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Protein Synthesis Initiation Factors from Human HeLa Cells and Rabbit Reticulocytes Are Similar: Comparison of Protein Structure, Activities, and Immunochemical Properties†

Marianne L. Brown-Luedi, Laurence J. Meyer, Susan C. Milburn, Peter Mo-Ping Yau, Susan Corbett, and John W. B. Hershey*

ABSTRACT: Five initiation factors, eIF-2, eIF-3, eIF-4A, eIF-4B, and eIF-5, were purified from human HeLa cells. Methods of protein fractionation and assays for initiation factors which had been developed for rabbit reticulocytes were found to be suitable for HeLa factors. The initiation factors from HeLa cells are similar to or indistinguishable from the corresponding rabbit reticulocyte factors with respect to specific activities, molecular weights as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and subunit structure. The molecular weight of eIF-3 particles from both species is about 410 000 as determined by equilibrium sedimentation analytical centrifugation. The partial protease fragmentation patterns of corresponding proteins also are similar and indicate that the primary sequences of the factors are related in the two species. Antisera raised in goats against rabbit eIF-3 and human eIF-2, eIF-4A, and eIF-4B cross-react with the cognate factors from both species. On the basis of immunoblotting techniques, eIF-4A is highly conserved, eIF-2α, eIF-3, and eIF-4B are somewhat less conserved, and eIF-2β is the least conserved of the proteins examined. The functional, structural, and immunological results are all consistent with the view that initiation factors from different mammalian cells are very similar.

Initiation of protein synthesis in mammalian cells is promoted by a large number of proteins called initiation factors. Elucidation of the roles played by these factors has been most extensive with those purified from rabbit reticulocytes. At least 10 different initiation factors have been purified and characterized (Schreier et al., 1977; Merrick, 1979; Benne & Hershey, 1978; Sonenberg et al., 1979; Dasgupta et al., 1979; Amesz et al., 1979). Rabbit reticulocytes are a good source of initiation factors because the cell lysate is an extremely active system for protein synthesis and large amounts of the cells can be obtained. However, the reticulocyte is a terminally differentiated cell which has lost its nucleus and is incapable of cell division. Thus, these cells are unsuitable for studying protein synthesis in different physiological states. We have chosen to study human HeLa cells grown in suspension culture. The cells are easily grown, may be synchronized, and can be infected by a number of animal viruses. Furthermore, the cost of growing the cells is comparable to that for obtaining rabbit reticulocytes. We report here the purification of five eukaryotic initiation factors (eIF)[1] from HeLa cells, eIF-2, eIF-3, eIF-4A, eIF-4B, and eIF-5, and show that they resemble closely the corresponding rabbit reticulocyte factors in all respects tested.

Materials and Methods

Growth of HeLa Cells. HeLa strain S3 cells were grown in 3-L spinner culture bottles (Bellco) in up to 2.5 L of Joklik's modified minimal essential medium supplemented with 5% fetal calf serum or calf serum (Flow Laboratories). The cells were harvested in exponential growth phase at a density of (7–8) $\times 10^5$ cells/mL by centrifugation, washed twice in PBS

† From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616. *Received November 16, 1981; revised manuscript received April 9, 1982.* Supported by National Institutes of Health Grant GM-22135 from the U.S. Public Health Service.

[1] Abbreviations: eIF, eukaryotic initiation factor; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; BME, 2-mercaptoethanol; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl.

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buffer (10 mM potassium phosphate, pH 7.2, and 150 mM NaCl), and stored as frozen cell pellets at −70 °C. Yields were routinely about 3 mL of packed cells per L of culture.

Preparation of Cell Lysate. Frozen cell pellets (50 g) were thawed rapidly and diluted with 100 mL of lysis buffer (10 mM Hepes, pH 7.5, 10 mM KCl, 1.3 mM magnesium acetate, 0.5 mM dithiothreitol, 67 μM EDTA, 20 μM hemin, and 2 mM PMSF). After the cells were allowed to swell 10 min on ice, they were lysed 20 s at full power with a tissumizer (Tekmar). Nuclei were pelleted by centrifugation in a Sorvall RC-3 centrifuge with an HG4 rotor for 3 min at 1800 rpm. The supernatant was removed with a syringe while avoiding the floating fatty layer. The nuclear pellet was suspended in 1 volume of lysis buffer and centrifuged and the supernatant removed and combined with the first. The supernatants were centrifuged for 15 min at 8500 rpm in the Sorvall SS-34 rotor, and the resulting supernatant was removed with a syringe. Glycerol (0.1 volume) was added to the lysate, called S10, which usually is 100–120 mL. To the S10 was immediately added 25 mL of settled hemoglobin–Sepharose resin to remove proteases. The suspension was stirred for 20 min at 0 °C and then filtered to remove the resin.

