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Review

Evolution of Invertebrate Deuterostomes and Hox/ParaHox Genes

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Abstract

Transcription factors encoded by Antennapedia-class homeobox genes play crucial roles in controlling development of animals, and are often found clustered in animal genomes. The Hox and ParaHox gene clusters have been regarded as evolutionary sisters and evolved from a putative common ancestral gene complex, the ProtoHox cluster, prior to the divergence of the Cnidaria and Bilateria (bilaterally symmetrical animals). The Deuterostomia is a monophyletic group of animals that belongs to the Bilateria, and a sister group to the Protostomia. The deuterostomes include the vertebrates (to which we belong), invertebrate chordates, hemichordates, echinoderms and possibly xenoturbellids, as well as acoelomorphs. The studies of Hox and ParaHox genes provide insights into the origin and subsequent evolution of the bilaterian animals. Recently, it becomes apparent that among the Hox and ParaHox genes, there are significant variations in organization on the chromosome, expression pattern, and function. In this review, focusing on invertebrate deuterostomes, I first summarize recent findings about Hox and ParaHox genes. Next, citing unsolved issues, I try to provide clues that might allow us to reconstruct the common ancestor of deuterostomes, as well as understand the roles of Hox and ParaHox genes in the development and evolution of deuterostomes.

Key words: invertebrate deuterostome, Hox, ParaHox, evolution

Introduction

Animal morphology along the body axes is greatly diverse, requiring both a system that confers positional character and a competence to respond to these positional cues. The Antennapedia-class Hox and ParaHox genes encode transcription factors that play crucial roles in controlling morphological development of animals. Hox genes have been noted for several striking properties, including their conserved roles in providing regional identities along the anterior–posterior (AP) axis and the spatial and/or temporal colinearity between their expression patterns along the AP axis and their positions within clusters on a chromosome (*1-3*). These factors generate the unique combinations of Hox genes expressed at the different AP axial levels during development; this arrangement is referred to as the "Hox code" (*4*). These observations lead to the hypothesis that the physical organization on the chromosome, expression pattern, and functions of the Hox genes are important for proper morphological patterning along the AP axis (*5*) and, in turn, for evolutionary changes in the animal body plan. The ParaHox cluster was first characterized in the cephalochordate amphioxus, in which three member genes, *Gsx*, *Xlox* and *Cdx*, are linked in a manner

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reminiscent of the Hox genes (*6*). The ParaHox cluster was regarded as an evolutionary sister of the Hox cluster; and both clusters were evolved from a putative common ancestral gene complex, the ProtoHox cluster, which is prior to the divergence of cnidarians and bilaterians (*6-8*). Subsequently, the Hox cluster was expanded by tandem duplications of the member genes during evolution; in contrast, the ParaHox cluster maintained a constant size of three genes.

The Deuterostomia comprise one of the major groups within the animal kingdom; it is a monophyletic group of animals (*9*) that belongs to the Bilateria, and a sister group to the Protostomia (**Figure 1**). Deuterostomes are characterized by having a "second mouth"; *i.e.*, during embryo development, the blastopore becomes the anus, whereas the mouth forms in a secondary anterior location. Deuterostomes can be further subdivided into two major clades: Ambulacraria and Chordata (to which humans belong) (*9-11*). Some authorities also include the newly described and enigmatic group Xenoturbellida (or Xenacoelomorpha even including acoelomorphs) (*12, 13*); however, I will not discuss this group here, mainly because their morphology is very divergent

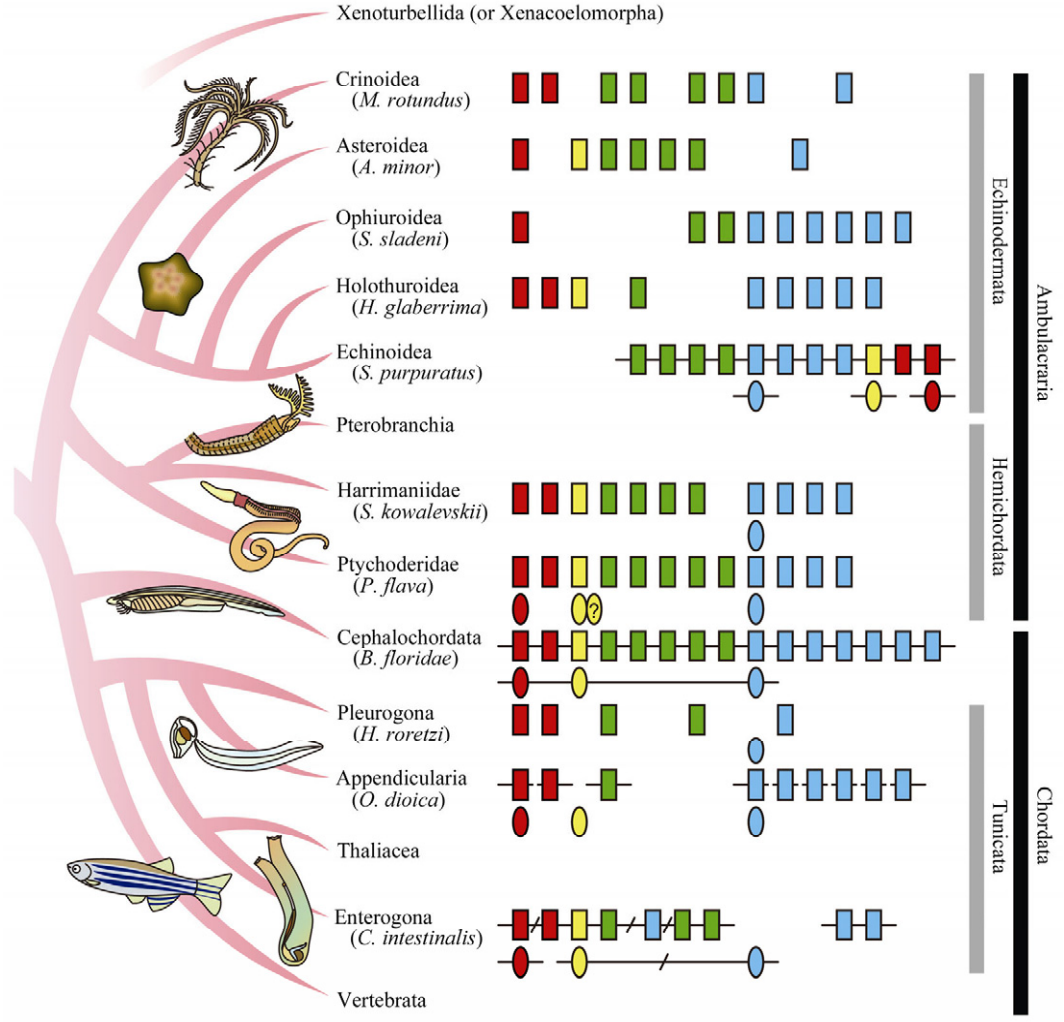

Figure 1 Deuterostome phylogeny with schematics of the genomic organization of Hox and ParaHox genes. Colored rectangles and ovals indicate Hox and ParaHox genes, respectively. Anterior Hox and Gsx are indicated in red; group3 and Xlox in yellow; central Hox in green; and posterior Hox and Cdx in blue, according to the nomenclature in a previous study (*7*). Lines passing under rectangles or ovals indicate clustered gene linkage on a chromosome. Slashes on the line represent a large gap between Hox or ParaHox genes, indicating disorganization of the cluster. Unconnected lines also indicate an unlinked situation. Rectangles and ovals without horizontal lines passing under them indicate the genes whose linkage has not been investigated. Information about vertebrate and xenoturbella (or xenacoelomorph) Hox and ParaHox genes is omitted.

