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Conserved Expression Pattern of *BMP-2/4* in Hemichordate Acorn Worm and Echinoderm Sea Cucumber Embryos

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ABSTRACT—The auricularia larva of sea cucumbers and tornaria larva of acorn worms share striking developmental and morphological similarities. They are regarded as not only an archetype of the nonchordate deuterostome larva, but also an archetype of the origin of chordates. Here we report the characterization and spatial expression patterns of the *BMP-2/4* genes of a hemichordate acorn worm (*Pf-bmp2/4*) and an echinoderm sea cucumber (*Sj-bmp2/4*). Both the *Pf-bmp2/4* and *Sj-bmp2/4* genes exhibited apparently conserved expression in the region of the coelomopore complex. This is in agreement with the homology between their basic larval body plans with respect to coelomogenesis and allows us to discuss the evolutionary counterparts of the coelomopore complex in chordates.

Key words: hemichordate acorn worm, echinoderm sea cucumber, *BMP-2/4*, coelomopore, conserved expression

INTRODUCTION

The phylogeny of deuterostomes, including chordates, hemichordates and echinoderms, has been a key subject of recent evo-devo studies (see Gee, 1996; Hall, 1998). Monophyly of the deuterostome phyla is supported by both morphological cladistic analyses (Schaeffer, 1987; Nielsen, 2001) and molecular phylogenetic analyses (Wada and Satoh, 1994; Turbeville *et al*., 1994; Cameron *et al*., 2000). Although the internal phylogenetic relationship among the deuterostome phyla still remains a controversial issue, recent molecular phylogenetic data appear to support a clade of nonchordate deuterostomes (echinoderms + hemichordates) (Wada and Satoh, 1994; Turbeville *et al*., 1994; Castresana *et al*., 1998; Cameron *et al*., 2000).

The pentametric radial morphology of adult echinoderms is secondarily derived both evolutionarily and developmentally, as echinoderm larval morphology is bilateral (Brusca and Brusca, 1990; Peterson *et al*., 2000). The larvae of echinoderms have long been recognized as variants of a common plan (Müller, 1853; also see Lacalli, 1993), and such larvae are sometimes called “dipleurulae” (Garstang, 1928; Jollie, 1973; see Nielsen, 2001). All the extant echinoderm classes except crinoids are known to form dipleurula-type larvae, such as the bipinnaria of sea stars, auricularia of sea cucumbers, and plutei of sea urchins and brittle stars (Brusca and Brusca, 1990). The dipleurula larva is characterized by a circum-oral ciliated band and tripartite enterocoelom (Nielsen, 2001).

Hemichordate enteropneusts or acorn worms are worm-like animals, with bodies clearly divided into three regions, proboscis, collar (mesosome) and trunk (metasome) (Brusca and Brusca, 1990). The phylogenetic position of hemichordates is unique, because they exhibit both chordate-like adult morphology and echinoderm-like larval morphology (Nielsen, 2001). Actually, hemichordates have been linked phylogenetically to the chordates for a long time (Bateson, 1885). The pharyngeal gill is a key characteristic, because it is observed in the bodies of both chordates at some stage and adult hemichordates (Nielsen, 2001). Recently, Ogasawara *et al.* (1999) showed that the differentiating gill expresses a *Pax1/9* subfamily gene in both chordates and hemichordates. This provides molecular evidence for the homology between the gills of these two phyla

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(reviewed by Tagawa *et al*., 2001). On the other hand, indirectly developing hemichordates develop through a planktonic tornaria larva stage, in which they possess a circum-oral ciliated band and tripartite enterocoelom, thus showing striking morphological similarity to the dipleurula larvae in echinoderms. Hence, the dipleurula has been regarded as an archetype of the larvae of both echinoderms and hemichordates (see Nielsen, 2001).

The tripartite coelomic architecture is a prominent characteristic shared by larvae of echinoderms and hemichordates. Their coeloms consist of an anterior coelom or protocoel, a middle coelom or mesocoel, and a posterior coelom or metacoel. In echinoderms, the protocoel, mesocoel and metacoel are usually called axocoel, hydrocoel and somatocoel, respectively (Brusca and Brusca, 1990). Ideally, echinoderms organize three pairs of coeloms, whereas hemichordates possess an unpaired protocoel, and paired mesocoels and metacoels. The actual larvae show considerable variations, for example, sea cucumber auricularia larvae lack the right axocoel and hydrocoel (Smiley, 1986). In both phyla, the protocoel opens a coelomopore exteriorly, and becomes integrated into an excretory organ, the axial complex of the adult.

