Endocrinology (*Baltimore*) (in press).
Williams, D., & Gorski, J. (1974) *Biochemistry 13*, 5537.
Wolff, M. E., Baxter, J. D., Kollman, P. A., Lee, D. L., Kuntz, I. D., Bloom, E., Matulich, D. T., & Morris, J. (1978) *Biochemistry 17*, 3210.
Yamamoto, K. R. (1974) *J. Biol. Chem. 249*, 7068.
Zaslavsky, B. Y., Miheeva, L. M., Mestechkina, N. M., & Rogozhin, S. V. (1982) *J. Chromatogr. 253*, 139.

Purification and Characterization of Protein Synthesis Initiation Factor eIF-4E from the Yeast *Saccharomyces cerevisiae*†

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ABSTRACT: A 24000-dalton protein [yeast eukaryotic initiation factor 4E (eIF-4E)] was purified from yeast *Saccharomyces cerevisiae* postribosomal supernatant by m^7GDP–agarose affinity chromatography. The protein behaves very similarly to mammalian protein synthesis initiation factor eIF-4E with respect to (i) binding to and elution from m^7GDP–agarose columns and (ii) cross-linking to oxidized reovirus mRNA cap structures. Yeast eIF-4E is required for translation as shown by the strong and specific inhibition of cell-free translation in a yeast extract by a monoclonal antibody directed against yeast eIF-4E.

The binding of eukaryotic ribosomes to mRNA is a multistep process involving mRNA cap recognition, scanning of the 5′ nontranslated region, and AUG start codon selection [for reviews, see Safer & Anderson (1978), Revel & Groner (1978), Kramer & Hardesty (1980), Maitra et al. (1982), and Perez-Bercoff (1982)]. These steps are catalyzed by 6 or more eukaryotic initiation factors (eIF's)[1] composed of a total of about 15 polypeptides. One of the earliest reactions, the recognition of the mRNA cap structure by the ribosome, is mediated by the factors eIF-4A (Grifo et al., 1982; Edery et al., 1983), eIF-4E (also termed 24K-CBP or CBP-I; Sonenberg et al., 1978, 1979), eIF-4F (also termed CBP complex or CBP-II; Tahara et al., 1981; Edery et al., 1983; Grifo et al., 1983), and very likely eIF-4B (Grifo et al., 1982; Edery et al., 1983). It requires the hydrolysis of ATP (Kozak, 1980) and may involve melting of RNA secondary structure in the 5′ proximal region (Sonenberg et al., 1981, 1983). Cap recognition is a regulatable step as has been clearly demonstrated for poliovirus-infected HeLa cells, where the factor eIF-4F is inactivated early in infection leading to a shut-off of host mRNA translation [for a review and references, see the introduction to Sonenberg et al. (1983)].

At present, we know very little about the functions of individual initiation factors and their subunits in cap recognition and cap binding and how their activities are regulated in uninfected cells. To answer these questions, we have begun to study mRNA binding to ribosomes in the yeast *Saccharomyces cerevisiae* where biochemical approaches can be combined with powerful genetic approaches. Here, we report on the isolation from *S. cerevisiae* of one of the factors involved in mRNA cap recognition, the initiation factor eIF-4E.

Experimental Procedures

Yeast Strains. The strain *S. cerevisiae* VdH2 used for the purification of eIF-4E was purchased from Clipfel and Co., Rheinfelden, Switzerland. The strain GRF-18 (α, Leu 2–3, 2–112, His 3–11, 3–15) used for the preparation of cell-free translation extracts and total RNA was obtained from Dr. A. Hinnen, Ciba Geigy AG, Basel, Switzerland.

Purification of Yeast Initiation Factor eIF-4E. (*A*) *Preparation of Postribosomal Supernatant.* One kilogram of *S. cerevisiae* VdH2 was suspended in 2 L of 50 mM phosphate buffer, pH 7.0, 150 mM KCl, and 1 mM EDTA and kept at 20 °C overnight with aeration and stirring. $Mg(OAc)_2$ and PMSF were added to 5 and 1 mM, respectively, final concentrations, and the cell suspension was cooled to 0 °C in ice/water. The cells were broken with glass beads (0.4-mm diameter) in an ethanol/dry ice cooled Dyno-Mill at 3000 rpm at a pumping speed of 7 L/h. The homogenate was centrifuged in a Sorvall GS3 rotor for 10 min at 8500*g* at 2 °C. From this supernatant, mitochondria were pelleted by centrifugation in a CEPA centrifuge at 60000*g* at 2 °C with a flow rate of 30 mL/min. To the postmitochondrial supernatant was added KCl to a final concentration of 0.5 M, and ribosomes were pelleted by centrifugation in type 35 rotors at 105000*g* for 19 h at 2 °C. The postribosomal supernatant (ca. 2 L) was frozen in aliquots at −70 °C.

