Isolation and Purification of Hen Oviduct Protein Synthesis Initiation Factors A2A and A2B†

J. Fielding Hejtmancik and John P. Comstock*

ABSTRACT: Two initiation factors, IF-A2A and IF-A2B, required for protein synthesis in a fractionated system have been isolated from hen oviduct. These factors were obtained from a 0.5 M KCl extraction of a nuclear-microsomal fraction of the oviduct. The crude extract inhibited protein synthesis, but, following DEAE-cellulose chromatography, activity was detected. Sephadex G-200 chromatography separated the activity into two active fractions, A2A and A2B. These factors have been characterized with respect to their activities in polyphenylalanine polymerization at a low Mg^{2+} concentration, AUG- and GTP-dependent Met-$tRNA_f$ binding to 40S ribosomal subunits, the hydrolysis of GTP, and natural messenger ribonucleic acid (mRNA) dependent protein synthesis. In all of these systems A2A and A2B were able to substitute for rabbit reticulocyte M2A and M2B. The molecular weights of A2A and A2B have been estimated by gel filtration chromatography to be 280 000 and 23 000, respectively. Sedimentation analysis on sucrose gradients showed A2A to have a sedimentation coefficient of 5.2 S. Combining these data, the molecular weight of A2A was calculated to be 125 000. These values are similar to those for corresponding reticulocyte proteins. Finally, in the presence of added ovalbumin mRNA, A2A and A2B stimulated protein synthesis on non-salt-washed hen oviduct and rabbit reticulocyte polysomes. Moreover, A2A- and A2B-dependent synthesis of ovalbumin was shown to occur on reticulocyte polysomes programmed with ovalbumin mRNA. This supports the conclusion that these factors are initiation factors for protein synthesis and not ribosomal structural proteins.

Factors capable of initiating protein synthesis have been described in several eucaryotic species (Prichard et al., 1970; McCroskey et al., 1972; Picciano et al., 1973; Schrier and Staehelin, 1973; Levin et al., 1973; Grummt, 1974). Generally, these factors have been extracted from crude polysomes or microsomes with high concentrations of salt (0.5–1.0 M). The most extensive investigative studies to date have been carried out with rabbit reticulocytes, although other animal and tissue sources have been used. Individual factors have been isolated from these extracts, and their functional and physical properties partially characterized. In vitro studies employing heterologous protein synthesizing systems have demonstrated varying degrees of interchangeability between corresponding factors or activities from these different sources (Picciano et al., 1973; Schrier and Staehelin, 1973).

In addition to other initiation factors,[1] two factors, 2A and 2B, are required for translation of both natural messenger RNA[2] and poly(uridylic acid) [poly(U)] on salt-washed ribosomes. IF-2A is required for binding of the initiator tRNA, Met-$tRNA_f$, to 40S ribosomal subunits in partially purified systems and is associated with a ribosomal-dependent GTPase activity. IF-2B is also required for binding of Met-$tRNA_f$ to the small ribosomal subunit (Shafritz et al., 1972). In addition to these activities there are indications that proteins similar to IF-2A and IF-2B are involved in the binding of 40S and 60S ribosomal subunits to form the 80S monomer (Grummt, 1974; Cashion and Stanley, 1974; Nombela et al., 1975). It has also been suggested from work with *Artemia salina* that these two proteins may be structural members of the 60S ribosomal subunit rather than initiation factors (Nombela et al., 1975). Initiation factors would be expected to associate and disassociate from the ribosome during each round of mRNA translation. Recently Merrick et al. (1975) have described the purification and physical properties of homogeneous IF-M2A from the rabbit reticulocyte.

Earlier reports have shown that administration of estrogen to immature chicks results in both morphological differentiation and biochemical specialization of the oviduct (Kohler et al., 1968; O'Malley et al., 1969). Following this stimulation the tubular gland cells synthesize large amounts of the specific protein ovalbumin. Although the production of this protein appears to be regulated primarily at the level of nuclear transcription (Harris et al., 1975), Palmiter (1972) has reported that a part of the biochemical differentiation of the estradiol-stimulated oviduct is expressed in a 200–300% increase in the rate of polypeptide initiation and a 40% increase in the rate of elongation. Studies from this laboratory (Means et al., 1971; Comstock et al., 1972a) have described the extraction by salt of a fraction (AvF) from estrogen-stimulated, chick-oviduct polysomes which stimulated the rate and extent of protein synthesis upon addition to salt-washed ribosomes. The AvF fraction could also stimulate poly(U)-directed polyphenylalanine synthesis while lowering the Mg^{2+} concentration optimum in the assay. The activity in these assays occurred in the presence of saturating amounts of tRNA, aminoacyl-tRNA synthetases, and elongation factors. These properties

† From the Department of Cell Biology, Baylor College of Medicine, and the Department of Medicine, Veteran's Administration Hospital, Houston, Texas 77030. *Received December 1, 1975.* This work was supported in part by the Center for Population Research, Research Grant Hd-7495.

* Address correspondence to this author at: Department of Cell Biology, St. Luke's Episcopal Hospital, Houston, Texas 77030.

[1] In this communication initiation factors will be designated according to the classification of Anderson and co-workers: IF(initiation factors)-M(mammalian) or -A(avian), followed by the designated factor number.

[2] Abbreviations used: DEAE, diethylaminoethyl; AUG, adenylyl-3′,5′-uridyl-3′,5′-guanosine 3′-phosphate; GTP, guanosine triphosphate; mRNA, messenger ribonucleic acid; tRNA, transfer RNA; poly(U), poly(uridylic acid); Met-$tRNA_f$, initiator methionyl-tRNA which can be formylated with *E. coli* transformylase; Na_2EDTA, disodium ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

are typical of initiation factors isolated from other sources. The activity of the AvF fraction was reported to increase twofold following isolation from estrogen-treated chicks (Comstock et al., 1972b). These initial studies did not determine whether specific initiation factors were stimulated. In order to make such determinations separation of the AvF fraction into individual activities was undertaken but required more material than could be obtained from chick oviducts. Work was therefore commenced using oviducts from mature hens.

The present study describes the isolation, partial purification, and characterization of protein synthesis initiation factors 2A and 2B from the oviduct of the hen. These factors were compared with the corresponding rabbit reticulocyte factors in terms of chromatographic behavior and interchangeability in assays for the initiation of protein synthesis. This paper presents additional evidence consistent with the interpretation that these proteins are not ribosomal structural proteins and are involved in the initiation of ovalbumin mRNA translation on hen oviduct polysomes.

Experimental Procedure

Unless otherwise noted, all procedures involving compounds with biological activity were carried out at 2–4 °C.