Preparation of Ribosomal Wash. The hemoglobin–Sepharose-treated S10 from 200 g of HeLa cells was layered over 4-mL cushions containing 20% glycerol in buffer (20 mM Tris-HCl, pH 7.6, 6 mM magnesium acetate, 50 mM KCl, 7 mM BME, and 1 mM PMSF) and was centrifuged 2 h at 55000 rpm in a Beckman Ti-60 rotor. The clear supernatant (S300) was removed, and the ribosomal pellets were resuspended in buffer and brought to 500 mM KCl and an A_{260} of 250. The suspension was layered over cushions containing the same buffer with 20% sucrose and was centrifuged as before. The supernatant was removed to yield about 275 mL of high-salt ribosomal wash (A_{280} = 12.8).

Purification of Initiation Factors. Initiation factors were purified from the ribosomal high-salt wash from 200 g of HeLa cells by essentially the same procedures developed for rabbit reticulocyte factors (Benne et al., 1979). The following changes were made: 1 mM PMSF was added to all buffers throughout the purification; Sephacryl S200 was substituted for Sephadex G-100 in step 8 and for Sephadex G-200 in step 11; the sequence of ion-exchange columns was changed for eIF-2 and eIF-5, which were separated first on DEAE-Sephacel and then further purified on phosphocellulose. Assays for HeLa factors were the same as those for reticulocyte factors (Benne et al., 1979); either reticulocyte or HeLa factors were used to complement the HeLa factor being assayed. Protein concentration was determined by the method of Bradford (1976) or Lowry et al. (1951). Rabbit reticulocyte initiation factors were prepared as described (Benne et al., 1979) without the use of PMSF or hemoglobin–Sepharose.

Analytical Ultracentrifugation. Equilibrium sedimentation was performed in a Beckman Model E ultracentrifuge with an AN-J rotor by the long-column meniscus-depletion method of Chervenka (1970). eIF-3 in buffer [20 mM potassium phosphate, pH 7.2, 7 mM BME, 0.2 mM EDTA, 0.2 mM PMSF, and 5% (v/v) glycerol] was centrifuged at 6000 rpm, and the absorbance at 280 nm was recorded with a photoelectric scanner and analyzed according to Yphantis (1964). Velocity sedimentation was performed in the buffer above by using an AN-D rotor at 7.5 °C. The peak of the schlieren pattern was used to estimate the position of the boundary; a value of 0.73 was used for J. After 18 min at 30000 rpm, the speed was increased to 40000 rpm. The viscosity of the buffer was determined independently. A microcomparator was used to measure photographic plates of the schlieren patterns.

