Hemichordate neurulation and the origin of the neural tube

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Abstract

The origin of the body plan of our own phylum, Chordata, is one of the most fascinating

questions in evolutionary biology. Yet, after more than a century of debate, the

evolutionary origins of the neural tube and notochord remain unclear. Here, we examine

the development of the collar nerve cord in the hemichordate *Balanoglossus simodensis*

and find shared gene expression patterns between hemichordate and chordate

neurulation. Moreover, we show that the dorsal endoderm of the buccal tube and the

stomochord expresses Hedgehog RNA, and it seems likely that collar cord cells can

receive the signal. Our data suggest that the endoderm functions as an organizer to

pattern the overlying collar cord, similar to the relationship between the notochord and

neural tube in chordates. We propose that the origin of the core genetic mechanisms for

the development of the notochord and the neural tube date back to the last common

deuterostome ancestor.

Introduction

The evolution of chordates has been investigated for more than a century; however, the origin of the chordate body plan remains controversial[1-8]. Establishment of the chordate body plan is achieved via several dramatic events, probably including dorsoventral inversion. The dorsoventral inversion was originally proposed as an inversion between protostomes and chordates by Geoffroy-St. Hilaire (reviewed by Gee[2]), and recent molecular studies have offered strong support that the inversion indeed occurred between non-chordates and chordates[9]. Establishment of the chordate body plan is also accompanied by the acquisition of novel organs, such as the neural tube and notochord. Hemichordates, together with echinoderms, and possibly Xenacoelomorpha, are the sister group of chordates[10]. Compared with the highly specialized radial form of the body plan of extant echinoderms, hemichordates may retain more of the ancestral form of deuterostomes and are consequently more suitable for comparison as an outgroup taxon[4]. Recent molecular developmental biology studies have shown startling similarities between the molecular architectures of the nervous systems between chordates and non-chordate invertebrates[11-13]. Hemichordates and

vertebrates share the genetic mechanisms for anteroposterior patterning of the neuroectoderm[11,13]. In addition, dorsoventral patterning within the central nervous system via BMP signaling is highly conserved between the annelid *Platynereis* and vertebrates, and thus the origin of the patterning mechanism predates the last common bilaterian ancestor[12]. In hemichordates, BMP signaling is also involved in the patterning of the dorsoventral axis of body regions[9], but there is no evidence that the nervous system of hemichordates has distinct dorsoventral patterning similar to protosotmes and chordates. Rather it is only recently that the hemichordates were revealed to possess a centralized nervous system[14]. The presence of a central nervous system in protostomes and hemichordates indicates that the centralization of the nervous system occurred before the divergence of protostomes and deuterostomes[14]. However, there is a critical difference in the nervous system of bilaterians. Only chordates have a tubular central nervous system along their anteriopposterior body axis. That is, the neural tube and its patterning are chordate novelties. Thus, the origin of the tubular central nervous system is a critical issue for elucidating the evolution of the chordate body plan.

Hemichordates have nerve cords in the dorsal midline and ventral midline. The

dorsal nerve cord is divided into the proboscis stalk region, the collar cord, and the

dorsal nerve cord in the trunk region[14]. The ventral nerve cord exists only in the trunk

region and is connected to the dorsal nerve cord via the prebranchial nerve ring. Among

them, only the collar cord has tubular organization and thus proposed to be a

homologous organ to the neural tube[4,8,15-17]. However, this homology is still

controversial[14,16], because the enteropneust collar cord, unlike the much more extensive

neural tubes of chordates, runs along only a relatively short stretch of the

anteroposterior axis[18]. In addition, the dorsal position of the collar cord is not consistent

with the dorsoventral axis inversion in the chordate lineage[9,18]. To investigate the

potential homology between the collar cord and neural tube, we here examine the

development of the collar cord in the enteropneust *Balanoglossus simodensis*, focusing

on the expression patterns of genes known to be critical for the early patterning and

formation of the chordate neural tube. We find conserved gene expression patterns

between neurulations of hemichordates and chordates. The present results suggest that

the origin of genetic mechanisms to form and pattern a tubular nervous system predates

the diversification of hemichordates and chordates.

Results

Hemichordate neurulation. In *B. simodensis* development, the neural plate is

visualized by the expression of the neural marker gene *BsimElav* at the dorsal midline

of the prospective collar region at the pre-metamorphic stage (~6 weeks

postfertilization) (Fig. 1a–e)[14]. The neural plate consisted of several layers of

elav-positive cells. Two days after settlement, the neural plate invaginated and the collar

cord formed in a similar manner as observed during chordate neurulation (Fig. 1f–o).

One week after settlement, the collar cord was separated from the epidermis and

neurulation was completed (Fig. 1p–t). Note that although some enteropneust species

lack an obvious tubular collar cord because the central lumen of the cord is filled with

cells, their collar cord still forms via invagination of the neural plate[16].

