

Chapter 3

Back Through Time: How Cnidarians and Basal Metazoans Shed Light on Ancient Nervous Systems

Hiroshi Watanabe

Abstract The origin of neurons and the evolution of the central nervous system (CNS) are not well understood. The physiological nature of primitive neurons has not been elucidated, and whether the CNSs of extant bilaterians originated with an array of nerve nets or with a primordial neuronal aggregation is unknown. The nervous systems of cnidarians, the closest sister branch to bilaterians, manifest similarities to bilaterian nervous systems, including developmental mechanisms and cellular features. For example, the cnidarian neurons are electrically excitable, communicating with other neurons or muscles via chemical synapses, and forming diffuse neural networks with significant condensations along the main body axis.

Recent genomic and gene expression data from cnidarians and other basal metazoans have provided hints to reconstruct the evolutionary history of neurons and the CNS. Genes involved in neuronal physiological functions are conserved among bilaterians, cnidarians, and even sponges. The latter possess sensory cells, but not neurons, providing insights into the origin of neurons. Accumulating evidence shows that cnidarians develop a neural condensation, a “semi-centralized nervous system (semiCNS),” composed of multiple neuronal cell types. Although the development and function of cnidarian nervous systems, especially the semiCNS, remain largely unexplored, numerous molecular signatures shared by cnidarians and bilaterians help us to understand early processes of neural centralization.

Keywords cnidarians • ctenophores • placozoans • sponges • Evolution • Protonuron • Nervous system

H. Watanabe (✉)

Evolutionary Neurobiology Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Kunigami, Okinawa 904-0495, Japan
e-mail: hiroshi.watanabe@oist.jp

3.1 Introduction

The anatomically and functionally organized network of a nervous system serves the operational center for animal behaviors. Regionalized condensations of neurons, including the brain of bilaterians, have essential roles for cognitive functions in which neurons process information about ambient stimuli and sometimes store it as individual experiences.

At a very early stage of animal evolution, neurons may have originated as unspecialized cells with sensory, neurosecretory, and contractile functions. These ancestral, multifunctional cells became segregated into distinct cell types with either specific sensory, neuronal, or contractile function (Mackie 1970). Neurons formed extended cellular processes, or neurites, connecting to a specific neighboring cells via synapses. This “neural” system seems to have evolved for a rapid and specific signal transmission from sensory cells to a certain specific cell clusters such as a contractile units of muscles and a ciliomotor systems. In contrast to cell–cell communication mediated by undirected diffusion of signaling chemicals, the directed and restricted mode of synaptic communication between connected neurons allows animals to execute coordinated body movements in response to specific environmental contexts.

The origin of the nervous system is one of the most exciting questions in biology. There has long been interest in the use of basal metazoans, animal lineages that diverged early in animal evolution, including poriferans, placozoans, ctenophores, and cnidarians (Fig. 3.1)—to understand the early evolutionary processes of animal-specific traits such as the nervous system. In recent years, thanks to sequencing of the basal metazoan genomes, evolutionary biologists have made spectacular advances in unveiling primitive neuronal components. Recent findings in the basal metazoans have also raised several important questions, including whether a nervous system arose only once, or multiple times, and whether neural condensations in bilaterian and cnidarian branches reflect a homologous ancestral nature or a paraphyletic neural characteristics. Answers to these questions are pivotal in reconstructing the molecular and cellular features of the nervous systems that existed in ancestral metazoans.

In this chapter, I first provide an overview of genetic repertoires of “neural” components found in basal metazoan genomes and anticipate the genetic and cellular natures of primordial neurons. I then focus on molecular and anatomical features and on physiological functions of the nervous systems in extant cnidarians. Finally, I discuss the nature of primordial neural assemblies that may have been present before divergence of the Cnidaria and Bilateria.

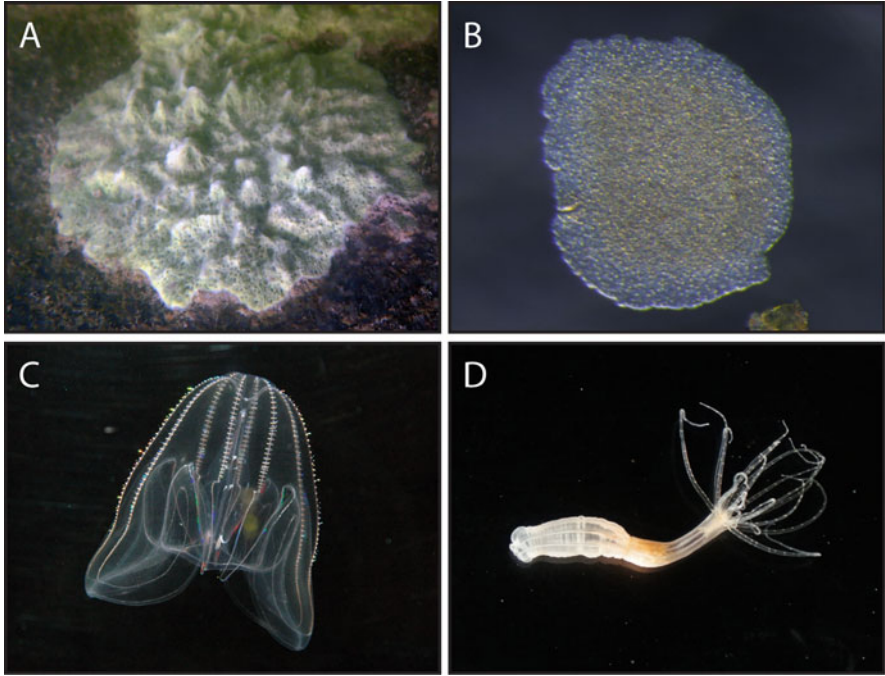


Fig. 3.1 Images of basal metazoans. (a) *Ephydatia fluviatilis* (Porifera). (b) *Trichoplax adhaerens* (Placozoa). (c) *Bolinopsis mikado* (Ctenophora). (d) *Nematostella vectensis* (Cnidaria). [Photographs courtesy of Dr. Noriko Funayama (*E. fluviatilis*), Dr. Hiroaki Nakano (*T. adhaerens*), and Ms. Noriko Ishikawa (*B. mikado*)]

3.2 Neural Gene Repertoires in Basal Metazoans

3.2.1 Poriferans

The phylum Porifera (sponges) comprises basal metazoans that do not possess bona fide neurons. Transcriptomic and genomic data from all four classes of poriferans (Hexactinellida, Demospongiae, Homoscleromorpha, and Calcarea) revealed that this basal metazoan lineage possesses surprisingly complex gene components believed to have been involved in the development and function of nervous systems (Table 3.1) (Simionato et al. 2007; Riesgo et al. 2014). Genomic analyses of *Amphimedon queenslandica* (Demospongia) have identified poriferan homologues for bilaterian neural genes such as *SoxB*, *Lhx*, and proneural basic helix-loop-helix (bHLH) transcription factors, *Elav/Musashi*-like RNA-binding protein (RBP) genes, and Notch signaling molecules (Richards et al. 2008; Larroux et al. 2008; Srivastava et al. 2010a; Fortunato et al. 2012; Richards and Degnan 2012). *AmqbHLH1*, a bHLH transcription factor gene that seems to belong to the atonal-related protein (Arp) superfamily, is expressed in globular cells of parenchymella larvae of *A.*

Table 3.1 Molecular and cellular features of sensory cells and neurons of metazoan animals

