Endo16, a Lineage-Specific Protein of the Sea Urchin Embryo, Is First Expressed Just prior to Gastrulation

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We have isolated and characterized a new endoderm-specific gene, designated Endo16, from a sea urchin gastrula stage cDNA library. Northern blot analysis and in situ hybridization experiments indicate that this gene is first expressed in the vegetal plate, a group of endodermal and mesenchymal precursor cells that are poised to invaginate in the first movement of gastrulation. Expression becomes progressively restricted to a subset of endodermal cells as development proceeds. To study the Endo16 gene product, a polyclonal antiserum was raised against bacterially expressed Endo16 protein. Indirect immunofluorescence experiments in midgastrula stage embryos reveal that the Endo16 protein is localized to the surface of endoderm and secondary mesenchyme cells. In Western blot experiments, the antiserum detects a small set of high molecular weight proteins ranging from 180 to >300 kDa. Analysis of the nucleotide-derived amino acid sequence from a partial Endo16 cDNA clone reveals a highly repetitive, extremely acidic protein segment that includes the Arg-Gly-Asp (RGD) tripeptide known to be important in cell binding domains of a number of extracellular proteins. Taken together, these data suggest that the Endo16 protein may be an adhesion molecule involved in gastrulation of the sea urchin embryo.

INTRODUCTION

The use of cloned gene probes and monoclonal antibodies (McAb) has facilitated the identification of an expanding set of lineage-specific molecules in the sea urchin embryo (reviewed in Davidson, 1986, McClay and Wessel, 1985). Knowledge of how expression of these molecules is regulated as well as what role the gene products play in development is crucial to understanding how this complex multicellular organism arises from a single cell, the fertilized egg. In mesenchyme blastula stage sea urchin embryos five distinct lineages can be identified on the basis of morphological criteria and lineage-specific markers: the oral and aboral ectoderm, the primary mesenchyme, the small micromeres, and the vegetal plate, which later gives rise to endoderm and secondary mesenchyme. It is the vegetal plate cells that perform the morphogenetic movements that accomplish gastrulation in these embryos. These movements are accompanied by changes in cell shape, pulsatile behavior, filopodial activity, and extensive cell re-packing (reviewed by Keller and Hardin, 1987). In a detailed analysis of sea urchin morphogenesis, Gustason and Wolpert (1967) proposed that all of the cell rearrangements which occur in the course of embryogenesis could be explained in terms of dynamic changes in cell adhesions and cell migrations. Since the time of their proposal, a growing body of evidence has been accumulating in support of the role of cell adhesion in sea urchin morphogenesis. Recently, several lines of experimental evidence implicate cell-cell and cell-matrix adhesions in sea urchin gastrulation. Inhibitors of N-linked glycosylation (Heifetz and Lennarz, 1979), proteoglycan synthesis (Karp and Solursh, 1974; Katow and Solursh, 1979), and collagen synthesis or processing (Butler et al., 1987; Wessel and McClay, 1987), as well as McAb fragments directed against the apical extracellular matrix protein hyalin (Adelson and Humphries, 1988), are all capable of preventing gastrulation. However, little is known about specific molecules expressed by vegetal plate cells and their descendants that participate in adhesive interactions involved in gastrulation.

In this report we describe the isolation and characterization of a new endoderm-specific gene designated Endo16. Using in situ hybridization and indirect immunofluorescence, we have investigated the spatial and temporal expression of the Endo16 transcript as well as localization of the Endo16 gene product. The results of these investigations, along with analysis of the nucleotide-derived amino acid sequence of the Endo16 cDNA clone, lead us to propose that the Endo16 protein may play a role in cell adhesion and gastrulation in the sea urchin embryo.

MATERIALS AND METHODS

Animals. Adult Strongylocentrotus purpuratus were purchased from Alacrity Marine Biological Services (Redondo Beach, CA). Maintenance of animals, spawn-
ing, fertilization, and embryo culture were as previously described (Pittman and Ernst, 1984).

