Molecular actions guiding neural regeneration in planarian

Yan-Fen ZHANG^{1,2,3}, Bo-Ping YE¹, Da-Yong WANG^{2,3}

¹*School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, China*

²*Department of Genetics and Developmental Biology, ³Key Laboratory of Developmental Genes and Human Disease, Ministry of Education of China, Southeast University, Nanjing 210009, China*

Abstract: Planarian is among the simplest animals that possess a centralized nervous system (CNS), and its neural regeneration involves the replacement of cells lost to normal ‘wear and tear’ (cell turnover), and/or injury. In this review, we state and discuss the recent studies on molecular control of neural regeneration in planarians. The spatial and temporal expression patterns of genes in intact and regenerating planarian CNS have already been described relatively clearly. The bone morphogenetic protein (BMP) and Wnt signaling pathways are identified to regulate neural regeneration. During neural regeneration, conserved axon guidance mechanisms are necessary for proper wiring of the nervous system. In addition, apoptosis may play an important role in controlling cell numbers, eliminating unnecessary tissues or cells and remodeling the old tissues for regenerating CNS. The bilateral symmetry is established by determination of anterior-posterior (A-P) and dorsal-ventral (D-V) patterns. Moreover, neurons positive to dopamine, serotonin (5-HT), and gamma-aminobutyric acid (GABA) have been detected in planarians. Therefore, planarians present us with new, experimentally accessible contexts to study the molecular actions guiding neural regeneration.

Keywords: neural regeneration; central nervous system; molecular mechanism; planarian

Neural regeneration is a fundamental problem of tissue homeostasis involving the replacement of cells lost to normal “wear and tear” (cell turnover), and/or injury^[1]. The process of neural regeneration can be divided into two parts: the regeneration of neurons, and the regeneration of scathing and ruptured axons. The freshwater planarian has been chosen as a model system to study this process because it is one of the simplest metazoans that possess tissue homeostasis and regeneration, and because it becomes relatively easy to molecularly manipulate this animal organism^[1]. Planarians are bilaterally symmetric metazoans of the phylum Platyhelminthes and capable of replacing large regions of missing structures throughout the regeneration. They were commonly found in freshwater streams and ponds where they predomi-

nantly prey upon insects and other invertebrates^[2]. Freshwater planarians are the most widely used Platyhelminthes in neural regeneration research as their totipotent somatic stem cells, called “neoblast”, can regenerate a functional nervous system from even a small portion of the body, and are easy to be cultured and handled in laboratory^[3-4]. In this review, we will state and discuss the recent studies on characterizing the nervous system at different levels and elucidating the molecular control of neural regeneration in the planarians.

1 Structure of the planarian nervous system

Although the exact relationship of the Platyhelminthes with other Lophotrochozoan phyla remains to be elucidated, planarians are among the simplest animals that possess a centralized nervous system (CNS). The planarian CNS consists of a brain (or cephalic ganglia) and a pair of ventral nerve cord (VNC) that run along with the length of the animals^[4], and the brain lies on the top of the VNC^[5]. In addition, the planarian brain and VNC can be considered as independent structures at both the molecular and morphological lev-

Corresponding authors: Da-Yong WANG and Bo-Ping YE
Tel: 86-25-83272314-817
E-mail: dayongw@seu.edu.cn; yebp2001@yahoo.com.cn
Article ID: 1673-7067(2008)-05-0329-09
CLC number: Q426
Document code: A
Received date: 2008-06-10

els^[5]. The brain is organized as a central neuropil surrounded by an outer layer of neuronal cell bodies, and exhibits a typical spongy texture^[4]. The nervous system is organized into bi-lobed cephalic ganglia connected to two VNCs, which are interconnected by commissural neurons^[2]. That is, along the length of the planarian VNCs, small clusters of neurons are grouped into regularly spaced ganglia with a spongy structure, and the transverse commissures extended from these ganglia connect both VNCs to form an orthogon. Moreover, many lateral nerves extend from the VNC and form branching bundles near the edge of the animal and are continuous with a fine marginal plexus^[4,6].

Sensory structures in planarians contain photoreceptors, chemoreceptors, and rheoreceptors^[2]. These sensory structures are found at the anterior end of the animal and send projections to the cephalic ganglia^[2]. A large number of different sensory nerves leave the brain and connect with the margins and sensory organs^[4,7]. In addition, bipolar neurons located in transverse commissures between the VNCs can function as interneurons^[7]. There are accessory or supporting glia-like cells between brain neurons and along the VNCs with little vesicles and cytoplasm^[7].

