Interaction of Bovine Mitochondrial Ribosomes with *Escherichia coli* Initiation Factor 3 (IF3)†

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ABSTRACT: Mammalian mitochondrial ribosomes are distinguished from their bacterial and eukaryotic–cytoplasmic counterparts, as well as from mitochondrial ribosomes of lower eukaryotes, by their physical and chemical properties and their high protein content. However, they do share more functional homologies with bacterial ribosomes than with cytoplasmic ribosomes. To search for possible homologies between mammalian mitochondrial ribosomes and bacterial ribosomes at the level of initiation factor binding sites, we studied the interaction of *Escherichia coli* initiation factor 3 (IF3) with bovine mitochondrial ribosomes. Bacterial IF3 was found to bind to the small subunit of bovine mitochondrial ribosomes with an affinity of the same order of magnitude as that for bacterial ribosomes, suggesting that most of the functional groups contributing to the IF3 binding site in bacterial ribosomes are conserved in mitochondrial ribosomes. Increasing ionic strength affects binding to both ribosomes similarly and suggests a large electrostatic contribution to the reaction. Furthermore, bacterial IF3 inhibits the Mg^{2+}-dependent association of mitochondrial ribosomal subunits, suggesting that the bacterial IF3 binds to mitochondrial small subunits in a functional way.

The translation system of mammalian mitochondria is unique in many aspects, including the composition and physical–chemical properties of the ribosomes (O'Brien, 1976; Matthews et al., 1982), the structure of the tRNAs (de Bruijn & Klug, 1983), and the requirements for some homologous soluble factors (Denslow & O'Brien, 1978, 1979; Eberly et al., 1985). Several properties of the ribosomes distinguish them from those of prokaryotes and the cytoplasm of eukaryotes. Having about the same mass as bacterial ribosomes, they contain only half as much rRNA and nearly twice as much protein, differences which affect their sedimentation coefficient and buoyant density (Hamilton & O'Brien, 1974; Sacchi et al., 1973). In addition, the mitochondrial r-proteins are unique and have no closely related homologues in bacterial or eukaryotic–cytoplasmic ribosomes that have been identified by electrophoretic mobility or by immunologic cross-reactivity (S. Pietromonaco and T. W. O'Brien unpublished observation; Matthews et al., 1982).

Despite many attempts in different laboratories, no reproducible in vitro translation system has yet been developed for mammalian mitochondria. Unlike mitochondrial systems from fungi (Grandi & Kuntzel, 1970; Richter & Lipmann, 1970; Piechulla & Kuntzel, 1983), the mammalian mitochondrial translation system has not been well characterized, and only limited information exists regarding the identity and properties of any specific protein factors involved. On the basis of several partial reactions of protein synthesis, including the fragment reaction (Denslow & O'Brien, 1978), poly(U)-dependent phenylalanine polymerization (Denslow & O'Brien, 1979; Ulbrich et al., 1980; Eberly et al., 1985), and GTPase activity (Denslow & O'Brien, 1979), mammalian mitochondrial ribosomes appear to share more homology with bacterial ribosomes than with eukaryotic–cytoplasmic ribosomes. Their homology with bacterial ribosomes is especially evident with respect to antibiotic sensitivity (Denslow & O'Brien, 1978) and the exchangeability of some soluble factors (Eberly et al., 1985). Mammalian mitochondrial ribosomes are able to utilize bacterial EF-Tu; however, unlike fungal mitochondrial ribosomes, this exchangeability does not extend to the other elongation factor, EFG, which must be of homologous origin (Denslow & O'Brien, 1979; Ulbrich et al., 1980; Eberly et al., 1985).

Nothing is known regarding the involvement of specific initiation factors in mammalian mitochondrial systems. In this study, we address this question with respect to the ability of bovine mitochondrial ribosomes to interact with one of the bacterial initiation factors, initiation factor 3 (IF3). We show that under several ionic conditions, *Escherichia coli* IF3 binds to the small subunit of bovine mitochondrial ribosomes with an affinity of the same order of magnitude as that exhibited for bacterial ribosomes, and with the same stoichiometry. We also show that IF3 prevents the Mg^{2+}-dependent association of mitochondrial ribosomal subunits to form monoribosomes, indicating that the bacterial IF3 disrupts the natural interaction of mitochondrial subunits to form monosomes. These results suggest that despite the vastly different physical properties of mammalian mitochondrial ribosomes, the binding site for IF3 is conserved and has essentially the same molecular features as those involved in the binding of IF3 to bacterial ribosomes. This work is another step toward defining the properties and requirements of the bovine mitochondrial translation system.

