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Cellular coexistence of two high molecular subsets of eEF1B complex

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Abstract The elongation factor eEF1B involved in protein translation was found to contain two isoforms of the eEF1Bδ subunit in sea urchin eggs. The eEF1Bδ2 isoform differs from eEF1Bδ1 by a specific insert of 26 amino acids. Both isoforms are co-expressed in the cell and likely originate from a unique gene. The feature appears universal in metazoans as judged from in silico analysis in EST-databanks. The eEF1B components were co-immunoprecipitated by specific eEF1Bδ2 antibodies. Quantification of the proteins in immunoprecipitates and on immunoblots demonstrates that eEF1Bδ1 and eEF1Bδ2 proteins are present in two subsets of eEF1B complex. We discuss and propose a model for the different subsets of eEF1B complex concomitantly present in the cell.

Keywords: Protein translation; Elongation factor; eEF1B; Sea urchin egg

1. Introduction

The first step of peptide chain elongation during eukaryotic translation is catalyzed by eEF1A, a G-protein responsible for delivering aminoacyl-tRNA to the ribosome on the A-site. The guanine nucleotide exchange activity upon eEF1A is supported by a macromolecular complex, eEF1B, which consists in a assembly of several subunits, the specific function of each being not fully elucidated (reviews in [1,2]). The canonical guanine nucleotide exchange subunit, eEF1Bα, was early demonstrated to be tightly associated to a protein named eEF1Bγ in the eEF1B complex of all eukaryotes from yeast to mammals review in [2]. The protein eEF1Bγ is usually considered as a structural subunit playing a role in the cellular localization of eEF1B. Noteworthy, eEF1Bγ has been the first identified physiological substrate [3] for CDK1, the kinase controlling entry into M-phase during the cell cycle (reviews in [4–6]). Surprisingly, it was found that, in addition to eEF1Bα, a second nucleotide exchange protein is present in the eEF1B complex of metazoans and plants (review in [1]). Strikingly, the supplementary exchange subunits of plants (eEF1Bβ) or of metazoans (eEF1Bδ), although they have guanine nucleotide exchange activity in their C-terminal domain, are not capable to functionally complement eEF1Bα deficient yeasts [7,8]. The most exciting feature came from the studies on metazoan eEF1Bδ. (i) The N-terminal domain of eEF1Bδ, which has no homology with any proteins present in the databanks, possesses a leucine zipper motif, a conserved secondary structure related to a protein–protein interaction [9]. (ii) The eEF1Bδ protein from vertebrates is a physiological substrate for CDK1 (reviews in [1,10]). Although physiological relevant function for this phosphorylation is not documented, a clue came from the finding that, in herpes virus infected cells, the eEF1Bδ protein is phosphorylated on the CDK1 site by the viral kinase in relation to the host protein synthesis shut-off and the viral mRNA preferential translation (review in [1]). (iii) The eEF1Bδ protein is responsible for the association of the valyl-tRNA synthetase in the eEF1B complex [11]. The presence of valyl-tRNA synthetase has been correlated to an inhibition of the valine encoding codon translation when the eEF1B complex was phosphorylated by CDK1 [12]. (iv) Finally, eEF1Bδ was reported to be a true oncogene in mammalian cells [13].

Considering the high structural complexity of eEF1B and the multiple potential regulation sites existing in the different subunits, eEF1B may have additional physiological roles besides the housekeeping nucleotide exchange function on eEF1A (review in [1]). Using the sea urchin embryo we have characterized a new isoform of eEF1Bδ, eEF1Bδ2 that coexists in the cell with eEF1Bδ1, increasing the known sophisticated physiological structure of the eEF1B complex. We demonstrate the concomitant presence of two subsets of eEF1B complex that differ in their eEF1Bδ composition. This feature, discovered in sea urchin may be universal among metazoans.

2. Materials and methods

2.1. Handling of animals and gametes

Sphaerechinus granularis sea urchins were collected in the Brest area (west Brittany). Animals and gametes were prepared and handled as already described [14].