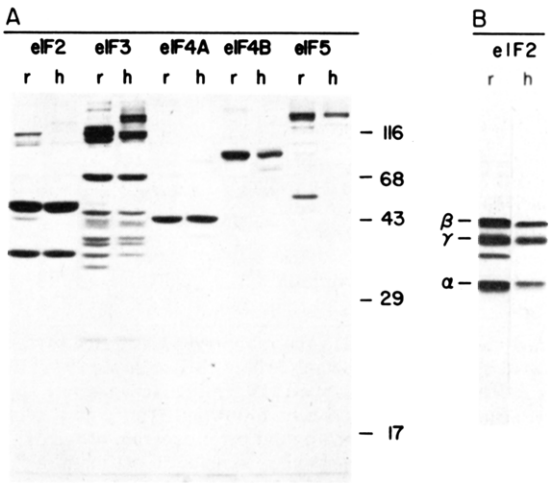

FIGURE 1: Comparison of rabbit and human initiation factors by $NaDodSO_4$–polyacrylamide gel electrophoresis. Initiation factors from rabbit reticulocytes (left lanes, labeled r) and human HeLa cells (right lanes, labeled h) were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis, and the gels were stained with Coomassie blue and photographed. (A) Five initiation factors were analyzed on 12.5% acrylamide slab gels (110 × 150 × 1.5 mm) according to Laemmli (1970). The following amounts of protein were analyzed for rabbit and human factors, respectively: eIF-2, 6.9 and 4.5 μg; eIF-3, 10.1 and 6.5 μg; eIF-4A, 1.1 and 1.6 μg; eIF-4B, 2.4 and 3.0 μg; eIF-5, 2.7 and 1.1 μg. (B) eIF-2 preparations from rabbits (8 μg) and humans (5 μg) were analyzed on a slab gel (110 × 150 × 1.5 mm) containing 15% acrylamide and 0.09% bis(acrylamide) according to Schreier et al. (1977).

Results and Discussion

Isolation and Purification of Initiation Factors. HeLa S3 cells were grown in suspension culture and harvested in exponential phase. Four 50-g lots of frozen cells were thawed and lysed, and a postmitochondrial supernatant (S10) was prepared rapidly. Precautions against the action of proteases were taken, since it is known that rabbit reticulocyte initiation factors may be partially degraded during their isolation (Meyer et al., 1981). PMSF was added to the lysis buffer, and the S10 was treated with a hemoglobin–Sepharose resin to remove proteases (Nakayama et al., 1977). The ribosomes in the S10 were pelleted by centrifugation and then resuspended in buffer containing 0.5 M KCl and pelleted again. The resulting supernatant, the high-salt ribosomal wash, was used as the source of the initiation factors. The above precedures are described in detail under Materials and Methods.

Methods previously developed for the purification of rabbit reticulocyte initiation factors (Benne et al., 1979) were used to isolate the HeLa factors. Assay procedures with complementing reticulocyte factors are satisfactory (see below) for monitoring the HeLa factor activities. The behavior of the HeLa factors during purification was essentially indistinguishable from that of the reticulocyte factors, so that documentation of the column elution profiles or sizing steps is unnecessary. Five initiation factors, eIF-2, eIF-3, eIF-4A, eIF-4B, and eIF-5, were purified in yields comparable to those obtained from rabbit reticulocytes. Analysis of these preparations and comparisons with the corresponding rabbit factors by $NaDodSO_4$–polyacrylamide gel electrophoresis are shown in Figure 1. No attempt has been made to isolate the small initiation factors, eIF-1 and eIF-4D; a single effort to purify eIF-4C was unsuccessful.

Biological Activities. Purified HeLa and reticulocyte factors were compared by using a number of different cell-free assays

Table I: Comparison of Specific Activities of Rabbit and Human Factors[a]

factor	assay	sp act. human	sp act. rabbit
eIF-2	ternary complex	1.12	1.50
	methionylpuromycin	0.30	0.38
	globin synthesis	18.6	19.6
eIF-3	globin synthesis	4.0	3.6
eIF-4A	globin synthesis	16.8	18.8
eIF-4B	globin synthesis	330	290
eIF-5	methionylpuromycin	10.8	10.0

[a] Each factor from human and rabbit cells was tested by the indicated assays as described elsewhere (Benne et al., 1979) by using reticulocyte factors as complementing factors where appropriate. Specific activities are the following: for ternary complex, picomoles of [^{3}H]Met-tRNA bound per microgram of factor added; for methionylpuromycin, picomoles of [^{3}H]methionylpuromycin synthesized per microgram of factor added; for globin synthesis, picomoles of [^{3}H]leucine incorporated into hot trichloroacetic acid insoluble protein per microgram of factor added. Protein concentration was determined by the methods of Bradford (1976) and Lowry et al. (1951) with bovine serum albumin as standard.

(Benne et al., 1979), and the results are reported in Table I. Three assays were used: ternary complex formation with [^{3}H]methionyl-tRNA and GTP (for eIF-2 only); [^{3}H]-methionylpuromycin synthesis, which utilizes washed ribosomal subunits and is stimulated by eIF-2, eIF-3, eIF-4C, and eIF-5; globin synthesis with globin 9S mRNA, which requires all of the identified initiation factors (except eIF-4D) for full activity. The specific activities for a given factor from the two cell sources are quite comparable within 25%. No consistent difference in activity was detected for any of the initiation factors in numerous assays, so we conclude that rabbit and human factors are functionally equivalent. Complete interchangeability is also seen when crude ammonium sulfate fractions from HeLa and reticulocyte high-salt wash fractions are compared in the assay for globin synthesis (results not shown).

