The elongation factor 1 A-2 isoform from rabbit: cloning of the cDNA and characterization of the protein


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ABSTRACT

Eukaryotic elongation factor 1 A (eEF1A, formerly elongation factor-1 α) is an important component of the protein synthesis apparatus. Here we report the isolation and characterization of the cDNA sequence encoding rabbit eEF1A-2, an isoform of eEF1A, as well as a structural and functional comparison of the two rabbit isoforms. Northern analysis of the expression pattern of eEF1A-2 showed that this isoform is expressed in skeletal muscle, heart, brain and aorta, while transcripts are not detected in liver, kidney, spleen and lung. In contrast, the previously characterized eEF1A-1 isoform is expressed in all tissues examined except skeletal muscle. We have recently purified eEF1A-2 from rabbit skeletal muscle. By partial amino acid sequencing and determination of the post-translational modifications of eEF1A-2 we found that both of the glycercylphosphorylethanolamine modifications observed in eEF1A-1 appear to be present in eEF1A-2. However, two of the residues found dimethylated in eEF1A-1 appeared to be trimethylated in eEF1A-2. A comparison of the enzymatic activity showed that eEF1A-1 and eEF1A-2 have indistinguishable activity in an in vitro translation system. In contrast, the GDP dissociation rate constant is ~7 times higher for eEF1A-1 than for eEF1A-2. The nucleotide preference ratio (GDP/GTP) for eEF1A-1 was 0.82, while the preference ratio for eEF1A-2 was 1.50.

INTRODUCTION

Peptide elongation factor 1 A (eEF1A, formerly elongation factor-1 α; 1) plays an important role in translation by catalysing GTP-dependent binding of aminoacyl-tRNA to the acceptor site of the ribosome. However, several studies have implied other functions of the protein as well. eEF1A has been shown to form complexes with tRNA synthetases (2), suggesting a possible function in the ‘channelling’ of components for protein synthesis. It is also found complexed with the mitotic apparatus (3) and in association with the endoplasmic reticulum (4). In addition, eEF1A has been demonstrated to bind and bundle actin (5) and to sever microtubules (6). Furthermore, it has been reported to act as an activator of phosphoinositol 4-kinase (7,8), to increase susceptibility towards UV-induced transformation (9) and to be highly expressed in various benign and malignant tumours (10,11). It may also play a part in ubiquitin-dependent degradation of Nε-acetylated proteins (12). Thus eEF1A may coordinate protein synthetic activity with other cellular events. This broad diversity of functions may also explain why eEF1A is such a well-conserved protein and why it constitutes 1–3% of the total cytoplasmic protein content of the eukaryotic cell (13).

Post-translational modifications such as methylation (14), phosphorylation (15,16) and addition of glycercylphosphorylethanolamine (14,17) have been found in eEF1A from different species. In contrast to conservation of the amino acid sequence, the modification pattern is not very well conserved (18,19). The glycercylphosphorylethanolamine modification has been found only in mammals. Likewise, the majority of the methylated sites differ between yeast and humans. Yet no function has been assigned to the modifications in mammals, but several suggestions have been put forward (20). In the fungus Macer racemosus an extensive methylation of eEF1A was suggested to be responsible for an increase in protein synthetic activity following sporulation (21).

In many organisms eEF1A belongs to a multigene family. Often these genes are expressed in a developmental- or tissue-specific manner, as in Xenopus laevis, in which three eEF1A genes are expressed at different developmental stages (22,23), and in Drosophila melanogaster, where two stage-specifically expressed eEF1A genes exist (24). In the mammalian genome many eEF1A-like sequences exist, but only two of these are currently known to be actively transcribed genes. The rest are suspected to be pseudogenes (25–27). In human (28,29), rat (30,31) and mouse (32,33) the cDNAs of the two actively transcribed isoforms of eEF1A have been cloned. The two eEF1A isoforms, referred to as eEF1A-1 and eEF1A-2, are differentially expressed in various tissues (28,29).

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expressed. eEF1A-2 is expressed tissue-specifically in terminally differentiated cells in skeletal muscle, brain and heart (29,34), whereas eEF1A-1 is expressed ubiquitously, although at a reduced extent in eEF1A-2-expressing tissues (34,35). Down-regulation of eEF1A-1 in eEF1A-2-positive tissue appears to coincide with up-regulation of eEF1A-2 expression.

