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Translational control genes in the sea urchin genome ☆

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Abstract

Sea urchin eggs and early cleavage stage embryos provide an example of regulated gene expression at the level of translation. The availability of the sea urchin genome offers the opportunity to investigate the "translational control" toolkit of this model system. The annotation of the genome reveals that most of the factors implicated in translational control are encoded by nonredundant genes in echinoderm, an advantage for future functional studies. In this paper, we focus on translation factors that have been shown or suggested to play crucial role in cell cycle and development of sea urchin embryos. Addressing the cap-binding translational control, three closely related eIF4E genes (class I, II, III) are present, whereas its repressor 4E-BP and its activator eIF4G are both encoded by one gene. Analysis of the class III eIF4E proteins in various phyla shows an echinoderm-specific amino acid substitution. Furthermore, an interaction site between eIF4G and poly(A)-binding protein is uncovered in the sea urchin eIF4G proteins and is conserved in metazoan evolution. In silico screening of the sea urchin genome has uncovered potential new regulators of eIF4E sharing the common eIF4E recognition motif. Taking together, these data provide new insights regarding the strong requirement of cap-dependent translation following fertilization. The genome analysis gives insights on the complexity of eEF1B structure and motifs of functional relevance, involved in the translational control of gene expression at the level of elongation. Finally, because deregulation of translation process can lead to diseases and tumor formation in humans, the sea urchin orthologs of human genes implicated in human diseases and signaling pathways regulating translation were also discussed.

Keywords: Sea urchin; Translational control; Cap-dependent initiation; Elongation factors

Introduction

mRNA translation, which has been considered for a long time as a "housekeeping" mechanism, is now recognized as a crucial regulatory step for gene expression in different physiological processes including development and cell cycle (Mathews et al., 2000; Wickens et al., 2000; Cormier et al., 2003; Le Breton et al., 2005). Early embryogenesis is highly dependent on translational regulatory cascades (Richter, 2000; Vasudevan et al., 2006). Although sea urchin eggs apparently contain all the necessary components for translation activity (Davidson et al., 1982), protein synthesis is low in unfertilized eggs and is stimulated rapidly following fertilization. This dramatic rise in protein synthesis is independent of mRNA transcription and ribosome biogenesis (Epel, 1967; Brandhorst, 1976). Therefore, sea urchin eggs and embryos provide an example of regulated gene expression that does not follow the classical theme of transcriptional control.

Furthermore, sea urchin eggs and embryos represent an elegant system to investigate the relationships between translational regulation and cell division during embryonic development. Unfertilized sea urchin eggs are arrested after completion of the meiotic divisions at the G1 stage. Fertilization triggers

☆ Complete names of species listed in the manuscript: *Asterina pectinifera*, *Bombyx mauri*, *Caenorhabditis elegans*, *Callinectes sapidus*, *Ciona intestinalis*, *Danio rerio*, *Dictyostelium discoidum*, *Drosophila melanogaster*, *Globodera rostochiensis*, *Homo sapiens*, *Mus musculus*, *Nematostella vectensis*, *Paracentrotus lividus*, *Saccharomyces cerevisiae*, *Sphaerechinus granularis*, *Strongylocentrotus purpuratus*, *Xenopus laevis*.

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entry into S-phase and completion of the first mitotic division of the embryonic development. De novo protein synthesis is dispensable for progression through S-phase but is required for the onset of the M-phase and subsequent embryonic cell cycles (Wagenaar, 1983; Dube, 1988). During early development stage, the cleavages of sea urchin are highly synchronized and synchronization is achieved naturally. In eukaryotes, the onset of mitosis is under the control of a heterodimeric complex composed of a cyclin-dependent kinase (CDK1) and a B-type cyclin (Nigg, 2001; O'Farrell, 2001; Fernandez-Guerra et al., this issue). The mitotic cyclins A and B were first discovered in sea urchin as key proteins which are synthesized and degraded during the M-phase of each cell cycle (Evans et al., 1983). The sea urchin genome project offers opportunity to obtain new insights into the translational regulation mechanisms. We focus on translational factors that have been already shown or suggested to play crucial role in proper cell cycle and development of sea urchin embryos.

Translation is a multistep and multifactorial pathway that can be regulated at many levels (Fig. 1). Translation rates are primarily regulated at the initiation level, a complicated process involving a large number of initiation factors (Hershey and Merrick, 2000; see Table 1). Among them, the eIF4 (eukaryotic initiation factor 4) group, a prime target for regulation (Cormier et al., 2003; Richter and Sonenberg, 2005), participates to the control of the translational activity dependent of the m^7GTP cap of the mRNA and plays an important role for cell cycle regulation following sea urchin fertilization (Cormier et al., 2001; Salaun et al., 2003, 2005). Several physiological conditions are associated to an inhibition of cap-dependent translation, such as stress, hypoxia, mitosis or apoptosis. An alternative translation, involving the direct entry of the ribosome

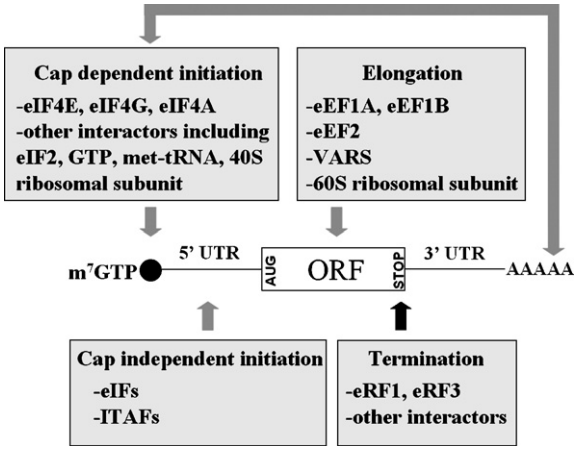

Fig. 1. Schematic representation of each step of translation (initiation, elongation and termination) with the involved translation factors. The mRNA is represented as follows: the cap m^7GTP structure, where cap-dependent translation is initiated; the 5′ untranslated region, containing structures implicated in IRES-dependent initiation; the open reading frame (ORF) starts with an AUG codon where ribosomes are competent for elongation up to the stop codon, when release factors promote termination; finally the 3′ untranslated region and poly (A) tail, where RNA-binding proteins modulate translation. The cap interacts with the poly(A) tail through association of cap-binding complex to poly(A)-binding protein (double arrow); this closed loop conformation is thought to enhance translation.

Table 1
Translation factors genes in *S. purpuratus* genome

Name	Other name	SPU #	Function	Tiling expression data
Initiation				
eIF1		016208	AUG recognition	+++
eIF1A		026084		+
eIF2alpha		003646	Binds GTP, Met-tRNA; GTPase	++
eIF2beta		009150		++
eIF2gamma		020412		++
eIF2Balpha		021689	GTP exchange activity on eIF2	++
eIF2Bbeta		004173		+++
eIF2Bgamma		005651		+
eIF2Bdelta		010743		+
eIF2Bepsilon		015443		++
eIF3a		014579	Multi-subunit complex, binds to 40S	++
eIF3b		022562	Ribosome, eIF4G, eIF1, eIF5	++
eIF3c		026863		+
eIF3d		006773		++
eIF3e	Int6	007226		++
eIF3f		001649		+
eIF3g		004443		++
eIF3h		023965		++
eIF3i	TRIP1	012216		+
eIF3j		013398		++
eIF3k		010303		+
eIF4A		023083	DEAD-box RNA helicase	++
eIF4B		004840	Stimulates helicase	++
eIF4H	WBSCR1	008411	Stimulates helicase	+++
eIF4E1		028477	Binds to m^7GTP cap, eIF4G	++
eIF4E2	4E-HP	016729		+
eIF4E3		025634		++
eIF4G		024859	Binds to eIF4E, eIF4A, eIF3, PABP	++
eIF5		018897	Stimulates eIF2 GTPase	+++
eIF5B		001393	Ribosome junction, GTPase	+
Elongation				
eEF1A		000595	Delivery of aa-tRNA, GTPase	+++
eEF1Balpha		015867	GTP exchange activity on eEF1A	+
eEF1Bdelta		000960		++
eEF1Bgamma		002587		++
eEF2		010829	Peptidyl-tRNA translocation, GTPase	++
Termination				
eRF1		023948	Recognizes STOP codon	++
eRF3		003213	Stimulates eRF1, GTPase	+++

Expression in the embryo was assessed from the tiling array data (Samanta et al., this issue). (−) <5, (+) 5–20, (++) 20–100, (+++) >100 arbitrary units.

at the vicinity of the start codon through an IRES (Internal Ribosome Entry Site), can overcome the general inhibition of translation by allowing synthesis of specific mRNAs (review in Holcik and Sonenberg, 2005; Le Breton et al., 2005). In sea urchin embryos, selective ribosomal recruitment of mRNAs occurs at mitosis, by a yet undetermined mechanism (Le Breton et al., 2003). Although less investigated, a regulation of protein

synthesis at the level of the elongation step was shown to be critical (Traugh, 2001; Browne and Proud, 2002; Le Sourd et al., 2006a) and was indeed reported to play a role during the first mitotic division following fertilization of sea urchin eggs (Monnier et al., 2001; Boulben et al., 2003). Translation termination is a potential regulatory step of gene expression (Welch et al., 2000), translation factors implicated in this process were also identified from the sea urchin genome. Translational regulation frequently involves the interaction between translation factors and interactors or the phosphorylation status of translational components. We therefore discussed these different interactors and the main kinases known to modulate the translational rate in physiological conditions and identified from the sea urchin genome project.

Materials and methods

The *Strongylocentrotus purpuratus* protein prediction set (GLEAN3) was made available at the Human Genome Sequencing Center at Baylor College of Medicine (HGSC-BCM, http://www.hgsc.bcm.tmc.edu/blast/) and searched by BLASTP (Altschul et al., 1990) using the known translation factors proteins, retrieved from the Swissprot or NCBI server. When no or partial sea urchin homolog was found in the GLEAN3 prediction set, the genome database (Whole Genome Shotgun, at HGSC-BCM) and EST databases (NCBI) were searched by TBLASTN. The identification of the sea urchin protein was based on the best score and confirmed by reciprocal blast on the NCBI databases. The predicted proteins were analyzed using the precomputed information in the sea urchin annotation and the GENBOREE tools available at the Human Genome Sequencing Center at Baylor College of Medicine. Expression in the embryo of the annotated sequences was assessed from the tiling array database (Samanta et al., this issue) made available in the GENBOREE tool.

The putative proteins were aligned with homologs from different species, retrieved from NCBI databases or from genome-specific databases (*Nematostella vectensis* at http://www.stellabase.org/ (Sullivan et al., 2006); *Ciona intestinalis* at http://genome.jgi-psf.org/Cioin2/; *Caenorhabditis elegans* at http://www.wormbase.org). Multiple alignments were done using ClustalW (Thompson et al., 1994). Phylogenetic tree were produced using neighbor joining methods using PhyloWin (Galtier et al., 1996) or MEGA 3.1 (Kumar et al., 2004).

The proteins containing a putative eIF4E-binding motif (Mader et al., 1995) were searched by screening the sea urchin predicted proteins sequences (GLEAN3 set) with [RK]-x(2)-Y-x(4)-L-[MLIVFWY] pattern using ScanProsite (Gattiker et al., 2002). The hits sequences were queried against Uniprot database with BLAST program (Altschul et al., 1990) and against the PFAM database using the hmmpfam program (Bateman et al., 2004).