Physical Characterization. The molecular weights of eIF-4A, eIF-4B, and eIF-5 derived from HeLa cells are indistinguishable from those exhibited by rabbit factors as measured by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Figure 1A). Similarly, the three subunits of eIF-2 from the two sources have comparable mobilities in a gel system which resolves these proteins (Figure 2B). The corresponding eIF-3 polypeptides also are similar, but all polypeptides are not identical. The most pronounced differences are in the components with molecular weights greater than 90 000. In reticulocytes, all but one of these polypeptides appear to be related to one another in primary structure (Meyer et al., 1981). The differences seen in the HeLa factor may therefore reflect a slightly different pattern or extent of proteolysis. A second major difference between the two species is the absence in the HeLa preparation of a polypeptide with a molecular weight of about 35 000. Both eIF-3 preparations contain a 24 000-dalton component which is presumed to be the "cap binding" protein (Sonenberg et al., 1979).

The molecular weight of the native form of eIF-3 was determined by analytical ultracentrifugation. Two preparations of rabbit eIF-3 and one preparation of HeLa eIF-3 were analyzed by equilibrium sedimentation, as shown in Figure 2A. All three preparations show linear data for the bulk of the protein, with nonlinear behavior toward the bottom of the cell. Least-squares analysis of the smaller particles reveals a molecular weight of 410 000 for both rabbit and HeLa preparations. The molecular weight range for the lesser amounts of material near the bottom of the cell is 800 000–1 000 000; this material may be aggregate forms of eIF-3. The preparations also were analyzed by velocity sedimentation as shown in Figure 2B. The average sedimentation coefficient calculated from the data is 14.2 S for HeLa eIF-3; a single component was seen in the schlieren patterns (see inset, Figure 2B). The value of 14.2 S is smaller than those estimated by sucrose gradient centrifugation (Schreier & Staehelin, 1973; Benne & Hershey, 1976) but is consistent with a globular protein complex of 410 000 daltons.

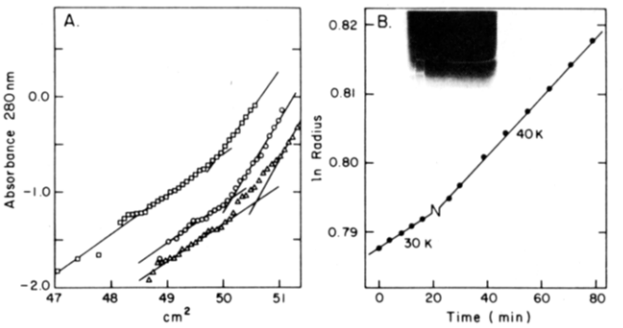

FIGURE 2: Analytical centrifugation of eIF-3 preparations. (A) Equilibrium sedimentation was performed as described under Materials and Methods: (□) 100 μg of rabbit reticulocyte eIF-3, pool A, described previously (Meyer et al., 1981); (○) 50 μg of rabbit reticulocyte eIF-3, pool B (Meyer et al., 1981); (△) 25 μg of HeLa eIF-3. Lines drawn are least-squares best-fit approximations grouping the data as follows: (□) first 26 points, last 8 (all points used); (○) first 15, last 12; (△) first 21, last 5. Molecular weights calculated from these lines are the following: (□) 402 000 and 810 000; (○) 410 000 and 980 000; (△) 420 000 and 1 020 000. (B) Velocity sedimentation was performed as described under Materials and Methods. The figure shows data with 2.5 mg of HeLa eIF-3. The inset shows a schlieren pattern taken at 39 min. The *s* values calculated from the data at 30 000 and 40 000 rpm are 13.8 S and 14.7 S, respectively.