Isolation and characterization of SpG16 cDNA clone. All enzymes were purchased from Promega Biotech or New England Biolabs except as indicated. All isotopes were purchased from Amersham. Standard molecular biology techniques were used (Maniatis et al., 1982). The 1.3-kb HindIII-BamHI fragment of the Caenorhabditis elegans col-1 gene (Kramer et al., 1982) (col-1 gene, gift of J. Kramer and D. Hirsch; subclone, gift of Dr. H. Boedtker) was nick-translated and used to screen a S. purpuratus gastrula stage ggtl0 cDNA library (gift of T. Thomas and E. Davidson) containing 10,000 recombinant clones. Hybridization conditions were 5X SSC, 5X Denhardt's, 0.1% SDS at 55°C. Washing was performed under the same conditions. Positive clones were plaque-purified and phage DNA was isolated and digested with EcoRI to determine insert size. One clone, λSpG16, contained the largest cDNA insert and this insert was subcloned into pUC8 to create the plasmid pEndo16 and into m13mp18 for restriction mapping and sequence analysis. Sequencing was performed using the Sequenase kit (US Biochemicals) according to the manufacturer's instructions. Asymmetric probes were prepared using standard sequencing reactions on Northern blot strips which were hybridized, washed, and exposed as described below.

Genomic Southern blot. Sperm was collected from a single spawned animal and DNA isolated by the method of Kedes et al. (1975). Ten micrograms of genomic DNA was digested with EcoRI or HindIII, electrophoresed on a 0.65% agarose gel, and blotted to nitrocellulose. The filter was hybridized overnight with nick-translated Endo16 at 65°C in 5X SSC, 5X Denhardt's, 0.1% SDS. Washing was done at 65°C in 1X SSC, 0.1% SDS. Autoradiography was for 24 hr using Kodak XAR-5 film and a DuPont Cronex intensifying screen.

Northern blot analysis. RNA from egg, 16-cell, 128-cell, 20-hr hatched blastula, 26-hr mesenchyme blastula, 36-hr gastrula, and 80-hr pluteus embryonic stages and adult tissues was prepared by the procedure of Chirgwin et al. (1979). Ten micrograms total RNA was electrophoresed on 1% agarose/formaldehyde gels and blotted to nitrocellulose. Nick-translated Endo16 and a control probe were used to probe filters. Hybridization and washing conditions were as for the genomic blot. Autoradiography was for 48 hr using Kodak XAR-5 film and a DuPont Cronex intensifying screen.

In situ hybridizations. The 550-bp EcoRI-HpaII fragment from pEndo16 was subcloned into the Sp6/T7 transcription vector pGem-7Zf (Promega Biotech). This construct was used to synthesize either sense or antisense RNA probes with the Riboprobe kit (Promega Biotech) according to the manufacturer's instructions with [35S]CTP (800 Ci/mmole, Amersham). Probes were synthesized to a specific activity of ~1 × 10^6 cpm/μg. The final concentration of probe in the hybridization solution was 3.3 × 10^6 cpm/ml.

Embryos were fixed, embedded in paraplast, sectioned, and spread onto polysine-coated slides as described by Cox et al. (1984). Hybridization and washing were performed according to the modifications of Hardin et al. (1988) for 35S probes except that no dehydration through a graded ethanol series was done preceding the formamide wash and 2X SSC was substituted for 0.3 M NaCl/20 mM Tris–HCl (pH 8.0). Slides were processed for autoradiography as described (Angerer and Angerer, 1981). Exposure time was 3 days for all sections shown, except pluteus stage which was for 9 days.

Fusion protein production and purification. To construct the Trp-Endo16 fusion protein, the 550-bp EcoRI-HpaII fragment of the Endo16 cDNA was ligated into EcoRI–ClaI digested pATH1 (Dieckmann and Tzagoloff, 1985) and transformed into Escherichia coli strain RRI. This arrangement fuses the Endo16 fragment in frame with the truncated E. coli TrpE gene under the control of the Trp promoter/operator sequences. Induction of the fusion protein was accomplished by growing cells overnight in M9 media supplemented with 0.5% casamino acids, 20 μg/ml L-tryptophan, and 50 μg/ml ampicillin. The cells were then diluted 1:10 in the same media minus tryptophan and grown for 1 hr at 30°C with vigorous aeration. At this time 5 μg/ml indoleacrylic acid (Sigma) was added to the cultures and incubation continued for 2 hr. For sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) analysis, 1 ml of culture was pelleted and resuspended in 50 μl of sample buffer (Laemmlli, 1970). Ten microliters of sample was loaded per lane. The fusion protein was purified by preparative gel electrophoresis. Gels were stained briefly in 4 M Na acetate (Higgins and Dahmus, 1979) and bands were excised and eluted by crushing and soaking overnight in 3 vol of 1 mM Tris–HCl, pH 7.5, 0.1 mM EDTA, 0.05% SDS. The sample was dialyzed against 10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, precipitated, and resuspended in phosphate-buffered saline (PBS). Protein concentrations were determined using the Bio-Rad protein assay kit.

