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Review

Cnidarians and the evolutionary origin of the nervous system

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Cnidarians are widely regarded as one of the first organisms in animal evolution possessing a nervous system. Conventional histological and electrophysiological studies have revealed a considerable degree of complexity of the cnidarian nervous system. Thanks to expressed sequence tags and genome projects and the availability of functional assay systems in cnidarians, this simple nervous system is now genetically accessible and becomes particularly valuable for understanding the origin and evolution of the genetic control mechanisms underlying its development. In the present review, the anatomical and physiological features of the cnidarian nervous system and the interesting parallels in neurodevelopmental mechanisms between Cnidaria and Bilateria are discussed.

Key words: Cnidaria, evolution, *Nematostella*, nervous system, neurogenesis.

Introduction

The function of the nervous system is to sense and relay fast information about surroundings. The network structure of neurons serves rapid signal transmission between sensory cells and a distant unit of specific cells, such as muscle. This rapid and restricted mode of signal transmission allows an animal to process multiple messages and respond appropriately. The appearance of the nerve cell is therefore one of the most prominent events in animal evolution. Recent comparative studies have revealed that a great deal of signaling molecules and transcription factors that are critically implicated in neural development are highly conserved among bilaterian animals. However, it is currently unknown how the genetic programs regulating development of the complex nervous system in modern bilaterians first came into place during the early evolution of Eumetazoa and how they further evolved in the diverse animal phyla.

In the present review, we focus on the nervous system of cnidarians, a clade widely regarded as the first class of organisms in animal evolution exhibiting a nervous system. The two main groups of Cnidaria, Anthozoa (sea anemones and corals) and Medusozoa (jellyfish including Hydrozoa, Scyphozoa and Cubozoa) separated early during cnidarian evolution (Fig. 1). Anthozoa is considered to be a representative of the basal group within the Cnidaria (Bridge *et al*. 1995; Medina *et al*. 2001; Collins 2002; Dunn *et al*. 2008). A recent molecular phylogeny based on > 300 orthologous protein sequences strongly suggests that the split is as ancient as that of the two main groups of Bilateria, protostomes and deuterostomes (Putnam *et al*. 2007). Here, we focus on the nature of the nervous system of cnidarian species ranging from anthozoans to medusozoans. The Cnidaria have a nerve net where the sensory and ganglionic neurons and their processes are interspersed among the epithelial cells of both layers, as an indication of a diffused nervous system. In addition to the nerve net, several cnidarian species have appeared to have a considerable degree of regionalization of the neural structure. The neural regionalization is most evident in the medusozoans that bear the elaborate eye-bearing sensory system such as rhopalia. However, these highly evolved sensory systems of medusozoans are thought to represent, at least in part, derived features. Thus, given the complexity and vast differences among nervous system organizations in Cnidaria, what can we learn from comparative studies about the ancient phase of the nervous system? Although insight from cnidarian research into the

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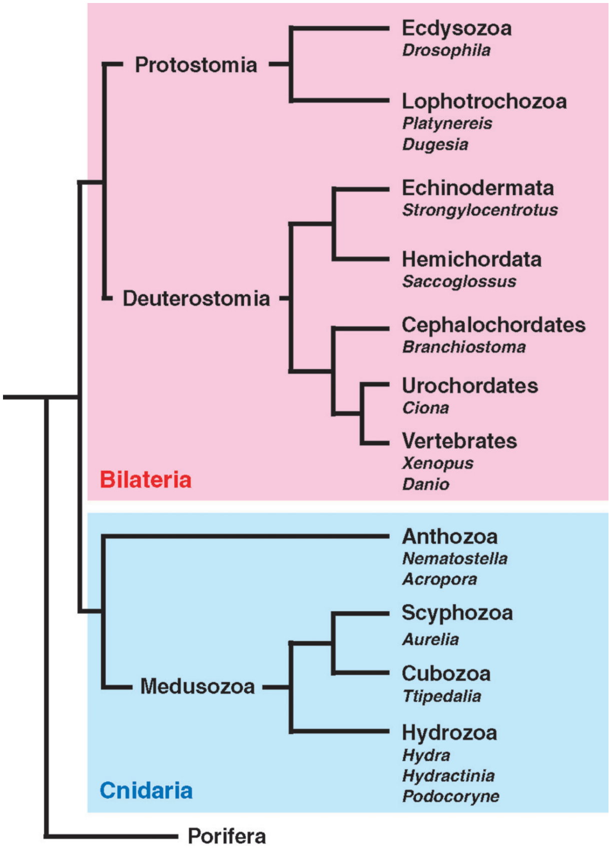

Fig. 1. Evolutionary relationships among metazoans. The overall phylogeny shown has been modified from Refs (Medina *et al*. 2001; Collins 2002; Dunn *et al*. 2008). Cnidaria is believed to have branched off the metazoan stem before the split of two main Bilateria phyla, Protostomia and Deuterostomia. Genera mentioned in the main text are listed under the phylum to which they belong.

mechanisms regulating the neural development have been very limited so far, histological and functional studies on a wide variety of cnidarian species and genomic data of *Hydra magnipapillata* (Hydrozoa) and *Nematostella vectensis* (Anthozoa) now shed light on the ancient mechanisms that have already been invented in the common ancestor of cnidarians and bilaterians, the 'ureumetazoan'. Comparative studies of nervous systems between Anthozoa and Medusozoa suggest that the mechanism(s) organizing the distribution of different neural cell types along the body axis predated Eumetazoa. In addition, the organization of the neural tracts observed among cnidarians indicates that the axon guiding and fasciculation machinery that are crucial for the proper development of bilaterian central nervous system (CNS) might be an evolutionarily primitive state of neural regionalization.

Here, we compare the genetic mechanisms regulating the neural development of Cnidaria and Bilateria. A great deal of recent genomic data revealed an unexpected complexity of cnidarian genetic repertoires and striking similarities to the Bilateria (Kortschak *et al*. 2003; Technau *et al*. 2005; Putnam *et al*. 2007). We discuss the primordial molecular features of the neurodevelopmental mechanisms that were probably already present in the common eumetazoan ancestor.

Cnidarian nervous system

A prevailing view of the cnidarian nervous system is that the neural network is simple and diffuse throughout the animal body as can be observed in the freshwater polyp *Hydra* (Hydrozoa). In *Hydra*, which has one of the simplest body plans among cnidarian species, neurons are located near the base of endodermal and ectodermal epithelial cells (epithelial nerve net) and are diffused along the main (oral-aboral) body axis. Their connecting processes extend to other neurons and to the muscle layer of epithelial cells.

Hydra has two classes of neural cells, nerve cells and nematocytes a cell type exhibiting mechanosensory functions with a remarkable level of complexity (David *et al*. 2008). Before reviewing recent work on the neural development of cnidarian animals, we will briefly address some important anatomical and physiological features and the molecular characteristics of the cnidarian nervous system.

