

Expression of Actin Genes in the Arrow Worm *Paraspadella gotoi* (Chaetognatha)

Etsuko Yasuda^{1,2}, Taichiro Goto¹, Kazuhiro W. Makabe²
and Noriyuki Satoh^{2*}

¹Department of Biology, Faculty of Education, Mie University, Tsu, Mie 514, Japan

²Department of Zoology, Graduate School of Science, Kyoto University, Sakyo-ku,
Kyoto 606-01, Japan

ABSTRACT—Arrow worms (the phylum Chaetognatha), one of the major marine planktonic animals, exhibit features characteristic to both deuterostomes and protostomes, and their ancestry therefore remains unknown. As the first step to elucidate the molecular bases of arrow worm phylogeny, physiology and embryology, we isolated cDNA clones for three different actin genes (*PgAct1*, *PgAct2* and *PgAct3*) from the benthic species *Paraspadella gotoi*, and examined their expression patterns in adults and juveniles. The amino acid sequences of the three actins resembled each other, with identities ranging from 86% to 92%. However, the patterns of the spatial expression of the genes were independent. The *PgAct1* gene might encode a cytoplasmic actin and was expressed in oogenic cells, spermatogenic cells, and cells in the ventral ganglion. The *PgAct2* and *PgAct3* genes encoded actins of divergent types. The former was expressed in well-developed muscle of the head (gnathic) region and trunk muscle cells, whereas the latter was expressed in muscle of the trunk and tail regions and oogenic cells. These results suggest that, similarly to other metazoans, the chaetognath contains multiple forms of actins, which are expressed in various manners in the adult and juvenile arrow worm.

INTRODUCTION

Arrow worms (the phylum Chaetognatha) are about 100 species of marine, largely planktonic animals, with the exception of a few benthic species (cf., Bone *et al.*, 1991). According to Brusca and Brusca (1990), they are bilateral deuterostomes, and are characterized by features including a trimeric body comprised of a head, trunk and postanal tail divided from one another by transverse septa, a body with lateral and caudal fins, a head with a pair of uniquely arranged eyes, and, around the mouth, sets of grasping spines and teeth used for prey capture. Arrow worms possess longitudinal muscles of an unusual type, arranged in quadrants rather than a circular arrangement, a complete gut anus at the ventral surface of the trunk-tail junction, and a central nervous system including a cerebral ganglion in the dorsal side of the head and a large ventral ganglion in the trunk. These animals are hermaphroditic and direct developers.

The phylogenetic status of chaetognaths is mysterious. They share some common characteristics with deuterostomes during their ontogeny; radial cleavage, a blastopore at the rear end of the body, and a postanal tail (Hyman, 1959; Brusca and Brusca, 1990; Willmer, 1990). However, the morphology

of adults - namely, a coelom without a peritoneum and the apparent lack of circular muscle in the body wall - suggests their similarity to pseudocoelomate groups (Willmer, 1990), although their coelomic condition is still debated (Shinn and Roberts, 1994). Their nervous system is more like that of protostomes (Rehkämper and Welsch, 1985; Goto and Yoshida, 1987). Moreover, even during ontogeny, they do not pass through the dipleurula stage that is seen in a few deuterostome phyla. Recent molecular phylogenetic studies using 18S rDNA (rRNA) sequences did not support the affinity of chaetognaths with deuterostomes (Telford and Holland, 1993; Wada and Satoh, 1994).

Despite such characteristics and their phylogenetic position, arrow worms have been a subject of very few molecular biological investigations. Our laboratory at the Mie University has recently succeeded in the maintenance of the benthic species *Paraspadella gotoi* by extending several generations. *P. gotoi* may provide an appropriate experimental system to investigate the molecular bases of the physiology, behavioral biology and developmental biology of the arrow worm. As the first step to elucidate the molecular basis, the present study was performed to isolate cDNA clones for actin genes from *P. gotoi*. Actin is a ubiquitous protein that is encoded by a multigene family in a variety of animals (Pollard and Cooper 1986; Rubenstein, 1990). There are two subtypes of actin muscle type and cytoplasmic type (Vandekerckhove and

* Corresponding author: Tel. +81-75-753-4081;
FAX. +81-75-705-1113.

Weber, 1984). Several kinds of muscle-type actin mRNA have also been shown in certain higher eukaryotes. The coding sequences of different members of the actin gene family are conserved, whereas the 5' and 3' non-coding sequences usually diverge and can be used as gene-specific probes (Shani *et al.*, 1981). Each actin isoform shows a distinct expression pattern specific to tissues or developmental stages (Sanchez *et al.*, 1983; Schwartz and Rothblum, 1981; Kusakabe *et al.*, 1995). The present study revealed that the arrow worm also contains multiple forms of actin, which show different patterns of spatial expression.

MATERIALS AND METHODS

Animals

Paraspadella gotoi Casanova (Casanova, 1990) was collected in the vicinity of Amakusa Marine Biological Station, Kyushu University, Kumamoto, Japan. *P. gotoi* belongs to the order Phragmophora with ventral transverse muscle bands (phragma) and is a benthic species. Adults specimens have been maintained in our laboratory at Mie University in a constant-temperature room at 17°C and fed with *Tigriopus japonicus*. They are cross-fertile and fertilization occurs internally. Juveniles were obtained by collecting laid eggs and were kept in a constant-temperature room at 23°C. The young hatched 2 days after egg-laying. Details of the culture method have been described elsewhere (Goto and Yoshida, 1997).

Isolation of RNA and construction of a cDNA library of *P. gotoi* adult

Total RNA was isolated from whole adult specimens (about 300 individuals) by the acid guanidinium thiocyanate-phenol-chloroform (AGPC) method (Chomczynski and Sacchi, 1987). Poly(A)⁺ RNA was purified by use of Oligotex-dT30 Latex beads (Roche Japan, Tokyo) according to the manufacturer's protocol. Complementary DNA was synthesized from the poly(A)⁺ RNA with a Zap cDNA Synthesis kit (Stratagene, La Jolla, CA, USA). Double-stranded cDNA was size-fractionated on a column of Sephacryl S-500 (Pharmacia Biotech, Uppsala, Sweden), and fractions that contained fragments more than 300 bp in length were collected. The double-stranded cDNA was cloned directly into the *EcoRI*-*XhoI* site of a Uni-ZAPXR vector (Stratagene). The titer of the amplified cDNA library was estimated to be 2.4×10^7 pfu/ μ l.