and their phylogenetic position is still debatable (*14-17*). Ambulacraria consists of the Echinodermata and the Hemichordata (*18*). Echinodermata is composed of five classes: Crinoidea, Asteroidea, Ophiuroidea, Holothuroidea and Echinoidea (*19*). Organisms in these classes share bilateral, ciliated dipleurula-type larva, and the characteristic pentameral adult body plan. Among the five extant classes, crinoids are regarded as the most basal group, based on fossil studies and molecular phylogenetic analyses (*20*). The phylum Hemichordata consists of two classes: Pterobranchia and Enteropneusta (*21*). The latter is known by the common name of acorn worms, which possess pharyngeal gill slits, bilateral body plan and clear AP and dorsal–ventral (DV) body axes. The Enteropneusta are therefore regarded to be an important existing animal to link between echinoderms and chordates. Recently constructed molecular phylogenies describe two main groups of enteropneusts: the Harrimaniidae in one lineage; and the Ptychoderidae and Spengelidae in the other (*22*). These two lineages have major life-history differences: harrimaniids are all direct developers, whereas the ptychoderids and spengelids are indirect developers with ciliated feeding tornaria-type larvae, often spending several months in the plankton stage before metamorphosing into juveniles (*22*). The pterobranchs, which are colonial and sessile organisms, might be derived from the enteropneust worms (*23*).

The phylum Chordata consists of three subphyla: Urochordata, Cephalochordata and Vertebrata (*24*). These groups are characterized by a hollow dorsal neural tube, a notochord, gill slits, endostyle, and a postanal tail, the first two of which are hallmarks of the chordate body plan (*25, 26*). Cephalochordates, known as amphioxus, and vertebrates share not only all the hallmarks of the chordate body plan, but also additional characteristics: gastrulation coordinated by an organizer (*27*), bilaterally paired somites, and adult morphology resembling a poorly cephalized fish. Thus, cephalochordates were long thought to be the closest invertebrate relatives of the vertebrates (*28*). However, recent genome analyses revealed that cephalochordates are closest to the chordate ancestors, leaving urochordates as a sister group of vertebrates (*29-31*). Urochordata, or Tunicata, is divided into three classes: the ascidians (sea squirts), which are further divided into Pleurogona and Enterogona; appendicularians (larvaceans); and thaliaceans (doliolids, salps and pyrosomids) (*32*). The ascidians have a sessile adult phase, while thaliaceans have a pelagic adult phase. These two types are generally known to develop into tadpole-like larvae, which have hallmarks of the chordate body plan at first and resorb the tail later through a drastic metamorphosis (*25, 33*); however, some species have secondarily lost the tadpole-like larval stage. On the other hand, larvaceans retain the tadpole-like body plan throughout their life span, using this tail for locomotion and the production of water currents for capturing foods in their tunic "house".

Invertebrate deuterostomes have been regarded as an important animal link between the protostome–deuterostome ancestor and vertebrates, and as the animal possessing basic and fundamental developmental programs of vertebrates. Recent cladistic studies, fossil studies and molecular phylogenetic studies support the idea that the main three groups of deuterostomes (echinoderms, hemichordates and chordates) diverged from a common ancestor during the Cambrian explosion around 520 million years ago (*22, 23, 28, 34*). The studies of Hox and ParaHox genes in recent decades, focusing largely on invertebrate deuterostomes, have slowly but surely provided insights into reconstruction of the common ancestor and the roles of these genes in development and evolution of deuterostomes. However, there are still many questions left unanswered. In this review, I will first summarize information from recent analyses about expression, genomic organization, and function of the Hox and ParaHox genes in invertebrate deuterostomes. Next, I discuss some unsolved issues regarding the evolution of the deuterostome body plan and the role of Hox and ParaHox genes in that process, and try to suggest some hints about potential solutions to the issues.

Hox Genes of Invertebrate Deuterostomes

Deuterostome Hox genes are generally classified into 14-15 paralogous subgroups on the basis of sequence similarity. Along the invertebrate deuterostome lineage, Hox clusters were shown to exhibit the loss of member genes and the dispersion of the clustered or-

ganization (*35, 36*); however, all members of invertebrate deuterostomes are regarded to have a single set of Hox genes.

In the sea urchin *Stronglyocentruotus purpuratus*, an echinoderm, eleven Hox genes have been identified (*37, 38*). The recent sequencing of bacterial artificial chromosome (BAC) clones from the genome of *S. purpuratus* revealed that the sea urchin Hox cluster is large, occupying 588 kb, and contains rearrangements both of transcriptional orientation and gene order (*38*). Whereas only two Hox genes are transcribed during the development of bilateral pluteus larva, ten Hox genes of *S. purpuratus* are transcribed during formation of the pentameral adult rudiment (*39*). Among them, *Hox7*, *8*, *9/10*, *11/13a* and *11/13b* exhibit spatially staggered expression in the somatocoels of mesodermal origin during the period of adult rudiment formation (*9*); however, the details of expression of the anterior member genes remain unknown. We have almost no data on function of sea urchin Hox genes, except for *Hox11/13b*, which may be involved in cell adhesion as well as in hindgut specification during larval development (*40*). In the most ancestral extant echinoderm, eight Hox genes have been identified in the stalked crinoid *Metacrinus rotundus*. Among these, *Hox5*, *7*, *8* and *9/10* exhibit spatially staggered expression in the mesodermal somatocoels of the bilateral auricularia larva (*41*); however, their genomic organization and function remain unknown. Although it was thought that echinoderms lacked the ortholog of *Hox4*, recent studies on asteroid and crinoid Hox genes demonstrated that the absence of *Hox4* from echinoids is a derived state (*41, 42*), and the ancestral echinoderm probably had a Hox gene complements not dissimilar to that of hemichordates.

Among hemichordates, Hox genes were relatively well studied in the harrimaniid species *Saccoglossus kowalevskii*, a direct-developing enteropneust. In this species, eleven Hox genes have been identified; these genes exhibit spatially staggered expression in the surface ectoderm during embryogenesis (*43, 44*). On the other hand, in the ptychoderid *Ptychodera flava*, an indirect-developing enteropneust, eight Hox member genes have been identified (*45*). Even so, it was unknown whether the ortholog of *Hox8* is absent in hemichordates; consequently, no consensus view about Hox gene complements in hemichordates has been established. Recently, however, we isolated the presumably full complement of twelve Hox genes, including the ortholog of *Hox8* in *Balanoglossus simodensis*, suggesting that the ancestral hemichordate had intact complements of ambulacrarian prototypical Hox genes (*46*). However, as regards to hemichordate Hox genes, there is no report of their genomic arrangement, developmental roles or expression pattern in the larval development.

