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REVIEW

Evolution of basal deuterostome nervous systems

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ABSTRACT

Understanding the evolution of deuterostome nervous systems has been complicated by the ambiguous phylogenetic position of the Xenocoelomorpha (Xenoturbellids, acoel flat worms, nemertodermatids), which has been placed either as basal bilaterians, basal deuterostomes or as a sister group to the hemichordate/echinoderm clade (Ambulacraria), which is a sister group of the Chordata. None of these groups has a single longitudinal nerve cord and a brain. A further complication is that echinoderm nerve cords are not likely to be evolutionarily related to the chordate central nervous system. For hemichordates, opinion is divided as to whether either one or none of the two nerve cords is homologous to the chordate nerve cord. In chordates, opposition by two secreted signaling proteins, bone morphogenetic protein (BMP) and Nodal, regulates partitioning of the ectoderm into central and peripheral nervous systems. Similarly, in echinoderm larvae, opposition between BMP and Nodal positions the ciliary band and regulates its extent. The apparent loss of this opposition in hemichordates is, therefore, compatible with the scenario, suggested by Dawydoff over 65 years ago, that a true centralized nervous system was lost in hemichordates.

KEY WORDS: Cephalochordate, Deuterostomes, Echinoderm, Hemichordate, Nervous system evolution

Introduction

Deuterostomes include at least the Chordata (vertebrates, tunicates and cephalochordates) and the Ambulacraria (echinoderms and hemichordates). The enigmatic Xenoturbellida has been placed either basally in the deuterostomes or together with acoel flatworms as the Xenocoelomorpha – the sister group of the Ambulacraria. Alternatively, the Xenocoelomorpha have been placed basal to the bilateria (reviewed in Achatz et al., 2013). *Xenoturbella* has a very simple body plan with a nerve net and is thought to have lost a number of ancestral features (Philippe et al., 2011). Therefore, it is unclear whether it is at all relevant to the evolution of deuterostome nervous systems. However, there is no question that echinoderms and hemichordates are deuterostomes. In most phylogenetic analyses, Ambulacraria are the sister group of the chordates (Blair and Hedges, 2005; Delsuc et al., 2008; Edgecombe et al., 2011). However, a recent analysis based on 586 nuclear genes places cephalochordates as the sister group of the Ambulacraria with high bootstrap support and cephalochordates plus Ambulacraria as the sister group of tunicates plus vertebrates (Moroz et al., 2014). Because the nervous systems of echinoderms, hemichordates and chordates are so very different, it has been controversial whether the ancestral deuterostome had a longitudinal nerve cord or a nerve net or a combination of both.

Several schemes for the evolution of deuterostome nervous systems have focused on the possible contribution of the larval nervous system to that of the adult (Figs 1, 2). All five classes of echinoderms (crinoids, holothurians, asteroids, ophiuroids and echinoids) have indirect development with pelagic larvae, although direct development has evolved independently in several lineages. Within the echinoderms, crinoids are basal to ophiuroids, asteroids, echinoids and holothurians. Adult echinoderms have in common a circumoral nerve ring plus five radial nerve cords (Fig. 2, Fig. 3C). The nerve ring and the outer parts of the nerve cord belong to the ectonerual compartment, whereas the inner layer of the nerve cords is the hyponeural system. These two systems were thought to be separate; however, fine-structural studies and histochemistry of neurotransmitters in sea cucumbers have indicated that both systems are derived from ectoderm and are interconnected (Mashanov et al., 2007; Hoekstra et al., 2012). Moreover, Echinoderms also have numerous ectodermal sensory cells.

In contrast, the nervous systems of adult hemichordates consist of both dorsal and ventral nerve cords plus a relatively neurogenic ectoderm (Figs 4, 5). The collar nerve cord undergoes neurulation, but there is no discrete brain such as that in chordates. Traditionally one or the other nerve cord of hemichordates has been proposed to be homologous to the chordate nerve cord. There are six families of hemichordates: Torquaratoridae, Ptychoderidae, Spengelidae, Harrimaniidae, Rhabdopleuridae and Cephalodiscidie. The first three have indirect development and the last three have direct development. Phylogenetic analysis groups Ptychoderidae and Torquaratoridae, but otherwise fails to resolve which of the five groups is basal. Therefore, it is impossible to know whether the hemichordate ancestor had direct or indirect development. However, the resemblance of the tornaria larva of enteropneust hemichordates to larvae of echinoderms has led to the suggestion that a pelagic larva is basal for the Ambulacraria (Amemiya et al., 2005; Nielsen, 2009).

Unlike echinoderms and hemichordates, chordates (amphioxus, tunicates and vertebrates) typically have direct development. Although the ascidian tadpole undergoes a drastic metamorphosis with loss of most of the nerve cord, the basal appendicularians retain the larval nervous system in the adult as do cephalochordates and vertebrates. Because of these differences in the mode of development, some researchers maintain that the deuterostome ancestor had direct development (Raff, 2008; Northcutt, 2005).

Larva-first theories of the evolution of deuterostome nervous systems

The idea that ancestral deuterostomes had a pelagic larva or even that the ancestral deuterostome was a pelagic, larval-like adult, has engendered larva-first theories of nervous system evolution. Garstang (Garstang, 1894) initially proposed that adults of the deuterostome ancestor resembled the auricularia larva of holothurian echinoderms (Fig. 1), although he later changed his mind (Garstang, 1928). Even so, the idea that the ancestral deuterostome resembled an echinoderm larva was revived by Davidson et al. (Davidson et al., 1995), but not widely accepted (Sly et al., 2003). According to Garstang, three evolutionary lines came from this ancestor. One line