In this report, we primarily describe a molecular examination of the similarity observed in these larval morphological features, by comparing the expression patterns of the *BMP-2/4* gene. Vertebrate *BMP-2/4* genes, and their ortholog in the fruit fly, *decapentaplegic* (*dpp*), are known to play a central role in dorso-ventral axis formation, although the vertebrate and fruit fly genes seem to have oppositely directed activities (Arendt and Nübler-Jung, 1994; DeRobertis and Sasai, 1996). Vertebrate *BMP-2/4* also exhibits multiple other functions in development and organogenesis, particularly in places where epithelial-mesenchymal interactions occur (Hogan, 1996). Here we report the cloning of *BMP-2/4* genes of an echinoderm sea cucumber, *Stichopus japonicus*, and a hemichordate acorn worm, *Ptychodera flava*. We also compared the spatial expression patterns of the genes to find their presumable plegiomorphic features.

MATERIALS AND METHODS

Animals and embryos

Mature adult *Ptychodera flava* were collected during December at a sand bar in Kaneohe Bay, Oahu, Hawaii (Hadfield, 1975). Natural spawning was induced as described (Tagawa *et al*., 1998a). Fertilized eggs were allowed to develop to the desired developmental stages at room temperature for a week.

In Japan, the sea cucumber *Stichopus japonicus* is cultivated for marketing. Collection of adult sea cucumbers from Mutsu Bay, induction of spawning by temperature shift, artificial fertilization and cultivation of embryos were performed by the staff of Aomori City Fisheries Technology Center, Aomori, Japan. These larvae were provided to us, and we cultured them at room temperature by feeding them algae in glass beakers containing seawater supplemented with 50 mg/l of streptomycin, in our laboratory at Kyoto University. Auricularia larvae began to change into doliolaria larvae as judged morphologically about 10 days after fertilization. They metamorphosed to juveniles about 3 weeks after fertilization (Shoguchi *et al*., 2000).

Molecular cloning

We first isolated cDNA clones for hemichordate *BMP-2/4* (*Pf-bmp2/4*) and then those for sea cucumber *BMP-2/4 (Sj-bmp2/4*) . PCR amplification was carried out using a *P. flava* gastrula cDNA library (Tagawa *et al*., 1998b) as template DNAs. The primer sequences were as follows: BMP-F: 5'-TGGGAYGAYTGGATHGT-NGC-3', BMP-R: 5'-GTYTGNACDATNGCRTGRTT-3', (where D = not C, H = not G, N = any, R = A or G, and Y = C or T), which correspond to the amino acid sequences, WDDWIVA and NHAIVQT, respectively. We screened the same cDNA library by probing with the thus-obtained PCR fragments labelled with [^{32}P]dCTP. Several cDNA clones were isolated. The phage cDNA clones were converted into plasmids. Isolated cDNA clones were sequenced on both strands using an automated DNA sequencer (ABI PRISM, Perkin Elmer).

Total RNA was extracted from 24-hr gastrulae of *S. japonicus* by the AGPC method (Chomczynski and Sacci, 1987). Poly(A)$^+$ RNAs were purified by use of Oligotex dT30 beads (Roche Japan). A cDNA library was constructed using a kit (ZAP-cDNA synthesis kit, Stratagene) following the supplier's instructions. cDNA clones for *Sj-bmp2/4* were isolated as in the case of those for *Pf-bmp2/4.*

Sequence comparison and molecular phylogenetic analysis

The putative amino acid sequences of the Pf-bmp2/4 and Sj-bmp2/4 proteins were deduced from their nucleotide sequences and aligned with other TGF-β superfamily gene product sequences. Their molecular phylogenetic relationships were analyzed by means of the neighbor-joining method using PHYLIP ver. 3.5 (Felsenstein, 1993).

Genomic Southern hybridization

High-molecular weight genomic DNA of *P. flava* was exhaustively digested with *Eco*RI, *Hin*dIII or *Pst*I and fractionated by electrophoresis. The DNA fragments blotted onto Hybond-N+ nylon membranes (Amersham) were hybridized with [^{32}P]-dCTP-labelled DNA probes and washed under specific conditions: hybridization, 0.5 M Na_2HPO_4, pH 7.2, 1 mM EDTA, 7% SDS at 65°C; washing, 2 x SSC, 0.1% SDS at 65°C. The probes were 0.9 kb in length containing the C-terminal conserved region of *Pf-bmp2/4.*

On the other hand, high-molecular weight genomic DNA of *S. japonicus* was exhaustively digested with *Bgl*II, *Eco*T22I, *Eco*RV or *Pvu*II, fractionated by electrophoresis, hybridized with [^{32}P]-dCTP-labelled DNA probes, and washed under the same conditions as mentioned above. The probes were 1.0 kb in length containing the C-terminal conserved region of *Sj-bmp2/4*.

