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# Regeneration of Brain and Dopaminergic Neurons Utilizing Pluripotent Stem Cells: Lessons from Planarians

Kaneyasu Nishimura[1,3], Yoshihisa Kitamura[2] and Kiyokazu Agata[1]
[1]*Department of Biophysics, Graduate School of Science, Kyoto University*
[2]*Department of Neurobiology, Kyoto Pharmaceutical University*
[3]*Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University*
*Japan*

## 1. Introduction

Cell-transplantation therapy for Parkinson's disease is close to becoming a reality thanks to the recent development of methods for the differentiation of dopaminergic neurons and/or dopaminergic progenitor cells from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) under *in vitro* conditions (Kawasaki et al., 2000, Perrier et al., 2004). There have been several reports concerning pre-clinical trial research for cell-transplantation therapy for Parkinson's disease with dopaminergic progenitor cells derived from either ESCs or iPSCs using rodent and non-human primate disease models before clinical trial (Björklund et al., 2002; Takagi et al., 2005; Wernig et al., 2008). Many researchers have contributed to improve the technology to create more efficient differentiation methods of donor cells for clinical applications (Chambers et al., 2009; Morizane et al., 2011). However, we still need to overcome many problems before such technology can be used in clinical settings. Even if we succeed in obtaining an optimized donor cell population for cell-transplantation, the rate of success of the transplantation may depend not only on the quality of donor cells but also on the host brain environment. One important issue is how to integrate dopaminergic neurons or dopaminergic progenitor cells into target regions after transplantation. However, we do not know what kind of donor cells will be efficiently integrated into the neural networks of the host brain. Also, we do not know whether fully differentiated neurons will really survive in the host brain. In addition, we need to know what state of the host brain environment will allow the participation of donor cells in the neural networks of the host brain. In order to solve such problems, planarians provide unique opportunities because they show robust regenerative ability based on their pluripotent stem cell system.

Planarians can regenerate lost tissues, including the nervous system, via their pluripotent stem cells (neoblasts) that are distributed throughout their body. In contrast, it is difficult for higher vertebrates to achieve the regeneration of the nervous system, in spite of their

possession of neural stem cells. The success of tissue regeneration requires not only the presence of proliferating stem cells as a source but also the presence of the regulatory system for stem cells. Knowledge gained about the planarian stem cell system can provide hints about how to conduct cell-transplantation therapy for regenerative medicine in the future.

In this chapter, we focus on two different regenerative phenomena utilizing the stem cell system in planarians. The first one is brain regeneration after decapitation. The second is brain neurogenesis after selective neuronal degeneration (without decapitation). Both of them are achieved by regulation of the pluripotent stem cells distributed throughout the body. We address the following questions: (1) what type(s) of cells recognize the loss of the organs or cells? (2) What signal(s) initiate the regeneration or neurogenesis? (3) What signal(s) are necessary for recruitment of stem cells to defined type(s) of cells and the replacement in the proper positions.

