

The 66 kDa component of eukaryotic initiation factor 3 interacts with globin mRNA and 18 S rRNA in preinitiation complexes

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The 66 kDa protein present in a complex with globin mRNA and 18 S rRNA [(1984) Eur. J. Biochem. 143, 27–33] has been reincorporated into functional eukaryotic initiation factor 3 (eIF-3) under conditions of protein synthesis. Additionally, two-dimensional polyacrylamide gel electrophoresis has been used to demonstrate the identity of the 66 kDa protein with the 66 kDa subunit of eIF-3.

Protein synthesis Initiation Eukaryotic initiation factor 3 mRNA mRNP

1. INTRODUCTION

During initiation of protein biosynthesis recognition of messenger RNA and its binding to the preinitiation complex determine the translational efficiency of individual mRNAs. Therefore investigations of the structure of preinitiation complexes should provide information concerning the mechanism by which messengers are selected for translation. In recent studies reovirus mRNA has been cross-linked to a 24 kDa cap binding protein [1] and to 18 S ribosomal RNA [2]. Cross-linking of complexes between native small ribosomal subunits of rabbit reticulocytes and globin mRNA demonstrates a close proximity between mRNA and (i) a 24 kDa cap binding protein, (ii) three subunits (111 kDa, 96 kDa and 66/64 kDa) of eukaryotic initiation factor 3 (eIF-3) and (iii) ribosomal proteins S1, S3/3a, S6, and S11 [3]. Thus, there is evidence for numerous interactions during binding of mRNA to the preinitiation complex. Information about the relative affinity between the individual components has been obtained by experiments in which polysomes of rabbit

reticulocytes, containing preinitiation complexes attached to the 5'-end of mRNA, were partially dissociated by incubation with 0.5 M LiCl and 0.5% SDS [4]. The resulting 22 S complex consists of globin mRNA, 18 S rRNA and a 66 kDa protein [4]. The intimate contact of the 66 kDa protein with both RNAs may serve to stabilize their interaction since mRNA and 18 S RNA possess only a low degree of complementarity [5]. Interestingly, the 66 kDa subunit of eIF-3 can be cross-linked both to globin mRNA [3] and to 18 S rRNA [6] and we now present evidence that the 66 kDa protein of the 22 S complex is identical with the 66 kDa subunit of eIF-3.

2. MATERIALS AND METHODS

Oligo(dT)-cellulose was purchased from PL Biochemicals, iodogen from Pierce and Warriner and carrier-free Na¹²⁵I from Amersham International. All glass and plastic ware was acid-washed and RNase-free, double glass-distilled water was used. Antibiotics were raised in sheep against rabbit reticulocyte eIF-3 and the isolated IgG fraction

(5.6 mg/ml) was kindly supplied by Dr J. Stahl (Central Institute of Molecular Biology, Berlin-Buch).

The 22 S complex was isolated as described in [4] and labelled according to [7]. For this purpose 0.5 A_{260} of the 22 S complex was iodinated in 20 μ l water with 3.7 MBq carrier-free Na^{125}I in the presence of 1 μ g iodogen. After 5 min at 20°C the solution was transferred into a tube containing 2 μ l of 20 mM dithiothreitol.

For two-dimensional electrophoresis of the labelled 66 kDa protein the 22 S complex was precipitated after labelling with 2 vols ethanol at -180°C . The precipitate was dried in vacuum and dissolved in 20 μ l lysis buffer containing 9.6 M urea, 2% NP-40, 2% Pharmalyte 3-10 (Pharmacia, Sweden) and 0.1 M mercaptoethanol. eIF-3 from rabbit reticulocytes (50 μ g), isolated as described in [8], was added and the proteins were separated using the two-dimensional system described in [9].