Results and discussion

Genes involved in the initiation step of translation

Translation initiation involves a complex biological machinery (review in Hershey and Merrick, 2000). Briefly, the cap structure of the mRNA interacts with the cap-binding protein eIF4E, which forms a stable ternary complex with eIF4A and eIF4G. eIF4A, is a DEAD family RNA helicase, thought to unwind secondary structures of the 5′ untranslated region of the mRNA. eIF4G is a scaffolding protein that interacts with eIF4E and eIF4A. eIF4G binds also to the poly(A)-binding protein, which interacts with the poly(A) tail, and this allows a closed loop conformation of the mRNA, thought to stabilize the interaction of the cap-binding complex and to facilitate recycling of ribosomes. A ternary complex comprising eIF2, GTP and initiator methionyl-tRNA, binds to the 40S ribosomal subunit to form the 43S complex together with other initiation factors. This complex is recruited to the mRNA via interaction of the multi-subunit eIF3 with eIF4G and scans the mRNA up to the start codon. Initiation codon recognition triggers eIF2-bound GTP hydrolysis, releasing of initiation factors and joining of the large ribosomal subunit. The ribosome is then ready for the elongation step. We identified in the sea urchin genome an ortholog of each translation factor as compared to the mammalian set, shown to be involved in the initiation step. Table 1 presents the translation factors and their role in each step of translation. Because in the sea urchin, the cap-binding protein eIF4E and its regulators have been implicated in the regulation of events following fertilization (Cormier et al., 2001), we will focus in this section on these actors.

eIF4E: three different family members in sea urchin

Recent genome-wide sequencing projects have shown the existence of several genes related to eIF4E in different organisms. Three eIF4E-family members have been described in mammals (Joshi et al., 2004), eight isoforms in *Drosophila melanogaster* (Hernandez et al., 2005) and five different eIF4E in *C. elegans* (Keiper et al., 2000), whereas only one eIF4E is present in *Saccharomyces cerevisiae*. The eIF4E-related proteins can be clustered in three families termed eIF4E1, eIF4E2 and eIF4E3. The structure of eIF4E1 proteins has been conserved through evolution: human and yeast eIF4E1 share 30% identity, and human eIF4E1 can rescue a yeast mutant. A C-terminus core domain of approximately 170 amino acids comprising 8 tryptophane residues is well conserved and is sufficient for cap recognition, binding to its inhibitor 4E-BP or to eIF4G and stimulation of cap-dependent translation (Marcotrigiano et al., 1997). eIF4E1 is the general cap-binding protein, whereas the role of the related eIF4E proteins is not fully understood. eIF4E2 shares 28% identity with eIF4E1 and has been reported not to interact with eIF4G in vitro (Rom et al., 1998; Joshi et al., 2004). RNAi knockout of *C. elegans* eIF4E2 (ife4) leads to pleiotropic effects and is correlated with a modification of the translation of a small subset (0.2%) of mRNA (Dinkova et al., 2005). *Drosophila* eIF4E2 (d4EHP) maintains the caudal mRNA in a translational inactive state, by interaction with both the cap structure and the mRNA-bound Bicoid protein (Cho et al., 2005). This suggests that eIF4E2 may be implicated in translation inhibition of specific mRNAs. Mouse eIF4E3 interacts with eIF4G, but not with 4E-BPs and is not ubiquitously expressed, suggesting that eIF4E3 could function as a tissue-specific translation factor (Joshi et al., 2004).

We have found three representatives of eIF4E in the sea urchin genome (Sp-4E1 SPU_028477, Sp-4E2 SPU_016729, Sp-4E3 SPU_025634) with an identity of 61%, 67% and 55%, respectively, with their mouse counterparts. The three sea urchins eIF4E share 30% identity one with each others. The C-terminus core domain of approximately 170 amino acids is conserved among the sea urchin eIF4Es (Fig. 2). The residues involved in cap structure recognition and in interaction with eIF4G and 4E-BP (Marcotrigiano et al., 1997, 1999) are

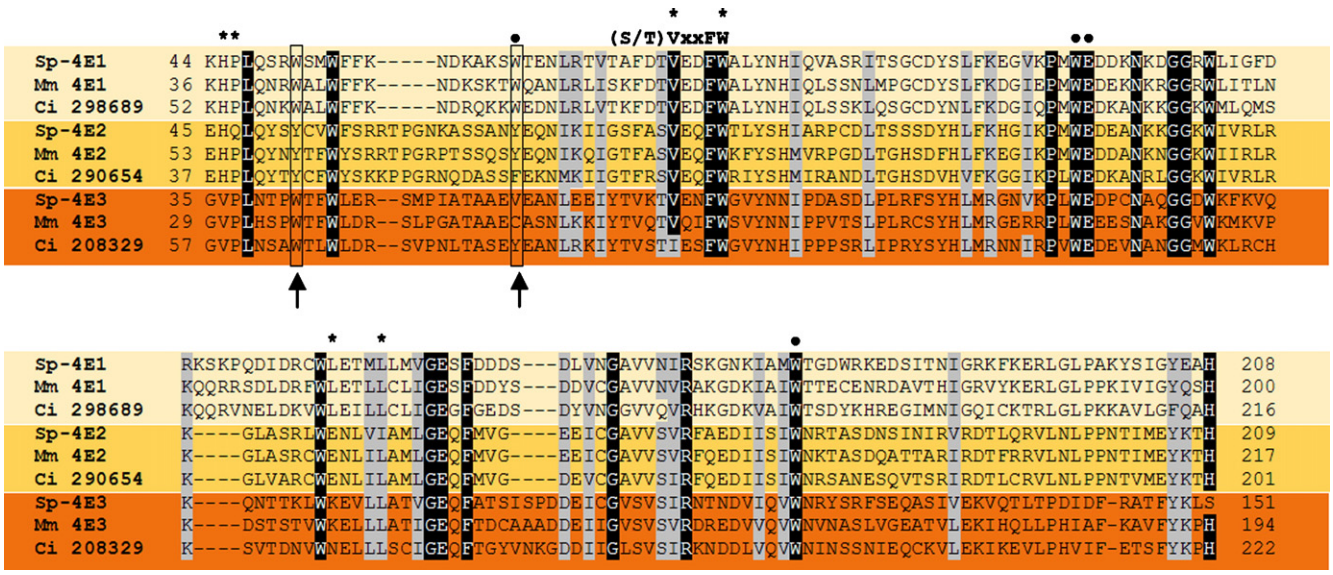

Fig. 2. Alignment of the core sequence of *S. purpuratus* (Sp) eIF4E isoforms with eIF4E proteins from *M. musculus* (Mm) and *C. intestinalis* (Ci). The core sequence corresponds to a ~170 amino acids region between two histidine residues (His36-200 in mouse eIF4E1). Identical and conserved residues greater than 85% of the sequences are shadowed in black and grey, respectively. The residues involved in eIF4E interaction with 4E-BP and eIF4G are indicated by a star, residues involved in cap structure recognition are labeled with a dot and the location of the conserved consensus sequence (S/T)VxxFW is noted above the alignment (all accession numbers are listed Table S1). The Trp43 and Trp56 residues used in the classification are indicated by an arrow. All sequences are grouped within subfamilies and boxed.

conserved in Sp-eIF4E1 (Fig. 2, residues indicated by stars and dots), suggesting that this protein may fulfil the *bona fide* cap-binding translation factor in sea urchin.

It has been suggested that eIF4E1 is present in all eukaryotes and eIF4E2 appears to be restricted to metazoans. eIF4E3 is well represented in chordates, is absent in *Drosophila* and nematodes (Joshi et al., 2004) and has been sporadically found in some other metazoans recently (Joshi et al., 2005). A fuller picture of eIF4E3 representation will be possible as more genome projects are available. Recently, Joshi et al. (2005) suggested a classification of eIF4E proteins in three classes based on comparison of the residues located at positions equivalent to Trp43 and Trp56 in human eIF4E1 in more than 200 species. Class I eIF4E contains Trp residues at both positions (and corresponds to eIF4E1). Class II eIF4E, corresponding to eIF4E2, possesses a Tyr residue instead of Trp43 and a hydrophobic residue (Tyr, Phe or Leu) at position 56. Class III eIF4E, like eIF4E3, contains a Trp43 and a Cys/Tyr residue instead of Trp56. The sea urchin eIF4E3 is the first echinoderm sequence available and contains a Val residue instead of a Cys/Tyr substitution at position 56, in an otherwise well-conserved sequence towards class III eIF4E. Because cnidarian and mollusks eIF4E3 sequences follow the class III substitution rules (Joshi et al., 2005), we asked whether the Val56 substitution was unique to *S. purpuratus* or was found in others species. Interestingly, a search in the NCBI EST databases lead to the retrieval of two echinoderms eIF4E3 sequences (sea urchin *Paracentrotus lividus* AM214046; starfish *Asterina pectinifera* DB388235) containing also a Valine at position 56 (Fig. 3A), suggesting that this substitution has occurred early in the echinoderm lineage and may be functionally critical. Phylogenetic comparison of eIF4E

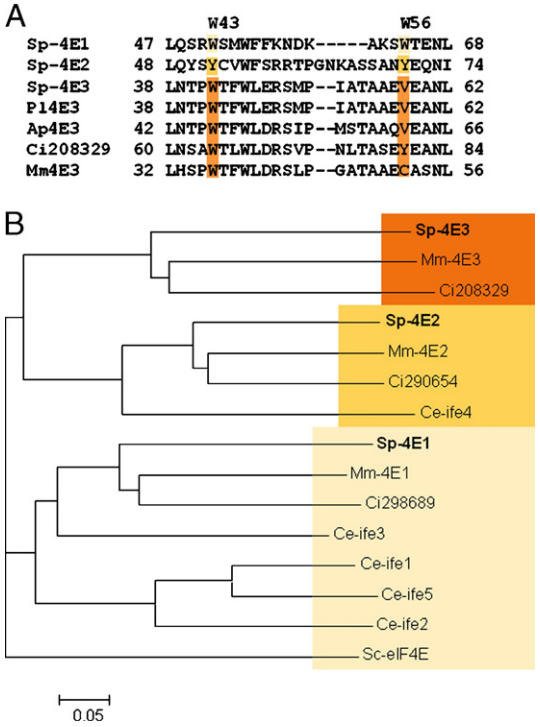

Fig. 3. (A) Trp56 is substituted by a valine residue in echinoderms eIF4E3. Sequence alignment of *S. purpuratus*, *P. lividus* and *A. pectinifera* eIF4E3 compared to other eIF4E proteins in the region containing Trp43 and Trp56 residues (as numbered in human eIF4E-1). (B) Clustering of *S. purpuratus* (Sp) eIF4E isoforms with eIF4E proteins from *M. musculus* (Mm), *C. intestinalis* (Ci), *C. elegans* (Ce) and *S. cerevisiae* (Sc) based on clustalW alignment (all accession numbers are listed Table S1). The three isoforms of *S. purpuratus* eIF4E (in bold) are distributed in each eIF4E class.

sequences from selected organisms suggests that eIF4E2 and eIF4E3 are closer to each other than to eIF4E1, and the three sea urchin proteins clustered within their respective subfamily (Fig. 3B), indicating that the diversity of cap-binding proteins is a more ancient characteristic than previously thought.

mRNAs for the three sea urchin family members are expressed in the *S. purpuratus* embryo, as assessed by the tiling expression data which detect transcribed genomic regions (Samanta et al., 2006, this issue) and in the *Sphaerechinus granularis* egg and early embryo by RT-PCR analysis (our unpublished data). Because cap-dependent translation during fertilization is regulated by association of eIF4E with its partners, the role of each of the three eIF4E proteins and their respective interaction with eIF4G and 4E-BP now have to be investigated in the sea urchin.

eIF4G

eIF4G is a modular scaffolding protein that plays a pivotal role in the assembly of mRNA-bound translation initiation factor and small ribosomal subunit. Only one eIF4G gene was detected in the sea urchin genome. The last two-third parts of the encoded protein is 36% identical with human eIF4GI and eIF4GII, the highest identity is found within the conserved domains shown to be implicated in the interaction of eIF4G with its partners. In mammals, eIF4G contains two conserved binding sites for the eIF4A RNA helicase and for eIF3 (MIF4G and MA3) and a C-terminus domain shown to interact with Mnk1. These three domains are well conserved (55%, 52% and 60%, respectively) within *S. purpuratus* eIF4G (Fig. 4). Although the overall identity with human eIF4Gs in the N terminal region is low, the eIF4E-binding site (YxxxxLΦ, where X is variable, and Φ is a hydrophobic residue) is conserved (Mader et al., 1995).