Whole-mount *in situ* hybridization

Whole-mount specimens were hybridized *in situ* basically as described by Tagawa *et al*. (1998b) and Shoguchi *et al*. (2000). Embryos were fixed in 4% paraformaldehyde in 0.5 M NaCl, 0.1 M MOPS, pH 7.5, on ice overnight. After a thorough wash with PBST (phosphate-buffered saline containing 0.1% Tween 20), the specimens were partially digested with 2 μg/ml proteinase K (Sigma) in PBST for 20 min at 37°C and post-fixed with 4% paraformaldehyde in PBST for 1 hr at room temperature. After a 1-hr prehybridization at 42°C, the specimens were hybridized with digoxigenin (DIG)-labeled antisense probes for about 16 hr at 42°C. The probe was synthesized following the instructions supplied with the kit (Boehringer Mannheim DIG RNA Labeling kit), and the hybridized fragments were reduced to about 200-500 nucleotides by alkaline hydrolysis. The probes were used at 0.5 μg/ml in the hybridization buffer. After hybridization, the specimens were washed and then

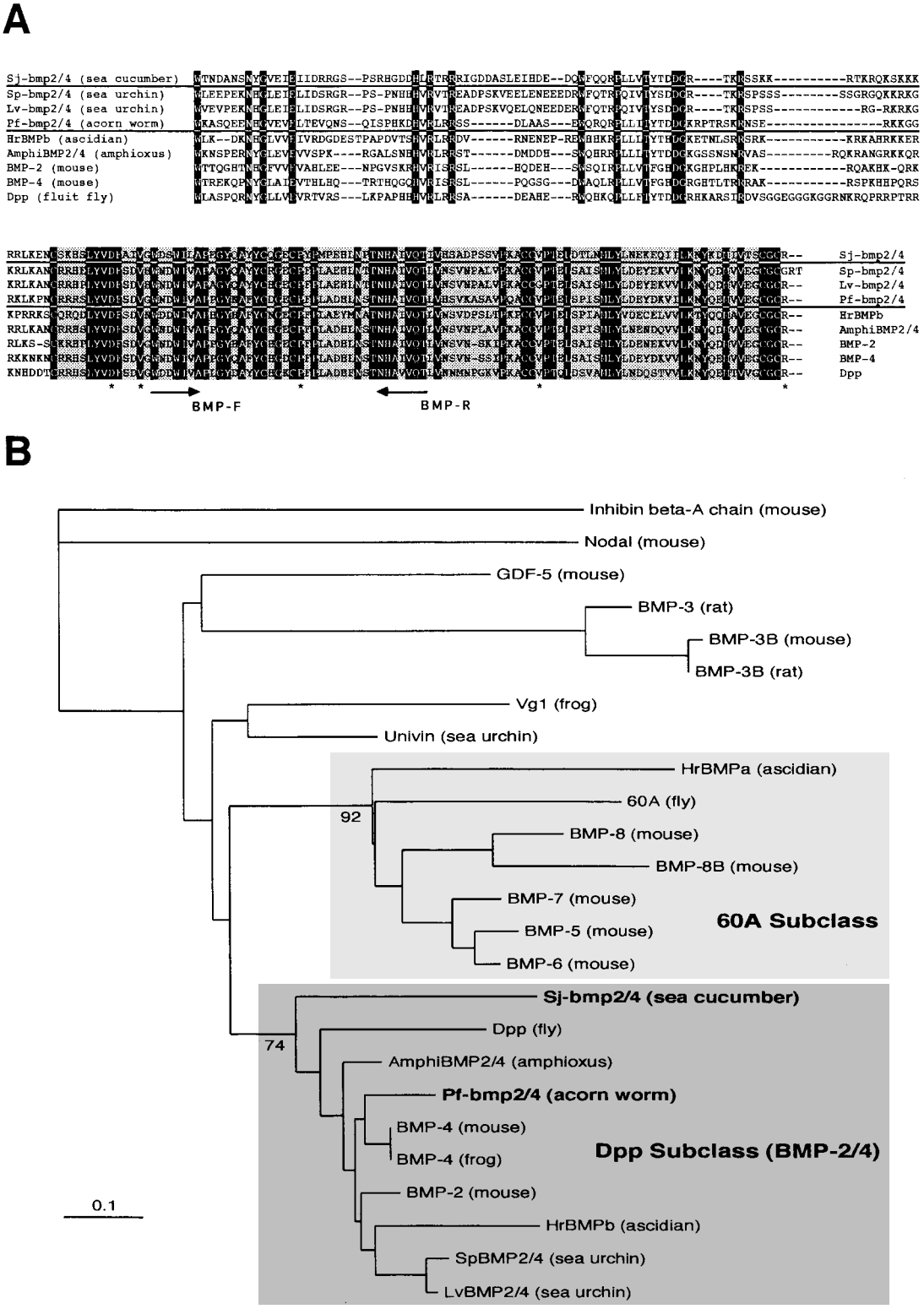

Fig. 1. (**A**) An alignment of the amino acid sequences of the C-terminal half of Sj-bmp2/4 and Pf-bmp2/4 (underlined) with those of other BMP-2/4 subclass members. High-lighted residues are identical among all members shown here. The five asterisks underneath the alignment show typical BMP-2/4 subclass-specific residues within the highly conserved C-terminal region, where other BMPs contain other amino acids. Arrows indicate sequences used for the degenerate oligonucleotide primers in the PCR cloning. The region used for the molecular phylogenetic analysis is shaded. (**B**) Molecular phylogenetic analysis of relationships among TGF-β superfamily members as deduced from the neighbor-joining method. Both Sj-bmp2/4 and Pf-bmp2/4 are grouped with the Dpp subclass (*Drosophila Dpp* and *BMP-2/4* gene products). Branch lengths are proportional to evolutionary distance corrected for multiple substitutions with the scale denoting 0.1 amino acid substitutions per site. The numbers indicate the relative robustness of each node as assessed by bootstrap analysis (100 replications).

digested with 20 μg/ml RNase A. After further washing, a signal was detected following the supplier's instructions.

RESULTS

Isolation and characterization of *Pf-bmp2/4* and *Sj-bmp2/4* cDNAs