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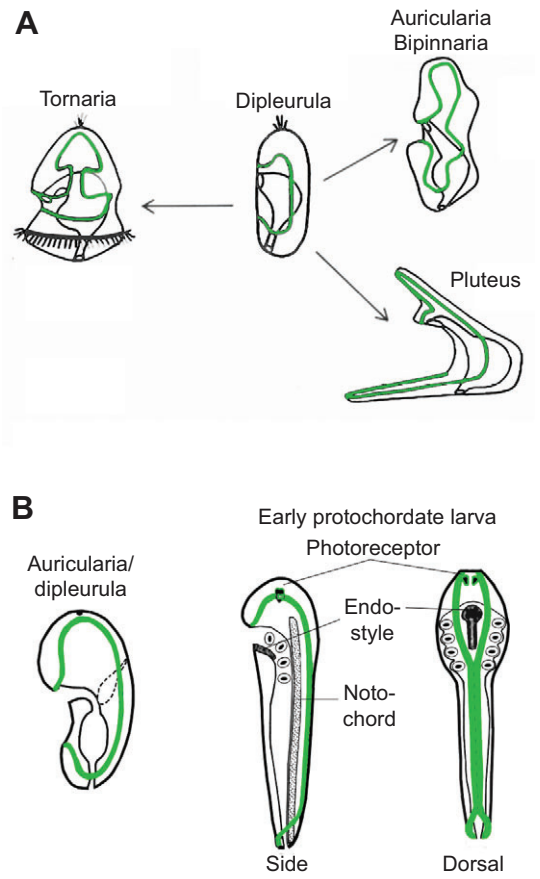

Fig. 1. A 'larva-first' scheme proposed by Garstang and Garstang. (A) The tornaria larva of hemichordates and echinoderm larvae (auricularia, bipinnaria, pluteus) are thought to have arisen from a dipleurula-type larva. (B) This dipleurula larva gave rise to protochordate larvae. In the view of the Garstangs, the protochordate-type larva of a sessile ascidian then gave rise to chordates by neoteny (Garstang and Garstang, 1926). The chordate nervous system evolved by fusion of the ciliary bands (green).

was proposed to evolve into modern echinoderms, a second line evolved into hemichordates and the third line evolved into chordates (Fig. 1). This common ancestor was characterized by a ciliary band looping around the mouth and a posterior anus. There were two anterior pits similar to the eyespots in some tornaria larvae of hemichordates. There was also an adoral ciliated band. The central nervous system (CNS) was an oval nerve ring directly underlying the circumoral ciliated ring. The circumesophageal nerve ring of adult echinoderms arose by ventral migration of the nerve-ring of this hypothetical ancestor. The pre-oral sense organs disappeared in echinoderms, but evolved into photoreceptors in chordates. In the evolution of enteropneusts from this hypothetical ancestor, the lateral halves of the circumoral ciliated band were divided into pre-oral and post-oral bands. The ciliated post-oral band fused to become the collar nerve cord.

Garstang envisioned that chordates evolved from this larva-like ancestor as the circumoral nerve ring migrated dorsally and the two sides fused in the dorsal midline. As they fused, a canal remained, which was internally ciliated and communicated with the gut near the blastopore (neurenteric canal) (Fig. 1B). The pre-oral pits became enlarged and turned into the optic vesicles of vertebrates. The adoral ciliated band evolved into the peripharyngeal bands and the marginal ciliated bands of the tunicate endostyle. Further, he speculated that 'A primitive Chordate tadpole may well have arisen from an elongated Auricularia-like larva which acquired the power of undulating from side to side'. The circumoral band and its underlying nervous system were regarded as predecessors of the medullary folds, and the adoral band with its ventral loop as the predecessor of the peripharyngeal band and endostyle. According to Garstang, a protochordate larva arose from this auricula by elongating and becoming muscular. The lateral halves of the circumoral band and its underlying nerve tract were brought into closer parallelism on either side of the dorsal mid-line. Somehow, an increase in yolk resulted in the larvae being hatched as muscular tadpoles with the circumoral band and associated nervous system and apical sense-organs being rolled up as a neural canal (Garstang, 1928). He argued that the chordate gill slits and the notochord came from adult pterobranch ancestors, which are sessile colonial hemichordates, and the endostyle and neural canal resulted from the further development of early larval characters. This theory had its roots in the idea that ontogeny recapitulates phylogeny. However, Garstang had serious doubts about recapitulation (Garstang, 1922; Garstang and Garstang, 1926).

The theory of Walter Garstang (Garstang, 1894) that echinoderms, enteropneust hemichordates and chordates evolved from an ancestor that resembled an auricularia larva (the dipleurula) has been seriously considered (Lacalli, 1994). However, it too suffers from the lack of persistence of the larval nervous system into the adult stage of echinoderms and hemichordates. More recently, Lacalli (Lacalli, 2010) reviewed Garstang's theory and the modifications of Romer (Romer, 1972) together with more recent molecular data and concluded 'dipleurula-type larvae are poor candidates to be transitional forms in any evolutionary scenario leading to advanced chordates'. Moreover, Lacalli noted that 'the molecular data has (sic) so far been effective in terms of reducing the number of viable hypotheses, but less so in generating new ideas about the true nature of the ancestral forms. The essential problem has not changed. It is one of recovering… some understanding of now extinct groups of organisms and it is not at all clear how to proceed'.

A variation on the theme of an echinoderm larva-like ancestor of deuterostomes, and in fact of all bilaterians, was proposed by Davidson and colleagues. According to this scheme, this larva-like adult developed 'set-aside cells', not unlike the adult rudiment of an echinoid larva. At some point prior to the Cambrian, these cells evolved into adult bilaterians and the former adult body plan persisted as a larva (Peterson et al., 2000). However, this theory also gave way to ideas that the pelagic larva was intercalated into the life history of a direct developing bilaterian ancestor (Sly et al., 2003).

Genes and development have inspired several ideas on evolution of deuterostome nervous systems

The discovery that *Hox* genes are co-linearly expressed in the hindbrain and spinal cord of vertebrates, cephalochordates and insects inspired three schemes for evolution of the echinoderm radial and circumoral nerves. One proposes the circularization of an ancestral nerve cord. The second argues that a circular nerve cord was formed by splitting of a longitudinal nerve cord down the center with the ends remaining attached (Fig. 3) and the third that the ancestral nerve cord duplicated five times and formed a ring at one end (Popodi et al., 1994).

