

Left-right asymmetric expression of *BbPtx*, a *Ptx*-related gene, in a lancelet species and the developmental left-sidedness in deuterostomes

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SUMMARY

The long-standing question of how asymmetric development or asymmetric body structures in lancelets (amphioxus) are phylogenetically related to the body plan of other animals is still untouched. Three anterior structures, the preoral pit, club-shaped gland and mouth, are remarkable asymmetric features in developing lancelets that all open on the left side of the body. A *Ptx*-related gene, *BbPtx* is the first identified transcription factor gene with an asymmetrical expression pattern in lancelets similar to that in vertebrates, and thus it may provide a clue for the above question. Expression of the *BbPtx* gene is first detected at the dorsal margin of the blastopore in early mid-gastrulae and then becomes restricted to the left anterodorsal wall of the primitive gut and to the developing left somitocoelomic system. Expression continues on the left side in the developing preoral pit, club-shaped gland and mouth as well as in the mesoderm at the caudal end. Unlike *D-Ptx1* in

Drosophila, *BbPtx* is not coexpressed with a *fork head* gene in lancelets; instead the two genes are expressed in a complementary fashion on the left side of the embryo. The expression pattern of *BbPtx* is not compatible with the calcichordate hypothesis of Jefferies, in which the proposed ancestor of chordates rotated its tail 90° counterclockwise in relation to the head/trunk. The expression of both *BbPtx* and vertebrate *Pitx2* in tissues derived from the coelom implies that the left-right asymmetric development has a common origin between cephalochordates and vertebrates. Considering the development of the coelom in deuterostomes, however, left-right asymmetric development involving *Pitx2*-related genes is rather likely to be a primitive character shared among deuterostomes.

Key words: Amphioxus, HNF-3 β , Deuterostome, Calcichordate, Coelom, BbPtx

INTRODUCTION

The study of lancelets can provide important clues to understanding the evolution of chordates. A number of genes shared with vertebrates have been studied developmentally in lancelets (Holland et al., 1992, 1995, 1996; Holland and Holland, 1996; Shimeld, 1997, 1999; Terazawa and Satoh, 1997; Glardon et al., 1998; Langeland et al., 1998). In these studies, the expression pattern of the lancelet gene has been found to be similar to that of the vertebrate counterpart, supporting the close affinity of cephalochordates to vertebrates. Phylogenetic systematics using molecular tools also support a view that the cephalochordate is the sister group of vertebrates (Turberville et al., 1994; Wada and Satoh, 1994) and have been cited frequently in the study of gene expressions.

In spite of the above studies, it is also true that lancelets retain many anatomical features whose phylogenetic relationships have yet to be elucidated (Gans and Northcutt, 1983; Yasui et al., 1998) and there are some examples of gene expression patterns that are quite different from vertebrates.

The study of *AmphiEn* in a Florida lancelet, *Branchiostoma floridae*, has demonstrated that metameric somites, which have been considered a shared character between cephalochordates and vertebrates, utilize the same molecular cue to establish the repeated pattern as in *Drosophila* (Holland et al., 1997). *AmphiBMP2/4* and *BbBMP2/4* present another example (Panopoulou et al., 1998; I. Semba, H. S., M. U. and K. Y., unpublished data). The vertebrate BMP4 protein plays an important role in establishing the dorsoventral polarity in mesoderm (Suzuki et al., 1994; Schmidt et al., 1995), whereas *AmphiBMP2/4* and *BbBMP2/4* do not apparently relate to this function. Likewise, molecular systematics can yield different results depending on the molecules to be utilized (Turberville et al., 1994; Nikoh et al., 1997; Naylor and Brown, 1998).

Left-right asymmetric development of lancelets is one of the most problematic features in investigating phylogenetic relationships. Three anterior structures, the preoral pit, club-shaped gland and mouth, show remarkable asymmetry in developing lancelets. Although their homology with organs or

structures in other taxa has long been discussed, a consensus has not yet been reached.

Ptx genes have been identified as a small group of bicoid-type homeobox genes in vertebrates (*Ptx1*, Lamonerie et al., 1996; Lanctôt et al., 1997; *Ptx2* (*Ptx2*), Gage and Camper, 1997; *Ptx3*, Smidt et al., 1997), *Drosophila* (*D-Ptx1*, Vorbrüggen et al., 1997) and *C. elegans* (*Unc-30*, Jin et al., 1994). Other than a common expression in cells of the central nervous system, *Ptx* genes are expressed in the stomodeum and the Rathke's pouch in vertebrates, and later in a subset of cells in the anterior and intermediate lobes of the hypophysis and in mesenchymal cells in the first pharyngeal arch. Accordingly, vertebrate *Ptx* genes were previously regarded as anterior-specific genes. Recently, it has been demonstrated that *Ptx2* (*Ptx2*), a member of *Ptx* genes, mediates the establishment of left-right asymmetry commonly in vertebrates under the control of a Shh-Nodal signalling pathway (Logan et al., 1998; Piedra et al., 1998; Ryan et al., 1998; Yoshioka et al., 1998). These findings suggest that *Ptx*-related genes may play a key role in the development of asymmetric structures, the focus of the present study.