The β-gal-Endo16 fusion protein was constructed by ligating the 794-bp Endo16 cDNA into EcoRI cut pUR288 expression plasmid (Ruther and Müller-Hill, 1983) and transformed into E. coli strain JM101. Induction of the fusion protein was accomplished by adding 0.5 mM isopropylthio-β-galactoside and growing at 37°C for 1–3 hr, and resulted in over production of a protein at the expected ~150 kDa size (data not shown).

Polyclonal antiserum. Preimmune serum was col-
lected from the rabbit before antigen injections began. The primary immunization consisted of 200 μg TrpE-Endo16 fusion protein emulsified in Freund's complete adjuvant injected intradermally. The animal was given two booster injections at 3-week intervals consisting of 150 μg TrpE-Endo16 fusion protein emulsified in Freund's incomplete adjuvant.

Western blot analysis. Total embryo protein lysates were prepared as described (Wessel and McClay, 1987); 25 μg of lysate was loaded per lane of 10% SDS–PAGE and electroblotted to nitrocellulose (Towbin et al., 1979). Filters were blocked in 5% dry milk/PBS for 1 hr, incubated in 1:100 dilution of preimmune or immune serum in PBS for 1 hr, washed in 5% dry milk/PBS for 1 hr, incubated in 1:7500 dilution of goat anti-rabbit alkaline phosphatase conjugate (Promega Biotech) for 1 hr, and washed again in 5% dry milk/PBS for 1 hr. The reaction was visualized by incubating in alkaline phosphatase substrate according to the manufacturer's instructions (Promega Biotech).

Indirect immunofluorescence. Embryos were settled onto polylysine-coated coverslips and fixed in 90% methanol/seawater on ice for 30 min. Embryos were then rehydrated to PBS through a graded methanol series. Embryos were incubated in 3% BSA/PBS for 15 min, 1:100 dilution of immune serum in PBS for 1 hr, 3% BSA/PBS 1 hr, 1:100 dilution of fluorescein-conjugated goat anti-rabbit antiserum (Polysciences) 1 hr, and 3% BSA/PBS 1 hr. The reaction was viewed and photographed under a Zeiss microscope equipped for epifluorescence using Kodak T-Max film at an ASA setting of 800.

EXPERIMENTAL RESULTS

Cloning and Sequence Analysis of the Endo16 cDNA

The 794-bp Endo16 cDNA clone was selected from a sea urchin gastrula stage Xgt10 cDNA library on the basis of low stringency hybridization to a C. elegans collagen type 1 probe. The restriction map and genomic representation of this clone are shown in Figs. 1a and 1b. The Endo16 cDNA probe hybridizes to a single DNA fragment in both EcoRI and HindIII-digested sea urchin genomic DNA, indicating that the Endo16 mRNA is transcribed from a single copy gene (Fig. 1b). The transcriptional orientation of this gene was determined by hybridizing strand-specific Endo16 probes to Northern blot strips of gastrula stage RNA. Only one of these two probes reacts with an mRNA transcript, 6.6 kb in length (Fig. 1c). Sequence analysis of the cDNA clone revealed that the entire 794-bp insert is an open reading frame which could encode 265 amino acids (Fig. 2A). Although the DNA sequence of the sea urchin clone does contain short regions of up to 65% similarity to the C. elegans gene (data not shown), the predicted amino acid sequence of the sea urchin clone does not contain any collagen-related sequence, including that of recently reported sea urchin collagens (Venkatesan et al., 1986; Saitta et al., 1989). However, the deduced amino acid sequence does display several noteworthy features. The sequence includes an Arg–Gly–Asp (RGD; boxed in Fig. 2A), which has been shown to be important in the cell binding domain of many extracellular proteins (reviewed in Ruoslahti and Pierschbacher, 1987). Two potential N-linked glycosylation sites are present (underlined in Fig. 2A), although the proline containing site is probably not utilized (Hubbard and Ivat, 1981). In addition the sequence contains six clustered copies of a 20-amino acid repeat (Fig. 2B), two of which are in tandem. Overall, this protein segment is very hydrophilic and extremely acidic, comprised of 30% glutamic and aspartic acid residues. A computer-assisted search, using the algorithm of Lipman and Pearson (1985), has not identified significant similarity of Endo16 with any sequences contained in the GenBank or EMBL nucleic acid databases, nor is the deduced amino acid sequence related to any protein sequence compiled in the NBRF or Institut Pasteur databases.

