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Expression of the Gene for Translation Elongation Factor 1 α -Related Protein during Development of the Sea Urchin *Anthocardaris crassispina*

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ABSTRACT—The cDNA for a protein that is related to translation elongation factor 1 α (EF-1 α) has been cloned from the sea urchin *Anthocardaris crassispina*. The sea urchin EF-1 α -related protein (AcEFP) seems somewhat unique in structure compared to EF-1 α of other organisms so that it is uncertain if AcEFP is a genuine EF-1 α . Still, it possesses many features of typical EF-1 α reported so far, suggesting that AcEFP is involved in protein synthesis or its regulation. Genomic Southern analysis indicated that the sea urchin genome contains one or at most two copies of the *AcEFP* gene. A single transcript of 2.2 kb is expressed ubiquitously in adult tissues examined and, during embryogenesis, zygotically after the blastula stage. Whole mount *in situ* hybridization showed that the *AcEFP* gene is also widely expressed in embryos, with relatively high expression in the gut and oral ectoderm, both of which are proliferating tissues in embryos. The expression of *AcEFP* was not affected in embryos by agents that destabilize the extracellular matrix (ECM), suggesting that expression of *AcEFP* is relatively independent of the integrity of ECM in sea urchin embryos.

INTRODUCTION

Protein synthesis involves a variety of different factors, among which elongation factor 1 α (EF-1 α) promotes the GTP-dependent binding of aminoacyl-tRNA to the acceptor site of the ribosome (Hershey, 1991). Besides its essential role in protein synthesis, there are several reports showing the involvement of EF-1 α in a number of other functions: complex formation with mitotic apparatus (Kuriyama *et al.*, 1990; Ohta *et al.*, 1990), interaction with cytoskeletal elements (Yang *et al.*, 1990; Shiina *et al.*, 1994), with the endoplasmic reticulum (Hayashi *et al.*, 1989), and with ribosomal subunits (Berchtold *et al.*, 1993). Furthermore, a possible implication has been inferred in cellular senescence and aging (Cavallius *et al.*, 1986; Shepherd *et al.*, 1989). All these facts suggest the coupling of protein synthesis and other cellular functions.

Meanwhile, it seems rather common that there are multiple EF-1 α genes or EF-1 α -related genes in a single organism. In *Xenopus*, four EF-1 α or related proteins have been identified so far (Deschamps *et al.*, 1991), which have different developmental expression patterns and/or different roles in development. This is also the case for the multiple EF-1 α genes in *Artemia* (Maassen *et al.*, 1985), *Drosophila* (Hovemann *et al.*, 1988), and mammals (Ann *et al.*, 1992).

Thus, different EF-1 α genes are regulated differentially and strictly in embryogenesis, serving a variety of aspects in embryonic cells. Since embryogenesis is a complicated process that requires strict regulation of protein synthesis, clarification of the expression and function of EF-1 α and its related proteins in embryos will contribute to our understanding of development.

The sea urchin is an excellent model animal for understanding early embryogenesis of animals, though information on EF-1 α is still limited despite a number of findings showing the important roles of translational regulation in sea urchin embryos (Brandis and Raff, 1978; Hille and Albers, 1979). There have been a few reports on sea urchin EF-1 α -related proteins. In one report, a partial cDNA was obtained, but the sequence was not shown (Peeler *et al.*, 1990). In another report, the cDNA sequence is also partial, and the gene product seems rather unique in structure compared to typical EF-1 α of other animals as described below (Kuriyama *et al.*, 1990). Ohta *et al.* purified a mitotic apparatus-associated protein that has EF-1 α -like activities and antigenicity, though no structural data were presented (Ohta *et al.*, 1990). Here, we show the cDNA structure for EF-1 α -related protein of the sea urchin *Anthocardaris crassispina* and its expression in embryos and adults.

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MATERIALS AND METHODS

Materials

Collection of gametes of *A. crassispina* and culture of embryos were performed as described before (Yamasu *et al.*, 1995).

Hybridization analyses

Total RNA was isolated from adult tissues or embryos at different developmental stages by the acid guanidinium phenol- chloroform method (Chomczynski and Sacchi, 1987). Southern and northern analyses were done according to the standard protocol (Sambrook *et al.*, 1989) using as probe the 5'-terminal cDNA fragment (+1 to +270/BamHI site) labeled with [α - 32 P]dCTP. Final wash was done with 0.2 \times SSC/0.1% SDS for 15 min at 65°C.

