Spatial expression of Hox cluster genes in the ontogeny of a sea urchin

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SUMMARY

The Hox cluster of the sea urchin Strongylocentrotus purpuratus contains ten genes in a 500 kb span of the genome. Only two of these genes are expressed during embryogenesis, while all of eight genes tested are expressed during development of the adult body plan in the larval stage. We report the spatial expression during larval development of the five ‘posterior’ genes of the cluster: SpHox7, SpHox8, SpHox9/10, SpHox11/13a and SpHox11/13b. The five genes exhibit a dynamic, largely mesodermal program of expression. Only SpHox7 displays extensive expression within the pentameral rudiment itself. A spatially sequential and colinear arrangement of expression domains is found in the somatocoels, the paired posterior mesodermal structures that will become the adult perivisceral mesoderm. No such sequential expression pattern is observed in endodermal, epidermal or neural tissues of either the larva or the presumptive juvenile sea urchin. The spatial expression patterns of the Hox genes illuminate the evolutionary process by which the pentameral echinoderm body plan emerged from a bilateral ancestor.

Key words: Hox, Strongylocentrotus purpuratus, Sea urchin, Gene expression

INTRODUCTION

The Hox gene cluster is a pan-bilaterian developmental patterning device, but thus far experimental evidence on its use refers almost entirely to chordates and arthropods. These are all direct developing animals that display a twofold symmetry around the adult anterior/posterior (A/P) axis. The single 500 kb Hox gene cluster of the sea urchin Strongylocentrotus purpuratus was recently cloned (Martinez et al., 1999) and the complete sequence of the ten-gene complex will soon be available (Cameron et al., 2000) and the fivefold radially symmetrical structure that is a definitive phyletic character of modern adult echinoderms is generated within the rudiment (Pearse and Cameron, 1991; Davidson et al., 1998; Peterson et al., 1997, 2000a). Many of the larval structures contribute no descendants to the adult body plan; for instance, most of the larval oral and aboral ectoderm, the pharynx, and the distal part of the intestine. All of those larval tissues are jettisoned during metamorphosis (Pearse and Cameron, 1991). We have previously shown that except for two of the Hox genes, viz. SpHox7 and SpHox11/13b, the Hox gene cluster of Strongylocentrotus purpuratus is not used at all to build the structures of the embryo, that is, to generate the basic feeding larva, while all of the eight Hox genes studied are copiously transcribed during the process of adult body plan formation, In the tissues that will give rise to the adult (Arenas-Mena et al., 1998). Here we describe the spatial expression of the five ‘posterior’ Hox genes during the early to mid stages of adult body plan function, the first observations of their kind on an indirect-developing, radially organized animal.

Molecular phylogeny confirms unequivocally the classically held supposition (Metschnikoff, 1881; Hyman, 1955) that echinoderms are the sister group of the hemichordates within the deuterostomes; the chordates are thus the sister group of the hemichordate plus echinoderm clade (Cameron et al., 2000; Wada and Satoh, 1994; Bromhan and Degnan, 1999). But while echinoderms are radially symmetrical, hemichordates (and chordates) and virtually all protostomes are bilaterally symmetrical. The radial symmetry of the echinoderms is therefore a derived feature, and the ancestor they shared with the hemichordates was bilaterally symmetrical. The initial bilateral arrangement of hemichordate (enteropneust) and echinoderm larval coeloms at early postembryonic stages is in fact remarkably similar (Peterson et al., 1997). Both groups essentially form three sets of coeloms: in echinoderms the anterior pair of coeloms is called the axocoels; the middle coeloms are the hydrocoels; and the posterior coeloms are the somatocoels. Consistent with expectation from its 5’ position in the S. purpuratus Hox gene cluster, we found that the SpHox11/13b gene is expressed exclusively in the somatocoels (Peterson et al., 2000a). This observation, combined with
paleontological evidence and current interpretations of skeletal structure in echinoderms (Mooi and David, 1997) enabled us to propose a reconstruction of the evolutionary process by which the echinoderm body plan was derived from its bilateral ancestral form (Peterson et al., 2000a). The fivefold symmetry of the echinoderm body first appears within a structure including the left hydrocoel, but the axis of fivefold symmetry is orthogonal to the original A/P axis (i.e. as if we had five arms positioned radially around our A/P axis). These changes in axial symmetry lead to a shift in the position of the coeloms so that they stack under one another. Thus, moving inward from the end of the body plan that derives from the original anterior end, still the site of the mouth, the sequence of coelomic derivatives is the left hydrocoel and left somatocoel, then the right somatocoel. Specific predictions as to Hox gene use follow from this interpretation, given that an ancestral function of the Hox gene cluster in bilaterians is A/P pattern specification, colinear with the gene order.

