Cloning and Sequencing of Complementary DNAs Encoding the \(\alpha\)-Subunit of Translational Initiation Factor eIF-2

CHARACTERIZATION OF THE PROTEIN AND ITS MESSENGER RNA*

Heidemarie Ernst†, Roger F. Duncan, and John W. B. Hershey

From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616

(Received for publication, August 26, 1986)

A clone encoding the \(\alpha\)-subunit of eukaryotic initiation factor 2 (eIF-2\(\alpha\)) was isolated from a \(\lambda\)gt11 expression library of rat brain cDNAs. The fusion protein expressed by the recombinant phage reacts with eIF-2\(\alpha\) antiserum except when the serum is preadsorbed with pure eIF-2. The translation of hybrid-selected HeLa cell mRNA produces two proteins which are indistinguishable from authentic HeLa eIF-2\(\alpha\) and its phosphorylated form when analyzed by electrophoresis in two-dimensional isoelectrofocusing/sodium dodecyl sulfate-polyacrylamide gels and by partial protein digestion. HeLa cell eIF-2\(\alpha\) mRNA migrates as a single band of about 1600 nucleotides. The rat cDNA insert was sequenced, and the region coding for eIF-2\(\alpha\) was identified. A human cDNA clone was obtained by hybridization screening with the rat cDNA, and its sequence was determined also. Both rat and human eIF-2\(\alpha\) proteins comprise 315 amino acids (36.1 kDa) and differ by only three amino acids. The eIF-2\(\alpha\) mRNA is found exclusively in polysomes containing growing HeLa cells. In serum-depleted cells which synthesize eIF-2\(\alpha\), the level of eIF-2\(\alpha\) mRNA is not changed, the average polysome size is reduced to 7, and little or no eIF-2\(\alpha\) mRNA is detected in the ribonucleoprotein fraction. These results are consistent with the view that eIF-2\(\alpha\) mRNA translation is very efficient compared to other mRNAs in the cell.

Initiation of protein synthesis in mammalian cells is mediated by a complex array of proteins called initiation factors. One of the initiation factors, eIF-2, forms a ternary complex with methionyl-tRNA and GTP and promotes the binding of the initiator tRNA to the 40S ribosomal subunits (1-3). eIF-2 comprises three nonidentical subunits, \(\alpha\) (36 kDa), \(\beta\) (38 kDa), and \(\gamma\) (52 kDa). In hemin-deprived rabbit reticulocyte lysates, inhibition of protein synthesis is due to the phosphorylation of the \(\alpha\)-subunit of eIF-2 (4). A correlation of eIF-2\(\alpha\) phosphorylation and translational repression also has been made in cells subjected to heat shock (5), serum deprivation (6), interferon treatment followed by virus infection (7, 8), and eIF-2\(\gamma\) likewise appears to occur in a covalently modified forms (12). It, therefore, is likely that many instances of translational control may involve the covalent modification of eIF-2 subunits.

The precise mechanism(s) whereby eIF-2\(\alpha\) phosphorylation results in the inhibition of initiation of protein synthesis has been recently resolved, in broad terms, though some details remain unclear. To function catalytically, eIF-2 must transiently associate with another initiation factor, eIF-2B (also called GEF or RF), which promotes a GDP/GTP exchange reaction on eIF-2 (4). Phosphorylation of eIF-2\(\alpha\) causes the formation of irreversible inactive complexes with eIF-2B, thereby preventing the reutilization of eIF-2. However, the effect of phosphorylation also may be detected later in the initiation pathway, possibly at the step where eIF-2 is released from the ribosome (13, 14). A satisfactory understanding of the mechanism of translational control by eIF-2 phosphorylation requires a precise knowledge of how the factor acts, but molecular details are lacking.

In order to approach the general problem of defining the mechanism of action of initiation factors, we have begun to clone the cDNA sequences for individual factor proteins. We report here the cloning and sequencing of both rat and human cDNAs for eIF-2\(\alpha\) mRNA. The availability of the clones enables us to begin more detailed studies on the structure, function, and expression of this important initiation factor protein.