Materials

Chromatography materials were purchased from the following suppliers: DEAE-cellulose, DE-23 (Whatman); Sephadex G-type gels (Pharmacia); hydroxylapatite, Hypatite C (Clarkson Chemical Company Inc.); Ultrogel, AcA-34 (LKB); and Agarose A-0.5m (Bio-Rad Laboratories). Radiochemicals were obtained from the following companies: [^{32}P]-γ-ATP (5.5 Ci/mmol), [^{32}P]-γ-GTP (14 Ci/mmol), [^{3}H]leucine (50 Ci/mmol), [^{3}H]alanine (18.3 Ci/mmol), [^{3}H]glycine (11.4 Ci/mmol), [^{3}H]valine (1.3 Ci/mmol), and [^{3}H]phenylalanine (16.1 Ci/mmol) (New England Nuclear Corp.); [^{14}C]phenylalanine (522 mCi/mmol) and [^{3}H]methionine (7.7 Ci/mmol) (Amersham/Searle); and [^{14}C]valine (260 mCi/mmol) (Schwarz/Mann). Poly(U), AUG, and *Escherichia coli* K-120 tRNA were supplied by Miles Laboratories. Dithiothreitol, ATP, GTP, phosphoenolpyruvate, and pyruvate kinase were purchased from Calbiochem and puromycin hydrochloride from Nutritional Biochemical Corporation. Catalase, aldolase, bovine albumin, ovalbumin, chymotrypsinogen A, myoglobin, cytochrome *c*, and monothioglycerol were obtained from Sigma Chemical Co. Immunological materials were purchased from the following suppliers: rabbit anti-ovalbumin serum (Cappel Laboratories, Inc.); goat anti-rabbit IgG (Miles-Yeda Ltd.). Ultrafiltration equipment and membranes were purchased from Amicon Corporation. Frozen hen oviducts were obtained from Pel-Freeze Biologicals.

Methods

Preparation of Rabbit Reticulocyte Salt-Washed Ribosomes and Initiation Factors M1, M2, and M3. These materials were prepared by methods described by Anderson and his co-workers (Merrick et al., 1974; Prichard and Anderson, 1974). Polysomes were separated from reticulocyte lysate by high-speed centrifugation, resuspended to 200 A_{260} units/ml, and washed for 30 minutes with 0.5 M KCl. The salt wash was then concentrated by $(NH_4)_2SO_4$ precipitation (0–70%) and gradient elution chromatography carried out on a DEAE-cellulose column. M1 was not adsorbed to the column (100 mM KCl). A 35–65% $(NH_4)_2SO_4$ fractionation was performed on this material. M3 eluted at 120–200 mM KCl, and M2 (A + B) eluted at 200–400 mM KCl. M2A and M2B were separated by Sephadex G-200 chromatography, with M2A appearing near the void volume and M2B being included. Samples were dialyzed, concentrated by ultrafiltration, and stored in liquid nitrogen. Salt-washed ribosomes were resuspended in standard sucrose solution—0.25 M sucrose, 0.1 mM Na_2EDTA (pH 7.0), and 1 mM dithiothreitol—at a concentration of 100 A_{260} units/ml and stored in liquid nitrogen. Twice-washed, reticulocyte ribosomes were obtained by suspending the salt-washed ribosome pellet in standard sucrose made 500 mM in KCl and mixing for 15 min. The suspension was then centrifuged at 105 000*g* for 90 min. The pelleted ribosomes were resuspended in standard sucrose solution to a concentration of 250 A_{260} units/ml and stored in liquid nitrogen.

Reticulocyte Supernatant Fraction. Reticulocyte lysate high-speed supernatant was prepared by procedures similar to those described by Moldave et al. (1971). The supernatant was adjusted to pH 5.0 with 1 N acetic acid and centrifuged at 12 000*g* for 12 min. The supernatant fraction was neutralized with KOH and applied to a Sephadex G-25 column equilibrated in 10 mM Tris-HCl (pH 8.0 at 4 °C). The void volume fractions were collected and made 1 mM in dithiothreitol. A 25–65% $(NH_4)_2SO_4$ fractionation was performed on this material and the fraction dialyzed overnight against buffer: 10 mM Tris-HCl (pH 7.5 at 4 °C), 1 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM Na_2EDTA (pH 7.0), and 100 mM KCl. The sample was applied to a hydroxylapatite column and eluted with a linear gradient from 5 to 600 mM KPO_4 (pH 7.2) in buffer: 25 mM KCl, 0.1% (v/v) monothioglycerol, and 10% (v/v) glycerol. Fractions with EF-2 activity were pooled and dialyzed overnight against buffer: 20 mM Tris-HCl (pH 7.5 at 4 °C), 10% glycerol, 0.1% monothioglycerol, 0.1 mM Na_2EDTA (pH 7.0), and 100 mM KCl, and frozen in liquid nitrogen. Fractions with EF-1 activity were concentrated by ultrafiltration with an XM-50 membrane, dialyzed for 6 h against buffer as above, and applied to an Agarose A-0.5m column. The EF-1 fractions were pooled, again concentrated by ultrafiltration, and stored in liquid nitrogen.

Preparation of tRNA. Hen oviduct tRNA was prepared from a homogenate of whole oviduct as described by Gilbert and Anderson (1970).

Preparation of [^{14}*C*]*Phe-tRNA. E. coli* tRNA K-120 was acylated with [^{14}C]phenylalanine by the procedure of Anderson (1969) using crude aminoacyl-tRNA synthetases from *E. coli* (Muench and Berg, 1966). The resulting [^{14}C]Phe-tRNA had a specific activity of 750 cpm/pmol. The tRNA was 1.3% acylated, and 90% of the counts were precipitable in cold trichloroacetic acid.

Preparation of [^{3}*H*]*Met-tRNA$_f$.* Rabbit liver $tRNA_f$ was isolated by reversed phase chromatography, RPC-5 (Pearson et al., 1971). [^{3}H]Met-$tRNA_f$ was prepared by methods similar to those used by Shafritz and Anderson (1970). [^{3}H]Met-$tRNA_f$ had a specific activity of 4930 cpm/pmol. The tRNA was 4.4% charged, and 43% of the counts were precipitated in cold trichloroacetic acid.

Isolation of Reticulocyte 40S Subunits. Reticulocyte lysate was centrifuged at 177 000*g* in a Beckman 60 Ti rotor for 2 h. The polysomal pellet was resuspended to 200 A_{260} units/ml in standard sucrose solution. A total of 600 A_{260} units was made 500 mM in KCl (by the addition of 4 M KCl), 2 mM in $MgCl_2$, and 0.1 mM in puromycin hydrochloride and the reaction mixture was incubated for 15 min at 37 °C. Five hundred microliters of this mixture was layered over 37 ml of a

10–30% sucrose gradient in buffer: 20 mM Tris-HCl (pH 7.5 at 4 °C), 2 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM Na_2EDTA (pH 7.0), and 500 mM KCl, in a Beckman SW-27 rotor, and centrifuged at 96 300*g* for 10 hours at 2 °C. 40S subunits were collected with an autoDensi-Flow IIC (Buchler Inst.), monitored at A_{260} with a UA-4 recording spectrophotometer (ISCO), diluted with an equal volume of buffer [10 mM Tris-HCl (pH 7.5 at 4 °C), 2 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM Na_2EDTA (pH 7.0), and 20 mM KCl], and collected by centrifuging for 6 h at 215 000*g* in a Beckman 60 Ti rotor. The subunits were resuspended to 50–100 A_{260} units/ml in buffer [0.25 M sucrose, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 0.1 mM Na_2EDTA (pH 7.0)] and stored in liquid nitrogen.