Diffused and regionalized neural networks in cnidarians

Although a complete picture of the cnidarian nervous system has not yet been obtained, studies on neuropeptides have greatly contributed to our understanding of the structural complexity of the nerve net among cnidarian species. Immunohistological studies have unveiled not only that the cnidarian nervous system is basically made up of a pervasive nerve plexus but also some cnidarian species bear condensed neurites and even neuronal cell bodies to form circular or linear tracts. In addition, it has been revealed that neurons expressing different neuropeptides are distributed in a polarized way with respect to the body axis (Koizumi *et al*. 2004).

Distribution of peptidergic neurons. The RFamide neuropeptide family has been studied most extensively among all cnidarian classes comprising Anthozoa (Fig. 2) (Grimmelikhuijzen *et al*. 1991; Anderson *et al*. 2004), Cubozoa (Anderson *et al*. 2004), Scyphozoa (Anderson *et al*. 2004), and Hydrozoa (Grimmelikhuijzen 1985; Grimmelikhuijzen *et al*. 1988; Plickert 1989; Grimmelikhuijzen *et al*. 1991; Koizumi *et al*. 1992; Moosler

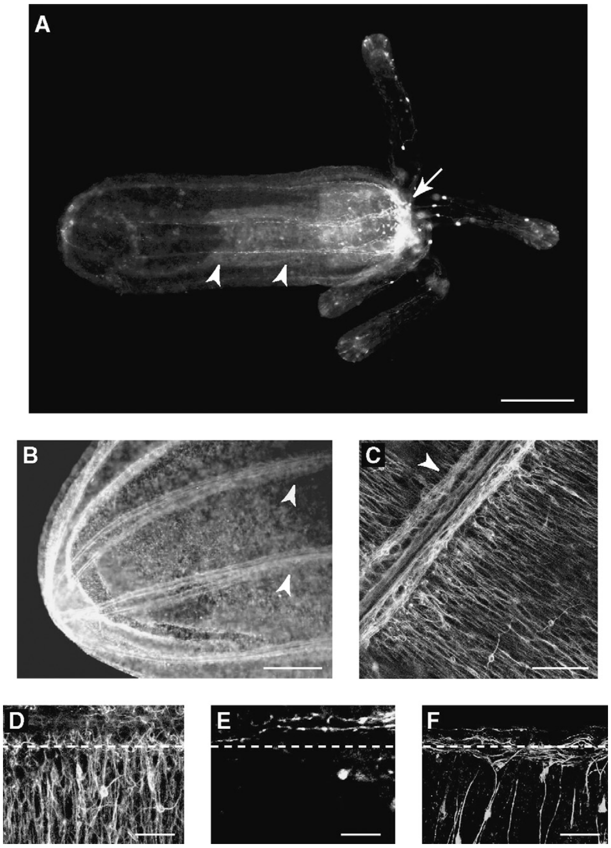

Fig. 2. The neural network of *Nematostella vectensis* (Anthozoa). (A) Distribution of RFamide-expressing neurons on the primary polyp. The RFamide-positive cell bodies are mainly localized at the mouth (Arrow) and oral part of the pharynx. The long and fine neurites run along the longitudinal axis of the polyp (Arrowheads). (B,C) Tyrosinated tubulin positive neural processes. Fasciculated neuronal processes are finely organized in the mesenterial endomesoderms (MEs) (Arrowheads) (B). The neural structures (Arrowhead) of MEs are connected each other with parallel neurites (C). (D–F) Dissection of physiological properties of the neural structure of the ME tissue. The neural structure of ME (D) comprises RFamide-positive neurites in the center of ME (E). LWamide-positive neurons compose parallel fibers and contact to the edge of the MEs (F). The edge of ME tissue is indicated in broken lines (D–F). Bars, 0.5 mm (A), 250 μm (B), 100 μm (C), 50 μm (D–F).

et al. 1996; Darmer *et al*. 1998; Mitgutsch *et al*. 1999; Anderson *et al*. 2004). Many features of the distribution of RFamide-positive neurons are common to all four cnidarian classes. The tentacles always contain the sensory cells and a loose plexus of the neuronal subpopulation at the base of the ectoderm (Mackie and Stell 1984; Mackie *et al*. 1985; Grimmelikhuijzen 1988; Anderson *et al*. 2004). The RFamide-positive neural network are also found in the ectoderm of manubrium, gonads and subumbrellar radial muscles of medusae in jellyfishes, where the epithelial muscle layers are well developed (Mackie and Stell 1984; Mackie *et al*. 1985; Grimmelikhuijzen 1988; Grimmelikhuijzen *et al*. 1991). In cnidarian polyps such as *Hydra* and *Hydractinia*, RFamide-positive sensory neurons are located in the ectoderm around the mouth opening (Grimmelikhuijzen 1985; Plickert 1989; Grimmelikhuijzen *et al*. 1991; Koizumi *et al*. 1992). RFamide-positive ganglion cells are located at the head region, tentacles and peduncle, the aboral quarter region of the body column (Grimmelikhuijzen 1985; Grimmelikhuijzen *et al*. 1991; Koizumi *et al*. 1992). LWamide has been demonstrated to be expressed in neurons of *Hydra*, *Hydractinia echinata* (Hydrozoan), *Podocoryne carnea* (Hydrozoa), *Anthopleura fuscoviridis* (Anthozoa) and *Nematostella vectensis* (Anthozoa) (Fig. 2) (Leitz and Lay 1995; Schmich *et al*. 1998a; Mitgutsch *et al*. 1999; Takahashi *et al*. 2003). LWamide-positive cell bodies are also located at the base of tentacles and in *Hydractinia* polyps LWamide-positive processes can form dense longitudinal bundles (Schmich *et al*. 1998a). In a hydrozoan jellyfish (*Podocoryne*), a dense manubrial nerve plexus and fibers in the ring and radial channels were reported to be LWamide-positive (Schmich *et al*. 1998a). Interestingly, another neuropeptide family member Hym-176 shows different expression pattern. Hym-176 is expressed intensely in a subset of neurons only in the *Hydra* peduncle, the aboral quarter region of the body column. The neurons in the gastric region and around the mouth opening show low level expression of this peptide (Yum *et al*. 1998a, 1998b; Fujisawa 2008).

Mesenterial nerve cords in anthozoan polyps. Within the Cnidaria, the Anthozoa is considered the sister-group to the Medusozoa (Bridge *et al*. 1995; Medina *et al*. 2001; Collins 2002). *Nematostella vectensis* (Anthozoa) shows linear tracts with condensed Tyr-tubulin-positive neurites (Fig. 2). The Tyr-tubulin-positive processes run in the parietal region of mesenterial endomesoderms (MEs) and between each MEs. MEs have long processes of RFamide-expressing neurons and the dense network of LWamide-positive neurites (Fig. 2).