Temporal and Spatial Expression of Endo16 Transcript during Embryogenesis

Northern blot analysis was used to examine the developmental expression pattern of the Endo16 mRNA transcript. The results of this experiment are shown in Fig. 3. The 794-bp cDNA hybridizes to a single 6.6-kb transcript that is not detected in egg or morula stages, but is first detected at the hatched blastula stage. The transcript increases in abundance by the mesenchyme blastula stage, continues to increase during gastrulation, and declines in abundance by the time the formation of the pluteus larva is complete. The Endo16 transcript was not detected in any adult tissue examined, including testis, ovary, intestine, coelomocyte, and tube foot RNAs. A reprobing of this blot with a sea urchin actin probe demonstrated the integrity of all the RNA samples (data not shown).

The embryonic distribution of Endo16 transcripts during development was determined by in situ hybridization. The results of these in situ hybridizations with radioactively labeled Endo16 antisense probes and developmentally staged embryo sections are shown in Fig. 4. In 28-hr mesenchyme blastula embryos, the autoradiographic signal is concentrated over the vegetal plate region. These cells are poised to invaginate in the first movement of gastrulation to form the archenteron, which later gives rise to the endoderm and secondary mesenchyme tissues. No signal above background is de-
tected over primary mesenchyme or ectodermal cells. In 36-hr gastrula stage embryos the Endo16 mRNA is detected in cells in all regions of the archenteron. By the 72-hr prism stage, the archenteron has begun constriction to form a tripartite larval gut. At this stage, hybridization is restricted to the posterior two-thirds of the gut (mid- and hindgut) which will become the larval stomach and intestine. No signal is detected over secondary mesenchyme cells at this stage. Interestingly, hybridization to 90-hr pluteus stage larvae shows labeling of stomach only. We did not obtain any tissue sections in which all three digestive tract regions were clearly identifiable. However, sections which included esophagus and stomach or stomach and intestine all showed labeling of stomach tissue exclusively. A representative example of control hybridizations with Endo16 sense probes is shown in Figs. 4A and 4B. In these controls, a low level of background signal is present evenly distributed over the tissue sections. These results demonstrate that the Endo16 gene is expressed in a spatially regulated, lineage-specific manner and that this expression becomes restricted during differentiation.

Production and Specificity of Polyclonal Antiserum

To facilitate study of the Endo16 gene product, polyclonal antibodies were generated. A bacterial fusion protein was produced using one of the pATH series of plasmid vectors and employed as the source of antigen. The plasmids contain a truncated E. coli trpE gene with a synthetic polylinker at the 3' end. A 550-bp restriction fragment of the Endo16 cDNA was subcloned into the polylinker of pATH1 so that the correct translational reading frame was preserved. Induction of the trp promoter by simultaneous tryptophan starvation and exposure to indoleacrylic acid results in the production of large quantities of the trpE fusion protein in cultures harboring the expression plasmid. Protein extracts from induced and uninduced cultures were analyzed by SDS-PAGE, and the appropriately sized fusion protein band was observed after staining with Coomassie blue (Fig. 5A). The fusion protein was purified by preparative gel electrophoresis and used for immunization of a Dutch Belted rabbit.