Since nothing is known about the molecular mechanism of hemichordate

neurulation or dorsoventral patterning of the nerve cord, we examined the expression of

genes involved in the formation and patterning of the chordate neural tube. In chordates,

BMP signaling performs essential roles in morphogenesis during neurulation and

dorsoventral patterning of the neural tube[19,20]. In the hemichordate *Saccoglossus*

kowalevskii, *bmp2/4* is involved in dorsoventral patterning of the body axis[9], but the

function of BMP signaling in the collar cord remains unclear. In *B. simodensis*,

BsimBmp2/4 was expressed in the dorsal surface of the neural plate during neurulation

and then was expressed in the dorsal part of the collar cord (Fig. 2a,b). We examined the

expression of several genes whose vertebrate homologs mark the neural plate border

and pattern the dorsal neural tube, including *dlx*, *pax3/7*, and *soxE*[21]. *BsimDlx* was also

expressed in the dorsal domain of the neural plate as *BsimBmp2/4* (Fig. 2c,e). After

neurulation, *BsimDlx* was expressed in the dorsal domain of the collar cord (Fig. 2d,f).

Compared with *BsimBmp2/4* and *BsimDlx*, *BsimPax3/7* and *BsimSoxE* had a different

expression pattern at the pre-metamorphic stage. The two genes were expressed at the

boundary between the neural plate and epidermis during neurulation (Fig. 2g,i). In the

later stage, *BsimPax3/7* and *BsimSoxE* were expressed in the dorsal domain of the collar

cord (Fig. 2h,j). The later expression of *BsimSoxE* was expanded more laterally in the

collar cord (Fig. 2j,k,l). We also found that a type A fibrillar collagen gene *BsimColA*

was also expressed in the dorsal part of the collar cord (Fig. 2m,n). In chordates,

pax2/5/8 is expressed in differentiating interneurons located laterally in vertebrates and

dorsolaterally in amphioxus[22,23]. In *B. simodensis*, the *pax2/5/8* ortholog *BsimPax2/5/8*

is expressed in the whole collar cord, except for the dorsal region of the neural canal

(Fig. 2o,p). In contrast to the conserved expression of these dorsal patterning genes, we

were unable to detect the expression of ventral marker genes, such as *pax6*, in the collar

cord (Supplementary Fig. S1). These results suggest that the molecular mechanisms of

the hemichordate collar cord and chordate neural tube patterning are only partly

conserved. In addition, expression of these genes at the neural plate border indicates that

hemichordates possess part of the gene regulatory network necessary for neural crest

induction[24]. We also examined the expression of these collar cord patterning genes at

the level of the trunk region, but we did not detect expression in either the dorsal nerve

cord or ventral nerve cord.

Dorsal endoderm of the buccal tube and the stomochord. Since Hedgehog signaling

from the notochord to the neural plate is essential for floor plate induction and for

patterning of the neural tube along the dorsoventral axis[25], we next examined whether a

similar type of cell exists in hemichordates. We found that *BsimHh* was expressed in the

stomochord progenitor and the dorsal endoderm of the buccal tube before settlement

(Fig. 3a). Expression was maintained in the stomochord and dorsal endoderm of the

buccal tube after settlement (Fig. 3b–d; Supplementary Fig. S2a). Notably, these

Hedgehog positive cells lay beneath the collar cord during metamorphosis. Thus, we

asked whether the collar cord has the potential to respond to Hedgehog signaling by

examining the expression of *patched* encoding the receptor for hedgehog. Before

settlement, *BsimPtc* was expressed in the mesoderm surrounding the

BsimHh-expressing endoderm and in the midline of the neural plate (Fig. 3e). *BsimPtc*

expression in the collar cord and mesodermal cells was maintained after collar cord

formation (Fig. 3f–h; Supplementary Fig. S2b). These results indicate that the anterior

endoderm (stomochord and dorsal endoderm of the buccal tube) functions as the source

of Hedgehog molecules, while collar cord and mesoderm cells have the potential to

respond to the signal (Fig. 3i–l).

To further test cell type similarity between the notochord of chordates and the

anterior endoderm of hemichordates, we examined another aspect of notochord function,

which is as a hydrostatic axial skeleton. For the notochord to function as a hydrostatic

axial skeleton, prominent vacuoles and a collagenous sheath are critical (Fig. 4a,b [26].

We examined the expression of *BsimColA*, the hemichordate homolog of the chordate

fibrillar collagen genes (clade A)[26]. *BsimColA* was expressed in the stomochord

progenitor of pre-metamorphic larvae and in the stomochord of juvenile worms (Fig.

4c−f). Expression was also detected in the dorsal endoderm of the buccal tube

(Supplementary Fig. S3). These expression patterns provide additional information for

cell type similarity between the anterior endoderm of hemichordates and the notochord.

Hemichordates have a skeletal element called the proboscis skeleton around the

stomochord. The proboscis skeleton forms during metamorphosis at the same time as

the stomochord (Fig. 4g−m). Thus, the proboscis skeleton is comparable with that of the

notochordal sheath. The dorsal endoderm of the buccal tube may also perform a similar

role in providing physical strength in the hemichordate collar region.

