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Eukaryotic elongation factor Tu is present in mRNA-protein complexes

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By two-dimensional gel electrophoresis, partial peptide mapping, and antibody binding we have shown that eukaryotic elongation factor Tu is in close contact with mRNA in rabbit reticulocytes. It can be crosslinked to mRNA by irradiating both polysomes and 40–80 S mRNA-protein complexes with short-wave UV light. To our knowledge this is the first case in which a known translation factor has been shown to be associated with mRNA in native ribonucleoproteins.

Elongation factor Tu; Protein synthesis; mRNA; RNA-protein complex; Cross-linking

1. INTRODUCTION

It has been known for some time that several eukaryotic translation factors, unlike their prokaryotic equivalents, are RNA-binding proteins [1–3]. However, they show little specificity in RNA binding, and in no case has it been shown that they are associated with any kind of RNA in cells. Therefore, the significance of this observtion remains unclear. eEF-Tu (also known as EF-1α) is an abundant basic cytoplasmic protein with a high degree of homology to prokaryotic EF-Tu [4,5]. Like its prokaryotic counterpart, eEF-Tu functions primarily in codon-dependent binding of aminoacyl tRNA to ribosomes [6,7]. However, unlike prokaryotic EF-Tu, it can bind in vitro to rRNA, mRNA, and certain synthetic polynucleotides [6–9]. eEF-Tu also binds GDP and GTP [6,7], and has considerable sequence homology with other GTP-binding proteins of unrelated function, such as transducin and the *ras* oncogene protein (review [5]). Furthermore, it is found in a complex with eukaryotic elongation factor Ts [6,7]. Its multiple binding specificities and high abundance raise the possibility that it has an additional function or functions which are not yet known. Here, we show that it is a component of native ribonucleoproteins, since it can be crosslinked to mRNA by irradiating polysomes and 40–80 S ribonucleoproteins with 254 nm UV light.

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Abbreviations: EF-1, elongation factor 1; EF-Tu, prokaryotic elongation factor Tu; eEF-Tu, eukaryotic elongation factor Tu; mRNP, messenger ribonucleoproteins; PAGE, polyacrylamide gel electrophoresis; 1D, one-dimensional; 2D, two-dimensional

2. EXPERIMENTAL AND RESULTS

To investigate the possible presence of eEF-Tu in mRNP UV-crosslinked mRNP proteins were radiolabeled with [^{14}C]formaldehyde and analyzed by 2D electrophoresis. Preparation of rabbit reticulocyte lysate, UV crosslinking, and [^{14}C]-formaldehyde labeling was done as described [10]. eEF-Tu was purified as in [11]. 2D gel analysis of mRNP proteins by non-equilibrium pH gradient electrophoresis in the first dimension and SDS-

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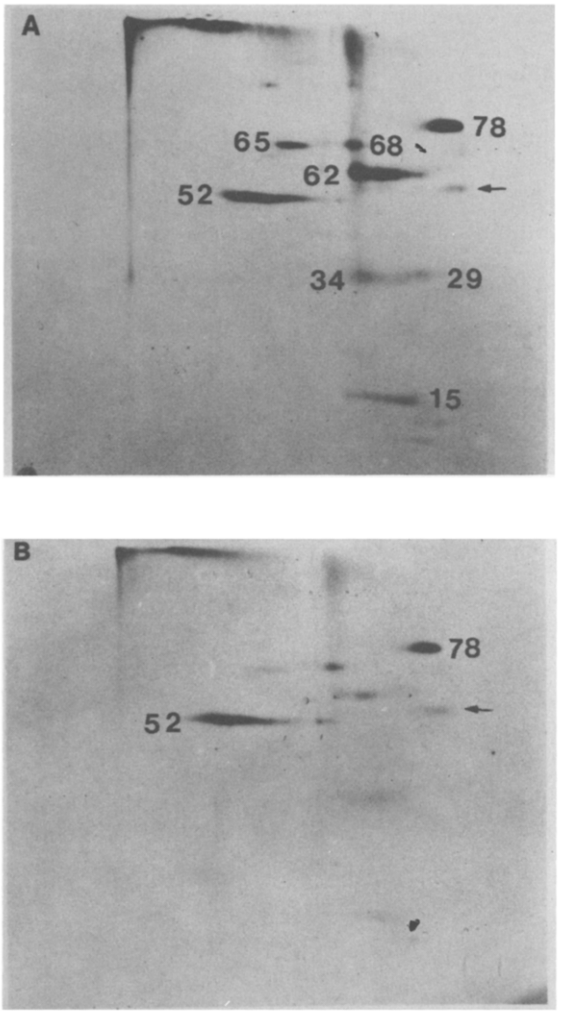