Whole mount *in situ* hybridization was performed as described previously (Onodera *et al.*, 1999) using as probe the entire *AcEFP* cDNA.

Sequence analysis

Nucleotide sequences of cDNAs and deduced amino acid sequences were analyzed by Genetyx-Mac (Ver. 9.0; Software Development Co., Ltd.). The following amino acid sequences of EF-1 α registered in GenBank were used for comparative analyses: *Homo sapiens* (EF-1 α -1, X03558; EF-1 α -2, X70940), *Xenopus laevis* (EF-1 α -S, M25697; EF-1 α -O, M75873; EF-1 α -O1, Z19545; 42Sp50, X56699), *Danio rerio* (X77689), *Drosophila melanogaster* (EF-1 α F1, X06869; EF-1 α F2, X06870), *Onchocerca volvulus* (M64333), *Arabidopsis thaliana* (X16430), *Tetrahymena pyriformis* (D11083), *Dictyostelium discoideum* (X55972), *Neurospora crassa* (D45837), *Saccharomyces cerevisiae* (M10992). In addition, the sequence for a EF-1 α -related protein (SU5 antigen) of another sea urchin *Strongylocentrotus purpuratus* deposited in the PIR data base (A37159) was also employed as well for comparison.

RESULTS

Screening of cDNA clones for the sea urchin EF-1 α -related protein

A PCR fragment with high similarity to EF-1 α cDNAs of other organisms was fortuitously obtained in our previous study searching for protein tyrosine kinase genes (Sakuma *et al.*, 1997). A λ ZAPII cDNA library from sea urchin prism embryos (Yamasu *et al.*, 1995) was screened using this fragment as probe. The longest cDNA clone obtained (pEF29) contains a fragment of 1754 bp and includes an ORF of 462 amino acids (aa) with an estimated size of 50.8 kDa (Fig. 1). Ten bp upstream to the initiation codon is seen an in-frame termination codon (data not shown), showing that the ORF codes for a full structure of the protein.

Structural analysis of the sea urchin EF-1 α cDNA

The deduced protein (AcEFP for EF-1 α -related protein of *A. crassispina*) shows significant homology to EF-1 α that has been reported so far (Table 1). Curiously, however, the similarities of the AcEFP protein to EF-1 α of other animals (78–81%) are not clearly distinguishable from those to EF-1 α of fungi (76–79%) in contrast to the higher similarities among typical EF-1 α of other animals such as vertebrates, arthropods, and nematodes (Table 1; additional data not shown; see also Fig. 2). Actually, though vertebrates and echinoderms including sea urchins constitute one phylogenetic group (deuterostome), EF-1 α of another group (protostome) show higher identities of more than 83% to vertebrate EF-1 α . This raises the possibility that AcEFP might be a specialized protein related to, but different from, the authentic EF-1 α .

In its entirety, however, the AcEFP protein shows all the features of typical animal EF-1 α (Fig. 1): 1) The 12-aa segment found in AcEFP from +214 to +225 with respect to the start codon is unique to animal and fungal EF-1 α sequences (Nordnes *et al.*, 1994). 2) The full complement of the three PKC phosphorylation sites, which is present only in those of animals and fungi (Nordnes *et al.*, 1994), is also seen in the sea urchin protein (+242, +316, +383). 3) The 2-aa insertion in the sea urchin protein (+158 to +159) has been seen in animal EF-1 α but not in fungal EF-1 α . 4) The actin binding sequence in human EF-1 α can also be seen in the sea urchin protein (Yang *et al.*, 1990). 5) Basic amino acids considered necessary for binding to tRNA can also be found in the sea urchin protein (Berchtold *et al.*, 1993). 6) The amino acid required for binding to the endoplasmic reticulum (Hayashi *et al.*, 1989) also occurs in the sea urchin protein (D at +306).

