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SpCoel1: a Sea Urchin Profilin Gene Expressed Specifically in Coelomocytes in Response to Injury

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SpCoel1 is a single copy gene that is specifically expressed in most of the coelomocytes of the adult purple sea urchin, *Strongylocentrotus purpuratus*. The 4-kb transcript from this gene has a relatively short (426 nucleotide) open reading frame (ORF) with long 3′ and 5′ untranslated regions. The ORF encodes a protein that has strong amino acid sequence similarity to profilins from yeast to mammals. Transcript titrations of SpCoel1 show significant increases per coelomocyte in animals that have been physiologically challenged. Increases in transcript levels are of similar magnitudes between animals receiving different treatments, such as injuries from needle punctures or from injections of foreign cells. The evidence presented here implies a molecular mechanism by which this lower deuterostome defense system responds to external insult, viz that an external "injury signal" activates a signal transduction system, which in turn mediates the alterations in cytoskeletal state that are required for coelomocyte activation.

INTRODUCTION

Coelomocytes, the immune effector cells of echinoderms, carry out many functions that are also performed by the cellular effectors of the immediate nonspecific defense systems of mammals. Thus, coelomocytes are capable of phagocytosis, chemotaxis, and expression of cytotoxic agents on activation in response to injury or invasion by foreign cells. There are four morphologically distinct types of coelomocyte in the purple sea urchin, *Strongylocentrotus purpuratus*. The majority of coelomocytes, 66.3 ± 11.6% (mean ± SD), are phagocytes; vibratile cells account for 13.9 ± 9.8%, colorless spherule cells account for 5.1 ± 4.6%, and red spherule cells account for 14.8 ± 8.5% (our unpublished data). Specific functions of the various coelomocyte classes are poorly defined. The phagocytes and the red spherule cells are apparently those that respond to injury, as indicated by their accumulation at injury sites (Coffaro and Hinegardner, 1977; Höbaus, 1979). The coelomic fluid is itself bactericidal (Wardlaw and Unkles, 1978; Service and Wardlaw, 1985), and echinochrome-A, contained within the red spherule cells, is a bacteriocidal agent (Service and Wardlaw, 1984). These cells degranulate in response to bacteria (Johnson, 1969). Various substances, cells, and microbes injected into the coelom of echinoderms are quickly removed by coelomocytes (Reinisch and Bang, 1971; Coffaro, 1978; Yui and Bayne, 1983). Clearance activities have also been observed in vitro where they are assigned to the phagocytic class of coelomocytes (Johnson, 1969; Messer and Wardlaw, 1979; Bertheussen, 1981). In addition, mixtures of adherent phagocytes from the same or different sea urchin species in culture display cytotoxic reactions (Bertheussen, 1979).

Coelomocytes have also been implicated in graft rejection. In sea urchins, as in other animals, surgically implanted body wall autografts heal in, whereas allografts and xenografts are rejected. An increase in the rejection rate of any secondarily implanted graft has been reported (Coffaro and Hinegardner, 1977). These results imply that the cellular rejection system in sea urchins can be generally enhanced by activation, but there is no evidence for recognition specificity as assayed by the effector functions that mediate rejection. Thus, a second set allograft is not rejected at a significantly faster rate than a third party allograft (Coffaro and Hinegardner, 1977). Nor do repeated injections into the coelom of a variety of substances, such as xenogeneic cells, proteins, viruses, and inert substances, result in detectable increases in clearance rates (Reinisch and Bang, 1971; Coffaro, 1978; Yui and Bayne, 1983). The immune defense systems of sea urchins are thus entirely nonspecific. Coelomocyte responses to challenge entail rapid alterations in motility, induction of phagocytic and encapsulation functions, and release of cytotoxic factors by degranulation. Similar cytological transformations

have been observed when coelomocytes are removed from coelomic fluid (Edds, 1977, 1980; Edds *et al.*, 1983). These responses involve redisposition of actin filaments and require a rapid transformation of the entire cytoskeletal organization.

This communication concerns a gene, SpCoel1, that appears to be involved in a sensitive response of *S. purpuratus* coelomocytes to minor injury to the organism. The transcript of the SpCoel1 gene encodes a polypeptide that displays striking amino acid sequence similarity to profilin, a small protein that has both actin and phosphatidyl inositol bisphosphate (PIP_2) binding functions (Reichstein and Korn, 1979; Lassing and Lindberg, 1985; Goldschmidt-Clermont *et al.*, 1991a,b). In mammalian cells, profilin appears to act at the intersection of signal transduction and cytoskeletal organization systems (Machesky *et al.*, 1990). We show that in response to challenge SpCoel1 transcripts increase sharply and specifically in coelomocytes. The SpCoel1 gene product thus may be involved in the protective response mechanisms of coelomocytes.

MATERIALS AND METHODS

Source, Handling, and Housing of Sea Urchins

Sea urchins used for this study were collected near Corona del Mar, California, from subtidal regions down to 30 ft. They were housed at the Caltech Kerckhoff Marine Laboratory (Corona del Mar, CA) in running, chilled, aerated sea tables under a scheduled light/dark cycle and fed kelp on a biweekly basis (Leahy *et al.*, 1978; Leahy, 1986).

RNA Isolation

Coelomocytes from adult sea urchins were drained from the animals after removal of the mouth structures, or "Aristotle's Lantern." The coelomic fluid was poured through sterile cheese cloth and mixed into 10 ml of cold Ca- and Mg-free sea water (CMFSW) (Humphreys, 1963) containing 30 mM EDTA, pH 8, salinity 32. The cells were pelleted and lysed by vortexing in 5 M guanidinium thiocyanate containing 50 mM NaOAc, 50 mM EDTA, and 5% beta mercaptoethanol. Other adult tissues, including ovary, testis, and gut, were dissected out of the animal, minced with razor blades, rinsed in filtered sea water, and lysed by vortexing in the guanidinium solution. After lysing, 0.1 vol of 30% sodium lauroyl sarcosine was added to each tissue sample, the DNA was sheared with a tight fitting dounce homogenizer, and the nucleic acids were isopycnically separated by centrifugation using a 5.7 M CsCl cushion containing 50 mM NaOAc and 50 mM EDTA. The pellets were washed in 70% ethanol, dried, resuspended in RNAse-free extract buffer (20 mM tris(hydroxymethyl) aminomethane [Tris], pH 8, 10 mM KCl, 3 mM $MgCl_2$, 0.5% sodium dodecyl sulfate [SDS]), extracted with phenol/Sevag (1:1) (Sevag solution is 24 parts chloroform, 1 part isoamyl alcohol), precipitated with 2.5 volumes of absolute ethanol, and resuspended in diethyl pyrocarbonate-treated water. Poly(A)$^+$ RNA was selected on an oligo(dT) cellulose column.

cDNA Library Construction

RNA was isolated from "activated" coelomocytes. For coelomocyte activation protocols, see below. Blunt-ended double-stranded cDNA was synthesized from poly(A)$^+$ RNA according to the manufacturers instructions (RNA Synthesis System Plus kit, Amersham, Arlington Heights, IL). The cDNA was either ligated to *Eco*RI linkers, size selected on a Biogel A-50 column (Bio-Rad, Richmond, CA) and ligated into lambda gt 10 phage, or ligated to *Bst*XI adapters (Invitrogen, San Diego, CA), size selected on a Select-5L spin column with a DNA fragment retention size of <271 base pairs (5′, 3′), ligated into pTZ18R-B plasmid (Invitrogen), and transformed into DH1αF′ bacteria (Invitrogen). The libraries were amplified once. The average insert size for the phage library was 1.7 kb and for the pTZ18R-B plasmid library was 1.4 kb.

SpCoel1 Coding Region Subcloned into the pET Expression Vector

The coding region of SpCoel1 (minus the first 20 amino acids) located in the Bsc9b cDNA between the *Kpn* I site and the 3′ end of the clone was subcloned into the *Bam*HI site of the pET-3a expression vector (Studier *et al.*, 1990) using specially constructed *Bam*HI to *Kpn* I linkers (from the Caltech Microchemical Facility) that restored seven amino acids to the 5′ end of the coding region. The construct was transformed into BL21(DE3) bacteria containing the pLysS plasmid that expressed a fusion protein of expected size. The fusion product included the first 11 amino acids of gene 10 and amino acid SpCoel1 sequence from amino acid 14 to the end of the sequence.

Rabbit Antiserum Against SpCoel1 Fusion Protein

The 15-kDa SpCoel1 fusion protein was isolated from the bacterial lysate by acrylamide gel purification. Two hundred micrograms were sonicated with Freund's complete adjuvant and injected intramuscularly in both hind legs of two New Zealand white rabbits. Subsequent booster injections were done with Freund's incomplete adjuvant subcutaneously on the rabbit's back. The rabbits were bled from the ear vein 10 d after the last injection. Blood was allowed to clot overnight at 4°C, the serum was recovered after centrifugation, and stored at −70°C.