It has been pointed out that the cephalochordate amphioxus retains a relatively intact Hox cluster organization, remarkably similar to that inferred for the direct ancestor of the vertebrates (*47*). The amphioxus *Branchiostoma floridae* has a single contiguous Hox cluster, containing an extra 14th and 15th member genes, spanning 470 kb (*48, 49*). Recent phylogenetic analysis suggested that the amphioxus and vertebrate *Hox14* genes were not orthologous, but arose independently through tandem gene duplications of *Hox13* genes (*50*). Moreover, the posterior Hox genes of ambulacrarians and chordates may result from two independent sets of tandem duplications (*38, 45, 46*). Thus, the true prototypical complement for deuterostome Hox gene members is still unclear. An initial expression study of amphioxus Hox genes revealed that *Hox1*, *3* and *4* exhibit spatial and temporal colinearity in the developing neural tube, although *Hox2* is exceptional (*51*). Furthermore, expression of amphioxus *Hox6* was described to break colinearity (*52*). On the other hand, a recent study showed that not only *Hox1*, *3* and *4*, but also *Hox2* and *6* join in spatial colinearity in the neural tube from the mid-neurula to larval stage, correcting the previous reports (*53*). However, expression patterns for more posterior member genes remain unknown. Functional studies were only conducted for *Hox1*, in which *Hox1* mediates retinoic acid (RA) signalling in establishing the posterior limit of the pharynx and regionalization of the hindbrain, as well as in specification of motor neurons (*53, 54*).

Among the urochordates, the larvacean *Oikopleura dioica* has two anterior, one central, and six posterior Hox genes. Although the locations of these genes on the chromosomes are unknown, genomic walking suggests that they are dispersed within the *O. dioica* genome (*55*), and the situation is the same even after recent draft genome sequencing (*56*); each Hox gene

is located on a different scaffold. Despite that, some *Oikopleura* Hox genes exhibit spatially staggered expression in the notochord, tail muscle, nerve cord, and epidermis (*55*). Among ascidians, Hox genes were most extensively studied in *Ciona intestinalis*. In this species, the draft genome analysis identified nine Hox genes (*57*). We revealed that some, if not all, *Ciona* Hox genes exhibit spatially staggered expression within the central nervous system (CNS) during larval development and in the gut of the juvenile. Nevertheless, nine *Ciona* Hox genes are dispersed on two chromosomes, with rearrangements in gene orders and transcriptional orientations (*58*). Among them, seven Hox genes, *Hox 1-6* and *10*, are distributed with some large gaps between them that contain many non-Hox genes, spanning approximately half the length of a chromosome, which could be roughly calculated as 5 Mb; *i.e.*, extraordinarily long in comparison to that of a typical higher vertebrate Hox cluster (*59*). Thus, urochordates like *Oikopleura* and *Ciona* provide some of the most extreme cases of Hox cluster disintegration reported to date. Significantly, in these species, the temporal staggering of Hox initiation is lost, while the spatial staggering is retained, supporting the hypothesis that the mechanisms producing temporal colinearity are likely the major constraining forces on gene cluster maintenance, *e.g.*, a shared regulatory mechanism, such as a shared enhancer(s) (*60, 61*). Interestingly, *lacZ* reporter constructs connected to the genomic fragments, including the flanking sequence of Hox genes and/or Hox gene sequence with introns, roughly mimic endogenous expression of each Hox gene, suggesting the loss of *cis*-regulatory element sharing in *Ciona* (*62*). Furthermore, surprisingly, our recent analysis suggested that the contribution of Hox genes to the larval development of *C. intestinalis* might be very limited, despite the fact that two Hox genes, *Hox10* and *12*, play important roles in neuronal and tail development, respectively (*63*).

ParaHox Genes of Invertebrate Deuterostomes

The ParaHox gene cluster was first discovered in the cephalochordate amphioxus, and is composed of members of three Hox-related homeobox gene families: Gsx, Xlox and Cdx. Gsx was described to be most similar to the anterior Hox genes, Xlox to group 3 Hox genes, and Cdx to the posterior Hox genes (*6, 7*). All invertebrate deuterostomes are regarded to have a single set of ParaHox genes.

Among echinoderms, ParaHox genes have been well studied in *S. purpuratus*. The sea urchin has three ParaHox genes, *Gsx*, *Xlox* and *Cdx*, but each ParaHox gene is located on a different genomic scaffold (>300 kb each), suggesting that they are not linked into a single coherent cluster (*64*). In spite of this, strikingly, the three member genes show both spatially and temporally staggered expression; *Gsx* is expressed first, followed by *Xlox* and finally *Cdx*, although *Xlox* and *Cdx* exhibit staggered expression in the larval gut, whereas transcripts of *Gsx* are detected in the ectoderm and not in the gut (*64*). A recent functional analysis of *S. purpuratus Xlox* and *Cdx* showed that these two genes interact in patterning of the larval hindgut (*65*).

Information about hemichordate ParaHox genes is limited. In *S. kowalevskii*, extensive EST screening identified only *Cdx* (*66*). On the other hand, in *P. flava*, four ParaHox member genes have been isolated: one *Gsx,* two *Xlox* and one *Cdx* (*45*). Furthermore, we recently reported the full complement of three ParaHox genes from *B. simodensis*, suggesting that the ancestral hemichordate had intact complements of the ambulacrarian prototypical ParaHox genes (*46*). However, the genomic arrangement, developmental expression and function of hemichordate ParaHox genes remain unknown.

In the amphioxus *B. floridae*, the three member genes are linked in a genomic region of 56 kb, with *Gsx* adjacent to *Xlox* in the same orientation, followed by *Cdx* on the opposite strand (*6, 67*). Of the invertebrates studied to date, the linkage of the three genes has been demonstrated only in amphioxus. The amphioxus ParaHox cluster exhibits both spatial and temporal colinearity. Initially *Cdx* is activated during mid-gastrulation in a ring around the blastopore, and then remains in a continuous domain in the posterior neuroectoderm and hindgut during neurulation and somitogenesis. *Xlox* expression commences slightly later than *Cdx* in the posterior endoderm and mesendoderm, but later becomes restricted to a more central

region of the gut in the developing larva, with transient expression in two cells of the neural tube approximately level with the anterior boundary of somite five (where the first pigment spot will form). The last ParaHox gene to be activated is *Gsx*, which is initially expressed in the neural tube during neurulation at the same location level as the transient expression of *Xlox*, followed by activation in the cerebral vesicle (*68*). Thus, interestingly, the temporal colinearity in the amphioxus ParaHox cluster along the AP axis is inverted with respect to the pattern in the Hox cluster (anterior to posterior) as well as with the observation in the sea urchin ParaHox genes (*Gsx* to *Cdx*) (*54*). I speculate that the temporal order observed in amphioxus and mouse (*Cdx2*, *Ipf1* and *Gsx1*) (*69-71*) is the conserved pattern, and the observation in sea urchin will be incidental, based on the hypotheses that temporal colinearity is the principal constraining force on cluster organization (*60, 61*); as long as this colinearity of only three genes is a subsistent system and has not occurred simply by chance. Developmental roles of amphioxus ParaHox genes are unknown.

