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THE EVOLUTIONARY ORIGIN AND PROCESS OF THE CENTRAL NERVOUS SYSTEM: COMPARATIVE GENOMICS APPROACH

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INTRODUCTION

Historically, it had been the most essential question to ask why we think we are ourselves. A famous phrase written by a French philosopher, René Descartes, in Latin '*Cogito, ergo sum*' (translated in English, 'I think, therefore I am'). This paradigmatic enigma given by Descartes about self-consciousness or self-recognition is still the central question for us today. Apparently there is no easy answer for this question from any perspective. However, we can question and resolve the biological problem of 'how our brains work and think' from the evolutionary standpoints, especially when we are able to study it at the genetic level.

Since the draft sequence of human genome project has been completed, biologists have focused on the post-genomics studies including proteomics, transcriptomics (gene expression profile), SNP (single nucleotide polymorphism), non-coding RNAs (eg. miRNA, siRNA), comparative genomics and etc. Among these studies, comparative genomics provide a powerful way to resolve the evolutionary questions (Koonin *et al.*, 2000). Sequence comparison across the species is a fundamental solution to understand the origin, the evolutionary differences between organisms and the complexity of biological systems. Particularly the currently advanced technology of second-

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generation sequencers such as 454, Solexa, SOLiD and Helicos allows one thousand human genomes to be sequenced just within two years. Therefore, we expect to have the number of genome sequences of various organisms increase significantly in the next few years. Taking advantage of comparative genomics, we attempt to understand the evolution of the central nervous system (CNS) or the brain of humans at the gene level. To do this, we address the following questions: (1) What is the origin of the nervous system (NS) as based on currently available sequence data and the advantage of homology search? (2) How old are the nervous system genes especially those that are also expressed in the human brain? (3) What kinds of genes are expressed in planarian, a primitive flatworm having the simplest brain?

Origin of nervous system – a nerve net

There are around 100 billion neurons in an adult human brain and they can be categorized according to the number of axonal processes extended from the perikaryon (the cell body). Depending on their localization in brain regions, these basic neuron types are further subdivided into specialized neuron types and also functionally diverse. Let us take pyramidal cells as an example, which are typically characterized by a spiny apical dendrite, a basal dendrite and a single axon. The morphologically identical populations of pyramidal cells are found largely in two distinct functional parts of the brain, neocortex and hippocampus. Further diversification of pyramidal cells can be found within the hippocampus in which a heterogeneous expression of genes is observed across the pyramidal cell layer (Lein *et al.*, 2007). The ancestor of the human brain is considered much simpler. It is believed to have a two-dimensional neural network that is somehow similar to the diffuse nerve net of basal phylum Cnidaria, having an average of less than 8000 neurons, no glial cells, no centralized nerve tissue and no anatomical compartmentalization (Holland, 2003, Telford, 2007) (Fig. 1, see p. 608). However, the neuroanatomical comparison has failed to show any homologous structures of the nervous system between human and Cnidaria, nor has the cell morphology given any clue due to the simplicity of cell types in cnidarians. Recently, gene expression data have proven the conserved body plan between vertebrates and cnidarians (Bode, 2001; Finnerty *et al.*, 2004; Kusserow *et al.*, 2005; Lengfeld *et al.*, 2009), suggesting that the origin of the body plan can be dated to the early Metazoa.

The phylum Cnidaria includes animals such as coral, sea anemone, sea pen, jellyfish and *Hydra* and they all share a sac-like body surrounded by two layers of epithelial cells (ectoderm and endoderm). They only have a single opening that functions as both mouth and anus. Cnidarians have the simplest nervous system named nerve net in which thousands of neurons make up a mesh-like network at both epithelial layers (Fig. 1 and 2A, see pp. 608-9). Jellyfish and certain species of *Hydra* (eg. *H. Oligactis*) also have neurons concentrate and form a ring around the mouth region (Fig. 1, see p. 608). This nerve ring is considered to be an intermediate structure between a nerve net and a ganglion. Since Cnidaria and Ctenophore are basal metazoans with the nervous system, many expect the origin of the nervous system to somehow resemble the cnidarian nerve net. From time to time hypotheses have been made to explain how a simple nerve net of cnidarians evolves into a bilaterian nervous system (Holland, 2003). Most ideas are based on the scenario proposed for the evolution of the bilateral body plan (Lacalli, 1995; Meinhardt, 2002; Holland, 2000; Martindale, 2005; Hejnol and Martindale, 2008). Holland (2003) has a good summary of all the hypotheses on the transformation of nerve net/nerve ring into brain and nerve cord. Among all, the majority of scenarios believe that Cnidaria has the ancestor-like nervous system and the bilaterian brain is originated from the nerve ring. Other minor scenarios either consider the whole cnidarian polyp as a brain or the nerve net is compressed to one side of the body axis and becomes the brain.