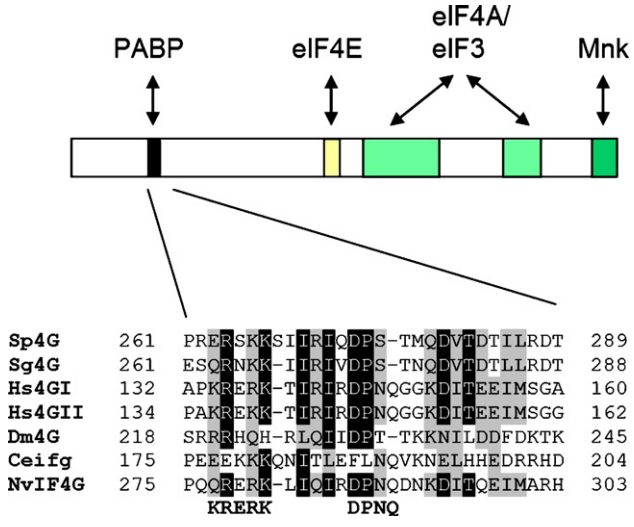

Fig. 4. Schematic structure of Sp-eIF4G protein, showing interacting regions with other proteins. Alignment of potential PABP-binding sites in eIF4G of sea urchin *S. purpuratus* and *S. granularis*, human eIF4GI and eIF4GII, *D. melanogaster*, *C. elegans* and *N. vectensis* eIF4G is shown. The residues implicates in the eIF4G-PABP interaction as determined in Imataka et al. (1998) are noted under the alignment (accession numbers are listed Table S1).

Human eIF4Gs were shown to interact with the poly(A)-binding protein (PABP) through a motif spanning amino acids 132–160 in the N terminus end of the protein. Mutating two sets of residues, KRERK and DPNQ within this motif abrogates the PABP-binding activity of eIF4G (Imataka et al., 1998). The N terminus end of sea urchin eIF4G contains a sequence that has a significant homology to the PABP-binding site (Fig. 4). It has been speculated that *D. melanogaster* eIF4G was not able to bind PABP because of the lack of a conserved motif (Hernandez et al., 1998). However, a putative PABP-binding motif in the fruit fly sequence (as well as in the *C. elegans* eIF4G) is highlighted here in comparison to the sea urchin motif (Fig. 4). Because the sequence of the PABP-binding site in eIF4G is not conserved between yeast and human, and our analysis suggests that it is less conserved in protostomes, we asked whether it was present in a cnidarian genome, *N. vectensis*. Strikingly, a predicted eIF4G protein from *N. vectensis* contains a PABP-binding site similar to human and sea urchin motifs (Fig. 4), suggesting that this interaction domain has emerged early in evolution.

The N-terminus region of *S. purpuratus* eIF4G is not conserved outside of the eIF4E- and PABP-binding sites. A strong homology (70% identity through the entire length) exists with a cDNA isolated from *S. granularis* eggs, Sg-eIF4G (AJ634049). In sea urchin eggs and embryos, Sg-eIF4G exhibits several isoforms that are post-translationally modified and associates to eIF4E following fertilization (unpublished data).

4E-BP

eIF4E-binding protein (4E-BP) is a small protein that inhibits cap-dependent translation by competing with eIF4G for a common binding site on eIF4E. Although three 4E-BP proteins (4E-BP1, 4E-BP2, 4E-BP3) exist in mammals, only one 4E-BP ortholog have been described in invertebrates (Bernal and Kimbrell, 2000; Cormier et al., 2001; Miron et al., 2001). A notable exception is the nematode *C. elegans*, where no 4E-BP homolog could be detected in the genome although five members of the eIF4E family are present. 4E-BPs phosphorylation status regulates its interaction with eIF4E: underphosphorylated 4E-BPs binds to eIF4E and inhibit cap-dependent translation, whereas hyperphosphorylated forms do not (Pause et al., 1994). In the sea urchin 4E-BP, degradation represents an additional level of control of this translational repressor. Following fertilization, 4E-BP is phosphorylated and rapidly degraded (Cormier et al., 2001; Salaun et al., 2003). The release of eIF4E from its repressor is correlated with a rapid increase in protein synthesis and is required for the onset of the first mitotic division of embryonic development (Salaun et al., 2005).

The Joshi/Jagus 4EBP database (http://umbicc3-215.umbi.umd.edu) shows 4E-BP sequences from protists, fungi and metazoa. In metazoa below urochordates only one form of 4E-BP is found per species. This database also gives sequences of EST encoding for 4E-BP from *S. purpuratus* and *S. granularis*. A search in the *S. purpuratus* genome reveals only one gene encoding for 4E-BP (SPU_005957). The predicted protein presents a core domain highly conserved (Fig. 5A) containing

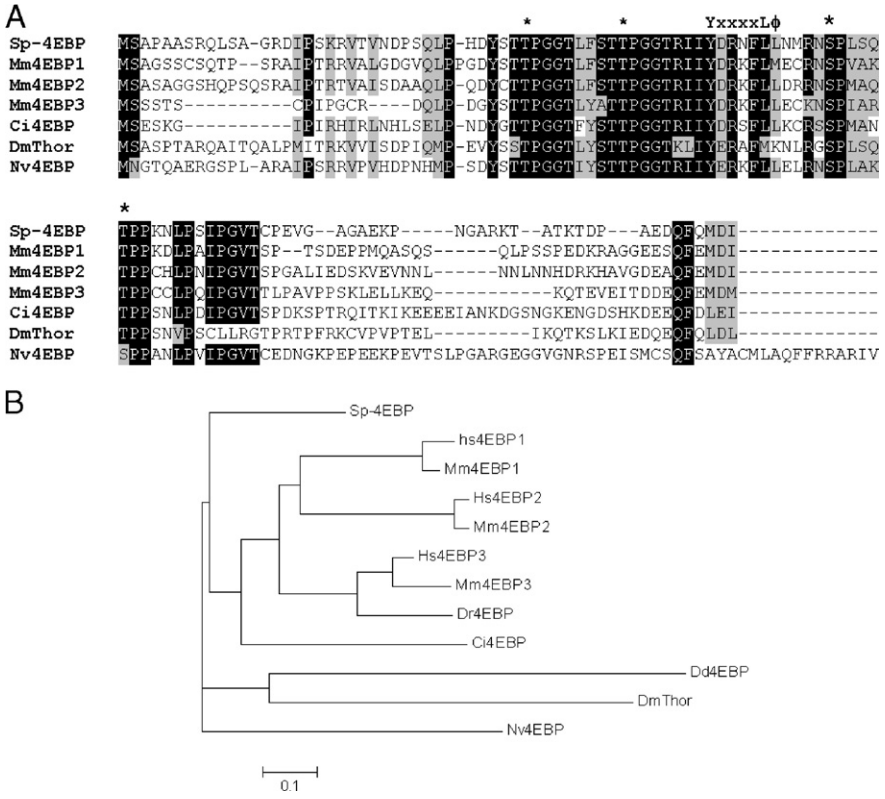

Fig. 5. (A) Alignment of sea urchin 4E-BP with *M. musculus*, *C. intestinalis*, *D. melanogaster* and *N. vectensis* 4E-BP proteins (full-length proteins). Identical and conserved residues in greater than 85% of the sequences are shadowed in black and grey, respectively. The conserved eIF4E-binding motif YxxxxLϕ is noted and the conserved phosphorylated serine and threonine residues are indicated by a star. (B) Consensus tree derived from a clustalW alignment of 4E-BPs from *S. purpuratus*, *Homo sapiens*, *M. musculus*, *Danio rerio*, *C. intestinalis*, *Dictyostelium discoidum*, *D. melanogaster* and *N. vectensis* (accession numbers are listed Table S1).

the eIF4E-binding motif (YxxxxLΦ) and the phosphorylation sites (Thr37, Thr46, Ser65 and Thr70, numbered according to *Mus musculus* 4E-BP1) involved in eIF4E-binding regulation (Gingras et al., 1999). Furthermore, conservation of these sites in radial animals such as *N. vectensis* (Fig. 5A) suggests a high level of selection for these regulatory sites and therefore of functional relevance. The consensus analysis from the alignment of 4E-BP from selected species suggests that 4E-BP gene duplication occurred recently in vertebrates (Fig. 5B).

Translational regulators including eIF4E interacting proteins

As discussed above, eIF4E/eIF4G assembly is prevented by eIF4E-binding proteins (4E-BPs), which are translational repressors that compete with eIF4G for interaction with eIF4E. Whereas 4E-BP interference with cap-dependent translation has an effect on a large spectrum of mRNAs, translation of specific mRNAs can be regulated through the association of proteins (or complexes) interacting both with the eIF4E and the mRNA (see below). Furthermore, several eIF4E proteins exists within an organism with different properties in translation regulation (see below). Besides its role in translation, eIF4E has been implicated in the nuclear export of a specific subset of transcripts (Topisirovic et al., 2003). Therefore, eIF4E is a major target for regulation of gene expression and plays an important role during embryogenesis of different organisms (Klein and Melton, 1994; Amiri et al., 2001; Cormier et al., 2001; Robalino et al., 2004).

The translation initiation factor 4E nuclear import factor (or 4E-T) is a nucleocytoplasmic shuttling protein that interacts with eIF4E both in the nucleus and in the cytoplasm, 4E-T mediates the import of eIF4E into the nucleus (Dostie et al., 2000). In the cytoplasm, 4E-T co-localizes with eIF4E and with mRNA decapping factors in P-bodies, to repress translation and enhance mRNA degradation (Ferraiuolo et al., 2005). A 4E-T homolog was indeed found in the sea urchin genome (SPU_024640) and contains the 4E-binding motif.

DEAD box RNA helicase p54/rck/DDX6, which is highly conserved through evolution, has been implicated in translation repression and mRNA decay. In *Xenopus* oocytes, p54 binds to CPEB (CPEB binds specific sequences in the 3′ untranslated region of mRNAs and mediates cytoplasmic polyadenylation, Hake and Richter, 1994; Stebbins-Boaz et al., 1996) on untranslated mRNAs and co-immunoprecipitates with eIF4E, although direct interaction is unclear (Minshall and Standart, 2004). In addition, p54 interacts with eIF4E and eIF4E-T in vivo in mammalian P bodies, where untranslated mRNAs are localized and degraded, suggesting a central role of eIF4E in the transition from translation to decay in the mRNA life (Andrei et al., 2005). p54/rck/DDX6 homolog in sea urchin (Sp-DDX6, SPU_023295) is 70% identical to its *Xenopus* and human counterpart and may fulfil similar roles.