Nerve ring in hydrozoan polyps and medusae. Immunohistological studies using anti-tubulin and anti-neuropeptide antibodies have demonstrated that cnidarian medusae and polyps possess an elaborate nerve ring, a circular structure of neural network, in addition to their diffuse nerve net (Grimmelikhuijzen and Spencer 1984; Grimmelikhuijzen 1985; Koizumi *et al*. 1992; Mackie and Meech 2000; Yi-Chan *et al*. 2001;

Mackie 2004; Garm *et al*. 2006; Garm *et al*. 2007). In some *Hydra* species, a nerve ring and related neural structures have been found near to the base of the hypostome or at the base of tentacles and between them (Matsuno and Kageyama 1984; Grimmelikhuijzen 1985; Koizumi 2007). *Aglantha digitale* (Hydrozoa) has been demonstrated to bear an elaborated ring nerve system. This nerve ring has been divided into at least seven subsystems with separate physiological properties and functions (Mackie and Meech 1995a, 1995b, 2000; Mackie 2004). Communication among the subsystems allows complex behavioral control such as swim contractions of the medusae (Mackie and Meech 1995b). The jellyfish generally tends to have a nerve ring with elaborate sensory systems and behavioral repertoire. Several cnidarian species including hydromedusae and cubomedusae bear sophisticated sensory complexes that often contain eyes (Singla 1974; Yamamoto and Yoshida 1980; Laska and Hündgen 1982; Singla and Weber 1982; Nilsson *et al*. 2005). Visually guided behavioral patterns are observed in these cnidarian classes and, especially in cubomedusae, these patterns are considerably complex (Hartwick 1991; Hamner *et al*. 1995; Matsumoto 1995).

Taken together, these observations distinctly demonstrate: (i) the cnidarian nervous system exhibits neuronal subsets that are unequally distributed along the body axis; (ii) some neuronal cell bodies in Cnidaria are condensed in specific body regions including the oral region (the pharynx in polyps and the manubrium in jellyfish), which is reminiscent to the regionalization in bilaterian neural tissues such as the mushroom body in *Drosophila* and the neuronal layers in the mammalian CNS; and (iii) cnidarians, especially medusozoans, often bear ring-shaped or longitudinally running fasciculated neuronal processes (neurites). These data indicate that the cnidarian nervous system is organized in a more complex way than previously assumed.

Evolutionary origin of bilaterian CNS

Anatomically and physiologically elaborated neural structures observed in cnidaria clearly reflect a considerable degree of regionalization of the cnidarian nervous system. Although these data have prompted several authors to propose that the nerve ring is the cnidarian CNS (Garm *et al*. 2007) that may be even homologous to the bilaterian CNS (Koizumi 2007), it still remains unclear whether these regionalized (centralized) neural networks in Cnidaria represent a homologous feature to the CNS of Bilateria. There is no reason why anatomical features of nerve tracts observed in cnidarians should necessarily be homologous to the CNS of bilaterians. Even if one accounts for a great degree of physiological complexity observed in the regionalized nervous system and eye-bearing sensory complex (Rhopalia) of cnidarians, an increasing number of examples indicating the evolutionary convergence in both nervous and sensory systems (Nishikawa 2002) make it difficult to simply compare cnidarian and bilaterian neural structures and to place the evolutionary origin of CNS as commonly discussed (Holland 2003). The idea that the rudimentary neural centralization observed in some cnidarians is an antecedent characteristic of the eumetazoan nervous system should be examined at least by comparative molecular analysis.

An additional hypothesis considering the origin of the CNS has recently arisen from the topological similarity of expression domains for several developmental genes in *Hydra* (Meinhardt 2002). In this hypothesis, foot and body regions of adult *Hydra* are assumed to be related to the bilaterian brain with respect to the anterior-posterior axis. In *Hydra* the aboral end expresses *CnNk2* (Grens *et al*. 1996), a homologue of the vertebrate forebrain marker, and the oral side of the body column expresses *Cnox-2* (Shenk *et al*. 1993) and *CnOtx* (Smith *et al*. 1999), homologues of vertebrate forebrain/midbrain and midbrain/hindbrain markers, respectively. It is interesting to consider that the topological similarity of these expression patterns in *Hydra* may reflect common intrinsic molecular properties involved in anterior-posterior patterning of a common ancestor of bilaterians. In fact, this view is supported by the expression patterns of *Wnt* genes and their antagonists, which may indicate that the bilaterian trunk was intercalated between the oral and aboral body region (Meinhardt 2002; Guder *et al*. 2006a, 2006b).

New expression studies in cnidarian species, however, reveal a more complex situation. The expression patterns of *Otx* genes in two anthozoans (*Acropora* and *Nematostella*) do not seem to correspond to that in *Hydra*. In *Acropora*, *OtxA-Am* is exclusively expressed in the gastrula only at the oral site of the blastopore, and later in undefined cells that are distributed at the oral half of the planula larvae (de Jong *et al*. 2006). In *Nematostella*, *NvOtxA*, *NvOtxB* and *NvOtxC* are initially expressed in invaginating mesendodermal cells of the gastrula, and can be later found at the oral and aboral side of planula larvae (Mazza *et al*. 2007). These recent findings, taken together, suggest that the expression patterns of cnidarian *Hox* genes along the oral-aboral body axis do not simply correspond to the anterior-posterior patterning of the bilaterian body axis (Ball *et al*. 2004; Chourrout *et al*. 2006; Ball *et al*. 2007). In addition to this point, only a similar topological overlap of gene expression observed in two species without landmarks to relate these gene functions to their anatomical and/or cellular characteristics does not necessarily indicate homology. In Leech, an *Otx* homologue *Lox22-Otx* has been expressed not only in the head ganglion of the CNS but also in the anterior part of foregut and ectoderm (Bruce and Shankland 1998). Also in deuterostomes, Otx has been demonstrated to be involved in gastrulation and mesendodermal specification as well as in neural patterning (Bally-Cuif *et al*. 1995; Ang *et al*. 1996; Rhinn *et al*. 1998; Li *et al*. 1999a; Harada *et al*. 2000; Davidson *et al*. 2002; Hinman *et al*. 2003). Thus, it is unclear whether Otx was already acquired for neural development at the early metazoan evolutionary phase. Functional analysis of cnidarian Hox genes is strongly required to settle these points.