Density Centrifugation of Coelomocytes on Percoll Step Gradients

Coelomic fluid was removed from a sea urchin and diluted into an equal volume of cold CMFSW-EDTA. The diluted cells were overlayered on a Percoll (Pharmacia, Piscataway, NJ) step gradient. The gradients were formed by overlayering decreasing concentrations of Percoll (dialyzed against CMFSW-EDTA over night) diluted in CMFSW-EDTA. The best range of Percoll concentrations for coelomocyte separations was 20%, 30%, 50%, 70%, and 100%. The gradients were spun at 118 × *g* at 4°C for 15 min without the brake. The layers of cells were unloaded from the top of the gradient.

RNA Blot Hybridizations

Hybridizations were carried out under standard conditions at 48°C with 50% deionized formamide, 5× SET (0.15 M NaCl, 30 mM Tris, pH 8, 2 mM EDTA), 1× Denhardt's solution (1% Ficoll, 1% bovine serum albumin, 1% SDS, 1% polyvinyl pyrrolidone), 20 mM phosphate buffer, pH 6.8, 200 μg/ml sheared denatured salmon sperm or calf thymus DNA, 200 μg/ml yeast transfer RNA. Final washes were carried out at 68–72°C in 0.3× SSC (1× SSC = 0.15 M NaCl, 15 mM Na citrate, pH 7) with 1% SDS.

^{32}P-rUTP-Labeled RNA Probes

Antisense RNA probes of known length from specific regions of the various cDNAs were generated on linearized templates with RNA polymerase T3 or T7 (New England Biolabs, Beverly, MA) incorporating ^{32}P-rUTP (800 Ci/mmo, from Amersham or New England Nuclear, Boston, MA) to the specific activity of 1.2×10^9 counts · min^{-1} · μg^{-1} of probe. Run-off transcripts were generated according to technical information from Promega Biotec (Madison, WI). After RNA synthesis, the DNA templates were removed by digestion with RNase-free RQ1 DNAse (Promega). The remaining RNA probe

was extracted with phenol/Sevag and spun through a G-50 Sephadex (Pharmacia) 1-ml column to remove unincorporated nucleotides. The riboprobes were heated to 80°C for 4 min before being used.

In Vitro Translation of SpCoel1

Run-off sense strand RNA was produced from cDNA clone Bsc9b (see Figure 1). The 5′ end of this clone is nt 491 of the full-length message. The transcript ended at the *Hin*dIII site, at nt 1911. This transcript includes the start and the following two stop codons (Figure 1). This message was added to wheat germ lysate (Amersham) with ^{35}S-methionine (Amersham) and incubated according to manufacturers instructions. The proteins were separated on a 4% stacking and 15% running SDS-polyacrylamide gel, stained with Coomassie, destained, dried, and autoradiographed for 2 d, together with markers and controls lacking SpCoel1 RNA.

Probe Excess Transcript Titration

Probe excess analysis of specific transcripts in varying amounts of total RNA from sea urchin tissues has been described in detail (Lee *et al.*, 1986). Numbers of transcripts per cell were derived by the calculations of Lee *et al.* (1986). Briefly, transcripts per cell = ab/cde, where as follows: a, cpm/pg of total RNA (a is the slope of the linear regression line); b, 1.5 pg RNA per cell (2.8 ng RNA/1800 cells per 72 h embryo) (Gouston and Wilt, 1981; Cameron *et al.*, 1989); c, cpm/pg of probe (specific activity of the probe); d, fraction of the probe represented in the message; and e, pg mass per transcript.

Coelomocyte Activation

Because sea urchins have been shown to increase their rejection rate of second set and third party allografts, we attempted to activate the coelocytes from which the cDNA library was constructed to increase the numbers of transcripts coding for gene products involved in the cellular defense responses of the animal. This was accomplished by injecting 100 μl of whole coelomic fluid (including proteins and cells) from a congeneric species *Stronglyocentrotus franciscanus* into the coelomic cavities of 16 adult *S. purpuratus* individuals. Injections were performed on days 1, 2, 3, 6, and 7; the cells were collected and pooled on day 9 and immediately processed for isolation of RNA.

Coelomocytes were also activated in a variety of other ways in the experiments in which SpCoel1 transcripts were measured. Sea urchins were injected with 50 or 150 μl of coelomic fluid from *S. franciscanus*; 50 μl of their own coelomic fluid was withdrawn and reinjected without removing the needle from the peristomial membrane or they were injured with 1 or 10 needle holes in the peristomial membrane per day. These treatments were carried out on days 1, 2, 4, and 5.

RESULTS

SpCoel1, a Coelomocyte-Specific cDNA Clone

This study began with the isolation of cDNA clones from a lambda gt10 library prepared from activated *S. purpuratus* coelomocyte poly(A)$^+$ RNA (see MATERIALS AND METHODS). The initial isolate, pSPT6, was selected on the basis of preliminary data that indicated that the transcript from which it derived is present at significantly higher levels in activated than in control coelomocytes obtained from undisturbed animals. Genome blots demonstrated that pSPT6 contains a single copy sequence. However, as indicated in Figure 1, pSPT6 includes only a section of the 3′ untranslated region (UT) of a coelomocyte message. Further clones were isolated using pSPT6 as a probe, and a composite

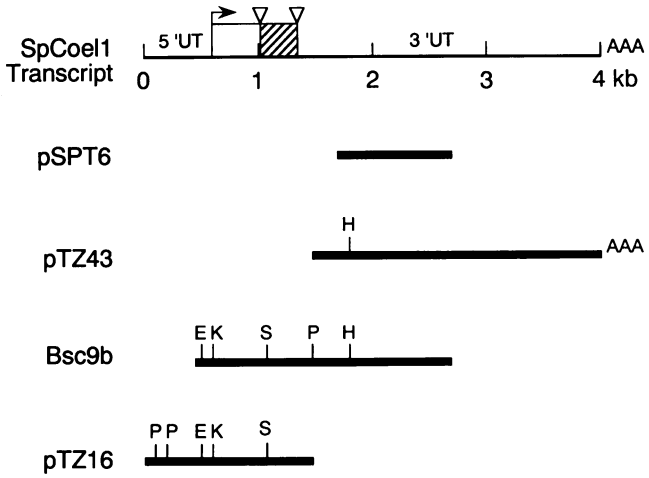

Figure 1. Map of SpCoel1 transcript from alignment of overlapping cDNAs. cDNA clones were obtained from an activated coelomocyte cDNA library (see MATERIALS AND METHODS). For sequencing, nested set deletions were generated by digestion with *Exo* III and mung bean nuclease, and the sequence was determined by the chain termination method (Sanger *et al.*, 1977; Messing *et al.*, 1983). The clones are shown aligned by restriction sites and are oriented so that the 5′ end is at the left. The full-length message is indicated at the top. The open region denotes the position of the ORF and the hatched region denotes an apparently cryptic ORF between the first two stop codons (see text). Arrow, start codon; triangles, stop codons; H, *Hin*dIII; R, *Eco*RI; K, *Kpn* I; S, *Sac* I; P, *Pvu* II; AAA, poly(A) tail.

map of the complete 4 kb transcript, which we have termed SpCoel1, is shown in Figure 1. The sequence of the SpCoel1 mRNA is given in Figure 2. An open reading frame (ORF) begins at nt 599 (as numbered from the 5′ end of the transcript), and the first stop codon is located at nt 1025. This defined an ORF of 426 nt, which encodes a rather small 15.3-kDa protein of 142 amino acids. A second stop codon does not occur until nt 1301. Were the stop signal at nt 1025 read through, the protein would then include 92 additional amino acids and its size would be 25.9 kDa. Numerous stop codons occur immediately after nt 1301. The stop codon at position 1025 is real and functional, however, at least in an in vitro translation assay. RNA was transcribed in vitro from clone Bsc9b (see Figure 1), which contains the whole of the relevant region, including some of the 5′ UT region, the start codon, the first and second stop codons, and about half of the 2.7 kb 3′ UT sequence. When added to a commercial wheat germ lysate, this RNA directed the translation of a 15.3-kDa protein, just as expected if translation indeed terminates at nt 1025.