EXPERIMENTAL PROCEDURES

Screening of cDNA Libraries—A rat brain cDNA library in \(\lambda\)gt11 (15) was kindly provided by Larry Fritz and Norman Davidson (California Institute of Technology). About 50,000 phages/150-mm plate were grown on a lawn of Escherichia coli Y1050 and screened according to the method of Young and Davis (16) with affinity-purified polyclonal antibodies prepared in rabbits against human eIF-2\(\alpha\) (California Institute of Technology). About 2.1 kb of cDNA and the cDNA insert, which was determined to be about 1.4 kb long, was subcloned into pUC18 (18) to yield pHr2a-1. The 1.4-kb rat cDNA fragment was purified from pHr2a-1 by EcoRI digestion, PAGE, and electrophoresis from the gel.

The 1.4-kb rat cDNA fragment was labeled by nick translation (19) to a specific activity of 106 cpm/\(\mu\)g of DNA and used to screen a human fibroblast cDNA library in \(\lambda\)gt11 (kindly provided by Barbara Wold, California Institute of Technology) by the method of Benton and Davis (20) with some modifications (25). Positive phages

* This work was supported in part by National Institutes of Health Grant GM22135. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by a fellowship from the Deutsche Forschungsgemeinschaft.

1 The abbreviations used are: eIF, eukaryotic initiation factor; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis; bp, base pair; kb, kilobase; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
were picked and plaque purified. The insert from one of these was isolated and cloned into pUC18 as described above to yield pH2a-1.

Hybrid Selection and Translation—Hybrid selection was carried out according to the method described by Parnes and co-workers (21) with minor modifications. The gel-purified 1.4-kb cDNA insert from pH2a-1 (0.45; 7-mm diameter) was bound to a nitrocellulose disc (Millipore HAWG-045; 7-mm diameter). Poly(A)* RNA (25 μg), isolated from the postnuclear supernatant of HeLa cells as described below, was hybridized to the disk for 12–16 h at 42 °C. Following extensive washing (10 × 5 min in 300 mM NaCl, 30 mM sodium citrate, 0.5% SDS, 5× SSC), the hybridized mRNA was eluted by boiling twice for 1 min in 200 μl of water containing 2 μg of rabbit tRNA followed by quick-freezing in liquid nitrogen. The mRNA from the combined eluates was precipitated with ethanol and translated in a nuclease-treated rabbit reticulocyte lysate as described by Jackson and Hunt (22). Reaction mixtures (25 μl) containing 20 μCi of [35S]methionine (specific activity, 1100 Ci/mm) were incubated for 60 min, lysophorized, and suspended in urea/Nonidet P-40/ampholyte lysis buffer and analyzed by isoelectric focusing/SDS-polyacrylamide gel electrophoresis (IEF/SDS-PAGE) as described elsewhere (23). The dried gels were exposed to Kodak X-Ormat AR film at −70 °C for 1–2 days.

Preparation of Poly(A)* RNA—HeLa strain 93 cells were grown exponentially in spinner cultures and harvested at a density of 5–6 × 10^6 cells/ml as described elsewhere (24). Washed cells were lysed by 4 ml of lysis buffer (10 mM Tris-HCl, pH 8.6, 140 mM NaCl, 1.5 mM MgCl2, 0.5% Nonidet P-40, 0.25 mg/ml benzonate, and 140 mM β-mercaptoethanol) and the lysate was clarified by centrifugation at 10,000 × g for 20 min. One volume of buffer (20 mM Tris-HCl, pH 7.5, 7.5 mM EDTA, 0.5 mM NaCl, 4% SDS, 10 μl urea) was added to the postnuclear supernatant, which was then treated twice with an equal volume of phenol/chloroform/isoamyl alcohol (24:24:1). RNA from the aqueous phase was precipitated with ethanol and then chromatographed on oligo(dT)-cellulose (two binding/elution cycles) as described (25) to isolate poly(A)* RNA (unbound fraction) and poly(A)* RNA (bound fraction). In some cases, the lysate clarification step was omitted, thereby leading to RNA preparations obtained from total cells.

Northern Blot Hybridization Analysis—RNA preparations were denatured by heating to 60 °C for 15 min in the presence of 50% deionized formamide and 6% formaldehyde, subjected to electrophoresis in 1% agarose/formaldehyde gels (26), and electrophoretically transferred to a nylon membrane (GeneScreen, New England Nuclear) (26). The 1.4-kb rat cDNA fragment obtained from pH2a-1 was labeled by nick translation (19) to 10^9 cpm/μg and hybridized to the blot at 44 °C for 16 h in hybridization buffer (50% formamide, 50 mM Tris-HCl, pH 7.5, 5 mM NaCl, 1% SDS, 0.1% sodium pyrophosphate, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% polyethylene glycol 100000, 100 μg/ml Herring sperm DNA). The membrane was washed at 65 °C as recommended (26), and RNA hybridizing with the labeled cDNA probe was detected by autoradiography using Kodak X-Ormat AR film at −70 °C with intensifying screens.

