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Original Article

Isolation and characterization of cDNA encoding a spicule matrix protein in *Hemicentrotus pulcherrimus* micromeres

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ABSTRACT A cDNA clone, termed pHPSMC, was obtained from the Japanese sea urchin *Hemicentrotus pulcherrimus*, and it was found to be highly homologous in sequence to the spicule matrix protein cDNA of *Strongylocentrotus purpuratus* (Sucov *et al., Dev. Biol. 120:* 507-519, 1987). During early embryogenesis, mRNA complementary to pHPSMC appeared in gastrulae and remained at a similar level until the pluteus stage. *In situ* hybridization revealed that the mRNA was localized exclusively in primary mesenchyme cells in gastrulae. pHPSMC mRNA was detected in micromeres *in vitro* after 48 h of culture, but it was not found in blastomeres immediately after isolation. These features suggested that pHPSMC represents the spicule matrix protein cDNA cognate in *Hemicentrotus pulcherrimus*. In the derived polypeptide, we detected a domain containing a tandemly repeated 13-amino acid sequence as did Sucov *et al.* (1987). Unexpectedly, the sequence of the repeated element was completely different from that originally reported for *Strongylocentrotus purpuratus*, but it was very similar to the corrected sequence that appeared recently (Katoh-Fukui *et al.*, Dev. Biol. 145: 201-202, 1991).

KEY WORDS: *Hemicentrotus pulcherrimus, spicule matrix protein, cDNA, micromeres, in situ hybridization*

Introduction

At the 16-cell stage of the sea urchin embryo, 4 micromeres arise at the vegetal pole. These micromeres are the source of primary mesenchyme cells, which in turn differentiate into spicule-forming cells of the larvae. Okazaki (1975) showed that micromeres can be isolated from the embryo and will differentiate autonomously in culture to form spicules. Thus, the micromere-lineage offers an excellent model for investigating initiation of lineage-specific gene expression associated with differentiation of spicule-forming cells. It may also contain clues for understanding the mechanism of determination, which presumably precedes explicit manifestation of the differentiated phenotype (Angerer and Davidson, 1984; Davidson, 1986).

Micromeres have been extensively studied morphologically (Hörstadius, 1975), but molecular analyses of differentiation in the micromere lineage have been reported only recently. These analyses show that several new micromere-specific proteins are synthesized (Kitajima and Matsuda, 1982; Harkey and Whiteley, 1983; Pittman and Ernst, 1984; Kitajima,1986; Matsuda *et al.*, 1988). In addition, monoclonal and polyclonal antibodies directed against these proteins as well as corresponding cDNAs have been prepared, providing new tools for characterizing the biochemical changes that underlie the differentiation process in micromeres (Carson *et al.*, 1985; Wessel and McClay, 1985; Benson *et al.*, 1986; Leaf *et al.*, 1987; Harkey *et al.*, 1988). Along these lines, Sucov *et al.* (1987) isolated a cDNA clone for a spicule matrix protein (SM50) in *Strongylocentrotus purpuratus*, and they found a unique domain composed of a tandemly repeated 13-amino acid element. *Strongylocentrotus purpuratus* is not available in Japan, and it was therefore necessary to search for a homolog of this gene in order to examine the control of its expression in micromeres of indigenous sea urchins.

We describe here the isolation of a cDNA clone, designated pHPSMC, from the Japanese sea urchin *Hemicentrotus pulcherrimus*, which is highly homologous to the *Strongylocentrotus purpuratus*

Abbreviations used in this paper: cDNA: complementary DNA; Denhardt's solution: Ficoll 0.1 g,polyvinylpyrrolidone 0.1 g, bovine serum albumin 0.1 g, H_2O up to 500 ml.; mRNA: messenger RNA; poly(A)$^+$RNA: polyadenylated RNA; poly(A): polyadenylic acid; pI: isoelectric point; dITP: inosine deoxyribonucleoside triphosphate; 7-deaza-dGTP: deoxy-7-deazaguanosine triphosphate; PBS: phosphate buffered saline; SDS: sodium dodecyl sulfate; SSC: 0.15 M NaCl, 0.015 M sodium citrate; SSPE: 0.18 M NaCl, 10 mM NaH_2PO_4 (pH 7.4), 1 mM EDTA (pH 7.4); FITC: fluorescein isothiocyanate; Mr: relative molecular mass.

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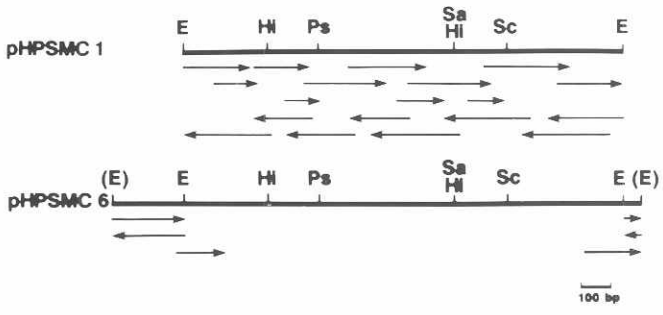

Fig. 1. Restriction map and sequencing strategy for pHPSMC1 and pHPSMC6. *Arrows denote the direction and length of the strand sequenced. E, EcoRI; Hi, Hinc II; Ps, PstI; Sa, SalI; Sc, SacI. (E) indicates an EcoRI site created during library construction by adaptor addition.*

clone. Expression of mRNA complementary to pHPSMC is developmentally controlled both *in vivo* and in isolated micromeres cultured *in vitro*. Immunocytochemical staining shows that its corresponding protein is associated with spicules of developing embryos. These results suggest that this cDNA represents the SM50 cDNA homolog in *Hemicentrotus pulcherrimus*. The derived polypeptide sequence also contains a domain with a repeated 13-amino acid element, but its composition is completely different from that originally reported for *Strongylocentrotus purpuratus*. The latter contained a frame shift that altered the amino acid sequence, because one nucleotide was not detected at position 706 during sequencing of the cDNA. Based upon this finding, a corrected nucleotide sequence of *Strongylocentrotus purpuratus* SM50 cDNA and its derived amino acid sequence were published (Katoh-Fukui *et al.*, 1991). Structural features of the protein encoded by pHPSMC, including its relative molecular mass and basicity, suggest that it may correspond to a spicule-associated protein previously identified in *Hemicentrotus pulcherrimus* micromere cultures (Kitajima and Matsuda, 1982; Matsuda *et al.*, 1988).