To examine Hox gene expression within the largely unexplored anatomy of the developing rudiment, we had to modify procedures for in situ hybridization and then reconstruct the patterns of gene use from serial sections. Owing to limitations of space, we are able to present only a small fraction of these in what follows. But while the results are indeed consistent with the evolutionary pathway proposed by Peterson et al. (2000a), many surprises nonetheless lay in wait for us: for the five Hox genes considered here we found that expression was largely mesodermal; we observed an unexpected sequence of Hox gene expression patterns within the somatocoel at cross orientation to the adult A/P axis; and we saw a remarkable number of apparent co-options of Hox gene use in developing structures that are special features of echinoderms.

MATERIALS AND METHODS

Culture of larvae

Sea urchin larvae were grown as described previously (Cameron et al., 1989). Cultures were kept at 16°C with constant stirring in filtered sea water at a density of 200 larvae/l. Feeding was provided 5 days after fertilization with Rodomus lens at about 3000 cells per ml.

Whole-mount in situ hybridization

Animals were fixed in 4% formaldehyde, 0.1 M MOPS (pH 7), 0.5 M NaCl for 1 hour at room temperature for later stages, defined as having several adult spines (as those in Fig. 3D-F). For earlier stages with pentameral rudiments (such as those in Fig. 2) a solution with both 2% paraformaldehyde and 2% formaldehyde was used in order to improve morphological preservation, despite some loss of hybridization signal intensity. After five washes in at least 10 volumes of 0.1 M MOPS, 0.5 M NaCl and 0.1% Tween-20 (MOPS buffer), the samples were stored indefinitely in 70% ethanol at −20°C. Rehydration was accomplished with three washes of 15 minutes each, in at least 10 volumes of MOPS buffer in 1.7 ml tubes. Hybridization was conducted in a solution consisting of 70% deionized formamide, 0.5 M NaCl, 0.1 M MOPS (pH 7), 1 mg/ml BSA (solubilized in water first) and 0.1% Tween-20. Two conditioning transfers to hybridization buffer preceded a 3 hour prehybridization at 50°C. Riboprobes containing digoxigenin-UTP were synthesized by conventional methods; for the specific Hox gene probes used see Arenas-Mena et al. (1998). It was found advantageous to employ a one week long hybridization period, using 0.1 ng/μl of riboprobe at 50°C (in the above hybridization buffer). After hybridization, samples were washed five times in MOPS buffer at room temperature to remove the probe, incubated for an additional 3 hours under hybridization conditions and washed three more times in MOPS buffer. The samples were blocked with 10 mg/ml BSA in MOPS buffer for 20 minutes at room temperature and then with 10% goat serum plus 1 mg/ml BSA at 37°C for 30 minutes in MOPS buffer. Incubation with a 1/1500 dilution of the alkaline phosphatase conjugated Fab fragments (Roche Molecular Biochemicals, Indianapolis, IN) was performed overnight at room temperature. The antibody was removed with five washes in MOPS buffer over an interval greater than 12 hours. After two washes in alkaline phosphatase buffer for a total of an hour, the reactions were developed by conventional methods with NBT/BCIP. Addition of 10% dimethyl formamide to the alkaline phosphatase development buffer greatly enhanced the staining reaction. The reaction was stopped by dilution in MOPS buffer.

Embedding, sectioning, viewing and photography

After the staining reaction, the specimens were embedded in Durcupan water-soluble medium for optical microscopy (Fluka, Milwaukee, WI). Serial sections of approximately 7.5 μm were obtained. Images of the sections were made with a Roche Camera system using Wimcam and Photoshop software.