Besides the CNS, planarians have several peripheral nerve plexuses to connect the main cords as a fairly diffuse and regular network of fibers with a small number of nerve cell bodies^[8]. The cells in this submuscular plexus are sparse, bipolar, or multipolar, and consist of spindle-shaped ganglion and sensory cells^[4,8]. Other nerve plexuses can be found around the gut diverticula or within the pharynx, or around the testis and the ovaries of sexual mature planarians^[8].

Therefore, the planarian CNS represents a conserved structure during animals' evolution, and studies on the planarian CNS regeneration will provide important cues for our understanding the regeneration in mammals and human beings.

2 Expression patterns of genes in intact and regenerating planarian CNS

2.1 Spatial expression patterns of genes in intact and regenerating planarian CNS

The first whole-mount *in situ* hybridization of a planarian gene was reported by Umesono *et al.* in 1997^[9]. The planarian CNS can be divided into several molecular and functional domains defined by the expression of different neural genes. Till now, more than twenty

neural genes have been identified in planarians. They include homologues of N-cell adhesion molecule (N-CAM), fibroblast growth factor receptor (FGFR), acetylcholine receptor, and synaptotagmin. Interestingly, some of these genes showed no sequence similarity to known genes^[10]. Among these genes, *PC2* (prohormone convertase 2), *GtPax6a* (*Girardia tigrina* Paired box 6a), *4307HH* (a nicotinic acetylcholine receptor α 7-1 subunit), *Gt-wnt-5* (a hybrid of *Wingless* and *Int* in *Girardia tigrina*), *DjPHM* (*Dugesia japonica* peptidylglycine a-hydroxylating monooxygenase), *Innexin3* (*Inx3*) and *Inx4*, *Smed-netR* (*Schmidtea mediterranea* netrin receptor), *DjCAM*, *Smed-roundabout A* (*Smed-roboA*), and *Dj-CHC* (*Dj-clathrin heavy chain*) are generally expressed in the planarian CNS^[6,11-19]. Genes of *Dj-otd/otxA* (*Dj-orthodenticle/orthodenticle homeobox*), *Dj-otd/otxB*, *ndk* (*nou-darake*), *DjFGFR1*, *DjFGFR2*, *DjXnp* (a novel member of the SNF2-like ATP-dependent chromatin remodelling genes), *DjFoxG* (planarian BF-1 homologue), *Dj-eya* (*Dj-eyes absent*), and *Dj-DSCAM* (*Dj-Down syndrome cell adhesion molecule*) are expressed in cephalic ganglia^[12,17,20-25]. Although most genes expressed in the cephalic ganglia are also expressed in the VNCs, genes of *nou-darake* and *DjDSCAM* are found specifically expressed in the brain^[4]. Genes of *Dj-otp* (*Dj-Orthopedia*), *Dj-nlg* (*Dj-noggin-like gene*), *1008HH* (a glutamate receptor), and *Gt-six3* (*Gt-sine oculis 3*) are specifically expressed in brain lateral branches, which project to the head margins^[9,12,26,27]. Even within the brain branches, *GiI α* (GTP-binding protein Gi α subunit) and *synaptotagmin VI* are mainly expressed in the distal half of those lateral branches^[12], whereas *Pax6*, *wnt-5*, a glutamate receptor gene (clone 1406), *1020HH* (encoding an unknown protein expressed in the central spongy region of the brain), and *eye53* are expressed in the central spongy region of the brain^[11-13,28]. It has also been reported recently that *DjWntA* is expressed in the posterior half of the brain, and *Dj-fzA* (*Dj-Frizzled receptor A*) can be detected in the anterior half^[28]. In addition, *1020HH* and *eye53* are supposed as neurotrophic factors required for synaptic functions between the photosensitive cells and their targets, and expressed surrounding the axons of the photosensitive cells^[29]. Therefore, the expression of these genes can be explored as useful neural markers to elucidate the mechanism of neural regeneration, and thus help us directly define the possibly functional domains in planarian CNS.