Experimental Procedures

Preparation of Mitochondrial Ribosomes. Mitochondrial ribosomes were prepared from bovine liver mitochondria as described previously (Matthews et al., 1982). Ribosomes were

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isolated by centrifugation in linear 10–30% sucrose density gradients in buffer A containing 100 mM KCl, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 10 mM triethanolamine, pH 7.5. Fractions containing the 55S ribosomes were pooled and concentrated by high-speed centrifugation. The 55S ribosomes were dissociated into subunits by sucrose density gradient centrifugation in the high-salt buffer B containing 300 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.5. Small and large subribosomal particles were pooled separately and concentrated by high-speed centrifugation. Ribosomal subunits were stored frozen at −70 °C.

Preparation of Bovine Cytoplasmic Ribosomes. Cytoplasmic ribosomes were prepared from bovine liver as described previously (Matthews et al., 1982). Subunits were derived from 80S ribosomes by sucrose density gradient centrifugation in 500 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 20 mM triethanolamine, pH 7.5. The subunits were concentrated and stored as described above.

Preparation of E. coli Ribosomes and IF3. E. coli ribosomes were prepared from *E. coli* K-12 as described previously (Denslow & O'Brien, 1978). Ribosomes were washed in a high-salt buffer containing 1 M NH_4Cl, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 10 mM Tris-HCl, pH 7.5. *E. coli* ribosomes were dissociated into subunits in a 10–30% sucrose zonal gradient in a buffer containing 0.5 M KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 20 mM triethanolamine, pH 7.5. Large and small ribosome subunits were pooled separately, concentrated by high-speed centrifugation, and frozen at −70 °C.

E. coli initiation factor IF3 was prepared from the 1 M NH_4Cl ribosomal wash and purified as previously described (Pawlik et al., 1981). The purified IF3 was radiolabeled by reductive methylation with [^{14}C]formaldehyde, according to the procedure of Jentoft and Dearborn (1979). The specific activity of the labeled IF3 (1110 cpm/pmol) was calculated by titration with *E. coli* small ribosomal subunits which bind 1 pmol of IF3/pmol of ribosome at saturation (Sabol & Ochoa, 1971; Thibault et al., 1972; Pon et al., 1972).

Binding of ^{14}C-Labeled IF3 to Ribosomal Subunits. Small subunits of mitochondrial (0.5 A_{260} unit, 39 pmol) or *E. coli* ribosomes (0.5 A_{260} unit, 38 pmol) were incubated with ^{14}C-IF3 in a 50–100-μL solution of 10 mM Tris-HCl, pH 7.5, 5 mM 2-mercaptoethanol, and variable amounts of KCl and $MgCl_2$ as indicated in the figure legends. All mixtures were incubated for 10 min at 30 °C. Longer incubations did not significantly increase binding. The samples were analyzed by sucrose density gradient centrifugation on 5-mL 10–30% linear gradients of the same ionic composition as the binding reaction. The gradients were centrifuged in a Beckman SW 50.1 rotor at 35 000 rpm for 4 h. The absorbance profiles of the gradients were continuously monitored at 254 nm as they were fractionated into scintillation vials. The samples were mixed with Aquasol scintillation fluid and counted in a Beckman LS 8000 liquid scintillation counter.

Alternatively, ribosome–IF3 incubation mixtures were analyzed by size-exclusion chromatography on a 0.5 × 15 cm column of Sephacryl S-200. Chromatography was carried out at 4 °C in the same buffer as used in each binding reaction with a column flow rate of 0.66 mL/min. As the material exited the column, absorbance was measured at 254 nm, and fractions of 5-drop aliquots were collected and counted as described above.

Equilibrium Binding Measurements. Sedimentation experiments under equilibrium binding conditions were performed with the Beckman airfuge (Howlett et al., 1978). Mitochondrial 28S and *E. coli* 30S subunits (24 pmol of each) were incubated with varying amounts of ^{14}C-IF3 in a buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM 2-mercaptoethanol, and KCl and $MgCl_2$ as indicated in the figure legends. The mixtures were incubated at 30 °C for 10 min in a final volume of 120 μL. Aliquots of 20 μL were removed from each incubation mixture prior to and after sedimentation of the ribosomes at 110000*g* for 30 min in the airfuge. The first aliquot is representative of the total IF3 concentration, and the second aliquot is representative of the concentration of free IF3 at equilibrium. These aliquots were counted by using Aquasol scintillation cocktail as described above. Correction was made for the partial sedimentation of ^{14}C-IF3 centrifuged under the same conditions but in the absence of ribosomes.

The multivariate secant method of nonlinear parameter estimation on SAS (SAS User's Guide, 1982) was used to analyze binding. The binding data were fitted to the single-site isotherm, $\bar{\nu} = nK_a x/(1 + K_a x)$, and best estimates for both the number of sites (n) and the binding affinity (k_a) were determined. Initial guesses for the parameters were varied over a wide range to assure that the final estimates were at a global minimum.