Discussion

We present the first evidence that the hemichordate collar cord is subdivided into

dorsoventral domains. Furthermore, we suggest that this patterning may be regulated by

Hedgehog signaling from the dorsal endoderm of the buccal tube (Figure 5). Complex

dorsoventral differentiation within the nervous system has been reported in protostomes,

such as the polychaete *Platynereis dumerilii*[12]. In *Platynereis*, the neuroectoderm is

subdivided into dorsoventral domains that match corresponding domains in the

vertebrate neural tube[12]. Moreover pattering genes of the neuroectoderm are sensitive to

BMP signaling[12]. The report indicates that the molecular mechanism responsible for

dorsoventral pattering of the neuroectoderm predates the divergence of protostomes and

deuterostomes. However, the mechanism patterns the neuroectoderm along the whole

body axis in *Platynereis* (Figure 5, protostome). In contrast, the mechanism is activated

within the neural tube to pattern it dorsoventrally in chordates (Figure 5, chordate). We

show here that the collar cord, the tubular nervous system in hemichordates, has

dorsoventral differentiation (Figure 5, hemichordates). Our present data suggest that the

patterning mechanism was already recruited to the tubular nervous system in the last

common deuterostome ancestor. Moreover, no evidence has so far been reported on the involvement of hedgehog signaling in the dorsoventral regulation of the protostome CNS. Our results suggest that recruitment of hedgehog signaling predates chordate ancestors and that the recruitment possibly contributed to the acquisition of a "tubular" nervous system. However, compared with the highly organized annelid and chordate CNS, differentiation along the dorsoventral axis within the collar cord is limited in hemichordates. The distributions of neurotransmitters and cell types of neurons are not regionalized along the dorsoventral axis in the collar cord[4,14]. We found that only some dorsal markers show restricted expression in the collar cord. This may be due to the secondary modification in the hemichordate lineage. Because enteropneusts burrow in mud, they have probably reduced a part of their locomotion system. Further analyses about the neuromuscular system of enteropneusts should help our understanding on this issue. Some ventral marker genes (*sim* and *chordin*) show expression in the ventral midline of the body of *S. kowalevskii*[9]. These observations suggest the possibility that the hemichordate nervous system extends dorsoventrally along whole body and the collar cord would be comparable only with the dorsal-most part of the neural tube.

Our results lead to the discussion on the homology between the chordate notochord

and the hemichordate anterior endoderm, the stomochord plus dorsal endoderm of the

buccal tube. The notochord has two functions: as a hydrostatic skeleton and a source of

neural tube patterning. The former was supported by the expression of fibrillar collagen,

and as noted by Bateson[27], the stomochord is rich in vacuoles. In addition, we show that

the dorsal endoderm of the buccal tube is also rich in vacuoles and expresses the

fibrillar collagen gene. Thus, the stomochord is quite able to function as a hydrostatic

skeleton and indeed functions as a mechanical support to resist pumping of the

pericardium[18]. The latter was supported from the *hedgehog* expression. In this sense, *ptc*

expression in the mesodermal cells is noteworthy. This suggests that the relationship

between the anterior endoderm and collar mesoderm is comparable with that between

the notochord–paraxial mesoderm in vertebrates.

In contrast, homology between the stomochord and notochord has been challenged

mainly from two aspects. The first comes from the differences in gene expressions

between the stomochord and notochord; genes that are expressed in the notochord and

its precursor. For example, the T-box gene *brachyury* plays an essential role in

notochord differentiation[28,29]. A previous report has shown that there is no *brachyury*

expression in the stomochord of hemichordates[30]. Our reexamination of *brachyury*

expression in *B. simodensis* did not identify expression in either the stomochord or the

dorsal endoderm of the buccal tube, while expression was detected in the posterior end

of the trunk (Supplementary Fig. S4).

The second challenge is from the dorsoventral inversion hypothesis. Because the

notochord develops from the dorsal part of the archenteron, according to the

dorsoventral axis inversion hypothesis, the homologous structure should occupy the

ventral side of the hemichordate endomesoderm. However, the stomochord develops as

an anterior protrusion from the dorsal wall of the buccal tube. Similarly, homology

between the hemichordate dorsal collar cord and chordate CNS is not consistent with

the dorsoventral inversion hypothesis. Rather, the ventral trunk nerve cord and the

pygochord, which are vacuolated cells in the posteroventral mesentery, were proposed

as homologous structures to the chordate notochord and CNS, respectively[31,32].

Based on these viewpoints, we consider two possible evolutionary scenarios. First,

in the deuterostome ancestors, hedgehog positive notochord/stomochord-like organs

occupy the endoderm midline both dorsally and ventrally. In hemichordates, hedgehog

positive cells are retained only in the anterodorsal part of the endoderm where it

patterns the dorsally located nerve cord. In contrast, in the chordate lineage, the

hedgehog positive cells are only retained ventrally. The ventral PNS of amphioxus[33] and

ascidians[34] may be evolutionary remnants, homologous to the dorsal nerve cord of

hemichordates.