Fig.1. 2D gel electrophoretic analysis of mRNP proteins. Polysomes and 40–80 S RNP were isolated from 5 ml reticulocyte lysate by sucrose density gradient centrifugation. They were irradiated at 254 nm for 16 min at 2000 μW/cm^2, and crosslinked mRNP were isolated by chromatography on oligo(dT)-cellulose. They were labeled with 10 μCi [^{14}C]formaldehyde (New England Nuclear, 50 mCi/mmol) digested with RNases, and subjected to electrophoresis. Unlabeled eEF-Tu (2 μg) was added to the samples as a marker, and unlabeled molecular mass markers were included in the second dimension only. After electrophoresis the gels were stained with Coomassie blue and fluorographed for 1 week. 10^5 and 5×10^4 cpm were applied to gels A and B, respectively. The basic end of the first dimension gel is at the right. The position of eEF-Tu is indicated by arrows. (A) Polysomal mRNP proteins. (B) 40–80 S mRNP proteins.

PAGE in the second was done by the procedure of O'Farrell et al. [12]. The results are shown in fig.1. Rabbit reticulocyte polysomal mRNP proteins include major species having sizes of 78, 62, 52, 34, 29, and 15 kDa [10]. The spots corresponding to these species were readily detected in the 2D gels (fig.1A). Some bands showed multiple spots or streaking in the first dimension suggestive of charge isomerism, particularly the 52 and 62 kDa bands. mRNP proteins also contain species of 68 and 65 kDa which are not always resolved in 1D SDS-PAGE [10]. However, these species gave 2 well-separated spots in a 2D gel (fig.1A). 40–80 S mRNP proteins, which are associated with mRNA not present in polysomes, are qualitatively similar to polysomal mRNP proteins in 1D gels, although some of the bands are reduced in intensity [10]. Similar results were obtained with 2D gels (fig.2B).

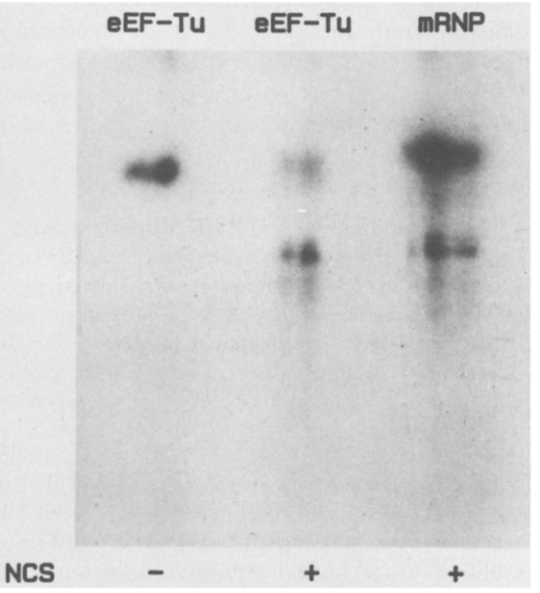

Fig.2. Partial peptide mapping of authentic eEF-Tu and the putative eEF-Tu spot from mRNP. Polysomal mRNP proteins were labeled and resolved in 2D gels as in fig.1, except that labeling was done with 500 μCi ^{125}I-labeled Bolton-Hunter reagent [13] (ICN, >1500 Ci/mmol). eEF-Tu was labeled and purified in a 1D SDS-10% polyacrylamide gel. About 500 dpm were applied to each lane. The gel was exposed for 10 days with an intensifying screen. eEF-Tu, eEF-Tu standard; mRNP, eEF-Tu spot from mRNP; NCS, *N*-chlorosuccinimide; −, not treated with *N*-chlorosuccinimide; +, treated with *N*-chlorosuccinimide.

The most prominent spots in the 40–80 S mRNP were those of 78 and 52 kDa. One clear spot was detected in 2D gels which had not been previously detected using 1D gels. Its position is indicated by an arrow. It was the most basic protein, and its apparent size was intermediate between those of the 52 and 62 kDa mRNP proteins (fig.1A,B). Its mobility was very similar to that of a stained marker of authentic reticulocyte eEF-Tu run in the same gel (not shown). This spot corresponded to a few percent of the polysomal mRNP proteins and a somewhat larger fraction of the 40–80 S mRNP proteins.