Assay of Mg^{2+}-Dependent Subunit Association. Mitochondrial 55S monoribosomes were dissociated into subunits in the high-salt buffer B, and the derived subunits were dialyzed in the presence or absence of IF3 against buffers containing 50 mM KCl, 5 mM 2-mercaptoethanol, 10 mM Tris-HCl, pH 7.5, and various Mg^{2+} concentrations, as indicated, for 2 h at 4 °C. The mixtures were then centrifuged on 5-mL linear 10–30% sucrose density gradients in a Beckman SW 50.1 rotor for 4 h at 32 000 rpm and analyzed as described above.

Results

E. coli IF3 Binds to the Small Subunit of Mitochondrial Ribosomes. The binding of *E. coli* IF3 to the small subunit of mitochondrial ribosomes was studied under different ionic conditions spanning the physiological range and was analyzed by using sucrose density gradient centrifugation (Figure 1). The ionic conditions chosen were within the range used to study this interaction with bacterial ribosomes (Pon et al., 1972; Subramanian & Davis, 1970; Goss et al., 1982; Weiel & Hershey, 1981). The ratio of KCl to Mg^{2+} concentrations in these buffers was maintained constant at 10 to minimize conformational changes of the ribosomal subunits under the different ionic conditions (Cox et al., 1976; Cox & Hirst, 1976). Bacterial IF3 binds well to the mitochondrial 28S subunits (Figure 1A) and to bacterial 30S subunits (Figure 1B) in buffers of moderate ionic strength. Most of the ^{14}C-IF3 added to the incubation mixture is bound to the subunits, and very little remains free at the top of the gradient, indicating a reasonably high association constant for the interaction of IF3 with both bacterial and mitochondrial ribosome subunits. These conditions, however, are unable to support the binding of IF3 to the small subunit of cytoplasmic ribosomes (Figure 1C,F), as was observed initially by Sabol and Ochoa (1971), suggesting that the binding site for IF3 is not conserved in cytoplasmic ribosomes. Under more stringent salt conditions, less IF3 associates with the small subunits of mitochondrial ribosomes (Figure 1D). This effect is also observed when *E. coli* 30S subunits are incubated with IF3 in this buffer (Figure 1E). The reduced binding of IF3 to the small subunits of both mitochondrial and bacterial ribosomes in the higher salt buffer suggests that a large component of the interaction of IF3 with ribosomal subunits is ionic in nature.