To determine the reactivity of the resulting antiserum against the sea urchin portion of the fusion protein, we produced a second fusion protein using the pUR series of plasmid vectors. These vectors allow the expression of protein coding sequence as C-terminal addition to E. coli β-galactosidase. Induction of the β-gal-Endo16 fusion protein resulted in overproduction of a protein of the expected ~150-kDa size as judged by Coomassie blue staining of whole cell extracts electrophoresed on SDS-PAGE (data not shown). Figure 5B shows the results of Western blot experiments reacting
FIG. 2. Sequence analysis of Endo16. (A) Nucleotide sequence and derived amino acid sequence of the Endo16 cDNA. The nucleotide sequence of the coding strand (as determined by the strand-specific hybridization, Fig. 1c) is shown. The entire 794-bp cDNA is an open reading frame encoding 265 amino acids. The sequence includes a potential cell attachment site (RGD, boxed) as well as two potential N-linked glycosylation sites (N x S/T, underlined). (B) Endo16 repeats. Six copies of a 20-amino acid repeat are present in the Endo16 sequence. Exact matches that are present in at least three repeats are boxed.

the anti-Endo16 serum with both fusion proteins. As anticipated, the serum reacts strongly with the Trp-Endo16 protein used in the immunization protocol. The serum also reacts very strongly with the P-gal-Endo16 fusion protein, indicating that the sea urchin portion of the Trp-Endo16 fusion protein elicited a strong immune response. The lower molecular weight bands present must be due to breakdown products of the fusion protein since they are not present in the lane containing the parent plasmid. Therefore, the majority of the immune response was elicited against the Trp-Endo16 fusion protein and little response was directed at any contaminants present in the preparation used for immunization.

To investigate the expression of the Endo16 gene product in sea urchin embryos, the polyclonal anti-serum was reacted with total sea urchin gastrula stage protein extracts in Western blot experiments. Preimmune serum, collected from the animal before immunization, was used as a control. As shown in Fig. 5C, the anti-Endo16 serum reacts with a small set of high molecular weight proteins, ranging from 180 to >300 kDa. These bands are not observed in the lane reacted with preimmune serum. It remains to be determined whether the multiple bands are the result of degradation, post-translational modification, or common epitopes shared by different proteins. The 6.6-kb Endo16 mRNA can potentially encode a primary translation product with a maximum size of 240-250 kDa. Therefore, degradation in the protein sample cannot account for the higher molecular weight bands observed.

**Immunolocalization of the Endo16 Gene Product in Gastrula Stage Embryos**

We reacted the anti-Endo16 serum with fixed, whole, midgastrula stage embryos in an effort to determine the spatial and cellular distribution of the Endo16 gene product. The reaction was visualized with fluorescein-conjugated goat anti-rabbit secondary antibody and observed with a fluorescence microscope. The results of this experiment, shown in Fig. 6, reveal that the Endo16
protein is localized to the surface of endodermal cells of the archenteron and secondary mesenchyme cells. The immunolocalization of the Endo16 protein to the surface of endodermal cells is corroborated by the in situ hybridization experiments, which detected significant levels of Endo16 transcripts in these cells. However, although the Endo16 protein appears to be present on the surface of secondary mesenchyme cells at midgastrula stage (arrows in Fig. 6), these cells do not contain detectable Endo16 transcripts (Figs. 4E and 4F). There are several possible explanations to account for the observation of Endo16 protein in association with these cells. At earlier stages of development, our in situ hybridization experiments have shown that the Endo16 gene is actively transcribed in vegetal plate cells and the archenteron, which includes the precursors of the secondary mesenchyme. Long-lived Endo16 protein, synthesized by the precursor cells, may persist in the cells through later stages of development. Alternatively, endodermal cells may be secreting the Endo16 protein, which subsequently binds to the surface of secondary mesenchyme cells by means of specific receptors expressed by the secondary mesenchyme.