Preparation of Reticulocyte Polysomes. Reticulocyte polysomes, free of 40S and 60S subunits, were isolated by the method of Lubsen and Davis (1974). One milliliter of lysate was layered over 4 ml of a 15–30% sucrose gradient [10 mM Tris-HCl (pH 7.5 at 4 °C), 1.5 mM $MgCl_2$, 10 mM KCl, and 2 mM dithiothreitol] and centrifuged at 189 000*g* for 90 min in a Beckman SW 50.1 rotor. Polysomes were collected in the pellet, while subunits were retained in the gradient.

Preparation of Oviduct Polysomes. Polysomes were prepared essentially by the technique of Palacios et al. (1972). Following centrifugation through 1.85 M sucrose, the polysomes were resuspended in standard sucrose solution, adjusted to a concentration of 120 A_{260} units/ml, and stored in liquid nitrogen.

Assays. Poly(U)-Directed Polyphenylalanine Synthesis from [^{14}C]Phe-tRNA [Poly(U) Assay]. One-hundred-microliter reaction mixtures were incubated at 37 °C for 2 min and contained 4 mM $MgCl_2$ (unless otherwise noted), 30 mM Tris-HCl (pH 7.2 at 25 °C), 100 mM KCl, 0.5 mM GTP, 3 mM phosphoenolpyruvate, 0.6 IU pyruvate kinase, 1 mM dithiothreitol, 0.18 A_{260} unit of poly(U), and 10 pmol of [^{14}C]Phe-tRNA. Assays were performed in the presence of saturating levels of reticulocyte EF-1 and EF-2, reticulocyte IF-M1, and reticulocyte or oviduct initiation factors 2A and 2B, as indicated in the individual figures. Reactions were stopped by the addition of 2 ml of cold 10% trichloroacetic acid and processed as previously described (Picciano et al., 1973). Radioactivity incorporated into protein was counted in Spectrofluor–toluene (efficiency 78% for carbon-14).

Exogenous mRNA-Directed Protein Synthesis. One-hundred-microliter reaction mixtures were incubated at 37 °C for 20 min and contained: 3.5 mM $MgCl_2$ (for polysomes) or 4.5 mM $MgCl_2$ (for salt-washed ribosomes), 20 mM Tris-HCl (pH 7.2 at 25 °C), 80 mM KCl, 0.375 mM GTP, 1 mM ATP, 3 mM phosphenolpyruvate, 0.6 IU pyruvate kinase, 1 mM dithiothreitol, 0.076 mM [^{14}C]valine (450 cpm/pmol), 0.04 mM of each of 19 unlabeled amino acids, 0.12 A_{260} unit of oviduct tRNA, saturating amounts of reticulocyte supernatant fraction, EF-1, EF-2, saturating amounts of globin or ovalbumin mRNA purified by the method of Rosen et al. (1975), and initiation factors as indicated in individual charts or figures. The reaction mixtures contained 0.2 A_{260} unit of either reticulocyte salt-washed ribosomes, reticulocyte polysomes, or oviduct polysomes, as indicated in individual legends. Radioactivity incorporated into protein was determined as cited above.

Ovalbumin Synthesis. Ovalbumin synthesis directed by ovalbumin mRNA on reticulocyte polysomes was identified by a modification of the immunological assay described by Wetekam et al. (1975). The incorporation of five tritiated amino acids (leucine, glycine, alanine, valine, and phenylalanine) into protein was carried out as described above. Ninety microliters of the incubation mixture was incubated overnight at 4 °C with a precipitate formed with a highly specific rabbit anti-ovalbumin serum and goat anti-rabbit IgG. The precipitate was washed as described, centrifuged through a sucrose layer, and finally dissolved in NCS prior to counting in spectrofluor–toluene. The binding capacity of the antibody complex was approximately 200 ng of ovalbumin determined with [*methyl*-^{14}C]ovalbumin. This was completely displaced by a 100-fold excess of unlabeled ovalbumin.

Met-tRNA$_f$ Binding to Reticulocyte 40S Subunits. Enzymatic binding of Met-tRNA$_f$ was performed by a modification of the nitrocellulose filter assay (Picciano et al., 1973). Fifty-microliter reaction mixtures were incubated at 25 °C for 3 min and contained: 20 mM Tris-HCl (pH 7.2 at 25 °C), 5 mM $MgCl_2$, 5 mM GTP, 100 mM KCl, 1 mM dithiothreitol, 0.25 A_{260} unit of AUG, 0.35 A_{260} unit of reticulocyte 40S subunits, 7.5 pmol of [^{3}H]Met-tRNA$_f$, and saturating amounts of reticulocyte IF-M1 and reticulocyte or oviduct initiation factors 2A and 2B where indicated. The reactions were stopped by the addition of 3 ml of ice-cold wash buffer [20 mM Tris-HCl (pH 7.2 at 25 °C), 100 mM KCl, 5 mM $MgCl_2$], immediately filtered on Millipore filters (type HA, 0.45 μm), and washed three times with 3 ml of the same buffer. The filters were dried and counted in Spectrofluor–toluene with an efficiency of 33% for tritium.

GTPase and ATPase Assays. [^{32}P]-γ-GTP or [^{32}P]-γ-ATP was diluted to a specific activity of 257 cpm/pmol for GTP and 1384 cpm/pmol for ATP. Fifty-microliter reaction mixtures were incubated at 37 °C for 10 min. Each reaction mixture contained: 20 mM Tris-HCl (pH 7.2 at 25 °C), 1 mM dithiothreitol, 5 mM $MgCl_2$, 100 mM KCl, 1 A_{260} unit of twice-washed reticulocyte ribosomes, [^{32}P]-γ-GTP or [^{32}P]-γ-ATP (1000 pmol), and factor additions as indicated in individual figures. Values reflect a rate of hydrolysis, for they were determined using factor concentrations for which GTP or ATP hydrolysis was a linear function of both protein added and time of incubation. The assay was linear for the hydrolysis of 500 pmol (50% total). The reaction was stopped by the addition of 500 μl of 0.1 N HCl, and then 200 μl of a suspension of 10 mM NaH_2PO_4, 0.1 N HCl, and 3% (w/v) Norite was added. The suspension was mixed and centrifuged at 1500 rpm in a Sorvall GLC-2. Five hundred microliters of the supernatant was counted in Spectrofluor–Triton X-100 (2:1).