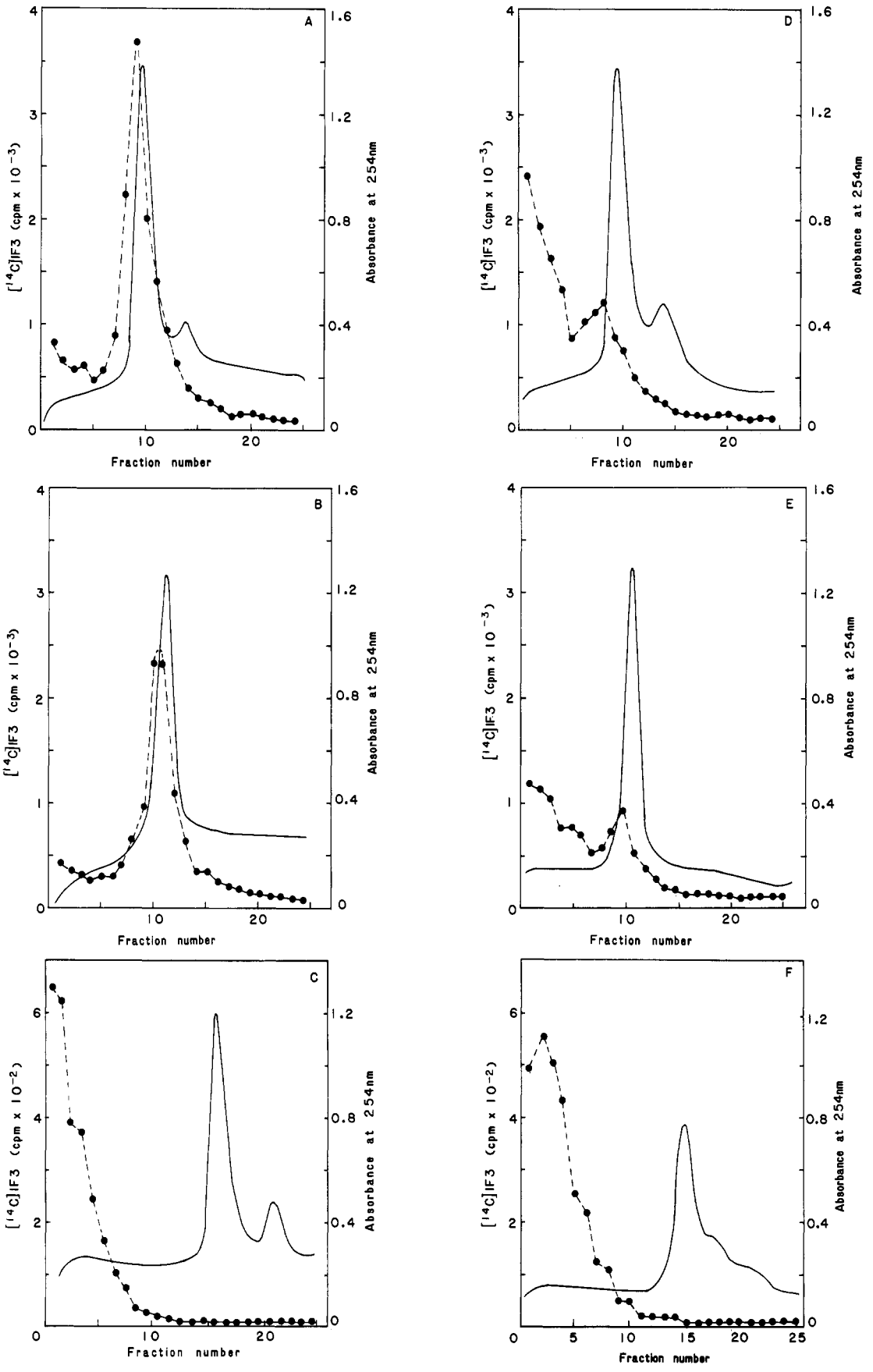

FIGURE 1: Binding of ^{14}C-IF3 to *E. coli* bovine mitochondrial and cytoplasmic subribosomal particles. The binding reaction was carried out as described under Experimental Procedures with 0.5 A_{260} unit of each subribosomal particle: (A and D) bovine mitochondrial small subunits with 13 500 cpm of ^{14}C-IF3; (B and E) *E. coli* 30S subunits with 10 000 cpm ^{14}C-IF3; (C and F) bovine cytoplasmic 40S subunits with 3000 cpm of ^{14}C-IF3. The buffer for panels A–C contained 50 mM KCl and 5 mM $MgCl_2$, and the buffer for panels D–F contained 70 mM KCl and 7 mM $MgCl_2$. Solid line, absorbance at 254 nm; dashed line, cpm of ^{14}C-IF3. Sedimentation in sucrose density gradients is from left to right.

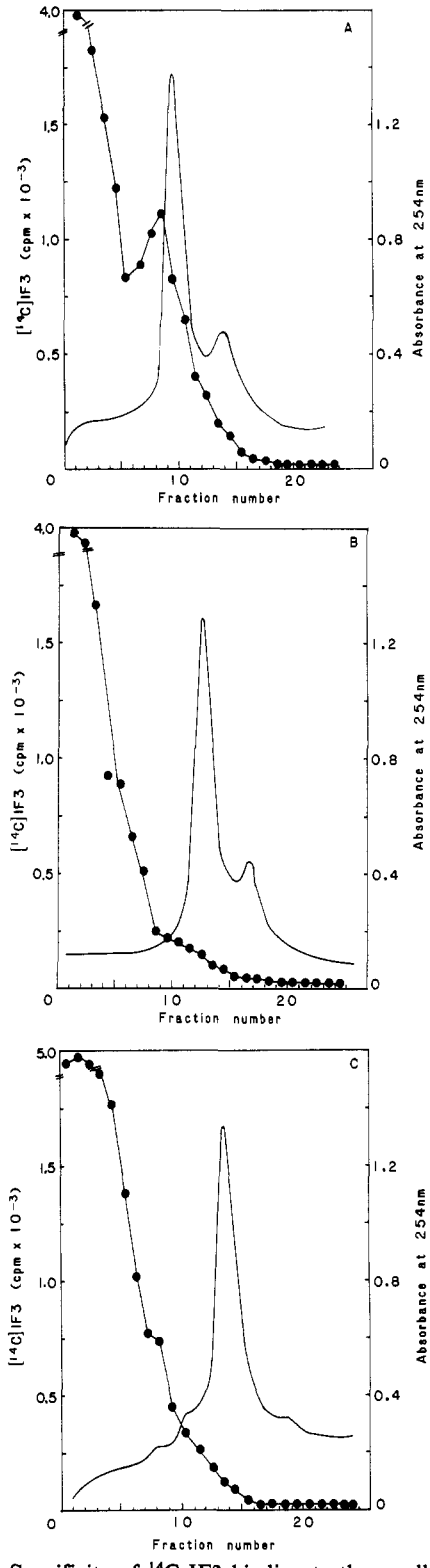

FIGURE 2: Specificity of ^{14}C-IF3 binding to the small subunit of mitochondrial ribosomes. *E. coli* IF3 was incubated with 35 pmol of small (A) or large (B) mitochondrial subunits in a buffer containing 70 mM KCl and 7 mM $MgCl_2$ or with 32 pmol of 55S monoribosomes (C) in a buffer containing 50 mM KCl and 10 mM $MgCl_2$ (to stabilize the monoribosomes). The samples were incubated for 10 min at 30 °C and were analyzed by sucrose density gradient centrifugation as described under Experimental Procedures. (—) Absorbance at 254 nm; (●) cpm of ^{14}C-IF3. Sedimentation is from left to right. Samples were centrifuged at 35 000 rpm for 4 h (A and B) or at 32 000 rpm for 4 h (C) in a Beckman SW 50.1 rotor.