The second possible scenario is co-option of hedgehog-dependent nerve patterning

originally establishing the anterodorsal part of the endoderm in the last common

deuterostome ancestor. This hedgehog-dependent nerve patterning may be activated on

the other side: the dorsal side of chordates. In this case, the stomochord and notochord

share only the genetic regulatory machinery for the hydrostatic skeleton and patterning

of the nervous system. It is notable that the *hedgehog* and *ColA* positive cells are also

found in the anterior endoderm in chordates[35,36], which may represent the homolog of

the hedgehog positive endoderm cells of hemichordates. In either scenario, the genetic

linkage between the hedgehog secreting cells and *brachyury* expression was established

in the chordate ancestors.

Several issues remain to be resolved to bridge the deep gap between the body plan of non-chordates and that of chordates. For example, we do not understand how metameric musculature was acquired or how coordinated development was established between metameric muscle blocks and the CNS in chordate ancestors. Further examination of hemichordate developmental biology will provide greater insight into the dramatic events in the evolution of the chordate body plan.

Methods

Animal collection. Adult acorn worms (*B. simodensis*) were collected along the rocky

seashore of the city of Shimoda. Induction of spawning, culture of larvae and induction

of metamorphosis were achieved in the laboratory[37].

Molecular cloning and phylogenetic analyses. Fragments of *bmp2/4*, *colA*, *elav*, *soxE*,

pax2/5/8, *pax3/7*, *pax6*, *dlx*, *hedgehog*, *patched* and *brachyury* were amplified using

degenerate primers (primer sequences and accession numbers are in Supplementary

Table S1). Gene orthologies were inferred using ML analyses (Supplementary Fig. S5)

and multiple alignments (Supplementary Fig. S6). Amino acid alignments were made

with ClustalX ver. 2.0[38]. Amino acid evolutionary models were selected using

Modelgenerator[39]. Maximum likelihood analyses were performed with PhyML ver.

3.0[40].

in situ **hybridization.** For section *in situ* hybridization, juvenile worms were treated

with 2% HCl in filtered sea water (FSW) to remove mucus at 4ºC, 5 min. Then,

specimens were fixed with 4% paraformaldehyde (PFA) in Mops buffer (0.1M Mops,

17

0.05M NaCl,) at 4ºC, over night. Frozen sections were air dried and washed with PBST

and fixed with 4% PFA/PBS for 10 min at room temperature (RT). Then, the slides

were washed with PBS and digested with 1 µg/ml proteinase K/PBS for 10 min at RT.

After a brief wash with PBS, the samples were post fixed in 4% PFA/PBS for 10 min at

RT. The slides were washed with PBS three times and acetylated in 0.1M

triethanolamine with 0.25% acetic anhydride for 15 min at RT and washed with PBS

three times. The slides were prehybridized at least 1 hr in hybridization solution (50%

formamide, 5× SSC, 5× Denhardt's, 200 µg/ml yeast RNA) at 60ºC and hybridized with

a DIG-labeled RNA probe at 60ºC at least 16 hr. The slides were washed in 50%

formamide/2× SSC for 60 min, 2× SSC for 30 min twice, 0.2× SSC for 30 min twice at

60ºC. Then, they were rinsed twice with maleic acid buffer (MAB), blocked 2%

blocking reagent (Roche, Indianapolis, IN, USA) in MAB for 60 min at RT and

incubated overnight at 4ºC with a 1:1,500 dilution of anti−DIG−AP antibody (Roche) in

blocking buffer. They were washed with MAB for 30 min four times and transferred

into AP buffer (100 mM Tris pH 9.5, 100 mM NaCl, 50 mM MgCl2, 2%

polyvinylalchol) and were developed by incubating the slides in NBT/BCIP (Roche) in

AP buffer until a signal was visible. The reaction was stopped in PBS, postfixed in 4%

PFA/PBS overnight, washed with PBS, and mounted with 80% glycerol. Then, they

were observed under a light microscope.

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End Notes

Author Contribution

N.M. and H.W. contributed to the design of experiments. N.M. performed sample collections and experiments. N.M. and H.W. discussed the results and wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Accession Codes: The sequence data have been deposited in GenBank/EMBL/DDBJ under accession numbers AB642245, AB642246, AB642247, AB642248, AB642249, AB642250, AB642251, AB642252.