Synaptic transmission and neurosecretion in cnidarians

Most hormones including neuropeptides found so far throughout the animal kingdom are short polypeptides. This may imply that the cnidarian nervous system comprising a wide variety of peptidergic neurons can be regarded as a neurosecretory system, which serves local and multidirectional diffusion of neuropeptides. One can also question the physiological importance of maintaining the structural consistency of the nerve plexus in some cnidarians such as *Hydra.* Here, nerve cells are in a steady state of production and loss by continuous displacement toward either the head or foot within a few weeks (Bode *et al*. 1986, 1988). In vertebrates, neurosecretory cells (NSCs) form a distinct population of nerve cells that bear vesicles distributed throughout the soma, neurites, and synapses, rather than being restricted to the synapse. Vertebrate NSCs release neuropeptides not only at the synapse but also anywhere along the cell body and neurites (Hartenstein 2006). The cnidarian nervous system has been demonstrated to show synaptic connections between, on the one hand, ganglion neurons and, on the other hand, sensory cells and epithelial muscular cells where synaptic vesicles are accumulated at the synapses (Westfall *et al*. 1971, 2002; Westfall 1973, 1987; Westfall and Kinnamon 1978, 1984; Kinnamon and Westfall 1982). Cnidarian neurons considerably accumulate neuropeptide-containing vesicles at the pre-synaptic area in nerve terminals and at en passant synapses (Koizumi *et al*. 1989; Westfall and Grimmelikhuijzen 1993; Westfall *et al*. 1995). This indicates that in Cnidaria the synapse-restricted release of neuropeptides may have a more distinctive role in serving directed signal transmission than undirected neurosecretion.

Directed neural conduction was observed in *Hydra*. Rushforth and Hofman have reported that during capture of prey the tentacle contraction is limited to the portion on the proximal side that is the side closer to the mouth (1972). This unidirectional contraction of tentacles is clearly mediated by neurons since tentacle contraction induced by capture of prey cannot be observed in nerve-free epithelial polyps (Shimizu 2002). In tentacles, synaptic vesicles have been shown to contain several neuropeptides comprising RF/RWamide peptides (Westfall and Grimmelikhuijzen 1993; Westfall *et al*. 1995). These indicate that in cnidarians the structural organization of the neural network is indispensable for neural regulation of behavior and that neuropeptides released from synaptic regions serve, at least in part, a role in the synaptic transmission of neuronal activity.

Physiological role of cnidarian peptidergic neurons

The activity of peptidergic neurons is required for several coordinated behaviors in cnidarians. Most of the so far characterized peptides have distinct myoactivities. LWamide neuropeptides Hym-248 has been reported in *Hydra* to induce the relaxation of the body column and tentacles (Takahashi *et al*. 2003). The relaxing effect of the exogenously applied LWamide peptide is also observed in epithelial hydra. This indicates that this neuropeptide can directly affect the muscular function of epithelial cells (Takahashi *et al*. 2003). *Hydra* neuropeptide Hym-176 peptide induces contraction of the peduncle (at 1 μM) and the body column (at 10 μM) of epithelial hydra (Yum *et al*. 1998b), indicating that the Hym-176 neuropeptide can work directly on the epithelial cells as well as LWamide. Although the detailed mode of function of the RFamide peptide family in cnidarians still remains obscure, several lines of evidence suggest that this family of neuropeptides can regulate the myoactivity and/or modulatory role of muscle contraction (McFarlane *et al*. 1987; McFarlane *et al*. 1991) in *Calliactis parasitica* (Anthozoa), the myoactivity of the body column and coordinated movement of the gut cavity in *Hydra* (Shimizu and Fujisawa 2003; Fujisawa 2008), and phototaxic behaviors of *Hydractinia echinata* (Katsukura *et al*. 2004) and *Tripedalia cystophora* (Cubozoa) (Plickert and Schneider 2004). In contrast to the empiric view on the neuropeptide functions among cnidarians, our knowledge of the evolutionarily conserved function(s) of neuropeptides is still fragmentary. There are only a few peptide families that are conserved from invertebrates to vertebrates. RFamide peptide was originally identified as a cardioexcitatory FMRFamide peptide in the nervous system of a Mollusk (Price and Greenberg 1977). A large number of RFamide peptides and their encoding genes have been identified on bilaterians; *C. elegans*, for instance, contains as many as 25 flp (RFamide-like peptide) genes (Li *et al*. 1999b; Kim and Li 2004). Three LWamide peptides have also been identified in *C. elegans*