Results

The cDNA library constructed from poly(A)$^+$RNA of prism stage of *Hemicentrotus pulcherrimus* embryos was screened with cDNA clone pHS72 that represents the spicule matrix protein (SM50) of *Strongylocentrotus purpuratus* (Sucov *et al.*, 1987). Screening of 2.5 x 10^4 cDNA clones gave 12 positive clones. Among several clones analyzed, the clone with the longest insert (1.5 kbp) was selected and designated pHPSMC1. Further screening of another cDNA library, freshly prepared from the same stage embryos of *Hemicentrotus pulcherrimus*, with pHPSMC1 as a probe, identified a clone with a longer insert (1.8 kbp). It was designated pHPSMC6. From the restriction maps of both cDNAs shown in Fig. 1, it was concluded that the two cDNA clones were derived from the same species of mRNA and represent a single cDNA, which was termed pHPSMC.

A total of 1787 bp of the pHPSMC cDNA sequence was determined according to the sequencing strategy shown in Fig. 1 (Fig. 2). Since the size of mRNA corresponding to pHPSMC cDNA was calculated to be 1.9 kb by Northern blotting (Fig. 6), the sequence shown in Fig. 2 was thought to represent almost the full length of the corresponding mRNA. This assumes that there are several hundred residues in a poly(A) tail, although the exact cap site was not determined in the present experiment. At the 3' end, a typical poly(A) addition signal, AATAAA, was detected and it was followed by a poly(A) sequence 19 nucleotides downstream. The longest possible open reading frame that could be discerned was composed of 407 amino acids (Fig. 2). Assignment of either a +1 or a -1 reading frame gave only short peptides, a peptide of 48 amino acids having methionine at an N-terminal end being the longest. The sequence, ACC*ATG*A, at the translation initiation site coincides fairly well with the optimum sequence, ACC*ATG*G, identified by Kozak (1986) for initiation by eukaryotic ribosomes. The validity of this assignment was also supported by the fact that antiserum against peptide synthesized based on derived amino acid sequence listed in Fig. 2 was immunoreactive *in vivo* (Figs. 9, 10).

The derived peptide sequence indicates a typical N-terminal signal peptide (von Heijne, 1983) and would have a relative molecular mass (Mr) of 41,000 after removal of signal peptide. We propose to call this protein HSM41. Fig. 3 shows the dot matrix analysis of the nucleotide sequence of pHPSMC cDNA (Staden, 1982). As indicated, pHPSMC mRNA is composed of non-repeated and repeated regions as is the case for SM50 mRNA (Sucov *et al.*, 1987). A similar sequence organization was found in both SM50 mRNA (Sucov *et al.*, 1987) and LSM34 mRNA, which encodes a spicule matrix protein in *Lytechinus pictus* (Livingston *et al.*, 1991). Highly homologous nucleotide sequences were present in the 5' and 3' non-repeated regions. In the 5' non-repeated region, *Hemicentrotus* showed a 94% homology with *Strongylocentrotus* and a 73% homology with *Lytechinus*. Similarly, in the 3' non-repeated region, *Hemicentrotus* was 90% homologous with *Strongylocentrotus* and 54% homologous with *Lytechinus*. Quantitative comparison of the central regions was difficult because of variations among repeats. The nucleotide sequence organization is reflected in the derived amino acid sequence of HSM41, which contains a tandemly repeated element of 13 amino acids. It has a consensus sequence of QPGFGNQPG(V/M)GG(R/Q/N) (Fig. 4). A hydropathy profile of the predicted protein shows that the repeated region is predominantly composed of hydrophilic amino acids while the N-terminal region is highly hydrophobic (Kyte and Doolittle, 1982) (Fig. 5). As in SM50 and in LSM34, a proline-rich region was also detected upstream of the repeated region in the HSM41 protein.

Developmental expression of pHPSMC mRNA

Expression of pHPSMC mRNA during early development was examined by Northern hybridization. Total cellular RNA from embryos at various developmental stages was examined for the presence of pHPSMC mRNA. No message was detected in unfertilized eggs, cleavage stage embryos or early blastulae. By contrast, an intense signal of 1.9 kb appeared at the gastrula stage 24 h after fertilization, and the signal intensity, hence the mRNA concentration, did not change appreciably thereafter through the pluteus stage (Fig. 6). In *Strongylocentrotus purpuratus*, Benson *et al.* (1987) and Killian and Wilt (1989) detected SM50 transcripts as early as 200-300 cell embryos. In our experiment a faint signal was observed in mesenchyme blastula (data not shown).