Does nervous system emerge from nowhere?

Yet, could the ancestor of the nervous system be simpler than a nerve net? The nerve cell (or neuron) is the fundamental unit of the nervous system. It is not found in the basal metazoans such as sponge and placozoan and thought to arise early in the Eumetazoa (a clade comprising all major animal groups except sponges and placozoans) (Fig. 3). The sponge has well-defined photosensory cells. At the posterior pole of demosponge larva, there appears a ring of monociliated, pigment-containing cells and these cells function as a photoreceptor and control the directional swimming of the larva (Leys and Degnan, 2001; Leys *et al.*, 2002). Unlike the sponge, placozoan contains four basic cell types and none of them is morphologically similar to the neuron or sensory cell. However, neural genes involved in neurosynaptic activity and biosynthesis are identified in the

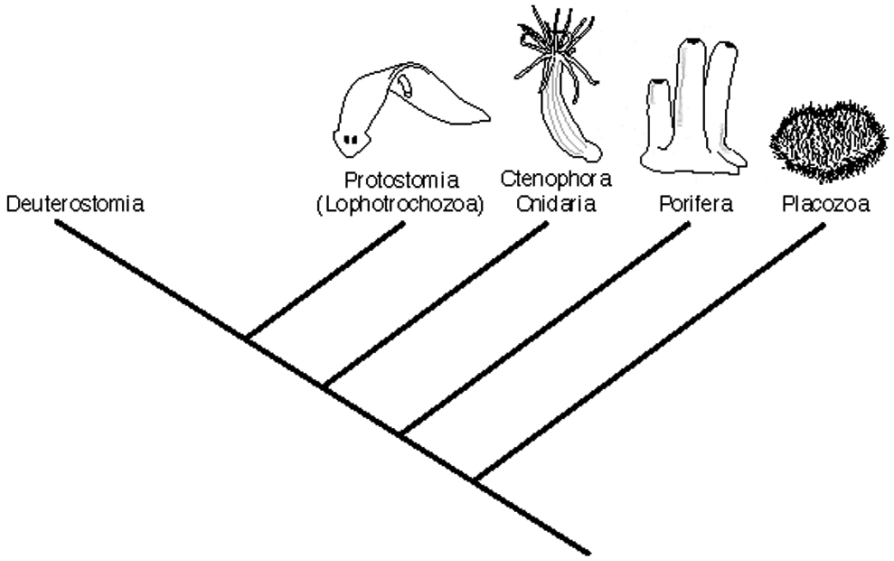

Figure 3. A classical taxonomy of basal metazoans.

placozoan genome (Srivastava *et al.*, 2008), and a *Proto-Pax* gene is expressed in a proliferating/differentiating region near the outer edge of placozoan cell body (Hadrys *et al.*, 2005). This molecular evidence suggests that neural genes predate the ancestor of nervous system. In fact, sponge and placozoan genomes encode a great deal of transcription factor genes that play a critical role in signaling pathway, embryogenesis and tissue specification of eumetazoan (Degnan *et al.*, 2005; Strivastava *et al.*, 2008). Therefore it is rather unlikely that the gene repertoire of the nervous system arises after the divergence of cnidarians but instead it emerges in the last common ancestor of Metazoa or even earlier. In other words, the repertoire of molecular factors that are essential for neuronal development and functions has already had a role in neuronal activities in the 'primitive cell' far before the emergence of the nerve cell in animals. The 'primitive cell' is referred to those having the potency but yet to develop into the neuron stem cell (Fig. 4). Later it evolves into two sister cells and one of them functionally diversifies into neuron stem cells. A similar view is found in a recent review, Arendt D. (2008) has proposed a scenario