In rabbits only the cDNA of eEF1A-1 has been cloned (36). In this study we report the cDNA sequence of rabbit eEF1A-2 and we characterize the expression pattern of eEF1A-2 and eEF1A-1 in different rabbit tissues. We have recently purified the eEF1A-2 protein from rabbit muscle (Kristensen, in preparation). Here we characterize the protein by partial amino acid sequencing, determination of the post-translational modifications and examination of guanine nucleotide binding affinities.

**MATERIALS AND METHODS**

The sequence reported in this paper has been deposited in the GenBank database (accession no. AF035178).

**Screening of cDNA library, subcloning and sequencing**

A random priming labelled full-length human eEF1A-2 cDNA (29) probe was initially used to screen a rabbit skeletal muscle 5′STRECH PLUS λgt11 cDNA library (Clontech). An EcoRI–EagI fragment from an isolated non-full-length eEF1A-2 cDNA clone (clone 20) was used to screen for recombinant plaques containing eEF1A-2 cDNA with 5′-UTRs (clone B).

Hybridization was performed in 6×SSC (20×SSC=3 M NaCl, 0.3 M sodium citrate, pH 7.0), 5× Denhardt’s, 0.1% SDS and 0.25 mg/ml total yeast RNA at 65°C. Washing was performed under high stringency conditions with several changes in 0.1×SSC and 0.1% SDS at 65°C.

Positive plaques were purified and inserts were subcloned into the pBluescript II KS(–) vector (Stratagene). Synthetic oligonucleotides were used for double stranded bi-directional sequencing by the dideoxynucleotide chain termination reaction (37).

**Northern blotting analysis**

Total RNA was purified from different rabbit tissues by the guanidinium thiocyanate/organic solvent method as described by Chomczynski and Sacchi (38). Samples of 15 μg purified total rabbit RNA were loaded onto a 1.4% agarose gel containing 1×MOPS and 2.2 M formaldehyde and run for 5 h at 80 V before the RNA was blotted onto a nylon membrane (Schleicher & Schuell) by an overnight capillary transfer procedure.

Probes recognizing eEF1A-2 were prepared by random labelling of a 269 bp EcoRI–NotI fragment specific for the 3′-UTR of eEF1A-2. An eEF1A-1-specific probe was obtained from an EcoRI/SstI digest of rabbit eEF1A-1 (36), giving rise to a 110 bp fragment specific for the 3′-UTR of eEF1A-1. Hybridization of the eEF1A-2-specific probe to RNA under low stringency washing conditions was used for normalization of the amount of loaded total RNA on the gel. The sizes of mRNAs recognized by the radioactive labelled probes was estimated by an RNA molecular weight marker (Omega).

Hybridization was performed overnight at 42°C in 5×SSC, 5× Denhardt’s, 0.1% SDS and 0.1 mg/ml total yeast RNA and the denatured probe. Washing at low stringency conditions was performed at 57°C in 0.1× SSC and 0.1% SDS. Washing under high stringency conditions was performed at 65°C with several buffer changes in 0.1× SSC and 0.1% SDS. Removal of the probe for rehybridization was carried out by boiling the membrane for 15 min in 0.1% SDS and complete removal of the probe was monitored by autoradiography.

**Amino acid sequencing**

Trypsin digestion, separation of the peptides and amino acid sequencing of rabbit eEF1A-2 were performed as described by Cavalli et al. (18). Briefly, 500 μg purified eEF1A-2 from rabbit muscle were digested at 37°C in a 1% (w/v) solution of trypsin (Boehringer Mannheim) in 50 mM Tris–HCl, pH 7.5, 150 mM NH₄Cl, 25% (w/v) glycerol, 0.1 mM EDTA and 10 mM β-mercaptoethanol. After 6 h more trypsin was added, to give a final concentration of 2%, and incubation was continued for 12 h at 37°C. The sample was then lyophilized and dissolved in 200 μl formic acid and 600 μl 0.05% trifluoroacetic acid (TFA). Separation was performed by HPLC using a C18 column and a 0.01% TFA/acetonitrile gradient.