	Porifera	Placozoa	Ctenophora	Cnidaria	Bilateria
Neurosecretory cells	+	+	+	+	+
Neurons	-	-	+	+	+
Transcription factor genes	Proneural bHLH (expr./func.)	+	+	+	+
	SoxB (expr./func.)	+	+	+	+
Neural synapses	Electric synapse (gap junctions)	-	-	+	+
	Synapses	-	+	+	+
Peptides	Conserved neuropeptides	+	- ^c	+	+
	Peptide-gated ion channels	+	+	+	+
Chemical transmitters	Chemicals	Glu, GABA, Gly, NA, AD, 5-HT ^d	Glu, GABA	Glu, GABA, Gly, DA, NA, AD, 5-HT, Ach	Glu, GABA, Gly, DA, NA, AD, 5-HT, Ach
	Genes	GAD, AAAH, AADC, DBH, AChE	GAD, AAAH, AChE	GAD, AAAH, AADC, DBH, ChAT, AChE	GAD, PH, TH, TpH, AADC, DBH, ANAT, HIOMT, ChAT, AChE
Neural function	-	-	+	+	+

^aGap junctions between small number of neurons have been found only in hydrozoans, but not the other cnidarian classes

^bAlthough no neuropeptides have been identified in poriferan genomes, cnidarian L-Wamide neuropeptide treatment has been found to trigger settlement of poriferan larvae (Whalan et al. 2012)

^cAlthough none of the evolutionary conserved neuropeptides (e.g., RFamide and LWamide) have so far been identified from ctenophore genomes, neuronal FMRFamide immunoreactivity has been observed (Jäger et al. 2011)

^dChemical transmitters known to modulate the contracting behavior of poriferan species

^eTrichoplax genome encodes genes involved in synthesis and vesicular transport of several chemical transmitters (NA, AD) (Srivastava et al. 2008)

queenslandica (Fig. 3.2). The globular cells, putative sensory cells in sponges, are located in the outer epithelium along the larval primary axis. Richards and colleagues found that the *AmqbHLH1* gene is coexpressed with genes for the Notch/Delta signaling pathway during cellular differentiation (Richards et al. 2008; Richards and Degnan 2012). In addition to the globular cells, the *A. queenslandica* larvae bear distinct sensory cells in anterior and posterior regions. In the anterior region, sensory flask cells develop in the outer epithelium to regulate larval metamorphosis (Nakanishi et al. 2015). A ciliated pigmented ring of photo-sensitive sensory cells develops at the posterior end of the larvae (Leys and Degnan 2001). Although genes specific to flask cells have not yet been found, pigmented ring cells express a number of genes that are involved in bilaterian sensory cell development, for example, proneural *Achaete-Scute homolog (Ash)*, *SoxB2*, *Pax2/5/8*, and *Lhx* (Fig. 3.2) (Larroux et al. 2006; Richards et al. 2008; Srivastava et al. 2010b; Degnan et al. 2015). Clear homologues of *SoxB* genes have been identified in the two demosponge species, *A. queenslandica* (Larroux et al. 2006, 2008) and *Ephydatia muelleri*, and in a calcareous sponge, *Sycon ciliatum* (Fortunato et al. 2012). In *S. ciliatum* and *A. queenslandica*, the *SoxB* genes are expressed in cruciform cells (putative sensory cells or their precursors) during larval development (Fortunato et al. 2012). Cruciform cells also express *Elav* and *Musashi*, as well as *Pax* and *Six* transcription factors involved in formation of bilaterian eyes and other sensory organs (Fortunato et al. 2014).

Phylogenetic analyses of poriferan genes have shown to be rich in molecular components involved in formation of the postsynaptic density (PSD) (Sakarya et al. 2007; Alié and Manuel 2010; Srivastava et al. 2010a; Riesgo et al. 2014). Genomes of all four poriferan classes contain PSD genes, with little variation among species, suggesting that these genes existed in the common poriferan ancestor. It should be noted that poriferan homologues for PSD components *Dlg*, *Homer*, *Grip*, *Cript*, and *Gkap* are coexpressed dominantly or exclusively in the sensory globular cells of *Amphimedon parenchymella* larvae (Sakarya et al. 2007). Concurrent expression of multiple postsynaptic gene homologues may support the existence of a macromolecular complex (Sakarya et al. 2007; Emes et al. 2008; Ryan and Grant 2009). The existence of PSD genes in poriferan genomes, however, does not necessarily connote the appearance of functional PSD in the common poriferan ancestor, because a significant number of PSD genes have also been identified even in unicellular organisms such as choanoflagellates (Alié and Manuel 2010; Burkhardt et al. 2014; Burkhardt 2015). Indeed, no clear morphological feature consistent with a PSD or a synapse has been observed in sponges.

These findings suggest that the genetic mechanisms giving rise to both sensory cells and neurons have a deep evolutionary root (Fig. 3.3). However, genetic and signaling mechanisms regulating early commitment and later differentiation of the poriferan sensory cells still remain largely unknown. Additionally, functional and molecular dissection of the PSD protein complex in the poriferan sensory cells will

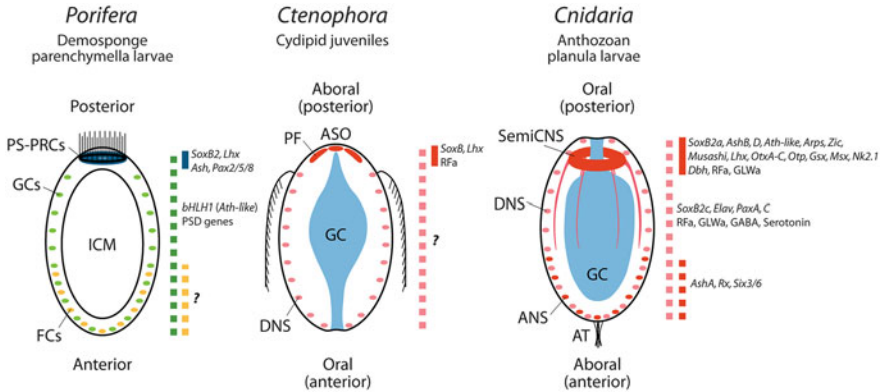


Fig. 3.2 The larval body plans of basal metazoans and expression patterns of neural markers. Regionalized expression of neural marker genes along the primary body axis of three basal metazoan larvae. In Porifera, the *blue line* indicates neurogenic gene expression in photosensitive pigmented ring cells. The *dashed green* and *dashed yellow lines* denote sensory globular cells and flask cells, respectively. The *red lines* in Ctenophora and Cnidaria indicate posterior neural aggregations from their diffuse nervous systems. The *dashed pink line* in Cnidaria shows pervasive expression of neural marker genes. Ctenophores possess a diffuse nervous system, whereas no expression of neural marker genes has been shown. The *dashed red line* in Cnidaria indicates expression of neural markers for aboral nervous system. *ANS* aboral nervous system, *ASO* apical sensory organ, *AT* apical tuft, *DNS* diffuse nervous system, *GC* gastric cavity, *FCs* flask cells, *GCs* globular cells, *semiCNS* semi-centralized nervous system, *PF* polar field, *PS-PRCs* photo-sensitive pigment ring cells

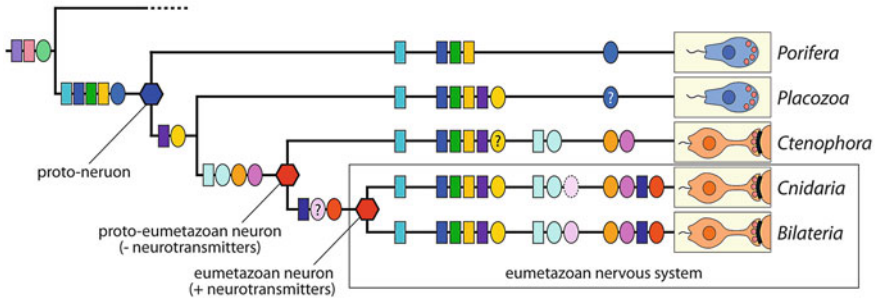
help us to explain how the “post”synaptic proteins are implicated in function of the sensory (usually “pre”synaptic) cells.