DNA Sequencing—The rat and human cDNA fragments were excised from the gel and purified by digestion with 1 unit of alkaline phosphatase, gel purified, and subcloned into M13mp9 (27) and M13mp19 (28) respectively. Clones carrying the inserts in both orientations were selected, and nested sets of overlapping deletions in the cDNAs were produced by the method of Dale et al. (29). These were sequenced by the dideoxynucleotide chain termination method of Sanger (30) with deoxyadenosine 5′-α-[35S]diphosphate ([α-35S]dATP) (31) to generate complete sequences on both strands. The Microgenie sequence analysis program (Beckman) was used to analyze the sequences.

Polysome Profile Analysis—Exponentially growing HeLa cells in spinner culture were transferred to 100-mm tissue culture dishes (~10 ml or 5 × 10^6 cells/dish) and analyzed following three different growth regimens (6): 1) exponential, harvested 1 h following plating; 2) serum-depleted, harvested 4 days after refeeding with incomplete medium; and 3) depletel-stimulated, 4-day depleted cells refed with fresh medium and harvested 1 h later. The preparation and analysis of cell lysates were performed at 4 °C. Cells were washed three times, then suspended in 900 μl of lysis buffer (20 mM Hepes-KOH, pH 6.8, 10 mM MgCl2, 100 mM KCl, 10 μg/ml cycloheximide, 1 mM EDTA), brought to 0.5% Triton X-100 and 1% deoxytritonethanol and the lysate was clarified by centrifugation at 8,000 rpm (Sorvall SS-34 rotor) and layered onto a 15–35% sucrose gradient in lysis buffer (no detergents), and centrifuged in a Beckman SW 41 rotor for 2 h at 35,000 rpm. Gradients were fractionated by bottom puncture using an Iso1 gradient fractionator and UV monitor, and 13 fractions (1 ml each) were collected. The fractions were extracted with phenol/ chloroform/isoamyl alcohol, ethanol precipitated, and analyzed by Northern blotting.

RESULTS

Isolation of a cDNA Clone Encoding eIF-2α—eIF-2α is a moderately abundant protein in HeLa cells, comprising 0.93% of the cytoplasmic protein molecules (11). If the relative mRNA abundance reflects the eIF-2α protein level, a cDNA expression vector library may be expected to express eIF-2α sequences at a frequency of about 10−4. A rat brain cDNA library in λgt11 was screened for eIF-2α by the antibody screening technique of Young and Davis (16) as described under “Experimental Procedures.” One recombinant phage (AHR2a-1) producing immunoreactive protein was isolated from extend recombinant phages and was plaque purified. When the anti-eIF-2α antibodies were preadsorbed with purified eIF-2α, the screening gave negative results which suggests that eIF-2α sequences are carried in the recombinant λgt11 phage.

The cDNA insert and adjacent vector sequences were excised from λHR2a-1 by KpnI-SstI digestion and subcloned into pUC18 to yield pH2a-1. The size of the cDNA fragment which is recovered from pHr2a-1 by EcoRI digestion is 1.4 kb (data not shown), which in principle is large enough to code for the entire eIF-2α protein. The cDNA corresponding to the predicted coding region is ~900 nucleotides.

Hybrid Selection and Translation—To obtain further evidence that the recombinant plasmid carries eIF-2α cDNA sequences, the purified rat cDNA insert from pHr2a-1 was used to hybrid select eIF-2α mRNA from a poly(A)* RNA preparation from HeLa cells as described under “Experimental Procedures.” Bulk poly(A)* and hybrid-selected mRNAs were translated in a nuclease-treated rabbit reticulocyte lysate, and the labeled products were analyzed by two-dimensional IEF/SDS-PAGE. Translation of total cytoplasmic poly(A)* RNA from HeLa cells produces numerous radiolabeled proteins (Fig. 1, panel A), one of which precisely co-migrates with a lysate protein detected by Coomassie Blue staining (labeled 2α in panel A). This protein co-migrates with purified HeLa eIF-2α (36 kDa) and reacts with anti-eIF-2α antiserum (112); data not shown). It, therefore, corresponds to authentic eIF-2α. Hybrid-selected mRNA produces two major spots of 36 kDa (panel C, indicated with arrows). The most basic spot co-migrates with this same Coomassie-stained rabbit reticulocyte eIF-2α spot. The other acidic spot appears in the gel where phosphorylated eIF-2α migrates (5). No protein products are seen in the absence of exogenous mRNA (panel B).