Samples were characterized by the Case Western Reserve University Molecular Biology Core Facility using an Applied Biosystems Inc. Model 477 A protein sequencer with on-line PTH analysis. For several of the peptides a mixture was obtained, although the major signal represented ~60–70% of the sequence. However, for positions where a weak signal was observed, an amino acid from a minor sequence appeared to be of approximately equal intensity. In these cases the amino acid ambiguity in the sequence is indicated (see Fig. 1). Note that in all cases the appropriate amino acid, based upon the cDNA sequence, was one of the alternatives. The identification of mono-, di- or trimethylsine is based upon the elution position of known standards. Identification of the glycerylphosphorylethanolamine modification is based upon the appearance at the appropriate glutamic acid residue of a small peak (~5% of the expected size) identified as glutamine. This characteristic was noted for rabbit eEF1A-1 where this protein was chemically sequenced and the modification characterized by mass spectrometry (14). The eEF1A-2 peptides were not characterized by mass spectrometry.

**Poly(U)-directed polyphenylalanine synthesis**

Poly(U)-directed polyphenylalanine synthesis was performed according to Cavalli et al. (18). Briefly, 15 pmol eEF1A-1 or eEF1A-2 were added to 125 μl factor mix consisting of 100 mM KCl, 20 mM Tris–HCl, pH 7.5, 1.0 mM GTP, 2.1 mM phosphoethanolamine (PEP), 0.3 U pyruvate kinase, 1 mM DTT, 10 mM Mg(CH₃CO₂)₂, 0.5 μM A₆₀ salt-washed sucrose cushion ribosomes (rabbit reticulocyte ribosomes), 4.0 μg eEF2 (rabbit reticulocyte), 9.6 μg poly(U) RNA (Boehringer Mannheim) and 19 pmol [14C]Phe-tRNA (Escherichia coli; Sigma). The reaction mixtures were incubated at 30°C. After 2–15 min 20 μl aliquots were withdrawn and added to 1 ml cold trichloroacetic acid, followed by heating to 90°C for 15 min before filtration through nitrocellulose filters (Schleicher & Schuell).

**Nucleotide binding**

**GDP binding.** Aliquots of 1 μM reticulocyte eEF1A-1 or eEF1A-2 were incubated for up to 20 min at 20°C in a buffer containing 50 mM Tris–HCl, pH 7.6, 100 mM NH₄Cl, 10 mM MgCl₂, 1 mM DTT, 50 mM KCl, 10 μg/ml bovine serum albumin (BSA) and 50 μM [3H]GDP (sp. act. 700–1500 d.p.m./pmol).
Comparison of the amino acid sequences of rabbit eEF1A-2 and eEF1A-1. The chemically determined amino acid sequences of eEF1A-2 peptides are shown as well, above the sequence of eEF1A-2. Non-ordered peptide sequences obtained from the amino acid analysis are underlined. *, location of an apparent glycerylphosphorylethanolamine modification; #, site of trimethylation (see Materials and Methods); ¤, an ambiguity in the chemical sequence was found (see text).

The reaction mixtures were then placed on ice for 5 min. A 100 µl aliquot of the assay mixture was then filtered through nitrocellulose and free [3H]GDP was removed from the filters by washing three times with 2 ml wash buffer (10 mM Tris–HCl, pH 7.6, 10 mM MgCl₂, 10 mM KCl). The time needed to reach equilibrium was determined for both eEF1A-1 and eEF1A-2.

GTP binding. This was performed as described for GDP binding, but with the addition of 0.55 mM PEP and 0.06 mg/ml pyruvate kinase to the incubation buffer and with 50 µM [3H]GTP (sp. act. 1533 d.p.m./pmol) instead of [3H]GDP.

Dissociation rate constants for eEF1A-1·GDP and eEF1A-2·GDP

The binary complexes eEF1A-1·GDP and eEF1A-2·GDP were formed by incubating 0.15 µM of either isoform with 10 µM [3H]GDP (sp. act. 700–1500 d.p.m./pmol) for 15 min at 20°C in a buffer containing 50 mM Tris–HCl, pH 7.6, 100 mM NH₄Cl, 10 mM MgCl₂, 1 mM DTT, 50 mM KCl, 10 µg/ml BSA and 15% glycerol. The eEF1A-1·GDP complex mixture was then placed on ice for 5 min. The dissociation reaction was started by adding a 500-fold excess of unlabelled GDP. Aliquots of 100 µl were withdrawn at different time intervals and filtered through nitrocellulose filters (Schleicher & Schuell), washed twice and counted.

