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**Evolutionary importance of translation elongation factor eEF1A variant switching:  
eEF1A1 down-regulation in muscle is conserved in *Xenopus* but is controlled at a post-  
transcriptional level**

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Abstract

Translation elongation isoform eEF1A1 has a pivotal role in protein synthesis and is almost ubiquitously expressed. In mice and rats that transcription of the gene encoding eEF1A1 is downregulated to undetectable levels in muscle after weaning; eEF1A1 is then replaced by a separately encoded but closely related isoform eEF1A2, which has only previously been described in mammals. We now show that not only is eEF1A2 conserved in non-mammalian vertebrate species, but the down-regulation of eEF1A1 protein in muscle is preserved in *Xenopus*, with the protein being undetectable by adulthood. Interestingly, though, this down-regulation is controlled post-transcriptionally, and levels of full-length eEF1A1 mRNA remain similar to those of eEF1A2. The switching off of eEF1A1 in muscle is therefore sufficiently important to have evolved through the use of repression operating at different levels in different species. The 3'UTR of eEF1A1 is highly conserved and contains predicted binding sites for several miRNAs, suggesting a possible method for controlling of expression. We suggest that isoform switching may have evolved because of a need for certain cell types to modify the well-established non-canonical functions of eEF1A1.

Key words

Translation elongation, eEF1A1, eEF1A2, *xenopus*

## Introduction

The translation elongation factor eEF1A is essential for protein synthesis. It delivers aminoacyl tRNAs to the A site of the ribosome in a GTP-dependent fashion. In mammals, there are two isoforms, eEF1A1 and eEF1A2, encoded by genes on different chromosomes; they are 92% identical and 98% similar at the amino acid level [1]. Both proteins are highly conserved, with only one amino acid difference between human and mouse eEF1A2 proteins [2]. The two proteins show similar translation activities, but have different relative affinities for GTP and GDP, with markedly different dissociation rate constants [3]. eEF1A1 has been implicated in a number of other “moonlighting” or non-canonical functions (reviewed in [4]), including actin binding and bundling [5], apoptosis [6] and tumorigenesis [7]. eEF1A2 is expressed at the RNA level in brain, heart and skeletal muscle in rats [8], rabbits [3] and humans [1]. Similarly, at the protein level, eEF1A2 expression is seen only in the brain, heart and skeletal muscle of rats, humans and mice [9], with minor sites of expression in pancreatic islets and colon crypts [10]. Deletion of the *Eef1a2* locus in mice results in the mutant wasted (*wst*) [11]. Mice homozygous for this mutation show neuromuscular defects, including a loss of muscle mass and neuropathological changes [12]. In humans, eEF1A2 has been shown to have oncogenic properties and has been implicated in ovarian and breast cancer [13, 14].

Although eEF1A1 is almost ubiquitously expressed, it is downregulated in those tissues in which eEF1A2 is expressed [15, 16], an effect which is seen most dramatically in muscle. In mouse muscle, levels of eEF1A2 mRNA increase after birth, whilst levels of eEF1A1 mRNA and protein decrease to undetectable levels by 25 days [9, 11] so that in this tissue at least, a complete switch occurs between eEF1A1 and eEF1A2. A similar situation is seen in rats,

with dramatic down-regulation of eEF1A1 mRNA in postnatal brain [16], muscle and heart [15].

There is no direct experimental evidence for an eEF1A2 orthologue in non-mammalian vertebrates. Indeed, in zebrafish and chicken it has been stated that only one eEF1A gene is present [17, 18]. In the frog *Xenopus laevis*, two major isoforms of eEF1A have been described. eEF1A-O is expressed in germ cells and early embryos [19, 20]. The somatically expressed eEF1A-S (the presumed orthologue of eEF1A1) was first described by Krieg *et al* [21]. It is expressed in adult liver [20] and in the germ cells of the gonad prior to metamorphosis [19]. At metamorphosis there is a switch from the somatic to the oocyte-specific form in the germ cells. At the amino acid level, the eEF1A-O and eEF1A-S proteins are 91% identical. eEF1A-S is said to be the only somatic form of eEF1A in *Xenopus* [20]. In spite of this, bioinformatic analysis suggested the presence of eEF1A2 orthologues in other vertebrates, based on their sequence similarity with mammalian eEF1A2 and potential tissue distribution. We now present the analysis of eEF1A2 in *Xenopus*; as in mammals, expression is limited to neurons and muscle and eEF1A1 (eEF1A-S) protein is absent from adult muscle. Importantly, and in contrast to the situation in mammals, eEF1A1 mRNA is still found in adult *Xenopus* muscle, so the tissue-specific down-regulation of eEF1A1 in postnatal muscle occurs post-transcriptionally whereas in mammals the same switch is regulated at the transcriptional level. The 3'UTR of eEF1A1 in both *X.tropicalis* and *X.laevis* contains highly conserved recognition sites for miR-133, a microRNA strongly implicated in muscle development [22], and we examined this miRNA as a candidate for control of eEF1A1 expression in *Xenopus*.