2.2. Cloning of sea urchin eEF1Bδ2 and eEF1Bγ

Full length *S. granularis* EF1Bδ2 (*Sg*EF1Bδ2) was cloned by RT-PCR from total RNA prepared from unfertilized eggs. The primers used for amplification, forward primer 5′CCGGAATTCATGGCACACCCACTGATGC3′ and reverse primer 5′CGCGGATCCCGGGAGGGTTGATGGGGG3′, were designed from, respectively, the N-ter and the C-ter coding sequences of the already characterized

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cDNA of *S. granularis* EF1Bδ (*Sg*EF1Bδ; EMBL No. Y14235) [15]. The PCR products were visualized by electrophoresis on 1% agarose/TBE gel. Two distinct bands were resolved around 800 and 880 bp. The upper band was excised from the gel, purified on Genelute column (Sigma), cloned in pCR2.1-TOPO vector (Invitrogen) and sequenced on both strands at the Genomer sequencing platform (Roscoff).

2.3. Cytoplasmic egg extracts

Egg extracts were prepared [14] from batches of packed unfertilized eggs (10–20 ml) homogenized in 2 vol of buffer containing 50 mM Tris–HCl, pH 7.4, 75 mM KCl, 50 mM sodium fluoride, 10 mM Na_2HPO_4, 2 mM EDTA, 10 mM ATP, 5 mM paranitrophenylphosphate, 100 μM orthovanadate, 0.3 mM Na-benzoyl-L-arginine methyl ester (BAME), 1 mM benzamidine, 5 μM soybean trypsin inhibitor, 0.3 mM *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), 1 mM 4-(2-aminoethyl)-benzene sulfonylfluoride hydrochloride (AEBSF), in the presence of 100 mM NaCl. Homogenates were clarified by centrifugation ($4000 \times g$ for 20 min). The cytoplasmic extracts were obtained by further centrifugation at $100000 \times g$ for 1 h. All steps were carefully carried out at 4 °C.

2.4. Antibodies generation

Guinea pig polyclonal antibodies against recombinant GST-*Sg*EF1Bδ (anti-eEF1Bδ$_{TOT}$) were obtained and used as already described [14]. Polyclonal specific antibodies against *Sg*EF1Bδ2 were obtained in guinea pig by the DoubleXP immunization protocol (Eurogentec) using the co-injection of two synthetic peptides designed from the 26 aminoacids insert specific sequence of *Sg*EF1Bδ2 (H_2N-CVQKSDGPASNLVSE-$CONH_2$ and H_2N-EIARARQNIQSSLSC-$CONH_2$). Antibodies against sea urchin eEF1Bγ were generated from the protein sequence determined from *Strongylocentrotus purpuratus* cDNA sequence obtained by two-hybrid (EMBL AJ973179). Two peptides designed from the coding sequence of *S. purpuratus* EF1Bγ (H_2N-KVPAFENGSGDTLFES-$CONH_2$ and H_2N-SNEQLRGTDD-LSKAQC-$CONH_2$) were synthesized and used to raise polyclonal antibodies in both guinea pig and rabbit by DoubleXP immunization protocol as above (Eurogentec). Two eEF1Bγ antibodies were obtained, a guinea pig serum and an affinity purified rabbit serum. Both antibodies were efficient for *Sg*EF1Bγ recognition (data not shown).

2.5. Western blot analysis

Proteins from the cytoplasmic extracts were resolved by 12% SDS–PAGE and transferred onto 0.22 μm nitrocellulose membranes as already described [14]. After Ponceau Red staining of the proteins, membranes were saturated for 1 h in Tris buffer saline (TBS) containing 1% bovine serum albumin and 0.1% Tween and probed with the indicated antibodies diluted 1:4000. After 2 h of incubation at room temperature and washing, the bound antibodies were revealed by chemiluminescence (ECL; Pharmacia Biotech) using HRP-conjugated secondary antibodies (Dako) diluted 1:5000 in TBS/BSA/Tween.

2.6. Immunoprecipitation experiments

Immunoprecipitations were performed in 1 ml with the eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$) recognizing both eEF1Bδ1 and eEF1Bδ2 proteins at a dilution of 1:200 and with the specific eEF1Bδ2 antibodies (anti-eEF1Bδ2) at a dilution of 1:1000 in buffer containing 50 mM Tris–HCl, pH 7.4, 500 mM NaCl, 1% IGEPAL (Sigma), 1% BSA, 50 mM sodium fluoride, 10 mM pyrophosphate, 100 μM orthovanadate, 10 mM β-glycerophosphate, 1 mM 4-(2-aminoethyl)-benzene sulfonylfluoride hydrochloride (AEBSF) for at least 2 h at 4 °C. The immune complex was recovered after 1 h incubation with 1% BSA-presaturated Protein A-Sepharose CL-4B beads (Sigma). The bound proteins were washed three times in BSA free buffer and twice in 50 mM Tris–HCl, pH 7.4, 500 mM NaCl. Proteins were resolved by SDS–PAGE and analyzed by silver staining (Amersham Biosciences kit) or by immunodetection as described above.