Expression of pHPSMC mRNA in cultured micromeres

The process of spicule formation can be reproduced *in vitro* in

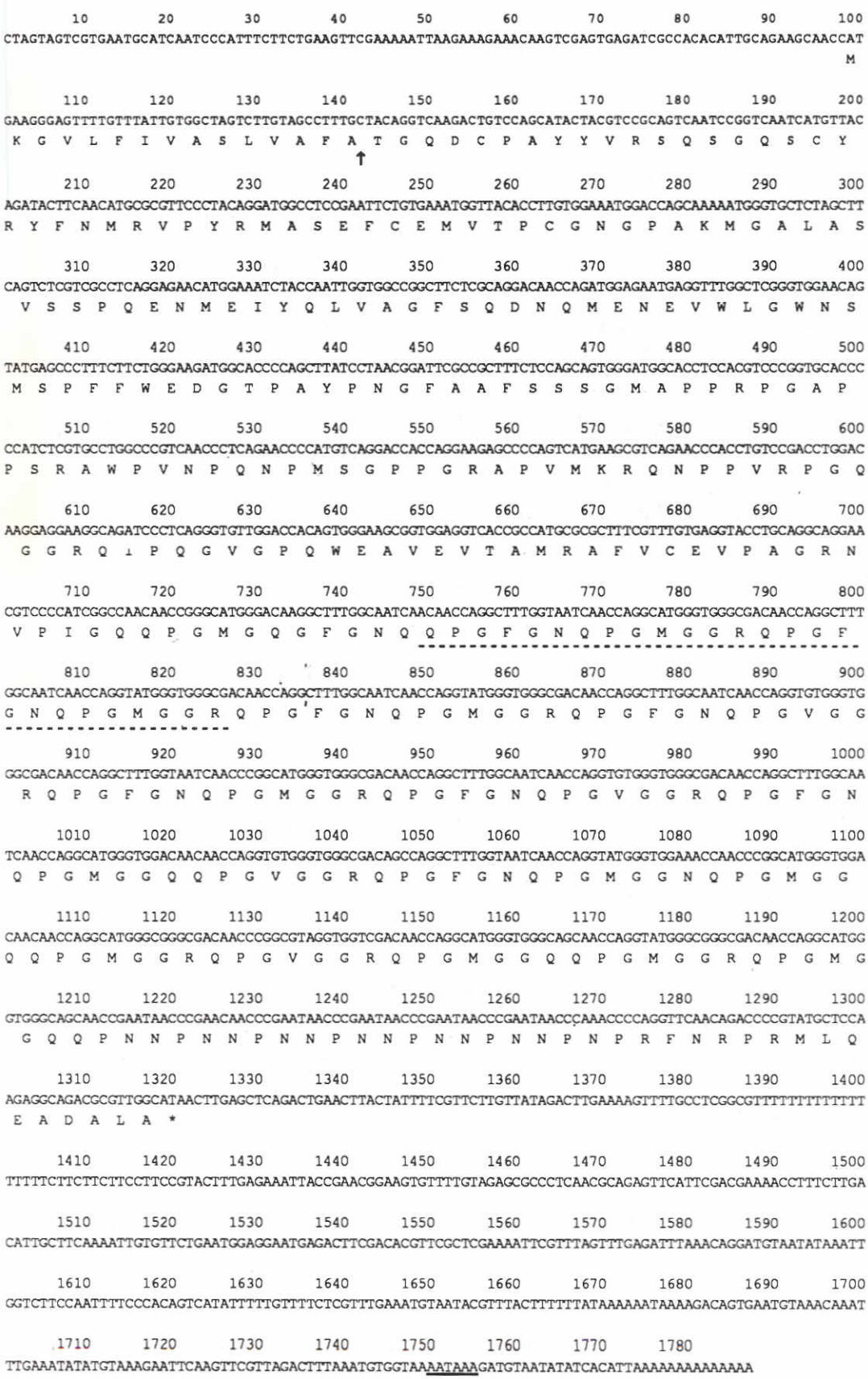

Fig. 2. Nucleotide sequence of the pHPSMC cDNA. *The derived amino acids are shown below the nucleotide sequence. Polyadenylation signal is underlined. Broken lines show a region of peptide used to raise antiserum. Arrow indicates the proposed signal peptide cleavage site.*

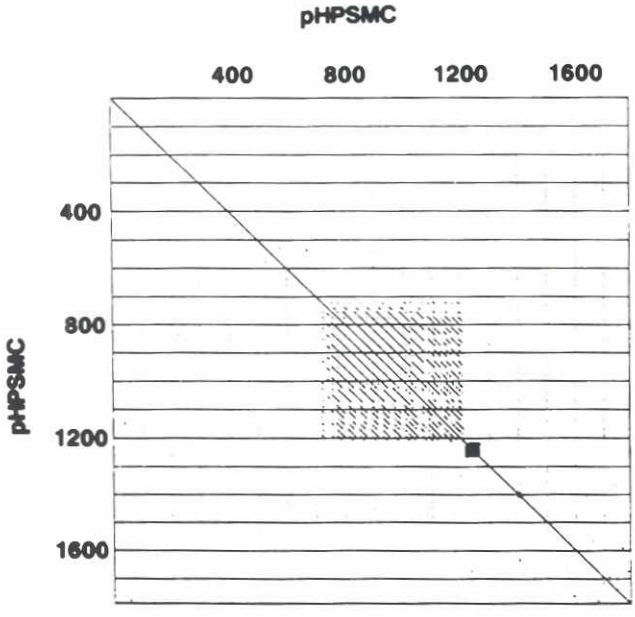

Fig. 3. Dot matrix analysis of pHPSMC cDNA sequences. *Analysis was performed by comparing 15 bases at a time, requiring 12 bases to be identical to register a dot.*

micromeres isolated from 16-cell embryos (Okazaki, 1975). When the expression of pHPSMC mRNA was examined in isolated blastomeres, no pHPSMC mRNA was detectable in micromeres or in a combined fraction of mesomeres and macromeres immediately after isolation (Fig. 7). After 48 h of culture, the descendants of micromeres began to form spicules, and concomitantly a strong signal was detected in micromeres. A signal of much weaker intensity (approximately 1/6) also was noted in cultured mesomeres and macromeres (Fig. 7). The latter signal may have resulted from contamination of mesomeres and macromeres by micromeres at the time of blastomere isolation. It is also possible that during the extended culture period, macromeres trans-differentiate into spicule forming cell lineages (Hörstadius, 1975).

These observations suggest that pHPSMC mRNA is expressed specifically in isolated and cultured micromeres that are actively forming spicules, and they are consistent with the idea that pHPSMC mRNA is involved in spiculogenesis. In this connection, pHPSMC represents a homolog of pHS72, and the HSM41 protein it encodes in *Hemicentrotus pulcherrimus* corresponds to SM50 of *Strongylocentrotus purpuratus* (Sucov *et al.*, 1987) and LSM34 of *Lytechinus pictus* (Livingston *et al.*, 1991).