Preparation of Hen Oviduct Initiation Factors A2A and A2B. Nuclear-Microsomal Wash. Frozen oviducts (400 g) were broken into small pieces and allowed to thaw in 2.5 volumes of buffer A: 50 mM Tris-HCl (pH 7.5 at 4 °C), 10 mM $MgCl_2$, 0.5 mM Na_2EDTA (pH 7.0), 0.25M sucrose, 0.1% monothioglycerol, and 35 mM KCl. The oviducts were homogenized in a Waring blender for 90 s at 30% of 120 V. This homogenate was centrifuged in a Beckman JA-14 rotor at 10 000*g* for 20 min. The supernatant was discarded, and the pellet containing nuclei, tissue fragments, and most of the rough endoplasmic reticulum (Tata, 1972) was resuspended in 2.5 volumes of buffer B: 50 mM Tris-HCl (pH 7.5 at 4 °C), 0.1 mM $MgCl_2$, 0.5 mM Na_2EDTA (pH 7.0), 0.25 M sucrose, 0.1% monothioglycerol, and 0.5 M KCl, by homogenization in a 200-ml cup Waring blender at 25% of 120 V for 120 s. The homogenate was gently mixed on a magnetic stirring plate for 1 h and centrifuged for 10 min at 10 000*g* in a Beckman JA-14 rotor. The supernatant was collected, filtered through four layers of cheesecloth, and centrifuged in a Beckman 35 rotor at 95 000*g* for 3 h. The clear zone of the supernatant was collected and concentrated by adding finely powdered

$(NH_4)_2SO_4$ containing 2% (w/w) $(NH_4)_2CO_3$ over 30 min until the solution was 90% saturated. The solution was stirred for 2 h and centrifuged at 10 000*g* for 20 min. The precipitate was collected and resuspended in buffer C [20 mM Tris-HCl (pH 7.5 at 4 °C), 1 mM $MgCl_2$, 0.1 mM Na_2EDTA (pH 7.0), 0.1% monothioglycerol] and dialyzed against 20 volumes of buffer C for 6 h. The solution was then dialyzed against 20 volumes of buffer C made 100 mM in KCl for 6 additional h. The solution was centrifuged in a Beckman JA-20 rotor at 7800*g* for 20 min and the supernatant designated as the nuclear-microsomal wash fraction.

Isolation of IF-A2 on DEAE-Cellulose. The nuclear-microsomal wash fraction was applied to a DEAE-cellulose (DE-23) column (1.2 × 20 cm) equilibrated with buffer C made 100 mM in KCl, and the column was washed with this buffer until no additional protein eluted. A linear gradient (400 ml total) from 100 mM KCl to 500 mM KCl in buffer C was run at 50 ml/h, and 4-ml fractions were collected. Protein elution was monitored at A_{280} with an ISCO UA-5 absorbance monitor, and conductivity was measured with a Radiometer conductivity meter, CD-2 (London Co.). Fractions containing IF-2 activity [poly(U)assay] were pooled, concentrated by ultrafiltration with a UM-2 membrane to a concentration of 50 mg/ml, and stored in liquid nitrogen.

Separation of IF-A2A and IF-A2B Activities on Sephadex G-200. The IF-A2 (A + B) sample (5.3 ml) from the step above was added to a Sephadex G-200 column (1.6 × 90 cm) which had previously been equilibrated with buffer C made 200 mM in KCl, and the protein eluted with the same buffer at a flow rate of 7 ml/h, collecting 4-ml fractions. Fractions showing IF-A2A or IF-A2B activity in the poly(U) assay were pooled and concentrated by ultrafiltration with a UM-2 membrane to a concentration of 10 mg/ml for IF-A2A and 20 mg/ml for IF-A2B. These samples were stored in liquid nitrogen.

Determination of Apparent Molecular Weight and Stokes Radius by Molecular Sieve Chromatography. Two milliliters of IF-A2 (30 mg/ml) isolated by DEAE-cellulose chromatography was applied to an upward flowing 1.6 × 90 cm analytical column of Ultrogel AcA-34. One milliliter of IF-A2B (10 mg/ml) obtained by G-200 chromatography was applied to a 1.6 × 61 cm column of Sephadex G-75. Both columns were equilibrated and eluted with buffer C made 200 mM in KCl at a flow rate of 8 ml/h. Two-milliliter fractions were collected. The void volume and total volume for each column were determined with the University Dye Kit (Pharmacia). Proteins of known molecular weight were used as standards: xanthine oxidase (bovine milk), 320 000; catalase (bovine liver), 220 000; aldolase (rabbit muscle), 158 000; lactic dehydrogenase (bovine heart), 135 000; bovine albumin, 67 000; ovalbumin, 46 000; chymotrypsinogen A (bovine pancreas), 25 000; myoglobin (whale skeletal muscle), 17 500; and cytochrome *c* (horse heart), 13 600 (Smith, 1970). Xanthine oxidase and lactic dehydrogenase were the gifts of Dr. Daniel Louie. Elution volumes were determined by absorbance at A_{280} with a UA-5 monitor (ISCO). Aliquots of 35 and 10 μl were analyzed for IF-A2A and IF-A2B activities, respectively, with the poly(U) assay. Stokes radii were obtained from published data (Siegel and Monty, 1966) or derived by the equation $A = kT/6\pi\eta D$ (A, Stokes radius; k, Boltzmann constant; T, absolute temperature; η, viscosity; and D, diffusion coefficient).

Determination of Sedimentation Coefficient. One hundred microliters of IF-A2A isolated by DEAE-cellulose and Sephadex G-200 column chromatography was layered on 5 ml of 5–20% sucrose gradients (containing buffer C made 200 mM in KCl). The samples were centrifuged in a Beckman SW 50.1 rotor at 189 000*g* for 16 h. Gradient fractions (0.3 ml) were collected with a Model 640 density gradient fractionator (ISCO). Proteins with known sedimentation coefficients were used as standards: aldolase, 8.8 S; bovine albumin, 4.27 S; ovalbumin, 3.54 S; and chymotrypsinogen A, 2.58 S (Smith, 1970). Elution volumes were determined by absorbance at A_{280} with a UA-5 monitor (ISCO). Aliquots of 20 μl were analyzed for IF-A2A activity with the poly(U) assay.

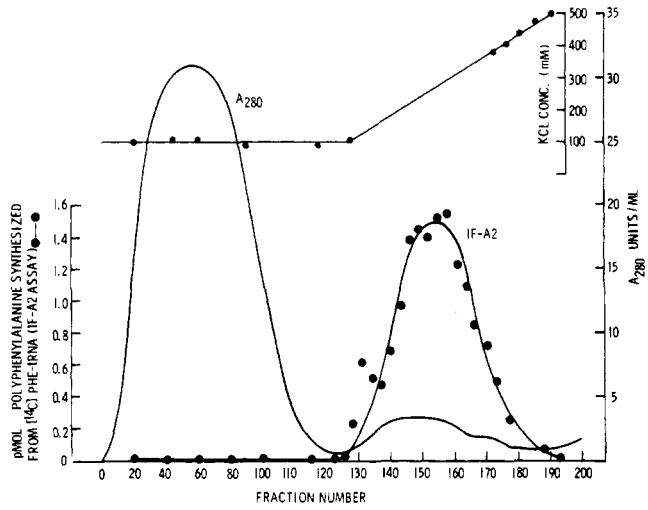

FIGURE 1: Isolation of hen oviduct IF-A2 by DEAE-cellulose chromatography. The chromatography procedures and the poly(U) assay are described in Methods. Aliquots (15-μl) of column fractions were assayed for IF-A2 activity.