Kuriyama *et al.* (1990) obtained monoclonal antibody (SU5) that recognizes a centrosphere protein of 50 kDa and cloned a partial cDNA for this antigen. The SU5 antigen also show significant similarity to EF-1 α , though the antigen has several distinct features such as a one amino-acid insertion at a site that corresponds to between +103 and +104 of AcEFP and lack of the first PKC target site among the three (Fig. 1). Despite these structural uniqueness, the SU5 antigen is most close to AcEFP (Figs. 1, 2, Table 1), suggesting that the SU5 antigen and AcEFP diverged during the evolution of the sea

Table 1. Comparison of eukaryotic EF-1 α proteins^a.

| | Homo ^b | Xeno ^c | Danio ^d | AcEFP | SU5 ^e | Droso ^f | Nem ^g | Yeast ^h | Arabi ⁱ |
|--------------------------|-------------------|-------------------|--------------------|-------|------------------|--------------------|------------------|--------------------|--------------------|
| Homo ^b | – | 95.9 | 92.0 | 80.7 | 72.5 | 84.4 | 83.6 | 80.7 | 75.9 |
| Xenopus ^c | 95.9 | – | 92.0 | 81.0 | 72.8 | 83.5 | 82.8 | 80.4 | 75.7 |
| Danio ^d | 92.0 | 92.0 | – | 81.0 | 73.4 | 84.4 | 82.8 | 81.7 | 75.7 |
| AcEFP | 80.7 | 81.0 | 81.0 | – | 76.6 | 80.5 | 78.2 | 78.7 | 72.5 |
| SU5 ^e | 72.5 | 72.8 | 73.4 | 76.6 | – | 70.9 | 71.0 | 71.0 | 62.4 |
| Drosophila ^f | 84.4 | 83.5 | 84.4 | 80.5 | 70.9 | – | 79.9 | 78.5 | 74.6 |
| Nematode ^g | 83.6 | 82.8 | 82.8 | 78.2 | 71.0 | 79.9 | – | 76.9 | 74.9 |
| Yeast ^h | 80.7 | 80.4 | 81.7 | 78.7 | 71.0 | 78.5 | 76.9 | – | 73.5 |
| Arabidopsis ⁱ | 75.9 | 75.7 | 75.7 | 72.5 | 62.4 | 74.6 | 74.9 | 73.5 | – |

^a Identities of amino acid sequences (%) are shown. ^b Homo, human EF-1 α -1. ^c Xenopus (Xeno), *Xenopus laevis* EF-1 α -S.

^d Danio, *Danio rerio* EF-1 α . ^e SU5, SU5 antigen. ^f Drosophila (Droso), *Drosophila melanogaster* EF-1 α F1. ^g Nematode (Nem), *Onchocerca volvulus* EF-1 α . ^h Yeast, *Saccharomyces cerevisiae* EF-1 α . ⁱ Arabidopsis (Arabi), *Arabidopsis thaliana* EF-1 α .

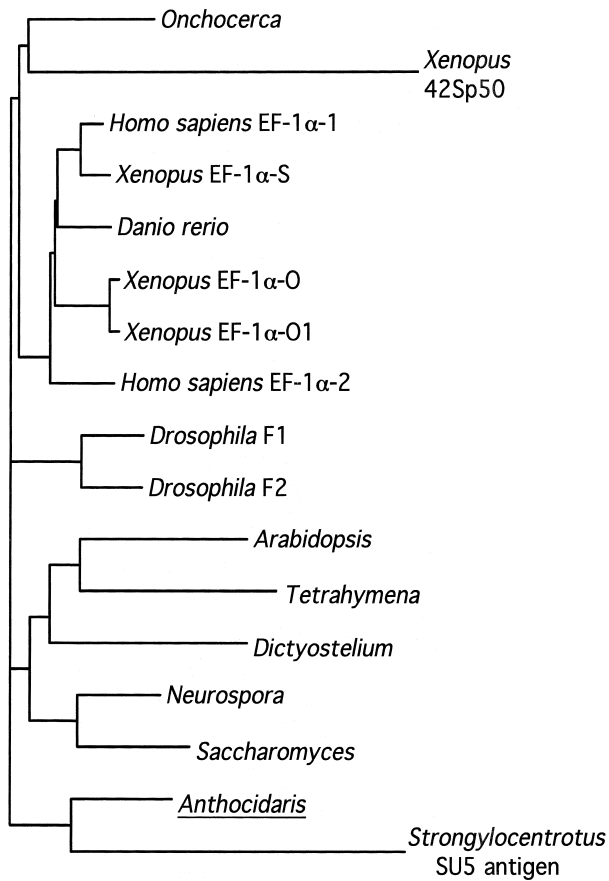


Fig. 2. Phylogenetic tree of EF-1 α and related proteins. The tree was built by the neighbor-joining method (Saitou and Nei, 1987).

urchin after the duplication of a common ancestor gene.