To obtain insights into evolutionary aspects of the anterior asymmetric structures, we have studied a cognate of vertebrate *Ptx* genes in Chinese lancelets. The identified gene, *BbPtx* showed a left-sided expression as seen in vertebrates. The left-sided expression first occurred in the prospective left lateral diverticular region, evaginating metameric mesoderm and the overlying ectoderm. From the region where the gene was expressed, the preoral pit, the duct of the club-shaped gland and the mouth are formed in which expression of the gene was maintained. Although the structures expressing the *Ptx*-related gene are derived characters, they are related to the formation of coelom like the lateral plate mesoderm in vertebrates. From the present results, we propose that the left-sided asymmetric development in cephalochordates and vertebrates has a common origin, that the origin could be traced back to the ancestor of deuterostomes and lastly, in lancelets, that the formation of mouth may be related to the coelomic formation.

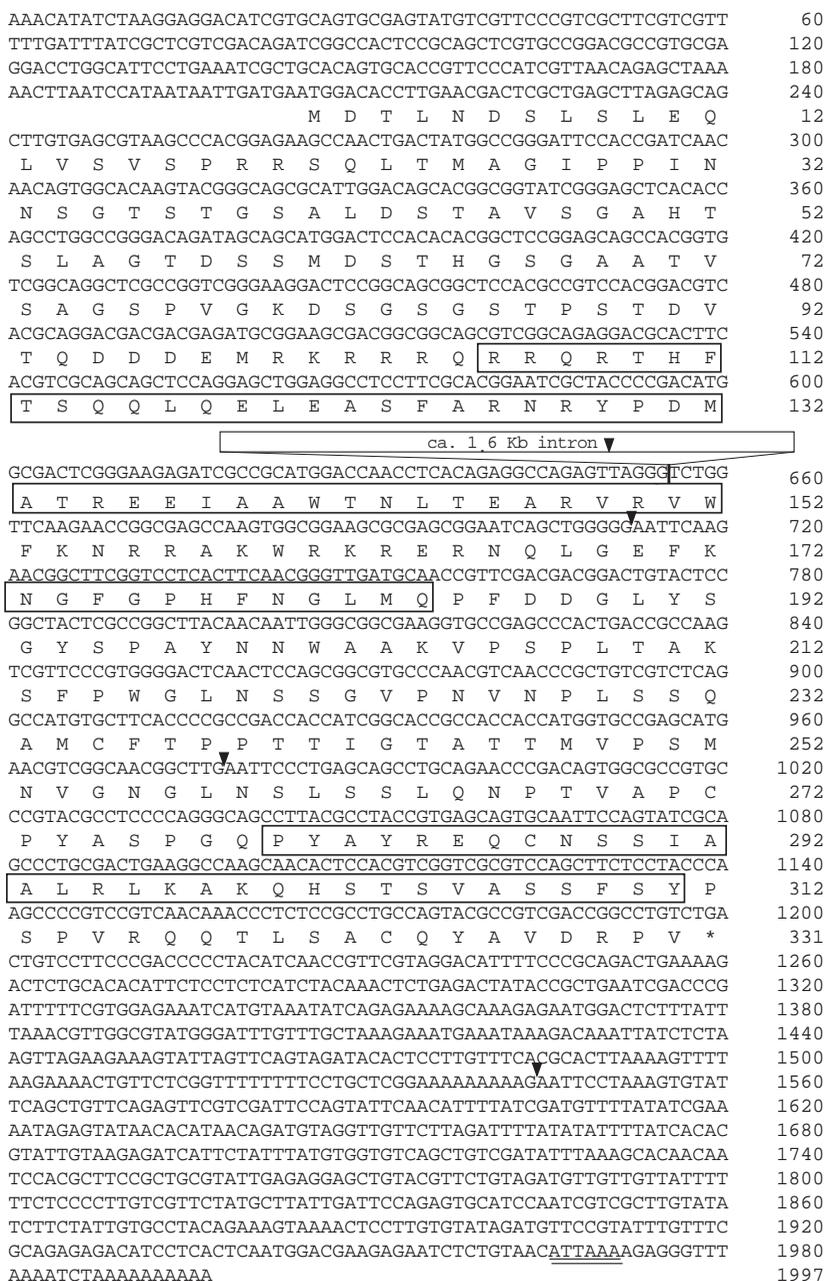
MATERIALS AND METHODS

Animals

Embryos and larvae of a Chinese lancelet species, *Branchiostoma belcheri tsingtauense*, were collected for in situ hybridization at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China in 1996-98 as described previously (Yasui et al., 1998). Embryos and larvae from initial gastrulae to 2-day-old larvae were staged according to Hirakow and Kajita (1991, 1994). The fixed materials were kept in 75% ethanol at -20°C until use. A mass of hatching neurulae were also collected and quickly frozen in liquid nitrogen for cDNA library construction.

cDNA library and screening *BbPtx*

A cDNA library was constructed in λgt11 vector (Amersham Pharmacia Biotech, Tokyo) using cDNA synthesized from poly(A)⁺ RNA isolated from the hatching neurulae. The library was screened with a PCR fragment of the homeobox region of *Hrgsc*, *goosecoid*-related gene of a Japanese ascidian, *Halocynthia roretzi* (Y. Ueno, Y. Shimojima and H. S., unpublished) as a probe under the same hybridization conditions as described previously (Yasui et al., 1998).