2.7. Protein quantification by densitometry

Proteins revealed by silver nitrate staining or ECL detection were quantified after digitization of the gels or the films, using the ImajeJ 1.34s program (Wayne Rasband, National Institutes of Health, USA). Results are expressed as mean of *n* experiments with standard deviation (S.D.).

2.8. Immunofluorescence experiments

Eggs and embryos were first extracted with buffer containing 10 mM EGTA, 25 mM MES, 0.55 mM $MgCl_2$, 1 mM 4-(2-aminoethyl)-benzene sulfonylfluoride hydrochloride (AEBSF), 1% IGEPAL (Sigma), 25% glycerol, pH 6.8, for 1 h at 4 °C and subsequently fixed in cold 90% methanol, 50 mM EGTA overnight at −20 °C as described [14]. After rehydration in PBS containing 0.05% Tween and saturation for 1 h in the presence of 1% BSA, cells were incubated, at room temperature, for 2 h with primary antibodies, followed by incubation for 1 h with fluorescent secondary antibodies. After washing, cells were mounted in Citifluor AF3 (Citifluor Ltd., London) and observed under a fluorescence microscope (Olympus BX61) using a 40× objective (UplanApo, numerical aperture 1.0, Olympus). Pictures were taken using a room temperature monochrome CCD camera (Diagnostic Instrument Inc.). Primary antibodies were used at a 1:100 dilution. FITC-linked secondary antibodies were diluted 1:100. When required, DNA staining was performed by addition of the dye bis-benzimide (1 μg/ml) during the last rinse after secondary antibodies incubation [14].

3. Results

3.1. Characterization of eEF1Bδ2 a new isoform of eEF1Bδ subunit

We have cloned and sequenced the full length of a sea urchin cDNA, referred as EMBL AJ973181, encoding for a protein identified to eEF1Bδ by sequence homology, including presence of a leucine zipper motif characteristic of the δ subunit of eEF1B complex [16]. The new form of eEF1Bδ identified here was compared to a previous form obtained by screening a cDNA sea urchin library [15] (EMBL Y14235). The new form contains an insert of 78 bp encoding for a 26 amino acid sequence while the 5′ and 3′ regions flanking the insert were 100% identical to the sequence previously reported. The corresponding two mRNAs therefore encode for two proteins that differ in 2 kDa in their molecular weights. We name eEF1Bδ1 the mRNA and the protein encoded by the shortest sequence and eEF1Bδ2 the mRNA and the protein encoded by the longest mRNA. The two proteins correspond to the doublet detected by Western blotting and arbitrary named eEF1Bδ1 and eEF1Bδ2 according to their molecular weights [14]. Additionally, the identification and nomenclature of the two proteins, match with the peptides found by microsequencing of the 35 and 37 kDa proteins and with the partial mRNAs previously obtained by RT-PCR [14]. The sequence identity of the two mRNAs encoding for eEF1Bδ1 and eEF1Bδ2 suggests that the two eEF1Bδ isoforms originate from alternative splicing of a unique pre-mRNA. A complete genome of the sea urchin *S. purpuratus* [17] is available at the National Human Genome Research Institute of Baylor College of Medicine-Houston, USA. The sequence from *S. purpuratus* shared 81.5% identity with the cognate sequence from *S. granularis* at the nucleotides level and 80.3% identity at the protein level. The analysis in silico of the sea urchin genome, using the Human Genome Sequencing resources (Baylor College of Medicine-Houston, USA), showed existence of a unique gene encoding for eEF1Bδ and containing the 78 bp insert specific for eEF1Bδ2 as a predicted full exon. Furthermore, two EST-sequences were found in *S. purpuratus* corresponding to each of the two mRNAs produced by alternative splicing. The sequence analysis in the complete genome thus supports that the two eEF1Bδ isoforms originate from alternative splicing of a unique pre-mRNA.