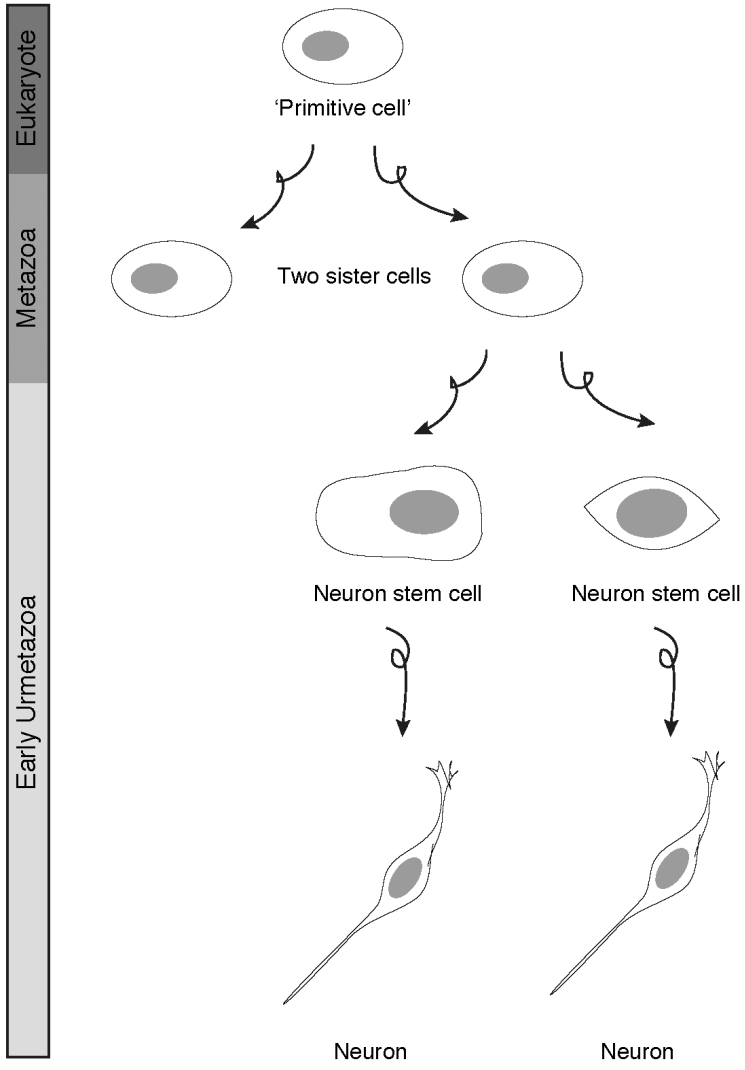

Figure 4. A scenario is proposed for the origin of the nervous system or neuron. A partial neural gene repertoire exists in the 'primitive cell' before the emergence of Metazoa. This 'primitive cell' evolves and diverges into two sister cells. One of the sister cells specializes its function via genetic modifications (eg. gene duplication, gene gain and loss, protein domain shuffling, horizontal gene transfer, etc) and further divides into two sister cells that contain similar potencies as neuron stem cells. Later in the evolution, one of two neuron stem cells might lose its ability to differentiate into a neuron, but the other one remains as a neuron stem cell (not illustrated in the figure).

of cell type evolution in which ancient metazoan cell types have multiple functions and later the cell type diversification within a species increases the number of functionally specialized cells. Arendt has further stated the importance of molecular signature (which refers to a set of differentiation and regulatory genes in sister cell types) in the evolutionary diversification of cell types. In fact, in our opinion, this molecular signature has to be imprinted in the ancient cell type before the emergence of sister cell types. Early sister cell types might retain the plasticity at the gene regulatory level. Not all cnidarians have neurons derived from one kind of cell types. The neurons of *Hydra* are differentiated from the interstitial cell (I-cell), a multipotent stem cell that lies between the ectodermal epithelial cells (David and Gierer, 1974). Nematostella, a marine cnidarian, lacks the I-cell and neurons are originated from the ectodermal and endodermal epithelial cells (stem cell lineages of cnidaria) (Extavour *et al.*, 2005; Marlow *et al.*, 2009). It seems plausible that the I-cell and epithelial cell are both sister cells and contain the same regulatory gene network that is capable of determining their cell fates and differentiating into neurons. Interestingly, this conclusion is supported by the observation that *Hydra* I-cell arose in the endoderm at the early embryonic stage, suggesting the endodermal origin of *Hydra* I-cell (Genikhovich, 2006).