The labelled 22 S complex was added to 0.5 ml rabbit reticulocyte lysate prepared according to [10], which had been supplemented with amino acids and energy mixture [11]. After adjusting the buffer concentration to 100 mM potassium acetate, 2 mM magnesium acetate and 20 mM Hepes-KOH, pH 7.6, the mixture was incubated for 30 min at 30°C. After dilution with 5 ml buffer containing 5 mM MgCl_2 , 10 mM Tris-HCl, pH 7.6, and 1 mM KI polysomes were pelleted for 3 h at $226000 \times g_{av}$. The pellet was resuspended in 0.2 ml buffer containing 0.5 M KCl, 5 mM MgCl_2 , 10 mM Tris-HCl, pH 7.6, 1 mM KI and 1 mM dithiothreitol (buffer A) and eIF-3 was separated by gradient centrifugation (10–20% sucrose in buffer A) for 16 h at $160000 \times g_{av}$ at 2°C using an SW 40 rotor (Beckman). Fractions containing eIF-3 were mixed with 0.1 ml anti-eIF-3 IgG solution and kept for 2 days in the presence of 0.5 mM phenylmethylsulphonyl fluoride at 2°C. The precipitate was collected by centrifugation for 10 min at $10000 \times g_{av}$. For identification of the labelled 66 kDa protein, the precipitate was dissolved in 1% SDS, 0.1 M Tris-HCl, pH 7.6, 1% mercaptoethanol and 30% glycerol, heated for 2 min at 90°C and separated by polyacrylamide gel electrophoresis according to [12] using a gradient gel (7–12% acrylamide, 0.2–0.32% bisacrylamide). The labelled protein band was visualised

by autoradiography using Kodak X-Omat film.

3. RESULTS

In order to determine whether the 66 kDa protein of the 22 S complex is identical with the 66 kDa subunit of eIF-3 both proteins were compared by two-dimensional electrophoresis. For this purpose the 66 kDa protein was labelled by iodination within the 22 S complex and coelectrophoresed with unlabelled eIF-3 from rabbit reticulocytes using the system described by O'Farrell [9]. The labelled spot with an apparent molecular mass of 66 kDa was superimposed with the 66 kDa subunit of eIF-3, which was localized by silver staining (fig.1).

Additional proof for the identity of the 66 kDa component of the 22 S complex with the eIF-3 subunit was obtained by an investigation of the functional properties of this protein. Reincorporation of the 66 kDa protein into the factor eIF-3 was assayed after addition of the 22 S complex containing labelled 66 kDa protein to a rabbit reticulocyte lysate and incubation under conditions of protein synthesis. After pelleting the ribosomes, eIF-3 was isolated by extraction with 0.5 M KCl, gradient centrifugation (fig.2) and immunoprecipitation. The immunoprecipitate contained labelled protein which on electrophoresis was found to be identical with the 66 kDa subunit of eIF-3 run in parallel (fig.3). The 66 kDa protein

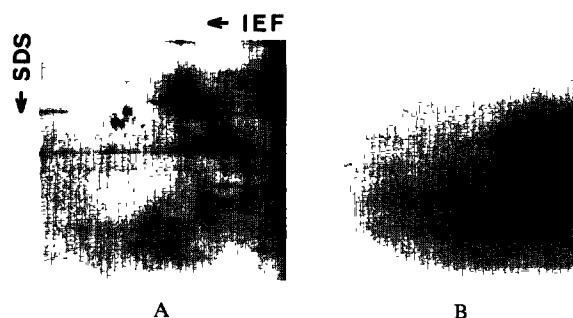


Fig.1. Characterization of the 66 kDa protein by two-dimensional electrophoresis. ^{125}I -labelled 66 kDa protein was coelectrophoresed with unlabelled eIF-3 from rabbit reticulocytes using the electrophoretic system described by O'Farrell [9]. (A) Pattern obtained by silver staining according to [19], (B) autoradiograph. The position of the 66 kDa protein is indicated by the arrow in panel A.

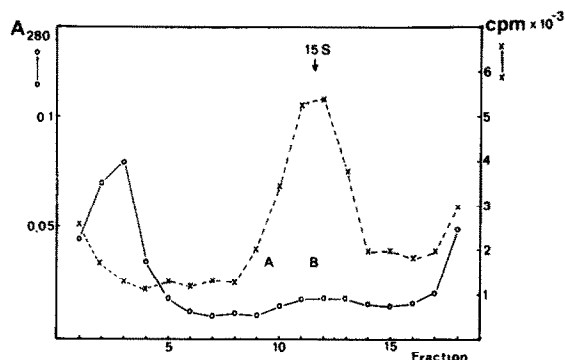


Fig. 2. Sucrose gradient fractionation of labelled eIF-3 isolated from polysomes. Labelled eIF-3 was obtained after incubation of the ¹²⁵I-labelled 22 S complex in a rabbit reticulocyte lysate, pelleting ribosomes, extracting eIF-3 with 0.5 M KCl and fractionation in a sucrose gradient as described in section 2. Gradient fractions A and B were used for immunoprecipitation of eIF-3.

could only be precipitated as a part of eIF-3 since the antibody preparation used in this experiment does not react with blotted 66 kDa or 64 kDa subunits of eIF-3 (Stahl, J., unpublished). Thus, if incubation of the 22 S complex with reticulocyte lysate was omitted, which prevents incorporation of the labelled 66 kDa subunit into eIF-3, no immunoprecipitation of the 66 kDa protein was observed.