Isolation and sequencing of cDNA clones for *P. gotoi* actin genes

The amino acid sequences of actins are highly conserved among eukaryotes (e.g., Kusakabe *et al.*, 1997). The primers were designed as follows: muscle forward, 5'-TG(C/T)GA(C/T)AA(C/T)GG(A/C/G/T)(A/T)(C/G)(A/C/G/T)GG(A/C/G/T)(C/T)T-3'; cytoplasmic forward, 5'-GT(A/C/G/T)GA(C/T)AA(C/T)GG(A/C/G/T)(A/T)(C/G)(A/C/G/T)GG(A/C/G/T)ATG-3'; and actin reverse, 5'-AA(A/G)CA(C/T)TT(A/C/G/T)C(G/T)(A/G)TG(A/C/G/T)AC(A/G/T)AT-3'. Using these oligonucleotide primers, we amplified target fragments from the first-stranded cDNAs which were obtained from RNAs of *P. gotoi* by means of reverse transcription-polymerase chain reaction (RT-PCR). Annealing was carried out at 37°C or at 42°C. Sequencing revealed that the amplified fragments were of actin genes.

Probing with candidate fragments random-labeled with [³²P]-dCTP (Amersham, Buckinghamshire, UK), we screened the cDNA library at moderate stringency conditions (hybridization; 5x SSPE, 0.5% SDS, 5x Denhardt's solution, 35% formamide at 42°C; washing; 2x SSC, 0.1% SDS at 37°C for 30 min twice and at 42°C for 30 min once; Sambrook *et al.*, 1989) and obtained many positive clones.

The nucleotide sequences were determined for both strands with a dye primer cycle sequencing FS ready kit and ABI PRISM 377 DNA

sequencer (Perkin Elmer, Norwalk, CT, USA).

In situ hybridization

In situ hybridization was carried out with both whole-mount specimens of newly-hatched juveniles and sectioned specimens of adults. All specimens were fixed in 4% paraformaldehyde in MOPS buffer (pH 7.5), 0.5 M NaCl. Fixed specimens were immersed in 80% ethanol and kept at -20°C until use. The probes were synthesized from the 3' untranslated region of the gene by following the instructions from the supplier of the kit (DIG RNA Labeling kit; Boehringer Mannheim, Mannheim, Germany).

Whole-mount specimens: After a thorough washing with PBT [phosphate-buffered saline (PBS) containing 0.1% Tween 20], the fixed specimens were treated with 2 μ g/ml proteinase K (Merck, Darmstadt, Germany) in PBS for 20 min at 37°C, and then they were post-fixed with 4% paraformaldehyde in PBS for 1 hr at room temperature. After a 1-hr period of prehybridization at 42°C, the specimens were allowed to hybridize with the digoxigenin-labeled antisense

| | | |
|------|--|------|
| 1 | GCACGAGAAAAGACGAGACGGCGCTAACGGTTTTCTCTGCGAGAAAAAAAATCCACTTTT | 60 |
| 61 | TAAACTTTTCTCTCCGCGAATTTTTTCGATCTTTTTAAAGCTCAAACAACAAATGTGC | 120 |
| | M C | |
| 121 | GACGAAGAAGTTGCCGCTTTGGTCGTGGCAATGGTTCGGGAATGTGCAAGGCCGGTTC | 180 |
| | D E E V A A L V V D N G S G M C K A G F | |
| 181 | GCCGGGACGACGACCCCGCGCGCTTCCCTCCATCGTGGGACGCCCGGTCATCAG | 240 |
| | A G D D A P R A V F P S I V G R P R H Q | |
| 241 | GGCGTCATGTGGCATGGGACAGAAGACTCTTATGTGGGAGACGAGGCCAATCCAAG | 300 |
| | G V M V G M G Q K D S Y V G D E A Q S K | |
| 301 | AGGGGCATCCTCACTCTGAATACCCCATCGAACACGGCATCTGCCAATCTGGGACGAC | 360 |
| | R G I L T L K Y P I E H G I V T N W D D | |
| 361 | ATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTTCGGTGGCCGACGAGGACAT | 420 |
| | M E K I W H H T F Y N E L R V A P E E H | |
| 421 | CCCGTCTGTGACGGAGGCCCTCTCAAGCCCAAGCAACAGGAGAGATGACGCGAG | 480 |
| | P V L L T E A P L N P K A N R E K M T Q | |
| 481 | ATCATGTTCCGAGACCTTCAACACCCCGCCATGTACGTGGCCATCCAAGCCGCTGTCT | 540 |
| | I M F E T F N T P A M Y V A I Q A V L S | |
| 541 | CTGTACGCCCTTGGCCGACCCGGTATCGTCTTGGACTTGGGACGCGCTTCCAC | 600 |
| | L Y A S G R T T G I V L D S G D G V S H | |
| 601 | ACCGTCCCATCTACGAGGGTTACGCCCTTCCCTCAGCCATCTTGGCTCTGGATTGGCC | 660 |
| | T V P I Y E G Y A L P H A I L R L D L A | |
| 661 | GGACGTGACTTGACCGACTATCTCATGAAGATCTTACCGGAGAGAGGCTACTCGTTCACC | 720 |
| | G R D L T D Y L M K I L T E R G Y S F T | |
| 721 | ACCACCCCGGACGGTGAAGATCGTCTGGCAATCAAGCAACAGCTCTGCTACGTGGCGTGT | 780 |
| | T T A E R E I V R D I K E K L C Y V A L | |
| 781 | GACTTCGAGCAGGATGGGACCCCGCCCTCCCTTCTCTTGGAGAGTCCCTACGAA | 840 |
| | D F E Q E M A T A A S S S L E K S Y E | |
| 841 | TTGCCCGACGGTCAAGTCAATCACCATCGGCAACGAGGATTTCCGCTGCCCGAGGCCCTC | 900 |
| | L P D G Q V I T I G N E R F R C P E A L | |
| 901 | TTCCAGCGCTTCTTCCGCGCATGGAATCTTCCGGCATCCACGAGTCACTACCTACAACCTCC | 960 |
| | F Q P S F L G M E S C G I H E T T Y N S | |
| 961 | ATCATGAAGTGGCGATCGACATCCGTAAGATCTTACGCCAACAACCGCTTGTCCGGT | 1020 |
| | I M K C D V D I R K D L Y A N T V L S G | |
| 1021 | GGCACCACCATGTACCCCGCATCCCGACCCGATCGAGAAGATCACAGCCCTGGCT | 1080 |
| | G T T M Y P G I A D R M Q K E I T A L A | |
| 1081 | CCCTCACCACATGAAGATCAAGATCATCCCTCCCGGAGAGGAAGTACTCCGCTCTGGATC | 1140 |
| | P S T M K I K I I A P P E R K Y S V W I | |
| 1141 | GGAGGTCATCTCCGCTCCCTGTCACCTTCCAACAGATGTGGATCTCCAAGCAAGAG | 1200 |
| | G G S I L A S L S T F Q M W I S K Q E | |
| 1201 | TACGACGATCTGCTCCGTCGATCGTCCACAGGAATGCTTCAATCTAACAAAACCTAA | 1260 |
| | Y D E S G P S I V H R K C F * | |
| 1261 | TGATTTTGACTTTAAAACCGCGCGCTCCCGACATTTTTTCTCTCTTTGAATTAAGA | 1320 |
| 1321 | AAITAGGAATCTGTTCCCTTTTCTTTTATTTATTTGCTAGTCCGTTTTTTTATCTCT | 1380 |
| 1381 | TTCCGAAAGATGTTTTTTTTTCCACTTCACTCCCTTGTATTCGGCTTTAAAACAACAA | 1440 |
| 1441 | AAAAATATATGATATGTTTCGAGTTTATTTGACCTTGTACCGCTTGGCAAAGGAATTAAA | 1500 |
| 1501 | ATTTGAAACATCTTCGAAACMTTCCATCCGCAATTAATTAATCTTATAAATAAGCCAAA | 1560 |
| 1561 | TTGATTTTTACAATTTCCCGCCCTACCAAAAATTTCCGTTACTGCTTTTTGGAGCGATT | 1620 |
| 1621 | CTTGACTATGATGATGAAATATAAGTCTTTTTAGCTTCAAATAAAAAAAAAAAAAA | 1677 |