Other schemes have been proposed that involve an ancestor (stem group echinoderm) with a longitudinal nerve cord and a pelagic larva with an apical nervous system, which is carried over into the adult (Fig. 2) (Burke, 2011). According to this scheme, the radial

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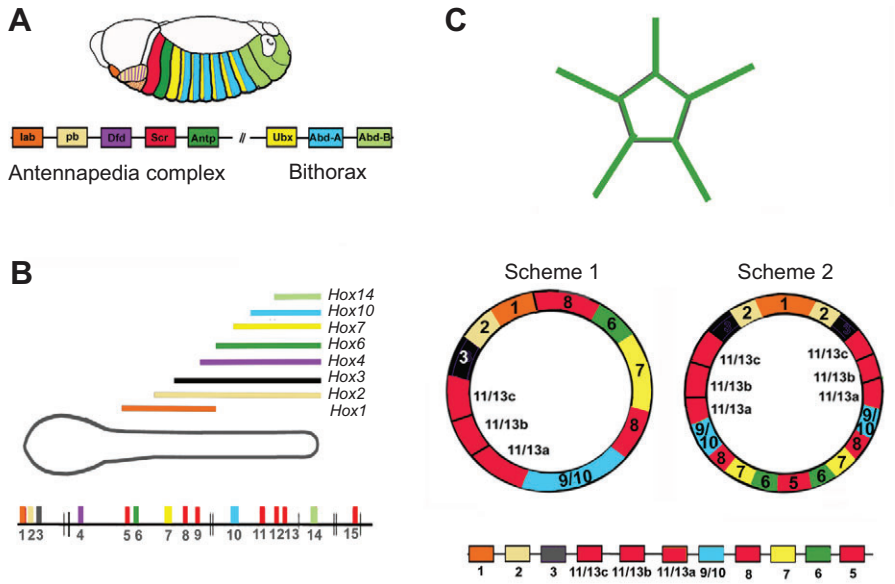

Fig. 2. Two schemes proposed by Popodi et al. for evolution of the echinoderm nerve cords. (A,B) *Hox* genes are co-linearly expressed in the developing CNS of flies and chordates. (C) Echinoderms have a circumoral nerve cord and five radial nerve cords (green). The *Hox* cluster has been rearranged with posterior genes in the middle of the cluster. In one scheme, an ancestral longitudinal CNS circularized and the two ends fused. In a second scheme, the ancestral linear nerve cord split in the center, but not at the ends, resulting in a duplication of the domains of *Hox* genes (Popodi et al.,1994). Unfortunately for these schemes, *Hox* genes are not sequentially expressed in the developing adult sea urchin CNS (Arenas-Mena et al., 1998; Arenas-Mena et al., 2000).

nerves evolved by duplication of the ancestral nerve cord, whereas the apical nervous system remains a separate entity in the adult. Only in the chordates did the two nervous systems unite with the anterior concentration of neurons becoming the brain. A variation on this theme is that a brain evolved from an anterior apical nervous system and a blastoporal nervous system. The former is neurosecretory and the latter involves motor centers. These two systems fused to form a forebrain in the bilaterian ancestor (Tosches and Arendt, 2013). A final scheme is that the deuterostome ancestor had parallel five nerve cords and an anterior brain, and these nerve cords evolved into the radial nerves of echinoderms (Kuznetsov, 2012).

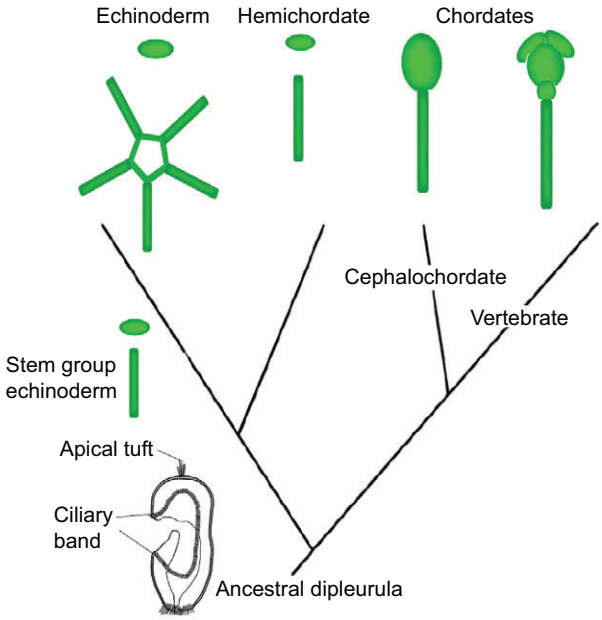

Fig. 3. 'Larva-first' scheme proposed by Burke. In this scheme, the ciliary band of a doliolaria larva gave rise to the longitudinal nerve cord of an ancestral Ambulacrarian and the apical tuft evolved into a separate anterior nervous system. In the echinoderm lineage, the nerve cord evolved into the circumoral nerve ring and radial nerves. These two nervous systems remained separate in hemichordates, but united in chordates. After Burke (2011).

Each of these schemes has major drawbacks. The schemes proposed by Popodi et al. (Popodi et al., 1994) (Fig. 3) were abandoned when it turned out that *Hox* genes are not expressed in the developing adult nerve cord of sea urchins, but are instead expressed in the coeloms (Arenas-Mena et al., 2000). This indicates that the echinoderm nerve cords are probably not homologous to those of chordates. Schemes deriving all or part of the echinoderm nervous system from a larval nervous system have the major problem that in protostome larvae and the larvae of echinoderms and hemichordates, the larval nervous system does not persist during metamorphosis (Nakano et al., 2010; Elia et al., 2009; Miyamoto et al., 2010). Moreover, at least one gene (*Onecut*, orthologous to *HNF6*), which is expressed in the apical organ of sea urchins, is not expressed in the chordate brain (Poustka et al., 2004). Even in a direct developing hemichordate, the embryonic nervous system appears to be transient; whether any of the neurons are incorporated into the adult nervous system is not known (Cunningham and Casey, 2014).