3.2.2 Placozoans

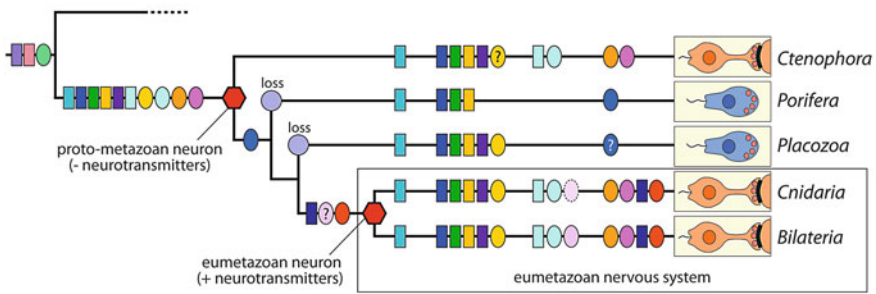
The phylum Placozoa contains at least 19 groups of disc-shaped marine invertebrates, a 1- to 2-mm in diameter. They are simple metazoans with two epithelial layers and some cell types interspersed in between, but they lack neurons. Recent studies on *Trichoplax* have described six somatic cell types comprising ciliated dorsal and ventral epithelial cells, lipophil cells, fiber cells, crystal cells, and gland cells (Smith et al. 2014).

Homologues of neurogenic *SoxB*, *Lhx*, and group A bHLH transcription factors related to *Ash* or *Arp* and to most components of synaptic vesicles and PSD, including synaptotagmin, have been identified in the genome of the placozoan, *Trichoplax adhaerens* (Fig. 3.3) (Srivastava et al. 2008; Gyoja 2014). Although the physiological functions and genetic signatures of placozoan cell types are largely unknown, immunostaining using anti-FMRFamide antibody revealed that this simple animal lacking neurons deploys RFamide neurosecretory cells in the

A Porifera-sister hypothesis



B Ctenophora-sister hypothesis



- | | | |
|---------------------------------|--|--|
| PSD component (DLG/Homer/Shank) | Glu/GABA | ancestral sensory neurosecretory cells |
| GAD/AAA/AADC | chemical transmitters (non-neural) | ancestral neurons (with neurite & triad synapse) |
| proneural bHLH/SoxB | RFamide | |
| neurexin | neurotransmitter (Glu) | |
| mGluR/iGluR | neurotransmitters (GABA, monoamines 5-HT, ACh) | |
| peptide-gated ion channels | triad synapse | |
| synaptotagmin | epithelial gap junction | |
| innexins | neuronal gap junction | |
| neuroligin | | |

Fig. 3.3 Two scenarios of neuronal evolution. Key genetic and physiological innovations underlying neural organization in metazoan evolution. *Rectangles* and *ellipses* indicate acquisition of selected gene families and physiological and cellular properties, respectively. The presence of neurosecretory cells and neurons with neurites and synapses are shown to the *right*, respectively. The emergence of postulated ancestral cell types is shown in hexagons. (a) In the conventional metazoan tree, the “porifera-sister hypothesis,” “proto-neurons” may have existed in the common metazoan ancestor. This postulates that ancestral cells may have resembled the neurosecretory cells lacking neurites and synapses that are seen in modern poriferans and placozoans. Genes for glutamatergic and peptidergic systems may already have been deployed for cell–cell communications in the common metazoan ancestor. After the common ancestor of Ctenophora/Cnidaria/Bilateria branched off, gap junctions (innexins), neurites, and synapses evolved. (b) In the ctenophora-sister hypothesis, one assumption is that gap junctions and these neuronal characteristics were deployed in the common metazoan ancestor. In this scenario, glutamatergic and probably peptidergic proto-metazoan nervous systems, gap junctions, neurites, and synapses have been lost in poriferan and placozoan lineages

marginal body region (Schuchert 1993a). Smith and colleagues have demonstrated that in the ciliated gland cells, an FMRFamide-like neuropeptide is coexpressed with synaptic vesicle proteins such as syntaxin, SNAP-25, and synapsin, suggesting neurosecretory functions of this cell type (Smith et al. 2014). Expression and function of the neurogenic transcriptional factors in the gland cells remain to be explored.

3.2.3 *Ctenophores*

Ctenophores, collectively known to as comb jellies, are a group of neuron-bearing marine invertebrates with controversial ancestry. They are thought to be genetically less complex, because ctenophore genomes only have a few Wnt and homeobox genes and apparently do not seem to encode any of the micro-RNA homologues that have been identified to date in cnidarians and bilaterians (Fortunato et al. 2015; Maxwell et al. 2012). Recent phylogenomic analyses and comparisons of genetic repertoires suggest that this orphan animal lineage is a sister group to all other extant metazoans (the ctenophora-sister hypothesis) (Fig. 3.3b) (Dunn et al. 2008; Ryan et al. 2013; Moroz et al. 2014; Whelan et al. 2015a). Other phylogenetic analyses, however, proposed that ctenophores and cnidarians form a clade with bilaterians in the *Eumetazoa* (animals with nerve and muscle cells) (Fig. 3.3a) (Philippe et al. 2009, 2011; Pick et al. 2010). With increasing transcriptomic and genomic data from basal metazoan species, systematic errors that can cause mis-positioning of basal metazoan taxa are now under careful scrutiny (Pisani et al. 2015; Whelan et al. 2015a, 2015b). Nevertheless, understanding the ctenophoran nervous system at the molecular and cellular level is essential to reconstruct the ancestral nervous systems.

Ctenophores have nerve nets with mesogleal fibers and tentacular nerves. These nerve nets exhibit numerous condensations associated with the apical sensory organs/polar fields and tentacle bulbs (Harbison 1985). Ctenophore genomes, as well as poriferan genomes, contain homologues of neurogenic transcription factors including *Lhx*, *bHLH*, *Six*, and *SoxB*, as well as neural RBP genes, *Elav* and *Musashi*, that are involved in early neural development of bilaterians. Poriferans and ctenophores have some genes for axon guidance molecules, including semaphorin, plexin, and an ephrin receptor, while others, such as netrin and Unc-5, are absent from the genomes of both phyla (Srivastava et al. 2010a; Ryan et al. 2013; Moroz et al. 2014). Most of the genes involved in the formation of bilaterian PSDs have been identified in both ctenophoran and poriferan genomes, but they lack certain genes, such as *Erbin* and *Neurologin* (Srivastava et al. 2010a; Riesgo et al. 2014; Ryan et al. 2013; Moroz et al. 2014). These comparative genomic data indicate that certain axon guidance molecules and scaffolding proteins were absent in the common ancestor of these basal metazoans.