In urochordates, the full complement of ParaHox genes, *Gsx*, *Xlox* and *Cdx*, has been identified in the *C. intestinalis* genome. An extensive mapping of BAC clones revealed that three genes are dispersed on two chromosomes: *Gsx* on chromosome 2q, and *Xlox* and *Cdx* on chromosome 14q (*72*). The latter two genes are approximately 240 kb apart, with head-to-head transcriptional orientation, and with many intervening genes between them. In *Ciona*, expression studies showed that *Gsx* is expressed in the posterior sensory vesicle from the mid-gastrula to tailbud stages (*73*); *Xlox* in the larval sensory vesicle, visceral ganglion and mesenchymal cells (*74*); and *Cdx* in the neural and posterior muscle lineage cells in mid-gastrula (*75*), the nerve cord, posterior epidermis and endodermal strand at the tailbud stage (*67*), and the nerve cord at the larval stage (*76*). Although expression patterns of *Ciona* ParaHox genes from metamorphosis onward are unknown, in another ascidian, *Herdmania curvata*, *Cdx* is expressed in the intestine of the juvenile as well as the posterior CNS during larval development (*77*), whereas expression patterns of *Gsx* and *Xlox* in this species remain unknown. An initial functional study of ascidian ParaHox genes was conducted for Cdx of third ascidian *Halocynthia roretzi*; the results suggested that *Halocynthia Cdx* is required for larval tail formation, probably by controlling ectodermal cell movement (*78*). In *C. intestinalis*, functional inhibition using a dominant negative *Cdx* under control of a *FoxD* promoter/enhancer produced a phenotype similar to that observed in the *Halocynthia* embryo in which *Cdx* function was suppressed. The authors concluded that *Ciona Cdx* is required for neural tube formation (*79*). Furthermore, a recent study aimed at establishing the gene regulatory network underlying CNS development of *Ciona* revealed several downstream genes under the transcriptional control of *Cdx* (*80*). The developmental roles of urochordate Gsx and Xlox have not yet been reported.

Hox Genes and Evolution of the Deuterostome Nervous System

It has been proposed that in the Bilateria, the ancestral function of Hox genes was in AP patterning and specification of ectodermal and neuroectodermal anlagen (*81, 82*). Indeed, the expression and function of Hox genes in *Drosophila melanogaster* and chordates are most evident in the ectodermal and neuroectodermal tissues, and Hox genes are associated with the development of CNS in many different taxa (*83-86*).

The ancestral character of the deuterostome nervous system is still enigmatic. Considering the similar ciliary band anatomy and apical organ structure in the echinoderm and hemichordate larva, this shared larval type apparently reflects the development of the ambulacrarian ancestor (*87*). However, Hox genes probably do not contribute to axial patterning of the neuroectoderm of the ambulacrarian larva, since no staggered expression of Hox genes has been observed in the neuroectoderm of echinoderm larvae (*39, 88*). The nervous systems of adult echinoderms and hemichordates are quite different. The centralized part of the echinoderm nervous system consists of a circumoral (or circumanal, in the case of crinoids) ring connecting five radial nerve trunks that run out along each arm of the animal (*24*). Again, staggered expression of Hox genes in the development of the adult nervous system has not been observed in *S. purpuratus*. However, it has been hypothesized that the cir-

cumoral and radial nervous system of echinoids may represent a small portion of the ancestral bilaterian CNS derived from a region anterior to much or all of the Hox patterning domain (*89*). As a corollary to this, the circumanal nervous system of crinoids would be expected to express more posterior patterning genes, including Hox genes. Thus, a study of the expression pattern of crinoid Hox genes during the development of the adult nervous system would contribute significantly to our understanding of the origin of the deuterostome nervous system and the evolution of the peculiar body plan of echinoderms.

In contrast to echinoderms, the major organizational feature of the hemichordate nervous system is that it is basiepithelial. It has been described that there is no CNS, and cell bodies are scattered throughout the epithelium (*24*). Lowe *et al* described the staggered expression of over twenty orthologs of neural patterning genes, including Hox genes in *S. kowalevskii*, in which Hox genes are expressed in circular areas in the ectoderm around the entire animal, with an AP arrangement nearly identical to that found in chordates (*43, 44*). This prompted the idea of ancient "skin brains", which proposes that the deuterostome ancestor had a nervous system that was not central but diffuse; in this model, the CNS evolved independently in the protostome and deuterostome lineages (*90, 91*). In contrast, in the 19th century, comparative anatomist Anton Dohrn proposed that the chordate CNS is homologous to that of protostome annelids and arthropods (*92*). Since that time, a growing list of genes has been proposed to play a conserved role in the patterning of CNS of *Drosophila* and of vertebrates. This led to the hypothesis that the CNS of arthropods and chordates are homologous (*93, 94*). Furthermore, Denes *et al* elegantly demonstrated that the mediolateral neural architecture of the developing trunk CNS of the annelid *Platynereis dumerilii* is quite similar to that of the developing vertebrate neural tube, supporting a common origin for nervous system centralization in the Bilateria (*95*). In addition to this, Nomaksteinsky *et al* recently showed that both juvenile and adult of the indirect-developing enteropneust *P. flava* in fact have a bona fide CNS, *i.e.*, dense agglomerations of neurons, forming two cords, ventral and dorsal, which merge anterior–dorsally at the level of the collar to form a chordate-like neural tube. Contrary to previous assumptions, the greater part of the adult enteropneust skin is non-neural, although elements of the peripheral nervous system (PNS) are found there. The authors proposed that the previously described "diffuse" nervous system present at earlier developmental stages in *Saccoglossus* is a transitory feature that may correspond to the larval nervous system of other enteropneusts (*96*). These findings have reopened the possibility that centralization of the nervous system predates the chordates; *i.e.*, the deuterostome ancestor had a centralized nervous system. This encourages more intensive comparative CNS research, especially the study of the roles of the axial patterning genes, including Hox genes, in the development of the enteropneust CNS. We have revealed that *P. flava* has intact complements of the twelve prototypical ambulacrarian Hox genes, and that all of them except for one are located on two contiguous BAC clones; *i.e.*, the cluster can be estimated to fit within 200-300 kb (unpublished data). As for the one exceptional Hox gene, I have not been able to isolate this gene from the two sets of BAC libraries. I speculate that this gene is dropped out of the BAC libraries over the course of several additional experiments, and that the full complement of twelve Hox genes will be clustered in the *P. flava* genome. If their gene order and transcriptional orientation are intact and the cluster has no intervening genes, the *P. flava* Hox cluster will be the most compact, clearly organized example in invertebrates studied to date, probably representing the ancestral character of the deuterostome Hox cluster. On the other hand, Duboule proposed that the Hox cluster was originally disorganized in the ancestral bilaterian as well as the ancestral deuterostome (*97*). Thus, in order to build a consensus view about the ancestral features of deuterostome Hox genes, and to cast in a clearer light the origin of the deuterostome body plan including the nervous system, we must conduct a comprehensive study of the genomics, expression, functions and regulation of the hemichordate Hox genes.