Figure Legends

Fig. 1. Neurulation of *B. simodensis*. External morphology, cross sections, *BsimElav* expression and schema of pre-metamorphic larvae (a–e), 2-day-old juveniles (f–o) and 1-week-old juveniles (p–t). (a) Dorsal view of a pre-metamorphic larva. Anterior is to the left. (b) A cross section of the collar primordium. (c) A high-magnification view of the boxed area in b showing the neural plate. (d) *BsimElav* expression in the neural plate. (e) Schematic illustration of the pre-metamorphic stage. (f) Dorsal view of a juvenile two days after settlement. (g) A cross section of the posterior part o the collar where the neural plate was invaginating. (h) A high-magnification view of the boxed area in g. (i) *BsimElav* expression in the invaginating neural plate. (j) Schematic illustration of the invaginating neural plate. (k) Dorsal view of a juvenile two days after settlemet. (l) A cross section of the middle part of the collar where tubular collar cord formed. (m) A high-magnification view of the boxed area in l. (n) *BsimElav* expression in the collar cord. (o) Schematic illustration of the newly formed collar cord. (p) Dorsal view of a juvenile one week after settlement. (q) A cross section of the collar. (r) A high-magnification view of boxed area in q showing the collar cord and the stomochord.

(s) *BsimElav* expression in the collar cord. (t) Schematic illustration of the dorsal part of

the collar. cc, collar cord; deb, dorsal endoderm of the buccal tube; np, neural plate; st

stomochord. Scale bars: 100 μm.

Fig. 2. Gene expression during neurulation in *B. simodensis*. The collar cord is indicated

by a dotted line. Asterisks indicate the position of the neural canal. (a) *BsimBmp2/4* was

expressed on the dorsal surface of the neural plate, mesodermal cells, which gave rise to

the blood vascular system (arrow) and developing gill slits (arrowheads) before

neurulation. (b) *BsimBmp2/4* was expressed in the dorsal part of the collar cord and

mesodermal cells (arrow). (c) *BsimDlx* was expressed in the dorsal surface of the neural

plate before neurulation. (d) *BsimDlx* was expressed in the dorsal part of the collar cord.

(e) Schematic illustration of *BsimBmp2/4* and *BsimDlx* expressions before neurulation.

(f) Schematic illustration of *BsimBmp2/4* and *BsimDlx* expressions after neurulation. (g)

BsimPax3/7 expression was detected in the border between the neural plate and

epidermis before neurulation. (h) *BsimPax3/7* was expressed in the dorsal par fo the

collar cord. (i) *BsimSoxE* expression was detected in the border between the neural plate

and epidermis before neurulation and the dorsal endoderm of the buccal tube. (j)

BsimSoxE was expressed in the dorsal and lateral parts of the collar cord. (k) Schematic

illustration of *BsimPax3/7* and *BsimSoxE* expressions before neurulation. (l) Schematic

illustration of *BsimPax3/7* and *BsimSoxE* expressions after neurulation. (m) *BsimColA*

was expressed in the dorsal part of the collar cord. (n) Schematic illustration of

BsimColA expression in the collar cord. (o) Expression of *BsimPax2/5/8* was detected

broadly in the collar cord, except for the dorsal region of the neural canal. (p) Schematic

illustration of *BsimPax2/5/8* in the collar cord. cc, collar cord; ep, epidermis; np, neural

plate. Scale bars: 50 µm.

Fig. 3. *Hh* and *Ptc* expression during *B. simodensis* neurulation. (a) *BsimHh* was

expressed in the anterior endoderm in pre-metamorphic larvae. (b) A longitudinal

section of a juvenile two days after settlement shows *BsimHh* expression in the

stomochord and the dorsal endoderm of the buccal tube. (c) A cross section of the collar

shows *BsimHh* expression in the dorsal endoderm of the buccal tube. (d) A cross section

of the collar shows *BsimHh* expression in the dorsal endoderm of the buccal tube. (e)

BsimPtc was expressed in the mesodermal cells surrounding the endoderm and the

midline cells in the neural plate. (f) A longitudinal section of two days old juvenile

shows *BsimPtc* expression in the mesodermal cells and collar cord cells. (g) A cross

section of the collar shows *BsimPtc* expression in the collar cord and mesodermal cells.

(h) A cross section of the collar shows *BsimPtc* expression in the collar cord and

mesodermal cells. Schematic illustrations of expression patterns of *BsimHh* and

BsimPtc in pre-metamorphic larva (i), two days old juveniles (j,k) and one week old

juvenile (l). cc, collar cord; deb, dorsal endoderm of the buccal tube; np, neural plate; st,

stomochord. Scale bars: 50 μm.

Figure 4. Morphogenesis and gene expression of the anterior endoderm during

metamorphosis of *B. simodensis*. Expression of the amphioxus fibrillar collagen gene

BbFCol1 at 14 h (a) and 22 h (b) postfertilization. (c) *BsimColA* expression was

detected in the anterior endoderm in pre-metamorphic larva. (d) A longitudinal section

of two days juvenile shows *BsimColA* expression in the stomochord (arrow) and the

dorsal endoderm of the buccal tube (arrowhead). (e) A cross section shows *BsimColA*

expression in the stomochord. (f) A longitudinal section shows *BsimColA* expression in

the stomochord (arrow) and the dorsal endoderm of the buccal tube (arrowhead). (g) A

longitudinal section of the anterior endoderm in a pre-metamorphic larva. (h) A

longitudinal section of the anterior endodermal region shows invagination of the

stomochord (arrow). (i) A cross section of the developing stomohocrd. (j) A longitudinal

section of the anterior endodermal region showing the stomochord (arrow) and the

proboscis skeleton (arrowhead). Schematic illustrations of longitudinal sections of

pre-metamorphic larva (k) and 2-day- (l) and 1-week-old (m) juveniles. Boxed areas in

k, l and m indicate positions of c and g, d and h, and f and j, respectively. cc, collar

cord; deb, dorsal endoderm of the buccal tube; nt, notochord; p, proboscis; ps, proboscis

skeleton; st, stomochord. Scale bars: 50 µm.