Fig. 1. Nucleotide and predicted amino acid sequences of the cDNA clone for the *PgAct1* gene. The sequence of the cDNA encompasses 1,677 bp including 18 adenyl residues at the 3' end. The ATG at the position 115-117 represents the putative start codon of the *PgAct1*-encoded protein. The asterisk indicates the termination codon. The nucleotide sequence will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the accession number (D45164).

or sense probe for at least 1 hr at 42°C. After hybridization, the specimens were washed and treated with RNase A and then washed again extensively with PBT. The samples were then incubated for 1 hr with 1:2000 Boehringer Mannheim alkaline-phosphatase-conjugated anti-DIG and treated for the development of color as indicated in the protocol from Boehringer. After dehydration, some of the specimens were cleared by placing them in a 1:2 mixture (v/v) of benzyl alcohol and benzyl benzoate.

Sectioned specimens: These specimens were embedded in polyester wax and sectioned at 10 µm. The *in situ* hybridization of sectioned specimens was carried out as in the case of whole-mount specimens.

RESULTS AND DISCUSSION

Isolation and characterization of a cDNA clone for *Paraspadella gotoi* actin genes

We first made first-stranded cDNAs against *P. gotoi* adult RNAs. Then, using oligonucleotide primers corresponding to

the shared amino-acid sequences of actins, we amplified target fragments from them by the RT-PCR reaction. We obtained 12 amplified fragments which were about 1.1-kb long and corresponded to the region from the amino acid position 10 to 375 of actins. Sequencing the fragments after subcloning them into pBluescript II SK(+) revealed that these 12 clones were subdivided into three types (Types-1, -2, and -3); 6 clones were of Type-1, 3 were of Type-2, and 3 were of Type-3.

With labeled clones, we screened 2.4×10^5 pfu of the *P. gotoi* adult cDNA library and obtained 42 positive clones. The partial sequencing of these clones revealed that 10 contained sequences identical to Type-1, 5 to Type-2, and 6 to Type-3. In addition to these three types, there were seven different types of 12 clones, which were not analyzed further. Of the clones belonging to each of the three types, the longest ones were entirely sequenced. Each of the longest clones contained a single open reading frame (ORF) that predicted actins.

```

1   GTTCGACGCATCTCTGATCCGACAGGAAATCTCGCCTCTTCTTTTGTGCTGCTT  58
59  TGAAGACAACAACAACAACCAACCAAGATGTGCGACGATGAGGAGTAGCGCGCTTG  118
      M C D D E E V A A L
119  GTCGTCGACAATGGATCGGGAATGTGCAAGGCCGGGTTCCGCGGGGACGACGCCCCCGC  178
      V V D N G S G M C K A G F A G D D A P R
179  GCCGCTTCCCTCCATCTCGGAAGCCCTCTCATGTGGGCGTCATGGTGGGCGTGGG  238
      A V F P S I V G R P R H V G V M V G M G
239  CAGAAGGACGCCATGTGGGGACGAGGCTCAGTCCAGGAGAGGATCTCTCTCTGAAA  298
      Q K D A Y V G D E A Q S K R G I L S L K
299  TACCCCATTGAGCAGCCATCGTACCACATGGGACGACATGAGAGATCTGGCATCAC  358
      Y P I E H G I V T N W D D M E K I W H H
359  ACCTTCTACAACGAGTGTGAGAGTCTCTCCGAGGAGCAACCCCGCTCCCACTCGGAGCG  418
      T F Y N E L R V S P E E H P A L H S E A
419  CCCCTCAACCTAAGGCCAACAGGGAGAAGATGACCCAAATCTGCTTCGAGGCTTTCAGC  478
      P L N P K A N R E K M T Q I C F E A F S
479  GCCCCCGCATGATGATGACCATTCAGGCTGTCTGTGCTGTAGCTTCGCGCCGACG  538
      A P A M Y V A I Q A V L S L Y A S G R T
539  ACCGGCATCTGTGCTGACACCGCGGACGVTGCTCAACCGCTCCCATCTACGAGGGT  598
      T G I V L D T G D G V A H A V P I Y E G
599  TACGCCCTCCCTCAGCCATCTCAGGCTCAGACTCGCCGGGAGGATCTCACCGACTAC  658
      Y A L P H A I L R L D L A G R D L T D Y
659  CTCATGAAGATCTCCACCGGAGGGGATATCTCTGTGACGACGCGCGAGGOGGAT  718
      L M K I L T E R G Y S F V T T A E R E I
719  GTCGCGACATCAGGAAAGCTCTGCTACGTGGCGCTCAGACTCGAAAACGAGATGAAC  778
      V R D I K E K L C Y V A L D F E N E M N
779  ATGCCAAATCTCTTGTGCTCGAGAACTCTCAGAGTTCGCCGAGCGTCAAGTCACT  838
      I A K S S S S L E K S Y E L P D G Q V I
839  ACCGTGCGCAACGAGCGATCTCCGCTGCCAGAGCCATCTCCAGCCTCTCTCTTGGGA  898
      T V G N E R F R C P E A I F Q P S F L G
899  ATGGAGATCTGCTGGTGTTCAGCGGGATGCTTCAACAGCATCATGAAGTCCGACATCGAC  958
      H E I V G V H E G C F N S I M K C D I D
959  ATCCGTAAGATCTCTACGCCAACACCGCTCTCTCGGAGGCCACCAATGTACCCCGGT  1018
      I R K D L Y A N T V L S G G T T M Y P G
1019  ATCGCCGACCCGATCGAAGGAGATCACCCGCTGGCTCCCTCCACAGATGAAGATCAAG  1078
      I A D R M Q K E I T A L A P S T M K I K
1079  ATCATGCTCCCGGAGAGGAAGTATTCGCTCTGGATCGCGGTTCCATCTGCGCTCC  1138
      I I A P P E R K Y S V W I G G S I L A S
1139  CTGTCCACTTCCAGGAGATGTGGATCTCCAGCAGGATACGACGAGGCCGCGCCGGSC  1198
      L S T F Q E M W I S K Q E Y D E A G P G
1199  ATCGTCCACAGGAAGTCTTCTAGAGAAACCGGGAGACTCCACCCAGCGTCTGATGTT  1258
      I V H R K C F *
1259  TGTAGCCGAGATTTTTTAAATTTTTTATATTTTAAATTTTTTGTATTTTTTTTTTTT  1318
1319  CGTATAAAGTTTTTCTGTATGAAAAAACAACATGCTTTTTAAAAAACAATCGCA  1378
1379  AACCTTGTCTGATTAATACTGTGACACAAACCTCAATGATGCTGCTGATGACA  1438
1439  TTCTGTACTGCTGATCAAAATCGAATGGTTCGATCCCTCTCATGCTCGCGATT  1498
1499  AAATTTATTTAAGTTCAAAAAAATAAAAAA  1534
    
```