In *Hydra*, I-cell and epithelial cell are having a relationship of sister cells as discussed above, while the differentiated products of I-cell, neuron and nematocyte (contain a stinging organelle that functions for prey capture and defense) can also be considered as secondary sister cells (Hwang *et al.*, 2007). This is not only because both are originated from the I-cell, but because they also both have specific expressions of *achaete–scute, prdl-b and COUP-TF* (Hayakawa *et al.*, 2004; Miljkovic-Licina *et al.*, 2004). The nematocyte has long been regarded as a sensory cell as it bears a mechano- and chemo-sensory receptor called cnidocil apparatus at the apical surface of cell.

In fact, the evolution of neural genes occurred far before the emergence of multicellular organisms, approximately 1,400 million years ago (Nei *et al.*, 2001). A genomic-scale analysis of nervous system (NS) specific genes shows that 35 out of 255 human NS specific genes (14%) appear prior to the split between metazoans and yeast (Fig. 5, see p. 610) (Noda *et al.*, 2006). Moreover in the same analysis, a sudden increase in the number of NS genes occurs before the emergence of vertebrates, and the majority of these NS genes are critical for protein binding or protein-protein interaction. Although the analysis is based on a small data set (255 human NS spe-

cific genes), the results support two conclusions: (1) A significant number of NS specific genes in yeast marks the ancestral complexity of the neural gene repertoire before the emergence of the nervous system, and (2) the evolution of the nervous system is mainly driven by the extensive gene gain.

The primitive brain of planaria

Although recent phylogenetic analyses have placed platyhelminth flatworms in the clade of Lophotrochozoa and not as basal to Bilateria (Fig. 3) (Baguñà and Riutort, 2004; Ruiz-Trillo *et al.*, 2004), it has a centralized nervous system that can be described as a 'primitive brain'. Planarian, a freshwater flatworm, contains a mass of cephalic ganglions in the head and a pair of ventral nerve cords (VNC) running parallel to the body axis (Fig. 1, p. 608). The cephalic ganglion has a bilobed structure with neuronal cell bodies that form the outer later (cortex) and nerve fibers that concentrate collectively in the inner core of the ganglion. Compared to the nervous system of cnidarians, the planarian central nervous system has evolved with several 'brain' features: (1) centralized neurons at cephalic region, (2) nerve cord, (3) neuron fibers surrounded by a layer of cortex, (4) lobes with commissural fibers, (5) glial cells, (6) motor neurons and (7) neurons with elaborated dendrites. Thus, the structure of the planarian nervous system has been well studied and characterized (Flexner, 1898; Oosaki and Ishii, 1965; Baguñà and Ballester, 1978). Not until recently, molecular tools including whole mount *in situ* hybridization, whole mount immunostaining, expression sequence tag (EST), microarray, and RNA interference are applied to study the detailed morphology and function of the planarian nervous system (Cebrià *et al.*, 2002a; Cebrià *et al.*, 2002b; Agata *et al.*, 1998; Mineta *et al.*, 2003; Nakazawa *et al.*, 2003).

What makes the nervous system (NS) complex?

In order to study the genes expressing in the planarian brain, we collect anterior tissue including the cephalic ganglia (above the neck) of planarians and conduct EST sequencing. Based on known NS genes, we have identified 116 genes out of 3101 that share significant homology to NS genes of other organisms (Mineta *et al.*, 2003). A further analysis of 116 NS-related genes has shown that more than 95% have their homologs

in humans, *Drosophila melanogaster* and *Caenorhabditis elegans*. These NS-related genes include those involved specifically in the brain morphogenesis and neural network formation, suggesting the possibility that the bilaterian central nervous systems are derived from a common origin.