To verify that this protein was actually eEF-Tu polysomal mRNP proteins were radioiodinated with ^{125}I-labeled Bolton-Hunter reagent, which, like [^{14}C]formaldehyde, labels amino groups [13]. eEF-Tu and ethanol precipitates of crosslinked mRNP were labeled in 8 M urea containing 0.1 M sodium borate (pH 8.5). The labeled eEF-Tu was separated from unreacted ^{125}I by chromatography on Sephadex G-25, and the mRNP were separated by rechromatography on oligo(dT)-cellulose. Labeled mRNP proteins were electrophoresed in a 2D gel, whereas eEF-Tu was electrophoresed in a 1D gel. The spots of interest were located by comparison with stained markers of eEF-Tu. Gel slices were cut out, treated with *N*-chlorosuccinimide, which cleaves at tryptophanyl residues, or control solvent, and electrophoresed in a second 12.5% gel as described [14]. The results are shown in fig.2. The first and second lanes, respectively, show an eEF-Tu marker not cleaved and cleaved with *N*-chlorosuccinimide. The third lane shows the putative eEF-Tu spot from mRNP after cleavage. The upper band, which presumably corresponds to uncleaved protein, migrated slightly slower than authentic eEF-Tu. However, the lower bands, which represent the cleavage products, appeared to be identical for the eEF-Tu standard and the spot

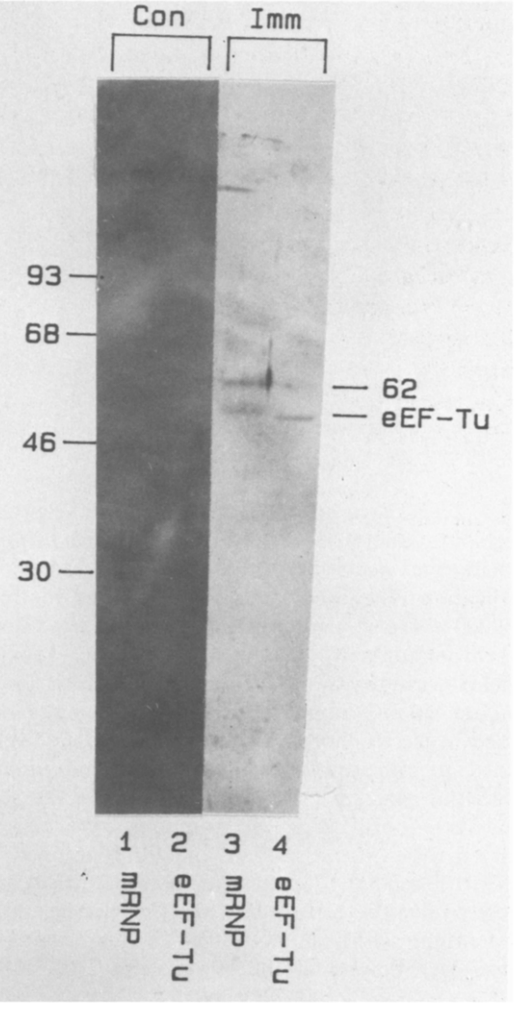

Fig.3. Western blot analysis of mRNP proteins with antibody against eEF-Tu. Duplicate samples of UV-crosslinked 40–80 S mRNP proteins from 1 ml reticulocyte lysate, 1 μg eEF-Tu marker, and ^{14}C-labeled molecular mass markers were electrophoresed in an SDS-10% polyacrylamide gel and transferred electrophoretically to nitrocellulose. The blots were blocked with 1% gelatin in 0.15 M NaCl, 10 mM Tris-HCl (pH 7.6), 1 mM EDTA (TBSE). They were incubated overnight with shaking at ambient temperature in sealed plastic bags with either the eEF-Tu antibody or control antibody in 10 ml TBSE containing 1% gelatin and 0.1% Triton-X-100. The control antibody was rabbit antibody against human IgG obtained from Worthington-Cappel. The eEF-Tu antibody has been described in [11]. In both cases antibody was affinity-purified by binding to the corresponding immobilized antigen. Both were used at 20 μg/ml. After washing with TBSE containing 0.1% Triton-X-100 the blots were incubated for 2 h in TBSE-Triton-gelatin containing a 1:500 dilution of peroxidase-conjugated rabbit anti-goat IgG (Cappel-Worthington). After washing again the blots were stained as described [17]. The positions of the marker proteins, whose sizes in kDa are indicated at the left, were determined by autoradiography. Lanes: 1,2, control antibody; 3,4, eEF-Tu antibody; 1,3, mRNP proteins; 2,4, eEF-Tu marker.

from mRNP. Other investigators have found that UV crosslinking to RNA results in a slight increase in the apparent size of proteins even after RNase digestion [15,16]. On the basis of these data it seems likely that mRNA in rabbit reticulocytes is associated with eEF-Tu.