(*B*) *Affinity Chromatography on m^7GDP–Agarose.* All steps were performed at 0–4 °C essentially as described by Edery et al. (1983). Fifty milliliters of postribosomal supernatant (750 mg of protein) was diluted to 150 mL with buffer A (20 mM Hepes–KOH, pH 7.5, 0.2 mM EDTA, 0.5 mM PMSF, 7 mM β-mercaptoethanol, and 10% glycerol) and added to 1 mL of m^7GDP–agarose [m^7GDP was coupled to adipic acid dihydrazide–agarose (from P-L Biochemicals)] by

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[1] Abbreviations: eIF, eukaryotic initiation factor; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline; kDa, kilodalton(s); Cl_3CCOOH, trichloroacetic acid; CBP, cap binding protein; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid.

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a modification of the procedure of Seela & Waldek (1975) (manuscript in preparation), and the slurry was gently shaken overnight. The slurry was then poured into a 1-mL column; the column was washed with buffer A containing 100 mM KCl, followed by 10 mL of buffer A containing 100 mM KCl and 0.1 mM GDP. Initiation factor eIF-4E was then eluted with buffer A containing 100 mM KCl and 0.075 mM m^7GDP. Typically, the m^7GDP eluate contained about 150 μg of eIF-4E in about 1 mL of buffer.

Purification of Rabbit Reticulocyte eIF-4E. Rabbit reticulocyte eIF-4E was purified as described previously (Sonenberg et al., 1979).

Preparation of Anti-eIF-4E Antibodies. (A) Polyclonal Anti-Yeast eIF-4E Antibody. A 0.5-mL sample (75 μg of protein) of purified yeast eIF-4E (for purity, see Figure 1, lanes 2 and 3) was mixed with an equal volume of Freund's complete adjuvant and injected intraperitoneally into two mice (Balb C/J, female). The injections were repeated after 2, 4, and 7 weeks with Freund's incomplete adjuvant. Blood was collected either from the tail vein or from the heart of a freshly killed mouse, allowed to clot, and clarified by centrifugation, and the serum was stored at −20 °C.

(B) Monoclonal Anti-Yeast eIF-4E Antibody. Mice were immunized as described above. Three days after the last immunization, spleen cells were fused with PAI mouse myeloma cells (Stocker et al., 1982; generous gift of Dr. T. Staehelin, Hoffmann-La Roche, Basel) as described (Staehli et al., 1980). Hybridoma culture supernatants were grown in Iscove's modified Eagle's medium (GIBCO) containing 10% fetal calf serum and tested by using a solid-phase enzyme-linked antibody assay as described earlier (Edery et al., 1983). Positive cultures were recloned by limiting dilution (Staehli et al., 1980).

For some applications (see figure legends), monoclonal antibodies were purified by affinity chromatography. Rabbit serum protein (30 mg) containing anti-mouse immunoglobulin antibodies was coupled to 1 mL of Affi-Gel 10 (Bio-Rad) according to the instructions of the manufacturer. Fifty milliliters of culture supernatant containing about 1 mg of monoclonal antibody was mixed with the affinity resin at 4 °C overnight. The resin was poured into a column and washed with TBS (20 mM Tris-HCl, pH 7.5, and 150 mM NaCl), and bound antibody was eluted with 10 mM HCl. Fractions containing protein were immediately neutralized with KOH and concentrated by lyophilization to a final concentration of about 1 μg/μL.