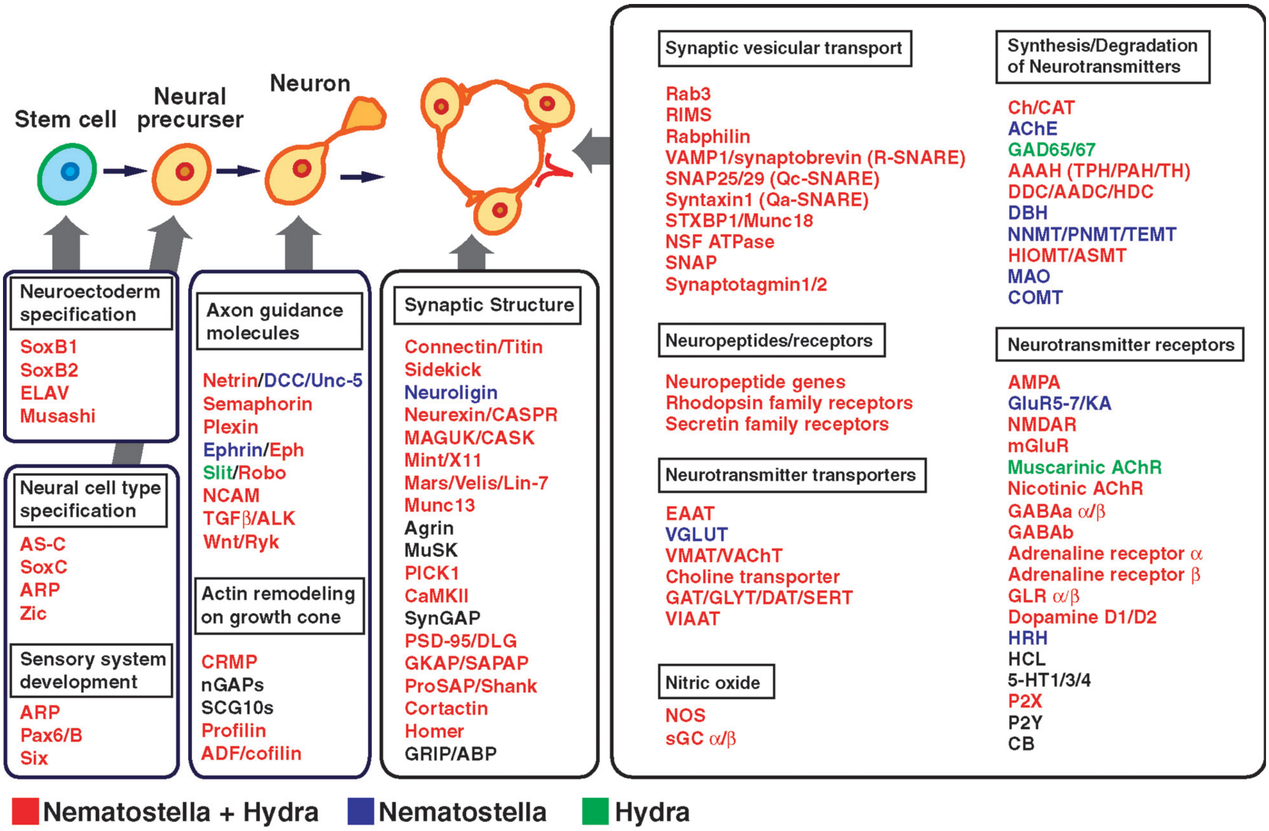

Fig. 3. Neural genes in the genomes of *Nematostella vectensis* and *Hydra magnipapillata*. tBLASTn and BLASTp searches of the National Center for Biotechnology Information (NCBI) trace archive of the *Nematostella* and *Hydra* (data generated by the Joint Genome Institute) were carried out by using bilaterian orthologues of neurogenic genes. Over 330 (*Nematostella*) and 280 (*Hydra*) genes have so far been identified by using reciprocal basic local alignment search tool (BLAST) to show the strong similarity for the bilaterian neural genes involved in neuroectoderm and neural cell type specification, neuronal migration, axon guidance, synaptic organization and transmission. Gene families found in both *Nematostella* and *Hydra* are shown in Red. The families found in *Nematostella* or *Hydra* are shown in Blue or Green, respectively. The families of neural genes that are not clearly annotated in both *Nematostella* and *Hydra* genomes are shown in Black.

(Husson *et al*. 2005). The gene encoding LWamide peptides is expressed in a small number of interneurons in *C. elegans*; however, the function of the gene in this nematode species is still unknown because mutations in the gene exhibit no obvious defect (Fujisawa 2008). The gene encoding LWamide peptide in other higher metazoans has not been discovered. The function of a peptide is generally determined by a motif of a few amino acids that is recognized specifically by its receptor and the rest of the peptide sequence can vary almost randomly. The diversity of peptides in many groups of metazoans thus makes it difficult to draw a simple picture in the context of evolution.

A genomic view of the cnidarian nervous system

In addition to the phylogenetic position of Cnidaria, expressed sequence tag (EST) and genome projects on *Hydra magnipapillata*, *Nematostella vectensis* and *Acropora millepora* (Anthozoa) (Kortschak *et al*. 2003; Technau *et al*. 2005; Putnam *et al*. 2007) made cnidarians become one of the most valuable model animals for comparative molecular studies and provide powerful tools for elucidating the origin of neurodevelopmental events. The comparative genomic studies have so far unveiled a great deal of complexity of the neural gene repertoire in the *Nematostella* and *Hydra* genomes (Fig. 3). One intriguing genetic feature of the Cnidaria is that these cnidarian classes, Anthozoa and Hydrozoa, possess an almost complete set of homologous genes that have critical roles in bilaterian neurodevelopmental events including neurogenesis, neuronal specification and neural network formation. Both of these cnidarian species bear functionally conserved transcription factors involved in neurogenesis and neural cell type specification, such as proneural basic helix loop helix (bHLH) factors, type B Sox genes (SoxB), zinc-finger protein genes, and neuron-specific RNA binding proteins (RBPs). Given the diversity of neural cell types in cnidarians, it might not be surprising that their genomes have such a complex gene set of regulatory molecules. In bilaterians, several neuronal RBPs control multiple steps of nuclear and cytoplasmic RNA processing including stabilization and translational repression of mRNAs to regulate neural cell fate decisions (Broadus *et al*. 1998; Okano *et al*. 2002; Sakakibara *et al*. 2002). The RBPs, such as Nova, have been demonstrated to bind to a subset of pre-mRNAs that encode components of inhibitory synapses (Ule *et al*. 2003). They are postulated to coordinately regulate the processing of multiple nuclear and cytoplasmic subsets of pre-mRNAs and mRNAs that encode products involved in the same regulatory pathways during neuron differentiation (Keene 2003). Therefore, RBPs are proposed to play pivotal roles in the execution of posttranscriptional operons by making ribonucleoprotein infrastructures regulating the flow of genetic information between the genome and the proteome (Keene 2001; Keene and Tenenbaum 2002). Because cnidarians have been demonstrated to bear different neuronal cell types as discussed above, it should be worth comparing the RBP binding sites in cnidarian mRNAs encoding neural genes and examining the expression and function of neural RBP orthologues such as Musashi and Elav to unveil the ancient molecular mechanisms that gave rise to various types of neurons.

In addition to these early neurodevelopmental genes, cnidarian genomes contain a wide variety of genes with a strong similarity to the molecules involved in extra- and intra-cellular regulation of axon path finding (Fig. 3). The function of the axon guidance molecules that are crucially implicated in the formation of complex neural architectures is conserved among protostomes and deuterostomes, indicating that the neural wiring control by these signaling pathways is the mechanism that predates Bilateria. As mentioned above, some cnidarians bear complex neural structures including an arrangement of functionally diverse neurons and a considerable degree of neurite fasciculation. It should be worth addressing the roles of these cnidarian axon guidance molecules to unveil their antecedent functions.

At the functional level, *Nematostella* and *Hydra* possess most genes required for axon targeting, synaptic structure formation, vesicular transport, and synaptic transmission (Fig. 3) (Watanabe and Holstein unpubl. data, 2008). Of particular interest are the neurotransmitter molecules. The genes for acetylcholine (ACh), catecholamine, and γ-aminobutyric acid (GABA) synthesis are present as well as the corresponding receptors (Muscarinic and Nicotinic ACh receptors, GABA receptors). Also epinephrine, dopamine and glycine receptor-like genes could be identified; however, some are only identified in *Nematostella,* indicating secondary gene loss in *Hydra*. Also, nitric-oxide synthase exists in *Hydra* and *Nematostella*. This indicates that intact neurotransmission by conventional neurotransmitters may play important roles in cnidarian neural functions as well as by neuropeptides, although it should be firmly established whether the systems in fact function in the nervous system or not. These findings, however, clearly indicate that much of the genetic complexity commonly assumed to have arisen much later in animal evolution is actually an ancestral feature.