Other proteins are known to interact with eIF4E in several species, and participate in the translational regulation of mRNAs involved in cell cycle and embryogenesis. In the sea

urchin genome, these proteins were not found or do not display an eIF4E-binding site. In *Drosophila*, the protein Cup bridges eIF4E to RNA-binding proteins Smaug or Bruno to inhibit translation of nanos or oskar mRNAs, respectively (Nakamura et al., 2004; Nelson et al., 2004). No Cup homolog was found in the sea urchin genome. In *Xenopus* oocytes, Maskin interacts with eIF4E and CPEB and maintains CPE-containing mRNAs in a translational inactive state (Stebbins-Boaz et al., 1999). Maskin homologs in other species, the transforming acidic coil coil domain proteins (TACC), do not bind eIF4E. A TACC homolog was found in the sea urchin genome (SPU_011724) and does not contain an eIF4E-binding motif. In *Drosophila*, the homeodomain-containing protein Bicoid directly interacts with eIF4E2 to inhibit translation of caudal mRNA, which encodes a transcription factor implicated in posterior segmentation (Cho et al., 2005). A Bicoid homolog in the sea urchin genome is homeodomain Sp-Hox11/13c protein (SPU_000388) that does not display the 4E-binding motif. Human Prh and Hoxa9 interact with eIF4E and regulate eIF4E-mediated mRNA transport, but for both the 4E-binding site is located in the N terminal region of the proteins (Topisirovic et al., 2003, 2005) that is lacking in their respective sea urchin homologs, Sp-Hex (SPU_027215) and Sp-Hox9/10 (SPU_002633) (Cameron et al., 2006). These regulations may be tissue or lineage specific and may not be conserved, as suggested by the sea urchin genome examination.

A common eIF4E-binding motif is used by a variety of proteins to interact with eIF4E, such as eIF4G and 4E-BP. The minimal eIF4E recognition motif was defined as YxxxxLΦ (where X is variable, and Φ is a hydrophobic residue). Most 4E-binding sites from eIF4G and 4E-BP homologs begin at a conserved basic residue three amino acids N-terminal to the invariant tyrosine. In order to screen the sea urchin genome for eIF4E partners, we carried out a search on the sea urchin genome for the presence of a motif defined as: (K/R) xxYxxxxLΦ (Mader et al., 1995). This screening lead to the isolation of 970 unique GLEAN predictions (see Supplementary Table S3), that can be clustered into ~350 groups depending on their PFAM motifs. Interestingly, the eIF4E-binding motif is conserved in ~120 homologous human proteins, suggesting high evolutionary conservation of this domain. Among the proteins, we found the already described 4E-binding proteins known to interact physically with eIF4E (eIF4G, 4E-BP, 4E-T). We noticed a number of protein kinases bearing the eIF4E motif, which could participate in the signal transduction pathway regulating translation in response to various physiological conditions (Table 2).

We also found an eIF4E-binding motif in five homeodomain proteins, including the Sp-pmar1b gene product (SPU_014722), involved in the early micromere specification pathway (Oliveri et al., 2002). Although homeoproteins are considered to act primarily as transcription factors, recent data suggest that they can also affect gene expression at a post-transcriptional level through modulation of eIF4E activity (Topisirovic et al., 2003, 2005; Cho et al., 2005). In the sea urchin as well, a relationship between homeoproteins and eIF4E seems to be a possible gene expression regulatory mechanism. Although the physical interaction of potential candidates with eIF4E has yet to be demonstrated, the proteins identified in this screen could represent potential new regulators of cap-dependent translation and probably of non-translation-related functions of eIF4E. Whether the translational regulatory function and mechanism of the cap-dependent interfering proteins are conserved, and how they regulate developmental decisions in the sea urchin embryo is now a challenging question.

Table 2
Protein kinases containing an eIF4E-binding consensus motif in *S. purpuratus* and in human

Protein name	SPU# (Sp) or Ac# (Hs)	Motif
Protein kinase C iota	SPU_000180	RGSYAKVLLV
	P41743	RGSYAKVLLV
NIMA related kinase 4	SPU_023369	KGSYGEVWLV
(NEK4)	Q6P576	KGSYGEVTLV
Polo-like kinase 2	SPU_000468	RLSYARHVLY
(PLK2)	Q9NYY3	RMEYALNMLL
TEK tyrosine kinase	SPU_028625	KFLYLLLPLL
	Q5TCU2	RKTYVNTTLY
Tau tubuline kinase 1	SPU_021174	KEKYDHRLLL
	Q5TCY1	KEKYEHRMLL
ERK5 (Mitogen-activated	SPU_025159	KDVYVVFDLM
protein kinase 7)	Q96G51	KSVYVVLDLM
Stress-activated protein	SPU_028478	KRAYREYILM
kinase JNK3	Q49AP1	KRAYRELVLM
Mitogen-activated protein	SPU_010118	KRTYRELRLL
kinase p38α	Q16539	KRTYRELRLL
ERK1 (Mitogen-activated	SPU_019715	RDVYIVQSLM
protein kinase 3)	P27361	RDVYIVQDLM
RIO1 kinase	SPU_004814	RRIYQKAKLV
	Q5W0K5	RRMYQDARLV
Consensus		R/KxxYxxxxLΦ

IRES-dependent translation

Although the majority of mRNAs are translated through cap-dependent initiation, an alternative cap-independent mechanism of translation has been uncovered. IRES (internal ribosome entry site) were initially discovered in virus (Pelletier and Sonenberg, 1988), but in the past few years, a growing list of cellular mRNAs were shown to be translated via an IRES-dependent translation. By polysome profiling analysis, 3% of the cellular mRNAs remain associated to polysomes during the general inhibition of cap-dependent translation. The mRNAs able to be translated in such conditions encodes for regulatory proteins implicated in cell growth, proliferation, differentiation or apoptosis (Stoneley and Willis, 2004; Komar and Hatzoglou, 2005). Indeed gene expression needs to be tightly controlled during these processes and this suggests that IRES-dependent translation allows for selective translation of specific mRNAs when global translation is repressed.

The mechanism controlling IRES-mediated translation remains unclear. IRES activity resides in sequences usually located in the 5′ untranslated region of the mRNA and requires the binding of canonical translation factors (eIF3, eIF4A, eIF4G, etc.), as well as RNA-binding proteins such as DAP5, Unr (upstream of N-ras), PTB (polypyrimidine tract-binding protein), PCBPs (polyC-binding proteins) and hnRNPK (heterogeneous nuclear ribonucleoprotein K) (Stoneley and Willis,

2004). These RNA-binding proteins are termed ITAFs, for "IRES *trans*-acting factors". DAP5, a protein homologous to the C-terminal end of eIF4G, is implicated in the IRES-dependent translation of several apoptosis related protein and in its own translation (Marash and Kimchi, 2005). DAP5 associates to eIF4A and eIF3, but not to eIF4E, suggesting that it can sustain translation when cap-dependent translation is inhibited. Cellular IRES-dependent translation has been mostly demonstrated in mammalian cells, but examples of mRNAs translated via IRES exist in *D. melanogaster* (Ye et al., 1997; Hernandez et al., 2004) and may be widely used throughout evolution. Although the function of ITAFs is probably not restricted to IRES-dependent translation, it is interesting to find in the sea urchin genome homologs for DAP5 (SPU_028861), Unr (SPU_010430), PTB (SPU_000961), PCBPs (3 genes SPU_005686, _027712, _027713) and hnRNPK (SPU_008011). It is then a challenging perspective to address the question of alternative translation in the sea urchin embryo.

Genes involved in the elongation step of translation

The elongation phase of protein synthesis depends on the selection of an aminoacylated-tRNA (aa-tRNA) according to the sequence of codons of the mRNA and on the formation of a peptide bond between a growing peptide and the incoming amino acid. This ribosomal process is assisted by two elongation factors consisting of eEF1A and eEF2 that are G-proteins and one known exchange factor, eEF1B (Merrick and Nyborg, 2000; see Table 1). Both eEF1A and eEF2 ensure GTP-dependent codon/anticodon recognition and translocation of the peptidyl-tRNA, respectively. Due to their essential role in the accuracy of translation, it is not surprising to find reasonable homology in the sequences and structures of the two G-protein elongation factors through evolution from prokaryotes to mammals (see Beane et al., this issue). The *S. purpuratus* genome contains a unique SpEF1A gene (SPU_000595), which encodes for a protein that has 77.8% identity with human eEF1A1 (NP_001393.1) and 77.2% with human eEF1A2 (NP_001949.1). The SpEF2 gene (SPU_010829) encodes for a protein that shares 75.1% identity with human eEF2 (NP_001952.1).

Regulation by eEF1B

Whereas no guanine nucleotide exchange factor is known for eEF2, eEF1A requires a factor to enhance the off-rate of GDP after GTP hydrolysis. The macromolecular factor eEF1B which serves this function in eukaryotes, increases in complexity through evolution (review in Le Sourd et al., 2006a). In fungi, eEF1B complex is made of two subunits, eEF1Balpha that possesses the nucleotide exchange activity and eEF1Bgamma that is assumed to be an anchorage protein. In plants, eEF1B comprises three subunits, the anchoring protein eEF1Bgamma in association with two nucleotide exchange proteins, eEF1-Balpha and eEF1Bbeta. In the metazoans eEF1B complex, the anchoring protein eEF1Bgamma is also associated with two guanine nucleotide exchange factors, eEF1Balpha which is homologous to the plant eEF1Balpha and eEF1Bdelta, which clearly differs from the plant eEF1Bbeta. The alpha, beta and delta subunits of eEF1B display a high homology in their C-terminus region, where the nucleotide exchange activity is, whereas only eEF1Bdelta contains a leucine zipper domain in its N-terminus (Guerrucci et al., 1999). Furthermore, one aminoacyl-tRNA synthetase, the Valyl-tRNA synthetase (VARS), was shown to be anchored to eEF1B in artemia and mammals (Bec et al., 1994; Brandsma et al., 1995). The sophisticated supramolecular structure of the eEF1B complex containing many phosphorylation sites, makes it a potential regulator of the elongation step (Le Sourd et al., 2006a). The complete sea urchin genome will give insights in the complexity of metazoan eEF1B structure and motifs of functional relevance.

Existence of two alternatively spliced cellular subsets of eEF1B complexes in sea urchin

Each subunit of the eEF1B complex appears to be encoded by a unique gene in the sea urchin genome. The sequences of the proteins encoded by the eEF1Balpha and the eEF1Bgamma genes (respectively SPU_015867 and SPU_002587) matched with the sequences deduced from the cDNAs that we recently obtained for each subunit (respectively AJ973180 and AJ973179). Annotation of the *S. purpuratus* genome leads to the characterization of a unique gene that encodes for the eEF1Bdelta protein (SPU_000960, Fig. 6). Studies on eEF1B in *S. granularis* demonstrated the presence of two eEF1Bdelta isoforms in the same eEF1B complex when purified from the embryos (Boulben et al., 2003). eEF1Bdelta1 (Y14235; Delalande et al., 1998) and eEF1Bdelta2 (AJ97318; Le Sourd et al., 2006b) cDNA encoded proteins are identical except for a 26 amino acids insert, which is present in the N-terminal domain of the eEF1Bdelta2 protein upstream of the leucine zipper motif. Remarkably, exon 2 from the *S. purpuratus* eEF1Bdelta gene encodes for a peptide matching exactly the 26 amino acids insert present in the *S. granularis* eEF1Bdelta2 isoform, thus alternative splicing of the transcript from the unique eEF1delta gene leads to the production of the two protein eEF1Belta isoforms (Fig. 6). Moreover, searches in the databank using NCBI resources revealed that the feature found in the sea urchin genome is of general significance in all metazoans from cnidarians to mammals, i.e., presence of one eEF1Bdelta gene leading by alternative splicing to two mRNA isoforms encoding for two proteins, eEF1Bdelta1 and eEF1B-delta2 (Le Sourd et al., 2006a). Two eEF1Bdelta proteins were co-expressed in sea urchin cleavage stage embryo and were found associated in two subsets of eEF1B complexes: one containing eEF1Bdelta1 and eEF1Bdelta2 in a 1:1 ratio, and the other containing only eEF1Bdelta1 (Le Sourd et al., 2006b). This structural organization of eEF1B complexes may be universal through evolution and therefore of functional relevance.