To test if other genes related to EF-1 α are expressed in sea urchin embryos, we performed RT-PCR on cDNA from gastrulae employing degenerate primers designed for the two regions (GEFEAG, QDVYKI; Fig. 1) which are highly conserved among EF-1 α of diverse arrays of organisms, and the PCR fragment of the expected size (ca.430 bp) was cloned into plasmid. Ten randomly selected clones showed essentially the same sequences as the *AcEFP* cDNA clone, demonstrating that *AcEFP* represents the main EF-1 α family gene at least in gastrulae of *A. crassispina*.

In the genomic Southern analysis (Fig. 3A), only one or a few intense bands were seen for different restriction enzymes, showing that there are at most two genes for *AcEFP* in the genome.

Expression of the *AcEFP* gene in embryos and adults

Zygotic expression of the *AcEFP* gene was first detected as a 2.2 kb band as early as in hatching blastulae and increased in amount until the early gastrula stage, when the expression leveled off and remained constant through the pluteus stage (Fig. 3B). Longer exposure revealed faint bands of the same size from the unfertilized egg through the cleavage stage (data not shown). The spatial regulation of the gene

in the embryo was addressed by the whole-mount *in situ* hybridization (Fig. 4). From mesenchyme blastulae (data not shown) to early gastrulae (Fig. 4B), the expression was observed rather ubiquitously. In later stages such as the prism stage, the transcript was still widely observed, though relatively intense expression was detected in the gut and oral ectoderm. In keeping with this, we observed a slight increase in the amount of the transcript after treatment with lithium ions, which are known to respecify the prospective ectoderm to endoderm in sea urchin embryos (data not shown). In adult sea urchins, the transcript of *AcEFP* exist in all the tissues examined (Fig. 3C), with relatively high expression in immature testes and low expression in coelomocytes. Generally speaking, the expression levels in adult tissues are lower than that in prism embryos.

ECM-independent expression of *AcEFP* gene during embryogenesis

To test if the *AcEFP* gene is regulated by external signals, embryos were treated with β -aminopropionitrile (BAPN) or *cis*-hydroxyproline, which destabilizes the collagen polymer, leading to disruption of the embryonic ECM (Wessel and McClay, 1987). Both treatments significantly reduced the expression of a gut-specific tyrosine kinase gene, *AcSrc1* (Onodera *et al.*, 1999), in a dose-dependent manner (Fig. 3D). In contrast, repression of *AcEFP* expression by hydroxyproline and BAPN was only limited. Thus, the expression of *AcEFP* does not depend strongly on the integrity of ECM in the embryo.

DISCUSSION

EF-1 α -related protein of the sea urchin

In the present study we have cloned a cDNA for a sea urchin protein that is highly related to EF-1 α . Comparison of this protein, *AcEFP*, with EF-1 α of other organisms renders it doubtful that *AcEFP* is the sea urchin EF-1 α . On the other hand, since the rate of base substitution is known to be rather high in the sea urchin genome (Hall *et al.*, 1980), the possibility cannot be excluded that *AcEFP* is the sea urchin EF-1 α . Actually *AcEFP* possesses all the features of EF-1 α known till now, and RT-PCR identified only the mRNA for *AcEFP* in gastrulae, favoring the view that *AcEFP* is the genuine EF-1 α . Though this issue awaits further study, it is highly likely that *AcEFP* is in some ways involved in protein synthesis.

Comparison with other EF-1 α -related proteins from sea urchins

From eggs of different sea urchin species, *Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus*, GTP-binding proteins of 51 kDa with EF-1 α -like activities and antigenicity were isolated as mitotic apparatus-associated proteins (Ohta *et al.*, 1990). This is not surprising since EF-1 α is now known to associate with the cytoskeleton (Shiina *et al.*, 1994; Yang *et al.*, 1990). It is possible that the 51-kDa protein corresponds to *AcEFP* of *A. crassispina*, though it should be addressed in