cDNA clones for *Pf-bmp2/4* (acorn worm *P. flava* - *BMP2/4*) and those for *Sj-bmp2/4* (sea cucumber *S. japonicus* - *BMP2/4*) were isolated by PCR amplification and by library screening. The insert of the longest clone for *Pf-bmp2/4* was 2,349 bp (DDBJ/GenBank/EMBL database accession number, AB028219), and encoded a predicted polypeptide of 405 amino acids. The insert of the longest clone for *Sj-bmp2/4* was 2,403 bp (DDBJ/GenBank/EMBL database accession number, AB057451), and encoded a predicted polypeptide of 422 amino acids.

Fig. 1A shows an alignment of the amino acid sequences of the BMP-2/4 subclass gene products within the conserved carboxyl-half. The five asterisks underneath the alignment show residues specifically conserved among the BMP-2/4 subclass members. Although Sj-bmp2/4 contained a relatively high proportion of amino acid substitutions, both Sj-bmp2/4 and Pf-bmp2/4 contained the BMP-2/4 subclass-specific residues.

We constructed a molecular phylogenetic tree for these gene products with other related TGF-β superfamily members (Fig. 1B). Both Pf-bmp2/4 and Sj-bmp2/4 were included in a group of the BMP-2/4 subclass, the clade of which was supported by a 74% bootstrap value. Taken together, these findings led us to conclude that these genes are orthologs of *BMP-2/4*.

The copy number of the genes in the genome

To determine the copy numbers of *Pf-bmp2/4*, a genomic Southern hybridization analysis was conducted. Fig. 2A shows a Southern blot probed with a DNA fragment of *Pf-bmp2/4* including a C-terminal conserved region. The hybridization condition in Fig. 2A showed low stringency which allowed a single cross-hybridized band in the lane of *Hin*dIII-digested *Halocynthia roretzi* (ascidian) genomic DNA (data not shown). Single bands were observed in the lanes from digestion by *Eco*RI or *Hin*dIII, while two hybridization bands were observed in the *Pst*I lane. The *Pf-bmp2/4* DNA fragment used for this assay contained an internal *Pst*I site. Therefore, this pattern of genomic Southern hybridization strongly suggests that *Pf-bmp2/4* is the only member of *Dpp* subclass of the TGF-β family in the *P. flava* genome.

Similarly we determine the copy numbers of *Sj-bmp2/4* by a genomic Southern hybridization analysis. As shown in Fig. 2B, single bands were observed in the lanes from digestion by *Bgl*II, *Eco*RV or *Pvu*II, while several hybridization bands were observed in the *Eco*T22I lane. Therefore, it is likely that *Sj-bmp2/4* is the only member of *Dpp* subclass