To determine whether existence of two eEF1Bδ isoforms, one of them containing the insert, was restricted to the sea urchin, we performed in silico searches using NCBI resources. In several metazoan organisms both forms of mRNAs were found. This was the case in hydra (Genbank CD680373 and CF780242), fishes (Takifugu; Genbank CA844665 and AL842751), amphibians (Xenopus; EMBL X66837 and Genbank BG017641), arthropods (Drosophila; RefSeq NP_723536 and NP_609361) and mammals (Mouse; dbj BAB 30841 and RefSeq NP_075729), strongly suggesting universality of the feature. Furthermore, accurate analysis in the human genome showed existence of a unique gene eEF1Bδ (named EEF1D) encoding for two pairs of mRNAs, as judged from EST search, with in each pair, one corresponding to the insert-containing form (RefSeq NM_001960 and NM_032378), and one corresponding to the insert-free form (Genbank BC094806 and BC000678). Therefore, existence of the two isoforms of eEF1Bδ, an insert-containing form and an insert-free form that originate from a unique gene appears to be a metazoan universal feature.

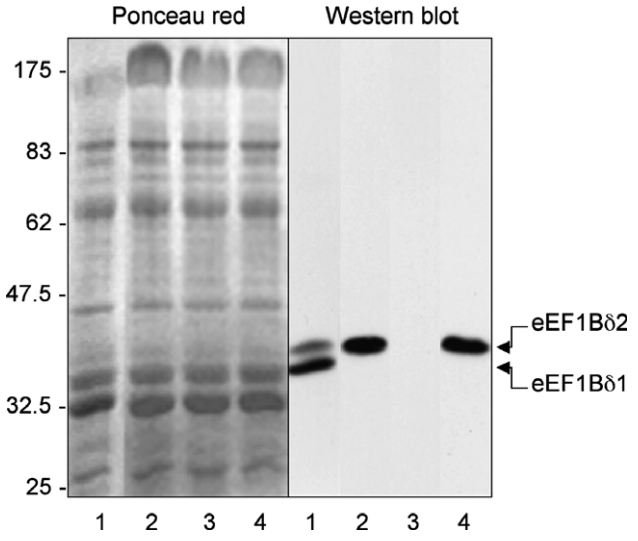

Fig. 1. Characterization of eEF1Bδ2 subunit in sea urchin eEF1B complex. Proteins (10 μg per lane) from cytoplasmic egg extracts were resolved on a 12% polyacrylamide gel and transferred onto a membrane for immunorevelation. Left panel, Ponceau Red staining of the membrane; Right panel, immunorevelation with (1) eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$), (2) eEF1Bδ2 specific antibodies, (3) eEF1Bδ2 specific antibodies pre-incubated with 1 μg of *S. granularis* eEF1Bδ2 derived-peptide (H_2N-CVQKSDGPASNLVSE-$CONH_2$), (4) eEF1Bδ2 specific antibodies pre-incubated with 1 μg unrelated-peptide (H_2N-SNEQLRGTDDLSKAQC-$CONH_2$). The positions of the molecular-weight markers run in parallel are indicated in kDa.

We have produced antibodies specific for eEF1Bδ2, using synthetic peptides matching the 26 amino acid insert of the *S. granularis* sequence and analyzed the proteins of cytoplasmic egg extracts by immunoblotting (Fig. 1). Comparable amounts of proteins were loaded on the immunoblots as judged by Ponceau red staining (Fig. 1 left). While a 35/37 kDa doublet was visualized with the polyclonal eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$; Fig. 1 right; lane 1) as previously shown [14], the eEF1Bδ2 antibodies recognized specifically the band at 37 kDa (Fig. 1 right; lane 2). When the eEF1Bδ2 antibodies were incubated with an excess of eEF1Bδ2-related peptide, the 37 kDa signal was totally abolished whereas an unrelated peptide had no effect (Fig. 1 right; lanes 3 and 4). Therefore, the eEF1Bδ2 antibodies appear to be highly specific for the eEF1Bδ2 protein allowing its analysis in the cells that contain both eEF1Bδ1 and eEF1Bδ2 proteins.