A number of hypotheses for the origin of the chordate nervous system have been proposed, and distinguishing between them will obviously depend on reaching a conclusion about the ancestral form of the deuterostome nervous system, as described above. Since this complex topic has been reviewed elsewhere

(*43, 98-100*), I do not cover it here. But in any case, one outstanding problem is determining whether Hox genes contributed to AP patterning of CNS of the chordate ancestor. Considering the clear staggered expression of five amphioxus Hox genes (Hox*1-4* and *6*), and the role of *Hox1* in regionalization of the amphioxus hindbrain as well as specification of motor neurons (*101*), the answer is probably "yes". In vertebrate gnathostomes, the specification of rhombomere segmental identities and neurons also depends on the highly organized expression patterns of the Hox genes (*85, 102-104*). However, Murakami *et al* proposed that the relationship between Hox gene expression domains and motoneuron identity may be an ancestral feature conserved throughout the AP axis of the chordate CNS, whereas the neuromeric compartments of the segmentation process are evolutionarily as well as developmentally independent. That is, the AP specification of branchiomotor neurons was already under control of a Hox code in the chordate ancestor, but the registration of hindbrain segmentation and Hox code regulation appeared in the gnathostome lineage (*105*). Indeed, the amphioxus hindbrain lacks obvious segments. Thus, in order to more precisely understand the role of Hox genes in CNS of the chordate ancestor, a functional assay for each amphioxus Hox gene will be an urgent task. On the other hand, in the tadpole larva of the ascidian *C. intestinalis*, the neural circuits of the motor system are simply composed of at most five pairs of cholinergic motor neurons in the visceral ganglion, and two pairs of GABA/glycinergic interneurons in the anterior nerve cord (*106*). However, we demonstrated that knock-down of *Ciona Hox1, 3* and *5* does not affect the development of these neurons (*63*), despite the fact that they precisely exhibit staggered expression in the visceral ganglion and anterior nerve cord, both of which are regarded as homologous with vertebrate rhombospinal region (*58, 107, 108*). The limited roles of ascidian Hox genes in larval CNS development are probably related to the ascidian's simple body plan and rapid, determinative embryogenesis, and may also be influenced by extensive genomic rearrangement and gene loss, including disintegration of the Hox cluster and loss of some Hox gene members (*63, 109*). Similarly, Seo *et al*, using data from larvaceans, correlated disintegration of Hox genes and loss of Hox gene members with size reduction and determinative development in the urochordate lineage (*55*). To confirm these scenarios, comprehensive studies of the Hox genes in a wider variety of urochordates, including functional study of larvacean Hox genes, will be required.

Basic Roles of Hox Genes in the Development of the Mesodermal Organs

Although the expression and function of Hox genes in *Drosophila* and vertebrates are most evident in the ectodermal and neuroectodermal tissues (*82*), Hox genes are also expressed in the mesoderm of *Drosophila* (*110, 111*) and vertebrates (*112-114*). In invertebrate deuterostomes, echinoderms are a particularly good example of staggered expression of Hox genes in mesoderm. In sea urchins, expression of five Hox genes in the mesodermal somatocoels at the late pluteus stages, *i.e.*, during formation of the pentameral adult rudiment, follows the curved gut underneath; thus it points in a curved AP direction (*9*). Does this mesodermal expression of Hox genes in sea urchins represent the ancestral character of deuterostomes?

In *Drosophila*, four Hox genes expressed in the visceral mesoderm of the midgut are crucial for gut morphogenesis (*110*). In vertebrates as well, Hox genes exhibit region specific expression in splanchnic mesoderm (*113, 114*). Based on these observations, staggered expression of sea urchin Hox genes in the larval somatocoels might reflect the ancestral role of Hox genes in the development of digestive tract. In echinoids, generally the stomach and most of hindgut survive metamorphosis and contribute to adult gut (*115*). Thus, this idea does not contradict the general view that Hox genes are used in patterning of the adult body plan rather than for development of the larva-specific structures (*116*). Here, I should note that the word "larva" requires careful handling. The sort of ciliated planktonic larvae of bilaterians termed as "primary larvae" by Jägersten (*117*), such as the trochophore larva of annelids or the dipleurula larva of echinoderms, are qualitatively different from the secondary larvae found in several taxa, such as arthropods, ascidians and vertebrates. Secondary larvae, as recognized by Jägersten, are modified adult (or juvenile) forms comparable to the adults of other bi-

laterians (*116*). Thus in this review, the term "indirect development" means that the organism grows into the primary larva stage before becoming an adult.

Getting back to the role of Hox genes in the echinoderm somatocoels, an alternative idea is that echinoderm Hox genes are involved in patterning the somatocoels along the AP axis. For instance, by comparing Hox gene expression patterns in sea urchin larvae and crinoid larvae, Hara *et al* proposed that staggered expression of medial and posterior Hox genes in the somatocoels along AP axis reflects an ancestral feature of echinoderms (or possibly of ambulacrarians), and that *Hox5* ortholog expression in the crinoid somatocoels will be associated with the differentiation of the chambered organ and reflects an ancestral trait involved in adult stalk formation (*41*). However, Omori *et al* recently showed that *Six3*, *Pax6*, *Otx* and *Hox* genes exhibit staggered expression in the archenteron and later in the coeloms along the AP axis during crinoid larval development (*118*). The staggered expression of the homeobox genes in the crinoid endomesoderm resembles strikingly the expression patterns of the gene orthologs of chordate and hemichordate in the neuroectoderm. From the results, Omori *et al* proposed that the stalked crinoids adopt *Six3*, *Pax6*, *Otx* and *Hox* genes as a patterning system of the larval endomesoderm, suggesting that a radical alteration of the expression and function of homeobox genes (*119*) has occurred in basal echinoderms (*118*). That is, Hox genes may be co-opted (*120*) to different roles in echinoderm development. Being highly suggestive, staggered expression of Hox genes has not been observed in the coeloms of direct-developing hemichordate *S. kowalevskii* (*44*), even though the coeloms of echinoderms and hemichordates are regarded as homologous (*121*). Therefore, expression study of Hox genes in the larval development of indirect-developing hemichordates, as well as functional assay of Hox genes in the echinoderm somatocoels, would give us a better understanding of the basic role of Hox genes in deuterostome mesodermal development.