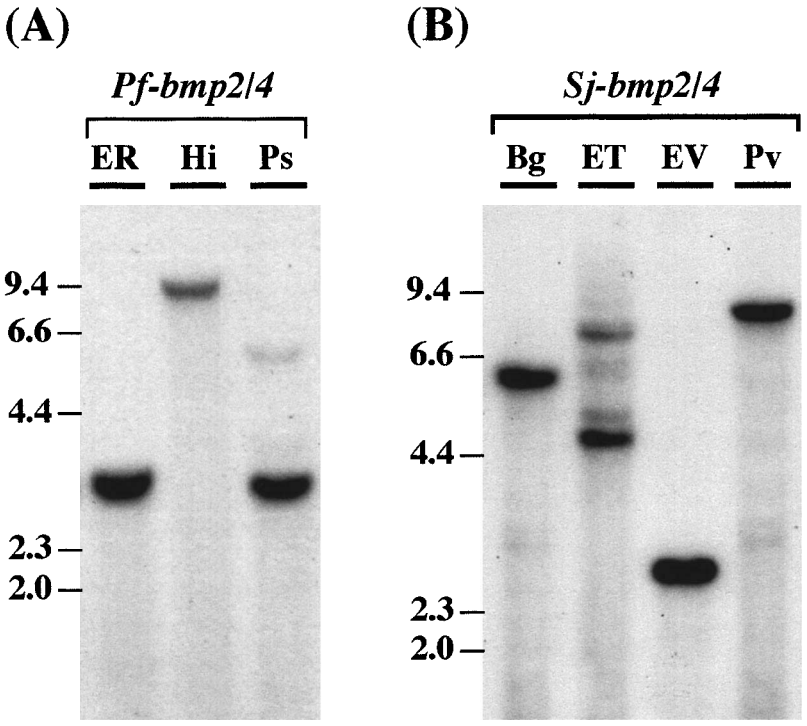

Fig. 2. (**A**) Low-stringency genomic Southern blot analysis of DNA from a single individual of *P. flava*. A Southern blot probed with a 0.9 kb DNA fragment of *Pf-bmp2/4* including a C-terminal conserved region. Each lane was loaded with 5 μg of restriction-digested *P. flava* genomic DNA. ER, *Eco*RI; Hi, *Hin*dIII; Ps, *Pst*I. (**B**) Low-stringency genomic Southern blot analysis of DNA from a single individual of *S. japonicus*. A Southern blot probed with a 1.0 kb DNA fragment of *Sj-bmp2/4* including a C-terminal conserved region. Each lane was loaded with 10 μg of restriction-digested *S. japonicus* genomic DNA. Bg, *Bgl*II; ET, *Eco*T22I; EV, *Eco*RV; Pv, *Pvu*II.