2.2 Temporal expression patterns of genes in intact and

regenerating planarian CNS The data from Reuter *et al.* suggested that the regeneration in planarians is the result of at least two processes: (1) an initial outgrowth of the original main longitudinal nerve cords that consequently send processes transversely, and (2) new nerve cells in front of the commissure differentiation from neoblasts and fasciculate with the fibers from the old VNCs giving rise to the new brain^[30]. The data from Cebrià *et al.* further indicated that the planarian CNS regeneration may have at least three developmental stages based on the assay of expression patterns from 12 neural markers^[31]. The early stage of CNS regeneration is defined by the appearance of the new brain primordium within the regenerative blastema. The new brain cells form two bilateral clusters do not connect to the preexisting VNC, which remain truncated in the stump region without apparent outgrowth, and these two bilateral cell clusters probably originate from the proliferation of neoblasts in the stump region and the subsequent migration of these neoblasts within the blastema. Two alternative mechanisms might be able to explain how neoblasts will be committed to brain cells as two bilateral clusters: (1) the regenerative blastema itself may possess bilateral cues to be sent to the neoblasts; or (2) it still cannot be dismissed that the VNC could have a role in induction of the bilateral brain primordium, although the brain primordium does not apparently appear from the outgrowth of the truncated VNC of the stump region^[31]. To date, no factor has been clearly defined to be responsible for the initial formation of the brain primordial tissue. The possible candidate genes for patterning of the brain primordial cells include *otd/Otx*, *DjwntA* and *DjzA*, and *Gtwnt-5*^[13,20,28]. *Otx*-related genes have been shown to define distinct mediolateral domains in the planarian brains^[20].

In the intermediate stage of CNS regeneration, the brain primordium differentiate into a small brain, with the two halves already connected in the most anterior part and with few lateral branches projecting to the new head periphery^[31]. At this stage, the VNC in the stump region will grow into the blastema and their association with the forming brain will be re-established^[31]. A clathrin heavy chain homologue has been shown to be required for this stage. After *DjCHC* RNAi, the brain primordial seemed to form normally, whereas the regenerated brain showed morphological defects^[19]. *DjCAM* and *DjDSCAM* are expressed in the mature CNS and appear in the brain primordial within the regenerative blastema 2 d after

amputation^[17]. In *DjDSCAM* knockdown animals, the gross morphology of the regenerated brain is aberrant with a disorganized neuropil and a reduced number of abnormal lateral branches, along with disorganization of the axonal tracts in branches, suggesting the essential role of *DjDSCAM* for proper regeneration of the neural network in the cephalic region^[17]. In addition, the functions of *netrin* may be related to the establishment of proper association between brain and VNC cells in the head region because of the expression pattern of this gene in the overlapping region of brain and VNC^[31].

In the late stage of CNS regeneration, the original CNS pattern is restored with a new spongy brain and VNC, which are connected through several transverse commissures running below them^[31]. Two novel genes named *eye53* and *1020HH* could be first detected within the blastema after 4–5 d of regeneration. After RNAi for *eye53* and *1020HH*, the animals regenerated a morphologically normal brain and visual system but did not show a normal photophobic response, suggesting that these genes are required for the functional recovery of the visual system^[29,31]. Therefore, although the basic structure is rebuilt at this stage, this new brain is probably not completely functional.

3 Axon guidance cues and neural regeneration

Conserved axon guidance mechanisms are necessary for proper wiring of the nervous system during early development of animals. During CNS regeneration, damaged axons need to project toward their original targets, and thus re-establish proper functional connections. Recent studies have addressed the functions of guidance cues during the planarian CNS regeneration. Netrins are the secreted molecules that act as chemoattractants or chemorepellents for guiding axons. In planarians, after *Smed-netR* RNAi, the association between the brain and the VNC was somewhat lost and patterns of the new brain and VNCs were disrupted: the VNCs formed a disorganized neural meshwork below the brain, and the new cephalic ganglia were shorter and wider and extended more laterally to the VNCs^[16]. After silencing the *Smed-netR*, the VNCs did not grow normally and showed disorganization similar to those observed in anteriorly regenerating VNCs^[16]. *Smed-net* and *Smed-netR* may play important roles in establishing the proper relationship between the cephalic ganglia and the VNCs, and are required for patterning of the nervous

system outside the cephalic region during regeneration^[16]. However, following *Smed-roboA* RNAi, the VNCs and new formed cephalic ganglia were not connected properly, and the development of a supernumerary pharynx and the production of ectopic, dorsal outgrowths with cephalic identity were observed during the anterior regeneration^[18]. Silencing of *Smed-roboA* resulted in a thinner anterior commissures, and in some cases, a complete loss of the commissure, suggesting a conserved role of the nervous system in pattern formation during blastema-based regeneration^[18]. Therefore, planarian *netrin/netR* and *robo* could be seen as having opposing effects during neural regeneration. Acting as repulsive cues for commissural axons at the midline and for cell migration, slit protein plays critical roles in axon guidance; and *Smed-slit* seems to display a more conserved function in planarian as a midline repellent^[32].

Moreover, clathrin-mediated endocytic signals are essential for axonal extension and proper CNS regeneration at the early stage of neural differentiation during regeneration^[19]. *DjCAM* is partially involved in axon formation, and *DjDSCAM* plays a crucial role in neuronal cell migration, axon outgrowth, fasciculation and projection^[17]. Nevertheless, it is not clear if the abnormal CNS regeneration is the direct result from axon-guidance defects or abnormal fasciculation or cell migration.