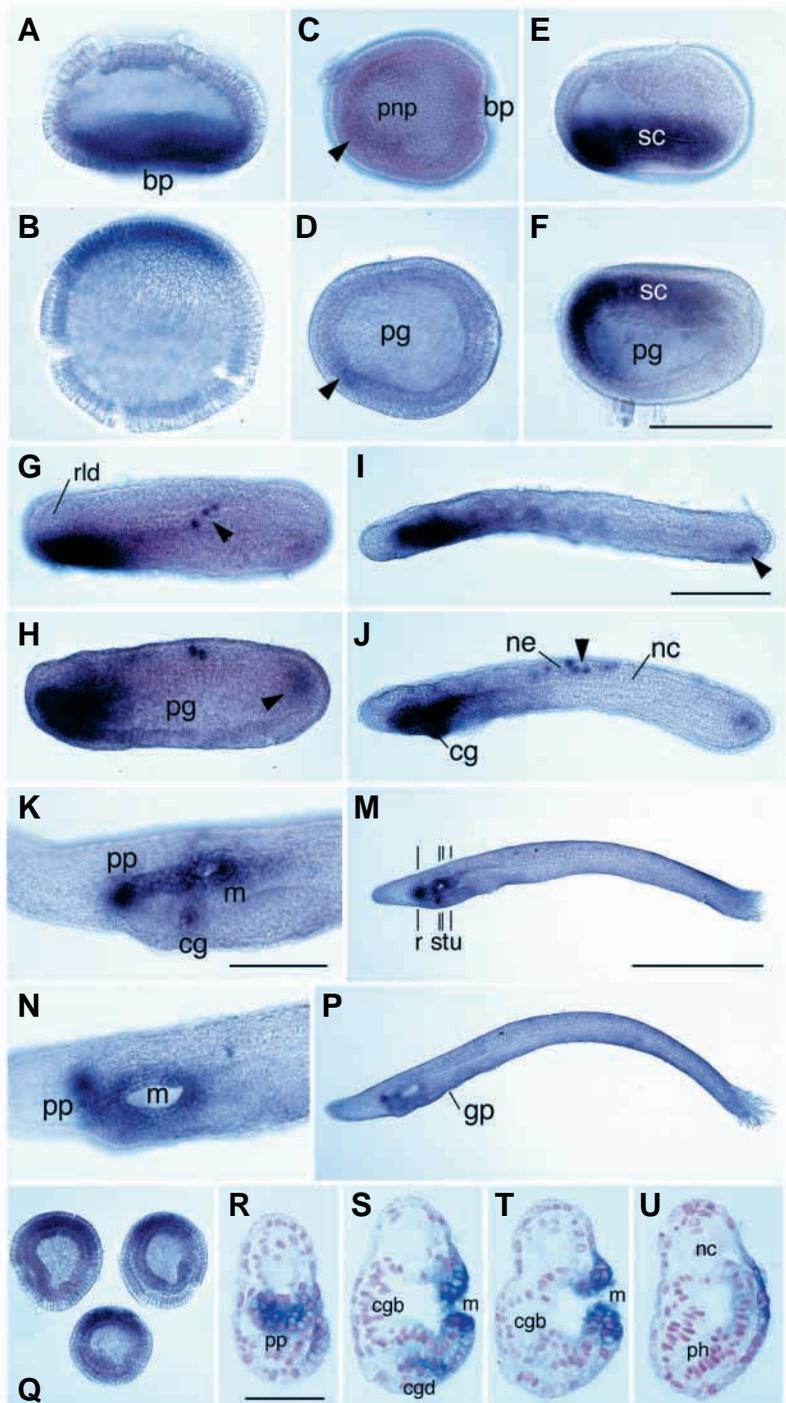
amino acid residues with a homeodomain of a bicoid class (Fig. 1).

The deduced amino acid sequence shows about 90% similarity in the homeodomain to other Ptx proteins and 60% similarity in a short motif in the C-terminal region conserved between mammalian *Ptx1* and *Pitx2* genes (Gage and Camper, 1997) (Fig. 2). Thus, we designate the gene corresponding to the cDNA *BbPtx* (*Branchiostoma belcheri Ptx*). To examine the relationship between the deduced BbPtx and other bicoid type proteins, an unrooted tree was drawn for homeodomains (Fig. 2) using the Neighbor-Joining method (Saitou and Nei, 1987). The deduced BbPtx protein

formed a cluster with other Ptx proteins including Unc-30 with a 100% bootstrap score. Within the cluster, although BbPtx is slightly more remote from the vertebrate members than *Drosophila* D-Ptx1, the bootstrap scores at the nodes of D-Ptx1 and BbPtx were 71% and 60%, respectively. It is noteworthy that several deduced proteins of protochordates are diverged further from vertebrate cognates compared with those of *Drosophila* (e.g., *AmphiD11*: Holland et al., 1996; *HrBMPb*: Miya et al., 1997; *Wnt1*: accession number AF061974).