Hemichordates have played a key role in theories of the evolution of the chordate CNS

Hemichordates have featured prominently in theories concerning evolution of the chordate CNS; however, they present particular problems because they have two nerve cords, plus a relatively neurogenic ectoderm but lack a discrete brain. Therefore, some people have postulated that the dorsal nerve cord is homologous to the chordate CNS, others have proposed homologies between the ventral nerve cord and the chordate CNS, and yet others have argued that neither hemichordate nerve cord is homologous to the chordate CNS, which evolved independently, perhaps by condensation of part of the ectoderm of a hemichordate-like ancestor. For example, Dawydoff (Dawydoff, 1948) regarded the hemichordate nerve cords as localized condensations of the sub-epidermal diffuse nervous system (Fig. 4). He stated that a true CNS, in a morphological sense, did not exist in enteropneusts (all hemichordates except the pterobranchs), which probably lost it in the course of evolution. This is still a viable idea, although subsequent authors have expressed every other opinion concerning the relationship of the hemichordate nerve cords to the chordate nerve cord. In contrast, Kaul and Stach (Kaul and Stach, 2010) suggested that neurulation of the collar nerve cord, which has a ventral neuropile, is homologous to that in

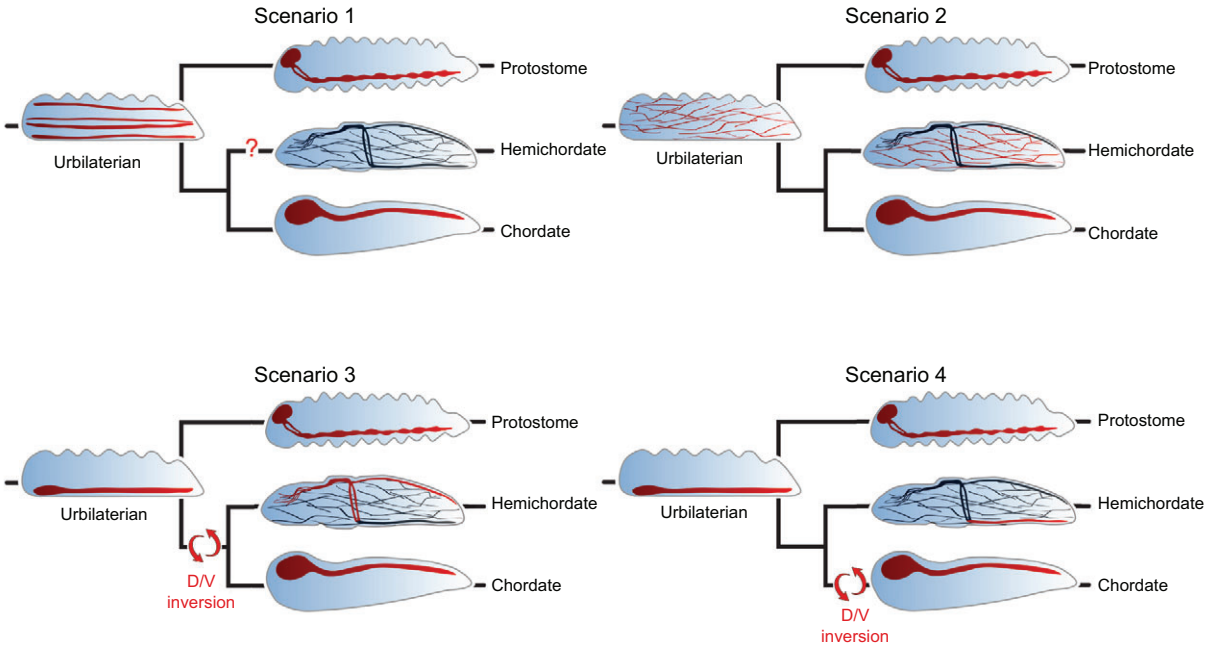

Fig. 4. Four hypotheses for evolution of bilaterian nerve cords. In scenario 1, the bilaterian ancestor had multiple nerve cords rather like a modern acoel flatworm. These nerve cords gave rise to nerve cords in bilaterians. In scenario 2, the ancestral bilaterian had a nerve net. Nerve cords in hemichordates, chordates and protostomes evolved independently. In scenario 4, the ancestral bilaterian had a ventral nerve cord, which gave rise to nerve cords in bilaterians. A dorsal-ventral inversion occurred at the base of the deuterostomes. In scenario 4, the dorsal ventral inversion occurred in the chordate lineage. From Holland et al. (Holland et al., 2013).

chordates and that a centralized nervous system preceded the origin of chordates. Nomaksteinsky et al. (Nomaksteinsky et al., 2009), who also examined the development of the nerve cords in the direct developer *Saccoglossus kowalevskii*, observed that the ectoderm of adults of this species was not very neural and suggested that the common ancestor of chordates and hemichordates had a CNS that was separate from a peripheral nervous system. However, they could not decide whether the dorsal or ventral nerve cord is the homolog of the chordate nerve cord.

Most recently Lowe and co-workers have focused on the nerve net in the larval ectoderm of *S. kowalevskii* as being the real nervous system (Lowe et al., 2003; Pani et al., 2012). The logical extension of this view is that the ancestral deuterostome had a nerve net and that the hemichordate and chordate nerve cords evolved independently (reviewed in Holland, 2003) (scenario 2, Fig. 4). In the direct-developing *S. kowalevskii*, the larval ectoderm, particularly in the proboscis, the most anterior of the three body divisions (proboscis, collar and trunk) contains numerous neurons. The larval ectoderm expresses homologs of many genes that mediate global patterning in chordates as well as homologs of some chordate genes with expression restricted to the CNS (Fig. 5). However, expression of genes such as *Hox* genes, *Pax6* and *Gbx* in the hemichordate ectoderm are not especially useful for inferring homologies with the chordate CNS, because expression of these genes in chordates is not limited to the CNS; they are also expressed in the general ectoderm, which contains many ectodermal sensory cells (Castro et al., 2006; Glardon et al., 1998). Even so, homologs of a number of genes that are expressed in the anteriormost CNS in chordates are expressed in the anterior proboscis ectoderm in *S. kowalevskii*. These include *Six3/6*, *Fgf8/17/18*, *Dlx* and *Rx* among others. *Otx* is expressed in the hemichordate ectoderm – very weakly in the proboscis and strongly in the collar and somewhat posterior to the collar (Lowe et al., 2003) – and in chordates is expressed in the neuroectoderm, but not in the general ectoderm (Tomsa and Langeland, 1999; Williams and Holland, 1996). These expression patterns lend credence to the idea that the proboscis ectoderm and chordate brain may be evolutionarily related.