Moreover, we also examined the gene expression in the anterior part (i.e. cephalic ganglia) of the planarian by using cDNA microarray containing 1,640 nonredundant genes (Nakazawa *et al.*, 2003). The use of planarian cDNA microarray has an advantage over the ESTs collection. Planarian cDNA microarray can be used to examine the novel genes expressed in the central nervous system. A total of 205 genes are differentially expressed in the anterior part and by using whole mount *in situ* hybridization, the top 30 genes show various regional expressions in the cephalic ganglia and the ventral nerve cords (Fig. 2B, **see p. xxx**). Many of the top 30 genes have an unknown function. The variety of expression patterns of the top 30 genes in the planarian brain demonstrates the highly organized nature and the complex neural activities of the planarian central nervous system.

In summary, the above data indicate that the planarian brain expresses genes related to those in the human central nervous system and it is also highly divided into distinct compartmentalized regions (i.e. functional domains) according to the gene expression patterns. One of the important features for a diffused nerve net (in *Hydra*) that evolved into a centralized nervous system (in planarian) requires the mechanism of axon guidance. Axon guidance allows the proper growth of the axon cone and the precise target reached by the axon. The axon guidance molecules in planarians such as *NCAM*, *slit, netrin and robo* play conserved and important roles in the maintenance of the nervous system architecture (Cebrià, 2007). RNAi interferences of these three genes in the planarian result in the failure to regenerate a normal brain. For example, no proper commissural connection is seen between cephalic ganglia and nerve cords in regenerating planarians after *Smed-roboA* RNAi (Cebrià and Newmark, 2007). Interestingly, sequences homologous to axon guidance genes such as *NCAM, robo, slit, netrin, Eph* receptor and NCAM are also identified in the *Hydra* genome (personal data). Thus, the emergence of axon guidance genes did not happen in the early ancestors of Bilateria but rather dates back to the Eumetazoa. It would be of great interest to know whether the axon guidance homologs of *Hydra* had conserved functions like those of planarians and other bilaterians. Perhaps the complexity of the central nervous system as compared to the nerve net is not due to the number of NS genes but to the dynamics regulation of the gene network. It should

be noted that the planarian also has lineage-specific NS genes. One of the examples is '*nou-darake*', a gene that belongs to the FGF (fibroblast growth factor) receptor family and is expressed in the cephalic ganglia and its surrounding tissues. The existence of '*nou-darake*' is important to restrict the brain tissue in the cephalic region (Cebrià, *et al.*, 2002a).

Concluding Remarks

For years, researchers have been struggling to isolate genes from an organism, to gain the genomic information of a gene and to compare the genes among many different species. Now with high-throughput sequencing, powerful analysis tools and large-scale data storage, we are able to collect a large amount of EST and genome data in a short time. These advanced approaches have found a solution to the current research on the evolution of the central nervous system. To understand the human brain, we believe that the study of lower organisms such as *Hydra* and planarian is essential and would provide useful knowledge of how the human brain evolves. In our studies, we predict that *Hydra* and planarian share at least half of their nervous system genes with humans. Clearly, many nervous system genes predate the emergence of Metazoa and the nervous system evolves as the gene network increases its complexity. For a future perspective, we believe that it is essential to construct a virtual 3D human brain. This 3D immersive environment would provide the gene expression map of each central nervous system gene against the anatomical, tissue- and single-cell levels of the human brain structure.