(C) Polyclonal Anti-Sheep Erythrocyte eIF-4E Antibody. Purified eIF-4E from sheep erythrocytes was prepared according to the protocol described for the purification of the 24-kDa CBP from rabbit erythrocytes (Tahara et al., 1981), except that the 0–40% ammonium sulfate fraction was loaded directly onto the cap analogue affinity column [see (B) under Purification of Yeast Initiation Factor eIF-4E] without prior sucrose density centrifugation. The preparation of the antibody was done according to Vaitukaitis (1981): approximately 50 μg of eIF-4E was mixed with 1.2 volumes of Freund's complete adjuvant and injected intradermally into the back of a white female rabbit (New Zealand, 3 kg) in about 20 different spots. The rabbit was bled 1 month later through the ear and serum prepared as described above.

SDS Gel Electrophoresis and Immunoblot Analysis. Polypeptides were fractionated on 15% SDS–polyacrylamide gels (Anderson et al., 1973) and either stained with Coomassie blue or silver (Oakley et al., 1980) or transferred to nitrocellulose sheets for immunoblot analysis (Towbin et al., 1979). Nitrocellulose sheets were reacted with antibody, and bound antibody was detected with peroxidase-conjugated rabbit anti-mouse antibody as described (Towbin et al., 1979). Bound rabbit anti-sheep erythrocyte eIF-4E antibody was detected with ^{125}I-labeled protein A: Nitrocellulose sheets were incubated for 30 min in TBS containing 0.5% bovine serum albumin, 0.1% Triton X-100, and 1 μg/mL ^{125}I-labeled protein A (1 mCi/mg of protein). The nitrocellulose sheets were then washed with TBS, dried, and exposed to Kodak X-Omat SO 282 film using intensifying screens at −70 °C.

Cross-Linking of Proteins to Reovirus mRNA. [^{3}H]-Methyl-labeled oxidized reovirus mRNA was incubated with protein fractions for 10 min at 30 °C, followed by the addition of $NaBH_3CN$ to reduce Schiff's bases and RNase A to degrade the mRNA (Sonenberg, 1981). The samples were fractionated by SDS–polyacrylamide gel electrophoresis, and labeled protein was detected by fluorography (Chamberlain, 1979).

Cell-Free Protein Synthesis in Yeast Extracts. S. cerevisiae S-100 extracts were prepared from strain GRF-18 according to Gasior et al. (1979) and treated with micrococcal nuclease as described (Pelham & Jackson, 1976), except that incubation was at 23 °C for 3–5 min. The composition of protein synthesis incubation mixtures (25 μL) was as described (Gasior et al., 1979) except that the S-100 made up 60% (v/v) of the total reaction mixture and cold methionine was replaced by 5 μCi of [^{35}S]methionine (1000 Ci/mmol). Where indicated, total yeast RNA (see below) was added to a concentration of 3–6 μg/25 μL. Incubation was at 23 °C for 30–60 min. Aliquots of incubation mixtures were processed as indicated in the figure legends.

Cell-Free Protein Synthesis in Rabbit Reticulocyte Lysate. Cell-free protein synthesis in rabbit reticulocyte lysate (20-μL incubation mixtures) was as described (Pelham & Jackson, 1976). 9S globin mRNA was added to a concentration of 0.5 μg/20 μL and [^{35}S]methionine (1000 Ci/mmol) to 4 μCi/20 μL.

Preparation of Total Yeast RNA. S. cerevisiae (strain GRF-18) was grown to a density of 3×10^7 cells/mL in YPD (1% yeast extract, 2% bactopeptone, and 2% glucose). Cells were washed twice in H_2O, and 1.5 g (wet weight) was resuspended in 5 mL of 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 100 mM LiCl, 1 mM EDTA, and 0.5% SDS. Twenty-gram glass beads (diameter 0.5 mm, washed in the same buffer) and 7 mL of phenol (equilibrated with Tris buffer at pH 8) were added; the mixture was shaken at 0 °C for 5 min and centrifuged at 16000*g* for 10 min at 0 °C. The water phase was collected, reextracted with phenol, and precipitated with ethanol; the precipitate was washed with ethanol, dried, and finally dissolved in H_2O at a concentration of 10 mg/mL. About 400 A_{260} units of total RNA were obtained from 1.5 g of cells.