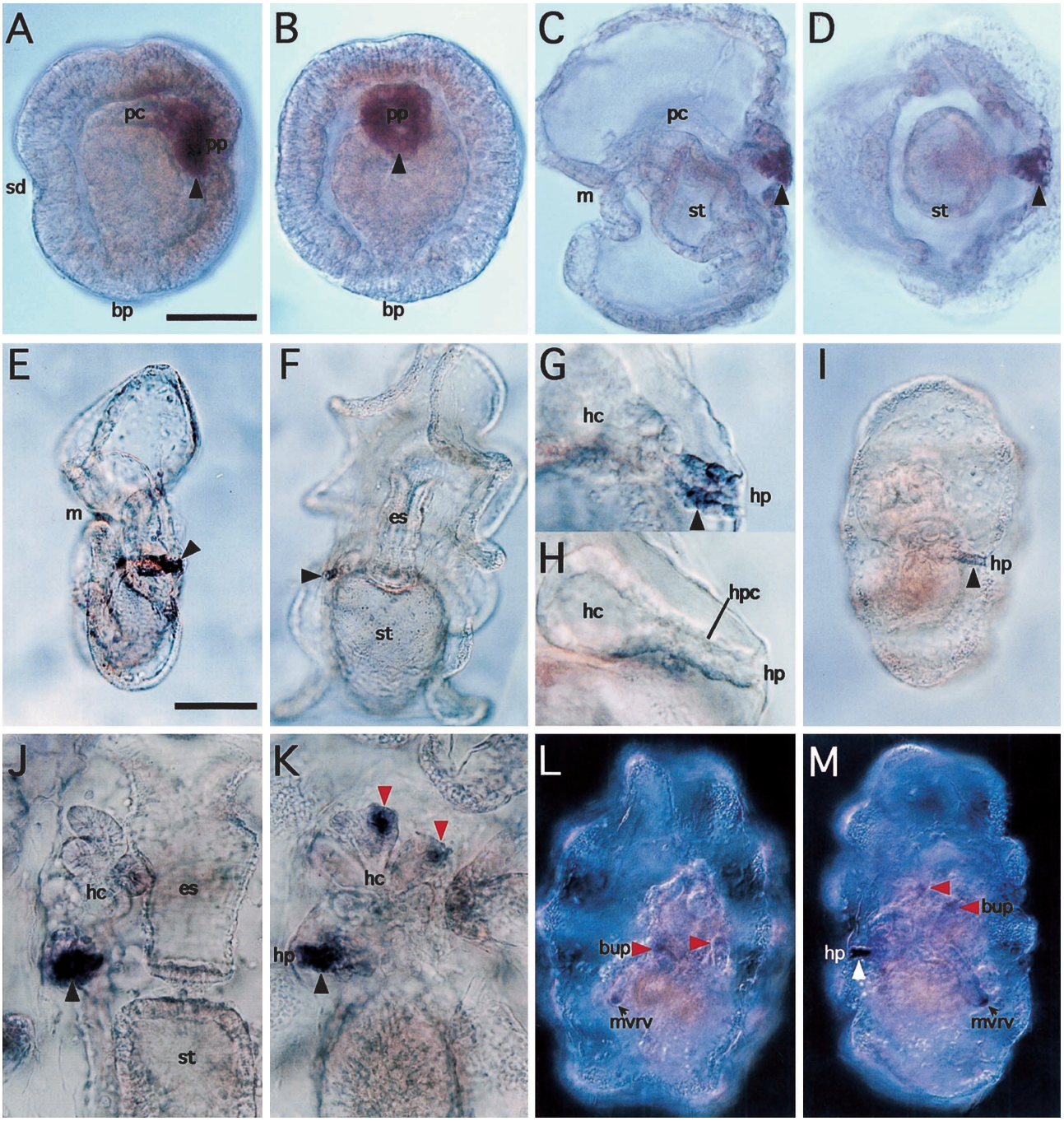

Fig. 3. (**A–D**) Spatial expression pattern of *Pf-bmp2/4*, as revealed by whole-mount *in situ* hybridization with a DIG-labeled antisense probe. (**A**, **B**) An early tornaria larva 48 hr after fertilization, (**A**) lateral view and (**B**) aboral view. A region of proboscis coelom contacting the aboral ectoderm shows a *Pf-bmp2/4* signal (arrowhead). bp, blastopore; pc, proboscis coelom; pp, proboscis pore; sd, stomodeum. Scale bar, 50 μm. (**C**, **D**) A four-day-old tornaria larva, (**C**) left lateral view and (**D**) posterior view. m, mouth; st, stomach. Signal in the proboscis canal persists (arrowhead). (**E–M**) Spatial expression pattern of *Sj-bmp2/4*, as revealed by whole-mount *in situ* hybridization with a DIG-labeled antisense probe. (**E**) An auricularia larva (48 hr), left lateral view. (**F–H**) An auricularia (94 hr), (**F**) dorsal view and (**G**, **H**) 4x further magnified images. (**H**) shows a negative control hybridized with a sense probe. The signals in the hydroporic canal were detected in the specimens with the antisense probe (**F**, **G**; arrowhead). (**I**) A doliolaria larva, left lateral view. The expression in the hydroporic canal continues (arrowhead). (**J**) A hydrocoel of an auricularia larva before metamorphosis. (**K**) A hydrocoel of a ciliary band remodeling larva during metamorphosis. Additional expression appears in the evagination regions of the hydrocoel (red arrowheads), anlage formation for the buccal podia, radial vessels, and polian vesicle. (**L**, **M**) A doliolaria larva, ventral view (**L**) and right lateral view (**M**). Signals were observed in the hydroporic canal (white arrowhead), mid-ventral radial vessel (arrow), and buccal podia (red arrowheads). The abbreviations are: bup, buccal podium; es, esophagus; hc, hydrocoel; hp, hydropore; hpc, hydroporic canal; m, mouth; mvrv, mid-ventral radial vessel. Scale bar in (E): 100 μm for (E), (F), (I), (L), (M); 50 μm for (J), (K); and 25 μm for (G), (H).

of the TGF-β family in the *S. japonicus* genome.

Developmental expression of the acorn worm *Pf-bmp2/4*

Cleavage of *P. flava* is holoblastic and radial, with the embryo developing into a hollow blastula (Hadfield, 1975; Tagawa *et al*., 1998a). Gastrulation begins approximately 18 hr after fertilization with the appearance of a cleft at the vegetal plate. The cleft rapidly extends to form the archenteron. Approximately 25 hr after fertilization, the enterocoelic pouch appears from the anterior swelling of the archenteron. Later, the pouch develops into a protocoel. At about 40 hr of development, an anterior prolongation of the protocoel occurs to make a connection with the apical plate and apical strand. The tubular evagination of the protocoel makes contact with the aboral (dorsal) wall of the embryo, opening exteriorly to form a proboscis pore or coelomopore (Fig. 3A, B). The tip of the archenteron contacts the thickened stomodeum on the oral (ventral) ectoderm, and eventually opens to form a mouth (Fig. 3A, C).

Pf-bmp2/4 mRNA was not detected above background level before the late gastrula stage (data not shown). In the late gastrula or early tornaria larva, distinct expression of *Pf-bmp2/4* began in the region where the protocoel (proboscis canal) attached to the dorsal ectoderm (Fig. 3A, B; arrowhead). This proboscis-canal-specific expression lasted into the later stage of tornaria (Fig. 3C, D; arrowhead). No *Pf-bmp2/4* expression was observed during embryogenesis, suggesting that it is not involved in axis formation.

