevisiae haploid strain dependent on expression of the S. pombe tif1 gene as its sole source of eIF4E. S. cerevisiae GEX1 (a/cdc33::LEU2 tps3–52 trp1) was transformed with the YCPSuper2 vector bearing S. pombe tif1. After sporulation, S. pombe tif1 complemented the CDC33::LEU2 disruption in 4 out of 17 tetrads (only 3 of which are shown here), yielding a ratio of viable:non-viable spores of 3:1. The remaining tetrads were 2 viable:2 non-viable.

Fig. 6. Two-hybrid analysis confirms the interaction between S. pombe eIF4E and p20. The interaction assay was positive for S. pombe eIF4E:p20 (column 2) and S. cerevisiae eIF4E:p20 (column 3) but not for human eIF4E:p20 (column 1). Typical test results are shown for three sets of three independent double transformants. In further experiments, quantitative β-galactosidase assays were performed on extracts from the three hybrid strains, revealing that the specific activities (expressed in Miller units, Ref. 45) for S. cerevisiae eIF4E/p20, S. pombe eIF4E/p20 and human eIF4E/p20 were in the ratio 1.0:0.8:0.001 (with the S. cerevisiae value normalized to 1.0).

The interaction assay was positive for S. pombe eIF4E and p20, as demonstrated by the β-galactosidase assay results. The specific activities for the interaction partners were 1.0, 0.8 and 0.001, respectively, normalized to 1.0 for the S. cerevisiae interaction.

Fig. 7. Photochemical cross-linking of yeast fractions to radioactive mRNA. A, α-32P-cap-labeled mRNA was irradiated together with recombinant S. pombe eIF4E (lanes 1 and 2), S. pombe S30 proteins that had been isolated by cap-analogue affinity chromatography (lanes 4 and 5), S. pombe S30 proteins prior to affinity chromatography (lanes 6 and 8), and S. cerevisiae S30 proteins (lanes 7 and 9). B, capped [α-32P]CTP-labeled mRNA was irradiated together with recombinant S. pombe eIF4E (lanes 1 and 2), S. pombe S30 proteins isolated using cap-analogue affinity chromatography (lanes 4 and 5), S. pombe S30 proteins (lanes 6 and 8), and S. cerevisiae S30 proteins (lanes 7 and 9). Some incubations contained 0.65 mM m7GTP (+). The molecular masses of protein standards (panels A and B, in both cases lane 3) are indicated in kilodaltons on the left side. Arrows on the right side indicate the positions of cross-linked bands referred to in the text.

The results obtained with S. cerevisiae indicate that the protein could be the S. pombe homologue of eIF4A, whose presence in the S. cerevisiae lanes could be confirmed by Western blotting of the cross-linked proteins (data not shown). The strength of cross-linking of the proteins in the 45-kDa region did not respond to the addition of cap-analogue, as would be expected of proteins whose binding is not cap-specific.

Quantitation of eIF4E Proteins in S. pombe and S. cerevisiae—A key issue related to eIF4E function is the abundance and availability (to ribosomes and mRNA) of this protein in the cell. In order to perform comparative quantitation, we raised polyclonal antibodies against S. pombe eIF4E. We then used Western blotting to assess the relative amounts of the respective eIF4E proteins in total cell extracts from S. pombe and S. cerevisiae (Fig. 8). By means of comparison with the staining intensities obtained with serial dilutions of the recombinant S. pombe (this work) and S. cerevisiae (18) eIF4E proteins, we estimated the relative amount of eIF4E as a function of total cell protein and per cell. The results (Fig. 8) revealed that S. pombe eIF4E has an even lower abundance than its counterpart in S. cerevisiae. Whether calculated on the basis of content per cell or as a function of total cell protein, we estimated that eIF4E is at least five times less abundant in S. pombe.

The cross-linking pattern of eIF4E proteins from S. pombe and S. cerevisiae was analyzed using radioactive cap-labeled mRNA. The results were compared with the staining intensities obtained with serial dilutions of recombinant S. pombe eIF4E (18) and S. cerevisiae eIF4E. The relative amount of eIF4E was estimated as a function of total cell protein and per cell. The results revealed that S. pombe eIF4E has an even lower abundance than its counterpart in S. cerevisiae.
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**A Novel Type of eIF4F Complex in *S. pombe***—In conclusion, we have described a new type of eIF4F in *S. pombe*. The cap-binding component eIF4E shows features typical of the mammalian proteins (H

Fig. 8. Quantitation of the relative amounts of eIF4E in *S. pombe* and *S. cerevisiae*. Serial dilutions of yeast extracts and recombinant eIF4E proteins were loaded on 14% SDS-polyacrylamide gels. Proteins from *S. cerevisiae* (A) were loaded as follows: 7.5, 15, and 30 ng of total cell extract in lanes 1-3; lanes 4-10, 0.5, 1, 5, 10, 50, 100, and 500 ng of recombinant *S. cerevisiae* eIF4E purified from *E. coli*. Proteins from *S. pombe* (B) were loaded in the same order and amounts as in A. Western blotting was then performed in order to allow quantitative comparison. The molecular weights of standard proteins (St) are indicated on the left-hand-side of each blot.

expected to provide new insight into the significance of the composition and function of this complex in eukaryotic translation.

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