Results

Purification of a 24 000-Dalton Protein. In an attempt to isolate cap binding protein(s) from the yeast *S. cerevisiae*, we fractionated postribosomal supernatant by m^7GDP affinity chromatography (Sonenberg et al., 1979; Experimental Procedures). The postribosomal supernatant was prepared in high-salt conditions (0.5 M KCl) to include ribosome-associated proteins. Analysis of the m^7GDP eluate from the affinity column by SDS gel electrophoresis reveals one major protein band with an approximate molecular weight of 24 000 (Figure 1, lanes 2 and 3). If more m^7GDP eluate was loaded onto the gel, minor polypeptide bands of higher molecular weight could usually be seen (especially one at an approximate

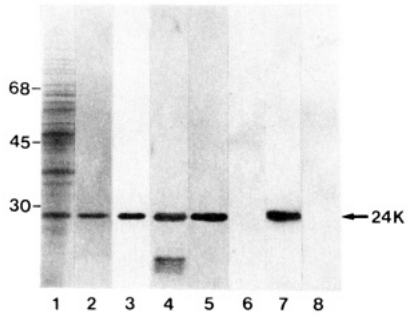

FIGURE 1: Reaction of purified yeast 24-kDa protein and rabbit reticulocyte eIF-4E with antibodies. Stained lanes cut from SDS–polyacrylamide gels (lanes 1–3) or the corresponding immunoblots (lanes 4–8) are shown. Lane 1, 75 μg of postribosomal supernatant, stained with Coomassie blue; lane 2, 1 μg of yeast eIF-4E, stained with Coomassie blue; lane 3, 1 μg of yeast eIF-4E, silver stained; lane 4, 1 μg of yeast eIF-4E, reacted with polyclonal mouse antibody (1:1000 dilution), peroxidase stained (Experimental Procedures); lane 5, 1 μg of yeast eIF-4E, reacted with monoclonal antibody (undiluted culture supernatant), peroxidase stained; lane 6, same as lane 5 but with 1 μg of rabbit reticulocyte eIF-4E; lane 7, 1 μg of rabbit reticulocyte eIF-4E reacted with rabbit anti-sheep erythrocyte eIF-4E antibody (1:500 dilution), decorated with ^{125}I-labeled protein A (Experimental Procedures), autoradiogram; lane 8, same as lane 7 but with 1 μg of yeast eIF-4E. Molecular weight markers are indicated by arrows on the left: bovine serum albumin, M_r 68 000; ovalbumin, M_r 45 000; carbonic anhydrase, M_r 30 000. The arrow on the right points to the position in the gel of yeast 24-kDa protein and rabbit reticulocyte eIF-4E.

molecular weight of 75 000 and one at 110 000). From 500 mg of postribosomal supernatant protein (Figure 1, lane 1), we routinely obtained about 100 μg of purified 24-kDa protein. Note that most of the protein appearing as a major band in the 24-kDa region of postribosomal supernatant (Figure 1, lane 1) is not our 24-kDa protein as judged by its inability to bind to the affinity column.

The close resemblance of the yeast 24-kDa protein with mammalian eIF-4E with respect to behavior on the m^{7}GDP affinity column and apparent molecular weight on SDS–polyacrylamide gels prompted us to investigate its possible role in mRNA cap structure binding in translation. We raised antibodies against the purified 24-kDa protein in mice and selected monoclonal antibodies which react exclusively with the 24-kDa component (Figure 1, lanes 4 and 5). These antibodies, however, do not react with rabbit reticulocyte eIF-4E (Figure 1, lane 6). The same result was obtained with the polyclonal antibody (result not shown). Conversely, polyclonal rabbit antibodies against sheep erythrocyte eIF-4E which react with rabbit reticulocyte eIF-4E (Figure 1, lane 7) do not react with yeast 24-kDa protein (Figure 1, lane 8), indicating that the yeast and mammalian proteins are immunologically distinct.

24-kDa Protein Cross-Links to mRNA Cap Structures. To examine the ability of yeast 24-kDa protein to recognize and bind to mRNA cap structures or cap analogues, cross-linking experiments with oxidized cap structure labeled reovirus mRNAs were performed (Sonenberg, 1981; Figure 2). The results show that the 24-kDa protein (i) interacts directly with the cap structures (Figure 2, lane 1) and (ii) binds specifically as indicated by the inhibition of cross-linking by the cap analogue m^{7}GDP (Figure 2, lane 2) but not by GDP (Figure 2, lane 3). Furthermore, the binding of the 24-kDa protein to cap structures is inhibited by the monoclonal antibody (Figure 2, lane 4). When the same experiment is done with rabbit reticulocyte eIF-4E (Figure 2, lanes 5–7), essentially the same results are obtained except that the monoclonal antibody does not inhibit cross-linking. On the basis of these