## Materials and Methods

### Frogs

Adult female *X. laevis* were bred at the University of Edinburgh and the MRC Human Genetics Unit, Edinburgh. They were euthanized using a Schedule 1 method. Stage 17, 25, 35 and 42 embryos were also obtained from the University of Edinburgh, and adult *X. tropicalis* muscle was obtained from the National Institute of Medical Research, Mill Hill, London.

### Bioinformatics

The mouse eEF1A2 mRNA sequence was used to search nucleotide databases at NCBI using BLAST. ClustalW analysis was performed using EBI services (<http://www.ebi.ac.uk/clustalw/index.html>) followed by Boxshade analysis ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)).

### mRNA RT-PCR

The RNeasy<sup>®</sup> Midi kit (Qiagen) was used to extract RNA from 150mg xenopus tissue, as per the manufacturer's instructions. The recommended modifications for extracting RNA from muscle were observed. RNA was reverse-transcribed using the Retroscript kit (Ambion). eEF1A2 PCR primers used in figure 2 were as follows: forward: 5'-GAAGGAAGGCAATGCAAAT-3'; reverse: 5'-TGCAGTATGGCAGTCAATA-3'. Primers used to amplify *X. tropicalis* eEF1A2 were: forward; 5'-TCGACAAAAGGACGATAGAG-3'; reverse: 5'-ATTGATITCCCACAATGAGGC-3'. Those used for *X. tropicalis* eEF1A1 were: forward; 5'-TCGACAAGAGAACCATCGAA-3'; reverse; 5'-GTTAACACCAACGATCAGT.

PCR primers for eEF1A1 (Figure 4) were forward: 5'-TAAAGAGGGATCTGGCAGC-3'; reverse: 5'-AGCAGTGTGACAATCCAAC-3'. Each reaction contained 5mM forward and reverse primers, 0.05mM dNTPs, 1.485M betaine (Sigma) and 1µl of either the reverse-transcribed or no-RT control RNA mixes, in a total volume of 50µl. Conditions were 95<sup>0</sup>C for 3 minutes, followed by 40 cycles for eEF1A2 and 30 cycles for eEF1A1 of 94<sup>0</sup>C for 30 seconds, 48<sup>0</sup>C for 30 seconds and 72<sup>0</sup>C for 30 seconds, followed by 10 minutes at 72<sup>0</sup>C, on an MJ Gradient Cycler. Full-length eEF1A1 was generated using the Expand High-Fidelity Plus kit (Roche), according to the manufacturer's instructions, with an annealing temperature of 50<sup>0</sup>C on an MJ Gradient Cycler. Primers used were: forward: 5'-AATCCACAATGGGAAAGGAA-3'; reverse: 5'-TTGGTCAAGTTGCCTCCCAC-3'.

### mRNA Real-time PCR

Real-time PCR analysis was performed on a Bio-Rad MyIQ using the following primers: eEF1A2 forward: 5'-GAAGGAAGGCAATGCAAAT-3'; reverse: 5'-GCTTTAGAATGCCGGTC-3'; eEF1A1: forward: 5'-AGGCTCCTTCAAGTATGCCT-3'; reverse: 5'-ATGCTCACGGGTTTGTCCAT-3'. *Rpl8* was used a reference gene, with the following primers: forward: 5'-TAAGCTTCGGGCTATCGACT-3'; reverse: 5'-GTGCCAACAGGGAGTACATT-3'. Standard curves were constructed, and the data analysed using a modified Pfaffl method and the MyIQ software.

### Sequencing

PCR products were cleaned up using ExoSapIT (USB), sequenced using Big Dye (ABgene) on an MJ gradient cycler, precipitated using ethanol and EDTA and analysed on an ABI 3100 capillary sequence analyser. Primers used were as for PCR, above.

