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Evolutionary origin and phylogenetic analysis of the novel oocyte-specific eukaryotic translation initiation factor 4E in *Tetrapoda*.

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Abstract

The transcriptionally active, growing oocyte accumulates mRNAs essential for early stages of development, the oocyte-to-embryo transition, in a stable, dormant form. Translational repression of mRNAs in eggs of various species is conferred by interactions, either direct or via intermediate proteins, of repressive factors bound to the 3'-UTRs with the proteins of the eukaryotic translation initiation factor 4E (eIF4E) family bound to the 5'-cap of the transcripts. Recently, a novel oocyte-specific eIF4E encoded by the *Eif41b* gene in mammals has been identified by our group. To further investigate this gene, the available cDNA libraries, as well as genome assemblies of non-mammalian vertebrates were surveyed. This analysis revealed that the *Eif4e1b* gene arose in *Tetrapoda* as a result of the ancestral *Eif4e* locus duplication. Unlike other known proteins of three subfamilies comprising eIF4E family (eIF4E1, eIF4E2 and eIF4E3), cDNA library evidence suggests that *Eif41b* locus has an oocyte-restricted expression across all classes of *Tetrapoda*. To further understand the role of eIF4E1B during oocyte maturation, injections of antisense morpholino nucleotides in the *X. tropicalis* fully-grown stage VI oocytes were performed. The resulted ablation of eIF4E1B protein led to significant acceleration of oocyte maturation after progesterone induction; morpholino-injected oocytes formed the metaphase plate 30 minutes faster than the control groups. These results suggest that eIF4E1B protein acts as a repressor in translational regulation of maternal mRNAs activated during, and required for, oocyte maturation.

Keywords

comparative genomics; eukaryotic translation initiation factor 4E; molecular evolution; oocyte maturation; translational control

Introduction

The early development of metazoans relies heavily on the maternal stores accumulated in the egg during its growth. Amassing of nutrients during oogenesis secures an embryo's independence from external energy sources and ensures rapid development during this vulnerable stage of an organism's life. Another vital role played by the egg is imparting the initial programs for gene expression upon the nascent embryonic genome. This role is particularly pronounced in viviparous mammals, whose oocytes have the minute amounts of yolk and embryos receive nutrition directly from mothers upon implantation. During the ooplasm-driven remodeling of parental genomes, transcription is barely, if at all, detectable. Thus, the initial embryonic genome is shaped by precise post-transcriptional and post-translational mechanisms, which concomitantly create the dynamic molecular environment as early development proceeds. Upon completion of this genome remodeling, re-initiation of

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transcription demarcates ‘embryonic genome activation’. Henceforth, the embryonic genome gradually assumes control of development from ‘maternal command’.

The molecules that drive embryogenesis at these earliest stages are still poorly understood. However, it is well-known that post-translational mechanisms, which govern initial embryonic development, rely on massive accumulation of transcripts during oogenesis. Indeed, during oocyte growth, many transcribed mRNAs are stored in the ooplasm as a ‘dormant’ form for translation at later stages and are deadenylated. Translational recruitment of these stored mRNAs is typically manifested by lengthening of their limited 3′ poly(A) tails (Bachvarova, 1992; Richter and Sonenberg, 2005). Spatio-temporal regulation of translational activation of maternal mRNAs deposited during oocyte growth is a key mechanism providing new proteins during the final stages of oogenesis (i.e., maturation), and first stages of embryogenesis in virtually all metazoans. One model for mRNA translational regulation, based upon extensive studies from diverse model organisms, proposes how transcripts may be translated in the time- and stage-dependent fashion (Richter and Sonenberg, 2005; Standart and Minshall, 2008). In this model, a regulatory protein can be bound to a cognate motif in the 3′-untranslated region (UTR) of mRNA. Concurrently, this RNA-binding protein may also be bound, either directly or via intermediate protein(s), to the eukaryotic initiation factor 4E (eIF4E), an mRNA 5′-cap-binding protein. In the ‘closed’ conformation, these protein-RNA interactions suppress translation by preventing formation of the pre-initiation complex. Certain signaling events, such as phosphorylation of a 3′-UTR-bound regulatory protein, result in a conformational change to the complex, leading to recruitment of poly(A) polymerases to the 3′ end of mRNA, formation of the translational initiation complex on the 5′ end and eventually, leading to translation.