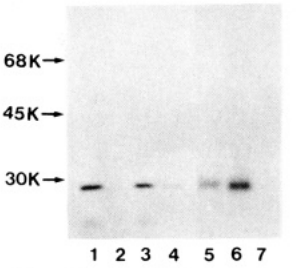

FIGURE 2: Cross-linking of yeast 24-kDa protein to reovirus mRNA cap structures. 24-kDa protein eluted from the m^{7}GDP–agarose affinity column was dialyzed against buffer A containing 100 mM KCl to remove the cap analogue m^{7}GDP. The experiment was performed in the presence of 1 mM ATP as described (Sonenberg, 1981) with 6×10^4 cpm of [^{3}H]methyl-labeled oxidized reovirus mRNA and 0.8 μg of yeast 24-kDa protein or 1 μg of rabbit reticulocyte eIF-4E. The samples were processed as described (Sonenberg, 1981). The autoradiogram is shown. Lanes 1–4, yeast 24-kDa protein; lanes 5–7, rabbit reticulocyte eIF-4E; lanes 1 and 5, no addition; lanes 2 and 7, plus 0.64 mM m^{7}GDP; lane 3, plus 0.64 mM GDP, lanes 4 and 6, plus 5 μg of affinity-purified monoclonal anti-yeast eIF-4E antibody. Molecular weight markers are indicated by arrows on the left (see legend to Figure 1).

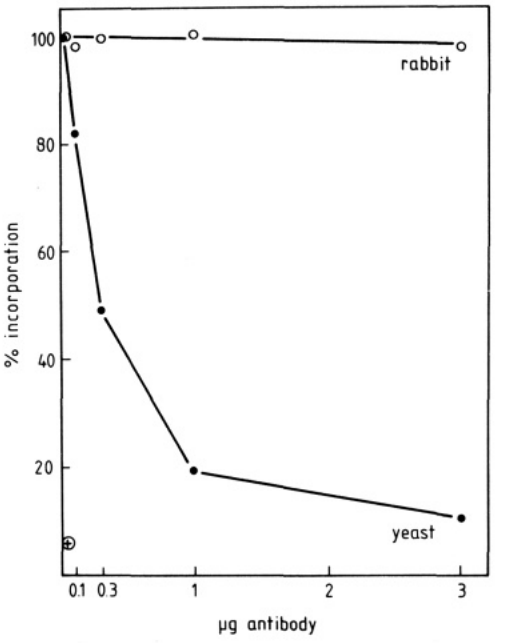

FIGURE 3: Effect of monoclonal antibody on in vitro translation. In vitro translation was performed in the yeast and rabbit reticulocyte system as described under Experimental Procedures. Affinity-purified monoclonal antibody (ca. 1 μg/μL) was incubated with complete assay mixtures minus mRNA at 0 °C for 15 min. Protein synthesis was started by the addition of 9S globin mRNA (rabbit reticulocyte lysate) or total yeast RNA (yeast extract), and incubation was for 30 min. Five-microliter aliquots of incubation mixtures were spotted on filter paper disks and washed for 5 min in 5% cold Cl_3CCOOH followed by boiling in 5% Cl_3CCOOH for 5 min. After being washed again in 5% cold Cl_3CCOOH for 5 min, filters were bleached in 10% H_2O_2 (for reticulocyte lysate only), rinsed with ethanol and acetone, and dried, and ^{35}S radioactivity was measured in a scintillation counter. (●) Yeast system; (○) reticulocyte lysate system; (⊕) yeast system minus mRNA and minus antibody.

data, we conclude that the 24-kDa protein is analogous to the rabbit reticulocyte 24-kDa cap binding protein. Henceforth, we will refer to this protein as yeast eIF-4E.