RESULTS

Stages of rudiment development and in situ hybridization

The derivation of the coelomic constituents of the imaginal rudiment from the embryonic mesodermal territories has been summarized earlier (Davidson et al., 1998; for morphological description of echinoid rudiment development see Pearse and Cameron, 1991; Hyman, 1955; Von Ubisch, 1913; MacBride, 1903). The main structural features of the developing larva that are relevant to our present purposes are illustrated in Fig. 1. In Fig. 1A the disposition of the larval coelomic sacs can be seen after about two weeks of larval development. The rudiment is beginning to form on the left-hand side of the stomach. Here the left hydrocoel (h) has assumed a thickened form, and it confronts the invaginating oral ectodermal pouch called the vestibule (v). These tissues soon unite and from their apposition many of the major oral structures of the adult body plan will derive. These include the radially organized water vascular and central nervous systems. The anterior coeloms or axocoels are also indicated in Fig. 1A, on the right-hand side indistinct from the hydrocoel (ax-h). Below the hydrocoels are the thin sheet-like somatocoelar sacs (s, in Fig. 1A), extending on both sides over the stomach. By homology with hemichordates, and by virtue of the observation that the 5′-most gene SpHox11/13b is expressed in the somatocoels, we identify the somatocoels as posterior structures (Peterson et al., 2000a). By the stage shown in Fig. 1B,C, the rudiment is more fully developed and the coelomic organization much more complex. Somatocoelar elements have grown up under the rudiment and are now interdigitated with hydrocoelar components, as illustrated in the following, and below this the somatocoels now extend widely around the stomach and intestine. Viewed face on from the left-hand side, as in Fig. 1C, the pentameral symmetry of the rudiment is clearly visible, in the five primary podia (pp) that can be seen protruding from it. In the center the adult mouth (am) will form: note that the oral
Larval Hox gene expression

When raised in the laboratory, *S. purpuratus* larvae develop at somewhat variable rates over the interval from the onset of feeding until competence to undergo metamorphosis is attained. Stages of larval development are therefore given here on the basis of anatomical progress rather than timing. Throughout this period the larval body per se undergoes much less change than does the rudiment, which grows from a few hundred cells to about 150,000 (Cameron et al., 1989). A stage usually attained by three weeks after fertilization in laboratory-raised *S. purpuratus* (Cameron et al., 1989) is the formation of a hydrocoelar torus, when pentameral symmetry first becomes evident. The most useful metric for subsequent stages of development is the number of inter-radial spines that have formed within the rudiment. Since the patterns of Hox gene expression are dynamic, it was very important to be able to compare results obtained at equivalent stages of rudiment development.

Single-stranded antisense RNA probes were obtained from the five genes so far identified at the ‘posterior’ end of the Hox cluster, i.e. from the 5’ end, viz. *SpHox11/13b*, *SpHox11/13a*, *SpHox9/10*, *SpHox8* and *SpHox7* (Arenas-Mena et al., 1998; Martinez et al., 1999). These were used for whole-mount in situ hybridization (WMISH) at all stages from embryogenesis through larval metamorphosis. After in situ hybridization, staining patterns in the larvae were examined in serial sections. Extensive alterations in procedures for WMISH were required for the larval samples, as detailed in Materials and Methods. Results obtained on the embryonic samples (not shown) merely confirmed what had been known earlier: only *SpHox11/13b* (Dobias et al., 1996) and *SpHox7* (Angerer et al., 1989) are expressed during embryogenesis, largely as described.
Fig. 2. Hox gene expression in larval somatocoels. Representative sections of larvae on which WMISH had been performed are shown (see Materials and Methods), using the indicated probes. The larvae have rudiments that start to acquire pentameral organization of their hydrocoels. Beneath each stained section (A-J) is a diagram illustrating the anatomy. The planes of the sections, with respect to the morphology of the larvae as a whole, are shown in the drawings (K-M), and the color code used in the anatomical diagrams is given in (N). Hybridization of each
Hox gene is shown in both a transverse (top), and a saggital (below) section: SpHox7 (A,B); SpHox8 (C,D); SpHox9/10 (E,F); SpHox11/13a (G,H); SpHox11/13b (I,J). Abbreviations are as in Fig. 1, except abn, abanal; abo, aboral; an, anal; ls, left somatocoel; o, oral; rs, right somatocoel.
Furthermore, the larval WMISH results are entirely consistent with the quantitative probe excess hybridization measurements of Arenas-Mena et al. (1998), using exactly the same probes; thus, it can be excluded that very high levels of transcript in just a few embryonic cells could have accounted for any of the results of those measurements. The WMISH patterns seen in metamorphosing animals were similar to those of advanced rudiment stages, and, with one exception, are not shown separately here.