To see whether the Chinese lancelet genome possesses genes similar to *Ptx* genes other than *BbPtx*, we performed genomic

Fig. 4. Expression patterns of *BbPtx* from gastrulae to 48 hour larvae detected by in situ hybridization. Anterior is to the left in all the panels, except for gastrulae (A,B,Q). (A) Probable dorsal view and (B) view from the blastopore of early mid-gastrula showing expression of *BbPtx* at the probable dorsal margin of blastopore (bp). (C) Dorsal view of late gastrula showing symmetric horseshoe-shaped expression at the probable anterior margin of the prospective neural plate (pnp) (arrowhead). (D) Dorsal view of an embryo at a transient stage from gastrula to neurula showing the first asymmetric expression in the anterodorsal endoderm of the primitive gut (pg) (arrowhead). (E) Dorsal and (F) left lateral views of early neurula showing strong left-sided expression in the somitocoelomic system (sc), endoderm and surface ectoderm. (G) Dorsal and (H) lateral views of late neurula showing cells expressing *BbPtx* in the CNS (arrowhead in G) and the left-sided expression at the anterior and posterior (arrowhead in H) regions. A similar pattern continues until the early larva stage (I, dorsal and J, lateral views). Cells expressing *BbPtx* in the CNS (ne) increase in number (arrowhead in J). (K) Magnification of the oral region and (M) lateral view of a 36 hour larva. Expression is restricted to the preoral pit (pp), the duct of the club-shaped gland (cg) and the mouth (m). (N) Magnification of the oral region and (P) a lateral view of a 48 hour larva showing that expression is disappearing at the rim of the mouth and the duct of the club-shaped gland but is retained in the preoral pit. (Q) View from the blastopore of mid-gastrulae. The outline of the blastopore indicates that expression at the margin of the blastopore is most likely at the dorsal. (R-U) Transverse plastic sections of a 36 hour larva. The level of the sections is indicated in M. The preoral pit (R), the duct (cgd) of the club-shaped gland (S) and the endoderm and ectoderm at the rim of the mouth (S-U) are expressing *BbPtx*. The body (cgb) of the club-shaped gland is negative (T). gp, gill pore; nc, notochord; ph, pharynx; rld, right lateral diverticulum. Bar, F, 100 μ m, also applies to A-H; I, 100 μ m, also applies to J; K, 50 μ m applies to N; M, 200 μ m applies to P and Q; R, 25 μ m applies to S-U.