The eIF4E family of proteins consists of three large subfamilies, eIF4E1, eIF4E2 and eIF4E3. Genomic sequencing and analysis of various model organisms, as well as the analysis of EST databases, suggest the existence of multiple eIF4E-encoding genes within each of these subfamilies in most organisms (Joshi et al., 2005). The reason for this redundancy is not clear. While it is assumed that most eIF4E proteins possess at least some 5′-cap binding activity, to date, the most extensively studied and characterized is the eIF4E1 subfamily. Until recently, only one mammalian eIF4E1 member, encoded by the *Eif4e* gene, has been identified. Our analysis of the fully-grown mouse oocyte cDNA library revealed another gene, *Eif4eloo* (*Eif4e1b* in current nomenclature), which, indeed, is a second *bona fide* member of this subfamily in mammals (Evsikov et al., 2006). Unlike its ubiquitously expressed paralog *Eif4e*, transcription of *Eif4e1b* gene appears restricted to growing oocytes. To date, the *Eif4e1b* gene has been identified in genome assemblies of various mammals (Evsikov et al., 2006), while transcripts encoding eIF4E1B proteins from different species, including *Homo sapiens*, have been identified in the large-scale survey of GenBank mRNAs and ESTs aimed to categorize all available information on various eIF4E family proteins across all eukaryotes (Joshi et al., 2005).

In order to understand the role of *Eif4e1b/Eif4eloo* in the oocytes, we sought to delineate the phylogenetic history of this gene. Indeed, a similar eIF4E1 homolog, with the expression pattern restricted to oocytes, testis and muscle, has been reported in the zebrafish, *Danio rerio* (Robalino et al., 2004). Interestingly, unlike ‘conventional’ eIF4E, this *D. rerio* eIF4E-1B protein cannot substitute for endogenous eIF4E in complementation assays using *eif4e*-deficient *Saccharomyces cerevisiae*; furthermore, it lacks the 5′-cap-binding activity (Robalino et al., 2004). Recently, a similar eIF4E1B protein with similar biochemical properties has been also identified in the frog, *Xenopus laevis* (Minshall et al., 2007). Here, we used available genome sequences and whole-genome assemblies of model organisms to scrutinize the evolutionary relationships among members of the eIF4E1 subfamily in vertebrates and to identify the non-mammalian orthologs of *Eif4e1b*. To investigate the functional role of

Eif4e1b as a regulator of translation during oocyte maturation, antisense morpholino oligonucleotides designed to suppress *Eif4e1b* mRNA translation were injected in immature, fully-grown *Xenopus tropicalis* oocytes.

Results and Discussion

To determine the potential orthologs of *Eif4e1b/Eif4eloo*, we have identified, using TBLASTN program, the ESTs of three vertebrates, *Anolis carolinensis*, *Gallus gallus* and *Xenopus tropicalis*, which share identity with *Mus musculus* eIF4E1B protein. The matching ESTs were next assembled into contigs, and the identities of resulting consensus cDNAs were established by aligning them, using BLAT, to the ENSEMBL genome assemblies of *A. carolinensis*, *G. gallus*, and *X. tropicalis*, respectively. As expected, some consensi aligned to the genomic regions of *A. carolinensis*, *G. gallus*, and *X. tropicalis* syntenic to the mammalian *Eif4e* gene, and the orthology was evidenced by colinearity of genes (*Metap1 – Eif4e – Tspan5*) in the genomes of all examined species (Fig. 1A). Another group of EST consensi aligned to the genomic regions of *A. carolinensis* and *X. tropicalis* that were clearly syntenic to the *M. musculus* locus (Fig. 1B: *Sncb – Eif4e1b – Tspan17*). Similarly, one *G. gallus* cDNA aligned to the syntenic locus downstream of *Sncb* gene (Fig. 1B: *Sncb – Eif4e1b – Unc5a*). Interestingly, *Tspan17* locus is apparently absent in this species as *Unc5a*, located immediately downstream of *Eif4e1b* in *G. gallus* (Fig. 1B), is located downstream of *Tspan17* in other species (not shown). Virtual Northern blotting (i.e., analysis of the EST sources for the consensi that comprised *Eif4e1b* cDNAs in *G. gallus*, *X. tropicalis* and *A. carolinensis*), identified that all *X. tropicalis* ESTs were from either oocyte, blastula or gastrula cDNA libraries, while *G. gallus* and *A. carolinensis* ESTs were from ovarian cDNA libraries. Due to the limited amounts of ESTs available for a marsupial, *Monodelphis domestica*, *Eif4e* and *Eif4e1b* loci in these species were identified using TBLASTN search of genome assembly using *M. musculus* eIF4E1B. This search revealed two candidate genes; further examination of adjacent loci authenticated these candidates as *bona fide* orthologs of *Eif4e* and *Eif4e1b*, respectively (Fig. 1A, 1B). In summary, we have identified the orthologs of the previously described mammalian *Eif4e1b* gene (Evsikov et al., 2006) in the genomes of representatives for all major classes of *Tetrapoda*. In all examined species, the origin of *Eif4e1b* gene is likely monophyletic, and its expression is commonly upregulated in oocytes and ovaries, suggesting conserved function(s) in female germline.