Figure 5. Schematic illustration of bilaterian nervous systems. A complex dorsoventral

patterning in the neuroectoderm was present in the common ancestor of protostomes

and deuterostomes (a). In the deuterostome lineage, a tubular neural structure was

acquired and the dorsoventral patterning was recruited to the tube. The tube was

induced and patterned by the endodermal signals in the ancestor (b). The endodermal

signal was activated (co-opted) to the newly evolved dorsal midline mesoderm, the

notochord, and the signals induced the neural tube (c). Drawing of a protostome

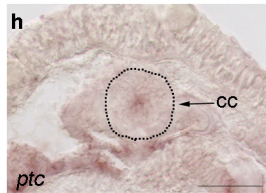modified from ref. 12.

pre-metamorphic	2 days		1 week

a b

f l g

k l g

p q

b c

g h

l m

q r

c np / deb

h deb / np

m cc / deb

r cc / st

d *elav* / np

i *elav* / np

n *elav* / cc

s *elav* / cc

e

j

o

t

epidermis | cell bodies (*elav*+) | neuropil | mesoderm | endoderm | stomochord

a *bmp2/4* — np

b *bmp2/4* — cc

c *dlx* — np

d *dlx* — cc

e ■ *bmp2/4 + dlx*   ■ *bmp2/4*