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Q P G F G N Q P G M G G R
Q P G F G N Q P G M G G R
Q P G F G N Q P G M G G R
Q P G F G N Q P G V G G R
Q P G F G N Q P G M G G R
Q P G F G N Q P G V G G R
Q P G F G N Q P G M G G Q
          Q P G V G G R
Q P G F G N Q P G M G G N
          Q P G M G G Q
          Q P G M G G R
          Q P G V G G R
          Q P G M G G Q
          Q P G M G G R
          Q P G M G G Q

                V     R
Q P G F G N Q P G   G G Q
                M     N
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Fig. 4. Amino acid sequence of the repeated region of HSM41 protein. *The consensus sequence is shown below.*

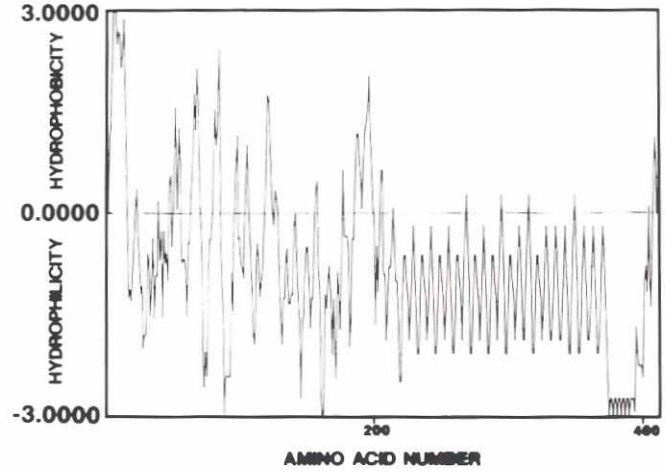

Fig. 5. Hydropathy profile of HSM41 protein.

In situ *localization of pHPSMC mRNA in a developing embryo*

During embryogenesis, descendants of micromeres are primary mesenchyme cells located within the blastocoel at the gastrula stage. *In situ* hybridization with an anti-sense pHPSMC probe revealed strong signals localized over the primary mesenchyme cells (Fig. 8b and d). The hybridization signal in other parts of the embryo was at background levels as shown by comparison with a control using a sense probe (Fig. 8f and h). Thus, pHPSMC mRNA was exclusively expressed *in vivo* in primary mesenchyme cells determined to differentiate into spicule-forming cells.

Localization of HSM41 polypeptide in a developing embryo

In order to examine the spatial distribution of the HSM41 polypeptide in embryos, a rabbit polyclonal antiserum was raised against a synthetic peptide corresponding to 26 amino acids from the repeated region of the gene (Fig. 2). In Western blots, this antiserum reacted with a polypeptide of Mr~40,000 that is present in prism stage embryos but not in unfertilized eggs (Fig. 9). The size

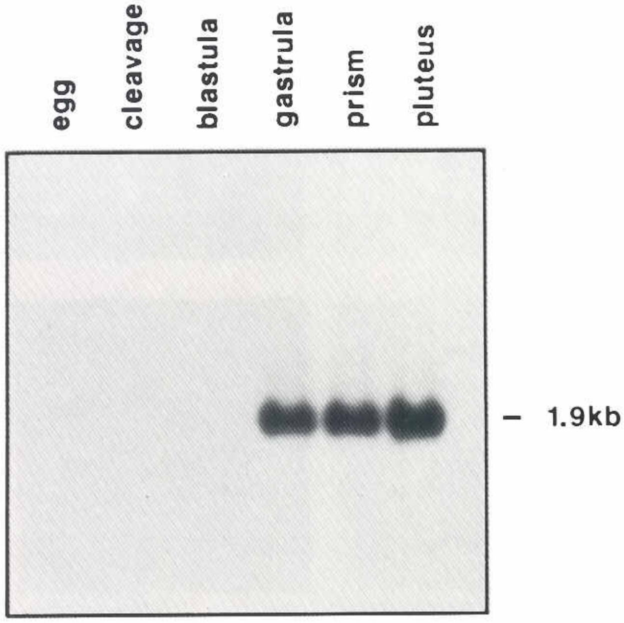

Fig. 6. Developmental Northern blot analysis of pHPSMC mRNA. *In each lane, 5 μg of total RNA from embryos of various developmental stages was electrophoresed through a denaturing formaldehyde gel composed of 2% agarose. RNA was transferred from the gel onto a Hybond-N nylon membrane, and the membrane was prehybridized in 50% formamide, 5x Denhardt's solution, 5x SSPE and 100 μg/ml salmon sperm DNA at 42°C for 3 h. Hybridization was performed in prehybridization solution supplemented with 0.1% SDS and ^{32}P-labeled probe (specific activity, 10^7 cpm/μg DNA) at 42°C for 48 h. The probe was an insert from a pHPSMC cDNA clone labeled with [α-^{32}P]dCTP by nick-translation. After hybridization, the filter was washed three times in 0.2x SSC, and 0.1% SDS at 42°C for 60 min.*

of this polypeptide agrees with that derived from the reading frame assignment and signal peptide cleavage described in Fig. 2. Staining of pluteus larvae with this antiserum revealed immunoreactivity associated with the spicules (Fig. 10).

Discussion

In this study, we cloned and sequenced a cDNA, termed pHPSMC, after screening a prism stage cDNA library with pHS72 as a probe. Our initial intention was simply to determine whether the SM50 cDNA homolog was present in a different sea urchin species, *Hemicentrotus pulcherrimus*. Screening of the library identified candidate clones that were found to be highly homologous to SM50 cDNA after nucleotide sequencing. Unexpectedly, the derived amino acid sequence, especially that of the repeated element thought to be the deposition site for $CaCO_3$ and $MgCO_3$, was completely different from SM50. It was puzzling that two proteins with supposedly similar functions had totally different functional domains. The disparity was reconciled when we independently sequenced the pHS72 cDNA clone. One nucleotide, a cytosine at position between 705 and 706, was missing in the published sequence (Sucov *et al.*, 1987). Sequencing of our genomic clone, pHPSMG, also confirmed this result (data not shown). After this change, the derived amino acid sequence of SM50 was very similar to that of HSM41 encoded by pHPSMC, and the consensus sequence of the repeated element was also very similar as shown below:

	F	N		V		R
SM50 (corrected)	QPGMG		QPG		GG	
	W	G		M		Q
				V		R
HSM41		QPGFGNQPG			GGQ	
				M		N

However, this correction resulted in two additional differences with published results for the SM50 protein of *Strongylocentrotus purpuratus*. The N-glycosylation site located at the C-terminal region (Benson *et al.*, 1986) disappeared after correction of the amino acid sequence, and the protein appeared to be basic instead of acidic (Benson *et al.*, 1986). These points were reexamined in