Although Pani et al. (Pani et al., 2010; Pani et al., 2012) did not claim structural homology between the hemichordate ectoderm and chordate CNS, they argued that homologous genetic programs mediated regionalization of the hemichordate CNS and the vertebrate brain. The implication was that as the chordate CNS evolved from the ectoderm of an ancestral hemichordate-like organism, the genetic programs partitioning the ectoderm into the anterior neural ridge (ANR), zona limitans intrathalamica (ZLI) and midbrain/hindbrain boundary [MHB, termed the isthmic organizer (ISO) in vertebrates] were carried over into the chordate CNS. This argument is tantalizing, but has some difficulties – chiefly that the domains of some key genes that partition the chordate CNS are not similarly expressed in the hemichordate ectoderm. For example, *Otx* and *Gbx* are expressed in domains that abut at the MHB/ISO in the amphioxus and vertebrate nerve cords (Castro et al., 2006), but in the *S. kowalevskii* ectoderm, their domains broadly overlap (Pani et al., 2012) (Fig. 5). In vertebrates, *Otx* and *Gbx* mutually repress one another and have been shown to position the MHB; they likely do so as well in amphioxus. Similarly, in vertebrates, the domains of *Fezf* and *Irx*, which mutually repress each other, abut at the ISO and in amphioxus abut about the midpoint of the diencephalon (Irimia et al., 2010). In *S. kowalevskii*, *Otx*, *Gbx*, *Fezf* and *Irx* are not expressed as they are in chordates (Pani et al., 2012) (Fig. 5). Therefore, although the similarities in gene expression and gene interactions between the hemichordate ectoderm and chordate CNS suggest some degree of inheritance from a common ancestor, it is unlikely that the ISO and MHB organizers were inherited from the common ancestor of Ambulacraria and chordates unless this ancestor, as discussed below, was more like a chordate than an ambulacrarian.

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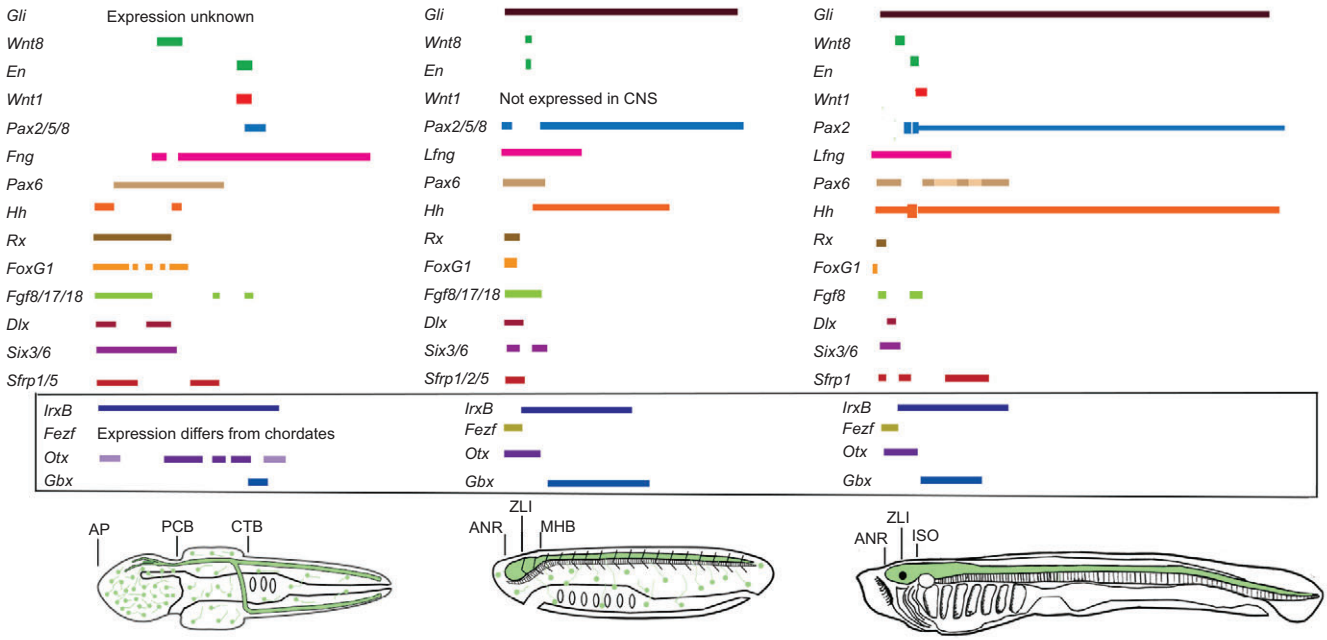

Fig. 5. Expression of anterior–posterior patterning genes in the ectoderm of the hemichordate *Saccoglossus kowalevskii* (left) and in the CNS of amphioxus (*Branchiostoma floridae*) (middle) and a generic vertebrate (right). In vertebrates, *Irx* and *Fezf*, which oppose each other define the zona limitans intrathalamica (ZLI) (Rodríguez-Seguel et al., 2009), whereas opposition between *Otx* and *Gbx* positions the midbrain–hindbrain boundary or isthmic organizer. These genes are comparably expressed in amphioxus, but not in the ectoderm of *S. kowalevskii*. Genes that specify the anterior neural ridge (ANR) are similarly expressed at the anterior tip of the CNS in amphioxus and vertebrates, and at the anterior end of the ectoderm in *S. kowalevskii.* Some other genes, such as *Wnt8*, are similarly expressed in the *S. kowalevskii* ectoderm and the chordate CNS, whereas others, such as *Wnt1* and *engrailed* are expressed at the collar–trunk boundary (CTB) in *S. kowalevskii,* at the boundary between fezf and Irx in amphioxus (the ZLI) and at the boundary between *Otx* and *Gbx* (the isthmic organizer, ISO) in vertebrates.