Further evidence that the translation products encoded by the hybrid-selected mRNA are eIF-2α proteins was obtained from partial protease digestion patterns. The [35S]methionine-labeled basic and acidic spots from the in vitro synthesis were excised from the gel, and the protein was digested with protease V8 as described in the legend of Fig. 2. The patterns for the two spots are strikingly similar (Fig. 2, lanes 1 and 2), indicating that the two spots represent different forms of the same protein. Their patterns also closely resemble the silver-stained V8 pattern of authentic eIF-2α (lane 0) obtained from a two-dimensional IEF/SDS-PAGE separation of eIF-2α subunits. A few minor bands are present in the silver-stained eIF-2α lane that are absent from the labeled in vitro synthesized protein, but most of the major bands are detected in both lanes. The V8 pattern from the hybrid-selected mRNA closely resembles previously published patterns for eIF-2α (12).
Cloning and Sequencing of cDNAs Encoding α-Subunit of eIF-2

The apparent phosphorylation of in vitro synthesized eIF-2α suggests that an eIF-2α kinase is active in the translation system sometime during the 60-min incubation. To further investigate this phenomenon, three translation assays programmed with hybrid-selected eIF-2α mRNA were incubated for a total of 60 min. The first served as a control; the second, sodium selenite, which inhibits the eIF-2α phosphorylation (33), was added at 45 min; and the third, alkaline phosphatase was added following the incubation. The three samples of [35S]methionine-labeled proteins were characterized by IEF/SDS-PAGE. The control sample (no treatments) produced the pattern shown in Fig. 1, panel C. In the sodium selenite-treated sample the ratio of phosphorylated to nonphosphorylated eIF-2α increases (Fig. 1, panel D). The eIF-2α and -2α(P) in the gel shown in panels C and D were not distinctly separated, but it can be estimated that in panel C about 50% of the radioactivity is in each spot, whereas in the sodium selenite-treated sample the eIF-2α(P) is much the larger of the two, comprising, we estimate, at least 75% of the

![Image](https://via.placeholder.com/150)

**Fig. 1. Hybrid selection/translation of eIF-2α.** Nuclease-treated rabbit reticulocyte lysates were programmed with exogenous mRNA preparations and products were analyzed by two-dimensional IEF/SDS-PAGE as described under "Experimental Procedures." The panels show autoradiograms of the complete gel (panel A) or a portion of the gels (panels B-E) as indicated by the rectangle corners drawn in panel A. The locations of eIF-2α and eIF-2α(P) are shown in panels C-E with arrows. Panel A, total poly(A)⁺RNA; panel B, no exogenous mRNA added; panel C, hybrid-selected mRNA; panel D, same as panel C, except that 50 μM sodium selenite was added to the translation mixture during the last 15 min of incubation; panel E, same as panel C, except that following incubation, the mixture was dialyzed overnight against buffer (10 mM Tris-HCl, pH 8.0, 1 mM MgCl₂, 1 mM CaCl₂, and 1 mM dithioerythritol) containing protease inhibitors (Boehringer Mannheim) for 30 min at 37 °C before gel analysis.

The apparent phosphorylation of in vitro synthesized eIF-2α suggests that an eIF-2α kinase is active in the translation system sometime during the 60-min incubation. To further investigate this phenomenon, three translation assays programmed with hybrid-selected eIF-2α mRNA were incubated for a total of 60 min. The first served as a control; the second, sodium selenite, which inhibits the eIF-2α phosphatase (33), was added at 45 min; and the third, alkaline phosphatase was added following the incubation. The three samples of [35S]methionine-labeled proteins were characterized by IEF/SDS-PAGE. The control sample (no treatments) produces the pattern shown in Fig. 1, panel C. In the sodium selenite-treated sample the ratio of phosphorylated to nonphosphorylated eIF-2α increases (Fig. 1, panel D). The eIF-2α and -2α(P) in the gel shown in panels C and D were not distinctly separated, but it can be estimated that in panel C about 50% of the radioactivity is in each spot, whereas in the sodium selenite-treated sample the eIF-2α(P) is much the larger of the two, comprising, we estimate, at least 75% of the