24-kDa Protein Is a Translation Factor. The availability of a yeast cell-free translation system (Gasior et al., 1979) and antibodies against yeast eIF-4E allowed us to test whether this protein is required for translation. Figure 3 shows that the affinity-purified monoclonal antibody (Experimental Procedures) inhibits translation in a mRNA-dependent yeast extract

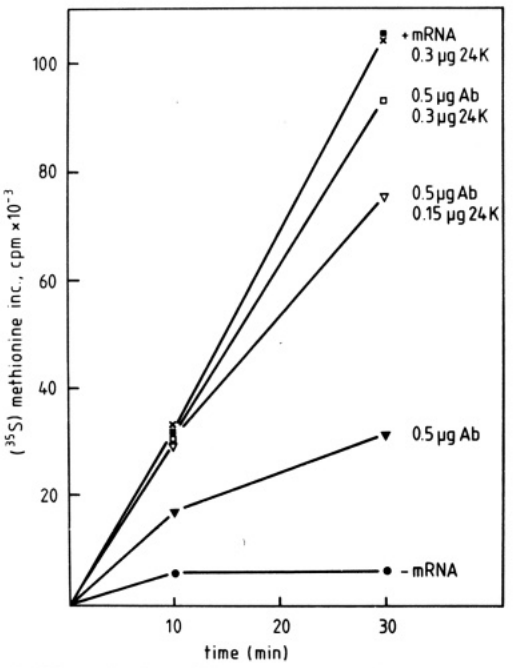

FIGURE 4: Neutralization of inhibitory activity of monoclonal antibody by 24-kDa protein (yeast eIF-4E). Affinity-purified monoclonal antibody (Ab) alone, 24-kDa protein (24K) alone, or monoclonal antibody and 24-kDa protein mixed together in TBS (20 mM Tris-HCl, pH 7.6, and 150 mM NaCl) was incubated for 15 min at 0 °C in a total volume of 3 μL. The total yeast translation mixture was then added and cell-free translation performed as described under Experimental Procedures and in the legend to Figure 3. (×) Buffer control (TBS); (■) plus 0.3 μg of 24-kDa protein; (□) plus 0.5 μg of antibody plus 0.3 μg of 24-kDa protein; (▽) plus 0.5 μg of antibody plus 0.15 μg of 24-kDa protein; (▼) plus 0.5 μg of antibody.

in a dose-dependent manner (closed circles). The same antibody preparation at the same concentrations has no effect on translation of 9S globin mRNA in rabbit reticulocyte lysate reactions containing similar amounts of ribosomes (open circles). This specificity of inhibition indicates that it is the antibody per se and not a contaminant (e.g., ribonuclease or protease) which inhibits translation. This conclusion is further supported by the experimental data presented in Figure 4 ([^{35}S]methionine incorporation in a yeast cell-free system) and Figure 5 (translation products of a yeast cell-free system). The mRNA-dependent yeast in vitro translation system is inhibited by the affinity-purified monoclonal anti-24-kDa (eIF-4E) antibody (Figure 4, +0.5 μg of antibody; Figure 5, lanes 1–5). Purified yeast eIF-4E (24 kDa) by itself has no effect in the system (Figure 4, +0.3 μg of 24 kDa). Some preparations gave moderate inhibition; however, stimulation was never observed. The inhibitory activity of the antibody preparation can be neutralized by preincubation of the antibody with purified eIF 4E (Figure 4, +0.15 μg of 24 kDa, +0.3 μg of 24 kDa, and Figure 5, lane 6). These data show that the monoclonal antibody inhibits yeast protein synthesis specifically and demonstrate that eIF-4E is an important component involved in translation of most if not all yeast mRNAs in the yeast cell-free translation system.

Discussion

We have purified from crude postribosomal supernatant a protein with an apparent molecular weight in SDS–polyacrylamide gels of 24000. The purification achieved by m^{7}GDP–agarose affinity chromatography of the 24-kDa protein was on the order of 2000-fold, and the purity as estimated from stained gels (see Figure 1) was ≥80%. The 24-kDa protein is very similar to mammalian protein synthesis initiation factor eIF-4E (Sonenberg et al., 1979) by several

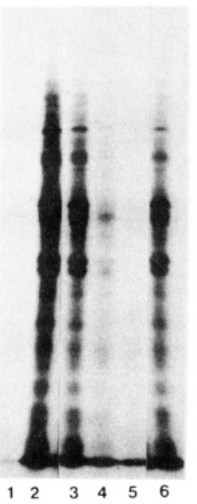

FIGURE 5: Analysis of translation products. Aliquots (10 μL) of in vitro translation reactions (Figure 4) were fractionated by SDS–polyacrylamide gel electrophoresis, and the gel was prepared for fluorography. The autoradiogram is shown. Lane 1, minus RNA; lanes 2–6, plus 3 μg of total yeast RNA; lane 2, buffer control (TBS), lane 3, 0.25 μg of antibody; lane 4, 0.5 μg of antibody; lane 5, 1 μg of antibody; lane 6, 0.5 μg of antibody plus 0.15 μg of 24-kDa protein.