### Western blotting

Tissues were homogenised in 0.32M sucrose with Complete Antiprotease Cocktail (Roche) and quantified using the DC protein quantification system (BioRad); 15 µg total protein extract was electrophoresed on a 10% SDS-polyacrylamide gel. This was then blotted onto Hybond-P membrane (Amersham Pharmacia Biotech) for 1 hour at 15V. Membranes were blocked overnight in blocking buffer (25% v/v Tween 20 and 5% w/v fat free powdered milk in PBS), incubated in primary antibody (1:200 in blocking buffer for eEF1A2-2; 1:500 for *A. salina* antibody; 1 in 1000 for GAPDH) for 1.5-2 hours, washed four times in PBS-T and incubated with secondary antibody for 1 hour at 1:500 dilution (HRP-conjugated anti-sheep, -rabbit or -mouse; Dako). Membranes were again washed in PBS and detected using enhanced chemiluminescence (ECL; Amersham Biosciences) according to the manufacturer's instructions. The eEF1A2-2 antibody was generated in-house (13), the *A. salina* antibody was previously described by Sanders *et al.* [23], and the GAPDH antibody was from Millipore.

### Immunohistochemistry

Tissues were fixed in 4% (w/v) paraformaldehyde for approximately 2 hours, paraffin-embedded and 4µm sections cut. Slides were rehydrated through a graded series of ethanols, subjected to antigen retrieval by microwaving at full power for 15 minutes in 0.01M citric acid (pH 6.0), cooled, washed in H<sub>2</sub>O and then treated in 3% v/v hydrogen peroxide for 10 minutes. They were again washed in H<sub>2</sub>O, washed in PBS for 5 minutes, and blocked in rabbit serum for 10 minutes (1:5 in PBS; Sigma). They were then incubated in anti-eEF1A2 antibody (1:100 in PBS), washed twice in PBS, incubated for 30 minutes in biotinylated anti-sheep antibody (1:500 in PBS; Vector), and finally incubated in StreptABC (Dako) for 30 minutes. They were again washed in PBS, and then incubated in diaminobenzidine (DAB;

Vector) for approximately two minutes. Slides were counterstained with haematoxylin (Surgipath), dehydrated and mounted in pterex (CellPath).

## Results

Initially we used BLAST to identify *X. laevis* ESTs which showed higher similarity to mouse eEF1A2 than to any other mammalian elongation factor, or any of the known *Xenopus* elongation factors. Subsequently, sequences have been deposited in NCBI for full-length cDNAs corresponding to *X.laevis* eEF1A2 (e.g. BC054279). ClustalW analysis of eEF1A1 and eEF1A2 protein sequences from *X. laevis*, *X. tropicalis* and mouse show two distinct clusters of sequences; those corresponding to eEF1A1, and those corresponding to eEF1A2. Mouse eEF1A1 and mouse eEF1A2 differ at 34 positions out of 463; of these differences, 22 are wholly conserved such that eEF1A2 from all three species has the same amino acid, as does eEF1A1 at the equivalent position (figure 1).

Using RT-PCR on RNA from a range of tissues from adult *X. laevis*, we analysed the expression pattern of eEF1A1 and eEF1A2 at the mRNA level. eEF1A2 was found in the tissues that would be predicted from analysis of the gene in mammals; that is, brain, muscle and heart (figure 2, panel A), suggesting that the putative *X. laevis* eEF1A2 is indeed a genuine orthologue of mammalian eEF1A2. eEF1A1 mRNA, unexpectedly, was present in all tissues examined, including adult muscle (figure 2 panel A). In order to confirm the specificity of the PCR, the products were sequenced. The sequence obtained was identical to the published eEF1A1 (eEF1A-S) sequence. Further PCR primers were used to amplify all regions of the gene; all were sequenced, and all corresponded to the published sequence (data not shown).

We then went on to analyse expression of the two genes at the protein level. Using an anti-eEF1A2 antibody we have generated (eEF1A2-2; [10, 14]), we show that eEF1A2 protein is

expressed in the same tissues as eEF1A2 mRNA (figure 2 panel B), that is, central nervous system (brain, spinal cord, optic ganglion) and muscle. No eEF1A2 was seen in liver, gall bladder, lung, kidney or spleen. We used a further antibody, eEF1A2-1, to investigate which cell-types express eEF1A2 using immunohistochemistry (figure 2 panel C). In the brain and spinal cord, expression was restricted to large neurons and localised to the cytoplasm, as seen in mammals [10]; strong staining was also seen in cardiac muscle.