Measurement of Protein and Nucleic Acid Content. Protein was assayed by a modification of the method of Lowry et al. (1951), first removing the various reacting materials in the buffer solutions by means of trichloroacetic acid precipitation. Bovine serum albumin was used as a standard. Nucleic acid content (A_{260} units) was estimated by determining the absorbance at A_{260} of 1 ml of solution in a 1-cm pathlength cell (Layne, 1957).

Results

Isolation of IF-A2A and IF-A2B from the Crude Nuclear-Microsomal, Salt-Wash Fraction. The nuclear-microsomal, salt-wash fraction did not display initiation factor activity and, indeed, significantly inhibited protein synthesis. Picciano et al. (1973) did not observe initiation factor activity in the crude salt wash of rabbit liver microsomes until after it was further purified by ammonium sulfate precipitation and DEAE-cellulose chromatography. Similar procedures were, therefore, carried out with the crude oviduct fraction. Column fractions were analyzed by the appropriate assays to detect activities similar to reticulocyte and liver IF-M1, IF-M2, and IF-M3. Only IF-A2 activity (assayed in the poly(U) assay in the presence of reticulocyte IF-M1) was found after ion-exchange chromatography. Figure 1 shows the chromatographic profile of the nuclear-microsomal, salt-wash fraction on DEAE-cellulose. The IF-A2 activity eluted as a broad peak between 150 and 350 mM KCl similar to the elution peak of rabbit reticulocyte or liver IF-M2 with DEAE-cellulose ion-exchange chromatography. The active oviduct fraction was further purified by Sephadex G-200 chromatography and was separated into two activities. As seen in Figure 2, IF-A2A activity appeared immediately after the void volume, while the

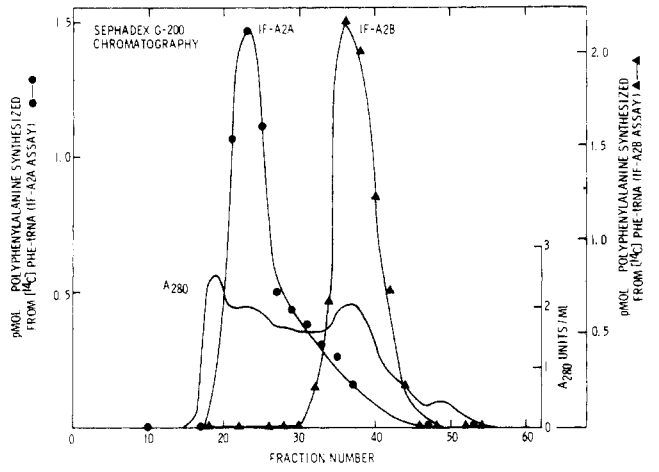

FIGURE 2: Sephadex G-200 chromatography of hen oviduct IF-A2. The chromatographic procedures and assay conditions are described in Methods. IF-A2A and IF-A2B were assayed by the poly(U) assay with the corresponding reticulocyte factor omitted. For the IF-A2A assay, 15-μl aliquots of the column fractions were added. A blank value of 1.1 pmol (minus IF-2A) was subtracted from the value of each fraction. For the IF-A2B assay 20-μl aliquots were added. A blank of 0.87 pmol (minus IF-2B) was subtracted.

TABLE I: Effect of IF-2A and IF-2B on [^{3}H]Met-tRNA$_f$ Binding to Reticulocyte 40S Ribosomal Subunits.[a]

Additions or Deletions	[^{3}H]Met-tRNA$_f$ Bound (pmol)
Complete	
none	0.18
+ M2A	0.41
+ M2B	0.38
+ A2A	0.27
+ A2B	0.28
+ M2A + M2B	0.75
+ A2A + A2B	0.64
+ M2A + A2B	0.75
+ A2A + M2B	0.95
−GTP	
+ M2A + M2B	0.60
+ A2A + A2B	0.38
−AUG	
+ M2A + M2B	0.38
+ A2A + A2B	0.22
−40S ribosomal subunits	
+ M2A + M2B	0.29
+ A2A + A2B	0.17

[a] Assay conditions are described in Methods. Oviduct nd reticulocyte factors were added in saturating amounts as indicated: M2A (0.6 μg), M2B (0.6 μg), A2A (4.9 μg), and A2B (12.8 μg).

IF-A2B was found to be included in the gel and eluted at approximately 2 void volumes. These chromatographic properties are similar to those exhibited by the corresponding rabbit reticulocyte or liver initiation factors at comparable stages of purification.

Molecular Weight Determinations of IF-A2A and IF-A2B. The molecular weight of each oviduct factor was estimated by molecular seive chromatography as described in Methods (data not shown). IF-A2A appears to be a large molecule with a molecular weight of 280 000 while the IF-A2B was found to be approximately 23 000. These values are in very close agreement with the molecular weights of the corresponding reticulocyte or liver factors when determinations were performed by similar methods. Both activities were assayed with the poly(U) assay in the presence of rabbit reticulocyte IF-M1 and either IF-M2A (for the IF-A2B assay) or IF-M2B (for the IF-A2A assay).

Initiation factor A2A was further characterized since homogenous IF-M2A has been recently reported by Merrick et al. (1975) to migrate atypically during molecular sieve chromatography. When the data obtained by analytical AcA-34 chromatography were treated by the method of Porath (1963), IF-A2A was determined to have a Stokes radius of 57 Å. Sedimentation analysis on 5–20% sucrose gradients showed IF-A2A to have a sedimentation coefficient of 5.2 S, a value in close agreement with that of the reticulocyte factor (Merrick et al., 1975). Assuming a value of 0.73 for the partial specific volume, the molecular weight of IF-A2A was calculated to be 125 000 from the relationship

$$M = [A(6\pi\eta N)S]/(1 - \bar{\nu}\rho)$$

(A, Stokes radius; η, viscosity; N, Avogadro's number; S, diffusion coefficient; $\bar{\nu}$, partial specific volume; and ρ density of the solution) (Siegel and Monty, 1966). This value for IF-A2A is similar to the molecular weight derived for reticulocyte IF-M2A (Merrick et al., 1975).

Avian and Mammalian Initiation Factors 2A and 2B Exchange Studies. In order to investigate the interchangeability of avian and mammalian initiation factors, exchange studies were performed in several assays. In addition to similar ion-exchange properties and physical size estimates, oviduct and reticulocyte initiation factors 2A and 2B had similar activities in several in vitro protein synthesis initiation systems. Initial studies employed poly(U)-dependent polyphenylalanine synthesis with reticulocyte, salt-washed ribosomes. The effect of increasing amounts of reticulocyte M2A and M2B or oviduct A2A and A2B in the poly(U) assay is shown in Figure 3A,B. The system, rate limiting in ribosomes, initially demonstrated a linear increase in activity upon addition of each initiation factor. The addition of greater amounts of M2B or A2B resulted in a plateau of activity while greater amounts of IF-2A, especially from the oviduct, resulted in inhibition. In this assay, saturating amounts of each factor gave a three-to fivefold stimulation of activity. The hen oviduct initiation factors generally gave 60 to 75% of the activity of the corresponding reticulocyte factors in this assay. Ten to twenty times as much oviduct protein as compared with reticulocyte protein from fractions of similar stages of purification was required to achieve saturation.