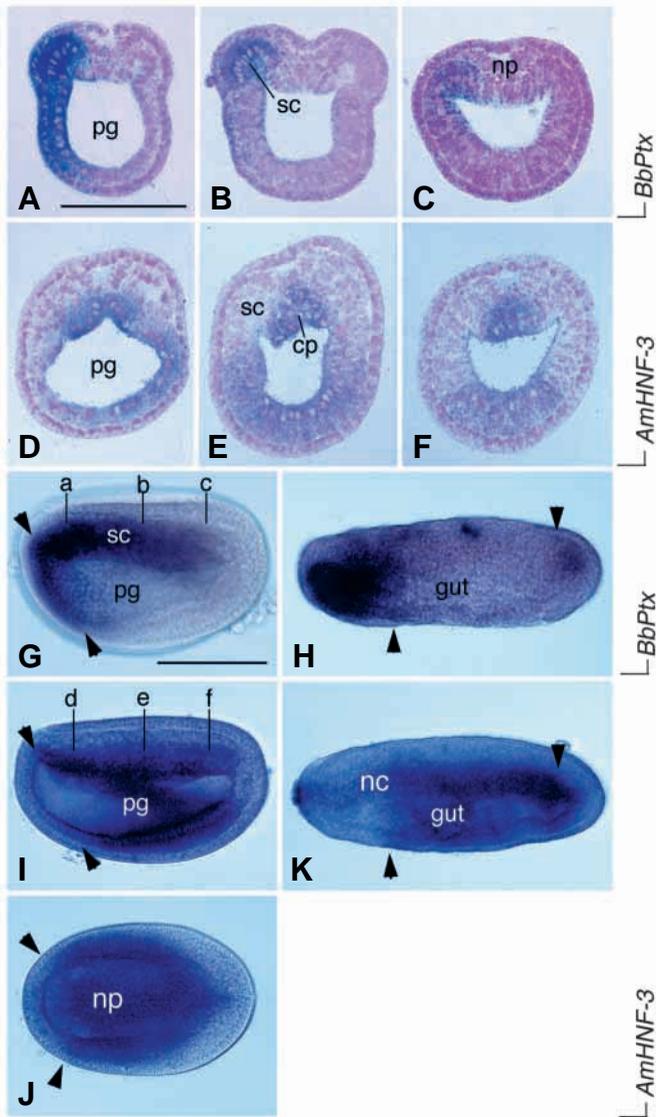


Fig. 5. Comparison between the expression patterns of *BbPtx* and of *AmHNF-3* in neurulae. (A-C) Transverse plastic sections of early neurulae showing the expression pattern of *BbPtx*; (D-F) expression pattern of *AmHNF-3*. The levels of sectioning are indicated in G for *BbPtx* and I for *AmHNF-3*. (G,H) Left lateral views of early and late neurulae probed with *BbPtx*, and (I-K) those with *AmHNF-3*. Compare A with D, B with E, C with F, G with I, and H with K. Expression of *AmHNF-3* in the anterior region is asymmetric (D,J), whereas in other regions it is symmetric (E,F). The expression pattern of *BbPtx* is complementary to that of *AmHNF-3* on the left side, although the ventral margin of the *BbPtx* domain in the anterior half (A,B) overlaps with the dorsal margin of *AmHNF-3* domain in the endoderm (D,E). As regards to comparison between H and K, see also figure 5e in Terazawa and Satoh (1997). cp, chordal plate; nc, notochord; np, neural plate; pg, primitive gut; sc, somitocoelomic system. Bar in A is 50 μ m, also applies to B-F, bar in G 100 μ m applies to H-K.

Southern blot analysis under reduced stringency conditions using a short DNA fragment from the *BbPtx* homeobox as a probe. As shown in Fig. 3, each lane yielded a single major hybridization band. The result was unchanged when stringent washing conditions were employed (data not shown). We

believe that the Chinese lancelet genome does not have any *Ptx*-related gene other than *BbPtx*.

Developmental expression of *BbPtx*

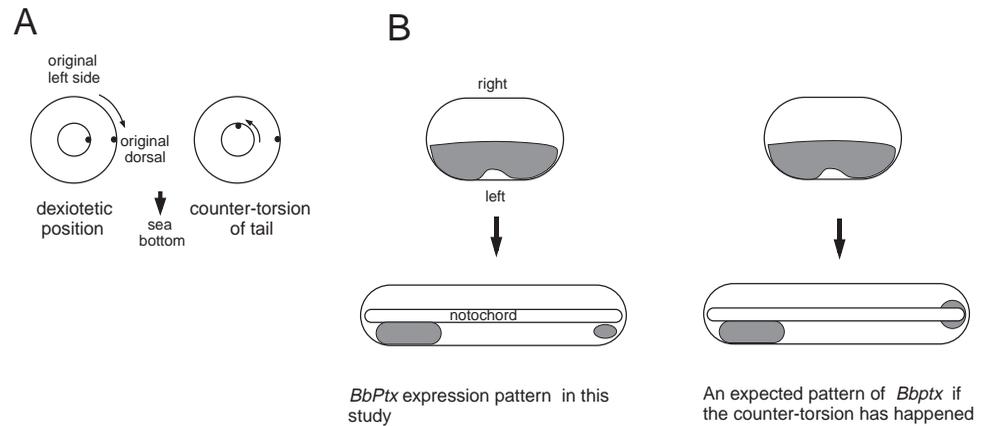
Hybridization signals in whole mounts were first detectable in the outer and inner layers at the margin of the blastopore in early mid-gastrulae (Fig. 4A,B). Although the primitive gut endoderm was colored weakly, we could not be certain if this represented weak expression or background. Although there is no useful marker to identify the dorsoventral polarity at this stage, judging from the shape of the blastopore in mid-gastrulae (Conklin, 1932) and the expression pattern in late gastrulae (Fig. 4C,Q), it is most likely that the initial expression of *BbPtx* was at the dorsal margin of the blastopore. In late gastrulae, expression was found as a horseshoe shape in the anterior region of the ectoderm, probably at the anterior margin of the prospective neural plate (Fig. 4C). *BbPtx* was expressed symmetrically until the late gastrula stage, which is similar to the mammalian cognates (Gage and Camper, 1997; Lanctôt et al., 1997). From the late gastrula stage, however, *BbPtx* expression was found in the anterodorsal wall of the primitive gut only on the left side, which was the first sign of asymmetric expression (Fig. 4D).

In early neurulae, metameric mesoderm evaginates from the dorsolateral wall of the primitive gut in an enterocoelic manner. In lancelet anatomy, the dorsolateral metameric mesoderm is called "somite" following vertebrate anatomy. However, we refer to it as the somitocoelomic system because so-called "somite" in lancelets contains both the somite and lateral plate portions of vertebrates and differentiates later into myotomes and coelomic walls. *BbPtx* transcripts were distributed in the evaginating somitocoelomic system and, anteriorly, they extended to the anterolateral margin of the primitive gut (Fig. 4E,F), where the left lateral diverticulum would appear later. The surface ectoderm overlying the anterolateral wall of the primitive gut that expressed *BbPtx* also exhibited expression (Fig. 4E). Accordingly, the expression domain was hook-shaped in the left lateral view (Fig. 4F). *BbPtx* also appeared in some cells in the central nervous system (CNS) in mid-neurulae (Fig. 4G,H). At the same time, the lateral expression separated into two domains, a large anterior and a small posterior regions (Fig. 4G,H). The posterior expression was coincident with newly forming somitocoelomic system. This expression pattern continued to the early larval stage (Fig. 4I,J), in which the number of cells expressing *BbPtx* in the CNS increased (Fig. 4J). Later, these cells in the CNS became distributed in a metameric fashion, although the expression became weak (data not shown).