On the other hand, what does the information on invertebrate chordate Hox genes tell us? In amphioxus, spatial colinearity is confined to the developing neural tube, and staggered expression has not been observed in the mesoderm. Based on this, Wada *et al* proposed that spatial colinearity of Hox genes was confined to the neural tube in the ancestral chordates (*51*). In urochordates, ascidian Hox genes do not exhibit staggered expression in the mesoderm during larval development or at the juvenile stage (*58*). In contrast, in the larvacean *O. dioica*, some, if not all, Hox genes exhibit spatially staggered expression in the notochord and tail muscle (*55*). Although expression of Hox genes in the notochord received less attention, Prince *et al* reported spatially staggered expression of Hox genes in the zebrafish notochord (*122*). As the notochord has a well-documented role in patterning adjacent structures (*123, 124*), the authors proposed that Hox genes may play an important functional role in the notochord, namely induction of localized expression of molecules with a role in patterning adjacent structures (*122*). If this expression pattern is the ancestral character in chordates, it is very interesting: the notochord is a direct descendant of the organizer region, which is capable of patterning an entire secondary axis (*125*). However, I wonder why staggered expression of amphioxus Hox genes has not been observed in the notochord. Further expression study of more 5′-Hox genes of amphioxus may enable us to address this issue and move the field forward. We face a similar situation in regard to Hox expression in the larvacean tail muscle. The staggered expression in the muscle reminds us of the well-known role of Hox genes in the paraxial mesoderm in vertebrates (*112, 126*). Furthermore, it was reported that, in *Drosophila*, *Antp*, *Ubx*, *abd-A* and *Abd-B* are involved in muscle pattern diversification cell-autonomously in the somatic mesoderm (*111, 127*). Likewise, in order to help us understand whether Hox gene expression in the larvacean muscle represents the ancestral role of Hox genes in chordates (or even in deuterostomes), again, we await expression study of the complete set of amphioxus Hox genes as well as functional study of larvacean Hox genes in development. On the other hand, I suppose that loss of staggered Hox expression in mesoderm of the ascidian tail may reflect a simple function (*e.g.*, only swimming) of the ascidian tail against putative sophisticated functions of the larvacean tail, which appears to have several distinct beating patterns (*109*).

Basic Roles of Hox Genes in the Development of the Endodermal Organs

Hox genes are also expressed in the endoderm of *Drosophila* (*128*) and vertebrates (*129*). In invertebrate deuterostomes, staggered expression of Hox genes in the endoderm was first reported in the intestine of the ascidian juvenile, in which *Hox10, 12* and *13* exhibit clearly staggered expression (*58*). Also in vertebrates, Hox genes exhibit staggered expression in the developing digestive tract (*129*). However, it was noted that the anterior expression limits of Hox genes in the endoderm do not always correlate with boundaries between organs. Furthermore, there is no evidence that Hox mutations cause homeotic transformations in the gut, although malformations have been observed (*129*). Thus, a systematic understanding of the role of these genes in vertebrate endoderm development is still lacking. However, in mouse, most Hox genes are expressed from the pharynx to the esophagus and intestinal endoderm, suggesting that they may play more essential roles in pharyngeal and intestinal development than in the formation of the other major endodermal organs (*69*). Intriguingly, a similar pattern was observed in *C. intestinalis*; *Hox1* is expressed in the larval trunk endoderm, which likely contributes to the esophageal region in adult (*130*), and the remaining genes expressed in the endoderm are *Hox10, 12* and *13* in the juvenile intestine (*58*). Furthermore, in amphioxus, *Hox1* expressed just posterior to the pharynx mediates the effect of RA signalling in setting the posterior limit of the pharynx (*54*). Therefore, the developmental roles (probably AP patterning) of Hox genes in the anterior and posterior endoderm may reflect the ancestral character, at least in chordates. In the hemichordate *S. kowalevskii*, *Hox1* is also expressed in the endoderm in hatched juvenile, and the domain of expression below the gill slit marks the posterior boundary of the pharyngeal endoderm (*44*). Aronowicz and Lowe pointed out that a similar expression domain is exhibited by *labial* gene of *Drosophila* (*44*). In *Drosophila*, *labial* is the only Hox gene expressed in the endoderm (*128*), where it specifies the most conspicuous cell type in the larval midgut, the so-called copper cells (*131*). Similarly, in the polychaete *Chaetopterus*, *Hox1* is highly expressed at the foregut–midgut boundary (*84*). Therefore, the role of *Hox1/lab* in endodermal patterning may be largely shared among bilaterians. With regards to posterior Hox genes, in the sea urchin *S. purpuratus*, *Hox11/13b* is expressed in the anus–hindgut region at the boundary between the ectoderm and the endoderm during embryogenesis, and is involved in cell adhesion at the endoderm–ectoderm boundary of the hindgut, as well as in specification of the hindgut via repression of several midgut-specific regulatory genes (*40*). Also in the development of the hemichordate *S. kowalevskii*, *Hox11/13b* and *11/13c* are expressed in the posterior-most endoderm (*44*). However, it is difficult to conclude that the role of Hox genes in patterning the posterior endoderm reflects the ancestral character of deuterostomes, because there is no evidence that ambulacrarian posterior Hox genes exhibit staggered expression as seen in ascidians and vertebrates, and the orthology of ambulacrarian *Hox11/13* to chordate *Hox11-13* remains unclear (see above). Clearly we need more information from further gene phylogenetic analysis and functional study of Hox genes in each animal in order to establish the basal role of deuterostome Hox genes in endodermal development.

Expression of ParaHox Genes in the Gut and Evolution of Deuterostomes

It has been proposed that in the Bilateria, the ancestral role of Hox genes was primarily in AP patterning and specification of ectodermal and neuroectodermal anlagen. Likewise, it was suggested that ParaHox genes might play a parallel role in AP patterning in the endoderm (or the digestive tract) of the developing embryo (*81, 82*). Based on the original work on ParaHox genes by Brooke *et al*, Holland hypothesized that the three ParaHox genes originated from the Proto-Hox cluster, and that these genes pattern the anterior, middle and posterior gut regions in a colinear manner in basal animals (*6, 81*). According to this hypothesis, Gsx played this role in the anterior gut development in basal animals, and lack of Gsx expression in the anterior gut of deuterostomes is then explained by loss of the primary mouth and evolution of a secondary "new mouth" (*81*). But what does the

"new mouth" mean? One must not confuse the advent of the "new mouth" with "mouth relocation", which likely occurred in the deuterostome lineage (or more precisely, in the chordates). Mouth relocation is central to the DV inversion hypothesis, which proposes that the ventral region of protostomes is homologous to the dorsal side of the deuterostomes (*132, 133*), and that in deuterostomes, the mouth is relocated to the former dorsal side. However, the available molecular data now strongly indicate that hemichordates share the DV orientation of protostomes (*134*). Additionally, the recent finding of right-sided expression of *pitx* and *nodal* in sea urchin larvae has been presented as evidence that echinoderms are also uninverted (*135*). Thus, it is chordates alone that seem to be inverted relative to both non-chordate deuterostomes and protostomes; the mouth of non-chordate deuterostomes opens on the ventral side, homologous to that of protostomes (*136*). Therefore, in deuterostomes, the term "new mouth" exclusively refers to the fate of the blastopore. That is, in deuterostomes, the blastopore becomes the anus and the mouth forms at a secondary anterior location. The data supporting Holland's hypothesis have been reported in the development of two polychaetes and one gastropod, in which *Gsx* is expressed in the developing mouth, the stomodeum (*137-139*), although the result from one polychaete species do not support such a model, since *Gsx* expression is limited to a restricted region of the forming brain (*140*). In the sea urchin *S. purpuratus*, *Gsx* is expressed in the ectoderm and not in the foregut (*64*). The latter data also might seem to support the Holland model, but it should be noted that the mouth of echinoderms opens on the same side as in protostomes, as described above. Furthermore, it has been suggested that the larval mouth regions of protostomes and deuterostomes are molecularly homologous, despite the difference in their larval gut ontogeny (*141*). Moreover, Martindale and Hejnol recently proposed that all oral openings are homologous across the Metazoa (except chordates) irrespective of the fate of the blastopore (*142*). In that case, why is *Gsx* not expressed in the *S. purpuratus* foregut? Alternatively, if the evolution of protostomy and deuterostomy would require reversal of gut polarity, why is *Gsx* not expressed in the deuterostome hindgut? Clearly, the homology of the mouth across bilaterians is still highly contentious (*142-145*), despite the fact that classification of the Bilateria into the protostomes and deuterostomes over the last 100 years was principally based upon the mode of formation of the mouth. Therefore, a wider range of deuterostomes as well as protostomes must be sampled to obtain a consensus on the basic role of Gsx and the other "mouth" genes deduced to be involved in early mouth specification and regionalization. We have demonstrated that in the indirect-developing hemichordate *P. flava*, three ParaHox genes: *Gsx*, *Xlox2* and *Cdx* are located on only a single BAC clone (unpublished data). If their gene order is intact and the cluster has no intervening genes, this will be the first example of an intact ParaHox cluster in non-chordate animals, probably representing the prototypical deuterostome (or even bilaterian) ParaHox cluster. If this is the case, it will be most interesting to observe how ParaHox genes are used in the development of *P. flava*.