4 Bone morphogenetic protein (BMP) and Wnt signaling in neural regeneration

BMP signaling mediates a number of developmental events. Reddien *et al.* investigated the functions of BMP1/Tolloid-like gene (*smedoloid-1*), SMAD4-like gene (*smedsmad4-1*), and BMP2/4/decapentaplegic (DPP)-like gene (*smedbmp4-1*) during planarian CNS regeneration in 2007 and found that injured animals lacking left-right symmetry reset their midline through modulation of BMP activity as an early and necessary event in regeneration^[33]. *Smedbmp4-1* was normally expressed at the dorsal midline, and BMP signaling was involved in the formation of new tissues at the midline of regeneration^[33]. Asymmetric fragments lacking a midline displayed new *Smedbmp4-1* expression prior to formation of a regenerative blastema, whereas asymmetric fragments containing the midline displayed expanded *Smedbmp4-1* towards the wound^[33].

Wnt proteins are a family of highly conserved secreted glycoproteins that regulate cell-to-cell interactions, and Wnt

signaling plays an important role in nervous system regeneration in planarians. The expression of Wnt ligands and other Wnt signaling-related genes, such as *Gtwnt-5*, have been reported in the brain and pharynx^[13]. *Gtwnt-5* was activated during regeneration and expressed in a subpopulation of the whole CNS of intact organisms, and its expression was preferentially found in the most external region of the CNS^[13]. The Wnt-dependent glycogen synthesis kinase-3 (GSK3) is known to be involved in both patterning and neuronal organization. The phenotypes in GSK3 RNAi planarians showed that the cephalic ganglia appeared smaller and disconnected, whereas the visual axons were ectopically projected, and the pharynx did not regenerate properly, suggesting that the GSK3s are essential for normal differentiation and morphogenesis of the nervous system^[34].

5 Fibroblast growth factor (FGF) signaling in neural regeneration

In order to identify and characterize molecules involved in the process of CNS regeneration in planarians, Cebrià *et al.* (2002) prepared microarrays containing 1 640 non-redundant transcripts derived from a head complementary DNA library, and found that *ndk* gene is highly and specifically expressed in both brain and non-brain tissues^[21]. The *ndk* gene encodes a putative FGFR-like molecule with two extracellular immunoglobulin-like domains and without the characteristic cytoplasmic kinase domains of this receptor family^[21]. After 15-d of regeneration, loss of function of *ndk* by RNAi would result in the formation of ectopic eyes consisting of both pigment and visual cells with projecting axons, and ectopic brain tissues were found differentiating in the posterior regions of planarian body and expressing specific genes of both lateral branches and the central region of the brain^[21]. While knockdown of either of FGFR homologues gene (*FGFR1* and *FGFR2*) in planarians did not cause any clear defects in regeneration. Triple-knockdown of these two genes plus *ndk* suppressed the *ndk* phenotype, indicating that *ndk* negatively regulates the FGFR signaling in the trunk, and ectopic brains are formed through FGFR signaling^[21,35]. In addition, the effects of *ndk* still could be observed when both its intracellular and transmembrane regions were deleted, demonstrating that the extracellular domain of NDK is sufficient for inhibition of the *Xbra* expression in *Xenopus*^[21]. Therefore, the data from Cebrià *et al.* suggest that *ndk* may

modulate FGF signaling in stem cells to restrict brain tissues to the head region of planarians. Cebrià *et al.* raised a hypothesis that NDK may restrict the diffusion range of brain-inducing factors (FGF or FGF-like molecules) from a putative source in the head to the rest of body in planarians, and loss of function of *ndk* then would allow these receptors outside of the head region to trigger the formation of ectopic brain [21].

6 Role of apoptosis in neural regeneration

Apoptosis is known as a highly organized process of cell death. It plays an important role during development by controlling the precise number of cells for different types of organs or tissues and directing the morphological reorganization. In planarians, the TdT-mediated dUTP nick-end labeling (TUNEL) assay and the expression of apoptosis-related genes can be used to evaluate apoptosis. TUNEL assays suggested that apoptosis could occur in (1) intact body and (2) head and tail regeneration pieces [36]. *Dugesia japonica* Caspase-like gene 3 (*DjClg3*) is expressed primarily in apoptosis cells as revealed by double detections together with TUNEL assay [36]. *DjClg3* is expressed in the cells of mesenchymal space and around the pharynx of the intact body, and its expression in regenerating piece could be observed in the blastema and less significantly in the brain [36]. The mesenchyme of planarian is known to be where the neoblasts are distributed to give rise to any kind of cell type, suggesting that the apoptosis may reduce the number of proliferating cells during normal development. The apoptosis is occurred in the head piece from onset of regeneration until the whole body can be fully developed, indicating that the neural cells in old tissues tend to undergo suicide during regeneration. Therefore, Hwang *et al.* hypothesized that the presence of apoptosis might have a role in controlling cell numbers, eliminating unnecessary tissues or cells, and remodeling the old tissues of regenerating body parts in planarians [36].