Development of the cnidarian nervous system

Development of peptidergic neurons

In cnidarian development neurons expressing neuropeptides appear at the larval stage (Thomas and Edwards 1991). The neuronal population, which is identified by expression of RFamide, progressively increases at peri-hatching stages of *Hydra* (Brumwell and Martin 2002). In *Halicordyle disticha* (Hydrozoa), *Podocoryne carnea* (Hydrozoa), *Pennaria tiarella* (Hydrozoa) and *Aurelia* sp. (Scyphozoa), RFamide-expressing neurons appear at planula larvae (Martin 1988; Martin 1992; Gröger and Schmid 2001; Seipel *et al*. 2004; Nakanishi *et al*. 2008). Although the physiological role of these neuropeptide-expressing neurons in planula larvae still remains to be clarified, recent studies on *Hydractinia* have unveiled an involvement in phototactic regulation of planula larvae and the metamorphosis to a primary polyp. Both, LWamide- and RFamide-positive neurons appear in the aboral (anterior) pole of developing and mature planula larvae (Plickert 1989; Leitz and Lay 1995; Gajewski *et al*. 1996). Planula larvae of some anthozoan species have been thought to have an aboral sensory structure called the apical organ (Widersten 1968), which has been implicated in perception and conveyance of environmental signals for searching appropriate substrate for settlement and consequent metamorphosis (Chia and Bickell 1978; Chia and Koss 1979). As LWamide- and RFamide-expressing cells in the aboral pole of the swimming larvae show an oval-like shape and the tip of one end of the cell body appears to be exposed to the outside of the larva, these neuropeptide-positive neurons are believed to directly receive environmental cues to regulate the onset of metamorphosis. The settlement and metamorphosis of cnidarian larvae can be induced by marine biofilms (Müller 1969; Morse and Morse 1991; Leitz and Wagner 1992). Interestingly, these neuropeptides are involved in the metamorphosis of planula larvae into polyps in various cnidarians (Leitz *et al*. 1994; Gajewski *et al*. 1996; Takahashi *et al*. 1997; Schmich *et al*. 1998b; Iwao *et al*. 2002; Katsukura *et al*. 2003, 2004). In addition, LWamide and RFamide peptides have been involved in the regulation of the creeping behavior of planula towards a light source (positive phototaxis), as the phototaxis of the planula was markedly promoted or suppressed by exogenously administered LWamide peptide or RFamide peptide, respectively (Katsukura *et al*. 2004; Plickert and Schneider 2004).

The sensory system of the cnidarian larva, which is made up, at least in part, by a rudimentary nervous system appears to allow orientation with respect to the information from suitable settlement sites and to light for coordinating the diurnal cycle of migration. These findings may indicate an ancient nature of signals mediated by neuropeptides that are expressed by neuronal/sensory cells, for example, in interconnecting the sensing of environmental cues and in the regulation of the behavioral response and developmental timing.

Conserved neurogenic gene functions

Although our current knowledge on the expression and function of genes specifying the neuronal subset in Cnidaria is fragmentary, a notable degree of conservation of amino acid sequences, and functional domain organizations between cnidarian and bilaterian neurogenic genes have indicated similar neurogenic functions.

Comparisons between deuterostomes and protostomes have revealed a high degree of conservation in the genetic control of neural development in Bilateria. Especially, functions of neural bHLH transcription factors that consist of the Achaete-Scute complex (AS-C) and Atonal-related protein (ARP) genes are strikingly conserved, as these genes have crucial roles in neuroectoderm and neuroblast specification during early embryogenesis of Bilateria (Hassan and Bellen 2000). To date, the counterparts for these bilaterian neurogenic transcription factors have been identified in a wide range of cnidarian species (Grens *et al*. 1995; Müller *et al*. 2003; Hayakawa *et al*. 2004; Lindgens *et al*. 2004; Seipel *et al*. 2004; Simionato *et al*. 2007). In *Drosophila*, there are two families of proneural bHLH genes, the AS-C and the Atonal superfamily, which not only determine neural fate but also specify the identities of different receptors and sense organs (Jan and January 1994; Jarman *et al*. 1995; Hassan and Bellen 2000; Kumar 2001; Hsiung and Moses 2002). In vertebrates, the AS-Cs and the ARPs are involved in the generation of different subsets of neurons of the CNS, peripheral nervous system (PNS), and development of sensory organs comprising retina and inner ear (Sommer *et al*. 1995; Casarosa *et al*. 1999; Fode *et al*. 2000; Tomita *et al*. 2000; Fritzsch and Beisel 2001; Inoue *et al*. 2001; Kumar 2001).

Proneural gene homologues are expressed in the neural cell progenitors in two hydrozoan species, *Hydra vulgaris* and *Podocoryne carnea* (Grens *et al*. 1995; Müller *et al*. 2003; Hayakawa *et al*. 2004; Seipel *et al*. 2004). *CnASH,* a hydra homologue for proneural bHLH transcriptional factor, is expressed in neural cell populations that give rise to nematocytes in the body column and in sensory neurons in the tentacles (Grens *et al*. 1995; Hayakawa *et al*. 2004). The amino acid sequence of the bHLH region of *CnASH* is highly conserved compared with that of other members of AS-C family proteins. In *Podocoryne*, Ash, an AS-C homologue, and Atl1, an ARP homologue, have been expressed in precursors for nematocytes and RFamide-positive neurons, respectively (Müller *et al*. 2003; Seipel *et al*. 2004). Although the proneural function of these bHLH genes in cnidarians still remains to be examined, ectopic expression of *CnASH* can rescue the neuronal specification in *Drosophila* mutants in which the endogenous *achaete* and *scute* genes were eliminated (Grens *et al*. 1995), indicating that cnidarian proneural genes including *CnASH* may have conserved activity in the commitment of progenitor cells to a specific neural fate.

Neural induction in Cnidaria

The genetic mechanisms mediated by neurogenic transcription factors regulating neural cell differentiation and cell type specification seems to be conserved between Cnidaria and Bilateria. The molecular nature of the neural inducer(s) among cnidarians is, however, still unclear. The identification of the neural inducing signal in cnidarians may provide important insights into not only the evolutionary origin of neural induction but also the molecular history of the centralized nervous system in bilaterians.

BMP/Chordin signaling. Bone morphogenetic protein (Bmp) signaling has been implicated in triggering regionalized neurogenesis in Bilateria; the CNS development is tightly coupled with the inhibition of Bmp2/4 signaling on the dorsal side in notoneuralians including vertebrates and amphioxus (De Robertis and Kuroda 2004; Levine and Brivanlou 2007; Yu *et al*. 2007) and on the ventral side in gastroneuralians including fly, spider, and flatworms (Bier 1997; Akiyama-Oda and Oda 2006; Mizutani *et al*. 2006; Molina *et al*. 2007; Orii and Watanabe 2007). In *Xenopus* development, most of the identified neural inducing molecules have the ability to inhibit Bmp signaling in common. The finding that Bmp inhibition in early CNS development is conserved between notoneuralians and gastroneuralians suggests that the Bmp-mediated neural inducing system had evolved before these animals diverged. This also led to a widely accepted 'default model', which proposes that ectodermal cells are destined to become neurons in the absence of any signal but are normally inhibited from a neural fate by Bmps expressed throughout the ectoderm. The organizer secretes Bmp antagonists, which block Bmp signaling in adjacent cells, allowing them to follow their default neural pathway (Hemmati-Brivanlou and Melton 1997; Weinstein and Hemmati-Brivanlou 1999; Munoz-Sanjuan and Brivanlou 2002). Consistent with this, misexpression of any of these antagonists leads to neural induction in *Xenopus* animal caps, while overexpression of Bmps has the opposite effect. However, recent studies in other vertebrates, ascidians, hemichordates and annelids have questioned the general notion of Bmp inhibition as a universal mechanism in the first phase of neural induction (Streit and Stern 1999; Darras and Nishida 2001; Stern 2005; Lowe *et al*. 2006; Denes *et al*. 2007). In chick, misexpression of Chordin or Noggin in the competent epiblasts does not induce neural tissue, and overexpression of Bmp4–7 in the prospective neural plate does not block neural induction (Streit *et al*. 1998). In addition, mouse mutants that lack Chordin, Noggin, or both cannot develop the most anterior neural structures, although the mutants develop neurons (McMahon *et al*. 1998; Bachiller *et al*. 2000; Mukhopadhyay *et al*. 2001). Recent findings demonstrated that the expression of the pan-neural maker was left unchanged in Bmp protein-treated embryos of ascidians, hemichordates and annelids (Darras and Nishida 2001; Lowe *et al*. 2006; Denes *et al*. 2007). These findings demonstrated that a dorso-ventrally distributed Bmp/Chordin axis is not always linked to the formation of a CNS.