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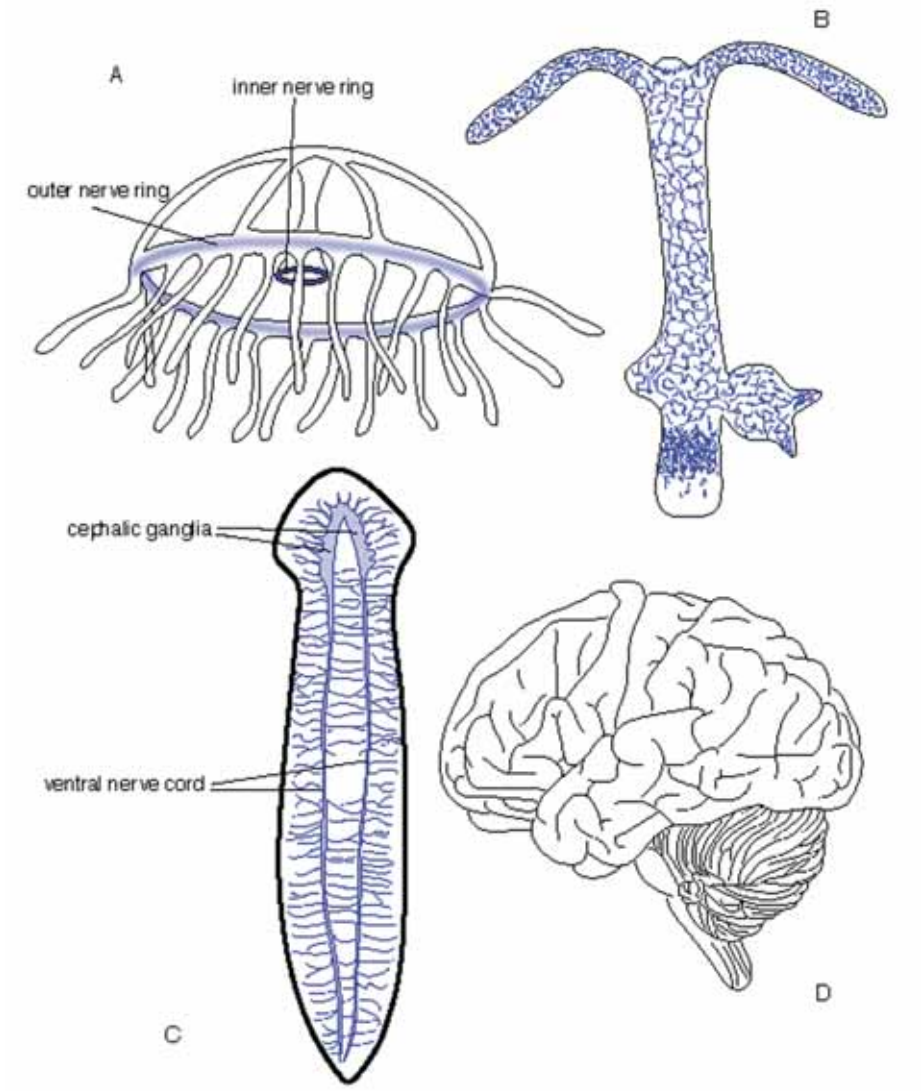

Figure 1. The nervous systems of jellyfish, *Hydra*, planarian and human. Both jellyfish and *Hydra* have a diffuse nerve net. (A) The inner and outer nerve rings of jellyfish are highlighted in blue. (B) In *Hydra*, neurons (blue) spread out in the ectoderm and the endoderm. Some *Hydra* species also possess a nerve ring at the mouth region. The central nervous system (blue) of planarians contains cephalic ganglia and ventral nerve cords. (D) Human brain. Note that the scale of the animal size in the figure does not refer to the relative size of the actual animal.