Developmental expression of the sea cucumber *Sj-bmp2/4*

S. japonicus embryos undergo an indirect development via the auricularia larval stage, and their early development is that of a type of deuterostome embryos (Imai *et al*., 1950; also see Smiley *et al*., 1991). The cleavage is radial, equal and holoblastic. The embryo develops into a coeloblastula approximately 10 hr after fertilization, and then hatches before gastrulation. Gastrulation begins approximately 17 hr after fertilization, as an involution from the vegetal plate. As the archenteron reached about halfway across the blastocoel, it made a right angle bend and grew out toward the blastoderm ventrally, and then fused with the stomodeum to open a mouth (data not shown). The invaginating enterocoel is single in *S. japonicus*, whereas it is paired in sea stars and sea urchins (Smiley *et al*., 1991). It then made contact with the aboral (dorsal) ectoderm to open a hydropore (dorsal pore or coelomopore). The hydropore was located just to the left of the mid-dorsal line of the aboral (dorsal) ectoderm (Fig. 3F, J).

After gastrulation, the embryo became an auricularia larva with a looped circum-oral ciliated band. The archenteron differentiated into three regions, esophagus, stomach and rectum. The enterocoel also divided into an anterior portion, i.e., the primordium of the left hydrocoel, and a posterior portion, i.e., the primordia of the left and right somatocoels. The left hydrocoel was linked to the opening of the hydropore by a hydroporic canal. In this species, the left axocoel was not clearly identifiable (in echinoderms, the left axocoel and left hydrocoel usually do not separate). No right axocoel or hydrocoel was formed (cf., Smiley *et al*., 1991). Fig. 3E shows a 48-hr early auricularia larva. In the early auricularia, *Sj-bmp2/4* expression commenced in the region of the hydroporic canal (Fig. 3E; arrowhead). This expression in the hydroporic canal continued in later auricularia larvae (94 hr; Fig. 3F, G; arrowhead). Fig. 3G shows the expression in the hydroporic canal under higher magnification (arrowhead), whereas Fig. 3H shows a sense-strand hybridization control.

Subsequently, the auricularia larva metamorphosed into a barrel-shaped doliolaria larva. Fig. 3I, M shows doliolaria larvae. The expression in the hydroporic canal persisted in this stage (Fig. 3I, M). The metamorphic morphogenesis was accompanied by shrinking of the body, traveling of the oral-anal axis, dynamic remodeling of the ciliated bands from a single continuous perioral loop into five serial latitudinal loops, and rapid growth of the hydrocoel (compare Fig. 3K with Fig. 3J) (Smiley, 1986). At this stage, new expression appeared in the evagination regions of the hydrocoel (Fig. 3K; red arrowheads). The hydrocoel grew around the esophagus to form a water vascular ring. The hydrocoel also produced pentametric buccal podia, pentametric radial vessels, and a single Polian lobe, as evaginations. The buccal podia grew anteriorly, whereas the radial vessels grew posteriorly between the evaginations of the buccal podia. The anterior tips of buccal podia seemed to express *Sj-bmp2/4* weakly (Fig. 3L, M; red arrowheads). Among the five radial vessels, the mid-ventral one grew faster than the other four (Smiley, 1986). The posterior tip of the growing mid-ventral radial vessel also seemed to express *Sj-bmp2/4* (Fig. 3L, M; arrow).

DISCUSSION

Conserved expression of nonchordate deuterostome *BMP-2/4* in the coelomic duct

The complex of coelomopore and pore-canal is the most characteristic structure among the nonchordate deuterostome larvae (Ruppert and Balser, 1986; Nielsen, 1995). It consists of a duct leading from a coelomic cavity to an external pore situated asymmetrically to the left of the dorsal midline. In *S. japonicus*, the coelomic evagination is formed from the archenteron during gastrulation, and it then curves dorsally, and makes contact with the dorsal ectoderm to form a hydropore (Smiley *et al*., 1991). *Sj-bmp2/4* is expressed in this hydroporic canal. In *P. flava*, the protocoel is pouched out from the archenteron, and then a tubular evagination extends from the protocoel dorsally, which opens as a proboscis pore at the midline of the dorsal ectoderm (Tagawa *et al*., 1998a). *Pf-bmp2/4* expression was observed only in this proboscis canal in the present study. We thus concluded that the *BMP-2/4* gene is expressed in the anterior coelomic canal in both the larvae of the sea cucumber

and acorn worm. This suggests that not only the larvae in other echinoderms may also express the *BMP-2/4* gene in the same region, but also that all the dipleurula larvae may share this expression.