Fig. 2. Nucleotide and predicted amino acid sequences of the cDNA clone for the *PgAct2* gene. The sequence of the cDNA encompasses 1,534 bp including 20 adenylyl residues at the 3' end. The ATG at the position 89-91 represents the putative start codon of the *PgAct2*-encoded protein. The asterisk indicates the termination codon. The nucleotide sequence will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the accession number (D45165).

```

1   CGCTCTCCAGCGTATTCGCCGAATCTTTCGACAGACGCGAAGTAAACCTCCAGGATG  58
      M
59  TCGCAGCAGGAGAAATCCGCGGCTGGTTCATCGATAATGGATCCGGAATGGTCAAGGCC  118
      C D D E E S A A L V I D N G S G H V K A
119  GGGTTCGCGCGGACGACGACGCCCGCGCGCTCTTCCCTCCGTCGTGCGAGCGCCCGGT  178
      G F A G D D A P R A V F P S V V G R P R
179  CACGTCAGCGTATGTTGGGCAACAAAGACGCTACGTCGGTACGAGGCTCAG  238
      H V S V M V G M G N K D A V V G D E A Q
239  TCCAAGAGAGGATCTCTCTCCCTGAAGTACCCCATCGAGCACGCGCATCTCACCACTGG  298
      S K R G I L S L K Y P I E H G I V T N W
299  GAGCATATGGAGAAATGTGGCATCACACTTCAACAACGAGCTCCGATCTCCCCCGAG  358
      D D M E K V W H H T F N N E L R I S P E
359  GAATCGCCCATCTCCACTCCGAGGCGCCCTCAACCCAGAGCAACAGGGAGAAGCTC  418
      E S P H L H S E A P L N P K S N R E K L
419  GTCAGATTTGTCTCGAGACTTCAACGCCCGCCCGTACCTCTGTATCCGAGCCGCTA  478
      V Q I V F E T F N A P A T Y V C I Q A V
479  CTCTCCCTGTACGCTCTGGCGTACGACCGCGATGGTGTGTGACATCGGAGACGGTGT  538
      L S L Y A S G R T T G M V L D I G D G V
539  TCCAAGGCTCCCATCTCCGAGGCTTCCGCTCAGCCATCTCCCTCAGCCATCTTCCGCTCGAT  598
      S H G V P I Y E G Y A L P H A I L R L D
599  TGGCCGAGCGGACTTACCGCATCTCATGAAGATCATGTCGGAGCTGGTACGCC  658
      L A G R D L T D Y L M K I M S E R G Y A
659  ATGGTCACCAACCGGACCGGAGATCTCCGCGACATCAAGAGAAAGCTTTGCTACGTG  718
      M V T T A E R E I V R D I K E K L C Y V
719  GCGCTGACTTCGAGCAGAAATGGCGACCGCCGCTCTCTCTCCATTCAGAAAGTCC  778
      A L D F E Q E M A T A A S S S S I D K S
779  TACGAGTTCGCGACGACAGATCTGACCGTCCGCAACGAGCGTTCCTCGTCCCGGAA  838
      Y E L P D G Q I V T V G N E R F R C P E
839  TCCATGTTCCGCGCAACTTCTCCGATGAAATCTCGGAATTCACGAGGATGCTTC  898
      S M F R P N F L G M E V V G I H E G C F
899  AACGGAATCATGAAGTGGACATCGACATCCGCAAGATCTCTACGCCAACACCGTCTTG  958
      N G I M K C D I D I R K D L Y A A N T V L
959  TCCGTTGGCACCACATGTACCCCGTATCGTACCGCGAGCAGAAAGATCACCGCC  1018
      S G G T T M Y P G I A D R M Q K E I T A
1019  CTGCTCCCTCCACATGAAGATCAAGATCACTGCTCCCGGAGGAGGATCTCCGCTC  1078
      L A P S T M K I K I I A P P E R K Y S V
1079  TGGATCGCGGTTCCATCTCGGCTCCCTCCACTCCAGGAGATGGATCTCCAG  1138
      W I G G S I L A S L S T G C A Q E M W I S K
1139  CAAGATACGACGAGGCTGGACCCGCTGACCGAATGCTTTTAAACGAATCCA  1198
      Q E Y D E A G P G I V H R K C F *
1199  CCGATTAACAACCCCACTAAACCGGCACACACAGAAAATAAATTTATGATTTAT  1258
1259  TTTTGTATCTTTTAAATTTTTTTCATTTTTTGTGATCTCTCGGTTGGGAATGAGTT  1318
1319  TCAAGATCTTTGCGTCCGACCGGAGAAAATACTGTGTCATCGAAACGAGAGCGAATC  1378
1379  TTTCCGCTTCTCGCAACAAAGACTTGAATTTTTTATTTTTTGAATTTGTCGAT  1438
1439  CGATTTTTAAAAAATATGTGCTGGATTTTTTCGGAATTAAGTTTCCACGAAAAA  1498
1499  AAAAAAATAA  1510
    
```

Fig. 3. Nucleotide and predicted amino acid sequences of the cDNA clone for the *PgAct3* gene. The sequence of the cDNA encompasses 1,510 bp including 18 adenylyl residues at the 3' end. The ATG at the position 56-58 represents the putative start codon of the *PgAct3*-encoded protein. The asterisk indicates the termination codon. The nucleotide sequence will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the accession number (D45166).