We then analysed expression by Western blotting. The samples shown in figure 2 were run simultaneously on duplicate gels, and the top panel Western shows eEF1A1 expression. We used an antibody raised against eEF1A from *Artemia salina* and found that it specifically recognised *X. laevis* eEF1A1 based on its reciprocal expression pattern with eEF1A2. eEF1A1 was expressed in all tissues examined, except adult muscle and optic ganglion (figure 2 panel B). All those samples that were negative for eEF1A1 were positive for eEF1A2, showing that the protein was present, with the exception of lung which came up negative for both eEF1A1 and eEF1A2. As in mammals, both eEF1A1 and eEF1A2 are found in whole extracts of brain and spinal cord. Thus, in *X. laevis*, the expression pattern of eEF1A1 at the protein level is the same as that seen in mammals, notably the absence of expression in adult muscle, in contrast to the results found at the RNA level.

To confirm that a full-length eEF1A1 transcript is generated in muscle long-range PCR was used to amplify the complete 1.6 kb transcript (figure 3 panel A). This too was sequenced, and again the sequence was identical to that of eEF1A1. We then wanted to rule out the possibility that the discrepancy between the RNA and protein results were due to obvious sensitivity differences between Western blotting and RT-PCR. Real-time PCR analysis was performed on cDNA generated from *X. laevis* liver and muscle. Whilst levels of eEF1A2

were undetectable in liver, levels of eEF1A1 and eEF1A2 mRNA were similar in muscle (figure 3 panel B), showing that if the transcript in muscle was being translated into a stable protein it would have been easily detectable by Western blotting.

As *X. laevis* are allotetraploid, any analysis could be complicated by the presence of paralogues, so we went on to establish whether eEF1A1 expression could be detected in muscle tissue from the diploid frog *X. tropicalis*. In adult *X. tropicalis* muscle, both eEF1A1 and eEF1A2 mRNA are present (figure 3 panel C). However, as in *X. laevis*, at the protein level only eEF1A2 is present in muscle (figure 3 panel D). The post-transcriptional repression of eEF1A1 in muscle is therefore conserved between *X. laevis* and *X. tropicalis*.

The 3'UTR of eEF1A1 is highly conserved throughout vertebrate evolution, and contains a predicted binding site for microRNA miR-133, which is crucial for muscle development. We therefore sought to establish whether the lack of eEF1A1 protein in muscle could be due to the actions of miR-133, potentially acting via translational repression. Since transgenesis was not possible, we used two *Xenopus* cell lines, XTC-2 [24] and A6 kidney epithelial cells, both of which we found to express high levels of eEF1A1 but only low levels of miR-133. Transfection of these cell lines with a miR-133 precursor led to many thousand fold upregulation of expression of miR-133 in comparison with cells transfected with a negative control miRNA, but had no appreciable effect on levels of eEF1A1 mRNA or protein when compared with cells transfected with a negative control miRNA at multiple time-points (data not shown).

## Discussion

We have demonstrated the presence of an orthologue of eEF1A2 (previously only described in mammals) in two non-mammalian vertebrates, *X. laevis* and *X. tropicalis*. As in mammals, expression of this eEF1A2 orthologue is confined to the central nervous system and muscle; within tissue from brain and spinal cord eEF1A2 is localised solely to neurons, again reflecting precisely the pattern seen in mammals [9, 10]. We have also demonstrated the absence of detectable eEF1A1 protein in adult muscle of *Xenopus*, again as seen in mammals. Unexpectedly though, eEF1A1 mRNA is still present in frog muscle, suggesting that downregulation of eEF1A1 has evolved through the use of a completely different mechanism from that in mammals. This suggests that eEF1A isoform switching in muscle is of critical importance for postnatal development. In mammals, regulation is almost entirely transcriptional. eEF1A1 mRNA is not detectable in adult mouse muscle by RT-PCR [11], or in rabbit muscle by Northern analysis [3]. In rat muscle, eEF1A1 is barely detectable by 30 days using RNase protection and Northern analysis [15]. By contrast, in both *X.laevis* and *X. tropicalis*, eEF1A1 mRNA is still present in abundance. Indeed the level of eEF1A1 mRNA in muscle is the same as that seen for eEF1A2 in muscle as measured by qPCR. The post-transcriptional regulatory mechanism seen in *Xenopus* species has clearly been quite strongly conserved, since estimates for the time of the *X.tropicalis/laevis* divergence range up to 110 mya. The mechanism by which eEF1A1 is regulated at the post-transcriptional level in *Xenopus* adult muscle is as yet unknown, and precise determination of the mechanism would be technically challenging, requiring transgenesis experiments followed by assays in fully developed animals.