Glutamate appears to be the best transmitter candidate for ctenophoran neuromuscular transmission (Table 3.1) (Moroz et al. 2014). Ctenophoran genomes

possess a gene related to glutamate decarboxylase (GAD) that synthesizes γ -aminobutyric acid (GABA). There is no clear genetic evidence supporting synthesis of any other bilaterian neurotransmitters. Immunohistochemical and biochemical analyses using *Pleurobrachia bachei* have failed to detect conventional neurotransmitters [e.g., monoamines and acetylcholine (ACh)] (Hay-Schmidt 2000; Moroz et al. 2014). While these data could be interpreted to mean that the ctenophoran nervous system evolved independently (Moroz et al. 2014), a substantial set of neuronal marker genes in the nervous systems of ctenophores and cnidarians/bilaterians imply a common evolutionary origin (Watanabe et al. 2014a; Marlow and Arendt 2014; Jékely et al. 2015). For example, *SoxB* and *Lhx* transcription factors and RFamide-like neuropeptides are expressed in neurons in the apical region of ctenophores (Fig. 3.2) (Jager et al. 2008, 2011; Simmons et al. 2012). In accordance with this hypothesis, ctenophores, cnidarians, and bilaterians, but not poriferans or placozoans, have neuronal synapses (Fig. 3.3) (Hernandez-Nicaise 1973).

The lack of unambiguous evidence for neurotransmitter use by ctenophores could argue for an independent origin of the ctenophoran nervous system; however, nonneural chemical transmitter localization and functions are observed among cnidarians and bilaterians. And in poriferans, many of the transmitters are involved in modulation of contractile behavior (see following). Thus, the absence of chemical neurotransmission may not be a reliable basis for falsifying the homology of nervous systems in early branching metazoans.

3.2.4 *Protoneurons: An Ancestral Neurosecretory Cells?*

Many primary ciliated larvae of marine invertebrates possess the apical sensory neurosecretory cells expressing RFamide and Wamide neuropeptides (Lacalli 1983; Dickinson and Croll 2003; Nielsen 2005; Tessmar-Raible et al. 2007; Byrne et al. 2007; Conzelmann et al. 2011, 2013; Conzelmann and Jékely 2012). Sensory neurosecretory cells have repeatedly been considered in different evolutionary contexts as ancestral neuronal cells or “protoneurons” (Víggh and Víggh-Teichmann 1982; Tessmar-Raible et al. 2007; Sakarya et al. 2007; Richards et al. 2008; Jékely et al. 2015). Since vertebrate and invertebrate deuterostomes possess an assembly of ciliated sensory neurosecretory cells contacting the cerebrospinal fluid in the lumen of the CNS (vertebrates) or ambient seawater (invertebrates), ciliated sensory neurosecretory cells have been regarded as a phylogenetically old neurosecretory cell type, the “protoneuron” (Víggh and Víggh-Teichmann 1982; Víggh et al. 2004). Detailed molecular and cellular dissections of the developing apical neurosecretory cell cluster of annelid larvae have suggested that the sensory neurosecretory cells already existed in the common bilaterian ancestor (Tessmar-Raible et al. 2007; Conzelmann et al. 2013; Tosches and Arendt 2013; Marlow et al. 2014; Nielsen 2015). RFamidergic sensory neurosecretory cells have also been identified in cnidarian planula larvae (Plickert 1989; Leitz and Lay 1995; Gajewski et al. 1996). Although peptidergic neurophysiological features of the ctenophoran

nervous system remain highly understudied, a rich neural gene repertoire, including peptide-gated ion channels (PGICs) in poriferan, placozoan, and ctenophoran genomes indicate that the conceptual protoneuron might be traced back to the sensory neurosecretory cell types that may have existed in the common metazoan ancestor (Fig. 3.3) (Smith et al. 2014; Jékely et al. 2015). This idea is supported by the fact that the RFamide neuropeptides are expressed exclusively in cnidarian nervous systems and placozoan neurosecretory gland cells. Since conventional chemical neurotransmitters don't seem to be neuron-specific in cnidarians, it would be useful to identify neuropeptides in neurons and sensory cells in sponges and ctenophores.

3.3 Cnidarian Nervous Systems

The Cnidaria is a large and successful phylum containing more than 9,000 species, and in phylogenetic terms, it represents the closest sister group to all bilaterians. The Cnidaria is divided into two major lineages: the Anthozoa (corals, sea anemones, and sea pens) and the Medusozoa, consisting of four classes: Hydrozoa (hydras and marine hydrozoans), Cubozoa (box jellyfish), Scyphozoa (true jellyfish), and Staurozoa (stalked jellyfish). Cnidarians usually have a life cycle containing a polyp stage and a medusa (jellyfish) stage (Bridge et al. 1992, 1995; Odorico and Miller 1997; Schuchert 1993b; Collins 2002; Collins et al. 2006). The Anthozoa are thought to retain ancestral characteristics, including 1) polyps that never metamorphose into medusae, 2) few derived genomic features, and conserved mitochondrial genome structure (circular in anthozoans and bilaterians, but linear in medusozoans), 3) nematocysts (the cnidarian-specific stinging apparatus) that are less elaborate and diversified than those of medusozoans (Pantin 1966; Willmer 1990; Bridge et al. 1995; Medina et al. 2001; Collins 2002; Dunn et al. 2008).

The privileged phylogenetic position of the Cnidaria as the closest sister group to the Bilateria has made these animals one of the most useful models for deciphering the genetic basis for the early evolution of sophisticated nervous systems, such as the central nervous system (CNS). In addition to the phylogenetic position of the Cnidaria, transcriptomic and genomic data from various cnidarian species, including the anthozoans *Nematostella vectensis* and *Aiptasia* sp. (sea anemones), *Acropora digitifera* and *Acropora millepora* (stony corals), and the hydrozoans *Hydra magnipapillata*, *Cladonema pacificum*, and *Clytia hemisphaerica*, have made the Cnidaria even more useful for comparative and functional molecular studies of the evolution of neurodevelopmental events (Kortschak et al. 2003; Technau et al. 2005; Putnam et al. 2007; Shinzato et al. 2011; Watanabe et al. 2014b; Baumgarten et al. 2015).

Comparative genomic analyses of neural genes using *Nematostella vectensis* (Anthozoa) and *Hydra magnipapillata* (Hydrozoa) have revealed extensive conservation of the neural gene repertoire, not only between these distantly related cnidarian species, but also among other metazoan lineages. In general, cnidarian

genomes possess a nearly complete set of homologous genes involved in bilaterian neurotransmission and neurodevelopmental processes, including neurogenesis, neuronal specification, and neural network formation (Fig. 3.3; Table 3.1) (for more detail, see Watanabe et al. 2009; Galliot et al. 2009; Galliot and Quiquand 2011).

Chemical synapses, in which signal transmission is effected by neuropeptides and small chemicals (acetylcholine, monoamines, and amino acids), are essential for both slow and fast transmission. Neuropeptides are related to peptide hormones and are often involved in slow transmission in bilaterian nervous systems. Among cnidarians, neuronal cells, mainly ganglionic neurons, have been shown to possess synapses with lucent-core and dense-core vesicles (Davis et al. 1968; Kinnamon and Westall 1981; Westfall and Kinnamon 1978, 1984; Kass-Simon and Pierobon 2007; Pierobon 2012). Small chemical neurotransmitters are generally packaged in small, lucent-core synaptic vesicles that are clustered in presynaptic terminals. Neuropeptides are generally stored in large, dense-cored vesicles that are distributed at presynaptic terminals and also along neuronal processes (for review, see Pierobon 2012; Fujisawa and Hayakawa 2012). These observations suggest that neurotransmission and neuromodulation mediated by both chemical transmitters and neuropeptides are involved in cnidarian neurotransmission.