The five rabbit and human initiation factors were characterized further by comparing partial protease fragmentation patterns of their polypeptides by the method of Cleveland et al. (1977). Proteins first were labeled with ^{125}I (Meyer et al., 1981) to increase the sensitivity of the method. The patterns of fragmentation with *Staphylococcus aureus* protease V8 (Figure 3A) of HeLa and rabbit reticulocyte eIF-4A, eIF-4B, and the three subunits of eIF-2 are strikingly similar. Digestions of eIF-2 and eIF-4A with elastase and subtilisin, respectively (Figure 3B), confirm that these proteins are closely related. eIF-5 suffers extensive degradation upon iodination, so its fragmentation patterns (Figure 3C) were identified by staining with a sensitive silver method (Oakley et al., 1980). Corresponding eIF-3 polypeptides (defined in Figure 3E) from rabbits and humans also give similar patterns (Figure 3D), although those from the high molecular weight polypeptides 1 and 2 are not identical. The evidence indicates that most of the corresponding factor polypeptides from human HeLa cells and rabbit reticulocytes are very closely related in primary structure.

Immunochemical Characterization. Antibodies were prepared by immunizing goats with highly purified preparations of eIF-3 from rabbit reticulocytes and with eIF-2, eIF-4A, and eIF-4B from human HeLa cells. The preparation and characterization of the antibodies are described in detail in the following paper (Meyer et al., 1982). We use the antisera here merely to compare the factors from the two species in order to determine whether the initiation factors share antigenic determinants. Anti-eIF-3 and anti-eIF-4B are precipitating antisera and form single precipitin lines when tested with their cognate antigens by Ouchterlony double immunodiffusion

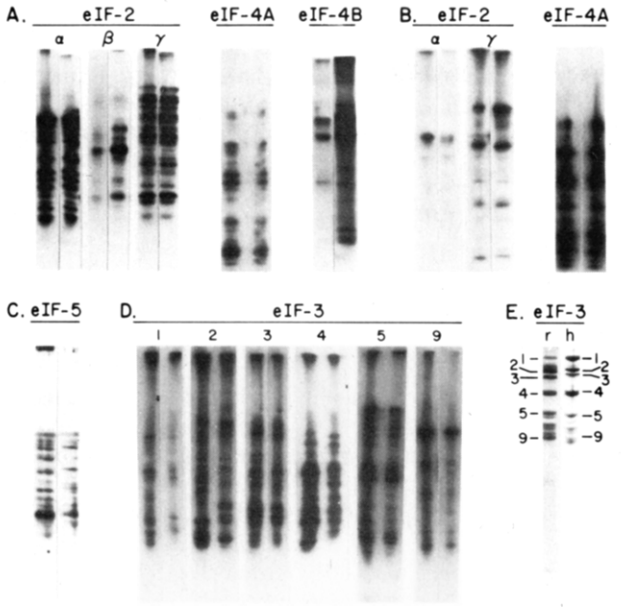

FIGURE 3: Partial protease digestion patterns of initiation factor proteins. Initiation factors were analyzed by limited digestion with proteases according to the method of Cleveland et al. (1977). Each factor preparation was iodinated (except eIF-5), protein was fractionated by $NaDodSO_4$–polyacrylamide gel electrophoresis as shown in Figure 1, and individual bands (containing about 1 μg of protein each) were excised for analysis as described in detail elsewhere (Meyer et al., 1981). The figure shows autoradiographs of the gels, except for eIF-5 (part C). In each of the double lanes, the rabbit factor is on the left, and the HeLa factor is on the right. (A) eIF-2, eIF-4A, and eIF-4B polypeptides were digested with *Staphylococcus aureus* protease V8 (35–175 ng/lane). (B) eIF-2 subunits were digested with elastase (3.5 μg), and eIF-4A was digested with subtilisin (3 ng). (C) Noniodinated eIF-5 (160 000 molecular weight bands, about 1.5 μg of each) was digested with protease V8; the gel was stained with silver (Oakley et al., 1980) and photographed. (D) eIF-3 polypeptides, numbered according to Benne & Hershey (1976) as shown in part E, were digested with protease V8 (100–150 ng). The gel lanes are identified at the top by the numbered polypeptides analyzed.