One possible explanation is that the highly conserved 3'UTR of eEF1A1, which shows no similarity with that of eEF1A2, is targeted by muscle-specific miRNAs. An attractive candidate was miR-133, but expression of precursor miR-133 resulting in the upregulation of both miR-133a and 133b in *Xenopus* cell lines failed significantly to affect the level of eEF1A1 mRNA in these cells. It is still conceivable that this miRNA is involved in the control of eEF1A1 expression in mature *Xenopus* muscle, but this would be harder to evaluate.

If the lack of expression was mediated at the level of translational repression then a candidate element would be the terminal oligopyrimidine (5'TOP) sequence which has been shown to be present in the 5'UTR of *X.laevis* eEF1A1 mRNA [25]. Control through the 5'TOP sequence in muscle might be expected to result in down-regulation of all 5'TOP-containing transcripts; this would presumably not occur in normal muscle since transcripts encoding most ribosomal components contain 5'TOPs. The transcriptional start site for *xenopus* eEF1A2 has not been determined, but there is no indication of anything more than a very short oligopyrimidine tract in the published cDNA sequence. An alternative mechanism would be the binding of a muscle-specific RNA-binding protein to the eEF1A1 transcript, resulting in suppression of translation. An attractive candidate for such a binding protein is the fragile X-related protein FXR1. It has been shown that eEF1A1 mRNA from *Xenopus* binds to the FMR1 protein, and that this binding is mediated through the RGG and KH2 domains of the protein [26]. FMR1, however, is widely expressed and is therefore unlikely to be mediating a muscle-specific down-regulation of eEF1A1. FXR1 on the other hand shares both the RGG and KH2 domains with FMR1 and is highly expressed in *X. tropicalis* muscle [27]; indeed, its expression pattern is very reminiscent of that of eEF1A2, as would be expected of a protein involved in eEF1A1 repression.

We hypothesise that frog muscle, with the growth and death programs that have to be executed during metamorphosis, has a need for a rapid-response mode of control of eEF1A1 expression, and that this is the reason for a post-transcriptional control mechanism operating in *Xenopus* species. The fact that eEF1A1 down-regulation during muscle differentiation occurs widely in vertebrates, and yet is controlled through completely different mechanisms in different species, suggests that eEF1A type switching is crucial for certain cell types. Although it is possible that the different properties of eEF1A1 and eEF1A2 relate solely to their activity in protein synthesis, it seems likely that the requirement for eEF1A-type switching relates to the moonlighting roles of eEF1A1 such as cytoskeletal remodelling, and the need for muscle and neuronal cells to avoid or to modify these moonlighting properties. The observation that eEF1A2 is expressed with such a restricted tissue distribution in such diverse species suggests that there may be a real need to avoid its expression in a wider context, tying in with the identification of eEF1A2 as a potential oncogene.

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*Xenopus tropicalis* eEF1A2 1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVL 60  
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*Xenopus laevis* eEF1A2 1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVL 60  
*Xenopus tropicalis* eEF1A1 1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVL 60  
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*Mus musculus* eEF1A1 1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVL 60

*Xenopus tropicalis* eEF1A2 61 DKLKAERERGITIDISLWKFETNKYYTTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGV 120  
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*Mus musculus* eEF1A1 121 GEFEAGISKNGQTRHALLAYTLGVKQLIVGNKMDSTPEPPYSCKRYDEIVKEVSTIYIKK 180