The next issue arises from the role of *S. purpuratus Xlox* and *Cdx*, both of which interact in patterning of the gut during embryogenesis (*65*). I agree with the authors that the results represent the shared role of ParaHox genes in deuterostomes (or even in bilaterians). As noted above, there is a widely held view that Hox genes are used primarily during the larval stage (with some apparent co-option during embryogenesis) in cells destined to become parts of the adult body plan, rather than for development of the larva-specific structures (*116*). On the other hand, such a situation has not been described with respect to ParaHox genes. The generality of Hox gene exclusion from embryogenesis in indirect developers is based on evolution of set-aside cells or the posterior growth zone, which was defined as tissues that do not contribute to larval fates and remain multipotent for use in the generation of adult structures (*144, 146*). However, for feeding larvae, development of the functional digestive tract must be completed once prior to the unfolding of the set-aside cells, *i.e.*, during embryogenesis. Therefore, the functional exclusion from embryogenesis in indirect developers will not be applicable to ParaHox genes. In protostome lophotrochozoans, every species in which expression of ParaHox genes has been investigated has non-feeding larva; and staggered ParaHox expression in the gut can be observed at the late larval stage at the earliest, in parallel with the

development of the digestive tract (*137-140*). Thus, it would be very interesting to investigate expression of ParaHox genes in the developing gut of the feeding trochophore larva, as well as the enteropneust tornaria larva. In addition, I speculate that the evolutionary sister gene clusters (Hox and ParaHox) performed some basic functions almost concurrently in basal direct-developing animals at some time in the primeval past. The advent of set-aside cells with adult body patterning mechanisms, including the Hox code, would be a key factor in the evolution of indirect development, and the origin of biphasic development is crucial to our understanding of the ancestral form of deuterostome development. But since this conundrum cannot be resolved exclusively using the information from the Hox and ParaHox genes, and this has been discussed elsewhere (*116, 144, 146-148*), I shall not go into it further here.

Expression of ParaHox Genes in the Neuroectoderm

As observed in vertebrates (*69, 149*), amphioxus (*6, 68*) and sea urchins (*64*), the role of ParaHox genes in the regionalization of the endoderm (except for Gsx, which is in question) is arguably shared among deuterostomes. On the other hand, we cannot overlook the expression of ParaHox genes in the neuroectoderm of these animals. By comparison of Gsx expression in the neuroectoderm in a variety of bilaterians, Hui *et al* hypothesized that the pattern of Gsx expression in the protostome–deuterostome ancestor was complex, with roles in eyes, neurosecretory cells and regionalization of the neural tube/column, and was secondarily reduced to small patches of expression in the anterior CNS in several lineages (*138*). Among deuterostomes, the chordates amphioxus (*6, 68*) and *C. intestinalis* (*73*), as well as possibly the sea urchin (*64*), have only small, restricted patches of expression in the anterior CNS, whereas vertebrates have more extensive and complicated expression patterns that are comparable to the polychaete *P. dumerilii* (*71, 138, 150-155*). This may mean that secondary simplification to narrow anterior CNS expression has occurred independently in the amphioxus, ascidian and possibly echinoderm (or ambulacrarian) lineages. Distinguishing whether the *Platynereis*–vertebrate comparison really does provide a better reflection of the ancestral condition than these simplified lineages requires a consensus regarding whether Gsx expression in the CNS is simple and anterior or complex and extended. More species need to be sampled in order to obtain a clearer consensus about the expression of Gsx, *e.g.*, crinoids, hemichordates and larvaceans, as well as expression during development of the adult echinoderm nervous system. Furthermore, it is also important to investigate the regulatory control of Gsx in protostomes and deuterostomes, in order to reveal whether there are or not comparable regulatory networks that would be consistent with conserved, complicated or simplified expression of Gsx in each lineage.

Among deuterostomes, neural expression of Xlox was demonstrated for chordates. Although expression of Xlox in the CNS has been observed in a very limited range of chordates, there seems to be two basic patterns of Xlox expression. Mouse has extensive and complicated expression pattern in the telencephalon, mesencephalon, cerebellar primordium, and area postrema within the medulla (*156*). In *C. intestinalis*, signals of immunostaning were localized in the sensory vesicle and visceral ganglion, which certainly correspond to the vertebrate fore-midbrain and hindbrain/spinal cord, respectively (*74, 107, 108*). Therefore, Xlox expression in the fore-midbrain and hindbrain in mouse and *Ciona* may represent the ancestral condition in chordates. In contrast, amphioxus *Xlox* exhibits strong but transient expression in two cells of the neural tube opposite anterior boundary of somite five, at a site probably comparable to a part of the hindbrain (*6, 157*). It is possible that amphioxus has lost *Xlox* expression in the fore-midbrain region. Alternatively, the hindbrain expression might reflect the basal condition of the ancestral chordates; in that case the fore-midbrain expression may represent a novelty that evolved specifically on the higher chordate lineage including urochordates. Just for information, in protostomes, two polychaetes (*P. dumerilii* and *Nereis virens*) and one gastropod (*Gibbula varia*) have *Xlox* expression in the cerebral ganglion, which shows *Otx* gene expression, and the ventral neuroectoderm, which shows Hox gene expression (*137-139, 158*), although in one polychaete species (*Capitella teleta*), *Xlox* expression is detected neither in the head nor in

the ventral neuroectoderm (*140*). Also perplexingly, in *N. virens*, three ParaHox genes exhibit spatially staggered expression in the ventral neuroectoderm (*137*). As in the case of Gsx, comprehensive studies in a broader range of taxa are clearly required in order for us to understand the basic expression patterns of Xlox and the impact of such a plasticity of expression on the evolution of nervous system patterning.