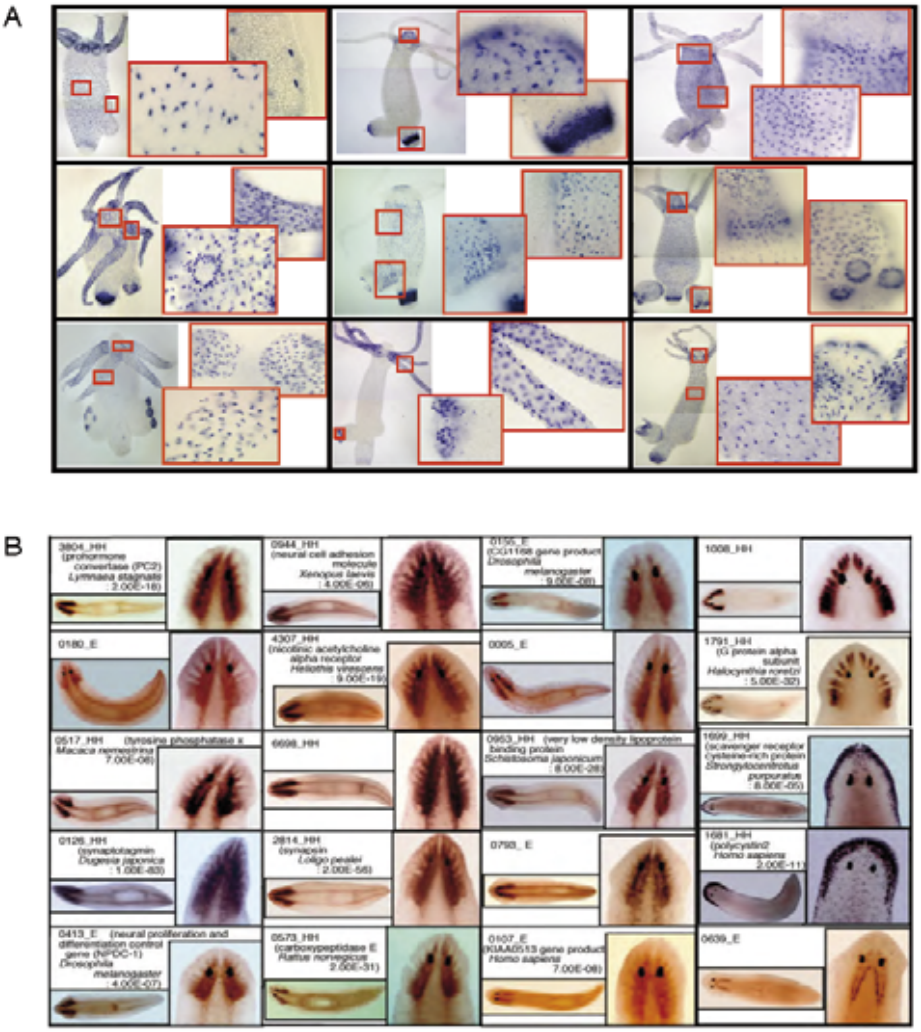

Figure 2. Various expression patterns of nervous system genes of *Hydra* (A) and planarian (B) are examined by whole mount *in situ* hybridization. The results have been published in Hwang *et al.* (2007) and Nakazawa *et al.* (2003).

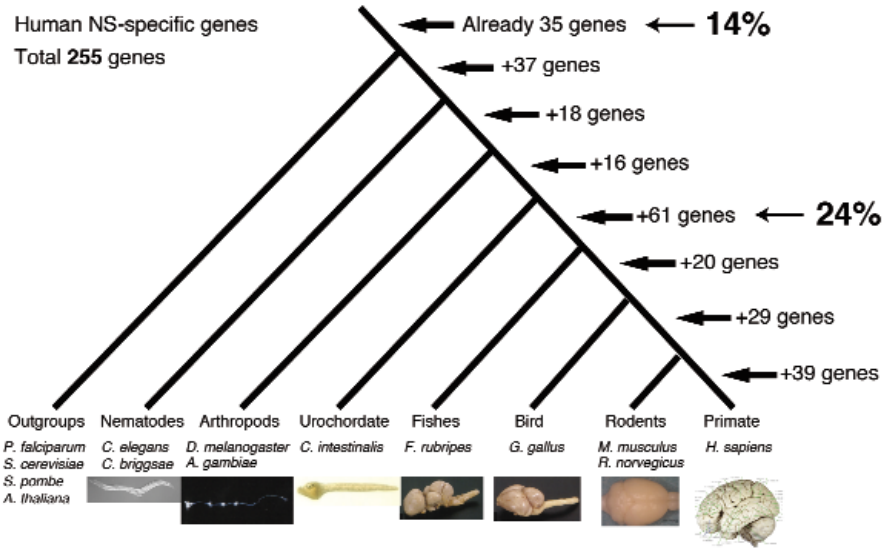

Figure 5. Schematic drawing of the emergence of human NS-specific genes. Out of 255 genes, 35 genes (14%) exist prior to the divergence yeast and human and 61 genes (24%) emerge after the divergence of urochordates and human. The data analysis of this figure is described in Noda *et al.*, 2006.