Our results shown here support the idea that echinoderms and hemichordates share a common body plan at the larval stage. However, we must note that we could find a similarity only between the fully-organized larval forms, but their developmental processes involved in organizing this final morphology may rather include a good deal more evolutionary divergence than previously thought. The diversity in this process may represent an aspect of the property of development known as a "developmental hourglass", also referred to in other phyla (Slack *et al*., 1993; Richardson, 1999), as vertebrate embryos show strikingly similar morphology only at the phylogenetic stage. If that is the case, the dipleurula larva represents a 'phylotypic' stage of the nonchordate deuterostomes. With respect to characterization of developmental regulatory genes, the data available about sea urchin embryos is the most detailed for all the nonchordate deuterostomes (e.g. Davidson *et al*., 1998). To construct a molecular map of the phylotypes of the nonchordate deuterostomes, comprehensive analyses of multiple taxa of nonchordate deuterostomes will provide us further evolutionary insights.

Insight into the chordate evolution

We discussed above the possibility that the echinoderm and hemichordate larvae may share *BMP-2/4* expression in the coelomopore complex. Interestingly, the amphioxus *BMP-2/4* ortholog, *AmphiBMP2/4*, is expressed in the developing paired anterior diverticula, although the expression in the left diverticulum disappears in early larva, and thus the organized preoral pit does not express *BMP-2/4* (Panopoulou *et al*., 1998; Table 1).

Regarding the coelomopore complex, its putative presence in chordates has long been argued (Goodrich, 1917; Nielsen, 2001; Table 1). The coelomopore complex consists of the left protocoel and the ectodermal invagination forming the coelomopore. In amphioxus, a pair of coelomic sacs are given off as lateral pouches at the anterior end of the archenteron. The right one expands to form the head cavity of the larva, whereas the left one makes an opening exteriorly in a preoral pit. Later in adults, Hatschek's pit is retained on the left side of the roof of the mouth (Goodrich, 1917). As for vertebrate head development, it has been reported that Rathke's pouch first establishes a connection with premandibular somites, based on observations in some cartilaginous fishes. This connection is transient: as development proceeds, it is lost (Goodrich, 1917; Ruppert, 1990). Therefore, the components of the coelomopore complex, i.e. the left protocoel plus the coelomopore, are regarded as follows: in amphioxus, the left anterior head coelom plus the preoral pit (Hatschek's pit in adult); in vertebrates, the premandibular somite (eye muscle in adults) plus Rathke's pouch (anterior pituitary in adults) (see Ruppert, 1990; Table 1).

Development of the vertebrate Rathke's pouch has been a good model system in which to investigate organogenesis with multiple inductive regulatory mechanisms. *BMP-2/4* genes carry a major part of the inductive signals for Rathke's pouch development. Recent studies suggest that Rathke's pouch is initially induced by the *BMP-4* signal from the neighboring ventral-diencephalon, whereas *BMP-2* plays an intrinsic role in the developing pituitary anlage (Takuma *et al*., 1998; Treier *et al*., 1998). Although the *BMP-2/4* expression in the putative chordate counterparts of the coelomopore is interesting, for now we cannot exclude the possibility that this similarity is due to a chordate-specific recruitment of *BMP-2/4*, since *BMP-2/4* possesses a wide range of developmental roles, even including roles in head development (Jones *et al*., 1991; Francis-West *et al*., 1994).

Rathke's pouch development is a well-characterized process, and a number of developmental regulatory genes have been shown to play specific developmental roles in it (Treier *et al*., 1998). Accordingly, analyses of the developmental roles of *Sj-bmp2/4* and *Pf-bmp2/4* in the coelomopore formation in the respective organisms, in relation to the roles of the homologs of such genes, may be important topics for further comparative studies. In addition, since the earlier observations were reported, Rathke's pouch development has not been described in much detail in relation to the

Table 1. The anterior coelomopore complex (left protocoel + ectodermal invagination) in deuterostome embryos and expression domains of *BMP-2/4*

	Anterior coelomopore complex*	Expression dmains of *BMP-2/4* in the region of anterior coelomopore complex	
Echinodermata	hydrocoel + hydropore	hydroporic canal and evagination wall of hydrocoel in *Stichopus japonicus*	this study
Hemichordata	protocoel + proboscis pore	proboscis canal in *Ptychodera flava*	this study
Urochordata	not identified		
Cephalochordata	left anterior head coelom + preoral pit	wall of Hatschek's diverticula in *Branchiostoma floridae*	Panopoulou *et al*., 1998
Vertebrata	premandibular somite + Rathke's pouch	*BMP2*: Rathke's pouch ectoderm and adjacent mesenchyme in *Mus musculus*	Treier *et al*., 1998

* The components of the anterior coelomopore complex were refered to the discussions of Nielsen (2001).

mesoderm (Gleiberman *et al*., 1999), and therefore we have little information about this structure as an enterocoelic pouch. Therefore, it may also be interesting to examine the expression of *BMP-2/4* or other regulatory genes expression in the head development of lampreys or cartilaginous fishes, because they are thought to preserve ancestral conditions better than do higher vertebrates (see Northcutt, 1993; Kuratani *et al*., 1999).

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