The left-sided asymmetric expression continued into the 48 hour larvae, the oldest stage examined in this study (Fig. 4G-P). Interestingly, the expression of *BbPtx* became restricted to the preoral pit, the duct of the club-shaped gland and the mouth, all of which are formed on the left side coincident with the region where *BbPtx* had been expressed (Fig. 4K-P,R-U). The surface ectoderm between the preoral pit and the mouth expressed the gene, connecting the two organs (Fig. 4K).

The preoral pit is derived from the left of the paired lateral diverticula. When it expanded into the right side after pinching off from the primitive gut, *BbPtx* was expressed throughout the epithelium (Fig. 4R). The club-shaped gland is an enigmatic organ. The gland body is derived from the right wall of the

Fig. 6. (A) Schematic illustrations showing the dexiotetic position of the calcichordate (left) and the position of the head/trunk and the tail of a putative ancestor of chordates in Jefferies' hypothesis in which a countertorsion took place in the tail (right). Large and small circles denote the head/trunk and the tail, respectively. Black dots indicate the original dorsal position of both the head/trunk and the tail. (B) The expression pattern of *BbPtx* observed in the present study and an expected expression pattern if the countertorsion of the tail took place in the ancestor of chordates.



primitive gut opposed to the mouth. It has two openings, one externally to the left side via a duct under the floor of the gut, and the other internally into the gut on the right side (Goodrich, 1930). *BbPtx* was expressed in the duct of the gland but not in the gland body on the right side (Fig. 4S,T). The mouth first opens on the left body wall at the level of the ventral margin of the myotome. Around the rim of the mouth, *BbPtx* expression was detected in both the ectoderm and endoderm (Fig. 4K-N,S,T). Signals for the transcripts extended slightly posterior to the mouth in the surface ectoderm (Fig. 4K). In the oldest larva in this study, intense expression of *BbPtx* continued in the preoral pit, but expression in other regions became weak or disappeared (Fig. 4P). There was no expression around the gill pores (Fig. 4N,P).

Relationships of spatial and temporal expression between *BbPtx* and *AmHNF-3*

In *Drosophila*, the initial expression of *D-Ptx1* is controlled by *fork head* gene activity (Vorbrüggen et al., 1997). To understand the relationship between a *fork head* gene and *BbPtx* in lancelet development, the expression pattern of *AmHNF-3* (Shimeld, 1997), a member of fork head family genes reported in Florida lancelets, was examined in Chinese lancelets.

Expression of *AmHNF-3* was not detected until late gastrula stage, which is later than the onset of *BbPtx* expression (data not shown). Previously, left-right asymmetric expression of *AmHNF-3* and *Am(Bb)fkH/HNF3-1* (Terazawa and Satoh, 1997) has not been noticed. In the present study, however, we found asymmetric expression at the neurula stage. *AmHNF-3* was expressed on both sides, but the anterior border of expression on the left side was located posterior to that on the right side (Fig. 5D,J). This is also observable in the previous report on expression of *Am(Bb)fkH/HNF3-1* (figure 6 in Terazawa and Satoh, 1997). The anteriormost expression of *AmHNF-3* on the left side appears to correspond to the posterior border of *BbPtx* expression (compare Fig. 5A with D). In the posterior part of the neurula, *AmHNF-3* was expressed in the chordal plate and the ventral region of the gut endoderm (Fig. 5E,F,K), whereas *BbPtx* was expressed in the evaginating somitocoelomic system and dorsolateral region of the gut endoderm (Fig. 5B,C,H). Expression domains of the two genes are also exclusive of each other in the posterior region of the embryo. Collectively, *BbPtx* and *AmHNF-3* were not expressed sympatrically from the beginning. Rather, they showed complementary expression patterns on the left side.

DISCUSSION

Variations in the temporal expression pattern of genes involved in left-right asymmetric development

In vertebrates, *Pitx2* is regulated sequentially by *Shh* and *Nodal* proteins (Logan et al., 1998; Piedra et al., 1998; Ryan et al., 1998; Yoshioka et al., 1998). Of these, a *Shh*-related gene, *AmphiHh* has been studied in lancelets (Shimeld, 1999). The left-sided expression of *AmphiHh* first appears in the anterior endoderm at the mid-neurula stage, later than that of *BbPtx*. The expression of *Shh* in vertebrates disappears prior to the onset of *Pitx2* expression on the left side and the manifestation of morphological asymmetries, whereas the left-sided expression of *AmphiHh* continues until 22 hours when asymmetric morphogenesis has already started. Unlike the vertebrate counterparts, *BbPtx* is coexpressed with *AmphiHh* for a rather long developmental period. The expression patterns of these two genes in lancelets suggest that the signalling pathway of the molecules involved in the establishment of left-right asymmetries has diverged between cephalochordates and vertebrates.