Intrinsic GTPase activity

eEF1A-1 or eEF1A-2 (1 µM) was incubated for 20 min at 20°C in a buffer containing 25 mM Tris–HCl, pH 7.8, 1 mM DTT, 10 mM MgCl₂, 100 mM KCl, 0.5 mM PEP and 0.06 mg/ml pyruvate kinase. The reaction mixtures were then held for 5 min at 30°C. The assay was started by adding [γ-32P]GTP (sp. act. 1533 d.p.m./pmol) to a final concentration of either 60, 90 or 120 µM. Samples of 20 µl were withdrawn at times from 0 to 110 min, stopped by addition of 1 vol ice-cold 1 M HClO₄ containing 3 mM KH₂PO₄ and extracted with 4 vol isopropyl acetate after addition of 3 vol 20 mM sodium molybdate. Scintillant was added to the extracted samples and these were counted.

RESULTS

Isolation and sequencing of the eEF1A-2 cDNA

In order to isolate the cDNA of eEF1A-2 from rabbits we screened 9 × 10⁵ recombinant plaques from a rabbit skeletal muscle 5′STRECH PLUS λgt11 cDNA library. The combination of sequence information from two different clones containing overlapping cDNA sequence resulted in determination of the complete coding sequence of the eEF1A-2 cDNA. Clone 20 displayed a 1713 bp long cDNA sequence containing 1380 bp coding sequence plus 333 bp of 3′-UTR and clone B contained a 998 bp stretch of eEF1A-2 cDNA sequence plus 24 bp of 5′-UTR.

The reaction mixtures were then placed on ice for 5 min. A 100 µl aliquot of the assay mixture was then filtered through nitrocellulose and free [3H]GDP was removed from the filters by washing three times with 2 ml wash buffer (10 mM Tris–HCl, pH 7.6, 10 mM MgCl₂, 10 mM NH₄Cl). The time needed to reach equilibrium was determined for both eEF1A-1 and eEF1A-2.
Figure 2. Northern blot analysis of the expression pattern of eEF1A-1 and eEF1A-2. (A–C) Aliquots of 15 µg/lane total RNA from eight different rabbit tissues: aorta, lung, brain, spleen, kidney, skeletal muscle, heart and liver. The cDNA probes were the 3′-UTR of eEF1A-2 (A) and the 3′-UTR of eEF1A-1 (B). Hybridization of the eEF1A-2 probe to 28S rRNA under low stringency conditions was used for normalization of loaded amount of RNA (C). The eEF1A-2 blot was exposed for 24 h, the eEF1A-1 blot for 18 h.

However, the presence of a signal indicates that terminally differentiated smooth muscle cells also express eEF1A-2. No signal for eEF1A-2 was observed in the other tissues examined. The eEF1A-1-specific probe hybridized to eEF1A-1 mRNA of ∼1.8 kb in total RNA isolated from liver, spleen, kidney, lung, aorta, brain and heart. No hybridization was observed in the lane of skeletal muscle, not even after a 4-fold extended exposure period (data not shown).

Post-translational modifications

eEF1A-1 is a protein which is highly conserved in eukaryotes. Surprisingly, the post-translational modifications of this protein are not well conserved. The rabbit eEF1A-1 isoform has been chemically sequenced and its post-translational modifications determined (14). To investigate the relationship between the two rabbit isoforms in this regard we decided to carry out an amino acid analysis and a partial chemical sequence determination of purified eEF1A-2 from rabbit skeletal muscle. Partial chemical sequencing showed the apparent presence of both of the glyceryl-phosphorylethanolamine modifications found in rabbit eEF1A-1 at the same positions in eEF1A-2 (Glu301 and Glu374) (Fig. 1). We also found Lys55 and Lys165 to be methylated. Both of these lysine residues are dimethylated in eEF1A-1 but trimethylated in eEF1A-2. Amino acid sequencing revealed that the digested peptides originated from eEF1A-2 (Fig. 1) and there was no trace of peptides originating from eEF1A-1. This was consistent with the results obtained from the Northern blot (Fig. 2), indicating that eEF1A-1 cannot be detected in skeletal muscle tissue. Two of the chemically sequenced peptides gave ambiguous results at positions 184 (Pro/Asn), 188 (Pro/Val), 194 (Gly/Ser) and 222 (Asp/Asn). As the amino acid residues in these positions deduced from the eEF1A-2 cDNA are Asn, Val, Ser and Asn respectively, we take these residues to be the correct ones.