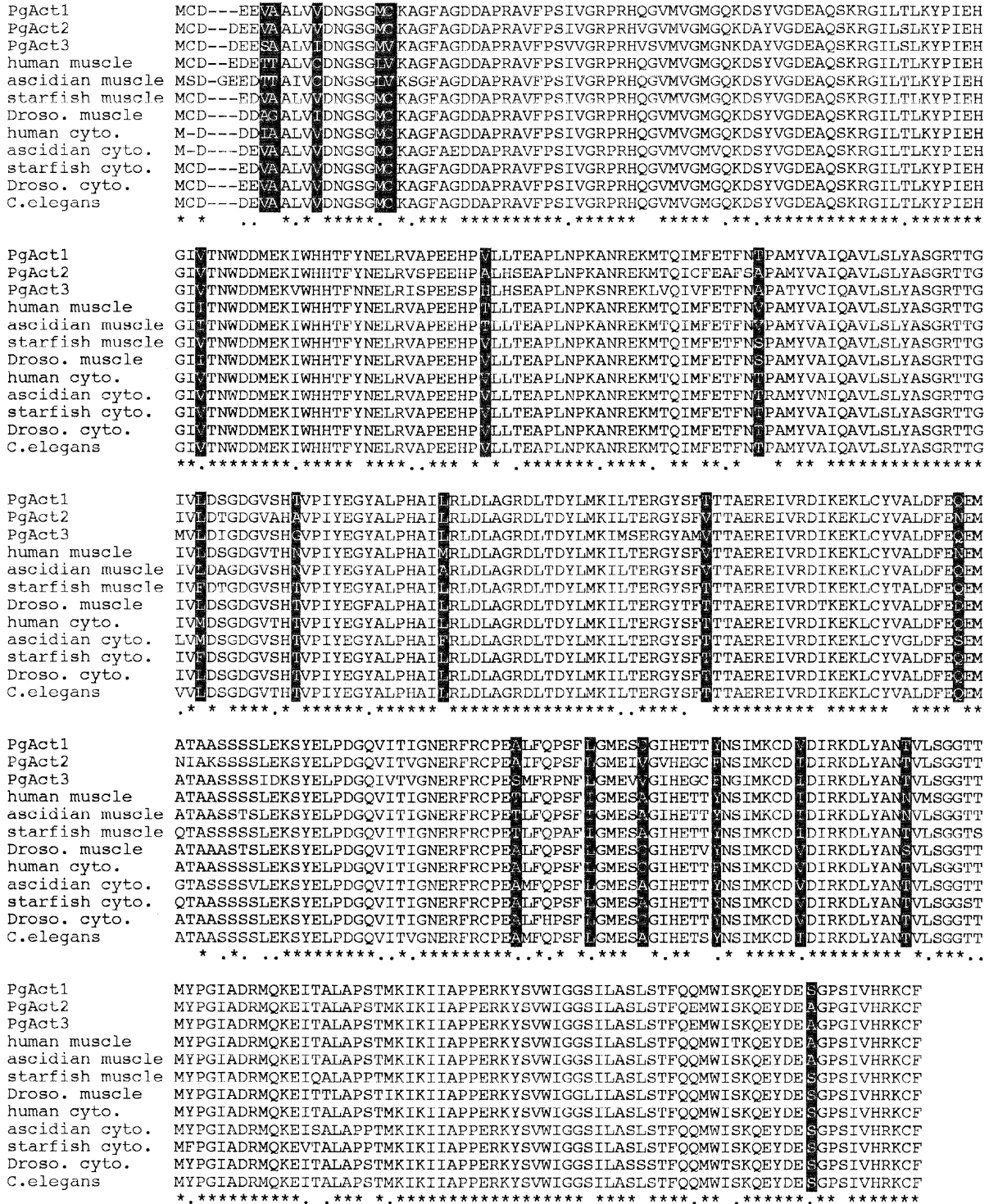


Fig. 4. Comparison of the amino acid sequences of PgAct1, PgAct2, and PgAct3 with actins of various metazoans. The amino acids are indicated with one-letter codes. Dashes indicate gaps introduced in the sequence to optimize the alignment. The amino acids at diagnostic positions indicating vertebrate actin types (cytoplasmic or muscle) are boxed (Vandekerckhove and Weber, 1978, 1984). The asterisks indicate the identity of amino acids, and the dots indicate high similarity. The sources, accession numbers, and references for the actin sequences are human α -skeletal muscle actin, M20543 (Taylor et al., 1988); ascidian larval muscle actin, D10887 (Kusakabe et al., 1992); starfish muscle actin, M26500 (Kowbel and Smith, 1989); *Drosophila* muscle actin, M18830 (Sanchez et al., 1983); human β -cytoplasmic actin, M10277 (Nakajima-Iijima et al., 1985); ascidian cytoplasmic actin, X61042 (Kovilur et al., 1993); starfish cytoplasmic actin, M26501 (Kowbel and Smith, 1989); *Drosophila* cytoplasmic actin, K00667 (Fyrberg et al., 1981) and *C. elegans* actin, X16796 (Krause et al., 1989).

We therefore named the corresponding genes *PgAct1* (*Paraspadella gotoi actin gene 1*) to Type-1, *PgAct2* to Type-2, and *PgAct3* to Type-3.

Figure 1 shows the nucleotide and deduced amino acid sequences of the cDNA clone for the *PgAct1* gene, which consisted of 1,677 nucleotides. The *PgAct1* cDNA contained a single ORF of 1,128 bp, which predicted a polypeptide of 376 amino acids. The calculated molecular mass of the predicted protein was 41.8 kDa.

The nucleotide and deduced amino acid sequences of the cDNA clone for the *PgAct2* and *PgAct3* genes are shown in Figs. 2 and 3. The *PgAct2* cDNA was 1,534 bp long and contained a single ORF of 1,128 bp, which predicted a polypeptide of 377 amino acids and had a calculated molecular mass of 42.3 kDa, while the *PgAct3* cDNA was 1,510 bp long and contained a single ORF of 1,128 bp, which predicted a polypeptide of 377 amino acids and 42.3 kDa.

Among these three actins, the amino acid identities were 91.5% between *PgAct1* and *PgAct2*, 86.2% between *PgAct1* and *PgAct3*, and 88.9% between *PgAct2* and *PgAct3*, although the identity of the nucleotide sequences of 3' untranslated region (UTR) was less than 50%.

Figure 4 shows a comparison of the amino acid sequences of *PgAct1*, *PgAct2* and *PgAct3* with actins of various metazoans. The comparison clearly indicated that the *PgAct1*, *PgAct2*, and *PgAct3* genes encode different types of actin. As shown in Fig. 4, the mammalian α -striated muscle actin is distinguishable from the β -cytoplasmic actin by a comparison of amino acid residues at the diagnostic positions (Vandekerckhove and Weber, 1978, 1984). Figure 4 suggests that *PgAct1* is of a type of cytoplasmic actin and that *PgAct2* and *PgAct3* are considerably divergent types of actin. Our molecular phylogenetic analysis comparing the amino acid sequences also suggested that *PgAct2* and *PgAct3* are closely related, while *PgAct1* is distinct from them, that *PgAct1* is a type of cytoplasmic actin, and that *PgAct2* and *PgAct3* are divergent types of actin (Yasuda *et al.*, unpublished data).