HNF-3 β has been thought of as a molecule playing a role in the establishment of left-right asymmetry in vertebrates (Levin et al., 1995). However, since the data were not very clear, the gene has not been included in discussions of asymmetric development. In the present study, we found an asymmetric expression pattern of *AmHNF-3*. Interestingly, the expression of *AmHNF-3* is apparently excluded from the expression domain of *BbPtx* during development. A similar observation has been made in the mouse, in which *HNF-3 β* protein is distributed solely at the right margin of the primitive streak in 8-8.5 dpc embryos (Yasui et al., 1997), when *Pitx2* is expressed on the left side (Piedra et al., 1998). The lancelet and mouse patterns suggest that *HNF-3 β* genes play a role in the asymmetric development of cephalochordates and vertebrates, probably controlled antagonistically to *Pitx2*-related genes. In contrast, initial expression of *D-Ptx1* is positively controlled by *fork head* in *Drosophila* (Vorbrüggen et al., 1997). Our present findings may be another example of variation in patterns of gene interaction between species.

Although there are variations in the temporal expression pattern, suggesting modifications of gene interactions, the fact that genes involved in left-right asymmetric development are conserved in cephalochordates and vertebrates strongly suggests that left-sidedness seen in the development of these

animals has a common origin. Modification of the role of each gene might have occurred but the left-sidedness was not perturbed.

Functional modifications of *Ptx* genes

In addition to the expression in the preoral pit, the duct of the club-shaped gland and the mouth, *BbPtx* was expressed in some cells of the CNS and posteriorly in the newly formed somitocoelomic system on the left side (Fig. 4G,J, arrowheads). The overall expression domains in the lancelet apparently reflect the sum of those of three *Ptx* genes in vertebrates (Gage and Camper, 1997; Lanctôt et al., 1997; Smidt et al., 1997). Of these, only expression in cells of the CNS is shared by *D-Ptx1* in *Drosophila* (Vorbrüggen et al., 1997) and *unc-30* in *Caenorhabditis elegans* whose expression is restricted to some neurons (Jin et al., 1994). Therefore, the original function of *Ptx* may be control of neuronal differentiation. In the cephalochordate-vertebrate lineage or more likely in deuterostomes, *Ptx* gene(s) might have gained additional functions in the left-right asymmetric development of coelom-derived structures including the lancelet preoral pit, club-shaped gland and mouth, as well as the vertebrate heart and the wall of alimentary canal.

Ptx genes also function in differentiation of the preoral pit in cephalochordates and the pituitary in vertebrates. The preoral pit in adult lancelets (then called Hetschek's pit) secretes materials similar to pituitary hormones in vertebrates (Nozaki and Gorbman, 1992). Since the *Ptx1* protein in the mammalian hypophysis has been shown to activate transcription of proopiomelanocortin genes that cause further differentiation of secretory cells (Lamonerie et al., 1996), the continuous expression of *BbPtx* in the preoral pit may play a similar role. It is interesting to note, however, that the lancelet *BbPtx* covers all of the functions alone whereas, in vertebrates, three copies, *Ptx1*, *Ptx3* and *Ptx2*, cooperatively fill up the domains corresponding to those of *BbPtx*.

BbPtx expression and calcichordate hypothesis

Although the expression patterns of *BbPtx* and *Pitx2* suggest a common origin of developmental left-sidedness in cephalochordates and vertebrates, it is still unknown how they acquired it. In this regard, Jefferies (1986; Jefferies et al., 1996) suggested in his calcichordate hypothesis that animals having calcite exoskeleton usually called carpoids were the stem group that raised echinoderms, cephalochordates, urochordates and vertebrates, all of which show left-side dominances during development. The common ancestor of these four groups once adopted dexiothetism, which means lying down on the right side, causing the left-side dominance (Jefferies, 1986). From the study of calcichordate fossils, he found a countertorsion of the tail in the animals prior to the split of cephalochordate lineage (Jefferies et al., 1996). The countertorsion led to the pre-dexiothetic position of the tail region, so that the original dorsal (= right side) returned to dorsal (Fig. 6A).

BbPtx was expressed in both the anterior and posterior ends of the body (Fig. 4E,G-J, arrowheads). If Jefferies' countertorsion model is true, the *BbPtx* expression domains at the anterior and posterior ends would behave differently; however, contrary to this expectation, both domains are kept on the left side (Fig. 6B).

Recently, concerted movement of cilia in the mouse node has been suggested to play a role in establishing left-right asymmetric development (Nonaka et al., 1998). It is well known that lancelet embryos swim forward with spiraling movement in the counterclockwise direction, which means that cilia on the surface move in concert. van Wijhe (1919) thought that the counterclockwise spiral physiologically caused the asymmetric development in lancelets. Although the movement of external cilia in lancelets seems difficult to relate to asymmetric development, developmental study of lancelets may offer some insights into the relationship between the structure of cilium and left-right asymmetric development.