criteria. These criteria are (i) behavior on the m^{7}GDP–agarose affinity column and in SDS–polyacrylamide gels, (ii) cross-linking to oxidized reovirus mRNA cap structures, and (iii) involvement in translation. When affinity-purified 24-kDa protein was analyzed by gel filtration, it behaved as expected for a monomeric 24000-dalton polypeptide. However, we find by gel filtration of crude postpolysomal supernatant followed by protein blotting of individual fractions and reaction with the monoclonal antibody (or cross-linking) that a minor part of the 24-kDa protein is in a fraction corresponding to a molecular weight of ≥130000 (unpublished results). This again is similar to what was found with mammalian eIF-4E (Tahara et al., 1981; Edery et al., 1983). On the basis of all these results, we suggest that the yeast 24-kDa cap binding protein be named eIF-4E.

Interestingly, we find with our antibodies that yeast and mammalian eIF-4E's are immunologically distinct. However, only elucidation of the primary structures of these proteins will tell how different they really are.

The existence in yeast of cap binding protein(s) and its (their) involvement in translation are not unexpected. It has been shown earlier that yeast mRNAs are capped (Mager et al., 1976; Scripati et al., 1976) and that cap analogues inhibit translation in vitro (Gasior et al., 1979; Tuite et al., 1980; Szczesna & Filipowicz, 1980). Our experiments identify the protein (or one of the proteins) involved. At present, we do not know how eIF-4E functions during translation, but preliminary translation experiments with a naturally uncapped mRNA (STNV RNA) show that its translation is also strongly inhibited in the yeast cell-free system by the monoclonal antibody and by m^{7}GDP (unpublished results). This indicates that yeast eIF-4E may be involved directly or indirectly in reactions other than just cap recognition.

The availability of purified yeast eIF-4E and antibodies directed against it should make it possible to study its function in protein synthesis, to isolate its gene(s), and to construct mutations.

References

Anderson, C. W., Baum, P. R., & Gesteland, R. F. (1973) *J. Virol. 12*, 241–252.

Chamberlain, J. P. (1979) *Anal. Biochem. 98*, 132–135.

