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Development of Nervous System in Lancelet (Amphioxus)

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The lancelet nervous system looks like that of vertebrates and has been regarded as a prototype of the latter. However, since the lancelet nervous system has important derivative characters, we have reinvestigated developmental pattern of peripheral nervous system and gene expression pattern of the CNS. About 32 genes expressed in the lancelet CNS have been studied so far, of which *sox1/2/3*, *soxC* and *neurogenin* are the earliest genes expressed at the mid-gastrula stage. Bipartite expression pattern in the CNS is depicted typically by *otx* and *wnt7* from the neurula stage. Another wnt gene, *wnt8* is expressed in segmental mesoderm with the anterior limit being coincident with that of *wnt7* domain. The gene expression pattern shows that neurogenesis starts as early as the mid-gastrula stage, and the anteroposterior regionalization takes place simultaneously or soon after. Although the early bipartite pattern is also found in vertebrate neural plate, it cannot be a synapomorphy because other invertebrates such as planarian and insects show similar patterns. In lancelet, the bipartite pattern seems to transform to cerebral vesicle (cv) in the anterior and nerve cord posterior to it. After the initial bipartite gene expression, the all region-specific genes except those expressed segmentally in the posterior nerve cord are expressed within the cv, the pattern of which is very difficult to correspond to the vertebrate pattern. Peripheral nerves develop anteroposteriorly with an exception of the first nerve. In the oral region, innervation pattern is modified significantly according to the development of the mouth, in which the mouth is first innervated with left 2nd to 4th nerves and then with 3rd to 8th immediately before metamorphosis. The left 3rd to 6th nerves, which may be related to motor element of feeding apparatuses, innervate contralaterally and make specific asymmetric innervation pattern during metamorphosis. Developmental pattern of the peripheral nerves in lancelet shows no prepattern of cephalic and spinal nerve types in vertebrates, instead it is characterized by intensive modification of innervattion pattern during development. Both from gene expression pattern and peripheral nerve development in lancelet, it is difficult to extract the characters homologous with vertebrate nervous system. To understand evolutional history of vertebrate nervous system, extensive studies that involve many groups of invertebrates are required.

The Nervous System of Hemichordates and Its Marker

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Hemichordates occupy a unique phylogenetic position between chordates and other invertebrates. Recent studies in molecular biology have underscored the importance of hemichordates in elucidating the evolution of not only chordates but also bilaterians. Adult hemichordates have pharyngeal gill slits and a dorsal hollow nerve chord, both of which are defining features of chordate anatomy. In contrast, the nervous system of adult hemichordates is composed primarily of intra-epidermal nerve networks, which, according to Bullock, are among the most primitive forms of neural organization found in bilaterians. Adult hemichordates exhibit both dorsal and ventral nerve cords, whereas unilateral cords are found among protostomes (ventrally) and chordates (dorsally). Further, in their embryogenesis and larval forms, hemichordates resemble echinoderms. With these unusual features in mind, we have explored the aspects of normal neural development in hemichordates, with simultaneous investigations at the immunohistochemical and molecular levels.

We have focused on the expression patterns of specific gene cascades that are conserved between the dorsal nervous system of chordates and ventral nervous system of protostomes. Our approach has been to isolate homologs of these genes across species and then compare their expression patterns at embryonic, larval, and early juvenile stages of development. Our results show that some genes are expressed in the course of normal neural development during the larval and early juvenile stages - and others not. Hemichordates constitute a sister group with echinoderms, having the same dipleurula larval form. Thus, the embryogenesis and the morphology of hemichordates and echinoderms are very similar until the larval stage. Our results also demonstrate that both immunohistochemical studies and the expression patterns of homologous genes can be used to compare the nervous systems of hemichordates and echinoderms at similar stages of development up to the larval stage. However, caution is warranted in comparing nervous systems across phyla during the transition from larval to adult stages of development. Exact standards for this comparison have not been established.

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These findings will set the stage for a discussion, which highlights the complexities of comparing gene expression patterns across species in the setting of divergent phylogenetic lines.

Biodiversity of Echinoderm Larval Nervous System

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The biodiversity of the neurogenesis is discussed in indirect developing larvae of all five classes of echinoderms. Neural structure is identified immunohistochemically with the neuron specific monoclonal antibodies that had been raised against adult radial nerve of starfish, the polyclonal antibodies against neurotransmitters, and by ultrastructurally observations with electronmicroscoy.
The neurons and neurites located in the ciliary bands are the most prominent and represent popular feature of nervous system in all types of the larvae. Most of them are catecholaminergic and some of them are serotonergic. The nerve cells show flask-shape cell bodies that are regularly spaced and interconnected by the tracts of neurites at the base of the ciliated cells. Fine intraepithelial networks of neurites originated from the neurons in the ciliary band are located in the epidermis of plutei of both echinoid and ophiuroid, doliolaria of crinoid, and in the oral epithelium of bipinnaria of asteroid.
The detailed location and the number of neurons are different among the types of larvae, although the paired bilateral cluster of serotonergic nerve cells are commonly located in the apical part of all types of larvae to constitute the apical ganglion (organ). Serotonergic neurites come up and run toward the posterior part of the larvae.
In feeding larvae, dopaminergic and serotonergic neurons are observed in a pair of lower lip region of the ciliary band. Nerve cells and neurites are also observed around the esophageal epithelia, sphincter of stomach and intestine and anal region in the larvae.
Nerve cells first differentiate at the apical epithelium at mid- to late-gastrula stage. They increase in number and are arranged into the ciliary band of pluteus, bipinnaria, auricularia and doliolaria larvae as the development proceeds. Neural cells project neurites, which exhibit distinctive growth-cone structure at the distal tip.
During metamorphosis, the adult rudiment of competent larvae of echinoid comes out inside out and the larval tissues are absorbed. The body axis of the juvenile is perpendicular to that of the larva. The metamorphosis of brachioralia of asteroid and ophiopluteus also goes through settlement, absorption of the larval organ and right angled axis shifts. The doliolaria of crinoid settles upside down and then the apical organ of the larvae is degenerated. On the contrary, holothurian doliolaria metamorphoses occurs without axis shift nor absorption of the larval epidermis. As far as four classes of the larvae investigated in this study, the adult neural tissues, the radial nerve with perioral nerve ring and nervous system of each primary podium develop independently in the adult rudiment. At the same time, a part of larval integument including nervous system is remodeled to the adult types, contributing to the juvenile structure. It still remains to be studied how the nervous system of integument derives from larval epidermis and the adult nerves, which differentiate independently, coordinate or reorganize in the juvenile.

Formation of Sea Urchin Nervous System: from *Hpwnt1* to 5-*Ht-hpr*

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Serotonergic nervous system is the first nervous system among others formed in sea urchin larvae during embryogenesis. A few number of serotonin cells detected by anti-serotonin antibodies appear near the apical tuft region in the oral lobe ectoderm at prism stage. The number increases rapidly between 36 hours post-fertilization (hpf) prism stage to 48hph 2-arm pluteus stage, and attains 8-10 cells (Yaguchi *et al*, 2000). Serotonin cells formed initially on both left and right side of an imaginary embryonic middle line (IEML) extend nerve fibers from basal side of the cells toward IEML in prism larvae, and form a neuroplexus at around IEML in plutei to form the apical ganglion. During the directional extension of nerve fibers from serotonin cells, *HpNetrin*, a *Netrin1* homologue cloned and sequenced from the larvae of *Hemicentrotus pulcherrimus*, is transcribed, implicating participation of this known extracellular nerve fiber guiding protein during the neuroplexus formation (Yasuda and Katow, 2004).

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