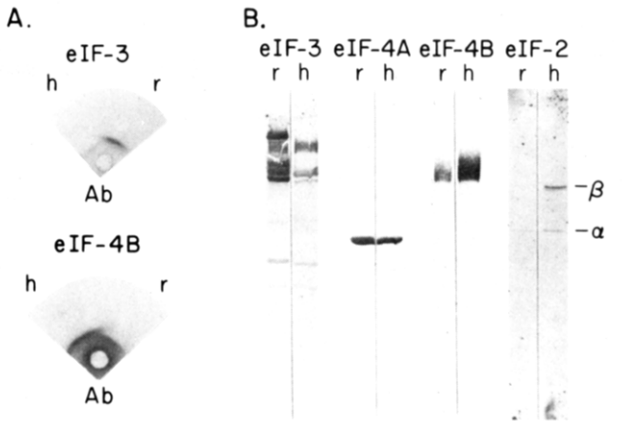

FIGURE 4: Immunochemical comparisons. (A) Ouchterlony double immunodiffusion. Plates were formed 1.8 mm thick with agarose (Bio-Rad) in 10 mM potassium phosphate, pH 7.2, 150 mM KCl, and 0.05% sodium azide. Wells (4-mm diameter) were cut 6 mm apart, and diffusion was performed at room temperature for 4–6 days. The plates were washed with 0.5 M NaCl and stained with Coomassie blue (Laurell, 1972). Wells contained the following: for anti-eIF-3, 20 μL of serum (Ab) and 10 μg each of rabbit reticulocyte eIF-3 (r) or human HeLa eIF-3 (h); for anti-eIF-4B, 20 μL of serum concentrated 2-fold by ammonium sulfate precipitation at 45% saturation (Ab), 8 μg of rabbit reticulocyte eIF-4B (r), and 10 μg of human HeLa eIF-4B (h). (B) Immunoblotting. Initiation factors were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis essentially as described in Figure 1A,B. The proteins were electrotransferred from the gel onto nitrocellulose sheets and like factors were treated with their cognate antiserum as described (Howe & Hershey, 1981; Meyer et al., 1982). The following amounts of protein from human and rabbit cells, respectively, were analyzed: eIF-3, 6.5 and 8.7 μg; eIF-4A, 1 μg of each; eIF-4B, 0.2 μg of each; eIF-2, 0.2 μg of each. The figure shows photographs of autoradiographs exposed for 1–3 days.

(Figure 4A). With antiserum made against rabbit eIF-3, a strong line forms with rabbit eIF-3, whereas with human eIF-3, a weaker line is observed along with a spur from the rabbit line, suggesting that some antigenic determinants present in rabbit eIF-3 are not present in the human factor. Similarly, anti-eIF-4B made against the human factor forms a strong line and spur with human eIF-4B and a weaker line with rabbit eIF-4B. The analyses indicate that the human and rabbit factors are antigenically related but that some determinants are missing in the factor not used for immunization. Anti-eIF-2 and anti-eIF-4A inconsistently form barely detectable precipitin lines with their cognate factors.

We also compared the four initiation factors by immunoblotting, a technique involving $NaDodSO_4$–polyacrylamide gel electrophoresis of the antigens, electrotransfer to nitrocellulose sheets, and treatment with antisera (Towbin et al., 1979). Each factor isolated from rabbit and human cells was analyzed with the cognate antiserum as shown in Figure 4B. We have modified the procedure (Howe & Hershey, 1981) and use ^{125}I-labeled rabbit anti-goat immunoglobulin G to detect the amount of specific antibody bound to proteins on the nitrocellulose. Identification of the specific polypeptides recognized by the antisera used here is documented in detail in the following paper (Meyer et al., 1982). We are concerned here only with the extent of cross-reactivity of the antisera with the corresponding factors from rabbits and human cells. With anti-eIF-2, the human eIF-2 preparation forms a strong band with the β subunit and a weaker band with the α subunit. The rabbit β subunit is much weaker, whereas the α subunit is nearly as intense. Since both eIF-2 preparations contained comparable amounts of α and β subunits (see Figure 1B), the results suggest that the β subunit is not so strongly conserved as the α subunit. With anti-eIF-3, most of the polypeptides of rabbit eIF-3 are recognized. Although the band patterns with the rabbit and human factors differ, especially in the high molecular weight region of the gel, the differences reflect differences in the protein subunits present in these preparations (Meyer et al., 1982). The similar overall intensities suggest that the eIF-3 preparations from rabbits and humans are fairly closely related antigenically. The band intensities observed with anti-eIF-4A are comparable, whereas with anti-eIF-4B the human eIF-4B band is stronger than the rabbit band. In summary, all four of the antisera cross-react with the corresponding factors from the two species. Strong conservation is indicated for eIF-4A, moderate conservation for eIF-2α, eIF-3, and eIF-4B, and weaker conservation for eIF-2β.