While these interpretations suggested that the modern *S. kowalevskii* larva might provide a proxy for the last common ancestor of vertebrates and Ambulacrarians and that chordate nerve cords had evolved independently, there are many caveats. First, very little is known about gene expression in the developing nerve cords of hemichordates. Cunningham and Casey (Cunningham and Casey, 2014) determined expression of several genes that are involved in specification of neural competence, neuronal specification and neuronal differentiation in embryos of *S. kowalevskii* from the gastrula stage (24 h) to the stage when the three body regions (trunk, collar and proboscis) are demarcated (72 h). In general, expression of these genes was relatively broad in the ectoderm of early embryos and became increasingly restricted to the nerve cords as development proceeded, although these genes continued to be expressed at high levels in the proboscis ectoderm. They concluded that the diffuse embryonic nervous system of *S. kowalevskii* is transient and reorganizes before hatching, into the centralized adult nervous system (Cunningham and Casey, 2014). Whether this is a true reorganization involving ectodermal neurons migrating into the nerve cords or a death of the ectodermal neurons and birth of new neurons in the nerve cords was not studied. Such information would be vital to an understanding of the evolutionary significance of the larval ectodermal nervous system in *S. kowaleveskii*. As Bullock (Bullock, 1945) noted, ‘the greater part of the adult enteropneust skin is non-neural’. Moreover, in an indirectly developing hemichordate, *Balanoglossus simodensis*, monitoring of apoptosis together with labelling of all neurons with an anti-synaptogamin antibody showed that the larval nervous system does not carry over into the adult even though the gut, mouth and anus do carry over (Miyamoto et al., 2010). As in *S. kowalevskii*, in *B. simodensis*, the adult nervous system begins to develop fairly early in embryogenesis and is completed in the young adults. These studies raise the possibility that the adult nervous system of hemichordates is more relevant than the larval system to an understanding of evolution of chordate central nervous systems. Thorough studies of development of adult hemichordate nervous systems, including determining the expression of a wide range of neuronal-specific genes (such as *VChat*, *GAD*, etc.), together with sections giving single-cell resolution, would be highly valuable.

A novel scenario for evolution of hemichordate nervous systems

It is virtually certain that vertebrate nerve cords evolved from the nerve cord in an amphioxus-like ancestor. Chordates have a CNS with a brain as well as a variety of ectodermal nervous structures (placodes in vertebrates; sensory cells in cephalochordates and tunicates). Similar patterns of gene expression in nerve cords of protostomes and deuterostomes have led some researchers to suggest that the ancestral bilaterian had a CNS with a fairly sophisticated brain (Strausfeld and Hirth, 2013a; Strausfeld and Hirth, 2013b; Tomer et al., 2010). If it is true that the ancestral bilaterian had a central nervous system with a brain, then it follows that the CNS became reduced in hemichordates and a second nerve cord evolved.

A clue as to how this might have happened lies in experiments in the cephalochordate *Branchiostoma floridae* involving manipulation of the levels of Nodal and bone morphogenetic protein (BMP), two secreted signaling proteins in the TGFβ superfamily. They signal through different, but related transmembrane receptors and act in opposition. It has been reported that they physically interact, preventing binding to their receptors (Haramoto et al., 2006). In amphioxus, opposition between Nodal and BMP signaling regulates