In the alkaline phosphatase-treated sample the relative amount of phosphorylated eIF-2α decreases to about 20–30% of the mass (Fig. 1, panel E). These results are consistent with our interpretation that the more acidic eIF-2α spot is due to phosphorylation, even though hemin is included in the lysate system. We have observed that translation begins to decrease after 45 min, even in the presence of hemin. A late activation of an eIF-2α kinase may account for the decreased activity of the lysate and the observed phosphorylation of the in vitro translated eIF-2α.

Characterization of eIF-2α mRNA—The size of eIF-2α mRNA in HeLa cells was determined by Northern blotting. Poly(A)⁺ RNA isolated from a postnuclear supernatant or from total HeLa cell extracts was subjected to agarose-formaldehyde gel electrophoresis, transferred to a nylon membrane, and probed with the labeled cDNA insert from pHr2α-1, as described under "Experimental Procedures." A single major band measuring about 1600 nucleotides is observed (Fig. 3, lanes a, b, and c). If the hybridization is conducted at a lower stringency (1 M NaCl, 41 °C) a minor band at about 4000 nucleotides can be detected as well (not shown). When poly(A)⁺ RNA is analyzed, no eIF-2α mRNA is detected (lane d). Since the coding region should comprise about 800 nucleotides (to encode the 36-kDa protein) and the poly(A) tract is likely about 100 nucleotides, the 1600-nucleotide eIF-2α mRNA appears to contain about 900 nucleotides of nontranslated sequence distributed between the 3′ and 5′ noncoding

![Image](https://via.placeholder.com/150)

**Fig. 2. Partial protease fragmentation analysis.** Following two-dimensional gel electrophoresis of a standard translation mixture containing hybrid-selected mRNA, the gel was stained briefly with Coomassie Blue, and spots corresponding to phosphorylated and nonphosphorylated eIF-2α were excised. The eIF-2α spot was also excised from another gel in which about 4 μg of purified HeLa eIF-2 was fractionated. The gel segments were analyzed by protease V8 digestion (0.25 pg/lane) during one-dimensional SDS-PAGE as described (12). Lanes 1 and 2 show an autoradiograph of the phosphorylated and nonphosphorylated forms of in vitro translated [35S]-labeled eIF-2α. Lane 0 shows a photograph of the gel containing purified eIF-2α stained by the silver method of Wray et al. (32). The major bands that line up (identical size proteolytic cleavage fragments) are indicated with horizontal connecting bars.
amino acids (this value includes the initiator methionine which is cleaved in the rabbit protein) with a mass of 36,111 daltons. The 27 bp of rat cDNA upstream of the initiator ATG contain no other ATG codon but nucleotides 1–11 may interact (one mismatch) with 26–36 in the mRNA. The 3′-nontranslated region is 401 bp long, possesses a polyadenylation signal, AATAAA (residues 1358–1363), and is directly followed by the poly(A) tail (not shown in Fig. 4).

Since we have previously characterized the eIF-2α protein from human (HeLa) cells, we were interested in analyzing and sequencing a human cDNA clone for eIF-2α as well. Toward this goal, the rat cDNA insert from pH2a-1 was excised, radiolabeled, and used as a hybridization probe to screen a human fibroblast cDNA library in λgt11 as described under “Experimental Procedures.” The cDNA insert from a plaque-purified isolate was subcloned into pUC18 to yield pH2a-1. A 1.9-kb cDNA insert in pH2a-1 was excised by digestion with EcoRI, gel purified, and subcloned into phage M13mp19 in both orientations for sequencing of both strands. A large portion of the human cDNA sequence is highly homologous to the rat cDNA, as shown in Fig. 4. The human cDNA also codes for a putative protein of 315 amino acids (36,115 Da), which differs from the rat sequence at only 3 residues. At the DNA sequence level, the rat and human coding regions show 93.4% homology, whereas the 5′- and 3′-nontranslated regions exhibit 82.1 and 80.8% homology, respectively. The human cDNA sequence contains an extra 17 base pairs interspersed at various sites in the 3′-nontranslated region (Fig. 4). Not shown in the figure are about 400 bp of human cDNA extending beyond the 3′-nontranslated region and 43 bp extending upstream from the rat 5′-nontranslated region. The extra 400 bp of downstream DNA cannot be eIF-2α cDNA since the total length of the sequenced insert is greater than the size of eIF-2α mRNA. This extra DNA presumably arose by a fusion of cDNAs during construction of the library and has not been investigated further.