4. DISCUSSION

The identity of the 66 kDa protein, complexed with 18 S rRNA and globin mRNA, and the 66 kDa subunit of eIF-3 confirms the important role of eIF-3 in mediating the interaction of mRNA and the small ribosomal subunit. Further information concerning other proteins which interact with mRNA has been obtained from structural studies of preinitiation complexes. Cross-linking of preinitiation complexes containing mRNA indicates a close proximity not only of mRNA and 18 S rRNA [2] but also of mRNA and ribosomal proteins S1, S3/3a, S6 and S11 [3], of the 5'-terminal cap structure of mRNA and a 24 kDa protein [1,3] and of mRNA and the 111 kDa, 96 kDa, and 66/64 kDa subunits of eIF-3 [3]. The multitude of components of the preinitiation complex in close association with

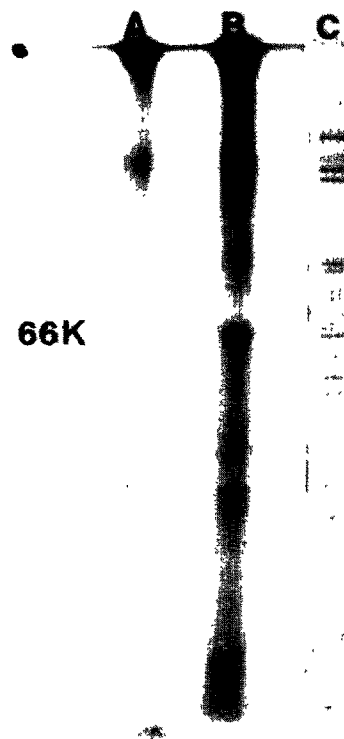


Fig. 3. Immunoprecipitation of eIF-3 from sucrose gradient fractions. eIF-3 fractions A and B (fig. 2) containing the ¹²⁵I-labelled 66 kDa subunit were immunoprecipitated and the factor subunits were separated according to [12]. Autoradiography demonstrates the presence of the 66 kDa subunit in fraction A (lane A) and fraction B (lane B); for comparison the complete pattern of eIF-3 subunits obtained by staining with Coomassie brilliant blue is given in lane C.

mRNA suggests that the formation of this complex is likely to be a multi-stage process. The sequence of events probably starts at the 5' cap structure and other nucleotides of the mRNA become involved subsequently in the interaction so that a total of about 60 residues are ultimately protected against nuclease degradation [13,14]. Within the complex pattern necessary for mRNA recognition the 66 kDa subunit of eIF-3 seems to mediate and possibly stabilize the interaction between mRNA and 18 S rRNA.

The binding forces between the three components are strong enough to protect the complex against dissociation by 0.5% SDS and 0.5 M LiCl.

It is of interest to compare the stability of the interaction of the 66 kDa subunit with that of the ribosomal proteins S3, S3a, S6 and S11, which are completely split off under the same conditions, although these ribosomal proteins can be also cross-linked to both 18 S rRNA [15] and globin mRNA [3]. This difference may be explained either by a stronger affinity of the 66 kDa subunit to the RNAs or by a higher stability of the protein against denaturing agents.

Contact between mRNA and the 66 kDa subunit is limited to the pre-initiation complex, since eIF-3 leaves the 48 S complex before 80 S initiation complex formation takes place [4]. In the context of the transient action of eIF-3 and reports on the differential stimulation of α/β globin chain synthesis by initiation factors [17], it is tempting to speculate that eIF-3 by means of its 66 kDa subunit may influence the selection of messenger RNAs for translation.

Summarizing the data it may be concluded that the eIF-3 promoted enhancement of mRNA binding to the preinitiation complex [18] and eventually the selection of individual messenger RNAs is mediated by the 66 kDa subunit of eIF-3.

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