These results suggest that the genome of *P. gotoi* contains a family of multiple actin genes and that among them, *PgAct1*, *PgAct2*, and *PgAct3* are major actin genes judging from the numbers of cDNA clones isolated. Because 6 of the 12 amplified fragments and 10 of the 42 positive cDNA clones corresponded to *PgAct1*, this gene may represent the most dominant actin gene of *P. gotoi*. *PgAct2* and *PgAct3* genes are also actively expressed in this animal, judging from the numbers of cDNA clones obtained.

Expression of *Paraspadella gotoi* actin genes

The expression of the arrow worm actin genes was examined by *in situ* hybridization with sectioned specimens of adults and whole-mount specimens of juveniles. To help clarify the gene expression, the anatomy of *P. gotoi* is diagrammatically shown in Fig. 5a. Their trimeric body is comprised of a head, trunk and postanal tail divided from one another by transverse septa. The head cavity is reduced by the complex cephalic musculature. The body musculature consists of four quad-

rants of well-developed dorsolateral and ventrolateral longitudinal bands. The digestive tract runs through the central part of the trunk region and is completed by the anus at the ventral surface of the trunk-tail junction. A major component of the central nervous system is a large ventral ganglion located in the trunk epidermis. Arrow worms are hermaphroditic; the wide coelom of the trunk region is occupied with oogenic cells, while that of the tail region occupied by spermatogenic cells. Adults were examined in cross-sections of the head, trunk, and tail regions.

The *PgAct1* gene. The *in situ* hybridization of adult specimens demonstrated intense signals in oogenic cells of the trunk region (Fig. 5c). Small oocytes showed stronger signals, whereas large mature oocytes showed weak signals (Fig. 5c). In addition, sections of the tail region revealed signals in the spermatogenic cells or spermatocytes (Fig. 5d). Therefore, the *PgAct1* gene is expressed in germ cells during the early phase of their formation.

Hybridization signals were also evident in the cytoplasm of the neuronal cells of the ventral ganglion (Fig. 5c) as well as in cells of the digestive tract including the pharynx in the head region (Fig. 5b) and intestine in the trunk region (Fig. 5c). However, signals were not so evident in longitudinal muscle cells (Fig. 5c, d).

The *in situ* hybridization of the whole-mount specimens of newly-hatched juveniles showed intense signals on the whole body except for the tail region (Fig. 5e). Strong signals were seen in the head region (Fig. 5e).

These results suggest that the *PgAct1* gene is expressed both maternally and zygotically. The gene is expressed in a variety of tissues, supporting the notion that *PgAct1* is a type of cytoplasmic actin.

The *PgAct2* gene. Cross-sections of the head region of the adults demonstrated that the *PgAct2* gene was actively expressed in well-developed muscle of the gnatha region (Fig. 6a). Hybridization signals were also evident on the epithelium lining the coelom near the ventral ganglion (Fig. 6b) and in cells of the sperm duct of the trunk region (Fig. 6b). However, signals were not detected in the pharynx (Fig. 6a, b) or germ cells (Fig. 6b, c).

The *in situ* hybridization of whole-mount specimens of newly-hatched juveniles showed intense signals in the head region, in particular in the gnathic region (Fig. 6d). Lateral cells in the trunk region also showed signals (Fig. 6d).

These results suggest that the *PgAct2* gene expression is zygotic, and primarily found in muscle cells.

The *PgAct3* gene. In contrast to the *PgAct2* gene, the *in situ* hybridization of sectioned specimens of adult tissues demonstrated intense signals in oogenic cells of the trunk region (Fig. 6f). The small oocytes showed very strong signals (Fig. 6f). Signals were also evident in longitudinal muscle cells of the trunk and tail regions (Fig. 6f, g). In the tail region, signals were evident in the mesentery (Fig. 6g). The *in situ* hy-