## 2. Pluripotent stem cells of planarians

The flatworm *Dugesia japonica* is a common species of freshwater planarian in Japan, and has been extensively used as an experimental animal for regeneration and neuroscience studies. When planarians are artificially amputated, they can regenerate their whole body from even very small fragments (is the smallest competent fragment reported was $1/279^{th}$ of the body; Morgan, 1898). This strong regenerative ability is supported by pluripotent stem cells called neoblasts. The neoblasts are the only mitotic cell population, and are distributed in the mesenchymal space throughout the body except for the region around the brain and the pharynx of *D. japonica* (Shibata et al., 1999; 2010) (Fig. 1). The neoblasts can differentiate in all types of cells and self-renew under both homeostatic and injured conditions. X-ray-irradiation induces selective elimination of proliferating stem cells in planarians, resulting in the loss of regenerative ability (Shibata et al., 1999; Hayashi et al., 2006). Therefore, X-ray irradiation is a powerful experimental tool for analyzing the stem cell system. We identified a *vasa*-like gene (*Djvlg*) as the first reported gene specifically expressed in neoblasts (Shibata et al., 1999). Recently, many reliable molecular markers for neoblasts, such as *piwi* homologue genes, have been identified (Fig. 1) (Salvetti et al., 2000, 2005, Orii et al., 2005; Reddien et al., 2005; Eisenfoffer et al., 2007; Shibata et al., 2010). Since pluripotent stem cells are the only proliferating and mitotic cell population, experimental methods using 5-bromo-2′-deoxyuridine (BrdU) (Newmark & Sánchez Alvarado, 1999), and immunostaining using anti-phosphohistone H3 (pH3) antibody (Hendzel et al., 1997; Newmark & Sánchez Alvarado, 1999) are also useful tools for staining neoblasts.Recently, the pluripotency of these cells was demonstrated by single Icell-transplantation experiments (Wagner et al., 2011). In addition, we found that pluripotent stem cells can be categorized into several cell populations by electromicroscopy analysis, suggesting that pluripotent stem cells are not a homogenous population, but may have heterogeneity like stem cell systems in higher animals (Higuchi et al., 2007). In addition, we recently developed single-cell PCR technology that is able to analyze the gene expression profile in individual cells at the single cell level (Hayashi et al., 2010). This method is a powerful tool for determining gene characteristics of not only pluripotent stem cells but also of tissues such as the nervous system.

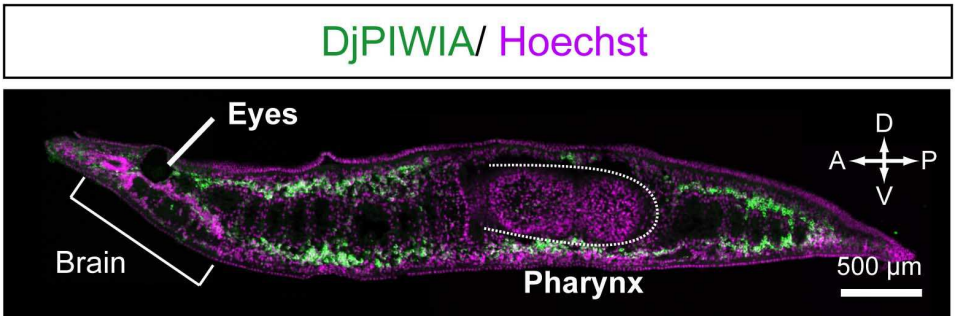


Fig. 1. Distribution of pluripotent stem cells of planarian *D. japonica.* Immunostaining using anti-DJPIWIA antibody (a marker of pluripotent stem cells) (Shibata et al., 2010) in a transverse section. Planarian stem cells are distributed in the mesenchymal space throughout the body.

## 3. Fundamental brain structure and function

Planarians have a simple body shape with cephalization, a dorso-ventral axis and bilateral symmetry, and are thought to be primitive animals, that acquired a central nervous system (CNS) at an early stage of evolution. The planarian CNS composed of a bilobed brain and a pair of ventral nerve cords (VNCs) (Agata et al., 1998; Tazaki et al., 1999). The brain is located in the anterior region of the body, and forms an inverted U-shaped structure (Fig. 2A). A pair of VNCs are located more ventral by relative to the brain, extending along the anterior-posterior (A-P) axis. The VNCs are a structure independent of the brain, although they are directly connected to it (Okamoto et al., 2005). The brain can be divided into several functional domains (Cebrià et al., 2002a; Nakazawa et al., 2003). The nine pairs of lateral branches of the brain project to the head margin, and function as the sensory system (Okamoto et al., 2005). A pair of eyes is located on the dorsal side of the brain, and the optic nerves forms the optic chiasm, and project to the dorso-medial position of the brain, which functions as the photosensory center (Sakai et al., 2000). The two main lobes of the brain consist of a mass of interneurons that function in the integration of multiple stimuli.