**DISCUSSION**

The results of Northern blot experiments demonstrate that the Endo16 gene is a member of the class of so-called “embryonic late genes” (Davidson, 1986) defined as not being significantly expressed in oocytes but activated during early development. It is estimated that not more than 10% of all sequences utilized during early sea urchin development fall into this class (Plytzanis et al., 1982). This pattern of expression is characteristic of spatially regulated genes (Davidson, 1986; Sargent et al., 1986) and in situ hybridization results confirm that Endo16 is expressed in a spatially regulated manner. Moreover, the gene is first transcribed in a group of endodermal and secondary mesenchymal precursor cells and transcription becomes progressively restricted to a subset of endoderm cells as development proceeds. Within the limits of resolution of our technique, we do not observe any differential expression of Endo16 among vegetal plate cells at early stages; however, we cannot completely rule out this possibility. Interestingly, the transcript has not been detected in any adult tissues examined, indicating that the function of the Endo16 gene product may be specific to embryogenesis.

Several endoderm-specific markers have been identified previously by McClay and colleagues using monoclonal antibodies generated against the insoluble fraction of sea urchin plutei and indirect immunofluorescence. Some of these antigens, for example, those recognized by McAbAA1a3 and McAbLL5f7 (McClay and Wessel, 1984), are first detected in all regions of the egg and early embryo and only become localized to the endoderm later in development. Others, such as those recognized by McAb7B3, McAb4Bl0 (McClay and Ettensohn, 1987), and the antigens designated Meso 2 and Endo 1 (Wessel and McClay, 1985), are already localized to the vegetal plate or endoderm when first detected at mesenchyme blastula or very early gastrula stages. A recently isolated endoderm-specific cDNA clone, designated Lv1.4 (G. Wessel, personal communication), is first detected in early gastrula stage embryos and is present only in the mid- and hindgut regions of the embryo at all stages of development. The pattern of Endo16 expression that we have observed is unique among endoderm-specific molecules; nevertheless it does share a feature common to the majority of endodermal markers described to date. With the exceptions of Endo 1 and Lv1.4, expression of endoderm-specific markers becomes progressively restricted to a subset of vegetal plate derivatives as the archenteron differentiates into a tripartite gut and secondary mesenchyme.

The portion of the Endo16 gene product encoded by the 794-bp cDNA displays several unique structural features which may provide clues to the biological function of the molecule. Overall, this region of the predicted Endo16 protein contains a very high density of negatively charged amino acids, with aspartate and
FIG. 4. Localization of Endo16 transcripts by in situ hybridization. Corresponding bright-field (A, C, E, G, I) and dark-field (B, D, F, H, J) photographs of hybridizations with single-stranded, radiolabeled sense (A, B) or anti-sense (C-J) Endo16 probes are shown. (A, B) Gastrula stage control. A low level of background hybridization signal is evenly distributed over the tissue section. (C, D) Mesenchyme blastula. Hybridization signal is detected over the vegetal plate region (v) of the embryo. No signal is present over primary mesenchyme cells (arrows) or ectoderm (e). (E, F) Gastrula stage. Hybridization signal is present over the entire archenteron at this stage. (G, H) Prism stage. Hybridization signal is restricted to the posterior two-thirds of the archenteron (mid- and hindgut) at this stage. Secondary mesenchyme cells (arrows) are not labeled. (I, J) Pluteus larva stage. Hybridization signal is limited to the stomach (midgut). Bar equals 50 μm.