3.2. The eEF1Bδ1 and eEF1Bδ2 proteins are present in two eEF1B complex subsets

Extracts were prepared from sea urchin unfertilized eggs. The extracts were immunoprecipitated (Fig. 2A) with the specific eEF1Bδ2 antibodies (anti-eEF1Bδ2; Fig. 2A; lane 1) or with the eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$) recognizing both eEF1Bδ1 and eEF1Bδ2 proteins (Fig. 2A; lane 3). In both cases, several proteins were detected using silver staining of the gels (Fig. 2A). As judged from immunoprecipitation experiments performed without egg extracts, the bands resolved at 48, 31 and 28 kDa corresponded to heavy and light chains of immunoglobins and the band at 60 kDa corresponded to

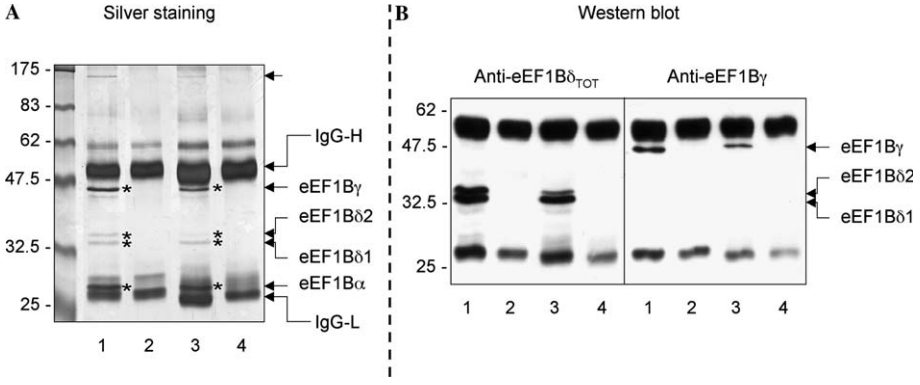

Fig. 2. Characterization of two subsets of eEF1B complex. Proteins (1 mg) from cytoplasmic egg extracts were immunoprecipitated and resolved on a 12% polyacrylamide gel. Figure A corresponds to silver nitrate stained gel and B to Western blots using eEF1Bδ antibodies (B; left panel: anti-SgEF1Bδ$_{TOT}$) or eEF1Bγ antibodies (B; right panel: anti-eEF1Bγ). In A and B: (1) immunoprecipitate using eEF1Bδ2 specific antibodies (anti-eEF1Bδ2; 1:1000), (3) eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$; 1:200), (2, 4) control immunoprecipitations performed with the respective antibodies in the absence of egg extracts. The position of eEF-1Bα, eEF-1Bδ1, eEF-1Bδ2 and eEF-1Bγ are arrowed and indicated by stars on the gel. The heavy and light chains of the IgG used for immunoprecipitation are indicated with arrows on the right of the panel. The positions of molecular-weight markers run in parallel are indicated in kDa.

bovine serum albumin (Fig. 2A; lanes 2 and 4). When the egg extracts were immunoprecipitated using the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), five bands were consistently and specifically detected at 150, 47, 37, 35 and 30 kDa (Fig. 2A; lanes 3). The 47 kDa protein corresponds to eEF1Bγ component, the 37 and 35 kDa doublet corresponds to the two eEF1Bδ components and the 30 kDa band to eEF1Bα subunit (formerly named eEF1β) as previously reported [14]. The 150 kDa protein was not identified although it may correspond to the valyl-tRNA synthetase (review in [1,10]).