Poly(U)-directed polyphenylalanine synthesis

To compare functional characteristics of the two isoforms eEF1A-2 and eEF1A-1 were purified using identical purification schemes (Kristensen, in preparation). This was done in order to avoid the danger of differences in amounts and identities of impurities (such as nucleotide exchange factors) brought about by the use of different purification procedures, which might confuse interpretation of the assays. In agreement with the high degree of identity between the two isoforms, eEF1A-1 and eEF1A-2 showed similar activity in an in vitro translation assay (Fig. 3). In view of the elements of uncertainty in the polyphenylalanine assay, we do not consider any difference to be significant.

Nucleotide binding and dissociation rate constants for eEF1A-1·GDP and eEF1A-2·GDP

Some differences between the relative affinities for GDP and GTP of the two isoforms were observed. We compared the ability of the two eEF1A proteins to bind guanine nucleotides, expressed as a ratio between the quantity of bound GDP and that of bound GTP when both isoforms are present in equal concentrations. The ratio [eEF1A·GDP]/[eEF1A·GTP] was found to be 0.82 ± 0.01 for eEF1A-1 and 1.50 ± 0.17 for eEF1A-2. The ratio between these was on average 1.8.

These data show that eEF1A-2 binds GDP more strongly than GTP, whereas the opposite is true for eEF1A-1. The data obtained are based on four individual assays where two different preparations of each isoform were used. Each assay was carried out with 15%
Acid substitution (Ser331→eEF1A-2) peptide sequences (31,33) reveals only a single amino acid sequences. Alignment with the two identical rat and mouse cDNAs and that the two proteins display 100% identical amino have taken place during evolution in the coding region of the human eEF1A-2 (29) corroborates that only wobble substitutions with skeletal muscle and heart.

To examine more closely the nucleotide binding profile of the two isoforms, we measured the rate of dissociation of prebound GDP from the two proteins. The dissociation of GDP from eEF1A-1 and eEF1A-2 was followed by diluting [3H]GDP with a 500-fold excess of unlabelled guanine nucleotide. Under these conditions, rebinding of [3H]GDP is negligible, thus allowing its dissociation to be followed by the filtration assay. This assay was performed with 15% glycerol present, in order to stabilize the eEF1A. Theoretically, the time-dependent decrease in the amount of eEF1A·[3H]GDP follows first-order kinetics. The dissociation rates for the two eEF1A proteins differ by a factor of 7.3, as shown in Figure 4. k_d for eEF1A-2 was found to be (0.45 ± 0.04) × 10^{-3} s^{-1}, whereas k_d for eEF1A-1 was (3.29 ± 0.43) × 10^{-3} s^{-1}.

**Intrinsic GTPase activity**

The intrinsic rates of GTP hydrolysis for eEF1A-1 and eEF1A-2 were measured under saturating substrate conditions to avoid side-effects caused by differences in guanine nucleotide affinities. A titration of GTP was performed beforehand to make sure that saturating conditions were obtained. As shown in Figure 5, the GTPase activities of eEF1A-1 and eEF1A-2 do not differ significantly. The hydrolysis rates were found to be 2.9 × 10^{-3} s^{-1} and 1.9 × 10^{-3} s^{-1} respectively.

**DISCUSSION**

Comparison of the deduced coding sequences of rabbit and human eEF1A-2 (29) corroborates that only wobble substitutions have taken place during evolution in the coding region of the cDNAs and that the two proteins display 100% identical amino acid sequences. Alignment with the two identical rat and mouse eEF1A-2 peptide sequences (31,33) reveals only a single amino acid substitution (Ser331→Ala). The nucleotide sequences of rabbit eEF1A-1 (36) and eEF1A-2 are 73% identical while the amino acid sequences are 92.3% identical. These results indicate that eEF1A-2 from different mammalian species are more related to each other than are the two isoforms of the same species.