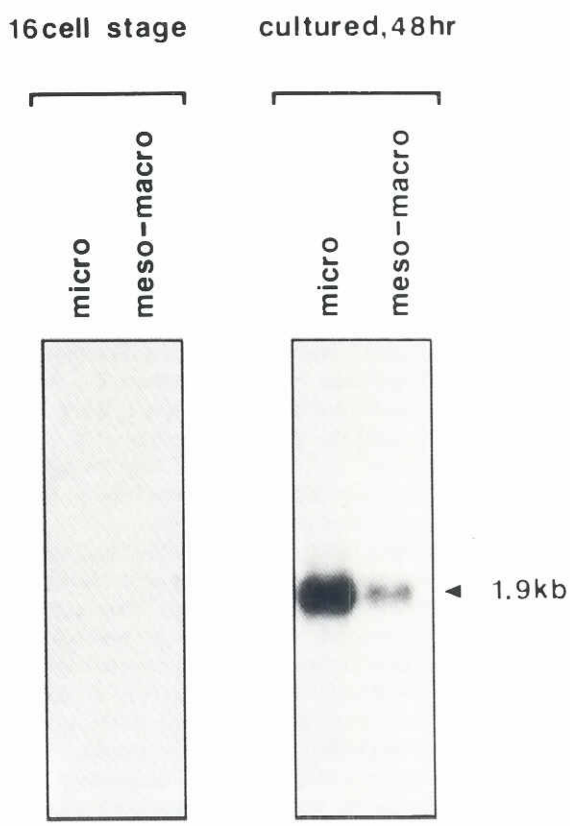

Fig. 7. Expression of pHPSMC mRNA in isolated and cultured micromeres. *At the 16-cell stage, embryos were dissociated into a micromere fraction and a combined fraction of mesomeres and macromeres. Total RNA was extracted from each fraction immediately after isolation and after 48 h of culture. 5 μg of RNA was applied to each lane. The conditions of hybridization and the probe were the same as in Fig. 6.*

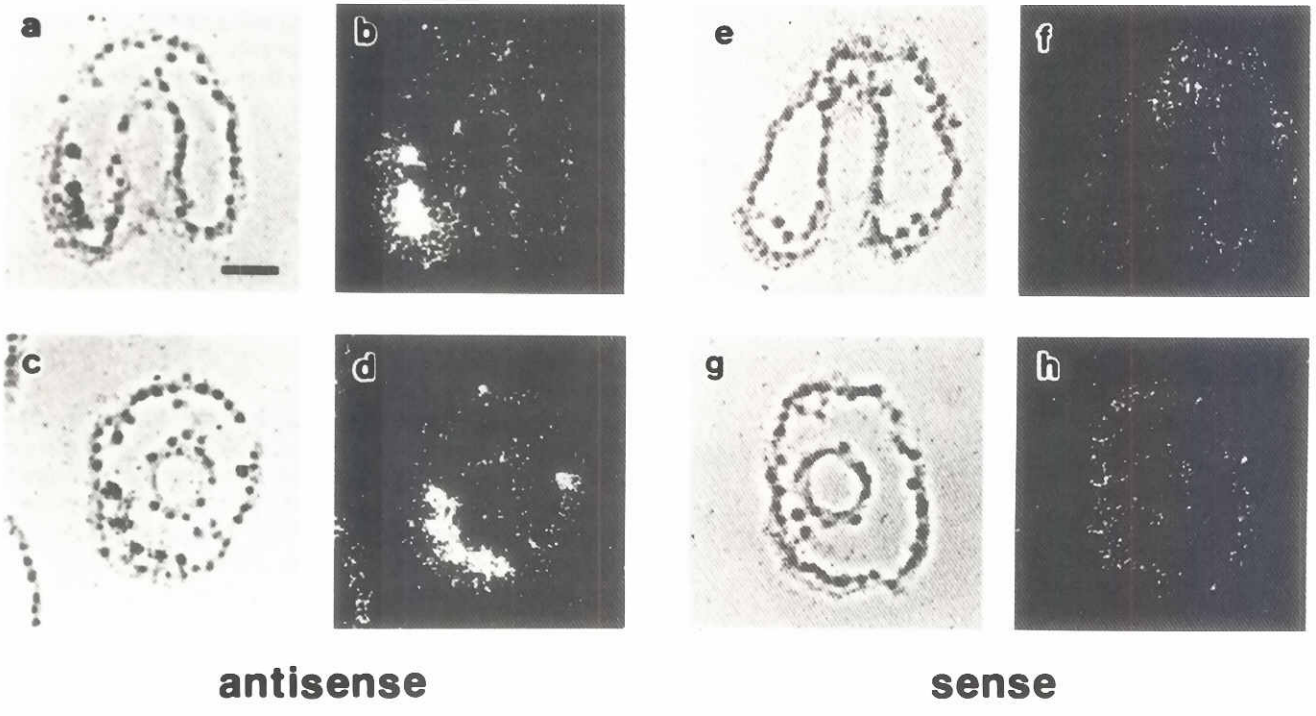

Fig. 8. *In situ* hybridization of pHPSMC mRNA in the gastrula embryo. (a, c, e and g) *Bright-field.* **(b, d, f and h)** *Dark-field. Bar represents 20 μm.*

Strongylocentrotus purpuratus, and SM50 turned out to be very basic and lacked N-glycosylation sites (Katoh-Fukui *et al.*, 1991). The pI of HSM41 protein in the present study was calculated to be 11. Structural analyses of the SM50 protein homolog of *Lytechinus pictus* provided further support for the corrected sequence (Livingston *et al.*, 1991). In *Lytechinus pictus*, there is a repeated element of 7 amino acids, GG(R/Q)QPGF, corresponding to about half the length of the repeats found in both *Hemicentrotus pulcherrimus* and *Strongylocentrotus purpuratus*. In this connection, it is interesting to note that *Strongylocentrotus purpuratus* and *Lytechinus pictus* diverged some 100 to 250 million years ago based on their actin gene sequences (Lee *et al.*, 1984), and that *Hemicentrotus pulcherrimus* taxonomically belongs to the same family as *Strongylocentrotus purpuratus* (Uchida, 1974).