Characterization of the eIF-2α Protein—The amino acid sequences of rat and human eIF-2α are highly homologous, differing at only 3 residues in the C-terminal half of the protein. The amino acid compositions of the human and rat proteins derived from the cDNAs are reported in Table I and are compared to that for rabbit reticulocyte eIF-2α determined by conventional amino acid analysis (34). The mole percents of most amino acid residues are very similar. A computer-generated hydrophilicity profile for rat eIF-2α is shown in Fig. 5. The protein contains many charged and hydrophilic residues and also exhibits a high degree of secondary structure as determined by the Garnier algorithm in the Microgenie™ program (not shown), most notably a long strongly acidic α-helix at the C terminus. A search of amino acid and nucleotide sequences in the GenBank data bank did not reveal other proteins or genes homologous to eIF-2α. Likewise, no sequences matching identified GTP binding domains were found.

Translational Control of eIF-2α mRNA—Previous results based on protein labeling suggest that the overall rates of eIF-2α and total protein synthesis decrease about 4–8-fold during serum depletion (35), reflecting a 3–5-fold decrease in the number of active ribosomes (35, 36) and an ~1.5-fold decrease in the elongation rate (37). The repression of eIF-2α synthesis could be due to (i) a repression of mRNA by conversion into inactive mRNP; (ii) reduced translational efficiency of eIF-2α mRNA in polysomes; or (iii) reduced levels of mRNA. These possibilities were examined using dot and Northern blot analyses of exponentially growing, serum-depleted, and refed serum-depleted HeLa cells (6, 35–37).

---

2 Fairwell Thomas, Millie Schaefer, and Brian Safer, personal communication.
To determine whether a decrease in eIF-2α mRNA concentration plays a role in controlling the amount of eIF-2α, a dot blot analysis was performed on cytoplasmic RNA from exponentially growing, serum-depleted, and refed cells. This is followed by autoradiography band densities to measure the amount of eIF-2α mRNA/cell. The amount of eIF-2α mRNA is found in fractions 12-13, corresponding to polysomes containing 10 or more ribosomes (precise polysome sizes cannot be determined for such large polysomes). There is no detectable eIF-2α mRNA at the top of the gradient where nontranslating mRNPs are found. These results indicate that eIF-2α mRNA is efficiently translated in exponentially growing HeLa cells.

In the serum-depleted cells (Fig. 6), the fraction of ribosomes in polysomes is reduced about 4-fold, to about 20% of the total. eIF-2α mRNA is found principally in fraction 10, corresponding to an average polysome loading of 7 ribosomes/mRNA. Again, little or no eIF-2α mRNA occurs in the slowly sedimenting subpolysomal region with free mRNP. This is somewhat surprising, since there is a substantial overall loss of poly(A)+ RNA from polysomes into free mRNP (36). The total amount of cDNA hybridizing to gradient fractions (measured by autoradiography band densities) was approxi-
amino acid analysis (34) is compared the cDNAs. 201 (314 amino acids, lacking the initiator methionine) deduced from the specific regulation of eIF-2α protein synthesis during polysomes was only 80-90% in the 1-h interval, as evidenced by the larger 80 S peak in panel c. This may account for the slightly reduced eIF-2α polysome size that is observed. Thus, the specific regulation of eIF-2α protein synthesis during serum depletion and refeeding appears to be due entirely to a specific modulation of the rate of initiation on the existing eIF-2α mRNA.