When planarians are exposed to some stimuli such as light-, chemo-, thermo- and mechano-stimulations, they can integrate different stimuli in the brain and decide on a response to these multiple stimuli. Planarians show light avoidance behavior known as negative phototaxis. We established a quantitative analytical method for this behavior that involves measuring the distance, direction, and speed of movement (Inoue et al., 2004). By using this method and RNA interference (RNAi), we showed that several molecules such as a planarian synaptosome-associated protein of 25 kDa (*Djsnap-25*) and a planarian glutamic acid decarboxylase (*DjGAD*) play important roles in photorecognition (Takano et al., 2007; Nishimura et al., 2008a). These results indicate that planarian behavior is regulated the molecular level via brain functions that are similar to mammalian brain functions.

### 3.1 Functional domain structure

We found that functional domains in the brain were defined by three *orthodenticle* and *orthopedia* homeobox genes (*DjotxA, DjotxB* and *Djotp*) that are exclusively expressed in

specific regions of the brain (Umesono et al., 1997; 1999). *DjotxA* is expressed in the optic nerves and medial region of the brain, which form a photosensory domain. *DjotxB* is expressed in the main lobes of the brain, which form a signal processing domain containing a variety of interneurons. *Djotp* is expressed in the lateral branches, which form chemosensory domains. The lateral side of the head region, where *Otx/otp* expression is not detected, contains mechanosensory neurons. In addition, A-P patterning of the brain was shown to be regulated by the expression of *wnt*-family genes (*DjwntA* and *DjfzA*) (Kobayashi et al., 2007). Whereas *DjotxA, DjotxB* and *Djotp* genes were shown to be expressed medio-laterally, *DjwntA* and *DjfzA* genes were expressed antero-posteriorly in the brain. *Wnt* family genes and *Otx/otp* family genes play important roles in domain formation in planarians, as in mammals.

DNA microarray analysis comparing the head region versus the body region of planarians identified many genes that are specifically expressed in the head region (Nakazawa et al., 2003; Mineta et al., 2003). Expression analysis based on whole-mount *in situ* hybridization revealed that many neural genes that are conserved in the vertebrate brain are also expressed in several distinct domains of the planarian CNS (Cebrià et al., 2002a; Mineta et al., 2003). These results indicate that the planarian CNS is functionally regionalized by discrete expression of neural-specific genes.

### 3.2 Variations of neurotransmitters

Recently, we revealed that planarians have various neural populations defined by neurotransmitters, such as dopamine (DA), serotonin (5-HT), γ-aminobutyric acid (GABA), octopamine (OA; a counterpart of noradrenaline of vertebrates) and acetylcholine (ACh) (Nishimura et al., 2007a, 2007b, 2008a, 2008b, 2008c, 2010; Takeda et al., 2008) (Fig. 2). Immunostaining with specific antibodies against these neurons enables us to visualize their cell morphology and localizations at the single-cell level (Fig. 2). These neurons are distributed in restricted regions in the planarian CNS. In addition, each neuron exclusively uses one neurotransmitter, and forms distinct neural networks in the planarian CNS.

These neurons have also distinct functions, such as locomotion activity and photorecognition. Combined RNAi and pharmacological approaches revealed that dopaminergic neurons positively regulate muscule-mediated behavior. Upregulation of the DA level induced by methamphetamine (DA releaser) caused hyperkinetic conditions such as screw-like hyperkinesia and C-like hyperkinesia, and treatment with DA receptor antagonists (sulpride and reserpine) and reduction of the DA level by RNAi suppressed these hyperkinetic conditions (Nishimura et al., 2007a). Moreover, although an increase of the ACh level by physostigmine (acetylcholinesterase inhibitor) treatment induced sudden muscular contraction, treatment with ACh receptor antagonists (tubocrarine and atropine) or reduction of the ACh level by RNAi extended these behavioral changes (Nishimura et al., 2010). Our histological analysis indicated that cholinergic neurons elongated at neighboring positions of the body-wall musculature (DjMHC-B-positive cells), but dopaminergic neurons did not elongate to the body-wall musculature. These results suggest that although both dopaminergic and cholinergic neurons regulate motor functions, cholinergic neurons act as motor neurons whilst dopaminergic neurons act as interneurons in planarians. These results also indicate that similar gene sets function in both the planarian CNS and the vertebrate CNS.