f

g *pax3/7* — ep, np

h *pax3/7* — cc

i *soxE* — ep, np

j *soxE* — cc

k ■ *pax3/7 + soxE*   ■ *soxE*

l

m *colA* — cc

n

o *pax2/5/8* — cc

p

pre-metamorphic	2 days		1 week

a *hh* — np

b *hh* — cc, st

c *hh* — cc, deb

d *hh* — cc

e *ptc* — np

f *ptc* — cc

g *ptc* — cc, deb

h *ptc* — cc

i

j

k

l

■ *hh*   ■ *ptc*

a BbFCol1 — nt

b BbFCol1 — nt
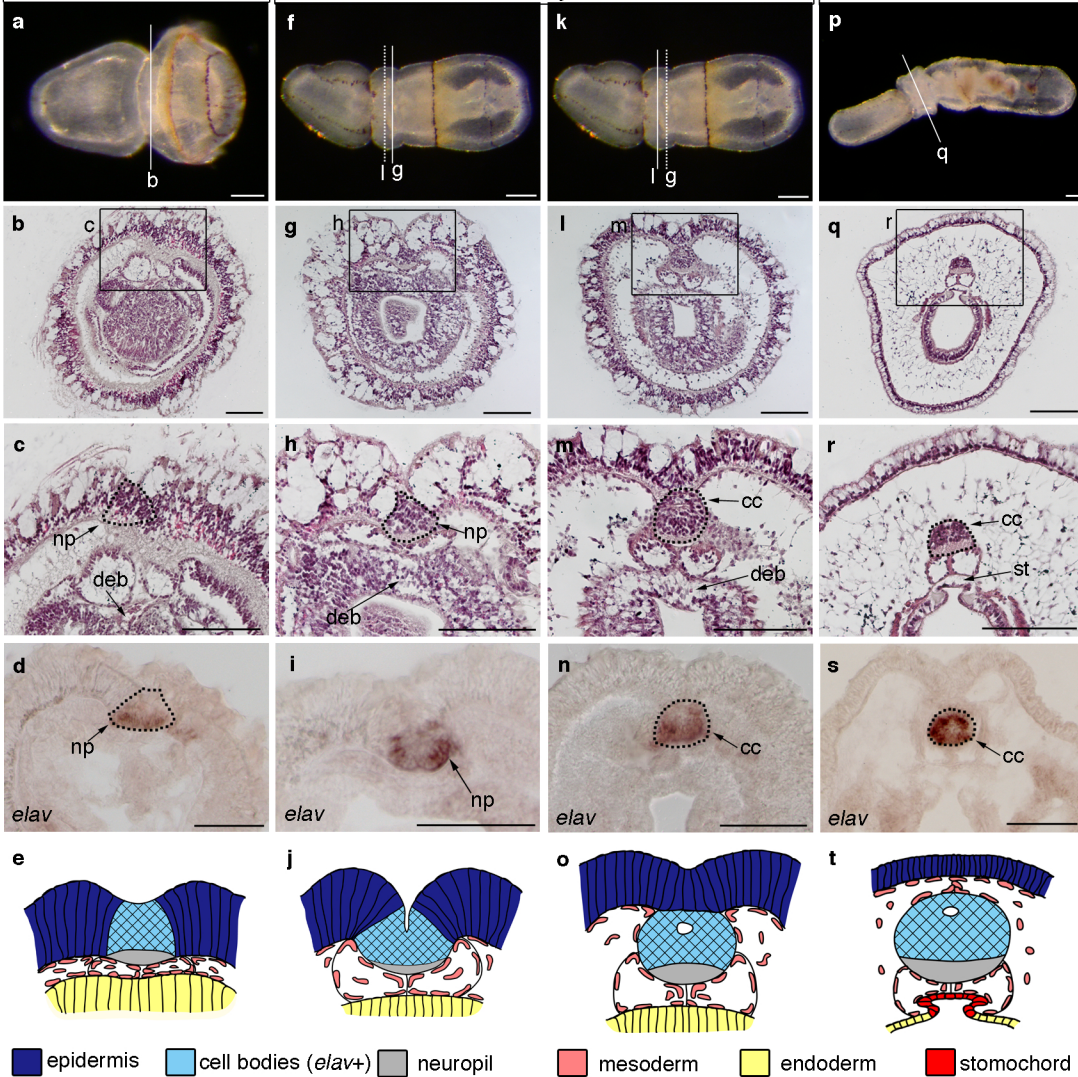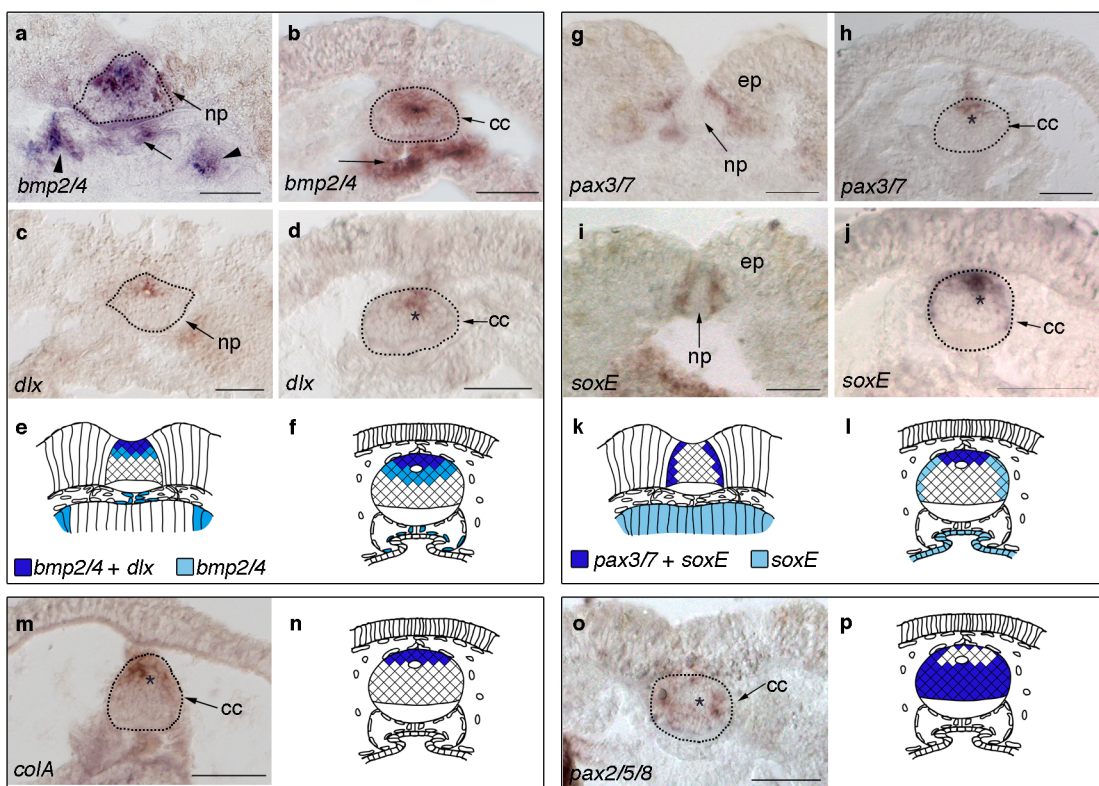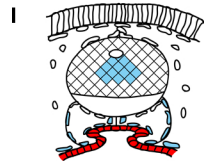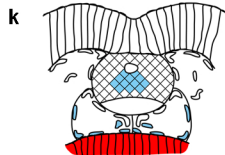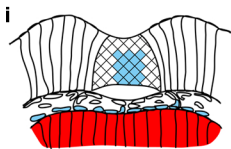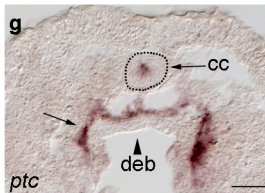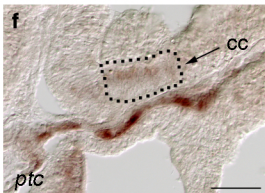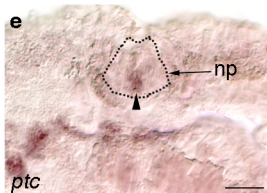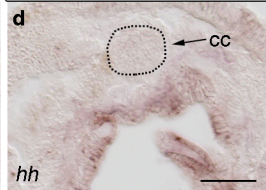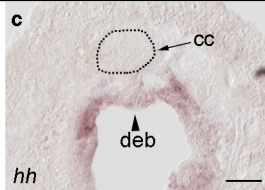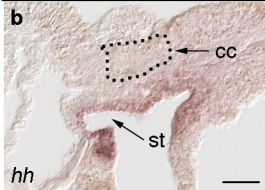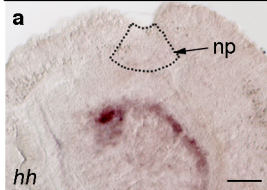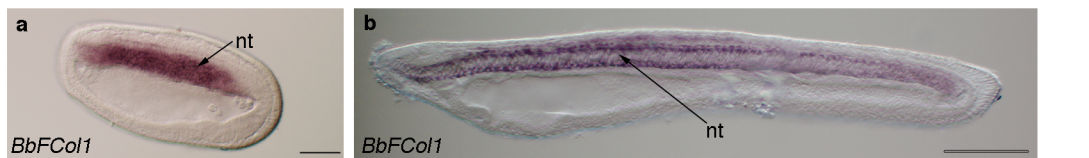
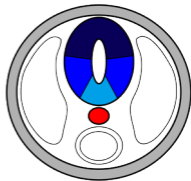| pre-metamorphic larva | 2 days juvenile | 1 week juvenile |