CDK1/cyclin B phosphorylation motif in eEF1B subunits

A potential translational regulatory role has been attributed to the eEF1B complex from its characterization as a CDK1/cyclin B physiological substrate in a number of vertebrates.

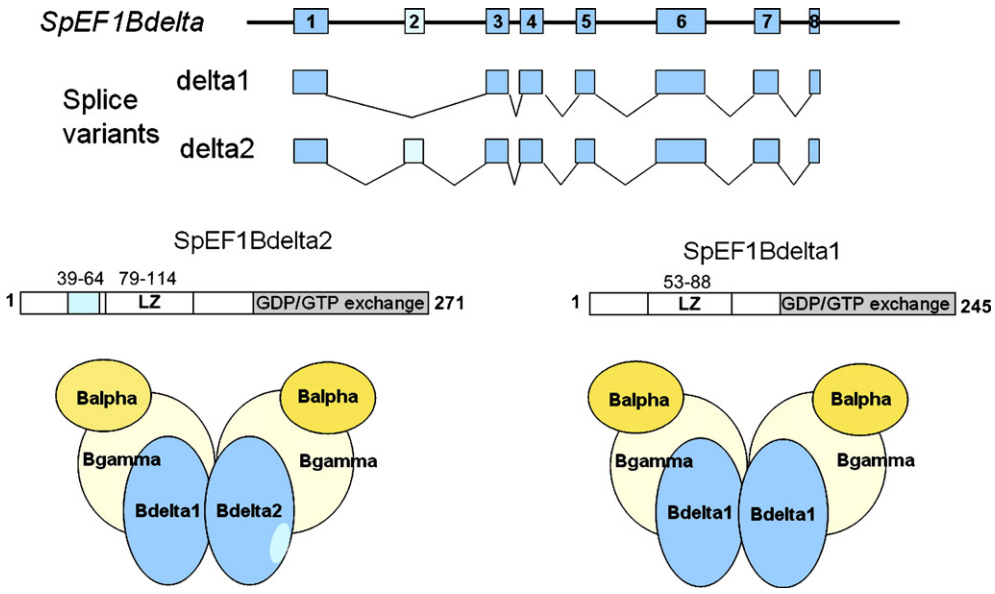

Fig. 6. The SpEF1Bdelta gene encodes for two eEF1Bdelta proteins. Two mRNAs originate from alternative splicing of the unique SpEF1Bdelta gene, comprising eight exons. The eEF1Bdelta1 and delta2 proteins are represented with their functional domains: the leucine zipper motif (LZ) and the GDP/GTP nucleotide exchange domain. The positions of each domain are numbered in the respective sequences as well as the eEF1Bdelta2 protein-specific insert encoded by exon 2. The lower part of the figure shows a schematic representation the two subsets of eEF1B supramolecular complexes.

Indeed, the eEF1Bgamma proteins from most deuterostomes contain a CDK1/cyclin B phosphorylation motif (review in Le Sourd et al., 2006a). Surprisingly, the sea urchin eEF1B gamma homolog does not. Conversely, the sea urchin eEF1Balpha was found to contain a CDK1/cyclin B phosphorylation motif whereas eEF1Balpha subunits from other deuterostomes do not. Analysis of all the known metazoan eEF1B sequences in the databases verified that most of the species in which no phosphorylation motif exists in the eEF1Bgamma sequence does contain this motif in the eEF1Balpha sequence. This is the case in a number of arthropods (*D. melanogaster*, *Bombyx mauri*, *Callinectes sapidus*) and in several nematodes (e.g., *Globodera rostochiensis*) (review in Le Sourd et al., 2006a). Because in all eukaryotes eEF1Balpha and eEF1Bgamma proteins are always found in association, the feature discovered in sea urchin that the eEF1B complex is phosphorylated by CDK1/cyclin B on one of its subunits depending on the species, appears universal and is suggestive of a conserved function through the eukaryotic kingdom.

Echinoderm eEF1B, a primitive form of complex devoid of Valyl-tRNA synthetase?

In vertebrates, Valyl-tRNA synthetase (VARS) is the only cellular aminoacyl-tRNA synthetase to be found associated within a macromolecular complex with eEF1B (Bec et al., 1994). Although the functional relevance of the association of VARS in eEF1B is not known, its universality in vertebrates suggests evolution constraints for maintaining this unique aminoacyl-tRNA in eEF1B. VARS is thought to be associated in eEF1B by interaction with the eEF1Bdelta subunit, through an extension of its N-terminal domain that is not present in VARS from fungi (Bec et al., 1994). The emergence of eEF1Bdelta appears in early metazoans (review in Le Sourd et al., 2006a) from gene duplication of an eEF1Balpha ancestor (Guerrucci et al., 1999). The sea urchin complete genome contains the eEF1Bdelta gene (see above) and does contain two genes (SPU_008058 and SPU_002908) encoding for two related (47% identity) proteins that share, respectively, 48% and 59% identity with human VARS2. However, the putative eEF1Bdelta anchoring N-terminal extension domain present in the vertebrates VARS2 was not found in the sea urchin sequences, neither in the two identified isoforms VARS isoforms, nor in the full genome assembly. Thus, sea urchin eEF1B would appear to be a model during evolution in which eEF1Bdelta is already present in the guanine nucleotide exchange complex whereas VARS anchoring is not yet realized.

Genes implicated in termination

Translation termination in eukaryotes is governed by two interacting factors, eRF1 and eRF3. eRF1 recognizes the stop codon in the A site of the ribosome and induces peptidyl-tRNA hydrolysis (Inge-Vechtomov et al., 2003). Sp-eRF1 (SPU_023948) shows a strong sequence identity with human and *D. melanogaster* eRF1 (83%) for all the three domains of eRF1 (Fig. 7): the N domain that participates in stop codon recognition, the M domain implicated in peptidyl transferase hydrolytic activity, the C domain necessary for phosphatase 2A and eRF3 binding.

eRF3 is a GTPase, which enhances the termination efficiency by stimulating the eRF1 activity in a GTP-dependent manner. eRF3 possesses a conserved C-terminal region that has a significant homology with eEF1A whereas the N-terminal domain of eRF3 proteins diverges. In yeast, the N terminal end of eRF3 (Sup35) bears a prion forming domain (Inge-Vechtomov et al., 2003). In mammals, two genes encode for

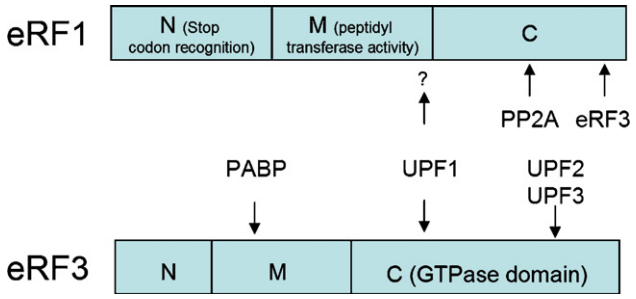

Fig. 7. Schematic structure of termination factor eRF1 and eRF3 and interaction with known partners. See text for details.

eRF3 proteins, GSPT1 and GSPT2 that differ in their N-terminal domains (Hoshino et al., 1998), which have no homology with yeast N-domain. Sp-eRF3 (SPU_003213) shows 68% and 49% identity in its C-terminal domain with human GSPT1 and yeast eRF3, respectively.

The translation termination complex is formed by additional proteins that interact with eRF1 or eRF3 (Fig. 7). eRF3 binds to the poly(A)-binding protein (PABP), an RNA-binding protein that plays a role in stabilization of mRNAs and promotes translation initiation (Mangus et al., 2003; Kahvejian et al., 2005). eRF3-PABP interaction enhances the efficiency of termination and mediates the coupling of termination and initiation of protein synthesis (Cosson et al., 2002a; Uchida et al., 2002). In vertebrates, two genes code for PABPC1 and PABPC2 (also called ePABP). PABPC2 is the major poly(A)-binding protein expressed during early development in mouse and *Xenopus* embryos (Cosson et al., 2002b; Seli et al., 2005). The sea urchin genome contains only one cytoplasmic PABP (SPU_015317), a common property of many invertebrates sequenced so far. In addition, translation regulation at the level of cytoplasmic polyadenylation is a well-documented mechanism in fly and *Xenopus* embryos (Groisman et al., 2002; Juge et al., 2002; Castagnetti and Ephrussi, 2003; Barnard et al., 2004) and starts to be uncovered in somatic cells (review in Le Breton et al., 2005). The sea urchin contains genes involved in cytoplasmic polyadenylation such as CPEB, Symplekin, CPSF (see Juliano et al., this issue; and Kelkar et al., unpublished results). Moreover, fertilization of sea urchin eggs is associated with an increased polyadenylation of mRNAs (Wilt, 1977).

UPF (up-frameshift protein) factors bind to eRF1 and eRF3. UPF are essential for NMD (nonsense-mediated decay), a phenomenon that provokes the rapid decay of premature-termination (nonsense) codons containing mRNAs (Culbertson and Neeno-Eckwall, 2005). Nonsense codons arise as a result of mutations or RNA splicing errors. NMD is a surveillance mechanism that detects and degrades nonsense codons bearing mRNAs that encode truncated proteins. In yeast, UPF proteins also stimulate the efficiency of translation termination (Maderazo et al., 2000). Upf1p interacts with both eRF1 and eRF3, whereas Upf2p and Upf3p interact only with eRF3. In mammalian cells, depletion of UPF1 and UPF2 by RNA interference (RNAi) has no measurable effect on nonsense suppression (Mendell et al., 2002). Sp-UPF1, Sp-UPF2 and Sp-UPF3 have been found in the sea urchin genome (respectively SPU_008215, SPU_017515, SPU_000502). Whether NMD factors are involved in the increase of translation termination efficiency in sea urchin remains to be determined.

In yeast, Dom34 and Hbs1, two proteins similar to the translation termination factors eRF1 and eRF3, respectively, are involved in the stop codon independent release of stalled ribosomes and trigger endonucleolytic cleavage of the mRNA (Doma and Parker, 2006). Dom34 (also called Pelota) is evolutionarily conserved and functions in meiotic and mitotic progression, probably through translation regulation (Adham et al., 2003; Xi et al., 2005). Sp-Pelota (SPU_009044) and Sp-HBS1 (SPU_020816) were found in the sea urchin genome. The conservation of the surveillance mechanism during elongation described in yeast remained to be determined.