In addition, because *DjCHC*-RNAi-treated neurons entered a TUNEL-positive apoptosis state, clathrin-mediated endocytic signals may be required for maintenance of neurons after synaptic formation in planarians [19].

7 Neurotransmitters and neural regeneration

In planarians, neurons positive for dopamine, serotonin (5-HT), and γ -aminobutyric acid (GABA) have been detected [37–39].

Dugesia japonica tyrosine hydroxylase (DjTH) and *Dugesia japonica* aromatic amino acid decarboxylase-like A (DjAADCA) are dopamine biosynthetic enzymes. The distribution of DjTH-positive neurons closely paralleled the amount of dopamine in planarian body parts, and dopamine, but not 5-HT, was almost completely lost in *DjTH*-knockdown planarians, indicating that tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis [37]. DjTH protein is co-expressed with DjAADCA in planarian CNS [37]. DjTH-expressing neurons could be classified into five types, *i.e.*, dorsal neurons and ventral neurons in the brain, neurons in the VNCs, head peripheral neurons, and epidermal neurons [37]. When the planarian body was amputated, the DjTH-positive neurons could be regenerated in the brain newly rebuilt from the tail piece at Day 3, and the DjTH-positive axonal and dendritic network in the CNS could be reconstructed at Day 5–7 [37]. The signal transmission of dopamine neurons may function mainly in the brain and moderately in the peripheral nervous system, and that central dopamine neurons can project to the end of the tail through the VNCs [37]. In addition, a *DjTPH* gene encoding tryptophan hydroxylase has also been identified, and it is mainly expressed in the nervous system, especially ventral nerve cords and eye pigment cells [38]. DjTPH antibody is a useful marker for serotonergic neurons, and DjTPH immunoreactivity could be clearly detected at commissure axonal connections in the ventral nerve cords [38]. The 5-HT was significantly decreased in *DjTPH*-knockdown animals, suggesting that *DjTPH* is required for 5-HT biosynthesis in planarians [38]. Also, immunostaining against GABA has been observed in the CNS and the subepidermal plexus of planarians [39].

For the possible actions of neurotransmitters on regeneration, the model from Baguña *et al.* suggested that different molecules from nerve cells could signal through receptors in planarian stem cells to control their proliferation either activating or inhibiting it. On the other hand, the same or different neural factors could act on already committed precursors cells to regulate their differentiation (Fig. 1) [40]. Nevertheless, the direct evidence to support this hypothesis are still absent in planarians.

8 Anterior-posterior (A-P) pattern and dorsal-ventral (D-V) pattern formation

In planarians, the bilateral symmetry is established by

Fig. 1 Possible actions of neurotransmitters on regeneration in planarian. nc, nerve cell; sc, stem cell; pc, precursor cell. Black and white circles indicate the same neural factors, and black circles indicate the different neural factors (adapted from Baguña *et al.*, 1989)^[40].

determination of the A-P and the D-V body axes. Wnt signaling also functions in axis formation, and is required for proper brain patterning during planarian brain regeneration^[28]. *DjwntA* is one of the Wnt homologues in planarian, and is expressed in the posterior region of the brain^[28]. RNAi knockdown of *DjwntA* made animals regenerate their heads at the anterior ends of the fragments, but formed ectopic eyes with irregular posterior lateral branches and brain expansion, which suggest that the Wnt signals may regulate the A-P patterning of the planarian brain^[28]. Moreover, the data from Kobayashi *et al.* (2007) also suggest that the *nou-darake/FGFR* signal may be involved in the brain rudiment formation at the early stage of head regeneration, and subsequently the *DjwntA* signal may function in A-P patterning of the brain rudiment^[28].