To further understand the evolutionary history of the *Eif4e1b* locus, we have performed the TBLASTN search of all *D. rerio* ESTs and cDNA sequences to detect the eIF4E-coding mRNAs in this species. Seven distinct eIF4E genes were identified, including previously reported *eif4e1a* and *eif4e1b* (Robalino et al., 2004; Suppl. Table 1). Multiple sequence alignment of corresponding proteins with eIF4E proteins of *Tetrapoda* using CLUSTALW confirmed that four *D. rerio* genes, *eif4e1a*, *eif4e1b*, *zgc:101581* (*Eif4e1_2*) and *zgc:110154* (*Eif4e1_3*) encode proteins of the eIF4E1 subfamily (Suppl. Fig. 1). To our surprise, while the zebrafish eIF4E1B is very similar to other eIF4E1B proteins and has a similarly restricted pattern of expression (Robalino et al., 2004), the *D. rerio eif4e1b* gene is not orthologous to either *Eif4e1b* or *Eif4e* loci of *Tetrapoda*. Indeed, alignment of *eif4e1b* cDNAs to the *D. rerio* genome identified the *eif4e1b* locus unambiguously (Fig. 1C). However, although the colinearity of flanking *Slc7a2* and *Casr* genes is well-conserved, the genomes of other *Actinopterygii* species (*Gasterosteus aculeatus*, *Oryzias latipes*, *Takifugu rubripes* and *Tetraodon nigroviridis*) lack any sign of eIF4E-encoding locus in the respective regions (Fig. 1C). Conversely, while the physical linkages of *Sncb* and *Tspan17* loci persist among these four fish species and *Tetrapoda*, there is no *Eif4e*-like gene residing in this region of any *Actinopterygii* genome as well (Fig. 1D vs. 1B). Together, these data indicate the lack of orthology between the *D. rerio eif4e1b* and the *Eif4e1b* of *Tetrapoda*.

Further TBLASTN search of fish genome assemblies using *D. rerio* eIF4E1A, eIF4E1B, eIF4E1_2 and eIF4E1_3 protein sequences revealed three ostensibly orthologous groups of eIF4E1-encoding genes in *Actinopterygii* (Fig. 2; Suppl. Table 1). The physical linkage of *Eif4e1_2* and *Metap1* loci in all five species indicated *Eif4e1_2* genes as ‘genuine’ orthologs of *Tetrapoda* *Eif4e* genes (Fig. 2B vs. Fig. 1A), while *Eif4e1_1* (Fig. 2A) and *Eif4e1_3* (Fig. 2C) loci are unique to *Actinopterygii* species. To test these conclusions, multiple sequence alignments of newly-identified eIF4E1_1, eIF4E1_2 and eIF4E1_3 proteins of *G. aculeatus*, *O. latipes*, *T. rubripes* and *T. nigroviridis* were performed to identify their relationships to the eIF4E1 family members of *D. rerio* and *Tetrapoda* (Suppl. Fig. 2). The results indicate that the eIF4E1_2 proteins of *Actinopterygii* are indeed the closest relatives of *Tetrapoda*'s eIF4E, while eIF4E1_1 and eIF4E1_3 are distinct clades of this subfamily. Furthermore, *eif4e1a* gene of *D. rerio*, absent in the (current) genome assembly, likely belongs to the *Eif4e1_1* group, while no close relative for *eif4e1b* gene could be identified in any other currently available *Actinopterygii* genome. This may be due, in part, to the taxonomy of *Actinopterygii*, as *D. rerio* has a very distant relationship to any of the other surveyed species (Suppl. Table 2). Indeed, several likely eIF4E1B-encoding sequences were identified previously among cDNA and EST sequences of other fish species (Joshi et al., 2005). However, absence of the genome assemblies or even any considerable genomic sequence data precludes the studies of orthological relationships for these additional *eif4e1b* genes.