| Amino acid | Rabbit protein | Rat protein | Human protein |
|------------|----------------|-------------|---------------|
| Ala        | 7.2            | 7.0         | 22            |
| Arg        | 6.4            | 8.0         | 25            |
| Asp        | 4.1            | 14          | 14            |
| Asn + Asp  | 12.6           | 11.2        | 35            |
| Asp        | 6.7            | 21          | 21            |
| Cys        | 1.7            | 1.6         | 5             |
| Gln        | 2.2            | 7           | 7             |
| Gln + Glu  | 15.7           | 14.3        | 45            |
| Glu        | 12.1           | 38          | 38            |
| Gly        | 4.9            | 4.1         | 13            |
| His        | 1.2            | 1.1         | 3             |
| Ile        | 5.6            | 7.0         | 22            |
| Leu        | 8.0            | 8.3         | 26            |
| Lys        | 8.7            | 6.7         | 21            |
| Met        | 1.3            | 2.5         | 8             |
| Phe        | 2.3            | 2.2         | 7             |
| Pro        | 3.6            | 3.2         | 10            |
| Ser        | 5.2            | 5.1         | 16            |
| Thr        | 4.3            | 4.9         | 15            |
| Trp        | 1.6            | 0.3         | 1             |
| Tyr        | 2.3            | 3.8         | 12            |
| Val        | 7.5            | 8.9         | 28            |

**Fig. 5.** Hydrophilicity profile of eIF-2α. The deduced amino acid sequence of rat eIF-2α was analyzed by the Microgenie™ sequence analysis program (Beckman) to generate a hydrophilicity profile. Hydrophilic regions of the protein are plotted above the line while hydrophobic regions are plotted below.

The amino acid composition of rabbit eIF-2α measured by standard methods is shown in Table I. The composition is approximately equal in exponentially growing and serum-depleted cells, consistent with the dot blot results cited above. The data indicate that translational repression for eIF-2α mRNA is accomplished in most part by reducing the specific initiation rate/mRNA, without changing the total number of messages engaged in translation. Note also that whereas the total number of polysomal ribosomes decreases about 4-fold, the total number of polysomal eIF-2α mRNAs shows no decrease. This provides further evidence that eIF-2α mRNA initiates translation quite efficiently.

When serum-depleted cells are refed fresh serum-containing medium, polysomes reform rapidly upon pre-existing mRNPs (35, 36) (also shown in Fig. 6, panel c). eIF-2α synthesis increases 3-5-fold (35). A Northern blot analysis of polysomes from refed cells was performed to directly assess the translation of eIF-2α mRNA (Fig. 6, panel c). The eIF-2α mRNA distribution in cells transferred to fresh 10% serum-containing medium for 60 min is similar to control cells: the larger eIF-2α polysomes containing 10 or more ribosomes reform in this interval. In this experiment conversion of ribosomes into polysomes was only 80-90% in the 1-h interval, as evidenced by the larger 80 S peak in panel c. This may account for the slightly reduced eIF-2α polysome size that is observed. Thus, the specific regulation of eIF-2α protein synthesis during serum depletion and refeeding appears to be due entirely to a specific modulation of the rate of initiation on the existing eIF-2α mRNA.

**Fig. 6.** Polysome profiles of eIF-2α mRNA. HeLa cell extracts were prepared and analyzed on sucrose gradients as described under "Experimental Procedures." The figure shows the absorbance profile of the gradient (-----) and a plot of the relative concentration of eIF-2α mRNA as determined by density scanning of the autoradiograms of Northern blots probed for eIF-2α sequences (-----). Sedimentation is from left to right. Panel a, exponentially growing cells; panel b, serum-depleted cells; panel c, refed serum-depleted cells.

**DISCUSSION**

It is our goal to understand the mechanism and role of the initiation factors in promoting and regulating the process of protein synthesis. Knowledge of their amino acid sequences and their transcription and translation rates should prove important to understanding these functions. Toward this goal, we are cloning and characterizing cDNAs for the initiation factors. We report here the cloning of cDNAs coding for the α subunit of eIF-2. In addition, the cloning of cDNAs for mouse eIF-4A (38), several subunits of mouse eIF-3, and yeast eIF-4E (the 24-kDa cap binding protein) have been reported. The identification of the cloned cDNA for eIF-2α is based on a number of independent criteria. Recombinant phage plaques synthesize a protein which reacts with eIF-2α antiserum except when the serum is preadsorbed with pure eIF-2. Hybrid selection and translation result in the synthesis of two proteins which co-migrate upon two-dimensional gel electrophoresis with purified eIF-2α and its phosphorylated form, eIF-2α(P). The two labeled translation products when examined by partial proteolytic digestion give fragmentation patterns indistinguishable from each other and from that of authentic eIF-2α mRNA.