D-V interaction is involved in the trigger of blastema formation, and the D-V positional cues may reside in differential cells. Whole-mount *in situ* hybridization indicated that *Smed-BMP* was expressed at the planarian dorsal midline, while *Smed-Smad1* was expressed throughout the mesenchyme and in the CNS^[41]. RNAi knockdown of *Smed-BMP* or *Smed-Smad1* resulted in disappearance of dorsal markers along with the ectopic expression of ventral markers on the dorsal side of the treated animals^[41]. Similarly, in *Djbmp* RNAi knockdown planarians, a bulge formed on the dorsal side with a dent in the middle of the bulge, and the body surface inside the dent was smoothed and less pigmented^[42]. Moreover, a duplication of CNS, one of the ventral expressions, differentiated dorsally after *Smed-BMP* or *Smed-Smad1* RNAi^[41]; and besides the original pair of ventral nerve cords, a pair of incomplete nerve cords in the dorsal side were formed

after *Djbmp* RNAi^[42]. Therefore, BMP signaling pathway plays a key role in the regeneration and maintenance of the DV axis in planarian. Nevertheless, the BMP signal appears not to be the D-V positional cue that triggers blastema formation, since the blastema was formed even in *Djbmp*-knockdown planarians^[42].

9 Conclusion

Although there was considerable interest in planarian regeneration during the first two-thirds of the twentieth century, a decline tendency for the planarian study was formed around the middle of the last century, and the researches in this field became restricted to just a few laboratories around the world. Over the last decade, along with the exploration of new tools including whole-mount immunostaining and *in situ* hybridization, RNAi, BrdU labeling of neoblasts, expression sequence tag (EST) collections, and sequencing of planarian genome, a new generation of scientist has revitalized the worldwide network of planarian regeneration laboratories.

In this review, we state and discuss the molecular control and the possible mechanisms for neural regeneration in planarians. To date, a series of genes have been identified to be involved in the spatial and temporal regulation of planarian neural regeneration, and some of these genes encode important signaling pathways, such as BMP, Wnt, and FGFR signal pathways^[9-31]. In addition, the dopaminergic, serotonergic and GABAergic neurons can be already labeled with specific antibodies^[37-39]. Moreover, Agata and Umesonono recently summarized the brain regeneration process, and raised

Fig. 2 Summary of planarian brain regeneration process (adapted from Agata K, Umesono Y, 2008)^[35].

a notion that the planarian brain regeneration can be further divided into five steps^[35]. The five steps are: (1) anterior blastema formation, (2) brain rudiment formation, (3) pattern formation, (4) neural network formation, and (5) functional recovery (Fig. 2)^[35]. The following genes were found to be involved in these steps: step (1) BMP/*noggin* signal, step (2) *nou-darake*/FGFR signal, step (3) Wnt signal and *otd/Otx* family genes, step (4) netrins and CAMs, and step (5) *1020HH* and *eye53*, indicating that the brain regeneration proceeds in a sequential manner accompanied by appropriate changes of gene expression^[35]. This classification, together with the obtained molecular evidence in planarians, contributes greatly to our understanding of the mechanisms of animals' neural regeneration, and suggests some useful pharmacological targets for the enhancement or regenerative capacities that are non-existing in humans.

By investigating the function of signaling pathways and their relationships in a variety of contexts, it will be possible to identify the molecular strategies by which the planarian neural regeneration is established. Though it is still an open question whether or not the study of planarians will uncover novel genetic or signal pathways, by providing a more complete picture of the molecular activities, the studies will be bound to bring us closer to elucidation of the general principles guiding the organization and order for neural regeneration in living organisms.

Acknowledgements: This work was supported by the Southeast University Foundation for Excellent Young Scholars (No. 4023001013) and the Program for New Century Excellent Talents in University.

References:

- [1] Sánchez Alvarado A. Regeneration and the need for simpler model organisms. *Philos Trans R Soc Lond B Biol Sci* 2004, 359: 759-763.
- [2] Reddien PW, Sánchez Alvarado A. Fundamentals of planarian regeneration. *Annu Rev Cell Dev Biol* 2004, 20: 725-757.
- [3] Saló E. The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). *Bioessays* 2006, 28: 546-559.
- [4] Cebrià F. Regenerating the central nervous system: how easy for planarians! *Dev Genes Evol* 2007, 217: 733-748.
- [5] Okamoto K, Takeuchi K, Agata K. Neural projections in planarian brain revealed by fluorescent dye tracing. *Zoolog Sci* 2005, 22: 535-546.
- [6] Agata K, Soejima Y, Kato K, Kobayashi C, Umesono Y, Watanabe K. Structure of the planarian central nervous system (CNS) revealed by neuronal cell markers. *Zoolog Sci* 1998, 15: 433-440.
- [7] Rieger RM, Tyler S, Smith JPS III, Rieger GE. Platyhelminthes: Turbellaria. In: Harrison FW, Bogitsh BJ (eds). *Microscopic Anatomy of Invertebrates*, Volume 3. New York: Wiley-Liss, 1991, 7-140.
- [8] Reuter M, Gustafsson MK. The flatworm nervous system: pattern and phylogeny. *EXS* 1995, 72: 25-59.
- [9] Umesono Y, Watanabe K, Agata K. A planarian orthopedia homolog is specifically expressed in the brain region of both the mature and regenerating brain. *Dev Growth Differ* 1997, 39: 723-727.
- [10] Nakazawa M, Cebrià F, Mineta K, Ikeo K, Agata K, Gojobori T. Search for the evolutionary origin of a brain: planarian brain characterized by microarray. *Mol Biol Evol* 2003, 20: 784-791.
- [11] Pineda D, Rossi L, Batistoni R, Salvetti A, Marsal M, Gremigni V, *et al.* The genetic network of prototypic planarian eye regeneration is Pax6 independent. *Development* 2002, 129: 1423-1434.
- [12] Cebrià F, Kudome T, Nakazawa M, Mineta K, Ikeo K, Gojobori T, *et al.* The expression of neural-specific genes reveals the