As a guide to the WMISH results, Table 1 lists the locations in the larva of the observed domains of Hox gene expression, and the Figs in which these are illustrated. Each of the genes is expressed in a unique pattern, but these patterns share one important feature: all five genes are expressed in the somatocoels.

### Somatocoelar expression patterns

The domains of expression of all five Hox genes in the somatocoels of late torus-stage larvae (i.e. slightly earlier than shown in Fig. 1B,C) are illustrated in Fig. 2. As an aid in interpreting the complex and unfamiliar anatomy of these larvae, color-coded drawings that represent the main features of the sections are shown beneath each. In general, the colored regions in these drawings are tissues that will contribute to the adult body plan (except for the anal area of the hindgut), while the black regions are jettisoned at metamorphosis (except for some local patches of the aboral ectoderm where adult test plates and spines). The diagrams in Fig. 2K-M are in the same orientation as the pictures in Fig. 1B,C. Note that the digestive tract is curved so that the intestine opens near the mouth (Figs 2L and 1C). The somatocoels, the main focus of Fig. 2, are in green. The expression patterns are shown in two aspects for each gene: in transverse sections in the top row of Fig. 2 (i.e. A,C,E,G,I) and in more or less sagittal sections in the bottom row (i.e. B,D,F,H,J). The planes of section are indicated in the diagrams (K-M).

SpHox7 was expressed only in the regions of the somatocoel nearest the larval mouth, at the opposite side from the anus and behind the pharynx (Fig. 2A, staining at s on larval left side). Expression is symmetrical, but the section is oblique and the equivalent region of the right somatocoel is not included. In Fig. 2B, note that the stained region is confined to the top of the somatocoel; as the diagram in Fig. 2M shows, the equivalent region on the right-hand side is again not included in the section. Other sites of SpHox7 expression (e.g. arrowhead in Fig. 2A) are considered below.

SpHox8 was expressed at the same stage in an abanal somatocoelar domain that overlaps that of SpHox7, but extended more towards the oral/aboral (O/Abo) midline of the larva, as viewed from either side. The domains of expression of this gene are shown in the transverse and sagittal sections of Fig. 2C,D. The expression extended in the oral direction right up to the margin of the somatocoel.

SpHox9/10 was expressed the most broadly in the somatocoels of any of the five genes. As shown in Fig. 2E, its expression extended most of the way around the somatocoelar circumference, excluding only the anal and abanal regions at the oral side, and in the sagittal section of Fig. 2F it can be seen to reach all the way down to the aboral end of the somatocoel (only the left somatocoel is included in this image).

SpHox11/13a was expressed towards the anal side of the somatocoels, along the intestine (Fig. 2G). Its domain of expression extends up but does not include the region overlain by the imaginal rudiment (red and blue in the diagram). In the sagittal section (Fig. 2H), the expression of SpHox11/13a can be seen to extend below the anal area all the way to the aboral vertex.

SpHox11/13b was expressed exclusively around the anus. The section shown in Fig. 2I passes through the anus, and the somatocoels can be followed from there all the way around to the opposite side; only the anal region is stained. The sections in Fig. 2J transect the intestine and anus, which can be seen to express the gene together with the adjacent somatocoelar tissue. SpHox11/13b begins to be expressed in the hindgut of the embryo, and this expression continues in the larva after feeding begins (Dobias et al., 1996; C. A.-M., A. R. C. and E. H. D., unpublished). Expression in the adjacent somatocoelar tissues is seen only after several days of larval development. For all the genes studied there was a general decrease in the intensity of the stain towards the aboral pole. This is shown by the broader area detected when using fixations that provide more intense staining (see Materials and Methods).