The Mg^{2+} concentration optimum for the poly(U) assay was determined to be 5–6 mM in the presence of saturating amounts of the oviduct initiation factors A2A and A2B. In the presence of salt-washed ribosomes, elongation factors, IF-M1, and either of these factors alone the Mg^{2+} concentration was found to be maximal at 9–10 mM (Figure 3C). These results indicate that the oviduct factors were capable of causing a shift to a low Mg^{2+} concentration for optimal polypeptide synthesis. This behavior is similar to that which has been observed for the rabbit reticulocyte and liver initiation factors (Shafritz et al., 1970; Picciano et al., 1973).

Comparison of activity in exogenous mRNA-directed globin synthesis is demonstrated in Figure 4. Here also, the hen oviduct initiation factors gave approximately 75% of the corresponding reticulocyte factor activity. In this assay, addition of the different factors gave a five- to sixfold stimulation of activity at saturating levels while addition of greater amounts of IF-2A resulted in inhibition.

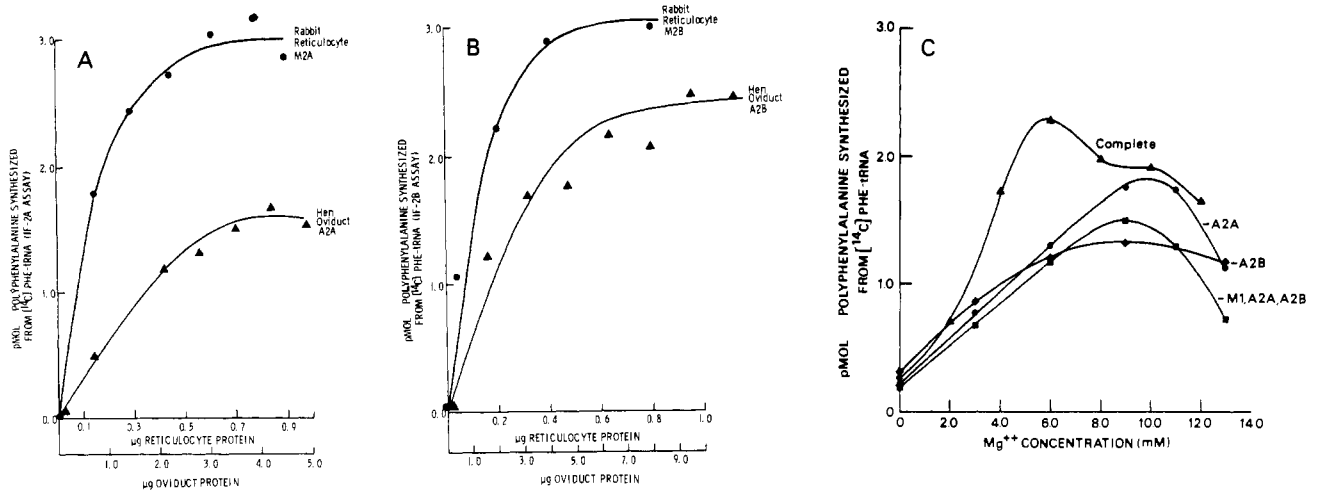

FIGURE 3: The effect of increasing amounts of IF-2A and IF-2B from rabbit reticulocyte or hen oviduct on polyphenylalanine synthesis and the determination of the Mg^{2+} concentration optimum for the hen oviduct factors. Activities were assayed as described in Methods with the appropriate reticulocyte factor deleted in each assay. (A) IF-2A assay: a blank of 1.1 pmol (minus IF-2A) was subtracted. (B) IF-2B assay: a blank of 0.75 pmol (minus IF-2B) was subtracted. (C) Mg^{2+} concentration optimum: saturating amounts of IF-A2A (2.8 μg) and IF-A2B (8 μg) were added.

TABLE II: Requirement for IF-2A and IF-2B in Natural mRNA-Directed Protein Synthesis on Polysomes.[a]

Additions	[14C]Val Incorporated (pmol)		
	Oviduct Polysomes	Reticulocyte Polysomes	Reticulocyte Salt-Washed Ribosomes
None	15.0	7.6	4.5
M2A	16.0	12.4	4.5
M2B	12.0	12.7	4.7
A2A	12.8	9.4	3.8
A2B	14.1	16.5	4.3
M2A + M2B	26.1	19.9	27.8
M2A + A2B	26.4	23.2	22.0
A2A + M2B	23.9	18.6	20.0
A2A + A2B	25.1	20.3	20.1

[a] Polysome isolation and incubation conditions are described in Methods. Purified ovalbumin or globin mRNA was added in saturating amounts to oviduct or reticulocyte polysomes (or salt-washed reticulocyte ribosomes), respectively. Initiation factors 2A and 2B were added in saturating amounts: M2A (0.45 μg), M2B (0.6 μg), A2A (2.8 μg), and A2B (8 μg). No blank values have been subtracted.

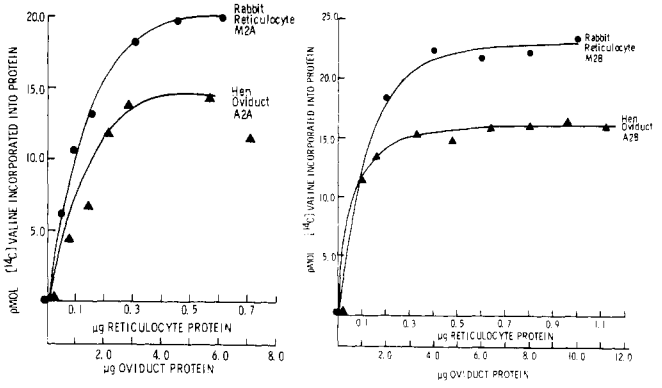

FIGURE 4: The effect of increasing amounts of IF-2A and IF-2B on globin mRNA-directed protein synthesis on salt-washed ribosomes. Activities were assayed as described in Methods with the appropriate reticulocyte factor deleted in each assay. (Left) IF-2A assay: a blank of 2.8 pmol (minus IF-2A) was subtracted. (Right) IF-2B assay: a blank of 2.6 pmol (minus IF-2B) was subtracted.

The results of substituting hen oviduct for rabbit reticulocyte initiation factors 2A and 2B in the AUG-directed Met-$tRNA_f$ binding assay are shown in Table I. There was complete interchangeability of factors in this assay. Factors from both sources also demonstrated dependence on GTP, AUG, and reticulocyte 40S ribosomal subunits. A greater dependence on GTP was found with the oviduct factors compared with that with the reticulocyte factors. This may be a result of the higher GTPase content in the oviduct materials. For the optimal binding of the initiator tRNA to ribosomes, a three- to fivefold dependence for either oviduct or reticulocyte IF-2A and IF-2B together, or a two- to threefold dependence for each factor separately was exhibited. Hot trichloroacetic acid treatment revealed that polypeptides were not synthesized under the conditions of the binding assay.