- structural and molecular complexity of the planarian central nervous system. *Mech Dev* 2002, 116: 199-204.
- [13] Marsal M, Pineda D, Saló E. *Gtwn-5* a member of the wnt family expressed in a subpopulation of the nervous system of the planarian *Girardia tigrina*. *Gene Expr Patterns* 2003, 3: 489-495.
- [14] Asada A, Orii H, Watanabe K, Tsubaki M. Planarian peptidylglycine-hydroxylating monooxygenase, a neuropeptide processing enzyme, colocalizes with cytochrome b561 along the central nervous system. *FEBS J* 2005, 272: 942-955.
- [15] Nogi T, Levin M. Characterization of innexin gene expression and function roles of gap-junctional communication in planarian regeneration. *Dev Biol* 2005, 287: 314-335.
- [16] Cebrià F, Newmark PA. Planarian homologs of netrin and netrin receptor are required for proper regeneration of the central nervous system and the maintenance of nervous system architecture. *Development* 2005, 132: 3691-3703.
- [17] Fusaoka E, Inoue T, Mineta K, Agata K, Takeuchi K. Structure and function of primitive immunoglobulin superfamily neural cell adhesion molecules: a lesson from studies on planarian. *Genes Cells* 2006, 11: 541-555.
- [18] Cebrià F, Newmark PA. Morphogenesis defects are associated with abnormal nervous system regeneration following roboA RNAi in planarians. *Development* 2007, 134: 833-837.
- [19] Inoue T, Hayashi T, Takeuchi K, Agata K. Clathrin-mediated endocytic signals are required for the regeneration of, as well as homeostasis in, the planarian CNS. *Development* 2007, 134: 1679-1689.
- [20] Umesono Y, Watanabe K, Agata K. Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Dev Genes Evol* 1999, 209: 31-39.
- [21] Cebrià F, Kobayashi C, Umesono Y, Nakazawa M, Mineta K, Ikeo K, *et al.* FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians. *Nature* 2002, 419: 620-624.
- [22] Rossi L, Deri P, Andreoli I, Gremigni V, Salvetti A, Batistoni R. Expression of *DjXnp*, a novel member of the SNF2-like ATP-dependent chromatin remodeling genes, in intact and regenerating planarians. *Int J Dev Biol* 2003, 47: 293-298.
- [23] Koinuma S, Umesono Y, Watanabe K, Agata K. The expression of planarian brain factor homologs, *DjFoxG* and *DjFoxD*. *Gene Expr Patterns* 2003, 3: 21-27.
- [24] Mannini L, Rossi L, Deri P, Gremigni V, Salvetti A, Saló E, *et al.* *Djeyes absent* (*Djeya*) controls prototypic planarian eye regeneration by cooperating with the transcription factor *Djsix-1*. *Dev Biol* 2004, 269: 346-359.
- [25] Ogawa K, Kobayashi C, Hayashi T, Orii H, Watanabe K, Agata K. Planarian fibroblast growth factor receptor homologs expressed in stem cells and cephalic ganglions. *Dev Growth Differ* 2002, 44: 191-204.
- [26] Ogawa K, Ishihara S, Saito Y, Mineta K, Nakazawa M, Ikeo K, *et al.* Induction of a *noggin-like* gene by ectopic DV interaction during planarian regeneration. *Dev Biol* 2002, 250: 59-70.
- [27] Pineda D, Saló E. Planarian *Gtsix3*, a member of the *Six/so* gene family, is expressed in brain branches but not in eye cells. *Gene Expr Patterns* 2002, 2: 169-173.
- [28] Inoue T, Kumamoto H, Okamoto K, Umesono Y, Sakai M, Sanchez Alvarado A, *et al.* Morphological and functional recovery of the planarian photosensing system during head regeneration. *Zoolog Sci* 2004, 21: 275-283.
- [29] Kobayashi C, Saito Y, Ogawa K, Agata K. Wnt signaling is required for anterior-posterior patterning of the planarian brain. *Dev Biol* 2007, 306: 714-724.
- [30] Reuter M, Sheiman IM, Gustafsson MK, Halton DW, Maule AG, Shaw C. Development of the nervous system in *Dugesia tigrina* during regeneration after fission and decapitation. *Invertebr Reprod Dev* 1996, 29: 199-211.
- [31] Cebrià F, Nakazawa M, Mineta K, Ikeo K, Gojobori T, Agata K. Dissecting planarian central nervous system regeneration by the expression of neural-specific genes. *Develop Growth Differ* 2002, 44: 135-146.
- [32] Cebrià F, Guo T, Jopek J, Newmark PA. Regeneration and maintenance of the planarian midline is regulated by a *slit* orthologue. *Dev Biol* 2007, 307: 394-406.
- [33] Reddien PW, Bermange AL, Kicza AM, Sanchez Alvarado A. BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. *Development* 2007, 134: 4043-4051.
- [34] Adell T, Marsal M, Saló E. Planarian GSK3s are involved in neural regeneration. *Dev Genes Evol* 2008, 218: 89-103.
- [35] Agata K, Umesono Y. Brain regeneration from pluripotent stem cells in planarian. *Philos Trans R Soc Lond B Biol Sci.* 2008, 363: 2071-2078.
- [36] Hwang JS, Kobayashi C, Agata K, Ikeo K, Gojobori T. Detection of apoptosis during planarian regeneration by the expression of apoptosis-related genes and TUNEL assay. *Gene* 2004, 333: 15-25.
- [37] Nishimura K, Kitamura Y, Inoue T, Umesono Y, Sano S, Yoshimoto K, *et al.* Reconstruction of dopaminergic neural network and locomotion function in planarian regenerates. *Dev Neurobiol* 2007, 67: 1059-1078.
- [38] Nishimura K, Kitamura Y, Inoue T, Umesono Y, Yoshimoto K, Takeuchi K, *et al.* Identification and distribution of tryptophan hydroxylase (TPH)-positive neurons in the planarian *Dugesia japonica*. *Neurosci Res* 2007, 59: 101-106.
- [39] Eriksson KS, Panula P. γ -Aminobutyric acid in the nervous system of a planarian. *J Comp Neurol* 1994, 345: 528-536.
- [40] Baguña J, Saló E, Romero R. Effects of activators and antagonists of the neuropeptides substance P and substance K on cell proliferation in planarians. *Int J Dev Biol* 1989, 33: 261-266.
- [41] Molina MD, Saló E, Cebrià F. The BMP pathway is essential for re-specification and maintenance of the dorsoventral axis in