SpCoel1 Amino Acid Sequence Comparisons

The SpCoel1 amino acid (a, a) sequence shown in Figure 2 was used to search the protein sequence database. The best match to the protein encoded by the SpCoel1 ORF is with the widely distributed protein profilin. As shown in Figure 3A, 72 of the 142 amino acids encoded

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GACACAGTGC GCCCTCTCCG AAGCAGCTCA TCCGCTCAAA TGTAGCGATG ATGAGGGTGA   60
TGGCTGCCAG CTGGGCCTTG GAGTCGCTAA TCTCATCCTC ATAGAGGAGA AGGCCTGTGA  120
CATGAACTCA TAGGCTACTG TCTCATGGTT CTCAAAGCCG ATCTCCCCAG CNGCGANNCG  180
TCTGTAGAAC AGTCGTAGAG GAANCTCCGG CTAGTTCGGC CTTGATCAGC GCTGTGATGG  240
TCTGGTGGCA GAACGTGAAG ATCTTCTGAC ATTTCTTCTC CCACTTATCA TCCTCTTCAC  300
CAAGCTCCTT GTATGTGAAA GCTAACCTGA ATGCAGAGAA TGTTAGGGGT GGTAATGTGT  360
ACTTTATCCT CTTGTTCCCT CCTACGCCAA AGTGTTTTCT TGCTGTGTTT AAAATCAGAT  420
ATTGCTGGTC TGCATCTTCT GCTTGTAACA GATTGATGTA GATCTAGGCT TAGACTGTGT  480
GTGAAACCGT ACCACTACCA CTAGGCCTAC CAGCGTTTAC ACATTTGTAC CGTACTATAC  540
TCGACGTCGG CGATTTACCC GAATTCGACG CTGTTTTCTT GTATTAACTT TCGTAAAC    598

ATG TCT TGG GAT TCA TAC GTC GAC AAT CTT ATA GCT CAG TCC AAA GAT     646
Met Ser Trp Asp Ser Tyr Val Asp Asn Leu Ile Ala Gln Ser Lys Asp     16

GCG TCA GGT ACC ACC CAT TGC GAC AAG GCC TGT ATA ATC GGT AAA GAT     694
Ala Ser Gly Thr Thr His Cys Asp Lys Ala Cys Ile Ile Gly Lys Asp     32

GGA TCT GCT TGG ACC ACG ATG CCG ACA TCC GAT ACC AGT AAC AAT TTA     743
Gly Ser Ala Trp Thr Thr Met Pro Thr Ser Asp Thr Ser Asn Asn Leu     48

AAG CTA ACC CCG GAA GAG ATG GCA AAT ATA GCA AAA TGT TTC AAG TCG     790
Lys Leu Thr Pro Glu Glu Met Ala Asn Ile Ala Lys Cys Phe Lys Ser     64

AAG GAT TTC GCA GCT TTC ATG TCC TCT GGT ATA TAT GTT AAC GGG ACA     838
Lys Asp Phe Ala Ala Phe Met Ser Ser Gly Ile Tyr Val Asn Gly Thr     80

AAA TAC CAA TTC TTA AGG GAA GAA GAC TCA AAG TTG GTG TTG GGG AAA     886
Lys Tyr Gln Phe Leu Arg Glu Glu Asp Ser Lys Leu Val Leu Gly Lys     96

AAG AAA GGT GAA GGA TCA CTC ACA TTG CAA AGC AGC AAG ACA GCG ATT     934
Lys Lys Gly Glu Gly Ser Leu Thr Leu Gln Ser Ser Lys Thr Ala Ile    112

GTA ATC GGT CAT TGC CCA GAA GGA GGC CAG CAA GGG AAT TTG AAT AAA     982
Val Ile Gly His Cys Pro Glu Gly Gly Gln Gln Gly Asn Leu Asn Lys    128

GCA GTT GGC GTA ATA GCA GAA TAT TTG GAG AGT TTG AGT ATG TAA TGT    1030
Ala Val Gly Val Ile Ala Glu Tyr Leu Glu Ser Leu Ser Met  * [Cys    143

ATG CCC AAC CAT AGG ATA TTG TAT ATG GCA AGA AGA TTT GGC TTG AGG    1078
Met Pro Asn His Arg Ile Leu Tyr Met Ala Arg Arg Phe Gly Leu Arg    159

TTG GAT GAC AGG AAA GGA GCT CAA GAA AAA CGG AAG ATT CAA GGA ATA    1126
Leu Asp Asp Arg Lys Gly Ala Gln Glu Lys Arg Lys Ile Gln Gly Ile    175

ATT TTA AGA ACA CCA TGT CTT CTC CTT GAC TTC ATG TGT TTG AAT GGC    1174
Ile Leu Arg Thr Pro Cys Leu Leu Leu Asp Phe Met Cys Leu Asn Gly    191

TGG TTA ACT GAA CAC ATC AGC TTT TTG GAG ATG TTA CAC TCA CTC ATA    1222
Trp Leu Thr Glu His Ile Ser Phe Leu Glu Met Leu His Ser Leu Ile    208

AAA ACT ATA CTT ACA TTT TAT ACA GCT ACA GGA TCA GAT CAA TTT AGT    1270
Lys Thr Ile Leu Thr Phe Tyr Thr Ala Thr Gly Ser Asp Gln Phe Ser    224

ATA AAG TTA TGT GAC AAA ACC CTT CTA GGA TAA AACTTAA AAGGGGCAAA     1320
Ile Lys Leu Cys Asp Lys Thr Leu Leu Gly] *                         234

CAAACTGTTT TATGTTTAAT TTGAAGAAAC AAAATATGTA TCTTGAGTAC ATGTGCAAGT 1380
GTTGGAAACA TCAATAGCTG TTATAAAGAA AAGAAACCCT AATCCATTAA ACATGACTGT 1440
TATTTAGTCT AAGGTAGTGG TTTACCTTGT ATAAAAACAT GCAACAGCTG TTCTAACTGT 1500
TAAATACCAA AAATAGAAAA TGTTATGAAA GTGATTTTGT AAAGAAGAAC ATCCTGTTTG 1560
AAAACTTTCC TCAAAACGAA GGATTTGGAA GATTTTTTTC TTTCTTTTGC TTATGGATTT 1620
AGGCATGTCA TCTAATAGCA ATTGGCTATT GTGTGCAGCA CTTCTAACAA ATATTATTCA 1680
ATTTTTTTCT CATCAGTTGT ACAATGTTGT CCATGAAGGC AATTAGATAG ATTAATTGCA 1740
TTTAAAAGAC ATAGAAATGC ATGATGTTAA CTACAGATTT TCATTTGTCA TAAAAATGCT 1800
ATGGAACCTA TGAATTTTTG TTTTATAAAG TTATTATGGT ACAATTCTGA TGCCATGCCA 1860
TTTTTCATGT TTGAAAATCT TTGTAATTCA TAAACTTGAT CTTAACCATT AAGCTTGTGA 1920
TTCTTAAAAT ACCAAGTACC TAAATTCTTA CATTTTAGCC AAGATAAAAT GAATTTTCAA 1980
AATACCATCC CTGGCTTCCT GAGAGAAAAA AGCATACCCC ATGATGCAAC TTGGATTTTT 2040
ACATTTTGTT TTCGTAGAGA GTAAATCATA AATTGCAACA TTACCCCCCA CGTTTGTGTN 2100
CTAGGATCTA GTCATGCATG GTTTCCCTTT TAACAGGGTA TGATTGCNGA AAACTTATNA 2160
GGCCCCAATT GCCCAAATTT CTACATGTTA GAAAACAGTA CAGAGTTTGC ATTGAATTTG 2220
TATATACATG TACATTTATA TATACATATA TATATATATA TATATATATA TATGTATTTA 2280
TACATGTATT ACGTTCTACC TAAATTTGTG GCTAGCGCAG GCGAGAAAAT AGCTACAATT 2340
CACTATTATG AATTAANATG ACTATCAAGA ACAAAAAAGA AAGTCCTTAA TGAAAGATAA 2400
TATATATCAC AGGAGAATTA AATTTTTCTT GAAATATTAC CTGAAATAAA ATTATGTTAT 2460
GTAAAAACAA CATTCTAGCC ATCTTGTTAA CGGCAAAAGC AATGTTTTAG GTATTGAGCA 2520
AAATCTATAC TATTGTCTCA CCAGATGATA AAAATCAAAT CCAAGAAAAT AAAATAAAAA 2580
ATAAATACAA AAAAAAAAAA AAAAA.....                                   2605
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Figure 2. SpCoel1 mRNA sequence and deduced amino acid sequence. The first ATG of the ORF occurs at nt 599. The ORF terminates with a stop codon located at nt 1025. A second stop codon occurs at nt 1301; both are denoted by an asterisk. The ORF is thus 426 nt in length (142 aa). If utilization of the initial stop codon at nt 1025 was in some way suppressed, the cryptic additional peptide indicated in brackets would be added to generate an ORF of 699 nt (234 aa).

by SpCoel1 (i.e., 51%) can be found in at least one of the four bona fide profilins at homologous positions in the protein. The comparison is anchored by agreement with all four profilins in 14 positions located throughout the protein. To achieve this alignment, three short regions of the sea urchin sequence were considered as "insertions" with respect to all four other profilins: a 7 amino acids sequence (positions 16–22 of SpCoel1), a 3 amino acids sequence (positions 45–47), and a 2 amino acids insertion (positions 66 and 67). On the other hand, the mouse and cow profilins possess a 16 amino acid insertion (at position 120 of the SpCoel1 sequence) with respect to the sea urchin, amoeba, and yeast profilins. All registry changes other than the three small sea urchin insertions utilized in the optimized alignment shown in Figure 3A are supported by occurrence of the same changes in at least two of the four profilins used for the comparison. Of the 72 shared amino acids resulting from this alignment, 23 are shared both with at least one mammalian profilin and at least one of the lower eukaryote profilins; 12 are shared only with one or more of the mammalian profilins; and 36 are shared only with one or more of the lower eukaryote profilins. Figure 3A indicates convincingly that SpCoel1 is a sea urchin profilin or a closely related protein. No other comparisons were discovered that approach the >50% level of similarity with a "consensus" protein that is revealed by the multiple profilin alignment illustrated.