The displacement of the ^{14}C-IF3 radioactivity peaks from the ribosome subunit absorbance peaks in Figure 1 probably

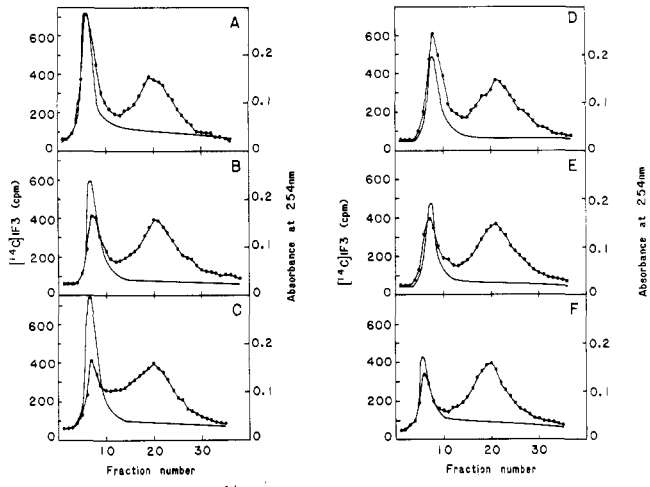

FIGURE 3: Analysis of ^{14}C-IF3 binding to mitochondrial (A–C) and *E. coli* (D–F) small subunits by Sephacryl S-200 column chromatography under three different ionic conditions. The buffer contained 5 mM 2-mercaptoethanol, 10 mM Tris-HCl, pH 7.5, and various KCl and $MgCl_2$ concentrations. (A and D) 50 mM KCl/5 mM $MgCl_2$; (B and E) 70 mM KCl/7 mM $MgCl_2$; (C and F) 100 mM KCl/10 mM $MgCl_2$. (—) Absorbance at 254 nm; (●) cpm of ^{14}C-IF3.

results from the dissociation of some IF3 during centrifugation. To reduce this effect, the interaction of IF3 with ribosome subunits was also investigated by using column chromatography (below).

Specificity of the Interaction of IF3 with the Small Subunit of Mitochondrial Ribosomes. To further characterize the interaction of IF3 with mitochondrial ribosomes, ^{14}C-IF3 was incubated with 28S subunits, with 39S large subunits, and with 55S monoribosomes (Figure 2). Under the conditions of this study, IF3 binds only to the 28S subunits (Figure 2A) and not to the 39S subunits (Figure 2B) or 55S monoribosomes (Figure 2C), indicating the specificity of this interaction. This result suggests that the binding site for IF3 on mitochondrial 28S subunits is not accessible on 55S monoribosomes, consistent with expectations that the IF3 binding site resides on the interfacial aspect of the small subunit.

Salt Dependence of the IF3 Interaction by Column Chromatography. In these experiments, 15–20 pmol of ribosomal subunits was incubated with approximately 10 pmol of ^{14}C-IF3 under three salt conditions in the physiological range in which the ratio of KCl to Mg^{2+} was held constant at 10. The ribosome–IF3 complexes were separated from free ^{14}C-IF3 by chromatography through a short (15 cm) column of Sephacryl S200. Figure 3 shows typical column chromatographic profiles for the three buffer conditions tested. Maximum binding to the mitochondrial 28S small subunits occurs in the lowest salt buffer (50 mM KCl/5 mM $MgCl_2$) tested. Less IF3 binds to the small subunits under the higher salt concentrations (Figure 3B,C), and more free IF3 is observed in the included volume, suggesting that the association constant varies with the ionic strength. The same kind of results are observed for the *E. coli* small subunits in buffers of increasing salt concentration (Figure 3D–F). The ratio of bound ^{14}C-IF3 per picomole of ribosome to free ^{14}C-IF3 is plotted in Figure 4 for each of the salt conditions tested. This ratio is a measure of the apparent association constant of IF3 for the small ribosomal subunits. At every salt concentration tested, the apparent association constant is slightly higher for the binding of IF3 to bacterial ribosomes than it is for mitochondrial ribosomes. Nevertheless, increasing the ionic strength of the incubation solution affects the binding of IF3 to mitochondrial and to bacterial ribosomes similarly. The observed destabilizing effect of salt on this interaction for both types of ribo-