Fig. 2 provides a very interesting general result: the patterns of expression of the five Hox genes formed a sequential series, but surprisingly the sequence extended bilaterally, more or less cross-wise in the larva with respect to its O/Abo axis. That is, the most 5’ of the genes, SpHox11/13b, was expressed in the anal area, and the more ‘anterior’ genes were expressed progressively towards the abanal side of the somatocoels, particularly at the top or oral side of the somatocoels (cf. diagrams in Fig. 7). An interpretation of this result is considered in the Discussion; but before proceeding thereto it is necessary to consider two additional parameters that progressively affect the somatocoelar expression pattern. This is a growing left-right asymmetry in the expression patterns, and other changes in somatocoelar expression patterns occur as development of adjacent structures proceeds.

In Fig. 3 the strong correlation is illustrated for several of the genes between expression levels and proximity of the rudiment. The first example is SpHox8 (Fig. 3A) where a strong
patch of somatocoelar expression was seen in the immediate vicinity of the rudiment, on the oral side. A striking asymmetry was generated (red arrowhead), which was entirely absent in sections below the level of the rudiment as seen in Fig. 2C, where SpHox9/10 expression appeared symmetrical. SpHox9/10 was also expressed asymmetrically at the torus stage, displaying enhanced activity near the rudiment (Fig. 3B). Later, at the stage when several adult spines have formed, the somatocoel has grown beneath the rudiment, and the specific region of the somatocoelar tissue underlying the hydrocoelar layer now displays a high level of expression, compared with the equivalent region on the right-hand side of the larva. This is illustrated in Fig. 3D (red arrowhead). But the same is not true of all the somatocoelar Hox gene expression patterns. Fig. 3E shows that Hox11/13a continued to be expressed in a perfectly symmetrical way, irrespective of the adjacent rudiment on the larval left side. Like SpHox7, SpHox8 and SpHox9/10, SpHox11/13b was also expressed asymmetrically as well, beginning at the torus stage (Fig. 3C) and continuing at the stage shown in Fig. 3F, which is similar to that of the larvae shown in Fig. 3D. It is interesting to note that the expression of this gene, which began precociously (in the endoderm) during embryogenesis, was no longer detectable in the anal region at the most advanced larval stages (not shown).

Somatocoelar expression of two of the Hox genes was also enhanced in the region directly adjacent to the stone canal, a
structure that connects the nascent water vascular system to the outside via the hydropore. Fig. 4 shows that expression of both the SpHox7 and SpHox8 genes is dramatically enhanced in the immediate vicinity of this structure. None of the five genes studied was expressed in the dental sacs, derivatives of the left somatocoel, where SpHox3 is used (Arenas-Mena et al., 1998).

**Hox gene expression outside the somatocoels**

The aspects of Hox gene expression so far considered are exclusively somatocoelar, i.e. exclusively mesodermal, and these are indeed the predominant (and for SpHox8 and SpHox9/10 the only domains of detectable transcription (Table 1)). As already noted, however, SpHox11/13b, the most 5’ of the genes studied, was also expressed in the endoderm, i.e. in the anal region and the distal part of the intestine during early rudiment stages (Fig. 21J). Restriction to the hindgut and anus is evident in early larvae (not shown), though this domain of endodermal expression fades out late in larval development. As summarized in Table 1, several kinds of developing peripheral structures that are destined to be carried forward into the adult stage also display expression of SpHox11/13b and SpHox11/13a in the larva. These genes are used similarly to one another outside of the rudiment proper in many small structures, particularly in epithelial cells that are derived from the aboral ectoderm and are associated with forming test elements (Figs 3F, 5). Note that in the high-magnification images of Fig. 5B,F the expression of these two genes is confined to the epithelial rather than mesodermal aspects of these structures. Similarly, SpHox11/13b is shown in Fig. 5C to be expressed in tissue that lies adjacent to the nascent adult test plate, probably from its position in the larva the madreporic plate. Other epithelial derivatives of the oral ectoderm in which these two genes are used include the vestibular margin (Figs 5A,D,E, 3F; and ectoderm lateral to or covering spines (Fig. 5D,E). These domains of expression have two factors in common: they are all ectodermal and they also occur in the immediate vicinity of particular forming structures, generally endoskeletal components, i.e. spines and test plates. These endoskeletal structures are specific morphological features of echinoids, and expression of SpHox11/13a and SpHox11/13b within them would seem clear examples of evolutionary co-option.