With the recent progress in the identification of conserved neurogenic genes in *Nematostella* (see above) (Simionato *et al*. 2007, Watanabe and Holstein unpubl. data, 2008), the deployment of the early neurogenic genes and their regulation can now be analyzed. In several anthozoans including *Nematostella* and *Acropora*, asymmetric expression of *Bmp2/4* and its antagonist *Chordin* becomes visible around the blastopore lip at the gastrula stage (Hayward *et al*. 2002; Finnerty *et al*. 2004; Matus *et al*. 2006; Rentzsch *et al*. 2006). In bilaterians, Chordin antagonizes Bmp activity on the opposite side of the dorsal-ventral axis in vertebrates and its homologue *short gastrulation* (*sog*) in *Drosophila*. In *Nematostella*, however, the Chordin orthologue is expressed on the same embryonic side in a partially overlapping manner. Unlike in frog*,* *Amphioxus* or fly, but similar to chick, mouse, ascidian, hemichordate or annelid, our preliminary experiment indicated that the first phase of neurogenic gene expression in *Nematostella* embryos is not inhibited by administration of the exogenous Bmp protein (Watanabe and Holstein unpubl. data, 2008). Although the detailed mode of Bmp function in cnidarian neural development has yet to be analyzed, this result casts more doubt on the simplest version of the default model as being evolutionary conserved and a sufficient explanation for neural induction.

There are several lines of evidence in vertebrates suggesting that Bmp inhibition may be a relatively downstream step in the developmental process of the CNS. In chick, although misexpression of Bmp4 in the prospective neural plate inhibits the expression of definitive neuronal markers (*Sox2* and *Sox3*) at a later developmental stage, it does not affect the early expression of *Sox3* (Linker and Stern 2004). In addition, prior treatment of chick epiblasts with grafted organizer tissue can induce responsiveness to Chordin by stabilizing the expression of the neuronal marker *Sox3* (Streit *et al*. 1998; Streit *et al*. 2000). Recent studies on *Xenopus* have demonstrated that inhibition of the BMP signal by Chordin and Noggin at an early developmental stage is necessary for the development of the anterior CNS structure, but not for pan-neural marker expression (Kuroda *et al*. 2004). These findings imply that additional signaling events are required for early neuronal induction processes.

FGF signaling. Fibroblast growth factor (FGF) signaling has been implicated in early neural induction of deuterostomes including ascidians and all vertebrate models tested so far (Darras and Nishida 2001; Imai *et al*. 2002; Bertrand *et al*. 2003; Böttcher and Niehrs 2005; Stern 2005). In chick, FGFs are sufficient to induce proneural markers and inhibition of FGF signaling suppresses neural induction by Hensen's node (Alvarez *et al*. 1998; Storey *et al*. 1998; Streit *et al*. 2000; Wilson *et al*. 2000). Several investigators have demonstrated in *Xenopus* that FGF signaling is required for the early neural induction (Launay *et al*. 1996; Hongo *et al*. 1999; Streit *et al*. 2000; Bertrand *et al*. 2003; Delaune *et al*. 2005). In zebrafish, ectopically activated FGF signaling during gastrulation has induced neuro-ectoderm (Kudoh *et al*. 2004; Rentzsch *et al*. 2004; Dee *et al*. 2007). Although FGF signaling pathway in major model ecdysozoans *Drosophila* and *C. elegans* seems to mainly be required for proper axon outgrowth and guidance (Garcia-Alonso *et al*. 2000; Bulow *et al*.

2004; Forni *et al*. 2004), the signaling in Lophotrochozoa has been implicated in a role in neurogenesis and anterior brain development of the planarians (Cebria *et al*. 2002; Ogawa *et al*. 2002; Mineta *et al*. 2003). A most parsimonious evolutionary scenario for the ancient FGF function on neural development is that the FGF signal pertained to the neural induction during early embryogenesis. This attractive scenario is supported if Cnidaria, a closest outgroup to Bilateria, has similar FGF function during the early neural developmental process. Unfortunately, however, the FGF involvement in cnidarian neurodevelopment has just started to be analyzed and quite a few experiments are still needed to answer this question. The expression of FGF signaling molecules has been analyzed on adult *Hydra* and developing *Nematostella* embryo. The FGF receptor (FGFR)-like transmembrane tyrosine kinase *Kringelchen* in *Hydra* has been expressed in ectodermal cells at the hypostome (oral end) and at the ring surrounding the bud base during bud development (Sudhop *et al*. 2004). Although the suppression of FGFR kinase activity resulted in inhibition of bud detachment, no data have addressed the effect on *Hydra* nervous system. Recent studies in *Nematostella* have demonstrated that this cnidarian species has 15 FGF ligand genes and two FGFR genes (Matus *et al*. 2007; Rentzsch *et al*. 2008). Among these FGF and receptor genes, *FGFa1*, *FGFa2* and *FGFRa* have been shown to be co-expressed in ectodermal cells at the aboral side at the gastrula stage, and later, at the planula stage, these aboral ectodermal expressions are more restricted to the small area on which the apical ciliary taft forms. Rentzsch *et al*. have elegantly demonstrated that these gene products tightly couple to the signal such as mitogen activating protein (MAP) kinase pathway regulating development of the apical ciliary tuft on planula larvae (2008). Additionally, they also found the involvement of FGF signaling pathway in the metamorphosis of planula larvae into primary polyps. In various cnidarian larvae, RFamide and LWamide peptides have been expressed in aborally located sensory cells and neurites and involved in the metamorphosis of planula larvae, which is induced by environmental cues released from biofilms (see above). Interestingly, many invertebrate protostome and deuterostome larvae possess apical sensory organs that are known to have chemosensory and/or mechanosensory roles with neuroendocrine functions involved in the metamorphosis induction (Lacalli 1981; Degnan *et al*. 1997; Kempf *et al*. 1997; Hadfield *et al*. 2000; Voronezhskaya and Khabarova 2003). In some deuterostomes, FGFs or FGF receptors have been expressed in the region of apical organ formation during embryonic development (Gerhart *et al*. 2005; Lapraz *et al*. 2006). Although there is no empirical evidence so far that the FGF signal functions on the sensory cell development during larval development among cnidarians, protostomes and deuterostomes, the crucial FGF role on *Nematostella* apical tuft formation suggests an ancestral FGF function that was already in place in the common eumetazoan ancestor and may give us the first chance to have an aspect of the neurosensory cell induction amenable to comparative studies.