Overall, our data strongly indicate rapid diversification of eIF4E1 subfamily occurred during the time of whole-genome duplication in vertebrates (Nakatani et al., 2007). This diversity was lost during the early phylogenesis of *Tetrapoda* lineage and resulted in the fixation of merely two loci, the ubiquitous *Eif4e* and oocyte-restricted *Eif4e1b*, in these taxa. It is plausible that multiple *Eif4e1b*-like loci existed in the common ancestor of *Actinopterygii* and *Tetrapoda*; some of them were asymmetrically retained in *Actinopterygii* (e.g., *eif4e1b* locus in *D. rerio*), while others, such as the ancestral locus that spawned *Eif4e1b* of *Tetrapoda*, were subsequently lost in other lineages. Whatever the case, this provides an excellent example of convergent molecular evolution for eIF4E1B-encoding genes which, while being phylogenetically distinct in *Actinopterygii* vs. *Tetrapoda*, endure selective pressure to encode functionally identical proteins, as well as to be preferentially expressed in female germline. In fact, this situation strongly resembles the ‘phylogenetic behavior’ of some oocyte-expressed genes in mammals that we discovered previously. For example, some critical maternal effect genes sustain asymmetrical reduplications, and presumably strong selective pressure, to result in the formation of multiple homologous, but not orthologous, genes belonging to several distinct families (e.g., *Nalp*, *Oasl*, *Oog* and others; Evsikov et al., 2006).

To address the function of eIF4E1B protein in the oocytes, morpholino antisense oligonucleotides (morpholinos) were injected to curb translation of its mRNA in the eggs of *X. tropicalis*. Unlike *Xenopus laevis* whose allotetraploid genome allows each protein to be translated from two different mRNAs and thus renders translational suppression experiments unfeasible, *X. tropicalis* was the most suitable model to perform such studies because it has a diploid genome and retains much of the advantages found in *X. laevis* as a model embryological system (Carruthers and Stemple 2006). Although *X. tropicalis* stage VI oocytes express all eIF4E-encoding genes identified in the genome of this species (Fig. 3A), we were interested in the specific functions for the oocyte-specific isoform. Injection of anti-*Eif4e1b* morpholinos into freshly harvested *X. tropicalis* fully-grown eggs *per se* did not result in any changes to oocyte morphology for up to 144 hours of culture. This result implies that eIF4E1B protein is dispensable at this stage and may function later in oogenesis, such as meiotic maturation, or during early development. To test this hypothesis, oocytes injected with anti-*Eif4e1b* morpholino oligonucleotides were induced to complete meiosis with progesterone and the rates of meiotic maturation, measured by the appearance of ‘white spots’ on animal poles, the gross morphological sign of metaphase spindle formation, were recorded. Since morpholinos only

inhibit translation of cognate mRNA, progesterone treatment was applied to fully-grown oocytes at 48 and 96 hours post-injection to ensure sufficient time for intrinsic protein turnover of any pre-existing eIF4E1B proteins. Two days after injection, anti-*Eif4e1b* morpholino treatment increased the number of matured oocytes at 3.5 hrs after progesterone treatment ($time \times treatment \chi^2=49.6$, $P<0.0001$) whereas no other time point was effected (Fig. 3B). Four days after treatment, 2-to-8-fold more oocytes receiving anti-*Eif4e1b* morpholino treatment ($\chi^2=62.24$, $P<0.0001$) completed maturation 30-60 min faster compared to all three control groups ($time \times treatment \chi^2=58.96$, $P<0.0001$), respectively (Fig. 3C). A similar effect of meiotic acceleration by 30-60 minutes was obtained with oocytes induced with progesterone at 144 hours post-injection (data not shown); however, in this case maturation in all groups was delayed up to ~7 hours, most likely due to the adverse effects of prolonged culture.