Even though the SpCoel1 sequence contains a genuine termination signal after nt 1025, it was interesting to explore sequence similarities in the ORF that follows nt 1025, because it is possible that this termination codon could be spliced out or suppressed in some cell types or stages of the life cycle not yet examined. This comparison, shown in Figure 3B, reveals a surprising 28% similarity, in almost perfect registry, with a portion of the human and rabbit low-density lipoprotein (LDL) receptor that includes the transmembrane sequence. Identities between SpCoel1 and the human and rabbit sequences are found throughout this portion of the sequence. The human and rabbit proteins include four amino acids, NPVY, that are located just inside the plasma membrane. The tyrosine (#805 in the rabbit) at the end of this diagnostic sequence element is necessary for binding the LDL receptor to clathrin-coated pits during internalization (Chen *et al.*, 1990). The SpCoel1 sequence lacks the NPVY element, except for the tyrosine, which occurs in the SpCoel1 sequence in exactly the same relative position as in the LDL receptor (Figure 3B, amino acid 215; this tyrosine is highlighted on a black background).

SpCoel1 Expression in Adult Tissues

An RNA blot hybridization study of SpCoel1 expression in adult tissues is shown in Figure 4 (see MATERIALS AND METHODS). Testis, ovary, and gut showed almost

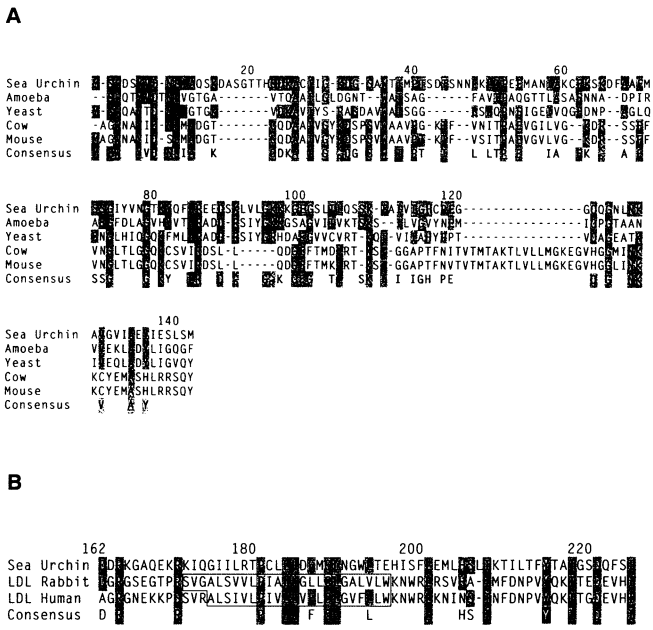


Figure 3. SpCoel1 encodes a profilin. Protein similarities were identified by searching the Protein Identification Resource data base Release 29.0, June 3, 1991 (National Biomedical Research Foundation). Amino acid sequence alignments were generated with the FAST A program (Pearson and Lipman, 1988), improved with the Macaw alignment program (Schuler *et al.*, 1991) for Windows 3.0 (Microsoft), and minor adjustments were then made by hand. Amino acid numbers refer to the SpCoel1 sequence. (A) Multiple alignment comparison with selected profilin sequences. Shaded letters denote matches to SpCoel1. Within the "consensus" sequence shown on the bottom row, an amino acid letter appears if there is at least one match to SpCoel1; the letter is shaded if the sea urchin sequence matches two or more of the four profilins and is underlined if the amino acid is found in all four profilins. The sequences on this table are from the following sources: Amoeba, *Acanthamoeba castellanii* profilin-I sequence from Ampe *et al.* (1988b) and Pollard and Rimm (1991); Yeast, *Saccharomyces cerevisiae* sequence from Oechsner *et al.* (1987); Cow, profilin sequence from Ampe *et al.* (1988a). The mouse profilin sequence is from Widada *et al.* (1989). In this comparison, R is considered equivalent to K, but no other equivalences between chemically similar amino acids have been accepted, and the comparison is thus a conservative one. Some pairwise similarity results for SpCoel1 versus other profilins are as follows: sea urchin (*Anthocideris crassispina*) 75%; sand dollar (*Clypeaster japonicus*) 73%; amoeba (*A. castellanii*) 37%; yeast (*S. cerevisiae*) 35%; slime mold (*Physarum polycephalum*) 35%; human (*Homo sapiens*) 23%; cow (*Bos bovis*) 21%; mouse (*Mus musculus*) 17%. These were obtained using the McCaw program (Schuler *et al.*, 1991) for Windows 3.0 (Microsoft). References for the additional sequences are as follows: for the sea urchin (*A. crassispina*) and sand dollar (*C. japonicus*), Takagi *et al.* (1990); for slime mold (*Physarum polycephalum*), Binette *et al.* (1990), Takagi *et al.* (1990); for human (*H. sapiens*), Kwiatkowski and Bruns (1988). (B) Comparison of the cryptic SpCoel1 ORF after nt 1028 with a portion of mammalian LDL receptor sequences. Shaded letters denote a match between SpCoel1 and either of the sequences listed. The boxed region denotes the amino acids located within the membrane in the mammalian sequences. The concensus sequence is shown on the bottom row. The black shaded Y, located in the concensus sequence, is found in all three sequences and in the LDL receptor is involved in receptor internalization (Chen *et al.*, 1990). The human sequence is from Yamamoto *et al.* (1984); the rabbit sequence is from Yamamoto *et al.* (1986).

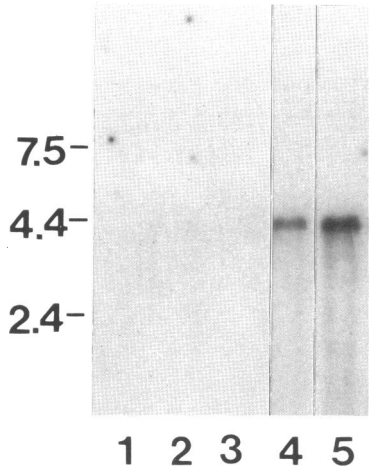


Figure 4. SpCoel1 blot hybridization to adult sea urchin RNAs. One microgram of poly(A)$^+$ RNA was electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde in 1× MOPS buffer (20 mM 3-[N-morpholino] propanesulfonic acid, 5 mM NaOAc, 1 mM EDTA, pH 7), blotted onto Genescreen Plus (Millipore, Bedford, MA), and probed with ^{32}P-rUTP-labeled antisense RNA under standard hybridization conditions (see MATERIALS AND METHODS). The probe spanned nt 1107 to 489 of the Bsc9b clone (*Sac* I site to the 5′ end of the cDNA clone, 609 nt long; see Figure 1). Lane 1, ovary; lane 2, testis; lane 3, gut; lane 4, coelomocytes; lane 5, activated coelomocytes. Marker sizes are shown on the left.

no expression compared with the coelomocyte samples. Control RNA gel blots and other evidence showed that these RNAs were not degraded, and the experiment indicates at least a major difference in profilin transcript concentration comparing coelomocytes with other cell types (this, of course, does not indicate the relative concentrations of stable profilin protein). On very long exposures, faint bands could be seen in the other adult tissues, which could have been due either to very low expression in those tissues or, possibly, to expression in migratory coelomocytes that were present in or on these tissues when they were isolated from the animal. Note that the band in the activated coelomocyte lane of Figure 4 is more intense than that for the normal coelomocytes (see below).