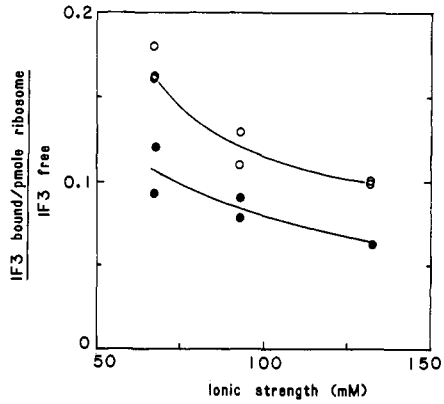

FIGURE 4: Plot of the ratio of cpm of ^{14}C-IF3 bound to cpm of ^{14}C-IF3 free as a function of ionic strength. All ionic species were taken into account. (○) *E. coli*; (●) mitochondria. The data are from Figure 3.

somes further indicates the ionic nature of this interaction.

Association Constant for the Interaction of IF3 with Mitochondrial 28S Subunits. We used the Beckman airfuge to study the binding of *E. coli* IF3 to mitochondrial 28S subunits under equilibrium conditions (Howlett et al., 1978). Fixed amounts of small ribosomal subunits from mitochondria (27 pmol) or *E. coli* (16 pmol) were titrated with aliquots of ^{14}C-IF3 under two salt conditions: 50 mM KCl/4 mM $MgCl_2$ and 70 mM KCl/7 mM $MgCl_2$. Figure 5 shows the binding isotherms. In all experiments, the binding data were analyzed by nonlinear regression as described under Experimental Procedures. Association constants (K_a) obtained from these fits, along with their 65% confidence intervals, are (4.8 ± 0.6) × 10^6 and (5.8 ± 1.0) × 10^6 M^{-1} for binding to mitochondrial and to *E. coli* small subunits in the 70 mM KCl/7 mM $MgCl_2$ salt condition and (1.9 ± 0.3) × 10^{-7} M^{-1} for binding to mitochondrial small subunits in the 50 mM KCl/4 mM $MgCl_2$ salt condition. These values lie within the range of association constants reported for *E. coli* small ribosomal subunits (Subramanian & Davis, 1970; Goss et al., 1982; Sabol et al., 1973). In all fits, the number of binding sites (n) converged to a value of 1.0 ± 0.2. From this analysis, mitochondrial 28S subunits appear to contain a single binding site for IF3 as do the bacterial 30S subunits (Sabol & Ochoa, 1971, 1973; Thibault et al., 1972; Pon et al., 1972; Weiel & Hershey, 1981). Also, as predicted by the gel permeation chromatography results, the binding constant of IF3 for mitochondrial 28S subunits increases severalfold under less stringent ionic conditions.

Although the confidence intervals overlap for the association constants of IF3 with *E. coli* and mitochondrial subunits in the 70 mM KCl/7 mM $MgCl_2$ salt condition, all three binding assay techniques employed in this study (sucrose density gradients, chromatography, and airfuge sedimentation) indicate that the affinity of IF3 for mitochondrial small subunits is slightly lower than that for the 30S subunits. Nevertheless, the close agreement between the affinity constants of IF3 for the two different ribosomes indicates that the binding site for IF3 is relatively well conserved in mitochondrial ribosomes.

Effect of IF3 on the Association of Ribosome Subunits from Bovine Mitochondria. To ascertain the effects of bacterial IF3 on the association of mitochondrial ribosome subunits, mixtures of large and small ribosomal subunits were incubated with IF3 in high-salt buffer B and then dialyzed against different buffers to promote subunit association (see Experimental Procedures). After being analyzed by sucrose density gradient centrifugation, the samples were compared to controls lacking IF3 (Figure 6). Clearly, IF3 hinders the