In order to further characterize the involvement of these factors in the initiation of protein synthesis, studies were carried out with native (non-salt-washed) polysomes which were isolated by sucrose gradient centrifugation to remove ribosome subunits. The activity of the reticulocyte and oviduct initiation factors with polysomes from the oviduct are displayed in Table II. A twofold dependence on factors M2A, A2A, M2B, and A2B was observed in this system. Reticulocyte polysomes also exhibited dependencies for these factors but demonstrated a larger activity with A2B alone. A greater dependency (fivefold) on each factor was present when reticulocyte, salt-washed ribosomes were used in this assay. These data are shown for comparison with the results obtained with the polysomes. Again, there appeared to be complete interchangeability between oviduct and reticulocyte factors in this assay system.

Data which document the participation of these factors in the initiation of protein synthesis are shown in Table III. In these experiments the ability of A2A and A2B to initiate the synthesis of ovalbumin on reticulocyte polysomes programmed by exogenous ovalbumin mRNA was utilized as a marker of initiation. Addition of the factors alone resulted in a twofold stimulation of total synthesis (radioactivity incorporated into hot trichloroacetic acid insoluble material). Total activity, however, was not reproducibly increased by the addition of

TABLE III: Initiation of Exogenous mRNA-Directed Ovalbumin Synthesis on Reticulocyte Polysomes.[a]

Additions	[^{3}H]Ovalbumin Immunoprecipitate (cpm)
None	1193
+ ovalbumin mRNA	1718
+ A2A, A2B	783
+ A2A, A2B, ovalbumin mRNA	4287
+ A2A, A2B, ovalbumin mRNA, 20 μg of ovalbumin	1535

[a] Polysome isolation and incubation conditions are described in Methods and footnote *a* of Table II. The immunological assay to determine ovalbumin synthesis is described in Methods. The data presented are representative of four separate experiments in which ovalbumin synthesis was demonstrated.

TABLE IV: Rate of Hydrolysis of GTP and ATP by IF-2A and IF-2B.[a]

Nucleoside 5′-Triphosphate	Additions or Deletion	pmol of [^{32}P]-γ-Nucleoside 5′-Triphosphate Hydrolyzed[b]
GTP	+ Ribosomes	
	+ M2A	147
	+ A2A	49
	+ M2B	0
	+ A2B	151
	+ M2A + M2B	120
	+ M2A + A2B	46
	+ A2A + M2B	50
	− Ribosomes	
	+ M2A	43
	+ A2A	38
ATP	+ Ribosomes	
	+ M2A	62
	+ A2A	42
	− Ribosomes	
	+ M2A	53
	+ A2A	53

[a] Incubation conditions are described in Methods. Reticulocyte twice-washed ribosomes (0.2 A_{260} unit) alone hydrolyzed 137 pmol of [^{32}P]-γ-GTP and 100 pmol of [^{32}P]-γ-ATP in this assay. This constant blank was subtracted from all rate points except the minus ribosome values. [b] Per μg of protein per 10 min.

exogenous ovalbumin mRNA. The complete system containing factors and ovalbumin mRNA resulted in a fourfold increase in the newly synthesized radioactive polypeptides measured in a highly specific immunological precipitation assay for ovalbumin. The bound radioactive ovalbumin was displaced from the primary antibody by a 100-fold excess of unlabeled ovalbumin. The percentage of ovalbumin synthesis (radioactivity of the anti-ovalbumin precipitate/radioactivity of the hot trichloroacetic acid insoluble precipitate ×100) was 0.58%. Similar results were obtained in four separate experiments.

Additional studies were performed in order to investigate the hydrolysis of nucleotide 5′-triphosphates, by fractions containing the avian initiation factors. In this assay, the hydrolytic properties of oviduct fractions were compared with those of reticulocyte fractions at similar stages of purification. As seen in Table IV, A2B and M2B were found to lack GTPase activity. They did not stimulate the GTPase activity shown by M2A or A2A. M2A displayed a ribosome-dependent GTPase activity as well as a lesser ribosome-independent ATPase activity. A2A exhibited similar GTPase activity as well as a lesser ribosome-independent ATPase activity. The GTPase activity of the A2A, however, demonstrated minimal ribosome dependence and was only slightly greater than the ATPase activity.

Discussion

Protein synthesis initiation factors showing activity similar to IF-2A and IF-2B have been described recently by several laboratories (Shafritz et al., 1972; Levin et al., 1973; Picciano et al., 1973; Gupta et al., 1973; Grummt, 1974; Cashion and Stanley, 1974; Suzuki and Goldberg, 1974). This paper describes the isolation of similar factors from the hen oviduct.

In an effort to study the mechanism and regulation of protein synthesis in a hormone sensitive system our laboratory has undertaken a program to identify and purify the factors involved with protein synthesis in the chicken oviduct. Previously a salt wash of oviduct polysomes from the estrogen-stimulated chick was shown to possess initiation factor activity similar to that found in the salt wash of rabbit reticulocyte polysomes. It was shown to stimulate the incorporation of amino acids into polypeptides using either natural mRNA or poly(U) as template at a low Mg^{2+} concentration and in the presence of saturating amounts of supernatant factors (Comstock et al., 1972a). Attempts were made to isolate the active components from this fraction but were unsuccessful due to the limited amount of tissue. Studies were undertaken therefore with the hen oviduct, a large organ under physiological hormone stimulation. In this communication it is shown that when a microsomal salt wash from hen oviduct is fractionated on DEAE-cellulose and Sephadex G-200, it is possible to isolate two initiation factors.

In both chromatographic behavior and in vitro activities these factors closely resemble initiation factors M2A and M2B found in the rabbit reticulocyte and rabbit liver (Picciano et al., 1973). Indeed, the hen oviduct and rabbit reticulocyte initiation factors 2A and 2B appear to be interchangeable to a high degree in a number of initiation assay systems. The molecular weights of oviduct IF-A2A and IF-A2B have been estimated by gel filtration chromatography to be 280 000 and 23 000, respectively. The value for IF-A2B is similar to that reported for the corresponding rabbit reticulocyte and liver factors (Picciano et al., 1973). Although rabbit reticulocyte IF-M2A and hen oviduct IF-A2A have similar molecular weight estimates by gel filtration chromatography, recent studies by Merrick et al. (1975) have shown that the molecular weight of IF-M2A is approximately 125 000 based on sodium dodecyl sulfate gel electrophoresis, Stokes radius, and equilibrium centrifugation. This value is much smaller than that determined by the gel filtration method. The anomalous value from molecular sieve chromatography is believed to result from the asymmetrical shape of the IF-M2A molecule. Similarly, by combining sedimentation analyses and gel filtration studies the molecular weight of oviduct A2A is calculated to be 125 000.