Northern analysis detected high levels of eEF1A-2 mRNA in total RNA purified from skeletal muscle, heart, and brain, as previously described for rats and humans (29,39). Furthermore, expression of eEF1A-2 was observed in RNA isolated from aorta, although the signal from this tissue is relatively faint (Fig. 2). Aorta is a complex tissue. Expression of eEF1A-2 in aorta might originate from the smooth muscle part of this tissue, which will limit eEF1A-2 expression to tissue composed of terminally differentiated brain and muscle cells. This will have to be verified by in situ studies. eEF1A-1 expression is observed in eEF1A-2-negative tissues such as liver, kidney, spleen, and lung and in eEF1A-2-positive tissues such as aorta, brain, and, to a lesser extent, in heart. eEF1A-1 was barely detectable in skeletal muscle, which is in agreement with previous observations for humans (29). Furthermore, this is substantiated by our amino acid sequencing analysis (Fig. 1). Experiments have shown that eEF1A-1 expression is down-regulated in myogenic, cardiomyogenic and certain neurogenic cells only when eEF1A-2 mRNA has accumulated late in the terminal differentiation process (34,35). In the brain only terminally differentiated neurons express eEF1A-2, while proliferating neurogenic cells only express eEF1A-1 (35). This leads to the suggestion that the presence of eEF1A-1 mRNA in heart, brain, and aorta mainly derive from remnants of eEF1A-2-negative cells in the tissue sample. This is corroborated by the fact that eEF1A-1 expression is higher in the heart than in the two more heterogeneous tissues, brain and aorta, as compared with skeletal muscle and heart. In situ hybridization should clarify this situation.

There are several possible reasons why two isoforms of eEF1A exist. One reason could be that the two isoforms have slightly different but overlapping functions. This could possibly be reflected in an altered pattern of post-translational modification...
of eEF1A-2 compared with eEF1A-1. To examine this we partially sequenced eEF1A-2 from rabbit muscle. The post-translational modifications of rabbit reticulocyte eEF1A-1 have been described by Dever et al. (14). At least four of the seven post-translationally modified amino acids in rabbit reticulocyte eEF1A-1 are also modified in eEF1A-2. Both of the two glycerclylphosphorylethanolamine modifications found in rabbit reticulocyte eEF1A-1 were found in eEF1A-2. This suggests that this modification plays an important role in muscle as well as in other tissues. Two of the methylated lysines in eEF1A-1 (Lys55 and Lys165) were also modified in eEF1A-2, but instead of the dimethylation found in eEF1A-1, these two sites in eEF1A-2 were trimethylated. The three additionally methylated lysines in eEF1A-2 were not examined in eEF1A-2. No function has yet been ascribed to these modifications, but if the speculation by Merrick et al. (20) is correct that the trimethylated lysine in eEF1A-1 could serve as a trimethyl donor in the synthesis of carnitine, then it seems reasonable that eEF1A-2 would be a better donor by having more trimethylated lysines than eEF1A-1, not least because muscles are fatty acid consuming tissues and therefore should be expected to need more carnitine.

For a further characterization of eEF1A-2 we compared the translational elongation activity of eEF1A-1 and eEF1A-2 in a poly(U) assay. The specific activities of eEF1A-1 and eEF1A-2 were similar (Fig. 3), as expected on the basis of the high degree of identity. Likewise, yeast eEF1A also has the same specific activity in the poly(U) assay, although it shares only 81% identity with rabbit reticulocyte eEF1A-1, does not contain the glycerclylphosphorylethanolamine modifications and has only a few of the lysine methylations found in rabbit reticulocyte eEF1A-1.

Unexpectedly, eEF1A-2 does not bind GDP and GTP with the same relative affinity as eEF1A-1. eEF1A-1 was reported to bind GDP and GTP with about equal affinity (40). In our hands eEF1A-2 showed a stronger relative affinity for GDP than did eEF1A-1. A similar nucleotide preference pattern is observed for E.coli EF1A (formerly EF-Tu) (41). The rate constant for dissociation of GDP for rabbit muscle eEF1A-2 was found to be $4.5 \times 10^{-9}/s$, whereas this constant for rabbit reticulocyte eEF1A-1 is $3.3 \times 10^{-5}/s$. In pig liver (42) the GDP dissociation rate changed as the concentration of NH$_4$Cl varied. $k_1$ (GDP) changed from $2.4 \times 10^{-3}$ to $22 \times 10^{-3}/s$ when the concentration of NH$_4$Cl was increased from 20 to 520 mM. eEF1A from calf brain did not show this dependence on NH$_4$Cl concentration (43). Furthermore, a lower $k_1$ of $9 \times 10^{-7}/s$ was found, resembling the $k_1$ value determined for eEF1A-2 in this report. This is not unreasonable, as both eEF1A-2 and eEF1A-1 are expressed in brain (Fig. 2). Therefore, it is likely that Crecet and co-workers actually worked with a mixture of the two proteins (43). Even though the values of $k_1$ (GDP) differ in the two rabbit eEF1A$_1$s, the $K_d$ value for binding of GDP may still be similar for the two isoforms. This will have to be examined in future studies. A possible prospect of this higher affinity of eEF1A-2 for GDP than for GTP might be the need for it to influence actin binding, as GTP inhibits binding of eEF1A to G-actin, whereas GDP does not have this effect (44).