When the egg extracts were immunoprecipitated using the specific eEF1Bδ2 antibodies (anti-eEF1Bδ2), again, five bands were consistently observed at 150, 47, 37, 35 and 30 kDa (Fig. 2A; lane 1). The bands immunoprecipitated by the specific eEF1Bδ2 antibodies at 47, 37 and 35 were further identified by immunoblot analysis using the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}) or using antibodies generated (see Section 2) against the eEF1Bγ protein (anti-eEF1Bγ). The immunoblot using eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}) revealed the presence of the two eEF1Bδ isoforms at 37 and 35 kDa in the immunoprecipitate obtained using the specific eEF1Bδ2 antibodies (Fig. 2B left panel; lane 1) comparable to the immunoblot of the precipitate obtained using the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}; Fig. 2B left panel; lane 3). The anti-eEF1Bγ antibodies revealed the presence of the eEF1Bγ component in both immunoprecipitates obtained using the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}; Fig. 2B right; lane 3) or using the specific eEF1Bδ2 antibodies (anti-eEF1Bδ2; Fig. 2B right panel; lane 1). Therefore, the 47 kDa component corresponds in both cases to eEF1Bγ while the 35 and 37 kDa proteins correspond to the two eEF1Bδ isoforms. We assume that the 30 kDa protein corresponds to eEF1Bα which is always associated to eEF1Bγ (reviews in [1,10]). Altogether, the specific eEF1Bδ2 antibodies co-precipitate all the components of eEF1B including eEF1Bδ1. Since the specific eEF1Bδ2 antibodies do not directly recognize eEF1Bδ1, its presence in the immunoprecipitate demonstrates that a subset of eEF1B complex contains both the eEF1Bδ1 and the eEF1Bδ2 components.

Immunoprecipitation experiments were performed using batches of embryos from different females. For each of the antibodies used, the eEF1B constituents were reproducibly observed in the same proportions. For note, while the eEF1Bδ2 antibodies were highly specific for eEF1Bδ2, the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}) were obtained from the recombinant eEF1Bδ-GST protein devoid of the 26 amino acid insert [14], therefore they may recognize each of the two eEF1Bδ isoforms with the same efficiency with respect to their identical primary sequence. Using the two types of antibodies, an interesting observation was consistently made. While the ratios between eEF1Bα and eEF1Bγ were comparable (0.47 and 0.43), the respective proportions of eEF1Bδ2 and eEF1Bδ1 were different according to the antibodies used for immunoprecipitation (compare in Fig. 2A; lanes 1 and 3 and compare in Fig. 2B left panel; lanes 1 and 3) suggesting that the pool of eEF1B in the cell is heterogeneous regarding eEF1Bδ composition. This point was addressed by accurate quantification of eEF1Bγ, eEF1Bδ1 and eEF1Bδ2 in the eEF1B complex depending on the antibodies used for immunoprecipitation. When egg extracts were immunoprecipitated using the specific eEF1Bδ2 antibodies, quantification of the silver stained gel showed that eEF1Bδ1 represented 49% and eEF1Bδ2 51% (S.D. = 7; $n = 6$) of the total amount of eEF1Bδ. Thus embryos contain a subset of cellular eEF1B that contains equal amounts of eEF1Bδ1 and eEF1Bδ2. The ratio of eEF1Bδ2 versus eEF1Bγ co-immunoprecipitated by the eEF1Bδ2 specific antibody was calculated to be 0.09 (S.D. = 0.02, $n = 4$). When the egg extracts were immunoprecipitated using eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), the ratio of eEF1Bδ2 versus eEF1Bγ was calculated to be 0.05 (S.D. = 0.03; $n = 3$). Thus, for an identical amount of eEF1Bδ2 immunoprecipitated, a higher amount of eEF1Bγ is co-immunoprecipitated by the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), implicating that this extra proportion of eEF1Bγ originates from a subset of eEF1B complex containing solely eEF1Bδ1 component. We conclude that two subsets of eEF1B complex coexists in the embryos, one containing the couple eEF1Bδ1–eEF1Bδ2 and the other containing solely eEF1Bδ1. To evaluate the potential presence of free eEF1Bδ1 in the cell that could have been immunoprecipitated by the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), we have evaluated the ratio of total eEF1Bδ components versus eEF1Bγ according to the antibody used for immunoprecipitation. The ratio was comparable in both cases, respectively, 0.21 and 0.22, thus discarding the eventuality of free eEF1Bδ1 excess in the cells, which is in agreement with previous findings (reviews in [1,10]).

When using eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), the proteins eEF1Bδ1 and eEF1Bδ2 in the immunoprecipitates represented, respectively, 71% and 29% (S.D. = 5; $n = 4$) of total eEF1Bδ. We further estimated the proportion of the two isoforms of eEF1Bδ in the whole cellular extracts, reflecting the whole pool of eEF1Bδ protein, by quantification of the Western blots. The proportion obtained was 69% and 31% for, respectively, eEF1Bδ1 and eEF1Bδ2 (S.D. = 8; $n = 10$) highly compatible with the proportions calculated from the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}) immunoprecipitation experiments.