To determine which type of coelomocyte expressed the SpCoel1 protein, rabbit antiserum was raised against an SpCoel1 fusion protein. Coelomocyte types were differentially enriched in Percoll step gradient fractions according to their intrinsic density differences, as described in MATERIALS AND METHODS. Proteins extracted from equal numbers of the major cell types in each fraction were separated by SDS-polyacrylamide gel electrophoresis, electroblotted, and incubated with the rabbit antiserum. Figure 5 shows that all four cell fractions produce the SpCoel1 protein. However, the phagocyte-enriched fractions appear to contain more than do the other types of coelomocyte (compare lanes 1 and 2 with lanes 3 and 4 on Figure 5). The small phagocytes (lane 1) appear to have more SpCoel1 protein than do the larger phagocytes (lane 2). The coelomocytes display only the 15.3-kDa protein, indicating that the first stop codon is indeed utilized by these cells and that a transmembrane region is not present in the protein.

To obtain a more detailed analysis of SpCoel1 representation in coelomocytes of different types, the rabbit antiserum was used for a cytological immunofluorescence study. As shown in Figure 6B, most coelomocytes produced the SpCoel1 protein. All of the red and the colorless spherule cells and all of the vibratile cells are uniformly positive. However, only some of the phago-

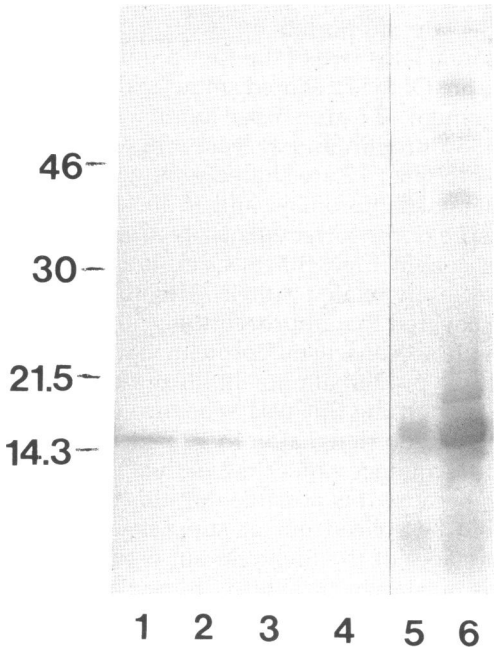


Figure 5. Immunological detection of SpCoel1 protein in coelomocyte fractions. Equal numbers (3×10^5) of the major coelomocyte type from each Percoll fraction were lysed by boiling in SDS sample buffer (4% SDS, 0.2% glycerol, 20% beta mercaptoethanol, trace of bromophenol blue). Samples were loaded onto an SDS-polyacrylamide gel (4% stacking gel, 15% running gel), electrophoresed at 25 mA for 2.5 h, and electroblotted onto an immulon-P filter (Millipore) in blotting buffer (20% methanol, 20 mM Tris, pH 8.8, 150 mM glycine, 0.05% SDS) at 0.3 A for 2 h at 4°C. The filter was subsequently incubated in blocking buffer (phosphate buffered saline with 1% normal goat serum, 1% bovine serum albumin [BSA], 0.1% nonidet P-40), followed by rabbit anti-SpCoel1 antiserum, diluted 1:40 in blocking buffer. The secondary antibody, goat anti-rabbit Ig labeled with alkaline phosphatase, was diluted 1:400 in Tris-buffered saline containing 3% BSA. The enzyme substrate reaction was run according to instructions from the Boehringer-Mannheim Genius kit. Lane 1, 20% Percoll fraction, mostly small phagocytes with some large phagocytes. Lane 2, 30% Percoll fraction, mostly large phagocytes with some small phagocytes and 2.4% contamination with other cell types. Lane 3, 50% Percoll fraction vibratile cells with 13.6% colorless spherule cells. Lane 4, 70% Percoll fraction, red spherule cells with 4.5% contamination of various cell types. Lane 5, gel-purified fusion protein used to inject the rabbits. Lane 6, bacterial lysate containing the fusion protein. Marker standards are shown on the left.

cytes displayed SpCoel1 protein, whereas some clearly do not. No fluorescence was observed over a subpopulation of the phagocytes, consisting of most of the large phagocytes and some of the small ones (compare Figure 6, A and B). Although it is not easily seen in Figure 6B, the small phagocytes stain more intensely than do the other cell types, as expected from the antibody blot shown in Figure 5. Because positive and negative cells are adjacent to each other on the same slide, it seems unlikely that any difference in fixation or treatment could account for failure of some of the large phagocytes to stain, although local effects cannot be formally excluded. The SpCoel1 protein is clearly not mounted on the cell surfaces, because unfixed live cells appear uniformly negative.

SpCoel1 transcripts were also detected in coelomocytes by in situ hybridization (Figure 6C). An antisense RNA probe made from clone Bsc9b was hybridized to Percoll separated coelomocytes. Transcripts are present in all cell types, including all of the phagocytes. Because some cells apparently fail to react at all with the antibody, while all contain the SpCoel1 message, this suggests that some of the phagocytes either do not translate the SpCoel1 transcript, have in some way eliminated the SpCoel1 protein, or that the determinants recognized by the antiserum are masked when the SpCoel1 protein is associated with other molecules.

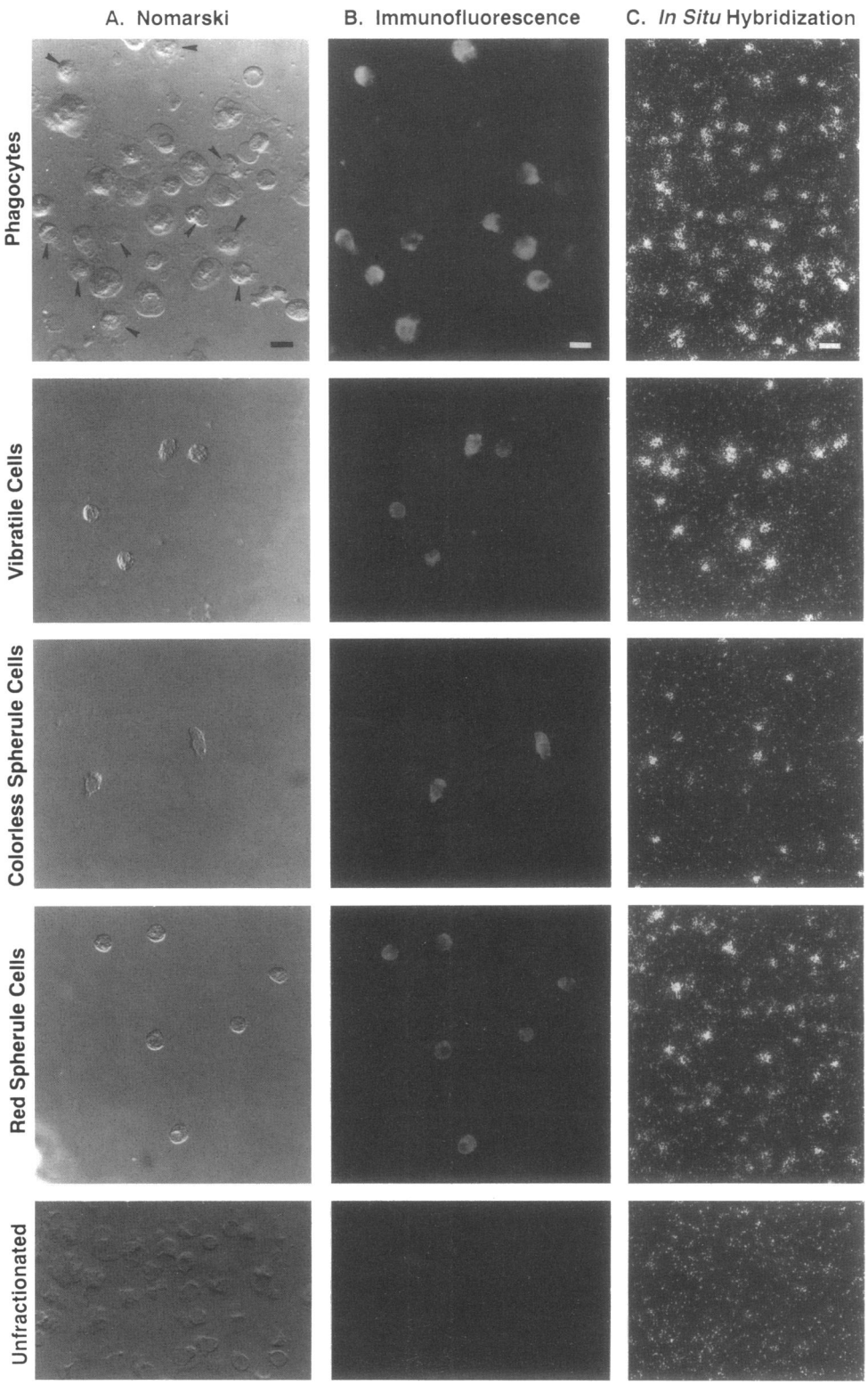
SpCoel1 Transcript Titrations

Preliminary analyses on coelomocyte RNA collected from sea urchins that had been injected with *S. franciscanus* coelomic fluid indicated that this treatment induces an increase in SpCoel1 transcript level, as compared with "unactivated" or "normal" coelomocytes (e.g., in Figure 4, compare lanes 4 and 5). In further experiments, we found that four injections of *S. franciscanus* coelomic fluid over 5 d resulted in a higher transcript level per cell than one injection alone. Preliminary cell count data indicated that there are no gross variations in coelomocyte populations in response to this physiological challenge. Nor have proliferative responses of mixed coelomocytes in vitro been found (Bertheussen, 1979). The increases in SpCoel1 transcripts after injection are thus due to changes in the number of transcripts per average responding cell, rather than to changes in the relative sizes of the coelomocyte populations.