c colA

d colA — g

e colA — st

f colA

g HE

h HE — c

i HE — st

j HE

k c,g

l cc, st, dce, d,h

m cc, st, ps, dce, f,j
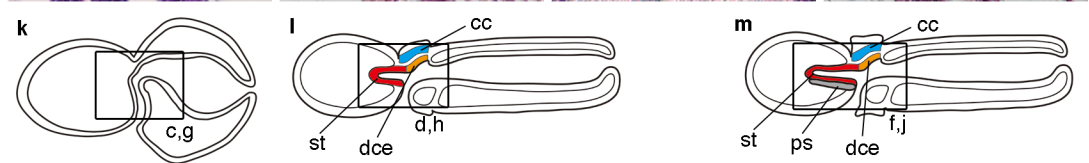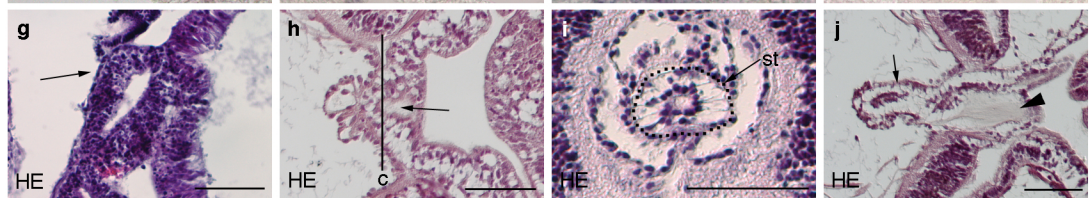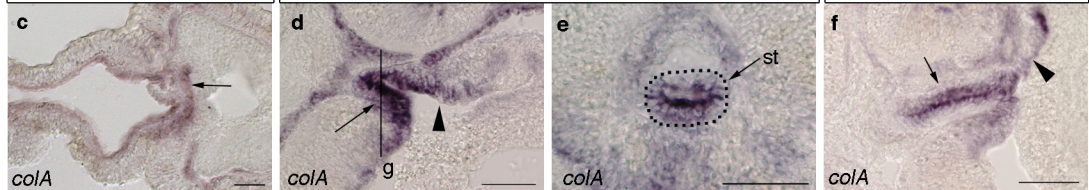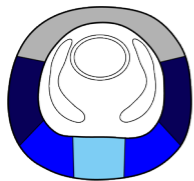
Protostome   Hemichordate   Chordate

c

b   Deuterostome

a   Urbilateria
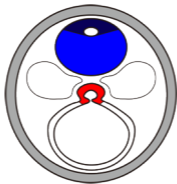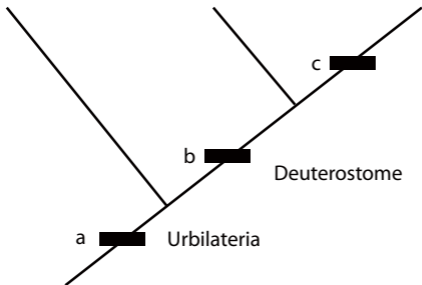
epidermis   ■ signal center (anterior endoderm and notochord)

■ dorsal domain   ■ lateral domain   □ ventral domain