regenerating and intact planarians. *Dev Biol* 2007, 311: 79-94.
[42] Orii H, Watanabe K. Bone morphogenetic protein is required for

dorso-ventral patterning in the planarian *Dugesia japonica*. *Dev Growth Differ* 2007, 49: 345-349.

涡虫神经再生的分子机制

张燕芬^{1,2,3}, 叶波平¹, 王大勇^{2,3}

¹ 中国药科大学生命科学技术学院, 南京 210009

² 东南大学基础医学院遗传与发育系, 南京 210009

³ 东南大学发育与疾病相关基因教育部重点实验室, 南京 210009

摘要: 涡虫是具有简单中枢神经系统的模式动物之一, 其神经再生过程包括受损细胞的替换以及细胞的再生过程。本文阐述并讨论了近年来利用涡虫进行神经再生研究取得的研究进展。目前, 参与涡虫神经再生相关基因的时空间表达图谱已被相对清晰地勾勒出来。骨形成蛋白和 Wnt 信号通路已被鉴定参与神经再生的调控。在神经再生过程中, 保守的轴突导向机制为神经系统网络的正确形成所必需。此外, 细胞凋亡在控制细胞数量、消除多余组织和细胞以及重新塑造再生的神经系统中发挥着重要作用。而且, 在涡虫中多巴胺能神经元、五羟色胺能神经元和 γ -氨基丁酸能神经元可以被有效地予以标记。因此, 涡虫为我们提供了一个可以在分子水平考察神经系统再生机制的新颖且具可操作性的实验研究平台。

关键词: 神经再生; 中枢神经系统; 分子机制; 涡虫