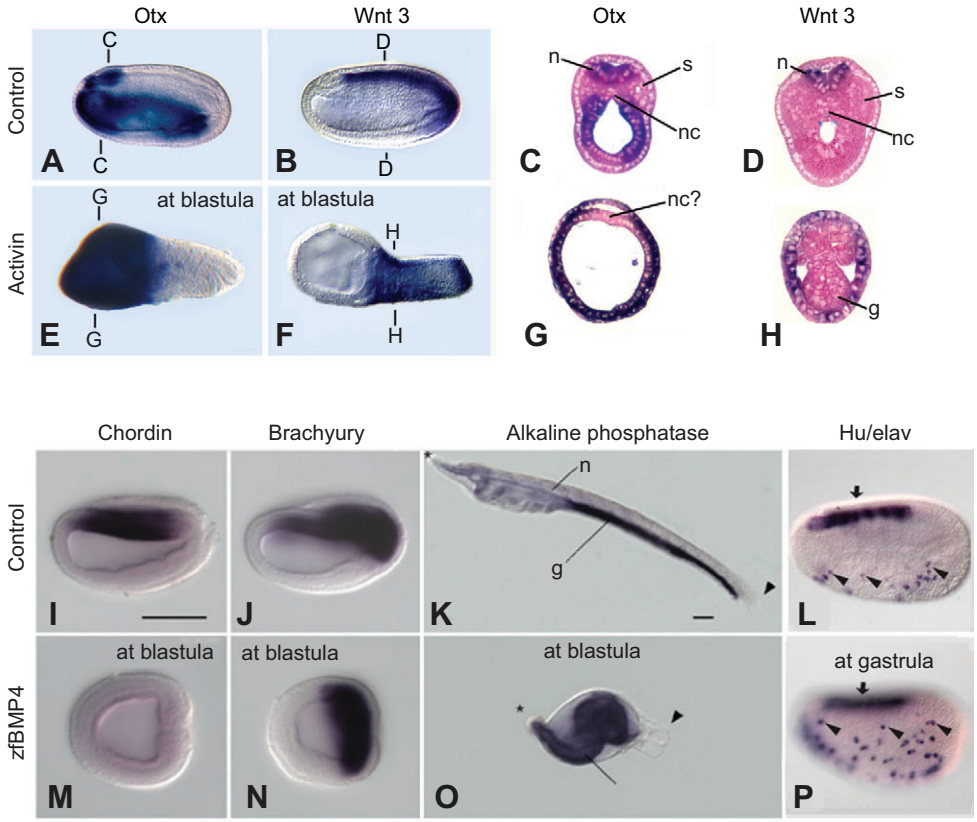

Fig. 6. A switch from a central nervous system to a 'skin brain' is effected in the basal chordate amphioxus (*Branchiostoma floridae*) by altering the ratio of the secreted TGFβ proteins, Nodal and BMP from the early blastula. Changing this ratio at the gastrula stage, affects development of the ectodermal sensory cells. (A–H) 10 ng ml^{-1} human activin applied to amphioxus embryos at the early blastula stage converts the entire ectoderm to neurectoderm and enlarges the forebrain region. (A,B) Control embryos at the neurula stage showing expression of *Wnt3* in the forebrain and endoderm and *Otx* in the hindbrain and spinal cord. (C,D) Cross-sections through neurulae at levels indicated in A and B. The neural tube has begun to round up. (E,F) Addition of activin eliminates the neural tube and converts the entire ectoderm to neurectoderm. *Otx* is expressed throughout the expanded anterior ectoderm and *Wnt3* throughout the entire posterior ectoderm. (G,H) Cross sections through levels indicated in E and F. (I–K,M–O) Addition of 250 ng ml^{-1} zebrafish BMP4 to amphioxus embryos at the blastula stage has the opposite effect as addition of activin at the same stage. (I–K) Control embryos. (I) *Chordin* is expressed in dorsal mesoderm and overlying neural plate. (J) *Brachyury* is expressed in the developing notochord and around the blastopore. (K) At the early larval stage, the gut endoderm (g) expresses alkaline phosphatase. Arrowhead indicates the tail; asterisk indicates the anterior tip of the larva. (M–O) Addition of zebrafish BMP4 at the gastrula stage eliminates all dorsal structures. *Chordin* is no longer expressed and *brachyury* expression is limited to tissue around the blastopore. (O) At the early larval stage, the head and all dorsal structures are eliminated. (L,P) When addition of BMP4 protein is delayed until the gastrula stage, the neural plate (arrows) is still specified as shown by expression of the pan-neural marker *Hu/elav*, but the number of ectodermal sensory cells (arrowheads) is increased and their position is dorsalized. g, gut; n, neural plate or neural tube; nc, notochord; s, somites. A–H from Onai et al. (Onai et al., 2010). I–K,M–O from Yu et al. (Yu et al., 2007). L,P from Lu et al. (Lu et al., 2012).

axial patterning in early development (Onai et al., 2010). The effects of manipulating the amount of Nodal and BMP are stage specific. Upregulation of Nodal signaling at the blastula stage dorso-anteriorizes amphioxus embryos, eliminating the neural tube and converting all the ectoderm into neuroectoderm (a skin-brain), whereas BMP2/4 signaling ventral-posteriorizes embryos, eliminating all dorsal structures, including the head and the entire CNS (Fig. 6) (Onai et al., 2010; Yu et al., 2007). However, if addition of BMP2/4 is delayed until the mid-gastrula stage, the CNS forms, but the number of ectodermal sensory neurons expressing *elav* is increased and they are positioned more dorsally (Onai et al., 2010; Lu et al., 2012).

Similarly, in sea urchin larvae, the secreted proteins Nodal and BMP2/4, which mutually repress one another, establish both dorso–ventral polarity and the position of the ciliary band. Upregulation of Nodal signaling shifts most of the ectoderm to a ventral fate; reduced Nodal signaling causes most ectoderm to become ciliary band. If BMP2/4 is reduced, an ectopic ciliary band forms (Molina et al., 2013). This suggests that Nodal vs BMP is a fundamental mechanism in deuterostomes for allocation of ectoderm to CNS or ectodermal sensory cells and for regulating where and how many ectodermal cells will be allocated to each compartment.

In fact, it is possible that a role for Nodal and BMP acting in opposition in the regulation of neural territories was present in the ancestral bilaterian (Fig. 7). In the annelid *Platynereis dumerilii*, BMP added at 24 hours post fertilization (between the protrochophore and early trochophore larva), did not change expression of the neural differentiation marker *elav*, but strongly upregulated expression of the sensory neuron marker *ath* in the lateral peripheral nervous system anlage and expanded the number of cells expressing the gene (Denes et al., 2007). This is comparable to addition of BMP at the late gastrula in amphioxus and supports the idea that BMP regulation of neuronal subtypes was present in the bilaterian ancestor. An apparent *Nodal* sequence has been described from the annelid *Capitella telata* (gb|ELT90528.1), and it would be most interesting to know how it is expressed in development and whether upregulation of Nodal signaling would have an effect on the CNS and/or peripheral