Groups of six sea urchins were used to analyze in more detail the time course of SpCoel1 transcript changes in response to various treatments (see MATERIALS AND METHODS). Probe excess titrations (Lee *et al.*, 1986) carried out on total RNA extracted from these pooled coelomocytes yielded the average values of SpCoel1 transcripts per cell shown in Figure 7. All experimental groups displayed elevated levels of SpCoel1 transcript, compared with controls, as early as 3 h after the final treatment. Animals receiving either puncture injuries or reinjected coelomic fluid maintained their elevated transcript levels at 24 h. SpCoel1 transcript levels had returned to normal by 3 d. The group receiving 50 μl of coelomic fluid from *S. franciscanus* displayed a peak transcript level at 24 h, and the level has returned to normal by 6 d.

The sea urchins used in this study were from wild populations collected subtidally between Corona del Mar and San Diego, California. Though they were held under carefully controlled conditions in our large-scale culture system (Leahy *et al.*, 1978) for several weeks before use, they could have retained significant physiological differences that might have affected our results. To examine individual responses, SpCoel1 titration measurements were carried out on coelomocytes prepared from single experimental and control animals treated as above. All the coelomocytes from an individual were needed for a single transcript titration measurement, and thus only one time point per animal could be examined. The coelomocytes were collected 1 d after the final treatment, i.e., the time at which the maximal response had been observed in the previous experiments (Figure 7). The results are displayed in Figure 8. Pairwise comparisons using Student's *t* test and Duncan's multiple range test were used to determine the significance of the differences observed between groups. The results of both tests showed that the experimental groups had significantly elevated mean transcript levels per cell, compared with controls, with a hierarchy of statistically significant differences among these groups. Animals receiving a single needle-hole injury per day, and those that had 50 μl of their own coelomic fluid withdrawn and reinjected, displayed levels of SpCoel1 per cell that were slightly above normal. (By Student's *t* test, the one-time injury and sham-injected distributions are both significantly different from the pooled controls at the $p = 0.02$ level of significance but are indistinguishable from one another.) When foreign cells were injected or if the animals were injured 10 times per day, a much greater significant difference was noted in comparison with the normals. (By Student's *t* test, the 10 times injury and the 150-μl *S. franciscanus* coelomic fluid injection distributions differ from the controls at $p \leq 0.005$ and $p \leq 0.0005$ levels of significance, respectively.) These data suggest that the sea urchin coelomocyte defense system is extremely sensitive to physiological challenges, that it is easily activated, and that it responds to varying amounts of injury but does not differentiate between different types of injury. The most likely interpretation is that the animals are only responding to injuries from the needles and irritations from the foreign cells.

In general, as Figure 8 shows, the results for the individual animals within an experimental group were coherent. However, within the group receiving 50 μl of *S. franciscanus* coelomic fluid, one animal had 138 transcripts per cell, by far the highest value seen in any of


A. Nomarski
B. Immunofluorescence
C. *In Situ* Hybridization
Phagocytes
Vibratile Cells
Colorless Spherule Cells
Red Spherule Cells
Unfractionated

the groups. This animal may simply have mounted a sharper response to *S. franciscanus* coelomic fluid than do most animals. Or, it may have been in a pathological or disturbed condition before the experiment was begun. Furthermore, because the surfaces of these animals cannot be "sterilized" with alcohol before being injected because they do not have a dead protective epidermal layer as do mammals, surface microbes may have been introduced during injections of foreign coelomic fluid. This could occasionally result in a combined response to the injury of injection, the foreign coelomic fluid, and unknown microbes. Whatever the cause, the occurrence of this result is interesting because it suggests that there are forms of stimuli that elicit far greater coelomocyte responses than those we have utilized. Identification of these stimuli would provide a more direct indication of the types of physiological challenge against which the coelomocyte defense reaction is actually armed.

DISCUSSION

We have identified a sea urchin gene, SpCoel1, that is expressed specifically in coelomocytes and at an enhanced level in response to injury. This is the first molecular parameter of lower deuterostome cellular defense systems so far reported. It is fascinating that SpCoel1 appears to encode an intracellular protein that in other organisms is believed to link cytoskeletal and signal transduction systems.

SpCoel1 and Other Profilins

Profilins have been cloned and sequenced from a variety of species. Although their amino acid sequences are similar, the transcript sizes for the other profilins are substantially smaller than that for SpCoel1, ranging in size from 0.5 kb in *Physarum* to 0.8 kb in humans, versus 4 kb for SpCoel1. None of the profilin mRNAs previously described include a 5′ untranslated region as long as 0.6 kb nor a 3′ trailer 2.7 kb in length. Pairwise amino acid comparisons of other profilins to SpCoel1 are summarized in Figure 3. The most closely related proteins are those from other echinoderms, which display a 73 to 75% similarity to SpCoel1. Profilins from *Acanthamoeba* and the fungi are ~35% similar, and individual mammalian profilins are ~20% similar.

It has been shown that profilins bind to actin and to polyphosphoinositides (Lassing and Lindberg, 1985,

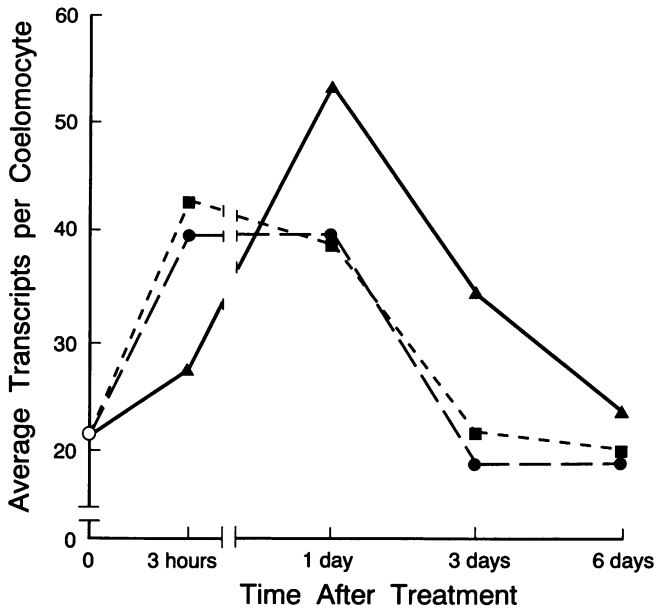


Figure 7. Average changes in SpCoel1 transcript levels in pooled coelomocytes after various treatments. Groups of six sea urchins were treated on day 1, 2, 4, and 5 as described in MATERIALS AND METHODS. The cells were collected and pooled after the last treatment at 3 h, 1 d, 3 d, or 6 d. Total RNA was isolated and the SpCoel1 transcripts measured per average cell by single strand probe excess titration. The accuracy of each titration was calculated from the linear regressions (correlation coefficient ≥0.9918). ○, normal sea urchins (this point is the average of 3 groups of 6 control sea urchins). ●, sea urchins injured with one needle hole in the peristomial membrane each day of treatment. ■, 50 μl of self coelomic fluid was withdrawn and reinjected without removing the needle each day of treatment. ▲, sea urchins were injected with 50 μl of coelomic fluid from *S. franciscanus* each day of treatment.