Kitajima and Matsuda (1982), Kitajima (1986) and Matsuda *et al.* (1988) studied the proteins synthesized in micromeres isolated and cultured from *Hemicentrotus pulcherrimus*. They found several proteins that were specifically synthesized in parallel with the formation of spicules. One of them was a basic protein with a Mr of 43,000 (Kitajima and Matsuda, 1982; Matsuda *et al.*, 1988). Because of the limited resolution of one-dimensional gel electrophoresis, Kitajima (1986) was unable to identify a protein of Mr 43,000 specifically in micromeres, but he repeatedly pointed out that the characteristic labeling profile of the Mr 43,000 protein band suggested the presence of protein(s) among Mr 43,000 molecular species that are involved in spicule formation. From its Mr, basicity and time of appearance, it is highly probable that this protein corresponds to the HSM41 protein. The slight difference in molecular weight, Mr 41,000 vs Mr 43,000, could be due to differences in experimental conditions associated with molecular weight determination in polyacrylamide gels.

The spicule matrix is thought to be composed of at least 10 proteins including some glycoproteins (Benson *et al.*, 1986, 1987). Some may be involved in primary mesenchyme cell migration, and some may provide sites for $CaCO_3$ and $MgCO_3$ deposition. It would,

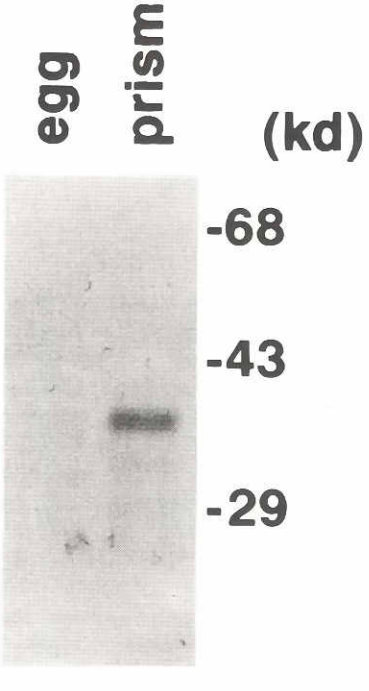

Fig. 9. Immunoblot analysis of HSM41 protein. *Proteins extracted from 700 unfertilized eggs and prism stage embryos were electrophoresed on a polyacrylamide gel and blotted onto a nitrocellulose filter. The filter was probed with an antiserum raised against a synthetic polypeptide corresponding to the repeated region of HSM41 protein.*

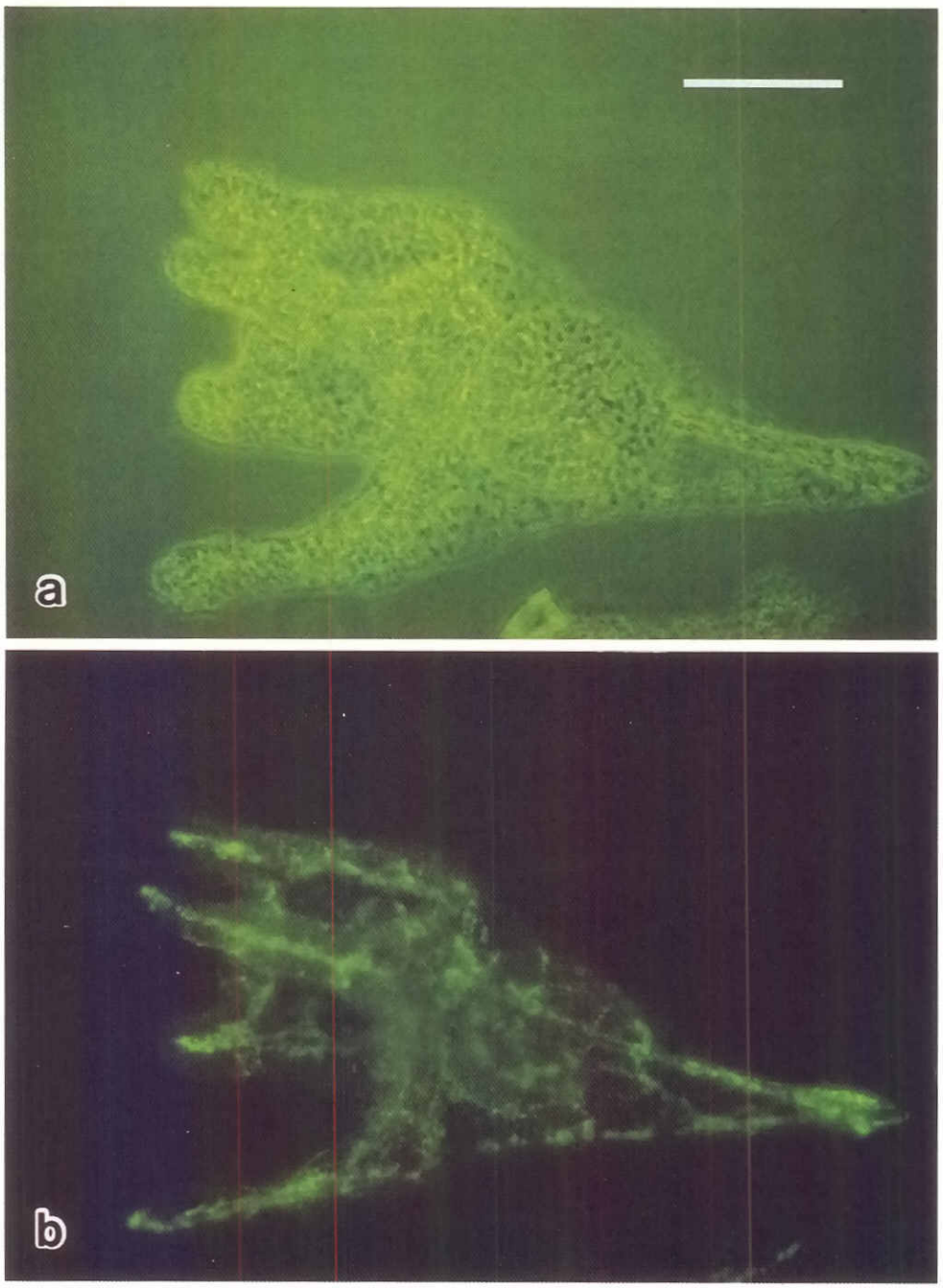

Fig. 10. Immunocytochemical staining of the pluteus stage embryo with the antiserum used in Fig. 9. (a) *Phase contrast micrograph.* **(b)** *Immunofluorescence micrograph. Bar represents 50 μm.*

therefore, be interesting to examine the structure of other spicule matrix proteins for features that may define their specific roles and their modes of interactions during spicule formation and biomineralization in general.