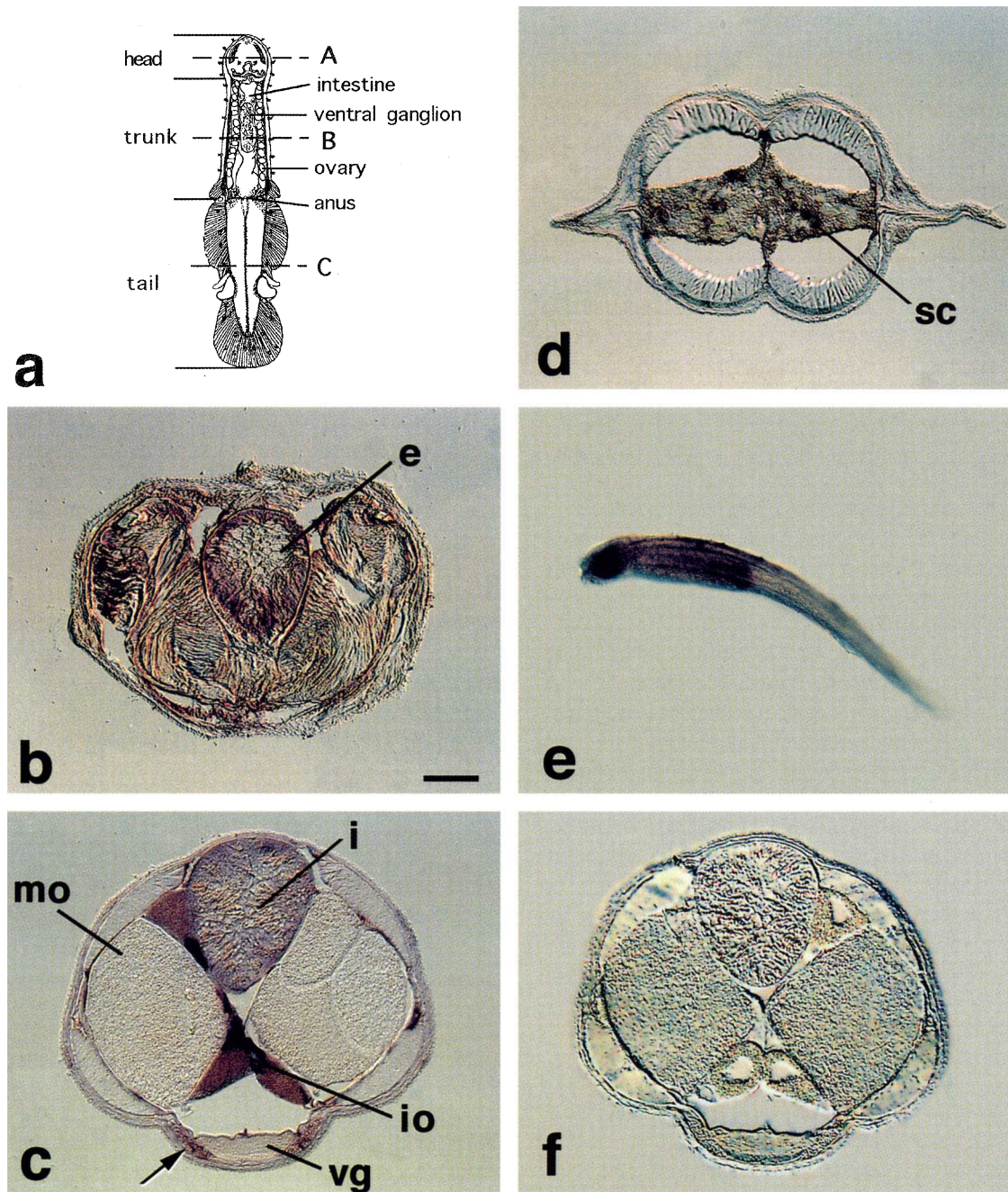


Fig. 5. Expression of the *PgAct1* gene. (a) Diagrammatic drawing of *P. gotoi* showing the anatomy and the sectioned lines; A, a section at the head region; B, a section at the trunk region; and C, a section at the tail region. (b) A cross-section of the head region of an adult, showing a weak hybridization signal in the esophagus (e). Scale bars = 100 μ m for all panels. (c) A cross-section of the trunk region of an adult, showing a strong signal in the immature oocytes (io) but not in the mature oocytes (mo). Signals were also evident in the intestine (i) and neuronal cells (arrow) in the ventral ganglion (vg). (d) A cross-section of the tail region of an adult, showing a hybridization signal in some of the spermatocytes (sc). (e) A whole-mount specimen of a juvenile, showing signals widely distributed in the head and trunk regions. (f) A control cross-section of the trunk region of an adult (corresponding to c) hybridized with a sense probe, showing no signals.

Fig. 6. (a-d) Expression of the *PgAct2* gene. (a) A cross-section of the head region of an adult, showing a strong hybridization signal in the muscle (m) of the gnatha region. Scale bars = 100 μ m for all panels. (b) A cross-section of the trunk region of an adult, showing signals in the seminiferous duct region (upper arrow) and in the epithelium lining the coelom of the ventral ganglion (lower arrow). (c) A cross-section of the tail region of an adult, showing no hybridization signal in this region. (d) A whole-mount specimen of a juvenile, showing distinct signals in the head muscle (left arrow) and trunk muscle (right arrow). (e-h) Expression of the *PgAct3* gene. (e) A cross-section of the head region of an adult, showing no hybridization signal in this region. (f) A cross-section of the trunk region of an adult, showing a strong signal in the immature oocytes (io) and muscle cells (arrow). (g) A cross-section of the tail region of an adult, showing a distinct signal in the muscle (m). Signals are evident in muscle cells (arrow). (h) A whole-mount specimen of a juvenile, showing strong signals over the entire body.

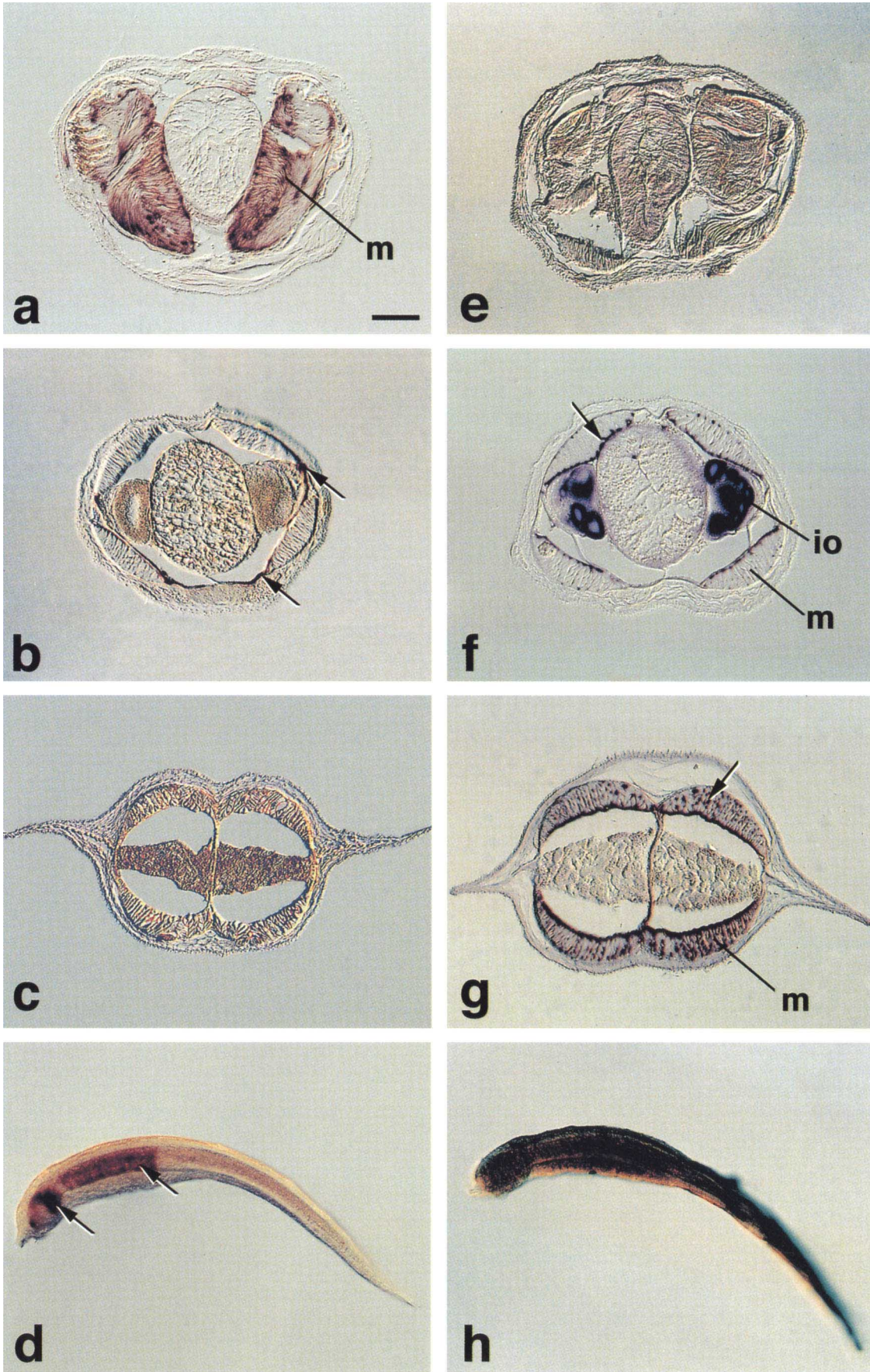


Table 1. Spatial expression of the three actin genes *PgAct1*, *PgAct2*, and *PgAct3* of the arrow worm *Paraspadella gotoi*

| | Adult | | | | | Juvenile | | | |
|---------------|-------------|--------------|---------|-----------|----------------|---------------|-------------|--------------|-------------|
| | head muscle | trunk muscle | oocytes | spermatid | neuronal cells | spermatocytes | head region | trunk region | tail region |
| <i>PgAct1</i> | – | – | +++ | – | ++ | + | ++ | + | – |
| <i>PgAct2</i> | ++ | – | – | + | – | – | +++ | ++ | – |
| <i>PgAct3</i> | – | ++ | +++ | – | – | + | ++ | + | ++ |