Interestingly, comparable acceleration of maturation was recently reported when the oocytes from another frog, *X. laevis*, were injected with an antibody that recognizes a likely ortholog of eIF4E1B in these species (Minshall et al., 2007). Overall, our data further underscores a novel, unexpected conclusion that the oocyte-specific eIF4E1B acts as a translational repressor of dormant maternal mRNAs. At first glance, this repressor activity is surprising, as all eIF4E1B and eIF4E proteins from *Tetrapoda* and *D. rerio* are identical at 98 out of 183 residues constituting the IF4E/CDC33 domain (Suppl. Fig. 3). Moreover, all the residues critical for 5'-cap mRNA binding and interactions with eIF4Gs or eIF4E-BPs are absolutely conserved among eIF4E1Bs and eIF4Es (Suppl. Fig. 3). Despite this striking sequence identity, our experimental data and the results from other laboratories suggest that functionally, eIF4E1B is very different from 'canonical' eIF4E. Indeed, assuming the functional identity among eIF4E1B proteins in vertebrates, the important characteristics of these proteins are weak mRNA cap and eIF4G-binding, and strong affinity to 4E-T (Minshall et al., 2007; Robalino et al., 2004), an eIF4E transporter protein encoded by *Eif4enif1* gene in mammals. Recently, a model based on the biochemical interactions of *X. laevis* eIF4E1B with 4E-T and CPEB in immature oocytes has been proposed (Standart and Minshall, 2008). In this model eIF4E1B, unlike 'canonical' eIF4E, behaves as translational co-repressor of CPE-containing maternal transcripts required for maturation, such as Cyclin B mRNA. Importantly, this proposed model may occur in mammalian systems as well because all three proteins are highly abundant in mouse oocytes (Evsikov et al., 2006; Villaescusa et al., 2006). In the future, studies of the biochemical interactions of eIF4E1B, which are beginning to be uncovered (Minshall et al., 2007; Robalino et al., 2004; Standart and Minshall, 2008), will hopefully explain the exact mechanisms and roles in translational control of maternal mRNAs.

Materials and Methods

Bioinformatics

National Center for Biotechnology Information (NCBI; Wheeler et al., 2008) BLAST engine was used for all GenBank searches. ESTs and cDNAs were downloaded from NCBI. EST sequences were assembled in consensus sequences as described (Evsikov et al., 2006), and the encoded protein sequences identified using ORF finder (NCBI). Searches against the genome assemblies for all species were performed using online search tools of ENSEMBL project (Flicek et al., 2008; <http://www.ensembl.org/>). The following genome assemblies were used: *Anolis carolinensis*, AnoCar1.0; *Danio rerio*, Zv7; *Gallus gallus*, WASHUC2; *Gasterosteus aculeatus*, BROAD S1; *Monodelphis domestica*, monDom5; *Mus musculus*, NCBI m37; *Oryzias latipes*, HdrR; *Takifugu rubripes*, FUGU 4.0; *Tetraodon nigroviridis*, TETRAODON 8.0; and *Xenopus tropicalis*, JGI 4.1. Multiple sequence alignments and their analyses were performed using MEGA 4.0 (Tamura et al., 2007). The protein sequences of eIF4E family members mentioned or identified in this study are provided in the Supplemental Table 3.

Antisense Oligonucleotides

To inhibit translation of *Eif4e1b* mRNA, the 25-mer morpholino antisense oligonucleotides, 'morpholinos' (Gene Tools LLC, Philomath, OR, USA) were used. The sequence of anti-*Eif4e1b* morpholino (5'CAGCTGCTGCCATTCTGCTATACAA-3') was designed according to manufacturer's recommendations using the 5'-end sequence of the *X. tropicalis* *Eif4e1b* mRNA containing the AUG start codon (EST AL890091 and the corresponding region of *X. tropicalis* genome assembly). This sequence does not match any mRNA but *Xt_Eif4e1b*, or any other region of the sequenced genome. The 'standard control morpholino' (5'-CCTCTTACCTCAGTTACAATTTATA-3') is designed towards a splice-generating mutation at position 705 β -globin pre-mRNA found in human thalassemia. This morpholino is commonly used as a control in egg microinjection experiments and does not have any known target or any significant biological activity in either *X. laevis* or *X. tropicalis*. Stock concentrations for both morpholinos were 1mM in sterile water. For injection, 10 nl of 200 μ M morpholino, or sterile water, was injected per oocyte.