Edery, I., Hümbelin, M., Darveau, A., Lee, K. A. W., Milburn, S., Hershey, J. W. B., Trachsel, H., & Sonenberg, N. (1983) *J. Biol. Chem. 258*, 11398–11403.
Gasior, E., Herrera, F., Sadnik, I., McLaughlin, C. S., & Moldave, K. (1979) *J. Biol. Chem. 254*, 3965–3969.
Grifo, J. A., Tahara, S. M., Leis, J. P., Morgan, M. A., Shatkin, A. J., & Merrick, W. C. (1982) *J. Biol. Chem. 257*, 5246–5252.
Grifo, J. A., Tahara, S. M., Morgan, M. A., Shatkin, A. J., & Merrick, W. C. (1983) *J. Biol. Chem. 258*, 5804–5810.
Kozak, M. (1980) *Cell (Cambridge, Mass.) 22*, 459–467.
Kramer, M., & Hardesty, B. (1980) *Cell Biol. 4*, 69–105.
Mager, W. H., Klootwijk, J., & Klein, I. (1976) *Mol. Biol. Rep. 3*, 9–17.
Maitra, U., Stringer, E. A., & Chaudhuri, A. (1982) *Annu. Rev. Biochem. 51*, 869–900.
Oakley, B. R., Kirsch, D. R., & Morris, N. R. (1980) *Anal. Biochem. 105*, 361–363.
Pelham, H. R. B., & Jackson, R. J. (1976) *Eur. J. Biochem. 67*, 247–256.
Perez-*Bercoff*, R., Ed. (1982) *Protein Biosynthesis in Eukaryotes*, Plenum Press, New York and London.
Revel, M., & Groner, Y. (1978) *Annu. Rev. Biochem. 47*, 1079–1126.
Safer, B., & Anderson, W. F. (1978) *CRC Crit. Rev. Biochem. 5*, 261–290.
Seela, F., & Waldek, S. (1975) *Nucleic Acids Res. 2*, 2343–2354.
Sonenberg, N. (1981) *Nucleic Acids Res. 9*, 1643–1656.
Sonenberg, N., Morgan, M. A., Merrick, W. C., & Shatkin, A. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4843–4847.
Sonenberg, N., Rupprecht, K. M., Hecht, S. M., & Shatkin, A. J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4345–4349.
Sonenberg, N., Guertin, D., Cleveland, D., & Trachsel, H. (1981) *Cell (Cambridge, Mass.) 27*, 563–572.
Sonenberg, N., Edery, I., Darveau, A., Hümbelin, M., Trachsel, H., Hershey, J. W. B., & Lee, K. A. W. (1983) *Proceedings of the 13th Linderström–Lang Conference on Translational and Post-translational Events* (Abraham, A. K., Ed.) pp 23–43, Humana Press, Clifton, NY.
Sripati, C. E., Groner, Y., & Warner, J. R. (1976) *J. Biol. Chem. 251*, 2898–2904.
Staehli, C., Staehelin, T., Miggiano, V., & Haering, P. (1980) *J. Immunol. Methods 32*, 297–304.
Stocker, J. W., Forster, H. K., Miggiano, V., Staehli, C., Staiger, G., Takacs, B., & Staehelin, T. (1982) *Res. Discl. No. 217*, 155–157.
Szczesna, E., & Filipowicz, W. (1980) *Biochem. Biophys. Res. Commun. 92*, 563–569.
Tahara, S. M., Morgan, M. A., & Shatkin, A. J. (1981) *J. Biol. Chem. 256*, 7691–7694.
Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Tuite, M. F., Plesset, J., Moldave, K., & McLaughlin, C. S. (1980) *J. Biol. Chem. 255*, 8761–8766.
Vaitukaitis, J. L. (1981) *Methods Enzymol. 73*, 46–52.

Cross-Linking of Streptomycin to the 50S Subunit of *Escherichia coli* with Phenyldiglyoxal†

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ABSTRACT: [^{3}H]Dihydrostreptomycin was covalently linked to the 50S subunit of *Escherichia coli* K12A19 with the bifunctional cross-linking reagent phenyldiglyoxal. The cross-linking was abolished under conditions that prevent the specific interaction of streptomycin with the ribosome. The binding primarily involved the ribosomal RNA and also a limited number of proteins, namely, L2, L6, and L17. This suggests that the binding domain for streptomycin is close to the peptidyl transferase center, in the valley between the central protuberance and the wider lateral protuberance of the 50S subunit. This domain faces the binding domain for streptomycin which we have previously characterized on the 30S subunit [Melançon, P., Boileau, G., & Brakier-Gingras, L. (1984) *Biochemistry 23*, 6697–6703]. Our results indicate that the 50S subunit is involved in the binding of streptomycin to the bacterial ribosome, in addition to the 30S subunit which is generally considered as the specific target of the antibiotic. They are consistent with the occurrence of a single binding site for streptomycin on the ribosome, comprised of regions of both subunits.

Streptomycin binds to a single site on the 70S ribosome of *Escherichia coli* and perturbs several steps of protein synthesis [reviewed in Vazquez (1979) and Wallace et al. (1979)]. It is generally assumed that the 30S subunit is the target of streptomycin in the 70S ribosome, since streptomycin binds tightly to the 30S subunit but not to the 50S subunit (Chang & Flaks, 1972; Schreiner & Nierhaus, 1973; Grisé-Miron & Brakier-Gingras, 1982) and resistance to or dependence on streptomycin results from mutations affecting the 30S protein S12 [Ozaki et al., 1969; Birge & Kurland, 1969; reviewed in Stöffler & Wittmann (1977)]. However, when it binds to the 70S ribosome, streptomycin induces conformational changes in the 50S subunit (Delihas et al., 1975; Martinez et al., 1978; Tritton, 1978), suggesting that it may also directly interact with this subunit.

In a previous study (Melançon et al., 1984), we characterized the binding site of streptomycin on the 30S subunit using the bifunctional cross-linking reagent phenyldiglyoxal. This binding site was located on the head of the 30S subunit, at the interface with the 50S subunit. In the present study,

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