3.3.1 Peptidergic Nervous Systems

Neuropeptides serve essential and pleiotropic neural functions in both cnidarians and bilaterians. The cnidarian nervous system is rich in neuropeptides, including the short amidated neuropeptides, RFamide and LWamide (Fujisawa 2008; Watanabe et al. 2009). These neuropeptides emerged before the Cnidaria/Bilateria evolutionary split (Jékely 2013). Among cnidarians, RFamide has been studied most extensively. The first sign of *Rfamide* expression can be detected in some cells of embryonic epithelium at the blastula stage (Marlow et al. 2009; Richards and Rentzsch 2014). Here, it contributes to formation of a loose plexus of the neurons at the base of the ectoderm. At the planula stage, RFamide-positive neurons are well developed around the anterior (aboral) region of hydrozoan and scyphozoan larvae (Fig. 3.4) (Martin 1988, 1992; Plickert 1989; Leitz and Lay 1995; Gajewski et al. 1996; Gröger and Schmid 2001; Seipel et al. 2004; Nakanishi et al. 2008; Marlow et al. 2009). In the more basal cnidarian group, sea anemones, the RFamidergic neurons form a neural cluster at the oral region in addition to the nerve net (Fig. 3.4) (Marlow et al. 2009; Watanabe et al. 2014a). Oral and pharyngeal development of the RFamidergic neurons at polyp and medusae stages has widely been observed among cnidarian phyla (Grimmelikhuijzen 1985; Plickert 1989; Grimmelikhuijzen et al. 1988, 1991; Koizumi et al. 1992; Mackie and Stell 1984; Mackie et al. 1985; Anderson et al. 2004). It probably constitutes a plesiomorphic neural character of cnidarians. The oral arrangement of the RFamidergic neurons in cnidarian polyps can be anatomically divided into a hypostomal accumulation of neuronal perikaria and ring-shaped neurite bundles (nerve ring) that formed between the hypostome

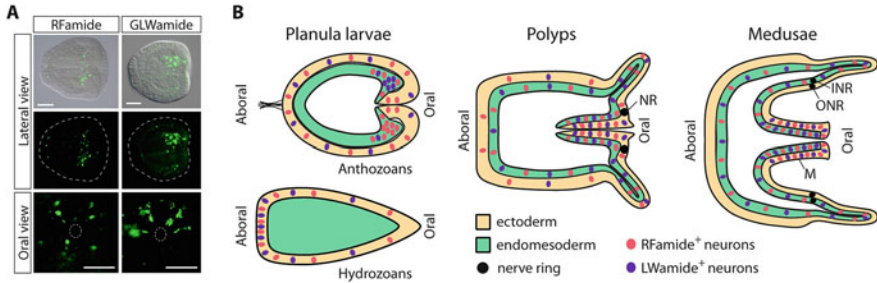


Fig. 3.4 Semicentralization of the cnidarian oral/blastoporal nervous system. **(a)** Lateral and oral views of neural subsystems expressing RFamide or GLWamide neuropeptides of *Nematostella* planula larvae. Note that these neural populations are localized mainly around the pharynx. GLWamidergic neurons exhibit an additional bias along the secondary axis, which is orthogonal to the primary oral–aboral axis. *Dotted white circles* in *lower panels* indicate the blastopore. *Bars* in *upper panels* (lateral view) 100 μm ; *bars* in *lower panels* (oral view) 50 μm . **(b)** Schematic views of regionalization of RFamidergic (RFamide⁺) and LWamidergic (LWamide⁺) neurons in the cnidarian life stages. Blastoporal condensation of these neurons develops in the planula larva stage of *Nematostella* (Anthozoa). The sensory function of the apical organ/tuft is not clear yet. Among medusozoans such as hydrozoans, neurons are developed predominantly in the aboral (or apical) region of the planula larvae. During the polyp and medusa stages, the oral/pharyngeal neural condensations become more conspicuous and nerve rings develop around the mouth opening (polyp) or at tentacle bases (medusa). *NR* nerve ring, *M* manubrium, *INR* inner nerve ring, *ONR* outer nerve ring

and tentacle bases (Mackie and Stell 1984; Mackie et al. 1985; Grimmelikhuijzen et al. 1988, 1991; Koizumi et al. 2014). These distinct neural architectures found in polyps could be related to a neuronal condensation at the manubrium (pharynx) and the nerve ring at the bell margin in medusae (Fig. 3.4). Neural expression of LWamide neuropeptide has also been demonstrated in a wide variety of cnidarian species (Leitz and Lay 1995; Schmich et al. 1998a; Mitgutsch et al. 1999; Takahashi et al. 2003; Watanabe et al. 2014a). In addition to the RFamidergic nervous system, LWamidergic neurons develop in the oral/pharyngeal neural condensation, and during the medusa stage, the nerve ring around the bell margin (Fig. 3.4) (Schmich et al. 1998a; Koizumi et al. 2014; Watanabe et al. 2014a). These expression patterns suggest that RFamide and LWamide have critical functions in the oral nervous system.

Activity of peptidergic neurons is required for various coordinated behaviors in cnidarians. Myoactivities of cnidarian neuropeptides have been well characterized in polyps. The RFamide neuropeptide family is believed to regulate the myoactivity and modulatory role of muscle contraction (McFarlane et al. 1987, 1991; Shimizu and Fujisawa 2003; Fujisawa 2008). A *Hydra* LWamide neuropeptide (Hym-248) is reported to directly induce relaxation of myoepithelial cells in the body column and tentacles (Takahashi et al. 2003). In addition to the myoactivity control, the RFamidergic and LWamidergic neurons have pivotal roles in medusozoans in positive and negative phototactic behavior (Katsukura et al. 2004; Plickert and

Schneider 2004). The cnidarian nervous system forms synaptic connections with both epitheliomuscular cells and neighboring neurons (Westfall et al. 1971, 2002; Westfall 1973, 1987; Westfall and Kinnamon 1978, 1984; Kinnamon and Westfall 1982). Dense-core, neuropeptide-containing vesicles accumulate in the presynaptic area in nerve terminals and at *en passant* synapses (Koizumi et al. 1989; Westfall and Grimmelikhuijzen 1993; Westfall et al. 1995), indicating that localized release of neuropeptides at synapses serves directed and restricted signal transmission. This idea finds support in the unidirectional neural conduction and partial contraction of tentacles in *Hydra* (Rushforth and Hofman 1972; Shimizu 2002). These findings, taken together, indicate that in cnidarians, the structural organization of the nervous system is indispensable for neural regulation of behavior during all life stages.

3.3.2 Classical Chemical Neurotransmitters

Although the cnidarian nervous system is rich in neuropeptides, accumulating physiological, biochemical, and ultrastructural evidence suggest that classical neurotransmitters and neuromodulators are also involved in neural function (Pierobon 2012). Cnidarian genomes possess a number of genes showing significant similarity to bilaterian genes for synthesis and degradation of classical chemical neurotransmitters, including acetylcholine (ACh), monoamines, GABA, and nitric oxide (NO). Homology searches of bilaterian genes for the chemical neurotransmitter receptors and transporters have identified a large number of putative cnidarian counterparts (Watanabe et al. 2009; Anctil 2009; Marlow et al. 2009; Chapman et al. 2010; Oren et al. 2014). Since many of these genes probably existed in the common poriferan and cnidarian ancestor, they seem to have deep evolutionary roots with non-neural functions. However, physiological and immunohistological data imply that small chemical transmitters and modulators play roles in the cnidarian neural functions.