Left-right asymmetry and coelomic development in deuterostomes

The vertebrate *Pitx2* regulates heart and visceral asymmetric development, being expressed in the lateral mesoderm and its derivatives (Harvey, 1998). The lancelet *BbPtx* is also expressed in the structures that manifest marked left-right asymmetric development. The vertebrate lateral mesoderm constitutes the wall of body cavities (coelom), and thus all the asymmetric organs in the body part are related to the formation of the coelom. As regards lancelet asymmetric structures expressing *BbPtx*, on the contrary, it is not easy to find out how these structures are related to the coelomic formation. This is because lancelets do not develop lateral mesoderm in a similar

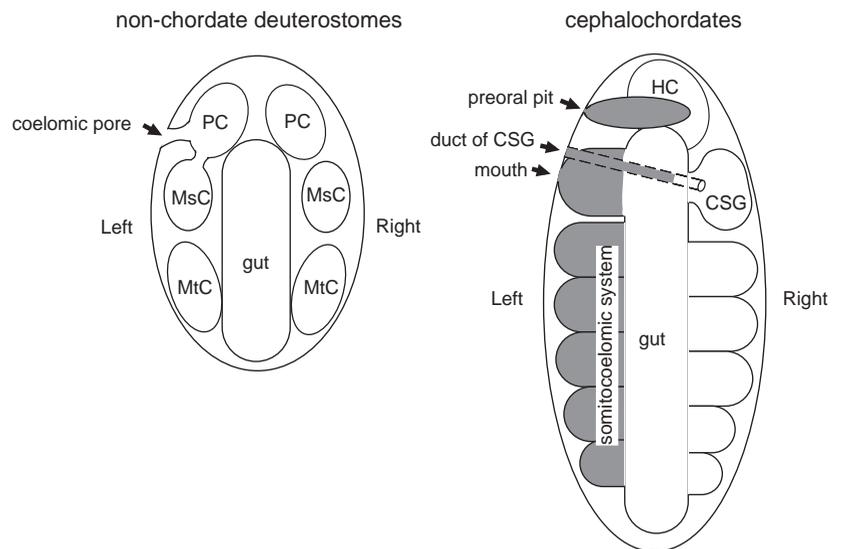


Fig. 7. A schematic illustration of coelomic systems compared between non-chordate deuterostomes and cephalochordates. In both animals, all the openings are located on the left side. In cephalochordates, the preoral pit, the duct of the club-shaped gland, and the mouth express *BbPtx* like the somitocoelomic system (shaded area), which suggests that all of these structures may be related to the coelomic formation. The preoral pit and head coelom in cephalochordates may homologize with the protocoel in non-chordate deuterostomes. Arrows denote openings to outside. CSG, club-shaped gland; HC, head coelom; PC, protocoel or axocoel; MsC, mesocoel or hydrocoel; MtC, metacoel or somatocoel.

manner to that of vertebrates. Instead, their so-called somites comprise not only somites but also the lateral plate mesoderm portions in which *BbPtx* is expressed. From studies of comparative embryology (Gislén, 1930) and recent analysis of the expression pattern of *Bbtwist* (Yasui et al., 1998), the lateral diverticulum has been regarded as an homologous structure with the protocoel (axocoels). Furthermore, Goodrich (1918) suggested that the proboscis pore in enteropneusts and the hydropore in echinoderms were comparable to the preoral pit in lancelets. These studies suggest that the asymmetric structures expressing *BbPtx* in lancelets are also related to the coelomic formation. Accordingly, the left-sided development mediated by *Pitx2*-related genes in lancelets and vertebrates is further support for their common origin.

Tripartite protocoel, mesocoel and metacoel have been regarded as the bauplan of coelomic system in deuterostomes (Masterman, 1897; Gee, 1996) and are found in extant non-chordate deuterostomes. In this coelomic system, intriguingly, the anterior left coeloms tend to open externally via coelomic pores, showing left-right asymmetry (Gislén, 1930; Gee, 1996). The expression pattern of *BbPtx* and the deuterostome bauplan of the coelomic system shed light on a possible origin of the lancelet mouth. Several attempts have been made to homologize the lancelet mouth with various structures in other animals from an anus (Gislén, 1930) to a gill slit (van Wijhe, 1919). At first glance, the expression pattern of *BbPtx* seems to support the affinity of the mouth to the vertebrate mouth and the preoral pit to the Rathke's pouch, because *Ptx1* gene is also expressed in the stomodeum and Rathke's pouch in the mouse (Lancôt et al., 1997). However, this suggested homology does not explain why they open on the left side in lancelets. The expression pattern of *BbPtx* indicates another possibility. It is expressed in the left side structures developed enterocoelically from the primitive gut. The mouth and the duct of the club-shaped gland are formed in the same fashion and open externally to the left side, as seen with coelomic pores in non-chordate deuterostomes. The common expression of *BbPtx* and the developmental similarity suggest that the lancelet mouth may have affinity to the coelomic pore (Fig. 7). The expression of *BbPtx* in the structures derived from the coelom including the mouth and the duct of the club-shaped gland in lancelets could bridge the left-right asymmetric development between non-chordate deuterostomes and vertebrates.

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