We further investigated the presence of eEF-Tu in mRNP by probing Western blots of mRNP proteins with an affinity-purified antibody raised in goats against purified rabbit elongation factor 1 [11]. UV-crosslinked mRNP proteins and an eEF-Tu marker were electrophoretically transferred to nitrocellulose. Antibody binding was detected with a rabbit anti-goat IgG second antibody which was conjugated to horseradish peroxidase. The blot was stained with 4-chloro-1-naphthol [17]. Fig.3 (lane 4) shows the eEF-Tu marker stained with antibody. Fig.3 (lane 3) shows the mRNP proteins stained by the antibody. There are two bands visible. The lower band is very similar in mobility to eEF-Tu. The slight retardation observed is characteristic of proteins UV crosslinked to RNA. The upper band corresponds in size to the 62 kDa mRNP protein. The binding of the eEF-Tu antibody to this protein was unexpected. This phenomenon has been further investigated, and the 62 kDa mRNP protein appears to have an epitope in common with eEF-Tu, although it is in other respects a different protein (Slobin, L.I. and Greenberg, J.R., submitted). A control antibody (affinity-purified goat antibody against human IgG) did not bind to the eEF-Tu marker or to any of the mRNP proteins (fig.3, lanes 1,2). The antibody-binding data are further evidence that eEF-Tu is associated with mRNA.

3. DISCUSSION

By the criteria of mobility in 2D gels, partial peptide mapping, and antibody binding eEF-Tu is among the proteins which can be crosslinked to mRNA by irradiating rabbit reticulocyte polysomes and 40–80 S RNP with UV light. UV crosslinking, which results from the action of short-lived free radicals and depends on a close and stable contact between molecules, has been widely used for demonstrating specific associations between nucleic acids and proteins (reviews [18,19]). eEF-Tu is similar in size to two other mRNP proteins of 52 and 62 kDa, and it cannot readily be resolved from them in 1D gels, especially since it is considerably less abundant. However, it appears to be the only distinct new band visible in 2D gels not previsouly detected in 1D gels.

These experiments were prompted by previous observations that eEF-Tu is an RNA-binding protein. It binds in vitro to mammalian rRNA, mRNA, and synthetic polynucleotides, but not poly(A) or poly(C) [6–9]. It has not yet been established whether eEF-Tu is associated with rRNA in vivo. However, our data make it likely that eEF-Tu is associated with mRNA in cells, since previous work showed that the proteins crosslinked to mRNA by irradiating polysomes in vitro under the conditions used in the present experiments were indistinguishable from those crosslinked to mRNA by irradiating intact cells [10]. The relatively low yield of crosslinked mRNP obtained by irradiating intact cells prevented successful use of this approach in the case of eEF-Tu.

The functional roles of mRNP proteins including eEF-Tu are still unclear, although eEF-Tu is involved in binding of aminoacyl tRNA to ribosomes in the presence of mRNA during peptide chain elongation [6,7], and it would not be surprising if it were in close enough proximity to mRNA for crosslinking to occur. This model does not explain its presence in 40–80 S RNP, however. 40–80 S RNP should contain relatively few elongation complexes, yet eEF-Tu appears to be a higher proportion of total mRNP protein in 40–80 S RNP than in polysomal mRNP. The binding site of eEF-Tu on mRNA is not known, but it is probably not associated with poly(A) since the purified factor does not bind to poly(A) [9].

Ovchinnikov et al. [1] showed previously that both reticulocyte elongation factors and some initiation factors are RNA-binding proteins. However, it has not been previously shown that eEF-Tu or any other translation factor is associated with mRNA in native ribonucleoproteins. The 2D gel analysis shown in fig.1 and the antibody-binding data suggested that eEF-Tu is not one of the more abundant mRNP proteins, although the eEF-Tu spot in fig.1 is comparable in intensity to some of the other spots, especially in 40–80 S mRNP. However, there is no clear relationship between apparent relative abundance and possible functional importance. For one reason, strong conclusions about relative abundances cannot be made from

UV-crosslinking experiments because of the possibility that different protein and nucleic sequences crosslink with differing efficiencies. For another, factors such as eEF-Tu may associate with mRNA only transiently during translation. In this case they would be present on the average in less than one copy per mRNA molecule, and they would be necessarily less abundant than mRNP proteins which are present in one or more copies per mRNA molecule.

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