In the exchange assays the lower activities of initiation factors isolated from the oviduct may be due to the presence of proteases or nucleases in these fractions, or to the presence of inhibitors of protein synthesis which may have been released from sequestration by homogenization of the tissues. The ribosome independent GTPase activity of the IF-A2A fraction

is due possibly to contamination by a nonspecific nuclease. Although the specific activity of the IF-A2A fraction in the GTPase assay is low, and the addition of ribosomes stimulates the activity only slightly, the total ribosome-dependent GTPase activity appears to be quite similar to that of the reticulocyte when the larger quantity of oviduct protein necessary to saturate the initiation system assays is considered. A similar nonspecific nuclease could account for the hydrolysis of ATP by IF-A2A. Merrick et al. (1975), however, have found that homogeneous IF-M2A possesses a rate of ATP hydrolysis one-fourth that of GTP. These questions can be resolved only by further purification of the oviduct factors. The role of GTP hydrolysis in the initiation reaction is not understood, although it may occur when the 40S and 60S ribosome subunits join (Merrick et al., 1975).

Oviduct IF-A2A as well as IF-A2B stimulate the AUG-directed binding of Met-tRNA$_f$ to the 40S ribosome subunit. These activities are similar to IF-M2A and IF-M2B as have been previously reported (Adams et al., 1975). More recently Merrick et al. (1975) have observed that, when homogeneous IF-M1 is used in the binding assay, both IF-M2A and GTP are no longer required. The IF-M1 which was used in these studies was not of that degree of purity and, therefore, the requirement for oviduct IF-A2A is uncertain.

Avian hen oviduct initiation factors A2A and A2B appear to be interchangeable with the corresponding mammalian reticulocyte factors. These factors show activity in poly(U)-dependent synthesis of polyphenylalanine, in lowering the Mg^{2+} concentration optimum, in binding Met-tRNA$_f$ to 40S ribosomal subunit, and in exogenous natural-mRNA-directed protein synthesis. Although the exact role of these factors in initiating protein synthesis is unknown, the corresponding factors from other systems are felt to act primarily in binding 40S and 60S subunits to form an 80S monomer in the presence of AUG and IF-M1. Perhaps then these proteins should be functionally interchangeable in so far as eucaryotic Met-tRNA$_f$ and ribosomes are similar in the different systems.

There is a more practical reason why IF-A2A and IF-A2B are interchangeable with their reticulocyte counterparts. This is due to the manner in which they were isolated. An oviduct initiation factor, no matter how active, would not be seen in the assay used to screen the crude salt wash and its purified fractions unless it did exchange with the corresponding reticulocyte initiation factor. Furthermore, the factors isolated by such techniques might be active only in the reticulocyte system, or in a system synthesizing protein on free polysomes and hence unrelated to the bulk of the membrane bound polysome synthesis of proteins for cellular export in the oviduct. To examine this problem, experiments were performed utilizing native oviduct polysomes. Since these polysomes presumably reflect the distribution of protein synthesis in the intact oviduct, and since they are programmed with purified ovalbumin mRNA, this assay system represents a more stringent test of initiation activity in the oviduct system. It does not, however, exclude the possibility that other factors which do not show characteristics typical of any reticulocyte factor might be present in the oviduct but undetectable in the assays used.

Recently experiments of Nombela et al. (1975) with *Artemia salina* ribosomes have suggested that IF-M2A and IF-M2B are intrinsic proteins of the ribosome 60S subunits which are extracted with high salt. Previously Lubsen and Davis (1974) have shown that, in the presence of saturating amounts of initiation factors, salt extracts from reticulocyte polysomes, stimulated further salt-washed polysomes but not unextracted (native) polysomes. The stimulation of natural-mRNA-directed protein synthesis by IF-2A and IF-2B reflects upon this question. The fact that these two factors from either source, the hen oviduct or rabbit reticulocyte, result in a two- to threefold increase in protein synthesis using polysomes isolated in a sucrose gradient in 2 mM Mg^{2+} and not salt extracted indicate that they are at least partially absent from ribosomes engaged in protein synthesis. The absence of these factors on polysomes indicates that they do join and leave the ribosome during its cycle. This, along with their activity in various initiation subreactions, implies that they are indeed protein synthesis initiation factors.

Additional studies are in progress to further purify these factors and determine more precisely their roles in protein synthesis. Work is also continuing to identify and isolate the other factors involved in protein synthesis from the microsomal salt wash and cytosol of the oviduct.

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Mode of Reconstitution of Chicken Erythrocyte and Reticulocyte Chromatin†

Robert A. Gadski and Chi-Bom Chae*

ABSTRACT: The mode of reconstitution of chicken erythrocyte and reticulocyte chromatin has been investigated. Chromatin was dissociated in 2 M NaCl, 5 M urea, and 0.01 M potassium phosphate (pH 7.2) and was dialyzed against various NaCl concentrations in 5 M urea and 0.01 M potassium phosphate (pH 7.2). Histone reassociation to DNA occurs with the binding of histone H5 at 0.5 M NaCl in 5 M urea, followed by histone H1 at 0.4 M NaCl in 5 M urea. All the classes of histones are reassociated with DNA at 0.2 M NaCl in 5 M urea and binding of all classes of histones is complete in 0.1 M NaCl and 5 M urea. Nonhistone proteins reassociate with DNA before and at the same time that histones reassociate with DNA. Binding of nonhistone proteins to DNA appears to be complete in 5 M urea and 0.01 M potassium phosphate (pH 7.2). There is also found in both erythrocyte and reticulocyte chromatin a nonhistone protein, present in relatively high concentrations, which remains associated with DNA in 2 M NaCl and 5 M urea. This tightly bound protein appears as one major band when chromatographed on sodium dodecyl sulfate-polyacrylamide gels, with a molecular weight of 95 000. This protein is soluble in phenol and sodium dodecyl sulfate but is insoluble in 5 M urea or 4 M guanidine hydrochloride. A fraction of reticulocyte nonhistone proteins was found to bind to DNA-cellulose in 5 M urea. The majority of these proteins elute at 0.15 M NaCl in 5 M urea but a significant fraction elutes at NaCl concentrations at which the bulk of the histones do not bind to DNA. The proteins that bind to free DNA have low molecular weights and do not show species specificity. Approximately 50% of the reticulocyte nonhistone protein does not bind to a DNA-cellulose column in 5 M urea and may require histones for complete reassociation.

Comparisons of native and reconstituted chromatin suggest that reconstituted chromatin has many of the same properties as native chromatin. RNA transcribed from reconstituted chromatin has been found to be similar to in vivo synthesized RNA (Gilmour and Paul, 1969), as well as RNA transcribed from native chromatin in vitro (Bekhor et al., 1969; Paul and Gilmour, 1968; Huang and Huang, 1969; Gilmour and Paul, 1970; Spelsberg et al., 1971). This is not only true for repetitive sequences of DNA but also for the transcription of a specific gene. The transcription of RNA from native and reconstituted avian reticulocyte chromatin (Barrett et al., 1974) and fetal liver chromatin (Paul et al., 1973) has shown similar (but not identical) fractional yields of globin-specific sequences. Also,

† From the Department of Biochemistry and Nutrition, University of North Carolina, Chapel Hill, North Carolina 27514. *Received January 6, 1976.* Supported by a grant (CA-17390) from the National Cancer Institute, National Institutes of Health.