Materials and Methods

Screening of the cDNA library

A lambda gt10 cDNA library was constructed from poly(A)$^+$RNA isolated from prism stage embryos of *Hemicentrotus pulcherrimus* essentially according to the method of Gubler and Hoffman (1983). An EMBL3 genomic library was constructed using sperm DNA from the same species as described (Kaiser and Murray, 1985). Both libraries were screened with pHS72, cDNA clone for SM50 (Sucov *et al.*, 1987), as a probe. The inserted DNA fragment in each selected clone was subcloned into plasmid pUC19.

Sequencing and computer analysis

Two cDNA clones, pHPSMC1 and pHPSMC6, and genomic clone pHPSMG were selected and subcloned into pUC19, and they were sequenced in both directions by the dideoxy nucleotide chain termination method (Sanger and Coulson, 1977). In some cases where adjacent bands of DNA on a sequencing gel became compressed and difficult to read, sequencing was carried out with the use of dITP or 7-deaza-dGTP (Gough and Murray, 1983;

Mizusawa *et al.*, 1986). The nucleotide sequence and the derived amino acid sequence were analyzed with GENETYX program (Software Development Co., Ltd. Japan) and DNASIS program (HITACHI SOFTWARE ENGINEERING Co., Ltd. Japan) on a PC-9800 microcomputer.

In vitro *culture of dissociated blastomeres*

Blastomeres were dissociated at the 16-cell stage, and they were separated into micromeres and a combined fraction of macromeres and mesomeres as described (Okazaki, 1975). Isolated blastomeres were cultured *in vitro* by the method of Kitajima (1986).

Isolation of RNA and Northern blot analysis

Total RNA was extracted from embryos at successive stages or from blastomeres cultured *in vitro* using the guanidinium thiocyanate/hot phenol procedure (Maniatis *et al.*, 1982), but treatment with proteinase K was omitted. To remove polysaccharides, ethanol-precipitated total RNA was dissolved in 7 M LiCl and the solution was kept at -80°C for 30 min. Then it was centrifuged at 16,000 rpm at 4°C for 20 min in a microfuge. The final RNA pellet was dissolved in distilled water. Northern blot analysis was carried out as described (Maniatis *et al.*, 1982). An insert from cDNA clone pHPSMC1 was labeled with [α-^{32}P]dCTP by nick-translation and used as a probe.

Immunocytochemical staining and immunoblotting

A polyclonal antiserum against a synthetic 26-amino acid peptide corresponding to the repeated element (Fig. 2, region with broken lines) was raised in a rabbit. The peptide was coupled to keyhole limpet hemocyanin to enhance its immunogenicity. Pluteus stage whole embryos were mounted onto a slide glass, fixed with 1% formaldehyde for 20 min at room temperature, and were treated with 0.1% Triton X-100 in PBS for 10 min at room temperature to render them permeable to antibody. The specimen was incubated with 300-fold diluted antiserum for 12 h at 4°C. After washing in PBS, the specimen was incubated with FITC-labeled goat anti-rabbit antibody for 2 h at 4°C. For immunoblotting, a collection of prism stage embryos was heated at 100°C for 3 min in SDS sample buffer and electrophoresed in a 10% polyacrylamide -1% SDS gel (Laemmli, 1970). The separated proteins were transferred electrophoretically to a nitrocellulose filter. The filter was incubated with a blocking solution (5% skim milk, Difco) in PBS for 2 h and then with 200-fold diluted antiserum at 4°C for 12 h. Detection of immunoreactivity was carried out by the biotin-streptavidin peroxidase method (Bonnard *et al.*, 1984).

In situ *hybridization*

An EcoRI fragment from a cDNA pHPSMC1 was inserted into a pT7T3-U19 vector (Pharmacia, Sweden) in both orientations. ^{35}S-labeled RNA probes, sense and anti-sense, were transcribed in the presence of [α-^{35}S]UTP (New England Nuclear) with T3 phage RNA polymerase from plasmid truncated with Hind III. *In situ* hybridization was performed as described (Angerer and Angerer, 1981; Hardin *et al.*, 1988), but with some modifications. Briefly, sections were prehybridized for 2 h at room temperature in hybridization buffer containing non-labeled α-thio-UTP (New England Nuclear). This step substantially reduced the non-specific background signal. Tissue sections on slides were coated with autoradiographic emulsion (Sakura NR-M2) and were exposed for 1 week. After processing, sections were viewed in a Zeiss microscope equipped with bright-field and dark-field optics. Photomicrography was performed with Kodak T-MAX 400 film at ASA 400.

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References

ANGERER, L.M. and ANGERER, R.C. (1981). Detection of poly A$^+$ RNA in sea urchin eggs and embryos by quantitative *In situ* hybridization. *Nucleic Acids Res. 9:* 2819-2840.

ANGERER, R.C. and DAVIDSON, E.H. (1984). Molecular indices of cell lineage specification in sea urchin embryos. *Science 226:* 1153-1160.

BENSON, S.C. BENSON, N.C. and WILT, F. (1986). The organic matrix of the skeletal spicule of sea urchin embryo. *J. Cell Biol. 102:* 1878-1886.

BENSON, S., SUCOV, H., STEPHENS, L., DAVIDSON, E. and WILT, F. (1987). A lineage-specific gene encoding a major matrix protein of the sea urchin embryo spicule. I. Authentication of the cloned gene and its developmental expression. *Dev. Biol. 120:* 499-506.