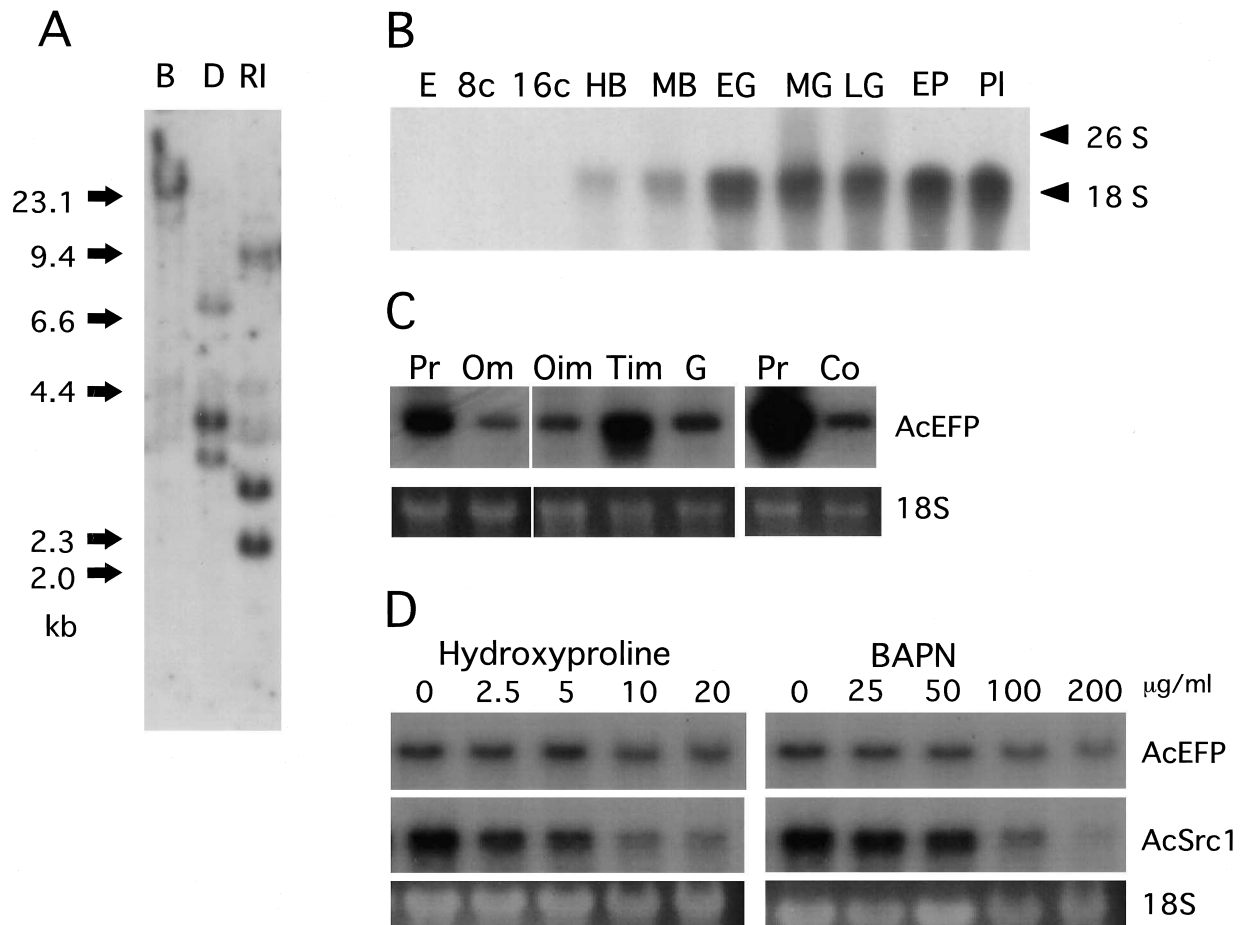


Fig. 3. Southern and northern analyses. **(A)** Genomic Southern analysis of *AcEFP*. Genomic DNA from sea urchin sperm was digested with *Bam*HI (B), *Dr*all (D), and *Eco*RI (RI). Two μ g digested DNA per lane were separated on a 1% agarose gel. **(B)** Developmental northern analysis. Five μ g of total RNA per lane were run on a formaldehyde agarose gel. E, unfertilized eggs; 8c, 8-cell stage; 16c, 16-cell stage; HB, hatching blastula stage; MB, mesenchyme blastula stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EP, early pluteus stage, PI, pluteus stage. **(C)** Expression of the *AcEFP* gene in adult tissues. One μ g of total RNA per lane were run on formaldehyde gels. Pr, prism embryos; Om, mature ovary; Oim, immature ovary; Tim, immature testis; G, gut; Co, coelomocytes. Patterns of 18S rRNA stained with ethidium bromide are shown to the bottom. For coelomocytes, the expression was significantly low and detected only after an extended exposure. **(D)** Effects of ECM destabilizer on the expression of *AcEFP*. BAPN or *cis*-hydroxyproline of the indicated concentration was added to the culture at 1 hr postfertilization. After culture till 24 hr postfertilization, RNA was obtained from embryos and subjected to the northern analysis. Membranes were sequentially hybridized with *AcEFP* and *AcSrc1* probe.