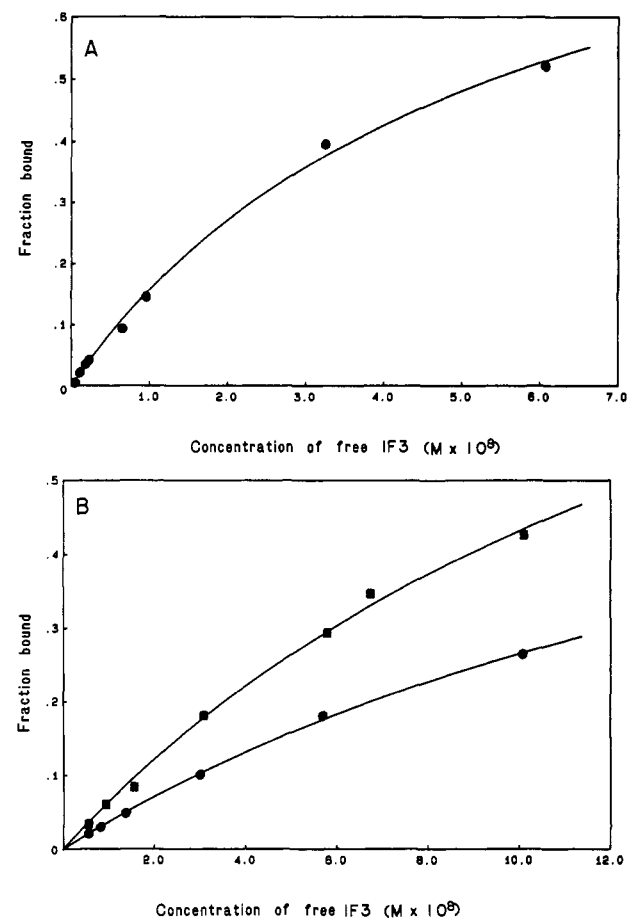

FIGURE 5: Binding isotherms for the interaction of IF3 with bovine mitochondrial small subunits (●) and *E. coli* small subunits (■). Subunits were incubated with various amounts of IF3 for 10 min at 30 °C, and the mixtures were separated by centrifugation in a Beckman airfuge as described under Experimental Procedures. The solid lines show the best fits to single-site isotherms for each experiment. (A) Binding isotherm for the buffer containing 70 mM KCl and 7 mM $MgCl_2$; (B) binding isotherms for the buffer containing 50 mM KCl and 5 mM $MgCl_2$.

association of mitochondrial subunits to form monoribosomes. Figure 7 displays this effect, with the fraction of ribosomes in the monoribosome peak plotted against the Mg^{2+} concentration. The bacterial IF3 can perturb the Mg^{2+}-dependent association of mitochondrial ribosomal subunits at concentrations of KCl and Mg^{2+} that are within the physiological range. This effect resembles that shown by IF3 for *E. coli* ribosomes (Subramanian & Davis, 1970; Goss et al., 1982; Chaires et al., 1981). Apparently, the bacterial IF3 binds to a single site on the mitochondrial small ribosomal particle (above) where it blocks subunit association, shifting the equilibrium between subunits and monoribosomes much as is observed with bacterial ribosomes.

Discussion

The present data indicate that the bovine mitochondrial ribosome has a binding site for eubacterial IF3. It is especially significant that this heterologous interaction occurs under physiological ionic conditions. The IF3 appears to bind to a single site on the small subunit of bovine mitochondrial ribosomes, probably at the interfacial region, as shown for bacterial ribosomes (Gualerzi & Pon, 1981), because the bound factor interferes with subunit association and because it does not bind to monoribosomes. The functional participation of *E. coli* IF3 in the subunit dissociation equilibrium of the mitochondrial ribosome requires that a reasonable degree of conservation exists at this step in the initiation cycle

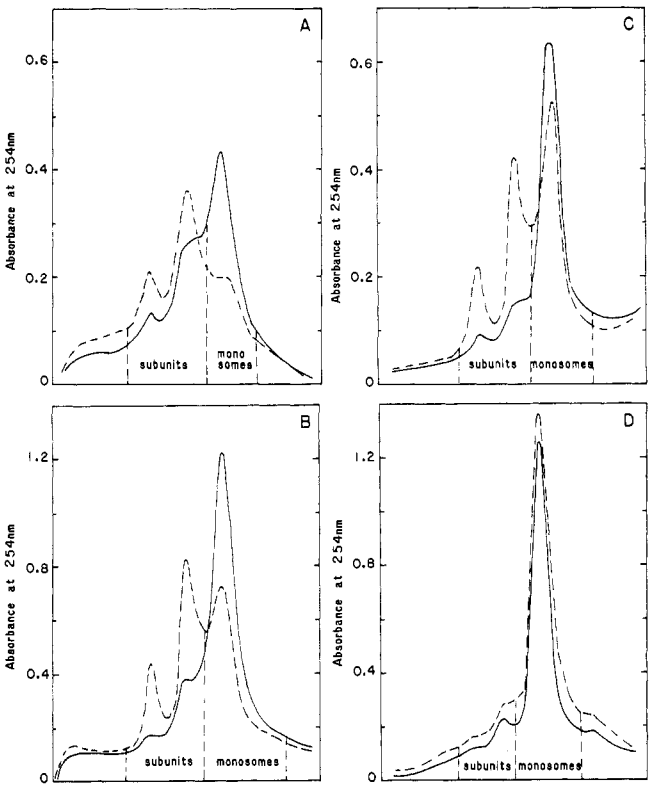

FIGURE 6: Effect of *E. coli* IF3 on the Mg^{2+}-dependent subunit association. 25–55 pmol (0.5–1 A_{260} unit) of dissociated mitochondrial ribosomes was incubated in the presence (dashed line) or in the absence (solid line) of *E. coli* IF3 in the high-salt buffer B for 10 min at 30 °C and then dialyzed into the indicated buffer containing 5 mM 2-mercaptoethanol, 50 mM KCl, 10 mM Tris-HCl, pH 7.5, and (A) 3 mM $MgCl_2$, (B) 4 mM $MgCl_2$, (C) 5 mM $MgCl_2$, and (D) 10 mM $MgCl_2$. Samples were analyzed by sucrose density gradient centrifugation as described under Experimental Procedures. Sedimentation is from left to right.