In conclusion, the comparison between the different complexes obtained by immunoprecipitation using either the specific eEF1Bδ2 antibodies or the eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}) demonstrated that several subsets of eEF1B complex coexist in the cells. The results demonstrate the existence of at least one subset of cellular eEF1B containing eEF1Bδ1 and eEF1Bδ2 in a 1:1 stoichiometry and existence of a complex containing only the eEF1Bδ1 isoform. They allow to propose a model for the different structural subsets of the factor eEF1B (see Section 4).

3.3. Early developmental changes in eEF1B components

We investigated developmental changes in eEF1Bδ. The expression of each isoforms remained constant from fertilization up to the pluteus stage (80 h after fertilization) as judged from Western blot analysis of embryo extracts (data not shown). We then investigated intracellular localization changes during the first cell cycle. Since it was shown that using eEF1Bδ antibodies (anti-eEF1Bδ_{TOT}), a subset of the eEF1B complex was subjected to cell cycle-directed localization changes [14], it was of interest to investigate potential specific changes of the eEF1B complex subset containing the eEF1Bδ2 component. The results showed highly comparable relocalization of the subsets of eEF1B complex during cell cycle (Fig. 3). The localization of the component eEF1Bγ of eEF1B was also analyzed, taking advantage of the generation of specific eEF1Bγ antibodies. The same cell-cycle-directed relocalization of eEF1Bγ was observed (Fig. 3). Therefore, all the subsets of eEF1B detected in our experiments undergo comparable cell-cycle directed localization changes.

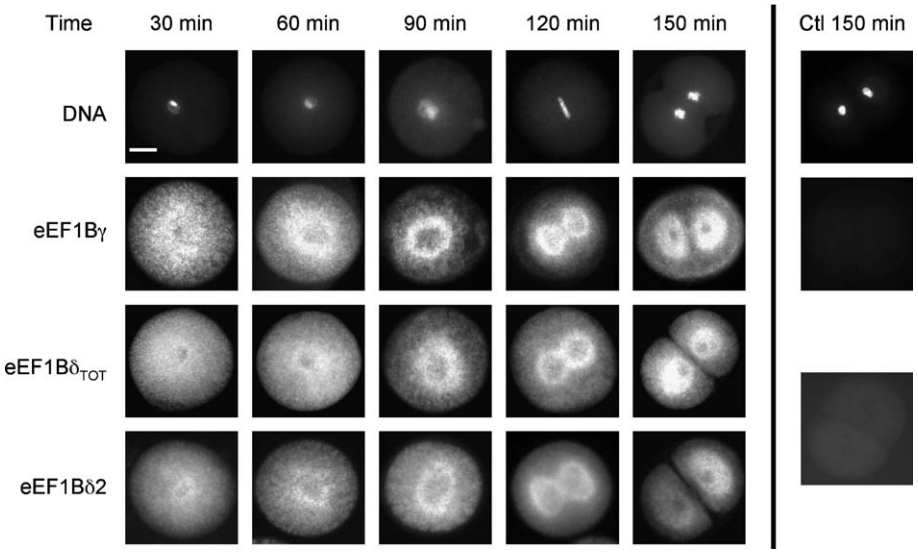

Fig. 3. Localization by fluorescence microscopy of the eEF1B subunits during sea urchin embryo first cell cycle. Progression through cell division was followed by DNA staining with the dye bis-benzimide (DNA). Embryos were sampled at the indicated times after fertilization and treated for immunostaining as indicated in Section 2. The subunit eEF1Bγ was detected using eEF1Bγ antibodies (eEF1Bγ), total eEF1Bδ was detected using eEF1Bδ antibodies (eEF1Bδ$_{TOT}$), eEF-1Bδ2 was detected using the specific eEF1Bδ2 antibodies (eEF1Bδ2). The right column corresponds to control staining using secondary antibodies alone (Ctl 150 min). Each photography is representative of the pool of embryos. Bar: 30 μm.

4. Discussion

Our results demonstrate the existence of different subsets of the guanine nucleotide exchange factor (eEF1B) known to be involved in the elongation step of protein synthesis (see Section 1). We have identified two isoforms for the eEF1Bδ subunit of eEF1B. The two isoforms eEF1Bδ1 and eEF1Bδ2 originate from a single gene by alternative splicing of a unique pre-mRNA and are concomitantly expressed in the cells. Immunodetection analyses show that eEF1B is present in the cell under several subsets differing in their eEF1Bδ isoform proportion.