Further such co-options are visible in the peripheral patterns of expression of SpHox7, which, as illustrated in Fig. 6 and Table 1, is the most diversely used of the genes studied. During embryogenesis SpHox7 is expressed in the vertex of the aboral ectoderm (Angerer et al., 1989), and this domain of expression persists far into larval development, as shown in Figs 6B,D, 4C. In the transverse sections in Fig. 6B,D, the intestine wall or surrounding mesenchyme can also be seen to express the SpHox7 gene (mesenchymal expression is also to be seen in Fig. 6F,E,H), while the adjacent somatocoels do not express this gene. In addition to these peripheral loci of expression, however, SpHox7 was also expressed within the rudiment, in a unique set of developing tissues, which change progressively. An interpretive diagram of relevant structures in the rudiment is shown in Fig. 6A and diagrams representing the section shown in Fig. 6B,F,H are given, respectively, in Fig. 6C,G,I. Two unidentified groups of cells on each side, lateral to forming podia (pp) at the base of the epineural folds expressed SpHox7, as shown in Fig. 6E. The SpHox7 gene expression patterns reported here could be or are associated with the radial central nervous system of the rudiment (see Fig. 6A). The radial nerve (yellow) arises at the base of a thickened domain of vestibular ectoderm (blue) derivatives, overlying the radial
canal (rc). The epineural canal (epc, Fig. 6A) overlies the radial nerve and it is formed after the fusion of the epineural folds (cf. Fig. 7I). In Fig. 6F,G the staining at locus 3 may lie within the radial nerve precursor cells or is at least immediately adjacent to them. Locus 4 of Fig. 6F (cf. A) may represent the central nerve ring, or it could involve elements of dental sacs (ds), which are somatocoelar derivatives (green in Fig. 6A,G,I). Staining in or directly around the radial nerve is also seen in Fig. 6H, again at locus 3 (cf. Fig. 6A and 6I). Staining at locus 2 here indicates SpHox7 expression in the epineural folds during the formation of the epineural canal (Fig. 7H). Expression in locus 1 (Fig. 6E,F) is initially continuous with the expression in locus 2, and perhaps also with locus 3. During the latest stages the expression in locus 1 becomes separated into domains that are pentamerally organized.

**DISCUSSION**

One way to summarize simply the complex patterns of posterior Hox gene expression described is to consider that they illustrate three separate issues. First, the developmental significance of SpHox11/13b expression in the hindgut; second, the many specific co-options to echinoderm-specific functions that are particularly evident for SpHox7; and third, the spatially sequential and dynamic somatocoelar expression pattern in which all five of the genes studied are used. SpHox11/13 is expressed intensively in the anal and adjacent hindgut endoderm of the larva, and, unlike its somatocoelar expression, this pattern of transcription is established far back in embryonic development (Dobias et al., 1996; C. A.-M., A. R. C. and E. H. D., unpublished). This can perhaps be understood in terms of a common feature of maximal indirect development: we pointed out earlier that while the mesodermal structures and central nervous systems of the adult body plan are always de novo products of the postembryonic developmental process in indirect development, the digestive tract or at least portions of it are often retained from embryogenesis (Peterson et al., 1997, 2000b). SpHox11/13b is the most 5′ of the Hox genes so far identified in the S. purpuratus Hox cluster (Martinez et al., 1999; however, additional posterior genes have been discovered in other echinoderms; Mito and Endo, 2000). Embryonic specification of the hindgut and anus apparently involves regional activation of SpHox11/13b, just as posterior Hox genes are expressed in the hindgut of other animals. This gene simply continues to be expressed in the anus and hindgut throughout most of larval development in S. purpuratus, i.e. so long as these structures remain functional.