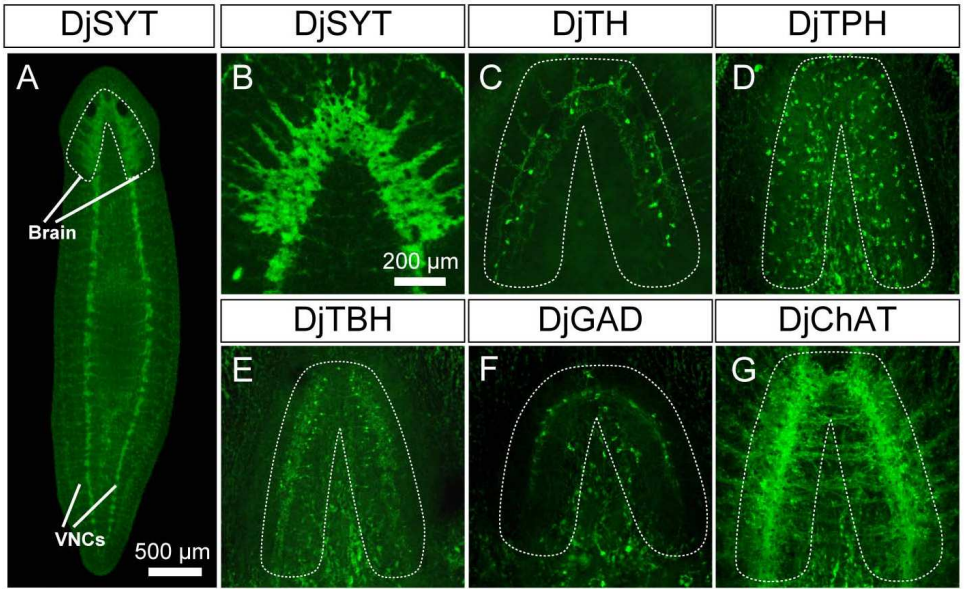


Fig. 2. The neural networks of neurotransmitter-synthesizing neurons. Distribution of pan-neural networks (DjSYT-positive neurons) of the whole body (A) and head (B). Distribution of dopaminergic neurons (DjTH-positive neurons) (C), serotonergic neurons (DjTPH-positive neurons) (D), octopaminergic neurons (DjTBH-positive neurons) (E), GABAergic neurons (DjGAD-positive neurons) (F), and cholinergic neurons (DjChAT-positive neurons) (G) in intact planarian head. White broken line indicates the outline of the brain (B-G).

## 4. Whole brain regeneration after head amputation

One of most interesting regeneration phenomena in planarians is that they can regenerate a functional brain from any portion of the body within 7-10 days after amputation, utilizing the pluripotent stem cell system. Although non-brain fragments just after decapitation show very little response external stimulation, they can restore normal behaviors such as feeding and negative phototaxis within one week. How can planarians regenerate their CNS not only morphologically but also functionally in one week? This regenerative process can be divided into at least five steps as defined by sequential gene expression alterations, which are similar to those in mammalian brain development (Agata & Umesono et al., 2008). That is, (1) anterior blastema formation, (2) brain rudiment formation, (3) pattern formation, (4) neural network formation, and (5) functional recovery (Fig. 3).