A model (Fig. 4) may be proposed to match our observations and estimations of the relative amounts of eEF1Bδ1 and eEF1Bδ2 in immunoprecipitation or immunoblotting experiments. The results indicate the coexistence of a subset of complex (Fig. 4, subset 1) containing the couple [eEF1Bδ1–eEF1Bδ2] in a 1:1 stoichiometry together with a subset of complex (Fig. 4, subset 2) containing the couple [eEF1Bδ1–eEF1Bδ1]. We have estimated the relative amounts of each eEF1B subset. When using the eEF1Bδ antibodies (anti-eEF1Bδ$_{TOT}$), the first subset of complex containing the couple [eEF1Bδ1–eEF1Bδ2] is immunoprecipitated in addition to the subset of complex devoid of eEF1Bδ2 and containing the couple [eEF1Bδ1–eEF1Bδ1]. The protein eEF1Bδ1 represents 70% of total eEF1Bδ and eEF1Bδ2 represents 30% of total eEF1Bδ. Since all eEF1Bδ2 is engaged in the couple [eEF1Bδ1–eEF1Bδ2], $70 - 30 = 40\%$ of eEF1B contains the couple [eEF1Bδ1–eEF1Bδ1], and consequently 60% of eEF1B contains the couple [eEF1Bδ1–eEF1Bδ2] (Fig. 4).

The complex eEF1B is assumed to be composed of a basic heteromer consisting in the association of four subunits, valyl-tRNA synthetase (ValRS), eEF1Bδ, eEF1Bγ and eEF1Bα [ValRS–eEF1Bδ–eEF1Bγ–eEF1Bα] in a 1:1:1:1 stoichiometry [10,11,18] or 1:1:2:1 stoichiometry [19]. Our results demonstrate the cellular existence of a macromolecular dimeric assemblage [ValRS–eEF1Bδ1–eEF1Bγ–eEF1Bα]–[ValRS–eEF1Bδ2–eEF1Bγ–eEF1Bα] and suggest by analogy the existence of a [ValRS–eEF1Bδ1–eEF1Bγ–eEF1Bα] dimer. This is in accordance with the models proposing dimerization of the heteromer [ValRS–eEF1Bδ–eEF1Bγ–eEF1Bα] [11,19–21]. Our results do not rule out existence of a [eEF1Bγ–eEF1Bα] multimer as proposed [10] and which would not have been revealed in the present analysis.

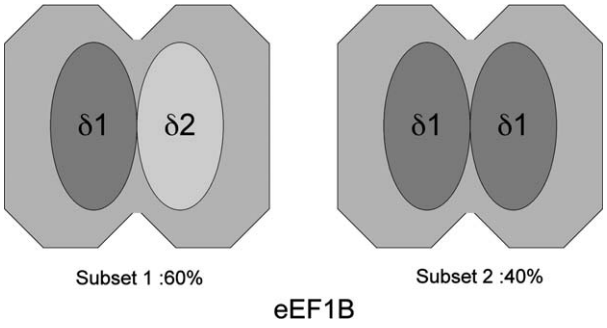

Fig. 4. Schematic model for the composition and the proportion of the eEF1B subsets. Subset 1 contains the couple [eEF1Bδ1–eEF1Bδ2] in a 1:1 stoichiometry. Subset 2 contains the couple [eEF1Bδ1–eEF1Bδ1] in a 1:1 stoichiometry. The grey polygons correspond to the grouped associated eEF1B subunits (eEF1Bδ, eEF1Bγ and Valyl-tRNA synthetase). The proportion of each eEF1B subset in the same cell is indicated.

The existence of the two isoforms of the protein eEF1Bδ first demonstrated here in sea urchin eggs, questions the respective roles for the proteins concomitantly expressed in the same cells and present under different subsets of eEF1B complex in vivo. The feature discovered in sea urchin is conserved in several if not all metazoan species including human and must then be of general significance among evolution. This further suggests an important specific function for each subset of complex related or not to protein synthesis elongation.

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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.febslet.2006.04.038.

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