Co-option of regulatory genes to the specific purposes of echinoderm development has evidently been a major, or the major, process in the evolution of this clade (Lowe and Wray, 1997). We here add several apparent new examples. For instance, both SpHox11/13b and SpHox11/13a are expressed in the vicinity of new structures associated with skeletal plates (Fig. 5). The most remarkable cases are afforded by SpHox7 (Fig. 6): this gene is expressed in many locations both within and peripheral to the rudiment, in ectodermal or epithelial components. SpHox7 is also used in the neuroectodermal tissues within which the pentameral ring nerve and the radial nerve system forms. This displays pentameral symmetry, by definition an echinoderm-specific character. The incidence of evolutionary co-option to clade-specific functions must be very great, considering how easy it is to find examples, that is, if one looks for it in organisms that differ in body plan from the arthropod and vertebrate examples that are most familiar to us (Lowe and Wray, 1997; this work).

**The somatocoelar ‘Hox vector’**

SpHox7, SpHox8, SpHox9/10, SpHox11/13a and SpHox11/13b are all expressed in the somatocoelar mesoderm. The most unexpected and on the face of it puzzling aspect of our results is that their expression domains form a bilateral, sequential set of spatial patterns that are not oriented in accord with either...
the O/Abo axis of the larva or A/P axis of the adult (Peterson et al., 2000a). The somatocoelar expression pattern is in the plane of the flat, sac-like somatocoelar mesodermal sheets (see Fig. 2K,L). On the left-hand side this pattern is orthogonal to the nascent A/P axis of the rudiment. But considered in light of the recent theory for the evolutionary origin of the echinoderm body plan put forth by Peterson et al. (2000a), this pattern turns out to be revealing rather than paradoxical.

In Fig. 7, we summarize the patterns of expression of the five posterior Hox genes in the somatocoelar tissues, both on
The major importance of Fig. 7 lies in the curved white arrows in the top pair of drawings. These illustrate the somatocoelar ‘Hox vector’, i.e. the orientation of the sequence of expression domains of genes located 3’ to 5’ in the Hox cluster. The sequence of expression domains is collinear with the order of the genes in the Hox cluster (Martinez et al., 1999). As the white arrows show, these sequential patterns extend continuously (SpHox7-SpHox11/13b) in an anal direction along a curved axis with respect to the morphology of the somatocoel that parallels the curvature of the digestive tract. Were the digestive tract and the accompanying somatocoels straightened out (see Fig. 2K,L), the arrangement of the Hox vector’ would be linear instead of curved. Such a straight digestive tract is found in the very similar larva of the sister group Hemichordates (Peterson et al., 1997), where a linear Hox vector within the posterior coeloms would be predicted.

**Evolution of the echinoderm body plan**

The common ancestor of echinoderms and hemichordates was almost certainly a bilaterally organized animal in which the posterior pair of coeloms terminated near the anus. We see that all of the posterior Hox genes are in fact expressed in the somatocoels. However, the pattern shown in Fig. 7 enriches our image of the evolutionary transformations that generated the pentameral structure that has been characteristic of most adult echinoderm body plans ever since the Early Cambrian. The original A/P orientation of the somatocoels that can safely be inferred for the bilateral common ancestor of echinoderms and hemichordates can still be seen in hemichordates. In the