---

1 Michael Altmann and Hans Trachsel, personal communication.
eIF-2α. The DNA sequences of the human and rat cloned cDNAs each code for a putative protein product which has an amino acid composition very similar to that for rabbit eIF-2α determined by classical amino acid analysis of the protein. Finally, the N-terminal amino acid sequence of rabbit eIF-2α precisely matches the derived human and rat sequences.

From the sequence of the human and rat eIF-2α cDNAs and from the N-terminal amino acid sequence of the rabbit protein, the coding region of the mRNAs can be identified unambiguously. The mRNAs code for proteins of 315 amino acids (36.1 kDa) which are likely processed post-translationally by removal of the N-terminal methionine. The sequence around the initiator codon of the human mRNA is AGAAUGGC, which possesses an A residue at position −3, but otherwise does not resemble initiation regions commonly found in eukaryotic mRNAs (39). The 27 bp of DNA upstream from the ATG do not likely represent all of the 5′-nontranslated leader sequence of the mRNA, since the 1377 bp of cDNA plus a poly(A) tail of about 100 nucleotides do not correspond to the overlapping portions of the 5′ leader sequences and 82.1% homology in the noncoding sequences of the human and rat cDNAs are somewhat less conserved, however. There is 82.1% homology in the 27 bp of DNA upstream from the initiator codon of the human mRNA, which is highly conserved amino acid sequence for eIF-2α proteins. The highly conserved amino acid sequence for eIF-2α is comparable to the density of ribosomes on the overlapping portions of the 5′ leader sequences and 80.8% homology in the 3′-nontranslated region. In addition, there are 17 fewer base pairs of DNA in the rat compared to the human cDNA in this latter region.

Several observations indicate that eIF-2α mRNA is translated efficiently. First, the size of the eIF-2α polyisome is 10 or more ribosomes/mRNA in exponentially growing cells. This corresponds to 80–100 nucleotides of coding sequence/ribosome and is comparable to the density of ribosomes on globin mRNA in rabbit reticulocytes. In HeLa cells an average-sized mRNA, measuring 1500 nucleotides (40), occurs in polyisomes containing 7–8 ribosomes (36) and encodes a 37,000-Da protein (37). In terms of overall size and coding length, eIF-2α mRNA is typical of most HeLa cell mRNAs, yet eIF-2α polyisomes are substantially larger than the average. Thus, eIF-2α mRNA likely initiates translation more efficiently than the average message in HeLa cells, even though the initiator codon consensus sequence is not particularly favorable. Second, no eIF-2α mRNA is detected in the sucrose gradient fractions where free mRNP particles are expected, indicating that all of the mRNA is active in translation. In serum-depleted cells which are 4- to 8-fold less active for overall protein synthesis, at least two-thirds of the ribosomes and a substantial portion of the mRNA are released from polyisomes into free subpolyosomal particles (6, 35–37). The eIF-2α polyisomes become considerably smaller (averaging about 7 ribosomes/mRNA). The eIF-2α polyisomes revert to their larger size when the depleted cells are fed medium with fresh serum. The increase in polysome size reflects the increased rate of eIF-2α synthesis, which is enhanced to the same extent as the bulk of HeLa protein (6). These results corroborate previous data indicating that initiation factor synthesis rates are not specially regulated in HeLa cells but suggest that the specific mechanisms used by eIF-2α to regulate translational repression (and reactivation) are different from those that regulate the bulk HeLa cell mRNAs.

Acknowledgments—We thank Drs. Larry Fritz, Nevis Fregien, Alan Golden, and Normen Davidson for providing the rat brain cDNA library and Barbara Wold for the human fibroblast cDNA library. Millie Schafer and Brian Safer for communicating the results of their N-terminal sequence analysis of eIF-2α prior to publication, and Dr. Gisela Heidecker for invaluable suggestions and fruitful discussions during early stages of the project.

REFERENCES
1. Jagus, R., Anderson, W. P., and Safer, B. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 109–112
2. Floyd, G. A., Merrick, W. C., and Traugh, J. A. (1979) J. Biol. Chem. 254, 277–286
3. Schafer and Brian Safer for communicating the results of their N-terminal sequence analysis of eIF-2α prior to publication, and Dr. Gisela Heidecker for invaluable suggestions and fruitful discussions during early stages of the project.