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).

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-3β, Deuterostome, Calcichordate, Coelom, BbPtx
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; Lantôt et al., 1997; Ptx2 (Ptx2), Gage and Camper, 1997; Ptx3, Smidt et al., 1997), Drosophila (D-Ptx1, Vorbriegen 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; Pedra 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 coelomonic 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 Hrsgc, gooseoid-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).

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**Fig. 1.** Nucleotide sequence of a cDNA clone for BbPtx and a deduced amino acid sequence of a putative BbPtx protein. The putative BbPtx protein is encoded by the nucleotide sequence from the nucleotide 205 through 1,200. Asterisk denotes termination codon. A putative polyadenylation signal is double-underlined. Boxed amino acid sequences are the homeodomain and a region conserved among mammalian Ptx proteins. A 1.6 kb intron is present in the homeobox. Arrowheads indicate EcoRI sites.
Asymmetric expression of \textit{BbPtx} in lancelets

**Genomic Southern analysis**

Pooled genomic DNA was prepared from 10 adult animals according to the method of Blin and Stafford (1976). The genomic DNA was digested with restriction enzymes, electrophoresed on a 0.8% agarose gel and transferred onto a Hybond N+ nylon filter (Amersham Pharmacia Biotech, Tokyo). For Southern analysis, PCR fragments were used as probes that were prepared using several primers flanking or inside of the homeobox region and the genomic DNA as a template. From this analysis and together with nucleotide sequence determination of the PCR fragments, it was found that the homeobox has an intron of about 1.6 kb between nucleotides 655 and 656 in Fig. 1. The filter was hybridized with a 32 P-labeled DNA fragment obtained by PCR, which corresponds to nucleotides 517-654 in Fig. 1, in 50% formamide, 5 \( \cdot \) SSPE, 5 \( \cdot \) Denhardt’s solution, 0.5% SDS and 20 \( \mu \)g/ml denatured salmon sperm DNA at 43°C for 20 hours. After hybridization, the filter was washed in 2 \( \cdot \) SSC and 0.2% SDS at 43°C for 20 minutes. Stringent washing was also performed under the conditions of 0.1 \( \cdot \) SSC and 0.2% SDS at 60°C for 30 minutes. The filter was exposed to X-ray film or an imaging plate, which was then subjected to the analysis using Bas2000 bioimaging analyzer (Fuji Film, Tokyo).

**RESULTS**

**Isolation and molecular characterization of \textit{BbPtx}**

Screening of the hatching neurula cDNA library using a PCR fragment of the ascidian \textit{Hrgsc} (Y. Ueno, Y. Shimojima and H. S., unpublished) homeobox as a probe yielded 10 candidates for a \textit{goosecoid}-related gene, out of which a sequence similar to vertebrate \textit{Ptx} genes was obtained. The cDNA was 1997 bp long (accession number: AF195616), encoding a protein of 331

\[ \begin{array}{cccc}
\text{BbPtx} & \text{RRQRTHFTSQQLQELEASFARNRPDMATREEIAAWTNLTEARVRVWFKNRRAKWRKRE} \\
\text{DmPtx1} & \text{----------------HTSS-------S------M-------------K----------} \\
\text{MmPtx1} & \text{-----------------T-Q-------SM-----V------P------K----------} \\
\text{MmPitx2} & \text{-----------------T-Q-------S------V-------------K----------} \\
\text{RnPtx3} & \text{---A-------------T-Q-------S------V----------G--K----------} \\
\text{Unc-30} & \text{---------H--T---NW-S--------C-----V-IS---P------K----------} \\
\text{Bicoid} & \text{--T--T---S-IA---QH-LQG--LTAPRLADLS-KLA-GT-Q-KI--K---RRHKIQS} \\
\end{array} \]

\[ \begin{array}{cccc}
\text{BbPtx} & \text{PY=AYREQCNSSIAALRLKAKQHSTSVASSFSY} \\
\text{MmPtx1} & \text{PYSVYRFQCNSSLAARLRLAAVTSVASSFSY} \\
\text{MmPitx2} & \text{PY=VYRFQCNSSLAARLRLAAVTSVASSFSY} \\
\end{array} \]

Fig. 3. Southern blot analysis of pooled 10 adult lancelet genomic DNA. Each lane contains 5 \( \mu \)g DNA digested with a restriction enzyme indicated at the top. Hybridization was carried out under reduced stringency conditions using a part of the \textit{BbPtx} homebox (corresponding to nucleotides 517-654 in Fig. 1) as a probe. λDNA digested with \textit{EcoRI} and \textit{HindIII} was used as a size marker.
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 Ptx2 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., AmphiDll: 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 μm, also applies to A-H; I, 100 μm, also applies to J; K, 50 μm applies to N; M, 200 μm applies to P and Q; R, 25 μm applies to S-U.
**Asymmetric expression of BbPx in lancelets**

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 BbPx 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). BbPx 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, BbPx 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. BbPx 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 only expresses BbPx transcripts (Fig. 4G,H). Accordingly, the expression domain was hook-shaped in the left lateral view (Fig. 4F). BbPx 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. 4J), in which the number of cells expressing BbPx 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 BbPx 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 newly forming somitocoelomic system. This expression pattern continued to the early larval stage (Fig. 4L), in which the number of cells expressing BbPx 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 BbPx 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 BbPx 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).

Asymmetric expression of BbPx in lancelets
primitives 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 lancelet, 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 this 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 somitocoelemic 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; Yoshio 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-3β 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-3β 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-3β 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
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. 4GJ, arrowheads). The overall expression domains in the lancelet apparently reflect the sum of those of three Ptx genes in vertebrates (Gage and Camper, 1997; Lancot 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 (Liu 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, Jefferyies (1986; Jefferyies 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 dextrothetism, which means lying down on the right side, causing the left-side dominance (Jefferyies, 1986). From the study of calcichordate fossils, he found a countertorsion of the tail in the animals prior to the split of cephalochordate lineage (Jefferyies et al., 1996). The countertorsion led to the pre-dextrothetist 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 Jefferyies’ 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 cilia 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

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**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 protocol (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 protocol, 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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