Conclusion

The comparative analyses of available cnidarian and other metazoan genomes with the upcoming data of the cnidarian gene functions are becoming particularly valuable for understanding the origin and evolution of the genetic programs involved in the development of the ancient nervous system. The comparative genomic studies have uncovered that Cnidaria possess an almost complete set of signaling molecules that have critical roles in bilaterian neurodevelopment, indicating that the genetic complexity is in fact ancestral.

The findings on slow-evolving bilaterians have now cast more doubt on the default model as a sufficient explanation for primordial neural inducing mechanisms. The analyses of neurogenic mechanisms across metazoan phylogeny are still in their nascent phase and the molecular nature of the earliest neural inducer even in Urbilateria still remains enigmatic (Fig. 4). We believe that the decipherment of neurogenic programs and the identification of neural inducing signal(s) of Cnidaria will provide crucial mechanistic insights not only into the elemental nature of the nervous system but also into the evolutionary histories of widely diverged metazoan nervous systems.

As has been mentioned in previous papers, a wide variety of cnidarian species have an anatomical and physiological regionalization of nerve cells and their neurites with respect to the body axis. This should be re-emphasized in the post-genome era on cnidarian biology. Among bilaterian animals, it seems undeniable that the Bmp signal is essential for patterning the neural tissue along the dorsoventral axis in their common ancestor (Arendt *et al*. 2008). However, the assumption arose from the data on an enteropneust *Saccoglossus* that the Bmp/Chordin-mediated centralization of the nervous system in both arthropods and chordates came up independently in these two groups after they diverged (Lowe *et al*. 2006; Lowe 2008) has yet to be supported by other model animals that should include cnidarians. It will, therefore, undoubtedly be worthwhile to decipher signaling pathways involved in the neural structural regionalization and/or polarized distribution of distinct neuronal cell types in Cnidaria.

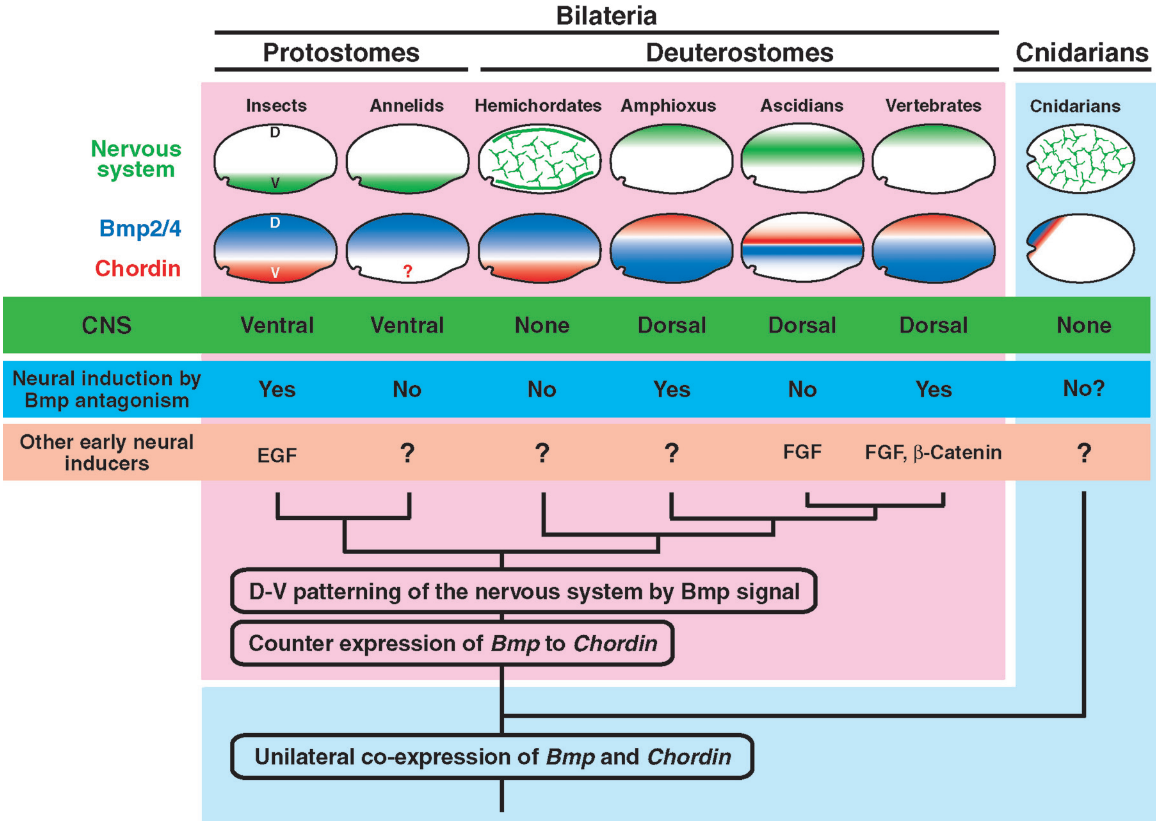

Fig. 4. Comparison of neural inducing signals among metazoan animals. Embryonic regions developing the nervous system (green), expressing *Bmp* (blue) or *Chordin* (red) are shown. In Bilateria, the *Chordin* expression in the dorsal side (vertebrates) or ventral side (*Drosophila*) of the embryo results in the suppression of *Bmp* expression at the opposite embryonic side. In vertebrates, *Bmp* signal has also been demonstrated to be suppressed by the fibroblast growth factor (FGF) and beta-Catenin pathways. The coordinated regulation allowed the bilaterians to establish a robust secondary Bmp/Chordin axis that is in turn used for dorsal-ventral patterning of the nervous system. The ancient neurodevelopmental role of *Bmp* signal still remains enigmatic because the functional importance of the unilateral embryonic *Bmp* expression in modern Cnidaria has not yet been determined. Several questions as to whether the Bmp involvement in the centralized neurodevelopment observed in some modern bilaterians and crucial roles of FGF and beta-Catenin signals that have been involved in vertebrate neurogenesis can be traced back to Ureumetazoa have so far been unanswered. The research on Cnidaria will provide relevant knowledge to address these important issues. EGF, epidermal growth factor.

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Conflict of Interest

No conflict of interest has been declared by H. Watanabe, T. Fujisawa or T. W. Holstein.

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