FIG. 5. Production of anti-Endo16 polyclonal antiserum. (A) Induction of the TrpE-Endo16 fusion protein. Whole cell lysates of JM101 cells harboring no plasmid (lanes 1 and 2), parental pATH1 plasmid (lanes 3 and 4), or the fusion construct (lanes 5 and 6) were prepared. Cultures were induced (lanes 2, 4, and 6) by simultaneous tryptophan starvation and exposure to indoleacrylic acid. Extracts were electrophoresed on 15% SDS-PAGE gels and stained with Coomassie brilliant blue. Molecular weight marker positions are indicated in kilodaltons on the left. Induction of cells harboring the fusion construct results in production of the expected 60-kDa fusion protein (arrow). This protein was subsequently purified and used in the production of a polyclonal antiserum (see Materials and Methods). (B) Specificity of the polyclonal antiserum. Samples were electrophoresed on 15% SDS-PAGE gels, electroblotted to nitrocellulose membranes and reacted with preimmune (lane 1) or immune (lanes 2-4) sera, as described under Materials and Methods. Molecular weight markers are indicated on the left. Lanes 1 and 2, 0.4 μg purified Trp-Endo16 fusion protein. Lane 3, whole cell extract prepared from E. coli JM101 strain harboring pUR288 β-gal expression plasmid. Lane 4, whole cell extract from JM101 harboring β-gal-Endo16 expression plasmid. The anti-Endo16 serum reacts strongly with both the Trp-Endo16 and the β-gal-Endo16 fusion proteins. (C) Detection of the Endo16 protein in sea urchin embryonic lysates. Whole cell extracts were prepared from gastrula stage embryos, electrophoresed on 10% SDS-PAGE gels, electroblotted to nitrocellulose, and reacted with the anti-Endo16 serum. Alkaline phosphatase-conjugated goat anti-rabbit secondary antibody was used to visualize the reaction. Lane 1, preimmune serum. Lane 2, anti-Endo16 serum. A small set of high molecular weight, cross-reacting proteins (180-300 kDa) are detected with the anti-Endo16 serum.

glutamate accounting for more than 30% of the amino acid residues. In addition, this domain of the molecule is rich in glycine and proline residues, suggesting a flexible, extended structure. This highly charged, flexible segment could be involved in calcium binding, much like
FIG. 6. Localization of the End016 gene product in whole embryos. Whole 36-hr gastrula stage embryos were fixed and reacted with preimmune (A) or anti-End016 serum (B) as detailed under Materials and Methods. The reaction was visualized with fluorescein-conjugated goat anti-rabbit secondary antibody and photographed under the fluorescence microscope. No fluorescent staining is observed with the preimmune serum. The anti-End016 serum appears to stain the surfaces of archenteron (a) and secondary mesenchyme (arrows) cells at this stage. Bar equals 50 μm.

the similarly structured extracellular proteins prothrombin and osteocalcin (Wylie and Vanaman, 1988). Alternatively, this region of the Endo16 protein may interact with other matrix components that carry positive charges or may modulate the binding of these molecules to negatively charged ligands in the matrix such as heparin sulfate proteoglycans. Interestingly, this portion of the Endo16 cDNA also contains a series of internal repeats—another structural feature commonly involved in physical interactions with other molecules. This 20-amino acid segment, which occurs as six clustered repeats, is not related to any known repeated sequences in other proteins. The predicted amino acid sequence of the End016 cDNA clone also includes the RGD tripeptide which occurs in the cell attachment domain of a number of extracellular proteins (reviewed in Ruoslahti and Pierschbacher, 1987). The importance of RGD cell attachment domains in both vertebrate and invertebrate morphogenesis has been revealed by experiments utilizing synthetic RGD-containing peptides to block cell attachment function by saturating receptors for the sequence (Boucaut et al., 1984; Springer et al., 1984; Naidet et al., 1987). Elucidation of the function of the RGD sequence in End016 awaits further experimental analysis. However, the inclusion of this sequence in a protein which appears to be localized to the cell surface leads us to postulate that it may serve as a cell attachment site in the Endo16 molecule.

It has been recently shown that active cell repacking is the primary mechanism responsible for the characteristic archenteron elongation in gastrulating sea urchin embryos (Ettensohn, 1985; Hardin and Cheng, 1986). This repacking requires extensive changes in cell-cell contacts and, as such, must involve molecules at the surface of archenteron cells. We have shown that the Endo16 molecule is first expressed in the vegetal plate cells just prior to the onset of morphogenesis and that the gene product appears to be localized at the surface of archenteron cells. These properties raise the intriguing possibility that Endo16 may be a cell surface molecule involved in gastrulation. Clearly, the pattern of expression, localization, and sequence characteristics of Endo16 suggest a possible role in morphogenesis. We plan to utilize the anti-Endo16 serum in future experiments designed to test the function of this molecule in cell adhesion and morphogenesis. Further sequence analysis of additional cDNA clones will be necessary to determine whether any regions of the Endo16 protein are related to previously described extracellular molecules or whether Endo16 may represent a novel class of such proteins.

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