### 4.1 The stem cell system for brain regeneration

The first step of head regeneration after decapitation involves wound healing and subsequently the formation of the blastema, which is defined by a mass of morphologically undifferentiated cells at the edge of the amputated site. Dorso-ventral attachment induces initiation of the expression of *noggin-like gene A* (*DjnlgA*) at the edge of the amputated site after wound healing, and this expression leads to blastema formation in the first step of

planarian regeneration (Ogawa et al., 2002). Mitotic cells are never observed in the blastema, in spite of the increasing mass of the blastema during regeneration (Wenemoser & Reddien, 2010; Tasaki et al, 2001a, 2001b). Recently, it was shown that the blastema cells are supplied from the postblastema region via mitosis from G2 phase-pluripotent stem cells, and that c-Jun-N-terminal kinase (JNK) is involved in this G2/M transition, and that extracellular signal-related kinase (ERK) is required for exit from the proliferative undifferentiated state during blastema formation (Tasaki et al., 2011a, 2011b). It is thought that BMP/noggin signal might be involved in activation of the ERK signal in cooperation with the JNK signal to form the blastema after wound closure.

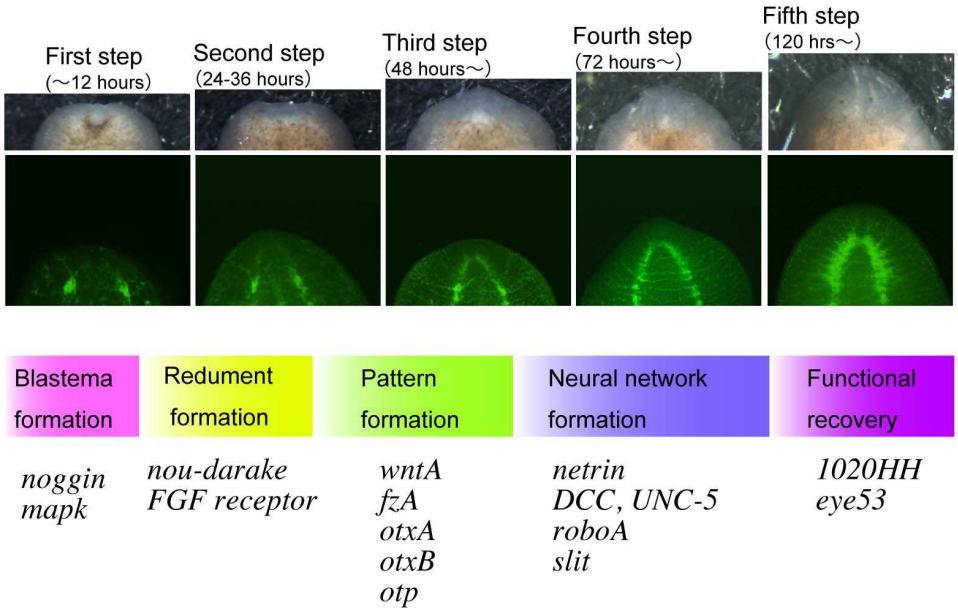


Fig. 3. Brain regeneration process after decapitation. This process can be divided into at least five steps according to sequential gene expression alterations. Abbreviations used; mapk, mitogen-activated protein kinase; FGF, fibroblast growth factor; DCC, deleted in colorectal cancer; UNC-5, uncoordinated-5; robo, roundabout.

After the formation of blastemas, the ERK signal is suppressed in the posterior blastema, but enhanced in the anterior blastema. Recently, we found that the hedgehog (Hh) signal has an important role in causing the difference between the anterior and posterior blastemas. In planarians, Hh is produced in the nervous system and Hh-containing vesicles might be transported from anterior to posterior along microtubules inside of the neurites (Yazawa et al., 2009). After amputation of the planarian body, Hh may be secreted from the posterior end of the amputated neurites, and then the Hh signal activates the Wnt signal in the posterior blastema to suppress the ERK signal and activate posterior-specific genes. In contrast, in the anterior blastema, the ERK signal forms a positive feedback loop to activate brain rudiment formation. A fibroblast growth factor receptor (FGFR)-like molecule, *nou-*

*darake* (*ndk*; meaning "brains everywhere" in Japanese), may have an important role in defining the region forming the positive feedback loop of the ERK signal in the anterior blastema (Cebrià et al., 2002b). The *ndk* gene was identified in *D. japonica* as a gene expressed in the brain rudiment at an early stage of brain regeneration. Interestingly, silencing of the *ndk* gene by RNAi induces the ectopic brain formation in all regions of the body. Thus, *ndk* is essential for defining the region where the brain rudiment is formed.