In this work, we show that initiation factors form rabbit reticulocytes and human HeLa cells are very similar with respect to biological activity and protein structure. Trachsel et al. (1979) purified seven initiation factors from mouse Krebs II ascites cells which closely resemble rabbit factors in functional assays and on analysis by $NaDodSO_4$–polyacrylamide gel electrophoresis. There are numerous additional reports of similar factors from a large variety of mammalian sources. In this work, we extend the analytical methods used for comparisons by including partial protease fragmentation mapping and immunochemical techniques. Whereas little or no differences between corresponding factors are detectable by $NaDodSO_4$–polyacrylamide gel electrophoresis and partial protease mapping, some loss of antigenic determinants is seen in these proteins. Nevertheless, the fact that each of the

cognate factors cross-reacts with that of the other species supports the view that these proteins are conserved in structure.

Acknowledgments

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Immunochemical Characterization of Mammalian Protein Synthesis Initiation Factors†

Laurence J. Meyer, Susan C. Milburn, and John W. B. Hershey*

ABSTRACT: Immunochemical techniques were used to investigate the molecular weight forms and levels of protein synthesis initiation factors (eIF) in crude cell lysates. Antisera were obtained from goats immunized with purified rabbit reticulocyte eIF-3 and human HeLa eIF-2, eIF-4A, and eIF-4B. The antisera were characterized by double immunodiffusion and immunoblotting techniques. Rabbit reticulocytes or HeLa cells were prepared and lysed rapidly into denaturing buffer containing sodium dodecyl sulfate. The lysates were analyzed by polyacrylamide gel electrophoresis and immunoblotting. With HeLa lysate and anti-eIF-4B, one intense band developed which corresponded in molecular weight with purified eIF-4B. Anti-eIF-2 and anti-eIF-4A produced much less intense bands corresponding to their cognate factors, and all three antisera produced minor bands as well. For improvement of the power of the analyses, antibodies specific for eIF-2, eIF-4A, and eIF-4B were affinity purified by extraction of the antibodies adhering to gel-purified initiation factors blotted onto aminobenzyloxymethyl paper. Each of these antibodies produced a single intense band with HeLa lysates. The eIF-4A and eIF-4B bands corresponded exactly with those against the purified factors, indicating that the isolated factors are the same size as those found in crude lysates. The eIF-2β band in the lysate was slightly higher than that of the isolated factor, which means that the β subunit in this eIF-2 preparation is partially degraded and is not identical with the in vivo form. The analyses further showed that the three factor proteins have no major precursor or degradation forms in lysates and that they are antigenically distinct from other abundant cellular proteins. Analyses of rabbit and HeLa lysates with crude anti-eIF-3 gave rather weak bands whose patterns were simpler than those obtained with purified eIF-3 preparations. The results are consistent with the view that many of the polypeptide subunits of eIF-3 are generated by proteolysis of high molecular weight precursor forms. The levels of eIF-3 in rabbit and HeLa lysates roughly estimated by rocket immunoelectrophoresis and immunoprecipitation are about 0.5 eIF-3 molecule per ribosome.

The initiation phase of protein synthesis in mammalian cells is promoted by a large number of proteins called initiation factors. The factors have been isolated and purified to near-homogeneity in order to identify the initiation factors and elucidate their mechanism of action. Up to 10 different initiation factors have been purified and studied (Schreier et al., 1977; Merrick, 1979; Benne & Hershey, 1978; Sonenberg et al., 1979; Dasgupta et al., 1979; Amesz et al., 1979). At least three of the eukaryotic initiation factors (eIF),[1] eIF-2, eIF-3, and eIF-5, may be isolated in multiple forms which appear

† From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616. *Received November 16, 1981; revised manuscript received April 9, 1982.* Supported by National Institutes of Health Grant GM-22135 from the U.S. Public Health Service.

[1] Abbreviations: eIF, eukaryotic initiation factor; BSA, bovine serum albumin; $NaDodSO_4$, sodium dodecyl sulfate; IgG, immunoglobulin G; Tris, tris(hydroxymethyl)aminomethane.

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