Figure 6. SpCoel1 expression in coelomocytes by immunofluorescence and in situ hybridization. (A) Four types of coelomocytes by Nomarski optics. Coelomocytes were removed from the animal, diluted into an equal volume of CMFSW-EDTA, and separated on a Percoll step gradient. (Cells used for negative controls, shown in the bottom photographs, were not fractionated.) The cells were then spun onto poly-1-lysine-coated slides using trunion carriers in an IEC refrigerated centrifuge at 576 × *g* for 5 min at 4°C, fixed immediately in cold 4% paraformaldehyde in CMFSW-EDTA for 2 min, and washed in CMFSW. These are paired photographs to the immunofluorescent photos in B. Arrow heads indicate the subpopulation of phagocytes that are positive for SpCoel1 protein expression. All cell types were photographed at 40× magnification; scale bar = 10 μM. (B) Immunofluorescent staining of coelomocytes. Separated fixed cells were preincubated in a humidified chamber at room temperature in blocking buffer (CMFSW-EDTA containing 10% normal goat serum, 1% BSA, 0.01% nonidet P-40) for 40 min, followed by a 1:200 dilution of rabbit anti-SpCoel1 in blocking buffer for another 40 min. The secondary antibody was goat-anti-rabbit IgG labeled with Cy3, diluted 1:120 in blocking buffer. The unfractionated cells in the bottom photograph were stained with normal rabbit serum diluted 1:200 in blocking buffer, followed by the goat anti-rabbit IgG secondary antibody as above. Cells were observed in an Olympus photomicroscope equipped with an Olympus exposure meter and an ultraviolet light source. Kodak ASA 400 print film was exposed for 2.5–5 s depending on staining intensity. All cell types were photographed at 40× magnification; scale bar = 10 μM. (C) In situ hybridization of SpCoel1 on coelomocytes. Hybridization of antisense riboprobes labeled with ^{35}S-rUTP to mRNA in density centrifugation separated coelomocytes was carried out according to Rothenberg *et al.* (1990). The bottom photograph shows background using the sense strand probe. Slides were autoradiographed for 6 d and photographed on black and white (ASA 64) film under dark field conditions. All cell types were photographed at 20× magnification and were from a different cell preparation than those seen in A and B; scale bar = 20 μM.

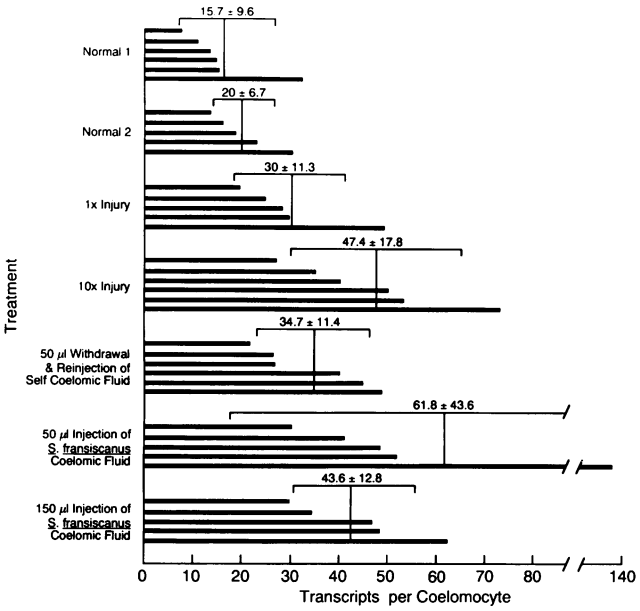


Figure 8. SpCoel1 transcript levels for individual sea urchins after various treatments. Individual sea urchins were treated on the same schedule as for the experiment shown in Figure 7, and the cells were collected 1 d after the last treatment. Total RNA was isolated and the SpCoel1 transcripts measured. The accuracy of the titrations for each sea urchin was calculated from the linear regressions (correlation coefficient ≥0.9649). Data from two experiments were combined and are shown together. One or 10 times injury is 1 or 10 needle-hole injuries in the peristomial membrane per day on each day of treatment. Means and SDs are shown for each group.

1988; Goldschmidt-Clermont *et al.*, 1991a). Variations from one profilin to another in binding to either actin or polyphosphoinositides, or both, seem to be based in part on the isoelectric point of the profilin. For the three profilin isoforms from *Acanthamoeba* (Ampe *et al.*, 1985, 1988b; Pollard and Rimm, 1991), profilin-IA and -IB are acidic (pI = 5.5) and bind well to actin, whereas profilin-II is basic (pI = 9) and binds moderately well to both actin and PIP_2, although it is probably mainly associated with PIP_2 (Machesky *et al.*, 1990). Profilin from human platelets is basic (pI = 9) and binds well to both. In addition to the overall pI of profilin, binding differences are also due to the amino acids found in positions 24, 50, and 66 as numbered in *Acanthamoeba* profilins (see Table 3 of Machesky *et al.*, 1990; Haarer and Brown, 1990). SpCoel1 is similar to profilin-II in these positions (31, 65, and 81 numbered according to Figure 3A) where all three are lysines. Profilin-II has histidine, lysine, and arginine, whereas profilin-I has leucine, asparagine, and histidine. SpCoel1 would be more likely to bind well to PIP_2, as does *Acanthamoeba* profilin-II, due to the lysine at position 65. However, the deduced isoelectric point of the SpCoel1 protein is acidic (pI = 6.1), suggesting that it should bind better to actin. Because only a single profilin or profilin-like gene can be detected in *S. purpuratus* (as in humans), the SpCoel1 protein might be expected to bind both actin and PIP_2 (Machesky *et al.*, 1990).

Possible Functions of SpCoel1

Profilins are thought to provide a regulatory linkage between the phosphatidyl inositol signal transduction pathway and cytoskeletal transformations, because binding of polyphosphoinositide metabolites is exclusive of binding to actin subunits (Lassing and Lindberg, 1985, 1988). Transient or prolonged interactions between profilin and actin can promote or block filament polymerization (Goldschmidt-Clermont *et al.*, 1991b). When bound to polyphosphoinositides, profilin also prevents their hydolysis by phospholipase C (Goldschmidt-Clermont *et al.*, 1990; Machesky *et al.*, 1990), except when the phospholipase C has been phosphorylated by tyrosine kinases such as are activated by growth factor receptors (Goldschmidt-Clermont *et al.*, 1991a). Profilin may thus regulate interactions between different signal transduction pathways, the stimulation of which cause changes in cytoskeletal form (Stossel, 1989; Goldschmidt-Clermont *et al.*, 1991b). In yeast, profilin mutants display defects in cell shape as well as a variety of other morphological abnormalities, and profilin interacts with signal transduction pathways that are activated by environmental nutrients (Haarer and Brown, 1990; Vojtek *et al.*, 1991).

The profilin-like SpCoel1 protein could be involved in biochemically similar aspects of the response mechanisms mounted by sea urchin coelomocytes on activation. Coelomocytes are sensitive to a variety of physiological challenges, including infection or injury (Coffaro and Hinegardner, 1977; Höbaus, 1979) or the presence of bacteria, foreign cells, or inert objects (Johnson, 1969; Bertheussen, 1981). The activation of coelomocytes involves dramatic changes in behavior that are controlled by changes in the actin cytoskeleton, which in turn may be initiated by signals from the cell surface. In this study, we demonstrate that SpCoel1 transcript concentrations per cell increase significantly in response to very minor puncture injuries made at peripheral locations remote from most of the coelomocytes. A diffusible injury signal must therefore be involved in the response we have measured. Perhaps, by analogy with previously studied systems, the coelomocyte profilin functions at the intersection between the intracellular system that informs coelomocytes that a remote injury has occurred, and the cytoskeletal reorganization involved in its response.

Evolutionary Implications

The echinoderm cellular defense system responds to challenges with a form of nonspecific cellular activation, which exhibits some similarities to the nonspecific primary line of cellular defense in mammals mediated by granulocytes, macrophages, and perhaps natural killer

cells. Sea urchins apparently survive quite well without immune recognition specificity. The effectiveness of their defense systems is suggested by the long evolutionary history of this taxon and by the current large population size of species such as *S. purpuratus*. Furthermore, these animals are individually long lived. Some have survived many years in our laboratory, and they display remarkable abilities to recuperate from infections and injuries. The intrinsic usefulness of this nonadaptive cellular defense systems is clearly rooted in coelomocyte responsiveness, of which we here provide an initial molecular parameter. Sea urchins share a deuterostome ancestry with the chordates, and their coelomocytes may function in a way that is homologous with some elements of the nonspecific primary cellular defense system of chordates. Perhaps the adaptive components of the vertebrate immune system also evolved from simpler migratory cells endowed with various effector functions activated in response to diffusible systemic signals, i.e., from cells much like the coelomocytes of modern echinoderms.

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REFERENCES

Ampe, C., Markey, F., Lindberg, U., and Vanderkerckhove, J. (1988a). The primary structure of human platelet profilin—reinvestigation of the calf spleen profilin sequence. FEBS Lett. *228*, 17–21.

Ampe, C., Sato, M., Pollard, T., and Vanderkerckhove, J. (1988b). The primary structure of the basic isoform of *Acanthamoeba* profilin. Eur. J. Biochem. *170*, 597–601.

Ampe, C., Vanderkerckhove, J., Brenner, S., Tobacman, L., and Korn, E. (1985). The amino acid sequence of *Acanthamoeba* profilin. J. Biol. Chem. *260*, 834–840.