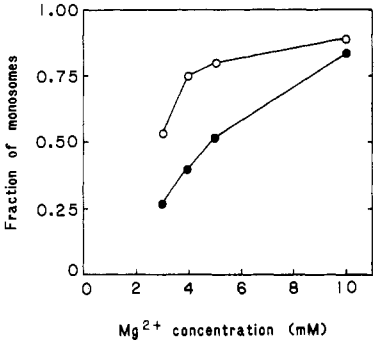

FIGURE 7: Titration curve for ribosomal subunit association as a function of Mg^{2+} concentration (●) in the presence of IF3 and (○) in the absence of IF3. The data come from the experiment in Figure 6.

in the two translation systems. For these reasons, we infer that IF3 is binding to a homologous site on bacterial and mitochondrial ribosomes. This binding contrasts with the inability of bacterial IF3 to bind to eukaryotic–cytoplasmic ribosomes [see Figure 1 and Sabol and Ochoa (1971)] and thus provides another example in which mammalian mitochondrial ribosomes share more functional homology with bacterial ribosomes than with their extramitochondrial counterparts. The fact that a binding site for eubacterial IF3 is conserved in bovine mitochondrial ribosomes suggests that mitochondrial ribosomes interact with an analogous initiation factor, which may play a similar role in mitochondrial protein synthesis.

The similarity in the association constants for the interaction of *E. coli* IF3 with bacterial and mitochondrial small ribosomal subunits points to a fairly well-conserved binding site for eubacterial IF3 in mitochondrial ribosomes. That the interaction of IF3 with both mitochondrial and *E. coli* ribosomes is sensitive to the ionic strength of the buffer further points to the similarity of the IF3 binding sites in both types of ribosomes and implies that the binding of IF3 to ribosomes involves ionic interactions. Early cross-linking experiments of IF3 to 16S rRNA in situ have shown that the factor is bound in the proximity of two separate sites: a major one (80%) within the 5′ half of the molecule and a minor one (≤20%) close to the 3′ end of the molecule (Pon et al., 1977). Very similar results have recently been obtained by Ehresmann et al. (1986), who identified a major cross-linking segment spanning from nucleotide 819 to nucleotide 859 and a minor cross-linking site between nucleotides 1506 and 1529 of the 16S rRNA. These two sites appear reasonably close to each other in a recently proposed three-dimensional model of the 16S rRNA (Dr. R. Brimacombe, personal communication) so as to make cross-linking of the 30S-bound factor to both of them a likely event. However, it must be noted that the stem and loop structure containing nucleotides 829–857 of *E. coli* 16S rRNA is absent in bovine mitochondrial 12S rRNA [for a review, see Brimacombe et al. (1983)]. Therefore, if the binding site(s) for IF3 on *E. coli* and on mitochondrial (small) subunits is (are) really conserved, then this stem and loop structure in *E. coli* rRNA is not likely to comprise part of the actual binding site for IF3.

The other site of potential interaction between IF3 and the 30S ribosomal subunit is the 3′-terminal stem and loop structure known as the "cloacin fragment" (Wickstrom, 1983; Wickstrom et al., 1986). The sequence and structure of this stem–loop region and the adjoining 14 nucleotides are highly conserved in ribosomes of bacteria, eukaryotic cytoplasm, and mitochondria (Zwieb et al., 1981; Van Knippenberg et al., 1984).

What are the structural features and/or nucleotide sequences conserved in bacterial and mitochondrial ribosomes that may contribute to the binding site for IF3? One way to approach this question is to identify those features or nucleotides common to those ribosomes able to bind eubacterial IF3 but which are altered in eukaryotic cytoplasmic ribosomes, unable to bind the eubacterial factor (Sabol & Ochoa, 1971). A prime candidate for this role is the G at position 1516 in *E. coli* 16S RNA, conserved in eubacterial, archaebacterial, and mammalian mitochondrial ribosomes (Zwieb et al., 1985; Brimacombe et al., 1983; Van Knippenberg et al., 1984; Gutell et al., 1985) which are able to bind IF3. In an otherwise highly conserved 3′-terminal stem–loop structure, the G_{1516} is replaced by a U in eukaryotic cytoplasmic ribosomes (Zwieb et al., 1981; Van Knippenberg et al., 1984). However, in rationalizing the reason for the failure of *E. coli* IF3 to bind to eukaryotic cytoplasmic ribosomes, one should not dismiss the possibility that access of IF3 to the cytoplasmic ribosomes may be precluded by some ribosomal proteins which mask, in part or completely, the rRNA domain which could be recognized by IF3.

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