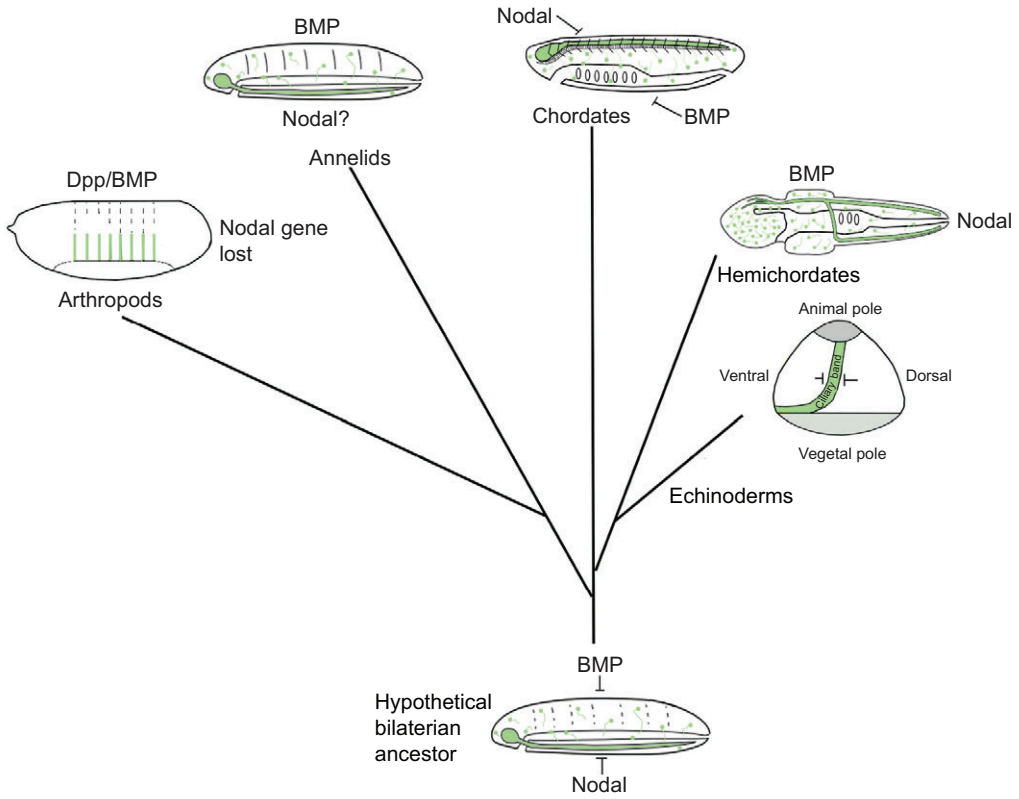

Fig. 7. Scheme for evolution of central nervous systems through opposition of Nodal and BMPs. The hypothetical bilaterian ancestor is envisioned as having a ventral nerve cord, the position of which is regulated by opposition of dorsal BMP signaling and ventral Nodal signaling. In sea urchin larvae, antagonistic Nodal and BMP expression positions the ciliary band. In the hemichordate *S. kowalevskii*, BMP is expressed dorsally and Nodal posteriorly (Wlizla, 2011). Upregulation of Nodal signaling posteriorizes embryos. Excess BMP has no effect on the number or position of ectodermal neurons. Chordates are inverted dorsal–ventrally with respect to the hypothetical bilaterian ancestor, annelids, arthropods, hemichordates and echinoderms. In the basal chordate amphioxus, opposition between Nodal and BMP signaling regulates development of a CNS and ectodermal sensory neurons. This is comparable to the situation in sea urchins, but differs from that in hemichordates, suggesting that the role of Nodal may have changed in hemichordates, shifting from a role in controlling centralization of the CNS to a role in posterior patterning. BMP is expressed dorsally in annelids and arthropods. Annelids have a *Nodal* gene, but expression is unknown, whereas arthropods have lost *Nodal*.

nervous system. Nodal signaling in protostomes has not received the attention it deserves because as *Drosophila* lacks a *Nodal* gene, it was assumed that *Nodal* was specific for deuterostomes. However, studies in molluscs showed that *Nodal* is indeed present in protostomes and mediates left–right patterning, as in chordates (Grande and Patel, 2009).

In contrast, in *S. kowalevskii*, although *BMP* is expressed dorsally, *Nodal* is expressed posteriorly. Moreover, excess BMP does not appear to affect the number of basiepithelial neurons in *S. kowalevskii*, and Nodal appears to posteriorize only the ectoderm (Lowe et al., 2006; Wlizla, 2011). This suggests that the antagonistic role of BMP vs Nodal in specification of neuronal territories was lost in hemichordates. If so, the CNS – in particular the brain – in the common ancestor of cephalochordates and Ambulacraria might have become reduced in hemichordates, with the brain becoming spread over the proboscis ectoderm. Decoupling of BMP and Nodal could then have allowed a second nerve cord to evolve in hemichordates. Although this scenario is pure speculation, the idea that Nodal vs BMP is a fundamental bilaterian mechanism for regulation of neural territories would be strengthened if it turns out to hold for protostomes, annelids in particular. Moreover, the surprising result that three of four phylogenetic trees published in connection with the ctenophore genome paper placed cephalochordates as sister group of the Ambulacraria (Moroz et al., 2014), strengthens the notion that the common ancestor of Ambulacraria and Chordata just might be more like a cephalochordate than a hemichordate. If so, the theory that the ancestral bilaterian had a CNS would be considerably strengthened.

Conclusions

Understanding how nervous systems arose in deuterostomes has been complicated by the uncertain phylogenetic positions of acoel flatworms, xenoturbellids and nemertodermatids (the Xenocoelomorpha). If they are basal to bilaterians as has been proposed (Hejnol et al., 2009), then the multiple nerve cords of an acoel-like ancestral bilaterian might have given rise independently to nerve cords in protostomes and deuterostomes (Fig. 4). However, if they are basal in the deuterostomes (Bourlat et al., 2006; Philippe et al., 2011), the ancestral urbilaterian might have had a longitudinal nerve cord and brain (De Robertis, 2008; Strausfeld and Hirth, 2013a). If so, then nervous systems in Xenocoelomorpha would be secondarily simplified and irrelevant for discussions of CNS evolution.

The key to understanding evolution of deuterostome nervous systems may lie in opposition between Nodal and BMP proteins. In the cephalochordate amphioxus and in echinoderm larvae, Nodal vs BMP regulates compartmentalization of the ectoderm into neural (CNS or ciliary band) and less-neural (containing sensory neurons) ectoderm. Indeed, it has been suggested that the hemichordate basiepithelial nerve net is really the equivalent of the peripheral

nervous system of chordates (Lu et al., 2012). If recent phylogenetic trees placing cephalochordates as sister group of the Ambulacraria (Moroz et al., 2014) hold up to scrutiny, the idea of a basal deuterostome with a longitudinal nerve cord would be strengthened. In that case, a well-developed CNS in a cephalochordate-like ancestor would have partially degenerated in hemichordates due to the decoupling of BMP and Nodal functions (Wlizla, 2011). A test of this theory would be to determine if the role of Nodal vs BMP in establishing position and identity of the neural ectoderm is conserved in annelids and was, therefore, probably present in the ancestral bilaterian.

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