Bertheussen, K. (1979). The cytotoxic reaction in allogeneic mixtures of echinoid phagocytes. Exp. Cell Res. *120*, 373–381.

Bertheussen, K. (1981). Endocytosis by echinoid phagocytes in vitro. I. Recognition of foreign matter. Dev. Comp. Immunol. *5*, 241–250.

Binette, F., Benard, M., Laroche, A., Pierron, G., Lemieux, G., and Pallotta, D. (1990). Cell-specific expression of a profilin gene family. DNA Cell Biol. *9*, 323–334.

Cameron, R.A., Britten, R.J., and Davidson, E.H. (1989). Expression of two actin genes during larval development in the sea urchin, *Strongylocentrotus purpuratus*. Mol. Reprod. Dev. *1*, 149–155.

Chen, W.-J., Goldstein, J., and Brown, M. (1990). NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. J. Biol. Chem. *265*, 3116–3123.

Coffaro, K. (1978). Clearance of bacteriophage T4 in the sea urchin *Lytechinus pictus*. J. Invertebr. Pathol. *32*, 384–385.

Coffaro, K., and Hinegardner, H. (1977). Immune response in the sea urchin *Lytechinus pictus*. Science *197*, 1389–1390.

Edds, K. (1977). Dynamic aspects of filopodial formation by reorganization of microfilaments. J. Cell Biol. *73*, 479–491.

Edds, K. (1980). The formation and elongation of filopodia during transformation of sea urchin coelomocytes. Cell Motil. *1*, 131–140.

Edds, K., Chambers, C., and Allen, R. (1983). Coelomocyte motility. Cell Motil. *3*, 113–121.

Goldschmidt-Clermont, P., Kim, J., Machesky, L., Rhee, S., and Pollard, T. (1991a). Regulation of phospholipase C-gamma 1 by profilin and tyrosine phosphorylation. Science *251*, 1231–1233.

Goldschmidt-Clermont, P., Machesky, L., Baldassare, J., and Pollard, T. (1990). The actin-binding protein profilin binds to PIP_2 and inhibits its hydrolysis by phospholipase C. Science *247*, 1575–1578.

Goldschmidt-Clermont, P., Machesky, L., Doberstein, S., and Pollard, T. (1991b). Mechanism of the interaction of human platelet profilin with actin. J. Cell Biol. *113*, 1081–1089.

Goustin, A. and Wilt, F. (1981). Protein synthesis, polyribosomes, and peptide elongation in early development of *Stronglyocentrotus purpuratus*. Dev. Biol. *82*, 32–40.

Haarer, B., and Brown, S. (1990). Structure and function of profilin. Cell Motil. Cytoskeleton *17*, 71–74.

Höbaus, E. (1979). Coelomocytes in normal and pathologically altered body walls of sea urchins. In: Echinoderms: Present and Past, ed. M. Jangoux, Rotterdam: A.A. Balkema, 247–249.

Humphreys, T. (1963). Chemical dissolution and in vitro reconstruction of sponge cell adhesions. I. Isolation and functional demonstration of the components involved. Dev. Biol. *8*, 27–47.

Johnson, P. (1969). The coelomic elements of sea urchins (*Strongylocentrotus*). III. In vitro reaction to bacteria. J. Invertebr. Pathol. *13*, 42–62.

Kwiatkowski, D., and Bruns, G. (1988). Human profilin. Molecular cloning sequence comparison, and chromosomal analysis. J. Biol. Chem. *263*, 5910–5915.

Lassing, I., and Lindberg, U. (1985). Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. Nature *314*, 472–474.

Lassing, I., and Lindberg, U. (1988). Specificity of the interaction between phosphatidylinositol 4,5-bisphosphate and the profilin:actin complex. J. Cell. Biochem. *37*, 255–267.

Leahy, P. (1986). Laboratory culture of *Strongylocentrotus purpuratus* adults, embryos, and larvae. Methods Cell Biol. *27*, 1–13.

Leahy, P.S., Tutschulte, T.C., Britten, R.J., and Davidson, E.H. (1978). A large-scale laboratory maintenance system for gravid purple sea urchins (*Strongylocentrotus purpuratus*). J. Exp. Zool. *204*, 369–380.

Lee, J., Calzone, F., Britten, R., Angerer, R., and Davidson, E. (1986). Activation of sea urchin actin genes during embryogenesis. Measurement of transcript accumulation from five different genes in *Strongylocentrotus purpuratus*. J. Mol. Biol. *188*, 173–183.

Machesky, L., Goldschmidt-Clermont, P., and Pollard, T. (1990). The affinities of human platelet and *Acanthamoeba* profilin isoforms for polyphosphoinositides account for their relative abilities to inhibit phospholipase C. Cell Regul. *1*, 937–950.

Messing, J., Crea, R., and Seeburg, P. (1983). A system for shot gun sequencing. Nucleic Acids Res. *9*, 309–321.

Messer, L., and Wardlaw, A. (1979). Separation of the coelomocytes of *Echinus esculentus* by density gradient centrifugation. In: Echinoderms: Present and Past, ed. M. Jangoux, Rotterdam: A.A. Balkema, 319–323.

Oechsner, U., Magdolen, V., and Bandlow, W. (1987). The cDNA and deduced amino acid sequence of profilin from *Saccharomyces cerevisiae*. Nucleic Acids Res. *15*, 9078.

Pearson, W., and Lipman, D. (1988). Improved tools for biological sequence analysis. Proc. Natl. Acad. Sci. USA *85*, 2444–2448.

Pollard, T., and Rimm, D. (1991). Analysis of cDNA clones for *Acanthamoeba* profilin-I and profilin-II shows end to end homology with vertebrate profilins and a small family of profilin genes. Cell Motil. Cytoskeleton *20*, 169–177.

Reichstein, E., and Korn, E. (1979). *Acanthamoeba* profilin. A protein of low molecular weight from *Acanthamoeba castellanii* that inhibits actin nucleation. J. Biol. Chem. *254*, 6174–6179.

Reinisch, C., and Bang, F. (1971). Cell recognition: reactions of the sea star (*Asterias vulgaris*) to the injection of amoebocytes of sea urchin (*Arbacia punctulata*). Cell Immunol. *2*, 496–503.

Rothenberg, E., Diamond, R., Pepper, K., and Yang, J. (1990). IL-2 gene inducibility in T cells before T-cell receptor expression. Changes in signaling pathways and gene expression requirements during intrathymic maturation. J. Immunol. *144*, 1614–1624.

Sanger, F., Nicklen, S., and Coulson, A. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Schuler, G., Altschul, S., and Lipman, D. (1991). A workbench for multiple alignment construction analysis. Proteins Struct. Funct. Genet. *9*, 180–190.

Service, M., and Wardlaw, A. (1984). Echinochrome-A as a bactericidal substance in the coelomic fluid of *Echinus esculentus* (L.). Comp. Biochem. Physiol. *79B*, 161–165.

Service, M., and Wardlaw, A. (1985). Bactericidal activity of coelomic fluid of the sea urchin, *Echinus esculentus*, on different marine bacteria. J. Mar. Biol. Assoc. UK *65*, 133–139.

Stossel, T. (1989). From signal to pseudopod. How cells control cytoplasmic actin assembly. J. Biol. Chem. *264*, 18261–18264.

Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990). Use of T7 polymerase to direct expression of cloned genes. Methods Enzymol. *185*, 60–89.

Takagi, T., Mabuchi, I., Hosoya, H., Furuhashi, K., and Hatano, S. (1990). Primary structure of profilins from two species of Echinoidea and *Physarum polycephalum*. Eur. J. Biochem. *192*, 777–781.

Vojtek, A., Haarer, B., Field, J., Gerst, J., Pollard, T., Brown, S., and Wigler, M. (1991). Evidence for a functional link between profilin and CAP in the yeast *S. cerevisiae*. Cell *66*, 497–505.

Wardlaw, A., and Unkles, S. (1978). Bactericidal activity of coelomic fluid from the sea urchin *Echinus esculentus*. J. Invertebr. Pathol. *32*, 25–34.

Widada, J., Ferraz, C., and Liautard, J.-P. (1989). Total coding sequence of profilin cDNA from *Mus musculus* macrophage. Nucleic Acids Res. *17*, 2855.

Yamamoto, T., Bishop, R., Brown, M., Goldstein, J., and Russell, D. (1986). Deletion in cysteine-rich region of LDL receptor impedes transport to cell surface in WHHL rabbit. Science *232*, 1230–1237.

Yamamoto, T., Davis, C., Brown, M., Schneider, W., Casey, M., Goldstein, J., and Russell, D. (1984). The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell *39*, 27–38.

Yui, M., and Bayne, C. (1983). Echinoderm immunology: bacterial clearance by the sea urchin *Strongylocentrotus purpuratus*. Biol. Bull. *165*, 473–486.