In vertebrates, most of our understanding of the function of Cdx genes is restricted to their role in paraxial mesoderm of mouse, where they have been shown to integrate FGF, RA and Wnt signals into coherent Hox gene expression (*159*). However, information has increasingly accumulated regarding the function of Cdx genes in CNS development. Among invertebrate deuterostomes, expression of Cdx in the neuroectoderm was reported only in ascidians and amphioxus. In the ascidian *H. roretzi*, *Cdx* expression begins at the mid-gastrula stage in the precursors of the lateral walls of the nerve cord. In mid-tailbud embryos, expression of *Cdx* is evident in the lateral walls of the nerve cord. The anterior border of *Cdx* expression in the nerve cord is at the junction of the trunk and the tail. To suppress the function of *Halocynthia Cdx*, two different techniques were employed: suppression at the RNA level using phosphorothiolated antisense oligonucleotide (PO), and suppression at the protein level using a dominant negative molecule. The shared suppression phenotypes included reduction of the length of the tail, inhibition of neural tube formation including body bent to the dorsal side unlike normal embryos, and delay or failure to complete gastrulation (*78*). In *C. intestinalis*, *Cdx* expression is similarly detected in the nerve cord, and functional inhibition using a dominant negative *Cdx* under control of the *FoxD* promoter/enhancer produced a phenotype similar to that observed in the *Halocynthia* (*79*). By contrast, loss of function of *Ciona Cdx* induced by antisense morpholino oligonucleotide (MO) seems to produce a much milder phenotype than that described above. Although the length of the trunk and tail appear slightly short, gastrulation and neural tube formation seem to progress normally (*80*). Furthermore, in *Ciona*, transcription of *Cdx* is up-regulated in the embryo injected with *Cdx* MO (*80*), whereas in *Halocynthia*, treatment with PO results in significant reduction of the transcription level of *Cdx* (*78*). The causes of these differences are not known, but the weaker phenotype with MO in *Ciona* might be due to incomplete inhibition of the *Cdx* function under the experimental conditions.

Nevertheless, the functional study of *Ciona Cdx* using MO, by Imai *et al* (*80*), provided some important insights into the role of *Cdx* in ascidian CNS development. The ascidian CNS comprises four compartments: the sensory vesicle, neck, visceral ganglion, and nerve cord (*108*). However, Cole and Meinertzhagen acknowledged that the posterior boundary of the visceral ganglion is poorly defined (*160*). According to Imai *et al*, A11.116 and A11.115 cells, which are the progenies of *Cdx* positive A9.29 cell, contribute to the nerve cord (*80*); in contrast, Cole and Meinertzhagen interpreted the progenies of A11.116 cell as the visceral ganglion, on the basis of anatomical data (*160*). In vertebrates, a landmark for the boundary between the spinal cord and hindbrain is the anterior limit of expression of *Hox5* (*59*); however in *Ciona*, *Hox5* exhibits a dynamic expression pattern in the progeny of A11.115 and A11.116 cells (*107*), and expressional overlapping with *Cdx* is still unknown. In any event, in *Cdx*-MO-injected embryos, *Engrailed*, *COE*, *Lhx3* and *Neurogenin*, all of which are normally expressed in the progenies of A9.30 cell, *i.e.*, the cells that contribute to the visceral ganglion, exhibit ectopic expression in A11.116 and A11.115 cells (*80*). That is, in *Ciona*, down-regulation of *Cdx* function results in posterior expansion of the visceral ganglion at the expense of the nerve cord, or at least posteriorization of the visceral ganglion. This reminds us that loss of *Cdx* function causes posterior expansion of the hindbrain at the expense of trunk and tail in zebrafish (*161, 162*). In this case, inhibiting the caudal-related genes *cdx1a* and *cdx4* in zebrafish embryos causes ectopic expression of genes (including Hox genes) that are normally expressed in the posterior hindbrain and anterior spinal cord, and leads to posterior, mirror-image duplication of posterior hindbrain and anterior spinal cord (*161, 162*). The authors concluded that *Cdx1a* and *Cdx4* repress posterior hindbrain-specific gene expression, including Hox genes, in the posterior neural tissue by modifying the competence of these tissues to respond to the Fgf and RA signals (*161*). By contrast, in *Ciona*, the posterior expansion of the visceral ganglion does not reach the

posterior end of the nerve cord, and the mirror-image duplication demonstrated in zebrafish has not been observed; however, we should keep in mind the difference between the phenotype induced by MO and that by the dominant negative *Cdx*. Furthermore, the effect of functional inhibition of *Cdx* on expression of Hox genes is not known in *Ciona* [I suspect that in *Halocynthia*, expression of *Hox1* in the neuroectoderm is not affected by treatment with PO (*78*)], although Cdx proteins are known to directly regulate the expression of the posterior Hox genes through direct binding to the *cis*-regulatory elements of the Hox genes (*163-167*). Thus, *Ciona Cdx* may play a role in inhibiting the posterior expansion of the visceral ganglion in the anterior nerve cord; however, the gene regulatory relationships among Fgf, Wnt, RA, Cdx and Hox in the posterior body seem to be different from those observed in vertebrates, as we showed previously (*63*). In fact, in *Ciona*, Nodal is an activator of *Cdx* expression (*79*), whereas in vertebrates expression of Cdx in the posterior region of developing embryos is regulated by extracellular signals, such as RA, Wnt, and FGF (*168*). Further exploration of the molecular network regulating ascidian tail development will be very interesting subject for future research.

In amphioxus, the neural *Cdx* expression seems to reach up to the anterior boundary of somite five at the earliest neurula stage. Thereafter, *Cdx* in neural tube has strong posterior expression, decreasing in a gradient toward the anterior. Later on, the anterior end of this gradient becomes definitely more posterior than the somite five level. Finally, after one week of development, anterior neural expression is down-regulated. This expression pattern is very similar to that of *Cdx-1* in mouse (*169, 170*). Meyer and Gruss indicated a possible relation between this retracting pattern of Cdx and the expression of clustered Hox genes (*169*), and Gaunt *et al* obtained the experimental results consistent with a model, in which anterior boundaries of Hox gene expression become positioned along a developing instructional Cdx protein gradient (*164*). Although it has been revealed that RA-signaling controls expression of five Hox genes in the amphioxus CNS (*53*), the presence of synergic regulation with *Cdx* and/or other signaling mechanisms cannot be ruled out.

Conclusion

Since discovery of the Hox cluster in *Drosophila* as the gene complex associated with homeotic transformation (*171-173*) and following findings of the homologous genes in vertebrates (*1, 3, 174, 175*), the conserved aspects of these genes have come under the spotlight. This attention has led to the notion that Hox genes play a central role in the AP patterning throughout animal phylogeny (*35, 176-179*). Subsequently, we have recognized that changes in Hox gene numbers, sequence, and regulation are responsible for body plan evolution and diversification (*36, 180*). Recently, it has been pointed out that there are significant variations in the level of organization of the Hox and ParaHox genes as well as their expression patterns through eumetazoans (*8, 97, 138, 140, 181-185*). As shown in this review, in invertebrate deuterostomes as well, there are notable differences in the physical organization on the chromosome, expression pattern, and functions of the Hox genes and ParaHox genes. In this review, I described clues that may help in reconstructing the common ancestor of deuterostomes, and in understanding the basic roles of Hox and ParaHox genes in the development of the body plan of the ancestral animal, as well as their roles in the subsequent evolution. Currently, however, information is still too limited to establish a clear view for them. As I have noted at the end of the every section, a number of critical tasks remain. I hope that the research subjects crystallized in this review will inspire future studies of deuterostome evolution and the roles of Hox and ParaHox genes in this process.

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