the future. Meanwhile, Kuriyama *et al.* obtained monoclonal antibody (SU5) which recognizes a centrosphere protein of 50 kDa and cloned a partial cDNA for this antigen (Kuriyama *et al.*, 1990). Despite its unique structural features, the SU5 antigen is most close to AcEFP (Figs. 1, 2, Table 1), suggesting that the duplication of the ancestor gene of AcEFP and SU5 antigen took place after the branching of the sea urchin or echinoderm from other phylogenetic groups in the deuterostome. Since SU5 antigen seems to have accumulated more amino acid substitutions than AcEFP, SU5 antigen might have acquired novel functions after gene duplication such as for the regulation of mitotic spindle formation in sea urchin cells. We failed to obtain cDNAs for any SU5-type protein from *A. crassispina* by the RT-PCR probably because it is not expressed abundantly at the gastrula stage or the primers

used here are not suitable for amplification of cDNA for such highly deviated proteins. Though cloning of another partial cDNA clone for sea urchin EF-1 α -related gene was reported previously, no sequence data was provided, making it impossible to be compared with AcEFP (Peeler *et al.*, 1990).

In fact, it is well-known that a given single organism contains multiple EF-1 α genes and related genes. In *Xenopus*, one of the four EF-1 α -related proteins (42Sp50) is considered involved in long-term storage of 5S RNA and aminoacyl-tRNA (Deschamps *et al.*, 1991; Viel *et al.*, 1991). It is possible, therefore, that the sea urchin also possesses multiple EF-1 α family proteins with different roles. Possibility of the presence of multiple genes closely related to *AcEFP* was, however, excluded by the result of the genomic Southern analysis (Fig. 3A). In adult sea urchins, the transcript of *AcEFP*