BONNARD, C., PAPERMASTER, D.S. and KRAEHENBUHL, J.P. (1984). The streptavidin-biotin bridge technique: application in light and electron microscope immunocytochemistry. In *Immunolabelling for Electron Microscopy* (Eds. J.M. Polak and I.M. Varndell). Elsevier Scientific Publishers, Amsterdam, pp. 95-111.

CARSON, D.D., FARACH, M.C., EARLES, D.S., DECKER, G.L. and LENNARZ, W.J. (1985). A monoclonal antibody inhibits calcium accumulation and skeleton formation in cultured embryonic cells of the sea urchin. *Cell 41:* 639-648.

DAVIDSON, E.H. (1986). Differential gene expression in embryonic cell lineages of the sea urchin. In *Gene Activity in Early Development* (Ed. E.H. Davidson). Academic Press, Orlando, FL, pp. 213-246.

GOUGH, J.A. and MURRAY, N.E. (1983). Sequence diversity among related genes for recognition of specific targets in DNA molecules. *J. Mol. Biol. 166:* 1-19.

GUBLER, U. and HOFFMAN, B.J. (1983). A simple and very efficient method for generating cDNA libraries. *Gene 25:* 263-269.

HARDIN, P.E., ANGERER, L.M., HARDIN, S.H., ANGERER, R.C. and KLEIN, W.H. (1988). Spec2 genes of *Strongylocentrotus purpuratus*. *J. Mol. Biol. 202:* 417-431.

HARKEY, M.A. and WHITELEY, A.H. (1983). The program of protein synthesis during the development of the micromere-primary mesenchyme cell line in the sea urchin embryo. *Dev. Biol. 100:* 12-28.

HARKEY, M.A., WHITELEY, H.R. and WHITELEY, A.H. (1988). Coordinate accumulation of five transcripts in the primary mesenchyme during skeletogenesis in the sea urchin embryo. *Dev. Biol. 125:* 381-395.

HÖRSTADIUS, S. (1975). Isolation and transplantation experiments. In *The Sea Urchin Embryo* (Ed. G. Czihak). Springer-Verlag, Berlin, Heidelberg, pp. 364-406.

KAISER, K. and MURRAY, N.E. (1985). The use of phage lambda replacement vectors in the construction of representative genomic DNA libraries. In *DNA Cloning* Vol. I (Ed. D. M. Glover). IRL Press, Oxford, pp. 1-47.

KATOH-FUKUI, Y., NOCE, T., UEDA, T., FUJIWARA, N., HASHIMOTO, N., HIGASHINAKAGAWA, T., KILLIAN, C.E., LIVINGSTON, B.T., WILT, F.H., BENSON, S.C., SUCOV, H.M. and DAVIDSON, E.H. (1991). The corrected structure of the SM50 spicule matrix protein of *Strongylocentrotus purpuratus*. *Dev. Biol. 145:* 201-202.

KILLIAN, C.E. and WILT, F.H. (1989). The accumulation and translation of a spicule matrix protein mRNA during sea urchin embryo development. *Dev. Biol. 133:* 148-156.

KITAJIMA, T. (1986). Differentiation of sea urchin micromeres: Correlation between specific protein synthesis and spicule formation. *Dev. Growth Differ. 28:* 233-242.

KITAJIMA, T. and MATSUDA, R. (1982). Specific protein synthesis of sea urchin micromere during differentiation. *Zool. Mag. 91:* 200-205.

KOZAK, M. (1986). Point mutation defines a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell 44:* 283-292.

KYTE, J. and DOOLITTLE, R.F. (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol. 157:* 105-132.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:* 680-685.

LEAF, D.S., ANSTROM, J.A., CHIN, J.E., HARKEY, M.A., SHOWMAN, R.M. and RAFF, R.A. (1987). Antibodies to a fusion protein identify a cDNA clone encoding msp130, a primary mesenchyme-specific cell surface protein of the sea urchin embryo. *Dev. Biol. 121:* 29-40.

LEE, J.J., SHOTT, R.J., ROSE III, S.J., THOMAS, T.L., BRITTEN, R.J. and DAVIDSON, E.H. (1984). Sea urchin actin gene subtypes, gene number, linkage and evolution. *J. Mol. Biol. 172:* 149-176.

LIVINGSTON, B.T., SHAW, R., BAILEY, A. and WILT, F. (1991). Characterization of a cDNA encoding a protein involved in formation of the skeleton during development of the sea urchin *Lytechinus pictus*. *Dev. Biol. 148:* 473-480.

MANIATIS, T., FRITSCH, E.F. and SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, New York.

MATSUDA, R., KITAJIMA, T., OHINATA, H., KATOH, Y. and HIGASHINAKAGAWA, T. (1988). Micromere differentiation in the sea urchin embryo: two-dimensional gel electrophoretic analysis of newly synthesized proteins. *Dev. Growth Differ. 30:* 25-33.

MIZUSAWA, S., NISHIMURA, S. and SEELA, F. (1986). Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. *Nucleic Acids Res. 14:* 1319-1324.

OKAZAKI, K. (1975). Spicule formation by isolated micromeres of the sea urchin embryo. *Am. Zool. 15:* 567-581.

PITTMAN, D. and ERNST, S.G. (1984). Developmental time, cell lineage and environment regulate the newly synthesized proteins in sea urchin embryos. *Dev. Biol. 106:* 236-242.

SANGER, F. and COULSON, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA 74:* 5463-5467.

SUCOV, H.M., BENSON, S., ROBINSON, J.J., BRITTEN, R.J., WILT, F. and DAVIDSON, E.H. (1987). A lineage-specific gene encoding a major matrix protein of the sea urchin embryo spicule. II. Structure of the gene and derived sequence of the protein. *Dev. Biol. 120:* 507-519.

STADEN, R. (1982). An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. *Nucleic Acids Res. 10:* 2951-2961.

UCHIDA, T. (1974). *Doubutsu Keitoubunruigaku*. Nakayama Shoten, Tokyo, pp. 288-324.

VON HEIJNE, G. (1983). Patterns of amino acids near signal-sequence cleavage sites. *Eur. J. Biochem. 133:* 17-21.

WESSEL, G.M. and McCLAY, D.R. (1985). Sequential expression of germ-layer specific molecules in the sea urchin embryo. *Dev. Biol. 111:* 451-463.

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