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The right and left sides, and at early (Fig. 7A,B) and late (Fig. 7C,D) stages. Early on, i.e. at the stage when the pentameral organization of the rudiment has just become visible, the patterns of expression are similar on the two sides, except that on the left side (Fig. 7A) the expression domain of SpHox7 and SpHox8 extend from the abanal end of the somatocoel more centrad, towards the young rudiment, and likewise the SpHox11/13b expression domain extends more centrad from the anal end. This results in a more complex set of overlapping expression domains on the left side near the rudiment compared with those on the right-hand side (striped regions of Fig. 7A versus Fig. 7B). The result is that for about 120° of its circumference, the rudiment confronts different combinations of Hox gene products in each quadrant. However, the rudiment develops in an independent pentamerally symmetrical manner; it is here that the fivefold organization of the echinoderm body plan is initially established. The adjacent tissues, including the digestive tract, skeletal elements and also the somatocoels, where the posterior Hox genes are expressed, display bilateral rather than pentameral organization. By late in rudiment development (Fig. 7C,D) right versus left asymmetries are much accentuated. For example, SpHox7 and SpHox8 expression display an asymmetrical stripe directly apposed to the position of the growing stone canal; these genes and SpHox9/10 now are expressed in somatocoelar mesoderm beneath the rudiment on the left-hand side; and in general most of the posterior Hox gene expression is lower on the right-hand side (white hatching in Fig. 7D), compared with the left.
evolutionary process leading to *S. purpuratus* this axis was evidently altered by a 90° shift in the digestive tract and the associated somatocoelar structures, so that what was originally at the tail of the animal is now on one side (thus, presumes or rather predicts, that in hemichordates the sequence of homologous *Hox* gene expression patterns will run directly along the A/P body axis). Part of the function served thereby is implied by the patterns of Fig. 7C,D. The definitive morphological change in the evolution of the echinoderm body plan is coelomic stacking (Peterson et al., 2000a) such that viewed from the oral surface of the pentaradial adult body plan (as in Fig. 7C), the left somatocoel comes to lie beneath (actually interdigitated within) the hydrocoel of the rudiment; further below the plane of the page, the right somatocoel comes to lie beneath the left. The roughly circular shape the somatocoels assume as they surround the digestive tract later in development, including its stubby intestine, facilitates this arrangement: were the gut removed, the coeloms would resemble a stack of coins. The morphological transformation would be much more different to conceive were the gut and somatocoels elongate linear structures. However, the price that had to be paid is also clear from Fig. 7: formation of the gut and somatocoels elongate linear structures. However, the posterior of the adult body plan requires formation of a new terminal hindgut and anus (as in Fig. 7C), the left somatocoel comes to lie beneath the lateral and anal surfaces of the adult, and correlated with this axis was this expression certainly represents the most basal of all of the posterior *Hox* gene functions during body plan development in *S. purpuratus*, except perhaps for the expression of *SpHox11/13b*, which in its remote ancestors marked the posterior terminus of the animal. This is to say that the most important and perhaps the most ancient developmental functions of these posterior *Hox* genes in this clade of animals are played out in mesodermal tissues. There is no detectable expression of *SpHox8-SpHox11/13b* in the progenitor field of the radial central nervous system of the adult body plan, while in both vertebrates (see review by McGinnis and Krumlauf, 1992) and invertebrate chordates (Wada et al., 1999) the cognate *Hox* genes are expressed in thoracic and posterior regions of the dorsal nervous system and in mesodermal derivatives. It will be interesting to determine whether these same genes are expressed in the dorsal (or ventral) nerve cords of developing hemichordates; if not, this co-option to CNS patterning of posterior *Hox* genes in chordate ancestors may have been an important specific aspect of chordate evolution. Since the hemichordates are the echinoderm sister group we predict that the posterior *Hox* genes of enteropneust hemichordates will be expressed in an A/P series in the metacoels, but without left-right asymmetry. In any case our observations do not sit well, i.e. parsimoniously, with the theory that the original, pleisiomorphic role of *Hox* genes in evolution was patterning of the A/P dimension of the central nervous system (Wada et al., 1999). As we have argued elsewhere (Davidson et al., 1995; Peterson and Davidson, 2000; Peterson et al., 2000b), a fundamental and novel event in bilaterian evolution must have been the appearance of mechanisms for patterning three-dimensional mesodermal structures. The creation of regulatory networks controlling regional specification of the mesoderm, which included genes of the *Hox* cluster, could have constituted a key step in this process.

We thank Dr Kevin Peterson for his extremely helpful review. This work was supported by the Stowers Institute for Medical Research; the National Science Foundation, Developmental Mechanism Program, Grant No. IBN-9604454; and the Fundamental Biology Research Program of the Life Sciences Division of the National Aeronautics and Space Administration/Ames Grant NAG2-1368.

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