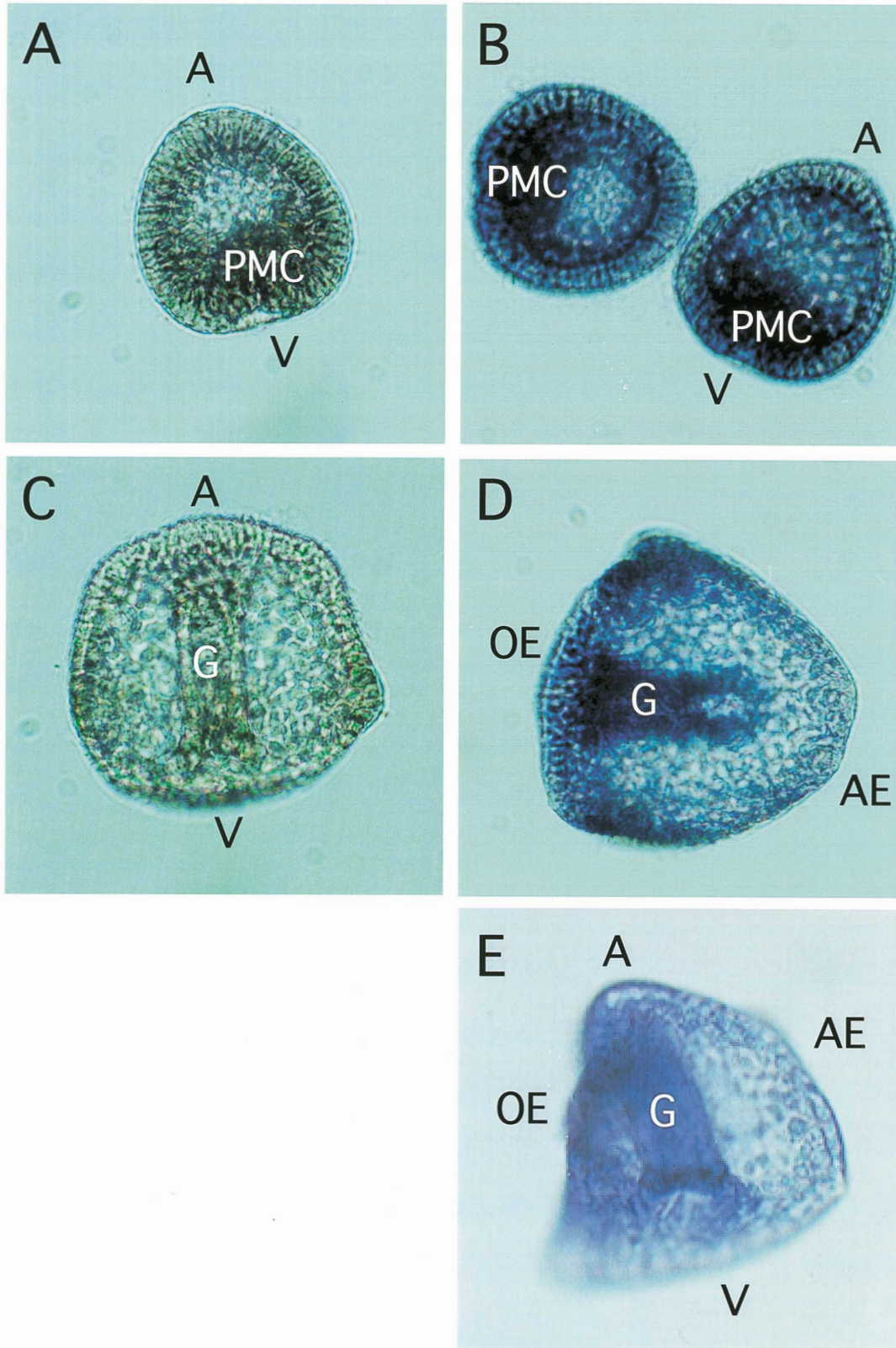


Fig. 4. Whole mount *in situ* hybridization analyses of *AcEFP* expression in sea urchin embryos. (A, C) An early gastrula (A, lateral view) or a prism embryo (C, frontal view) was stained using sense probes as negative controls. (B, D, E) An early gastrula (B, lateral view) or prism embryos (D, anal view; E, lateral view) were stained using antisense probes. A, animal pole; AE, aboral ectoderm; G, gut; OE, oral ectoderm; PMC, primary mesenchyme cells; V, vegetal pole.

exists in all the tissues examined (Fig. 3C), with relatively high expression in immature testes and low expression in coelomocytes. In embryos, *AcEFP* is expressed also ubiquitously, with some bias to the gut and oral ectoderm. Its universal expression is in line with a view that *AcEFP* is involved in various aspects of protein synthesis *per se* or its regulation. The bias of the expression to the gut and oral ectoderm is known for SpS24, a small ribosomal subunit protein in the sea urchin *S. purpuratus*, which is also involved in protein synthesis (Angerer *et al.*, 1992). Both tissues are highly proliferative in sea urchin embryos (Angerer *et al.*, 1992 and references therein), and may require active translation for their growth. Among sea urchin genes expressed during embryogenesis, some are known to depend on ECM for its full expression (*Spec1*, *LpS1*) while others (*SM50*, *LyUSF*) do not (Benson *et al.*, 1991; George *et al.*, 1996). Our present result suggested that the expression of *AcEFP* is partially independent of the external signal. In fact, we observed that *AcEFP* expression still persisted, though at a reduced level, when embryos were dissociated into cells and cultured successfully (unpublished data).

Translational control in sea urchin embryos

Translational control plays important roles during sea urchin embryogenesis, especially in the enhancement of protein synthesis after fertilization. Total rate of protein synthesis increases more than 100-fold (Regier and Kafatos, 1977), and enhancement of the rate of translational elongation was estimated to be 2.5-fold (Hille and Albers, 1979). Since *AcEFP* belongs to the EF-1 α family, it will be involved in the protein synthesis itself or its regulation. Thus, cloning of the *AcEFP* cDNA will certainly contribute to the elucidation of this long-standing issue of the sea urchin development.

NOTE

The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB029058.

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