*Xenopus tropicalis* eEF1A2 181 IGYNEATVPEFVPISGWHDGNMLEPSNMPFWKGWKVERKEGNANGVLEALDITLPPSR 240  
*Mus musculus* eEF1A2 181 IGYNEATVPEFVPISGWHDGNMLEPSNMPFWKGWKVERKEGNANGVLEALDITLPPSR 240  
*Xenopus laevis* eEF1A2 181 IGYNEATVPEFVPISGWHDGNMLEPSNMPFWKGWKVERKEGNANGVLEALDITLPPSR 240  
*Xenopus tropicalis* eEF1A1 181 IGYNEATVPEFVPISGWHDGNMLEPSNMPFWKGWKSRKEGSSSGTLLLEALDITLPPSR 240  
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*Xenopus tropicalis* eEF1A2 241 PTDKPLRLPLQDVYKIGGIGTVPVGRVETGLKPGMVVTFAPVNTTEVKSVMHHEALS 300  
*Mus musculus* eEF1A2 241 PTDKPLRLPLQDVYKIGGIGTVPVGRVETGLKPGMVVTFAPVNTTEVKSVMHHEALS 300  
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*Mus musculus* eEF1A1 241 PTDKPLRLPLQDVYKIGGIGTVPVGRVETGLKPGMVVTFAPVNTTEVKSVMHHEALS 300

*Xenopus tropicalis* eEF1A2 301 EALPGDNVGFNVKNSVKDTRRGNVCGDSKADPEEAAAGFTAQVILLNHPPQISAGYSPV 360  
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*Mus musculus* eEF1A1 301 EALPGDNVGFNVKNSVKDTRRGNVAGDSKNDPEEAAAGFTAQVILLNHPPQISAGYAPV 360

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*Xenopus laevis* eEF1A2 361 IDCHTAHIACKFAELKEKIDRRSGKKLEDNPKSLKSGDAAIVDMTPGKPMCVESFSQYYP 420  
*Xenopus tropicalis* eEF1A1 361 IDCHTAHIACKFAELKEKIDRRSGKKLEDNPKSLKSGDAAIVDMTPGKPMCVESFSQYYP 420  
*Xenopus laevis* eEF1A1 361 IDCHTAHIACKFAELKEKIDRRSGKKLEDNPKSLKSGDAAIVDMTPGKPMCVESFSQYYP 420  
*Mus musculus* eEF1A1 361 IDCHTAHIACKFAELKEKIDRRSGKKLEDGPKSLKSGDAAIVDMTPGKPMCVESFSQYYP 420

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*Mus musculus* eEF1A2 421 LGRFAVRDMRQTVAVCVIKNVEKKSAGAGKVTKSAQKAQKA 463  
*Xenopus laevis* eEF1A2 421 LGRFAVRDMRQTVAVCVIKNVEKKSAGAGKVTKSAQKAQKA 463  
*Xenopus tropicalis* eEF1A1 421 LGRFAVRDMRQTVAVCVIKNVEKKAAGAGKVTKSAQKAQKA 463  
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Figure 1