Glutamate is the most common excitatory neurotransmitter among bilaterians. The *Nematostella* genome has a large number of genes for metabotropic and ionotropic glutamate receptors (mGluR and iGluR, respectively) (Anctil 2009). Among four classes of the iGluRs, including NMDA, AMPA, Delta, and kainate receptors, *Nematostella* genes show greater similarity to the NMDA and AMPA classes. Immunohistochemical studies indicate that the cnidarians have several neuronal and sensory cell populations expressing iGluR-like proteins (Kass-Simon and Scappaticci 2004). Glutamate accumulates in processes of these neural cells (Martin 2004; Delgado et al. 2010). Glutamate and its agonists, NMDA, AMPA, and kainite, appear to have an excitatory functions in control of epitheliomuscular cell contraction and discharge of the nematocysts (Kass-Simon et al. 2003; Scappaticci et al. 2004; Scappaticci and Kass-Simon 2008). Other excitatory neurotransmitters, such as serotonin, dopamine, and adrenaline, as well as neuromodulatory molecules, including NO and carbon monoxide (CO), are involved in cnidarian neural functions (Kass-Simon and Pierobon 2007; Pierobon 2012). Cnidarians possess homologues to bilaterian genes such as choline acetyltransferase (ChAT), acetylcholinesterase

(AChE), and nicotinic ACh receptor subunits (Anctil 2009). ACh induces musculoepithelial contraction in cnidarians (Lentz and Barnett 1961; Scappaticci and Kass-Simon 2008).

The *Nematostella* genome includes receptors for inhibitory transmitters, GABA and glycine. Sequences of GABAB and glycine receptors show a higher degree of similarity to bilaterian receptors than do GABA_A sequences, which are more distantly related to their bilaterian counterparts and which lack certain amino acid residues important for GABA binding (Anctil 2009). Immunohistochemical analyses for GABA have demonstrated the neuronal localization of this transmitter in sea anemones and cubozoan medusae (Martin 2004; Marlow et al. 2009; Delgado et al. 2010), implying a role in cnidarian neural function. This idea is supported by data from the sea fan, *Eunicella cavolini* (Anthozoa), in which GABA signaling molecules such as GAD, the enzyme that produces GABA, the vesicular GABA transporter (VGAT), and the GABAB receptor are expressed in neuronal cells (Giroi et al. 2007). Glycine and taurine, a glycine receptor agonist, also localize in cnidarian neurons. A taurine-like immunoactivity in the ganglia and sensory neurons was reported from anthozoan and scyphozoan species (Carlberg et al. 1995; Anctil and Minh 1997; Nakanishi et al. 2008). Neurophysiological functions of glycine and taurine have been analyzed in *Hydra*, and both chemicals are involved in the peristaltic contraction of the epitheliomuscular cells and in the chemosensory response (Pierobon et al. 2001; Ruggieri et al. 2004; Kass-Simon and Pierobon 2007). An interesting finding is that taurine can function as an excitatory neuromuscular transmitter in cnidarians (Carlberg et al. 1995; Anctil and Minh 1997). Taken together, it appears that these classical neurotransmitters had already been used in the nervous system before the Cnidaria/Bilateria cladogenesis (Fig. 3.3; Table 3.1).

3.3.3 Nonneural Functions of Classical Transmitters

While a large variety of ultrastructural, biochemical, and neurophysiological data from cnidarians indicates that the conventional neurotransmitters and neuromodulators are involved in neural control of cnidarian behavior, nonneural localization and functions of these chemicals have also been reported (for review, see Kass-Simon and Pierobon 2007). Glutamate, for instance, has been detected in nonneural cells in the oral/pharyngeal region of sea anemones (Anctil and Carette 1994; Oren et al. 2014). Expression patterns of genes for GAD and AChE suggest that GABA and ACh can also be metabolized in nonneuronal epithelial cells (Denker et al. 2008; Takahashi and Hamaue 2010; Oren et al. 2014). Nonneuronal metabolism of ACh is likely because the gene encoding the choline transporter (ChT) was expressed in epithelium of *Hydra* polyps that were depleted of all neural cells (Chapman et al. 2010).

Interestingly, molecular evidence suggests that receptors for glutamate and GABA were present before plants and animals diverged (Lam et al. 1998; Moroz

2001). Glutamate and GABA act as important chemical messengers in organisms lacking neurons, such as poriferans, plants, and even unicellular protists, in which these molecules are involved in contraction, feeding, sensory systems, and development (Lam et al. 1998; Moroz 2001; Bouche et al. 2003; Davenport 2002; Elliott and Leys 2010). Clearly, glutamine- and GABA-based transmission systems predated the development of the nervous systems (Fig. 3.3) (Parker 1910; Jones 1962; Pavans de Ceccatty 1974a, 1974b, 1979; Mackie 1970, 1979, 1990; Nickel 2004). In poriferans, some classical transmitters control contraction behaviors regulated by contractile cells, such as myocytes/actinocytes (Boury-Esnault and Rützler 1997) and/or pinacocytes (Nickel et al. 2011). mGluRs and iGluRs have been identified in poriferan genomes (Perovic et al. 1999; Srivastava et al. 2010a; Riesgo et al. 2014; Burkhardt et al. 2014). Glutamate treatment of adult poriferans induced contraction and propagation of a stereotypical behavior, inflating and deflating the canal system (Ellwanger et al. 2007; Elliott and Leys 2010). Recent phylogenetic studies have demonstrated that poriferans have genes involved in synthesis of monoamines (dopamine, adrenaline, and serotonin) and ACh, that include GAD, DOPA decarboxylase, tryptophan hydroxylase (TpH), and dopamine β -hydroxylase (DBH) (Srivastava et al. 2010a; Riesgo et al. 2014). Consistent with the existence of the GAD and TpH/DBH genes, GABA and serotonin have been detected in poriferans, where they stimulate and modulate contractions (Ellwanger and Nickel 2006; Ellwanger et al. 2007; Ramoino et al. 2007, 2011; Elliott and Leys 2010).

Genomic and transcriptomic analyses have tentatively identified genes for the synthesis, degradation, and transport of classical transmitters in basal metazoans; however, sequence data for these enzymes are not sufficiently conclusive to confirm their substrates and catabolites, thereby decreasing the reliability of transmitter prediction, especially in lower metazoans. For example, in cnidarian genomes, genes related to bilaterian choline/carnitine acetyltransferase subfamilies have been found. However, detailed analyses of the amino acids involved in substrate discrimination have obscured whether the catalytic efficiency of these cnidarian enzymes favors choline more than carnitine as a substrate (Chapman et al. 2010). One should exercise great caution when considering genes of the aromatic amino acid hydroxylase (AAAH) family, including TH and TpH, and of the methyltransferase family, including phenylethanolamine *N*-methyltransferase (PNMT). Since a large genetic repertoire for chemical neurotransmitter synthesis exists in poriferans, more detailed sequence analysis of these basal metazoan homologues and biochemical examination of their real catabolites will help us to understand the ancient means of intercellular communication and the evolutionary history of neurotransmitters.