Signaling pathways in translation control

The activities of several translation factors are regulated through phosphorylation/dephosphorylation, and the kinases involved are under the control of signal transduction pathways (Fig. 8). The PI3K and FRAP/mTOR pathways target the phosphorylation of 4E-BP and its affinity to eIF4E (Raught et al., 2000). In addition, FRAP/mTOR affects phosphorylation status of eIF4G, which is speculated to induce conformational changes of the protein necessary for full activity (Raught et al., 2000). Through its activation of ribosomal S6 kinase, the mTOR pathway leads to phosphorylation of ribosomal protein S6 and helicase eIF4B (Tee and Blenis, 2005). The MAPK pathway activates Mnk1 which binds to eIF4G and phosphorylates eIF4E (Pyronnet et al., 1999). Initiation is also regulated by phosphorylation of eIF2 by eIF2 kinases. Phosphorylation of the alpha subunit of eIF2 at a conserved serine (Ser-51 in mammals) is a widely used mechanism of translational control in many organisms (Fig. 8). Phosphorylated eIF2alpha has an increased affinity to its guanine nucleotide exchange factor eIF2B, leading to the sequestering of eIF2B as an inactive complex with eIF2 and GDP. Translation is then inhibited because of the decreased overall rate of guanine nucleotide exchange on the remaining unphosphorylated eIF2. A family of eIF2 kinases has been identified, sharing sequences and

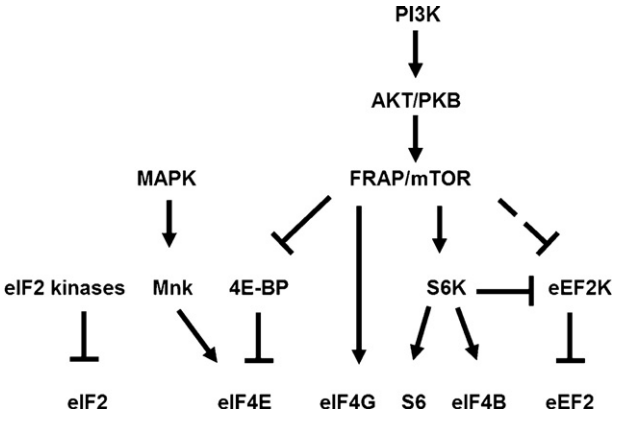

Fig. 8. Signaling pathways affecting the translation apparatus. The sea urchin genes orthologs of the kinases phosphorylating translation factors eIF2, eIF4E, eIF4G, eIF4B, eEF2 and ribosomal protein S6 are listed Table 3.

structural features in their catalytic domains, but with unique flanking regulatory regions allowing for distinct control pattern, in response to different stresses. In mammals, four kinases have been identified: GCN2 activated upon amino acid starvation, pancreatic eIF2alpha kinase (PERK) which inhibits protein synthesis in response to endogenous endoplasmic reticulum stress, heme regulated kinase (HRI) which couples translation to heme deprivation and oxidative stress and double-stranded RNA protein kinase (PKR) involved in antiviral pathways.

Sea urchin eIF2alpha (SPU_003646) possesses the conserved residue equivalent to the Ser-51, suggesting a possible control of translation at this level. We identified three eIF2 kinases in the sea urchin genome: Sp-GCN2 (SPU_004272), Sp-HRI (SPU_009270) and Sp-PERK (SPU_026724). No PKR homolog is found in the genome. PKR and HRI were previously thought to be restricted to mammals because GCN2 and PERK are the only eIF2 kinases present in *D. melanogaster* and *C. elegans* genomes; however, the recent discovery of HRI-related kinases in lower eukaryotes (Zhan et al., 2002) suggested a loss of this enzyme during evolution. The finding of HRI in the sea urchin genome, along with GCN2 and PERK, reinforces the early divergence of eIF2 kinases.

In mammals, translational regulation can occur through eEF2 phosphorylation by the highly substrate-specific calcium calmodulin-dependent eEF2 kinase (review in Browne and Proud, 2002). As a result of eEF2 phosphorylation on a Thr residue (Thr56 in human eEF2, conserved in the sea urchin homolog), the peptide chain elongation is inhibited. The elongation process is targeted via the eEF2 kinase by various signaling pathways (Browne and Proud, 2002). The PIP3 signaling pathway increases intracellular Ca2+ levels and activates the calmodulin-regulated eEF2 kinase. The cAMP signaling pathway through activation of PKA, phosphorylates and activates eEF2kinase. Finally, AMP-activated kinase was shown to activate eEF2 kinase, although the molecular mechanism involved in this case is not clear (review in Browne and Proud, 2002). Conversely, the FRAP/mTOR pathway via S6kinase, the MAP kinase pathway via p90rsk1 kinase and the stress kinase activated pathway via the p38 SAPK have been implicated in the regulation of elongation by phosphorylating eEF2 kinase on several inhibitory sites (review in Proud, 2004). Because the inhibition of eEF2 kinase leads to activation of eEF2 and therefore increases the elongation rate, an analysis of this regulation in sea urchin is interesting in the context of protein synthesis regulation during the early development. The *S. purpuratus* genome contains one eEF2 kinase gene (SPU_011641) sharing 45% identity with the human eEF2 kinase. Both the site of calmodulin binding (Trp85 as numbered in the human sequence) and the residue target for PKA phosphorylation (Ser500) are conserved in the sea urchin eEF2 kinase sequence. Other regulatory residues remain unknown, as not all the mammalians target sites are conserved. No eEF2 kinase homolog was found in the *S. cerevisiae* or *D. melanogaster* genomes, although it exists in *C. elegans*. It is therefore of importance to test the function of the sea urchin eEF2 kinase homolog to unreveal the evolutionary origin of eEF2 regulation by phosphorylation.

Protein synthesis in sea urchin eggs is stimulated upon fertilization, translational control is exerted through multifactorial mechanisms, including mRNA recruitment into polysomes and increased rates of translation initiation and elongation (Brandis and Raff, 1979; Hille and Albers, 1979). The stimulation in the rate of protein synthesis in sea urchin egg extracts is observed by the addition of exogenous eIF2, eIF2B or eIF4 (Colin et al., 1987; Huang et al., 1987; Dholakia et al., 1990; Akkaraju et al., 1991), suggesting that their activities must rise after fertilization. The sea urchin orthologs of the kinases affecting the activity of the translational apparatus are found in the sea urchin genome (Table 3) and the signal transduction pathways involving cascades of kinases are mostly conserved (Bradham et al, this issue). The PI3K pathway in sea urchin is involved in the fertilization process and is specially important in regulating translation at this step. Following fertilization of the sea urchin egg, 4E-BP is phosphorylated and rapidly degraded in a rapamycin sensitive FRAP/mTOR pathway, this degradation coincides with dissociation of 4E-BP from eIF4E (Cormier et al., 2001; Salaun et al., 2003). Furthermore, conservation of the phosphorylatable residues in Sp-4E-BP (Fig. 5A) reinforces the hypothesis that this regulatory pathway is conserved. The recycling of eIF2 is also an important regulatory step of protein synthesis upon fertilization (Dholakia et al., 1990; Akkaraju et al., 1991), and genomic data obtained from this analysis are interesting to analyze the signaling pathways linking fertilization to eIF2 activity regulation. Uncovering the actors of the signaling pathways will help apprehend better the regulatory events of translation.

Translational control implication in cancer and diseases

Translational control has been shown to be crucial in the regulation of gene expression and plays a primary role in cell-

Table 3
Genes for translation factors kinases in *S. purpuratus* genome

Name	Other name	SPU#	Function	Tiling expression data
Mnk		006947	eIF4E kinase, binds to eIF4G	++
FRAP/mTOR		006053	Phosphorylates and inactivates 4E-BP, acts upstream of S6K and eEF2K	++
eIF2 kinase 1	HRI	009270	Phosphorylate eIF2alpha and inhibits translation in response to various signals	+
eIF2 kinase 3	PERK	026724		+
eIF2 kinase 4	GCN2	004272		−
eEF2 kinase		011641	Phosphorylates eEF2 and inhibits translation, CaCAM-binding domain	+
$p70^{S6}$ kinase		011124	Phosphorylates ribosomal protein S6, eIF4B, eEF2 kinase	+++

cycle progression and cell differentiation. It has been known for many years that the aberrant levels of the factors involved in mRNA translation can contribute to diseases, mostly to cancer and tumors. Several reports demonstrate that aberrant expression of translation factors (including eIF2, eIF3, eIF4A, eIF4G, eEF1A and eEF1B) is a widespread feature of tumor development (review in Hershey and Miyamoto, 2000; Ejiri, 2002). Cell proliferation is associated with enhanced rates of protein synthesis which increase in response to treatment with cytokines, growth factors, hormones and mitogens (Cormier et al., 2003; Ruggero and Sonenberg, 2005). Oncogenic activity of translation factors was first suggested by the finding that eIF4E overexpression leads to the transformation of NIH-3T3 fibroblasts (Lazaris-Karatzas et al., 1990). Malignant transformation as a result of eIF4E overexpression may be explained by a specific effect on a subset of mRNAs containing long and structured 5′ UTRs, encoding proteins involved in cell growth and proliferation (e.g., growth factors and their receptors, signaling polypeptide, transcription factors and cyclins). Levels of the translation repressor 4E-BP protein increase rapidly in breast cancer lines and lymphoma when treated with TRAIL (Morley et al., 2005). Hypoxia, which impacts on malignant tumor progression, is associated with increased levels of 4E-BP in sea urchin embryos (Le Bouffant et al., 2006). The rapid degradation of 4E-BP following fertilization of sea urchin eggs (Cormier et al., 2001) provides an exquisite model system to analyze the post-translational turnover regulation of 4E-BP.

Recently, mutations in genes of the translational control machinery have been linked to several human diseases, highlighting the significance of this regulatory mechanism. Mutations in any of the five subunits of eIF2B are linked to the inherited brain disease, leukoencephalopathy with vanishing white matter (VWM), and mutations in three subunits are implicated in ovarioleukodystrophy (Abbott and Proud, 2004). Mutation in the EIF2AK3 gene, encoding for the endoplasmic reticulum eIF2alpha kinase (PERK), has been linked to the Wolcot–Rallison syndrome (Delepine et al., 2000). Deletion of WBSCR1 gene, which encodes for eIF4H, a novel initiation factor related to eIF4B, is associated to Williams–Beuren syndrome (Osborne et al., 1996; Richter-Cook et al., 1998). Misregulating the activity of translational control proteins may also have dramatic effects on human health. Felty syndrome and systemic lupus erythematosus are associated to high titer of antibodies directed against eEF1A1 and eEF2 kinase, respectively (Ditzel et al., 2000; Arora et al., 2002). In Alzheimer disease, the levels of phosphorylated forms of mTOR, 4E-BP and eEF2 kinase proteins were significantly increased, and total eEF2 significantly decreased, possibly leading to an up-regulation of the Tau mRNA translation (Li et al., 2005).

Translational control genes implicated in these human diseases possess an ortholog in the sea urchin genome, usually with minimal redundancy (see Tables 1 and 3). These translation-factor proteins are highly conserved throughout evolution. Therefore, sea urchin eggs and embryos offer a system in which to study the regulation of translation with a molecular, biochemical and developmental perspective and data obtained in this model system will give insights on the regulatory mechanisms of translation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2006.07.036.

References

Abbott, C.M., Proud, C.G., 2004. Translation factors: in sickness and in health. Trends Biochem. Sci. 29, 25–31.

Adham, I.M., Sallam, M.A., Steding, G., Korabiowska, M., Brinck, U., Hoyer-Fender, S., Oh, C., Engel, W., 2003. Disruption of the pelota gene causes early embryonic lethality and defects in cell cycle progression. Mol. Cell. Biol. 23, 1470–1476.

Akkaraju, G.R., Hansen, L.J., Jagus, R., 1991. Increase in eukaryotic initiation factor 2B activity following fertilization reflects changes in redox potential. J. Biol. Chem. 266, 24451–24459.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Amiri, A., Keiper, B.D., Kawasaki, I., Fan, Y., Kohara, Y., Rhoads, R.E., Strome, S., 2001. An isoform of eIF4E is a component of germ granules and is required for spermatogenesis in *C. elegans*. Development 128, 3899–3912.

Andrei, M.A., Ingelfinger, D., Heintzmann, R., Achsel, T., Rivera-Pomar, R., Luhrmann, R., 2005. A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. RNA 11, 717–727.

Arora, S., Yang, J.M., Craft, J., Hait, W., 2002. Detection of anti-elongation factor 2 kinase (calmodulin-dependent protein kinase III) antibodies in patients with systemic lupus erythematosus. Biochem. Biophys. Res. Commun. 293, 1073–1076.

Barnard, D.C., Ryan, K., Manley, J.L., Richter, J.D., 2004. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell 119, 641–651.