Oocyte collection, microinjection and maturation assays

Follicle-cell-free *X. tropicalis* stage VI oocytes were obtained by collagenase treatment (2% type I collagenase, 0.1% SBTI, 0.1% BSA in 1 $\times$ Modified Ringer's [MR] medium: 100 mM NaCl, 1.8 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 5 mM Na-HEPES; pH adjusted to 7.8 with NaOH) for 1 hour at room temperature with gentle shaking (De Sousa and Masui, 1990). Oocytes were washed 10 times in 100 mM potassium phosphate buffer (28 mM K₂HPO₄, 72 mM KH₂PO₄, pH 6.5), 5 times in 1 $\times$ MR, and sorted on a 100 mm agarose-coated Petri dish in 1 $\times$ MR. Oocytes were placed in the OR3 medium (50% Leibovitz's L-15 medium, 15 mM HEPES, 100 μ g/ml gentamycin, 50 U/ml mystatin, 50 U/ml penicillin, 50 μ g/ml streptomycin) for 2-3 hours at 25°C and processed for microinjection (Gallo et al., 1995). Batches of 25 oocytes were placed in 35 mm plastic Petri dishes with a piece of nylon mesh (0.8-1 mm openings) attached to the bottom. Before injection, level of OR3 medium was adjusted to 200-400 μ m above oocytes, so they were held in place by the mesh on the bottom and surface tension on top. Injections were performed with a Picospritzer apparatus (Parker Hannifin Co., Cleveland, OH, USA) using needles pulled from glass borosilicate capillaries (outer diameters of needle tips were 17-19 μ m). After injection of morpholino or water (or no injection control), the oocytes were cultured for 48, 96 or 144 hours in OR3 in agarose-coated Petri dishes at 25°C. Maturation was induced in oocytes by incubation in 1 μ g/ml progesterone in OR3 for 6-10 hours at 25°C. The meiotic progression was monitored by counting the number of oocytes with the clearly defined white spot on the animal pole every 30 minutes beginning at 3 hours after the start of progesterone treatment. Fifty oocytes per experimental group (anti-*Eif4e1b* morpholino-injected, control morpholino-injected, water-injected and uninjected) in two independent experiments conducted on different days were used.

Gene Expression

RNA was isolated from *X. tropicalis* fully-grown oocytes (a generous gift from Dr. Laurinda Jaffe, University of Connecticut Health Center, Farmington, CT, USA). RNA was treated with RNase-free DNase (Quiagen) and used for cDNA first strand synthesis (Superscript III, Invitrogen). Conditions for PCR were optimized using FailSafe PCR system (Roche). The PCR amplification program was: 97°C 1 min; 35 cycles of 95°C/30 sec; 58°C/30 sec; 72°C/45 sec; 72°C 10 min; 4°C infinity. PCR products were separated on 1% agarose gels, stained with ethidium bromide and visualized by UV transillumination. Sequences of primers used for RT-PCR studies are listed in Suppl. Table 4.

Statistics

Descriptive statistics (mean and SEM) were reported as the percent of mature oocytes at each time interval. To detect statistical differences for the effects of treatment, time, and the interaction of time $\times$ treatment on the total number of matured oocytes, an ordinal logistic fit model was used to calculate the χ^2 likelihood ratio tests, with differences considered significant at $P < 0.05$ (JMP 7 software, SAS Institute, Cary, NC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Conservation of genomic structure for Eif4e and Eif4e1b loci in Tetrapoda

Colinearity of genes underscores the common phylogenetic origins for *Eif4e* (a) and *Eif4e1b* (b) in tetrapods. (c) The *Danio rerio eif4e1b* locus is not conserved among *Actinopterygii* genomes. (d) While the colinearity of *Sncb* and *Tspan17* orthologs persists across vertebrates, fish genomes lack the *Eif4e1b* locus. Orthologs shown in the same color; non-orthologous genes shown in black.

Figure 2. Actinopterygii genomes contain multiple members of eIF4E1 subfamily
Danio rerio expresses at least four members of this family (Suppl. Table 1), three of which have orthologs in other sequenced fish genomes. Genomic arrangement of loci for putative orthologs of zebrafish *eif4e1a* (*Eif4e1_1*) (a), *zgc:101581* (*Eif4e1_2*) (b) and *zgc:110154* (*Eif4e1_3*) (c) genes. Orthologs shown in the same color; non-orthologous genes shown in black.

Figure 3. Gene expression of Eif4e1b and its function in oocytes of *Xenopus tropicalis*
(a) RT-PCR detection of eIF4E-encoding genes (*Xt_Eif4e1b*, *Xt_Eif4e*, *Xt_Eif4e2a*, *Xt_Eif4e2b*, *Xt_Eif4e3*), *Xt_Gpcr13*, *Xt_Odc* as positive controls of cDNA quality and H₂O as a negative control. **(b, c)** Effects of blocking eIF4E1B translation on the resumption of meiosis and maturation in oocytes exposed to anti-*Eif4e1b* morpholino or control for two and four days.