3.3.4 *Electrical Synapses and Gap Junctions*

In addition to chemical neurotransmission, there are electrical synapses in hydromedusae that fire synchronously, serve pacemaker functions and coordinate contraction of the epitheliomuscular cells (Campbell et al. 1976; Passano and McCullough

1963; Shimizu and Fujisawa 2003; Takaku et al. 2014). Electric synapses are specialized and physically connected transcellular channels enabling rapid bidirectional communication between two neighboring cells. In *Hydra*, gap junctions form between ectodermal cells and between endodermal cells, and mediate electrical coupling of these epitheliomuscular cells (Hand and Gobel 1972; Wood 1977, 1979; Fraser et al. 1987). In medusae, striated muscle-like cells, forming the innermost layer of the bell that are not directly connected to neurons, are coordinated so that contraction occurs by epithelial conduction through gap junctions (Mackie 1990). Electron microscopic studies have shown that in hydrozoans certain neurons are connected with gap junctions (Westfall et al. 1980; Takaku et al. 2014).

Gap junctions are formed by two unrelated gene families, innexins in protostomes and connexins in deuterostomes (Phelan 2005; Scemes et al. 2007). Although connexins do not exist in the cnidarian genomes sequenced to date, 17 and 8 genes encoding innexins have been identified in the genomes of the hydrozoans, *Hydra* and *Clytia* (Chapman et al. 2010), respectively. In *Hydra*, innexin-1 is expressed in ectodermal epithelial cells, suggesting that the innexin-1 is a component of the ectodermal gap junctions (Alexopoulos et al. 2004; Chapman et al. 2010). Recently, characterization of innexin-2 has shown that it forms neural gap junctions and coordinates spontaneous contraction of the body column (Takaku et al. 2014). In hydromedusae, expansion of innexin genes has probably been accompanied by synchronous epithelial conduction via gap junctions (Mackie and Passano 1968; Spencer 1974; Anderson 1980).

In contrast to hydrozoans, anthozoans and scyphozoans do not seem to have gap junctions and no empirical evidence has so far suggested any neuronal electrical coupling in their nervous systems (Mackie et al. 1984; Mackie 1990). Only one innexin/pannexin-like gene is present in the *Nematostella* genome, and it is absent in the other anthozoan, *Acropora digitifera* (Shinzato et al. 2011), suggesting that the innexin genes in hydromedusae have undergone independent expansions after their divergence from other cnidarian lineages. The existence of electrical synapses between neurons is therefore not a common feature in Cnidaria. Although poriferans and placozoans do not have proteins that form gap junctions, the ctenophore genomes encode multiple innexin genes (Moroz et al. 2014). These gene products probably form many gap junctions in ciliated cells of the apical organ and in endodermal cells of the comb plates (Satterlie and Case 1978). This suggests that gap junctions were established between epithelial cells, but not neurons, before the Cnidaria and Ctenophora diverged.

3.4 Anatomical and Physiological Features of the Cnidarian Nervous System

The Cnidaria are often used in textbooks to show a primitive nerve net, a neuronal network connected by a mesh of neurite processes. In the freshwater polyp, *Hydra* (Hydrozoa), which shows the simplest body plan and nervous system among

cnidarian polyps, neurons are connected to other neurons and to the epitheliomuscular cells, forming a diffuse neural meshwork throughout the animal body (Westfall et al. 2002). The nerve net is composed of sensory cells and interneuronal ganglion nerve cells. Sensory cells have elongated cell bodies with a ciliary cone at the apical end. Ganglion cells extend neurites at the basal end of epithelial cells (Davis et al. 1968; Kinnamon and Westall 1981; Westfall and Kinnamon 1978; 1984). Immunohistochemical studies of neuropeptides have demonstrated that the nerve plexus is composed of neuronal subtypes expressing distinct neuropeptides that are distributed in a polarized way with respect to the body axis (Koizumi et al. 2004).

In addition to the diffuse nervous system, cnidarians also possess regionally restricted and condensed nervous systems (Figs. 3.2, 3.4). A cluster of neurosecretory cells with sensory functions develops on the aboral (apical) side, mainly among lecithotrophic (yolk-feeding) larvae. In the oral region, a neuronal accumulation with organized neurite fasciculations, called a “nerve ring” or “oral nervous system,” has been observed in planktotrophic (plankton-feeding) larvae and polyps (Koizumi 2007; Koizumi et al. 2014; Watanabe et al. 2009; Marlow et al. 2009; Layden et al. 2012). Although anthozoans bear neuronal condensation at the oral region (mouth and pharynx), ring-shaped neural architecture is observed more clearly in hydrozoans at the base of the oral tip (hypostome) of polyps, as in *Hydra oligactis*; it is most evident at the bell margin of medusae. In cubozoan medusae, the nerve ring connects to the elaborate visual sensory system at the tentacle bulbs (Mackie 1990; Koizumi et al. 2014).

3.4.1 Aboral Nervous Systems and Apical Sensory Organs

The apical sensory organ, an anterior cluster of ciliated sensory neurosecretory cells (Richter et al. 2010), has widely been observed in the ciliated larvae of marine invertebrates (Lacalli 1983; Nielsen 2005; Tessmar-Raible et al. 2007; Conzelmann et al. 2011; Dickinson and Croll 2003; Byrne et al. 2007). In primary larvae of marine invertebrates, the apical organ comprises sensory cells with neurosecretory characters, and is assumed to help control of larval swimming behavior and metamorphosis (Chia and Bickell 1978; Chia and Koss 1979; Hadfield et al. 2000; Conzelmann and Jékely 2012; Conzelmann et al. 2013). In annelids, an apical sensory cell cluster comprises neurosecretory cell types expressing several neuropeptides, including RFamide and Wamide (Conzelmann et al. 2011, 2013; Conzelmann and Jékely 2012). These neuropeptides regulate swimming depth and settlement of pelagic larvae (Conzelmann et al. 2011, 2013). Similarly, in cnidarians, the aboral ectoderm of planula larvae harbors RFamidergic and LWamidergic sensory cells (Plickert 1989; Leitz and Lay 1995; Gajewski et al. 1996). These ciliated neuropeptide-positive cells are thought to have a sensory neurosecretory function. Although sensory functions of the apical tuft/organ formed at the aboral pole of the planula larvae remain obscure, RFamidergic and LWamidergic sensory neurons in the aboral half in various cnidarian larvae appears to be involved in the metamorphosis of free-

swimming planula larvae into benthic polyps (Chia and Bickell 1978; Chia and Koss 1979; Leitz et al. 1994; Gajewski et al. 1996; Takahashi et al. 1997; Schmich et al. 1998b; Iwao et al. 2002; Hatta and Iwao 2003; Katsukura et al. 2003, 2004; Erwin and Szmant 2010; Takahashi and Hatta 2011). Since settlement and metamorphosis of cnidarian pelagic larvae are induced by marine biofilms (Müller 1969; Morse and Morse 1991; Leitz and Wagner 1992), neuropeptide-expressing sensory cells are probably implicated in perception of environmental signals from suitable sites on the benthos. The aboral neurosecretory system of cnidarian larvae appears also to allow orientation toward light for coordinating the diurnal cycle of migration. RFamide and LWamide peptides are involved in control of the creeping behavior of planulae toward a light source (positive phototaxis), as the phototaxis of the planulae was drastically suppressed or promoted by exogenous RFamide peptide or LWamide peptide, respectively (Katsukura et al. 2004; Plickert and Schneider 2004).