Bateman, A., Coin, L., Durbin, R., Finn, R.D., Hollich, V., Griffiths-Jones, S., Khanna, A., Marshall, M., Moxon, S., Sonnhammer, E.L., Studholme, D.J., Yeats, C., Eddy, S.R., 2004. The Pfam protein families database. Nucleic Acids Res. 32, D138–D141.

Bec, G., Kerjan, P., Waller, J.P., 1994. Reconstitution invitro of the valyl-transfer RNA synthetase-elongation factor (EF) 1 beta gamma delta complex-essential roles of the NH2-terminal extension of valyl-transfer RNA synthetase and of the EF-1 delta subunit in complex formation. J. Biol. Chem. 269, 2086–2092.

Bernal, A., Kimbrell, D.A., 2000. *Drosophila* Thor participates in host immune defense and connects a translational regulator with innate immunity. Proc. Natl. Acad. Sci. U. S. A. 97, 6019–6024.

Boulben, S., Monnier, A., Le Breton, M., Morales, J., Cormier, P., Belle, R.,

Mulner-Lorillon, O., 2003. Sea urchin elongation factor 1delta (EF1delta) and evidence for cell cycle-directed localization changes of a sub-fraction of the protein at M phase. Cell. Mol. Life Sci. 60, 2178–2188.

Brandhorst, B.P., 1976. Two-dimensional gel patterns of protein synthesis before and after fertilization of sea urchin eggs. Dev. Biol. 52, 310–317.

Brandis, J.W., Raff, R.A., 1979. Elevation of protein synthesis is a complex response to fertilisation. Nature 278, 467–469.

Brandsma, M., Kerjan, P., Dijk, J., Janssen, G.M., Moller, W., 1995. Valyl-tRNA synthetase from Artemia. Purification and association with elongation factor 1. Eur. J. Biochem. 233, 277–282.

Browne, G.J., Proud, C.G., 2002. Regulation of peptide-chain elongation in mammalian cells. Eur. J. Biochem. 269, 5360–5368.

Cameron, R.A., Rowen, L., Nesbitt, R., Bloom, S., Rast, J.P., Berney, K., Arenas-Mena, C., Martinez, P., Lucas, S., Richardson, P.M., Davidson, E.H., Peterson, K.J., Hood, L., 2006. Unusual gene order and organization of the sea urchin hox cluster. J. Exp. Zool. B Mol. Dev. Evol. 306, 45–58.

Castagnetti, S., Ephrussi, A., 2003. Orb and a long poly(A) tail are required for efficient oskar translation at the posterior pole of the *Drosophila* oocyte. Development 130, 835–843.

Cho, P.F., Poulin, F., Cho-Park, Y.A., Cho-Park, I.B., Chicoine, J.D., Lasko, P., Sonenberg, N., 2005. A new paradigm for translational control: inhibition via 5′-3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. Cell 121, 411–423.

Colin, A.M., Brown, B.D., Dholakia, J.N., Woodley, C.L., Wahba, A.J., Hille, M.B., 1987. Evidence for simultaneous derepression of messenger RNA and the guanine nucleotide exchange factor in fertilized sea urchin eggs. Dev. Biol. 123, 354–363.

Cormier, P., Pyronnet, S., Morales, J., Mulner-Lorillon, O., Sonenberg, N., Belle, R., 2001. eIF4E association with 4E-BP decreases rapidly following fertilization in sea urchin. Dev. Biol. 232, 275–283.

Cormier, P., Pyronnet, S., Salaun, P., Mulner-Lorillon, O., Sonenberg, N., 2003. Cap-dependent translation and control of the cell cycle. Prog. Cell Cycle Res. 5, 469–475.

Cosson, B., Couturier, A., Chabelskaya, S., Kiktev, D., Inge-Vechtomov, S., Philippe, M., Zhouravleva, G., 2002a. Poly(A)-binding protein acts in translation termination via eukaryotic release factor 3 interaction and does not influence [PSI(+)] propagation. Mol. Cell. Biol. 22, 3301–3315.

Cosson, B., Couturier, A., Le Guellec, R., Moreau, J., Chabelskaya, S., Zhouravleva, G., Philippe, M., 2002b. Characterization of the poly(A) binding proteins expressed during oogenesis and early development of *Xenopus laevis*. Biol. Cell 94, 217–231.

Culbertson, M.R., Neeno-Eckwall, E., 2005. Transcript selection and the recruitment of mRNA decay factors for NMD in *Saccharomyces cerevisiae*. RNA 11, 1333–1339.

Davidson, E.H., Hough-Evans, B.R., Britten, R.J., 1982. Molecular biology of the sea urchin embryo. Science 217, 17–26.

Delalande, C., Monnier, A., Minella, O., Geneviere, A.M., Mulner-Lorillon, O., Belle, R., Cormier, P., 1998. Developmental regulation of elongation factor-1 delta in sea urchin suggests appearance of a mechanism for alternative poly (A) site selection in gastrulae. Exp. Cell Res. 242, 228–234.

Delepine, M., Nicolino, M., Barrett, T., Golamaully, M., Lathrop, G.M., Julier, C., 2000. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat. Genet. 25, 406–409.

Dholakia, J.N., Xu, Z., Hille, M.B., Wahba, A.J., 1990. Purification and characterization of sea urchin initiation factor 2. The requirement of guanine nucleotide exchange factor for the release of eukaryotic polypeptide chain initiation factor 2-bound GDP. J. Biol. Chem. 265, 19319–19323.

Dinkova, T.D., Keiper, B.D., Korneeva, N.L., Aamodt, E.J., Rhoads, R.E., 2005. Translation of a small subset of *Caenorhabditis elegans* mRNAs is dependent on a specific eukaryotic translation initiation factor 4E isoform. Mol. Cell. Biol. 25, 100–113.

Ditzel, H.J., Masaki, Y., Nielsen, H., Farnaes, L., Burton, D.R., 2000. Cloning and expression of a novel human antibody-antigen pair associated with Felty's syndrome. Proc. Natl. Acad. Sci. U. S. A. 97, 9234–9239.

Doma, M.K., Parker, R., 2006. Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. Nature 440, 561–564.

Dostie, J., Ferraiuolo, M., Pause, A., Adam, S.A., Sonenberg, N., 2000. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5′ cap-binding protein, eIF4E. EMBO J. 19, 3142–3156.

Dube, F., 1988. Effect of reduced protein synthesis on the cell cycle in sea urchin embryos. J. Cell. Physiol. 137, 545–552.

Ejiri, S., 2002. Moonlighting functions of polypeptide elongation factor 1: from actin bundling to zinc finger protein R1-associated nuclear localization. Biosci. Biotechnol. Biochem. 66, 1–21.

Epel, D., 1967. Protein synthesis in sea urchin eggs: a "late" response to fertilization. Proc. Natl. Acad. Sci. U. S. A. 57, 899–906.

Evans, T., Rosenthal, E.T., Youngblom, J., Distel, D., Hunt, T., 1983. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 33, 389–396.

Ferraiuolo, M.A., Basak, S., Dostie, J., Murray, E.L., Schoenberg, D.R., Sonenberg, N., 2005. A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay. J. Cell Biol. 170, 913–924.

Galtier, N., Gouy, M., Gautier, C., 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. Comput. Appl. Biosci. 12, 543–548.

Gattiker, A., Gasteiger, E., Bairoch, A., 2002. ScanProsite: a reference implementation of a PROSITE scanning tool. Appl. Bioinformatics 1, 107–108.

Gingras, A.C., Gygi, S.P., Raught, B., Polakiewicz, R.D., Abraham, R.T., Hoekstra, M.F., Aebersold, R., Sonenberg, N., 1999. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev. 13, 1422–1437.

Groisman, I., Jung, M.Y., Sarkissian, M., Cao, Q., Richter, J.D., 2002. Translational control of the embryonic cell cycle. Cell 109, 473–483.

Guerrucci, M.A., Monnier, A., Delalande, C., Belle, R., 1999. The elongation factor-1 delta (EF-1 delta) originates from gene duplication of an EF-1 beta ancestor and fusion with a protein-binding domain. Gene 233, 83–87.

Hake, L.E., Richter, J.D., 1994. CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. Cell 79, 617–627.

Hernandez, G., del Mar Castellano, M., Agudo, M., Sierra, J.M., 1998. Isolation and characterization of the cDNA and the gene for eukaryotic translation initiation factor 4G from *Drosophila melanogaster*. Eur. J. Biochem. 253, 27–35.

Hernandez, G., Vazquez-Pianzola, P., Sierra, J.M., Rivera-Pomar, R., 2004. Internal ribosome entry site drives cap-independent translation of reaper and heat shock protein 70 mRNAs in *Drosophila* embryos. RNA 10, 1783–1797.

Hernandez, G., Altmann, M., Sierra, J.M., Urlaub, H., del Corral, R.D., Schwartz, P., Rivera-Pomar, R., 2005. Functional analysis of seven genes encoding eight translation initiation factor 4E (eIF4E) isoforms in *Drosophila*. Mech. Dev. 122, 529–543.

Hershey, J.W., Merrick, W.C., 2000. Pathway and mechanism of initiation of protein synthesis. In: Sonenberg, N., Hershey, J.W., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 33–88.

Hershey, J.W.B., Miyamoto, S., 2000. Translational control and cancer. In: Sonenberg, N., Hershey, J.W., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 637–654.

Hille, M.B., Albers, A.A., 1979. Efficiency of protein synthesis after fertilisation of sea urchin eggs. Nature 278, 469–471.

Holcik, M., Sonenberg, N., 2005. Translational control in stress and apoptosis. Nat. Rev., Mol. Cell Biol. 6, 318–327.

Hoshino, S., Imai, M., Mizutani, M., Kikuchi, Y., Hanaoka, F., Ui, M., Katada, T., 1998. Molecular cloning of a novel member of the eukaryotic polypeptide chain-releasing factors (eRF). Its identification as eRF3 interacting with eRF1. J. Biol. Chem. 273, 22254–22259.

Huang, W.I., Hansen, L.J., Merrick, W.C., Jagus, R., 1987. Inhibitor of eukaryotic initiation factor 4F activity in unfertilized sea urchin eggs. Proc. Natl. Acad. Sci. U. S. A. 84, 6359–6363.

Imataka, H., Gradi, A., Sonenberg, N., 1998. A newly identified N-terminal amino acid sequence of human eIF4G binds poly(A)-binding protein and functions in poly(A)-dependent translation. EMBO J. 17, 7480–7489.

Inge-Vechtomov, S., Zhouravleva, G., Philippe, M., 2003. Eukaryotic release factors (eRFs) history. Biol. Cell 95, 195–209.

Joshi, B., Cameron, A., Jagus, R., 2004. Characterization of mammalian eIF4E-family members. Eur. J. Biochem. 271, 2189–2203.

Joshi, B., Lee, K., Maeder, D.L., Jagus, R., 2005. Phylogenetic analysis of eIF4E-family members. BMC Evol. Biol. 5, 48.

Juge, F., Zaessinger, S., Temme, C., Wahle, E., Simonelig, M., 2002. Control of poly(A) polymerase level is essential to cytoplasmic polyadenylation and early development in *Drosophila*. EMBO J. 21, 6603–6613.

Kahvejian, A., Svitkin, Y.V., Sukarieh, R., M'Boutchou, M.N., Sonenberg, N., 2005. Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. Genes Dev. 19, 104–113.

Keiper, B.D., Lamphear, B.J., Deshpande, A.M., Jankowska-Anyszka, M., Aamodt, E.J., Blumenthal, T., Rhoads, R.E., 2000. Functional characterization of five eIF4E isoforms in *Caenorhabditis elegans*. J. Biol. Chem. 275, 10590–10596.