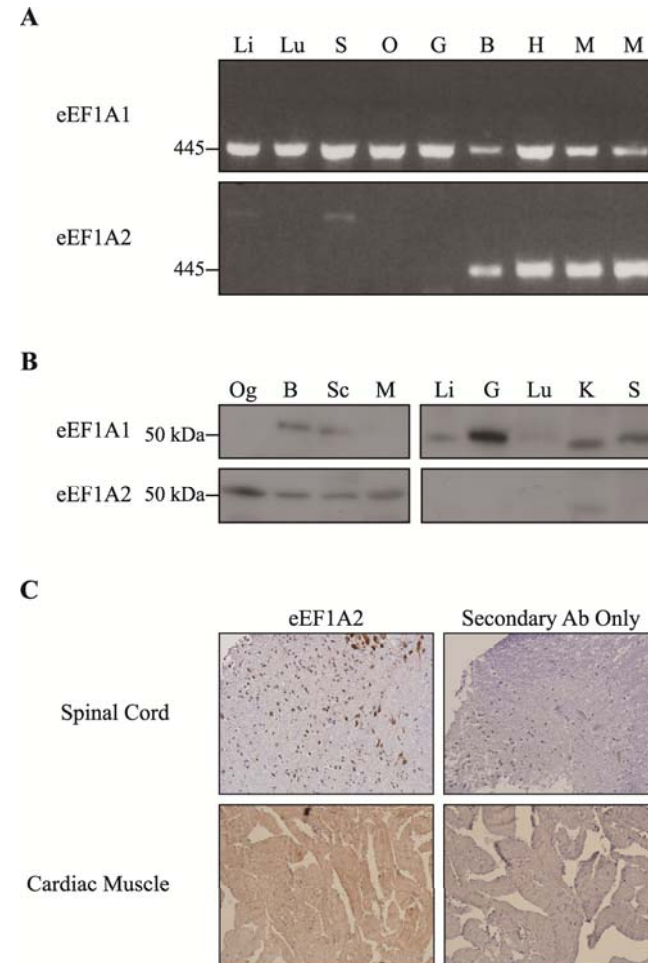
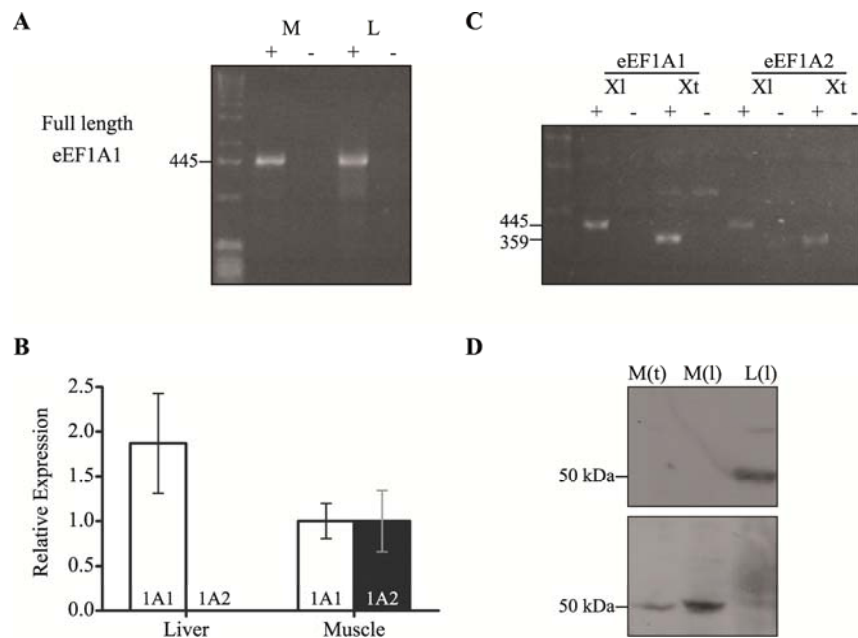


Figure 2



**Figure 3**

Figure legends

Figure 1

Figure 1 shows the results of ClustalW analysis of eEF1A1 and eEF1A2 from *X.laevis*, *X.tropicalis* and mouse; the results were coloured using Boxshade. Xt is *Xenopus tropicalis*, Xl is *Xenopus laevis*, m is mouse; 1A1 is eEF1A1, 1A2 is eEF1A2. An asterisk denotes the position of the serine residue present in eEF1A2 from all species that is predicted to be a phosphorylation site.

Figure 2

Panel A: RT-PCR of eEF1A1 (top panel) and eEF1A2 (bottom panel) in RNA from a range of tissues taken from adult *X.laevis*. Li= liver, Lu= lung, S= spleen, O= oocytes, G= gall bladder, B= brain, H= heart, M= muscle (two independent tissue samples). No-RT controls were carried out and were negative for all tissues for both genes (data not shown).

Panel B: Western blots of eEF1A1 (top) and eEF1A2 (bottom). Gels were run in duplicate using the samples of same protein extract at the same time. Og= optic ganglion, B= brain, Sc= spinal cord, M= muscle, Li= liver, G= gall bladder, Lu= lung, K= kidney, S= spleen.

Panel C: Immunohistochemistry for eEF1A2 on spinal cord and cardiac muscle from *X.laevis*, together with negative controls. eEF1A2 shows widespread expression in cardiac muscle but is expressed in only in neuronal cells in spinal cord.

Figure 3

Panel A: RT-PCR of eEF1A1 in *X.laevis* muscle and liver using primers that amplify full-length mRNA. Lanes labelled with – are no-RT controls.

Panel B: Real-time RT-PCR results for eEF1A1 and eEF1A2 in liver (showing expression of eEF1A1 only) and muscle (showing equal levels of expression of eEF1A1 and eEF1A2).

Units of expression are relative to the internal control, *Rpl8*.

Panel C: Analysis of expression of eEF1A1 in adult muscle from *X.tropicalis*. B shows RT-PCR of eEF1A1 and eEF1A2 in muscle from *X. tropicalis* (Xt) and *X.laevis* (Xl) together with negative controls (-RT). All PCR products are of the predicted size.

Panel D: two Western blots, run in parallel, for eEF1A1 and eEF1A2. M(t) is muscle tissue from *X. tropicalis*, M(l) is muscle tissue from *X. laevis* and L(l) is liver from *X. laevis*.