3.4.2 *Oral/Pharyngeal Nervous Systems*

Immunohistochemical analyses of neuropeptide-expressing neurons have shown that, in addition to their aborally localized neurosecretory cells, cnidarians develop elaborate nerve structures in the oral region. Because the basic cnidarian body plan shows radial symmetry with a single mouth opening, the oral nerve plexus sometimes shows an annular architecture called ‘nerve ring’ (Figs. 3.2, 3.4) (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, 2007; Satterlie 2011). The highest level of morphological and physiological elaboration of the nerve ring has been observed especially at the bell margin of medusae (Koizumi et al. 2014). A nerve ring comprising neuronal subsets with distinct neurophysiological functions was found in most medusae investigated so far. The nerve ring of *Aglantha digitale* (Hydrozoa), for example, has been divided into at least seven subsystems with distinct physiological properties (Mackie and Meech 1995a, 1995b, 2000; Mackie 2004). Communication among the subsystems allows complex behavioral control, including swim contractions of the medusae (Mackie and Meech 1995b). Several medusae bear a nerve ring connected to a sophisticated eye-bearing sensory complex at the base of the tentacles (Singla 1974; Yamamoto and Yoshida 1980; Singla and Weber 1982; Laska and Hündgen 1982; Nilsson et al. 2005). Visually guided behavioral patterns are observed in these cnidarian classes, and especially in cubomedusae, these patterns are quite complex (Hartwick 1991; Hamner et al. 1995; Matsumoto 1995). Ring-like neurite bundles and a neuronal condensation at the oral side have been reported from polyps and even from planula larval stages (Matsuno and Kageyama 1984; Grimmelikhuijzen 1985; Koizumi 2007; Marlow et al. 2009; Watanabe et al. 2014a). Immunostaining of neuropeptides and gene expression analyses of neurogenic genes demonstrate that neuronal differentiation starts in the blastula epithelium and progressively increases on the oral side during larval developmental stages. This oral neurogenic domain

develops into a semi-centralized nervous system (semiCNS) on the oral side of the planula larvae and primary polyps that comprises several subsystems (Fig. 3.4). The cnidarian oral nervous system is therefore regarded as a considerable degree of neuronal condensation and has been regarded as the beginning of the bilaterian CNS (Holland 2003; Davis et al. 1968; Koizumi 2007; Tosches and Arendt 2013; Holland et al. 2013; Marlow et al. 2014; Watanabe et al. 2014a; Nielsen 2015). More detailed comparisons of the bilaterian CNS and the cnidarian semiCNS are needed, however.

3.5 Development of Cnidarian Nervous Systems

Cnidarian homologues for the proneural bHLH genes, *Ash* and *Arp*, as well as *SoxB* genes, have been identified in a wide range of cnidarian species and are expressed in the neural cell progenitors (Grens et al. 1995; Müller et al. 2003; Hayakawa et al. 2004; Lindgens et al. 2004; Seipel et al. 2004; Magie et al. 2005; Simionato et al. 2007; Layden et al. 2012; Watanabe et al. 2014a). In *Nematostella*, a series of gene function analyses of these neurogenic transcription factors and *Elav1* have unveiled genetic mechanisms essential for differentiation of ectodermal and endodermal neurons. The first sign of neurogenesis in *Nematostella* is the salt-and-pepper-like expression of the neurogenic transcription factors *NvSoxB2c* (also called *NvSoxB2* or *NvSoxB(2)*), *NvAth-like (NvArp3)*, and *NvAshA*, and various neural markers, including *NvElav1* and *Rfamide*, in blastula epithelium (Magie et al. 2005; Marlow et al. 2009; Layden et al. 2012; Nakanishi et al. 2012; Richards and Rentzsch 2014; 2015). The patterns of *NvSoxB2c* and *NvAshA* expression suggest that the early embryonic epithelium of *Nematostella* has the potential to generate various neuronal cell types that form a diffuse nerve net (Fig. 3.2) (Magie et al. 2005; Layden et al. 2012). Gene function analyses indicate that they are required for the development of *NvElav1*-positive neuronal populations in the endoderm and *NvRfamide*-positive cells in the lateral ectoderm (Nakanishi et al. 2012). During gastrulation, the expression of transcription factors, such as *NvRx* and *NvAshA*, is localized mainly in the aboral half of the embryos, indicating that these genes may be involved in development of the sensory cells/neurons in the aboral region. The genetic mechanism responsible for development of the aboral nervous system is still unclear. *Six3* is a homeodomain transcription factor with a central role in the development of anterior sensory and neural structures in bilaterians (Steinmetz et al. 2010). In *Nematostella*, the *NvSix3/6* gene is expressed in the aboral region where the aboral sensory cells and neurons develop, suggesting that the *NvSix3/6* has an evolutionarily conserved role in demarcating the anterior neurosensory region, both in Cnidaria and Bilateria (Sinigaglia et al. 2013). Although an inhibition of the *NvSix3/6* gene in *Nematostella* embryos did not have a significant effect on *NvRfamide* expression, its possible involvement in expression of aboral neural genes *NvRx* and *NvAshA* has not been explored.

In addition to their aborally-biased nervous systems, the oral region of *Nematostella* larvae expresses a number of neurogenic genes and markers (Fig. 3.2) (Magie et al. 2005; Shinzato et al. 2008; Marlow et al. 2009; Nakanishi et al. 2012; Layden et al. 2012; Watanabe et al. 2014a). The blastopore region of the gastrulae develops into a prominent oral neurogenic domain, showing dominant or exclusive expression of *NvSoxB2* homologues, *NvAsh* and *NvArp* homologues, and *NvMusashi* and *NvRfamide*. Early neurogenic markers for, and later differentiation of, neuropeptide-expressing neurons in the oral nervous system are severely reduced or absent in the embryos injected with morpholino antisense oligonucleotides against early oral neurogenic transcription factors such as *NvSoxB2a*, *NvAshB*, and *NvAth-like/NvArp3* (Watanabe et al. 2014a), which suggests that the blastoporal side of the early embryos has a distinct neurogenic capacity. It has been shown that the development of the oral nervous system is dependent on β -catenin and bone morphogenetic protein (BMP) signaling pathways. The function of these signaling activities in development of the cnidarian oral nervous system is highly reminiscent of their functional patterning of the bilaterian the CNS (Watanabe et al. 2014a).

3.6 Outlook

Accumulating molecular and cellular evidence has led many researchers to propose that the Urbilateria, the last common bilaterian ancestor, had a condensed nervous system with more or less specific anatomical and/or physiological features (Hirth et al. 2003; De Robertis 2008; Tomer et al. 2010; Strausfeld 2010; Strausfeld and Hirth 2013; Bailly et al. 2013; Holland et al. 2013), whereas others favor the idea that the bilaterian CNS started with a diffuse nerve net (Gerhart et al. 2005; Pani et al. 2012; Arendt et al. 2016). Since the Cnidaria is the closest sister group to bilaterians, it is important to decipher molecular and cellular features of diffuse and regionalized components of the cnidarian nervous system. Expression analyses of CNS genes in *Nematostella* suggest that the aboral sensory system and the oral nervous system of cnidarians are related to the anterior part of the bilaterian brain, including sensory organs, and the posterior part of the brain with trunk nervous systems, respectively (Tosches and Arendt 2013; Marlow et al. 2014; Arendt et al. 2016). Recent gene function data suggest that rudimentary centralization of the oral nervous system might be an antecedent characteristic of the bilaterian CNS (Watanabe et al. 2014a). However, more detailed functional analyses of CNS genes in the Cnidaria are required to better explain how the first step in nervous system centralization may have been accomplished. Studies on nervous systems of cnidarians and ctenophores, as well as the sensory systems of poriferans and placozoans, are also strongly needed to reconstruct the early evolution of the nervous system.

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