Klein, P.S., Melton, D.A., 1994. Induction of mesoderm in *Xenopus laevis* embryos by translation initiation factor 4E. Science 265, 803–806.

Komar, A.A., Hatzoglou, M., 2005. Internal ribosome entry sites in cellular mRNAs: the mystery of their existence. J. Biol. Chem. 280, 23425–23428.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5, 150–163.

Lazaris-Karatzas, A., Montine, K.S., Sonenberg, N., 1990. Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5′ cap. Nature 345, 544–547.

Le Bouffant, R., Cormier, P., Mulner-Lorillon, O., Belle, R., 2006. Hypoxia and DNA-damaging agent bleomycin both increase the cellular level of the protein 4E-BP. J. Cell Biochem. 99, 126–132.

Le Breton, M., Belle, R., Cormier, P., Mulner-Lorillon, O., Morales, J., 2003. M-phase regulation of the recruitment of mRNAs onto polysomes using the CDK1/cyclin B inhibitor aminopurvalanol. Biochem. Biophys. Res. Commun. 306, 880–886.

Le Breton, M., Cormier, P., Belle, R., Mulner-Lorillon, O., Morales, J., 2005. Translational control during mitosis. Biochimie 87, 805–811.

Le Sourd, F., Boulben, S., Le Bouffant, R., Cormier, P., Morales, J., Belle, R., Mulner-Lorillon, O., 2006a. eEF1B: at the dawn of the 21st century. Biochim. Biophys. Acta 1759, 13–31.

Le Sourd, F., Cormier, P., Bach, S., Boulben, S., Belle, R., Mulner-Lorillon, O., 2006b. Cellular coexistence of two high molecular subsets of eEF1B complex. FEBS Lett. 580, 2755–2760.

Li, X., Alafuzoff, I., Soininen, H., Winblad, B., Pei, J.J., 2005. Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. FEBS J. 272, 4211–4220.

Mader, S., Lee, H., Pause, A., Sonenberg, N., 1995. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. Mol. Cell. Biol. 15, 4990–4997.

Maderazo, A.B., He, F., Mangus, D.A., Jacobson, A., 2000. Upf1p control of nonsense mRNA translation is regulated by Nmd2p and Upf3p. Mol. Cell. Biol. 20, 4591–4603.

Mangus, D.A., Evans, M.C., Jacobson, A., 2003. Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. Genome Biol. 4, 223.

Marash, L., Kimchi, A., 2005. DAP5 and IRES-mediated translation during programmed cell death. Cell Death Differ. 12, 554–562.

Marcotrigiano, J., Gingras, A.C., Sonenberg, N., Burley, S.K., 1997. Cocrystal structure of the messenger RNA 5' cap-binding protein (eIF4E) bound to 7-methyl-GDP. Cell 89, 951–961.

Marcotrigiano, J., Gingras, A.C., Sonenberg, N., Burley, S.K., 1999. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. Mol. Cell 3, 707–716.

Mathews, M.B., Sonenberg, N., Hershey, J.W., 2000. Origins and principles of translational control. In: Sonenberg, N., Hershey, J.W., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 1–31.

Mendell, J.T., ap Rhys, C.M., Dietz, H.C., 2002. Separable roles for rent1/hUpf1 in altered splicing and decay of nonsense transcripts. Science 298, 419–422.

Merrick, W.C., Nyborg, J., 2000. The protein synthesis elongation cycle. In: Sonenberg, N., Hershey, J., Mathews, M. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 89–125.

Minshall, N., Standart, N., 2004. The active form of Xp54 RNA helicase in translational repression is an RNA-mediated oligomer. Nucleic Acids Res. 32, 1325–1334.

Miron, M., Verdu, J., Lachance, P.E., Birnbaum, M.J., Lasko, P.F., Sonenberg, N., 2001. The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in *Drosophila*. Nat. Cell Biol. 3, 596–601.

Monnier, A., Morales, J., Cormier, P., Boulben, S., Bellé, R., Mulner-Lorillon, O., 2001. Protein translation during early cell divisions of sea urchin embryos regulated at the level of polypeptide chain elongation and highly sensitive to natural polyamines. Zygote 9, 229–236.

Morley, S.J., Coldwell, M.J., Clemens, M.J., 2005. Initiation factor modifications in the preapoptotic phase. Cell Death Differ. 12, 571–584.

Nakamura, A., Sato, K., Hanyu-Nakamura, K., 2004. *Drosophila* cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. Dev. Cell 6, 69–78.

Nelson, M.R., Leidal, A.M., Smibert, C.A., 2004. *Drosophila* Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. Embo J. 23, 150–159.

Nigg, E.A., 2001. Mitotic kinases as regulators of cell division and its checkpoints. Nature Rev. Mol. Cell Biol. 2, 21–32.

O'Farrell, P.H., 2001. Triggering the all-or-nothing switch into mitosis. Trends Cell Biol. 11, 512–519.

Oliveri, P., Carrick, D.M., Davidson, E.H., 2002. A regulatory gene network that directs micromere specification in the sea urchin embryo. Dev. Biol. 246, 209–228.

Osborne, L.R., Martindale, D., Scherer, S.W., Shi, X.M., Huizenga, J., Heng, H.H., Costa, T., Pober, B., Lew, L., Brinkman, J., Rommens, J., Koop, B., Tsui, L.C., 1996. Identification of genes from a 500-kb region at 7q11.23 that is commonly deleted in Williams syndrome patients. Genomics 36, 328–336.

Pause, A., Belsham, G.J., Gingras, A.C., Donze, O., Lin, T.A., Lawrence Jr., J.C., Sonenberg, N., 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature 371, 762–767.

Pelletier, J., Sonenberg, N., 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334, 320–325.

Proud, C.G., 2004. mTOR-mediated regulation of translation factors by amino acids. Biochem. Biophys. Res. Commun. 313, 429–436.

Pyronnet, S., Imataka, H., Gingras, A.C., Fukunaga, R., Hunter, T., Sonenberg, N., 1999. Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. EMBO J. 18, 270–279.

Raught, B., Gingras, A.C., Sonenberg, N., 2000. Regulation of ribosomal recruitment in eukaryotes. In: Sonenberg, N., Hershey, J.W., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 245–293.

Richter, J.D., 2000. Influence of polyadenylation-induced translation on metazoan development and neuronal synaptic function. In: Sonenberg, N., Hershey, J., Mathews, M. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 785–806.

Richter, J.D., Sonenberg, N., 2005. Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature 433, 477–480.

Richter-Cook, N.J., Dever, T.E., Hensold, J.O., Merrick, W.C., 1998. Purification and characterization of a new eukaryotic protein translation factor. Eukaryotic initiation factor 4H. J. Biol. Chem. 273, 7579–7587.

Robalino, J., Joshi, B., Fahrenkrug, S.C., Jagus, R., 2004. Two zebrafish eIF4E family members are differentially expressed and functionally divergent. J. Biol. Chem. 279, 10532–10541.

Rom, E., Kim, H.C., Gingras, A.C., Marcotrigiano, J., Favre, D., Olsen, H., Burley, S.K., Sonenberg, N., 1998. Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. J. Biol. Chem. 273, 13104–13109.

Ruggero, D., Sonenberg, N., 2005. The Akt of translational control. Oncogene 24, 7426–7434.

Salaun, P., Pyronnet, S., Morales, J., Mulner-Lorillon, O., Bellé, R., Sonenberg, N., Cormier, P., 2003. eIF4E/4E-BP dissociation and 4E-BP degradation in the first mitotic division of the sea urchin embryo. Dev. Biol. 255, 428–439.

Salaun, P., Boulben, S., Mulner-Lorillon, O., Belle, R., Sonenberg, N., Morales, J., Cormier, P., 2005. Embryonic-stage-dependent changes in the level of eIF4E-binding proteins during early development of sea urchin embryos. J. Cell Sci. 118, 1385–1394.

Seli, E., Lalioti, M.D., Flaherty, S.M., Sakkas, D., Terzi, N., Steitz, J.A., 2005. An embryonic poly(A)-binding protein (ePAB) is expressed in mouse oocytes and early preimplantation embryos. Proc. Natl. Acad. Sci. U. S. A. 102, 367–372.

Stebbins-Boaz, B., Hake, L.E., Richter, J.D., 1996. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in *Xenopus*. EMBO J. 15, 2582–2592.

Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., Richter, J.D., 1999. Maskin is a CPEB-associated factor that transiently interacts with elF-4E. Mol. Cell 4, 1017–1027.

Stoneley, M., Willis, A.E., 2004. Cellular internal ribosome entry segments: structures, *trans*-acting factors and regulation of gene expression. Oncogene 23, 3200–3207.

Sullivan, J.C., Ryan, J.F., Watson, J.A., Webb, J., Mullikin, J.C., Rokhsar, D., Finnerty, J.R., 2006. StellaBase: the *Nematostella vectensis* genomics database. Nucleic Acids Res. 34, D495–D499.

Tee, A.R., Blenis, J., 2005. mTOR, translational control and human disease. Semin. Cell Dev. Biol. 16, 29–37.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Topisirovic, I., Culjkovic, B., Cohen, N., Perez, J.M., Skrabanek, L., Borden, K.L., 2003. The proline-rich homeodomain protein, PRH, is a tissue-specific inhibitor of eIF4E-dependent cyclin D1 mRNA transport and growth. EMBO J. 22, 689–703.

Topisirovic, I., Kentsis, A., Perez, J.M., Guzman, M.L., Jordan, C.T., Borden, K.L., 2005. Eukaryotic translation initiation factor 4E activity is modulated by HOXA9 at multiple levels. Mol. Cell Biol. 25, 1100–1112.

Traugh, J.A., 2001. Insulin, phorbol ester and serum regulate the elongation phase of protein synthesis. Prog. Mol. Subcell. Biol. 26, 33–48.

Uchida, N., Hoshino, S., Imataka, H., Sonenberg, N., Katada, T., 2002. A novel role of the mammalian GSPT/eRF3 associating with poly(A)-binding protein in Cap/Poly(A)-dependent translation. J. Biol. Chem. 277, 50286–50292.

Vasudevan, S., Seli, E., Steitz, J.A., 2006. Metazoan oocyte and early embryo development program: a progression through translation regulatory cascades. Genes Dev. 20, 138–146.

Wagenaar, E.B., 1983. The timing of synthesis of proteins required for mitosis in the cell cycle of the sea urchin embryo. Exp. Cell Res. 144, 393–403.

Welch, E.M., Wang, W., Peltz, S.W., 2000. Translation termination: it's not the end of the story. In: Sonenberg, N., Hershey, J., Mathews, M. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 467–485.

Wickens, M., Goodwin, E.B., Kimble, J., Strickland, S., Hentze, M.W., 2000. Translational control of developmental decisions. In: Sonenberg, N., Hershey, J.W., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 295–370.

Wilt, F.H., 1977. The dynamics of maternal poly(A)-containing mRNA in fertilized sea urchin eggs. Cell 11, 673–681.

Xi, R., Doan, C., Liu, D., Xie, T., 2005. Pelota controls self-renewal of germline stem cells by repressing a Bam-independent differentiation pathway. Development 132, 5365–5374.

Ye, X., Fong, P., Iizuka, N., Choate, D., Cavener, D.R., 1997. Ultrabithorax and Antennapedia 5′ untranslated regions promote developmentally regulated internal translation initiation. Mol. Cell. Biol. 17, 1714–1721.

Zhan, K., Vattem, K.M., Bauer, B.N., Dever, T.E., Chen, J.J., Wek, R.C., 2002. Phosphorylation of eukaryotic initiation factor 2 by heme-regulated inhibitor kinase-related protein kinases in *Schizosaccharomyces pombe* is important for resistance to environmental stresses. Mol. Cell. Biol. 22, 7134–7146.