In order to explain why there is a difference in the affinity of eEF1A-1 and eEF1A-2 for guanine nucleotides we examined some of the amino acid differences between eEF1A-1 and eEF1A-2. Some of the differences are conservative substitutions and would not be expected to cause any change in nucleotide binding/dissociation. The amino acid change that is most likely to influence nucleotide binding is found at position 197. In eEF1A-1 this residue is an asparagine, whereas in eEF1A-2 it is a histidine. The crystal structure of EF1A GDPNP from Thermus aquaticus (45), used as a model for eEF1A structure, indicates that this histidine residue is very close to the guanine nucleotide. The serine residue at position 194 makes a hydrogen bond to the guanine ring. The amino acid at position 196 in eEF1A is a tryptophan, which stacks onto the guanine base by hydrophobic interaction. It seems likely that the change of residue 197, which lies in an area that is important for nucleotide binding, could explain the different nucleotide dissociation rates of the two proteins. In both D.melanogaster eEF1A isoforms and in two of the X.laevis gene products residue 197 is also a histidine. At least one case Xenopus eEF1A0 has a higher GDP association rate constant than that found for eEF1A-1 from rabbit reticulocytes (46). In contrast, there is no obvious explanation for the differing guanine nucleotide preference.

The intrinsic rates of GTP hydrolysis were about identical for eEF1A-1 and eEF1A-2, which implies that the differences between their sequences have no significant influence on their GTPase activity. (The above-mentioned difference in rate constants for GDP dissociation is assumed not to influence measurement of GTP hydrolysis, i.e. release of GDP from eEF1A was not rate limiting; the dissociation rate constants were measured at 0°C, whereas GTP hydrolysis is measured at 30°C and it is easily shown that the dissociation rates at 30°C can be expected to exceed the hydrolysis rates observed at this temperature.) The amino acids proposed to play a role in the GTPase activity of EF1A (formerly EF-Tu), His84 (47), Asp86 and Arg58 (48), are also conserved in both eEF1A-1 and eEF1A-2, thus providing a structural explanation for the observed conservation of intrinsic GTP hydrolysis rate.

At present it is not clear why there are two isoforms of eEF1A. Our results would indicate that in vitro eEF1A-2 is functionally equivalent to eEF1A-1 and thus should provide the eEF1A function necessary for protein synthesis in terminally differentiated muscle and brain cells. The only biological difference is the difference in the off-rate for GDP, which would suggest a greater dependence on the nucleotide exchange factor eEF1B for eEF1A-2 than for eEF1A-1. This difference in affinity for GDP may play a role either in protein synthesis (although this is not apparent from the in vitro studies) or in one of the many non-translational roles suggested for eEF1A (see Introduction). Although the data are not available for eEF1A, it is anticipated that the large and dramatic change in conformation that occurs with bacterial EF1A upon hydrolysis of GTP will also occur with eEF1A. The increase in the time possible for eEF1A to exist as an eEF1A GDP complex may influence the ability of eEF1A to participate in non-translational events in the cell. Of course, it is possible that development/evolution of the muscle and neural cell lineages has led to a tissue-specific isoform of eEF1A, as has happened with many metabolic enzymes. Consistent with this possibility is the recent cloning of an isoform of eEF1B (formerly EF-1β) which displays a similar expression pattern to that of eEF1A-2 (49). However, to date there have been very few other ‘muscle-specific’ isoforms of the 40 or so polypeptides that constitute the initiation, elongation and termination factors for eukaryotic protein synthesis.
Clearly, additional study is required to address the issue of why there are muscle-specific isoforms of some of the translation factors.

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