–, not expressed; +, weakly expressed; ++, moderately expressed; +++, strongly expressed

bridization of whole-mount specimens of newly-hatched juveniles showed intense signals on the whole body (Fig. 6h).

These results suggest that the *PgAct2* gene is primarily expressed in the longitudinal muscle as well as oocytes.

The regions with expressions of *PgAct1*, *PgAct2* or *PgAct3* are summarized and compared in Table 1. As is evident in the table, the patterns of spatial expression of the three actin genes are not identical but rather are specific for each gene. It is highly likely that each of these genes has its own function.

ACKNOWLEDGMENTS

We thank Dr. T. Kusakabe for his help with molecular phylogenetic analysis. This research was supported in part by a Grant-in-Aid for Specially Promoted Research (No. 07102012) from the Ministry of Education, Science, Sports and Culture of Japan to N.S.

REFERENCES

- Bone Q, Kapp H, Pierrot-Bults AC (1991) *The Biology of Chaetognaths*. Oxford Univ Press, Oxford, UK
- Brusca RC, Brusca GJ (1990) *Invertebrates*. Sinauer, Sunderland, Mass, USA
- Casanova J-P (1990) A new species of *Paraspadella gotoi* (Chaetognatha) from the coastal waters of Japan. *Proc Biol Soc Wash* 103: 907–912
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159
- Fyrberg EA, Bond BJ, Hershey ND, Mixter KS, Davidson N (1981) The actin genes of *Drosophila*: protein coding regions are highly conserved but intron positions are not. *Cell* 24: 107–116
- Goto T, Yoshida M (1987) Nervous system in Chaetognatha. In "Nervous System in Invertebrates" Ed by MA Ali, NATO-ASI series, Plenum Press, pp 461–481
- Goto T, Yoshida M (1997) Growth and reproduction of the benthic arrow worm *Paraspadella gotoi* (Chaetognatha) in laboratory culture. *Invet Reprod Develop* 32: 201–207
- Hyman LH (1959) The enterocoelous coelomates—phylum Chaetognatha. In "The Invertebrates Vol 5, Smaller Coelomates Groups" McGraw-Hill, pp 1–71
- Kovilur S, Jacobson JW, Beach RL, Jeffery WR, Tomlinson CR (1993) Evolution of the chordate muscle actin gene. *J Mol Evol* 36: 361–368
- Kowbel DJ, Smith MJ (1989) The genomic nucleotide sequences of two differentially expressed actin-coding genes from the sea star *Pisaster ochraceus*. *Gene* 77: 297–308
- Krause M, Wild M, Rosenzweig B, Hirsh D (1989) Wild-type and mutant actin genes in *Caenorhabditis elegans*. *J Mol Biol* 208: 381–392
- Kusakabe T, Makabe KW, Satoh N (1992) Tunicate muscle actin genes: structure and organization as a gene cluster. *J Mol Biol* 227: 955–960
- Kusakabe T, Hikosaka A, Satoh N (1995) Coexpression and promoter function in two muscle actin gene complexes of different structural organization in the ascidian *Halocynthia roretzi*. *Dev Biol* 169: 461–472
- Kusakabe T, Araki I, Satoh N, Jeffery WR (1997) Evolution of chordate actin genes: Evidence from genomic organization and amino acid sequences. *J Mol Evol* 44: 289–298
- Nakajima-Iijima S, Hamada H, Reddy P, Kakunaga T (1985) Molecular structure of the human cytoplasmic β -actin gene: interspecific homology of sequences in the introns. *Proc Natl Acad Sci USA* 82: 6133–6137
- Pollard TD, Cooper JA (1986) Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu Rev Biochem* 55: 987–1035
- Rehkämper G, Welsch U (1985) On the fine structure of the cerebral ganglion of *Sagitta* (Chaetognatha). *Zoomorphol* 105: 83–89
- Rubenstein PA (1990) The functional importance of multiple actin isoforms. *BioEssays* 12: 309–315
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory, New York
- Sanchez F, Tobin SL, Rdest U, Zulauf E, McCarthy BJ (1983) Two *Drosophila* actin genes in detail: Gene structure, protein structure and transcription during development. *J Mol Biol* 163: 533–551
- Schwartz RJ, Rothblum KN (1981) Gene switching in myogenesis: Differential expression of the chicken actin multigene family. *Biochemistry* 20: 4122–4129
- Shani M, Nudel U, Zevin-Sonkin D, Zakut R, Givol D, Katcoff D, Carmon Y, Reiter J, Frischauf AM, Yaffe D (1981) Skeletal muscle actin mRNA. Characterization of the 3' untranslated region. *Nucl Acids Res* 9: 579–589
- Shinn GL, Roberts ME (1994) Ultrastructure of hatching chaetognaths (*Ferosagitta hispida*): phylogenetic implications. *J Morphol* 219: 143–163
- Taylor A, Erba HP, Muscat GE, Kedes L (1988) Nucleotide sequence and expression of the human skeletal alpha-actin gene: evolution of functional regulatory domains. *Genomics* 3: 323–336
- Telford MJ, Holland PWH (1993) The phylogenetic affinities of the Chaetognaths: a molecular analysis. *Mol Biol Evol* 10: 660–676
- Vandekerckhove J, Weber K (1978) Mammalian cytoplasmic actins are the products of at least two genes and differ in primary structure in at least 25 identified positions from skeletal muscle actins. *Proc Natl Acad Sci USA* 75: 1106–1110
- Vandekerckhove J, Weber K (1984) Chordate muscle actins differ distinctly from invertebrate muscle actins. The evolution of the different vertebrate muscle actins. *J Mol Biol* 179: 391–413
- Wada H, Satoh N (1994) Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc Natl Acad Sci USA* 91: 1801–1804
- Willmer P (1990) *Invertebrate Relationships: Patterns in Animal Evolution*. Cambridge Univ Press, Cambridge, UK

(Received July 29, 1997 / Accepted August 20, 1997)