After formation of the brain rudiment, the Wnt and bone morphogenic protein (BMP) signaling pathways may regulate pattern formation of the brain along the A-P (Kobayashi et al., 2007; Gurley et al., 2008; Petersen & Reddien, 2008) and D-V (Molina et al., 2011; Gavino & Reddien, 2011) polarity, respectively. In conclusion, stem cells may be regulated by various signals in spatial- and temporal manners to form a functional brain.

### 4.2 Axon guidance and neural network formation during brain regeneration

New brain neurons have to project toward appropriate target sites to reconstruct their neural networks during regeneration. Recently, several axon guidance molecules, including netrin, uncoordinated-5 (UNC-5), deleted in colorectal cancer (DCC), slit, and roundabout (robo) were identified as key molecules regulating axon guidance during eye and brain regeneration in planarians (Cebrià & Newmark 2005, 2007; Cebrià et al., 2007; Yamamoto & Agata, 2011). It is known that netrin is a secreted protein that regulates the direction of axon growth by chemo-attractive and repulsive responses mediated by two types of receptor, UNC-5 and DCC (Hong et al., 1999). Slit is also a secreted protein, and acts as a chemo-repulsive factor for commisure axons by binding to robo in various animals (Brose et al., 1999). RNAi-mediated functional analysis revealed that the silencing of these guidance molecules caused abnormal neural network formation in the CNS and optic nerves during regeneration.

### 4.3 Functional recovery after completion of whole brain regeneration

In order to analyze the brain function during brain regeneration, we focused on negative phototaxis behavior. We found that there is a time gap between morphological and functional recovery. Although the optic nerves were reconstructed within 4 days after decapitation, negative phototaxis behavior began to recover from 5 days after decapitation (Inoue et al., 2004). Interestingly, two genes, *1020HH* and *eye53* genes, were activated just after completion of the morphological recovery (Cebrià et al., 2002c). Silencing of either *1020HH* or *eye53* caused a defect of the complete recovery of negative phototaxis. These findings suggest that these genes might be involved in the functional recovery, and morphological regeneration and functional regeneration can be distinguished according to their respective gene expression alterations (Inoue et al., 2004).

## 5. Neurogenesis after selective neuronal lesioning

Recently, we established an experimental model system for selective neuronal elimination to analyze the neurogenesis after selective neuronal lesioning without amputation. For this, we employed 6-hydroxydopamine (6-OHDA)-induced lesioning. 6-OHDA is a cytotoxic substance that induces dopaminergic neuronal cell death, and is widely used for killing dopaminergic neurons and creating parkinsonian animal models (Ungerstedt & Arbuthnott,

1970; Schwarting & Huston, 1996; Nass et al., 2002; Parish et al., 2007). In rodents, the nigro-striatal dopaminergic system is acutely and selectively degenerated by 6-OHDA-microinjection into the substantia nigra, and never recovers the missing neurons (Ungerstedt & Arbuthnott, 1970; Schwarting & Huston, 1996). We succeeded in selective degeneration of dopaminergic neurons in planarians, like that in higher animals. Interestingly, we found that planarians can regenerate only the dopaminergic neurons within 14 days after 6-OHDA-incuded selective dopaminergic neural degeneration (Fig. 4A). Although it has been reported that dopaminergic neurons are also regenerated during the head regeneration process after decapitation (Nishimura et al., 2007a; Takeda et al., 2009), our findings with 6-OHDA are the first showing that planarians are able to regenerate dopaminergic neurons after the selective degeneration of only dopaminergic neurons in the brains of non-amputated animals (Nishimura et al., 2011). According to our observations, dopaminergic neurons were completely degenerated and this degeneration was accompanied by reductions of DA content and locomotion activity within 24 hours after

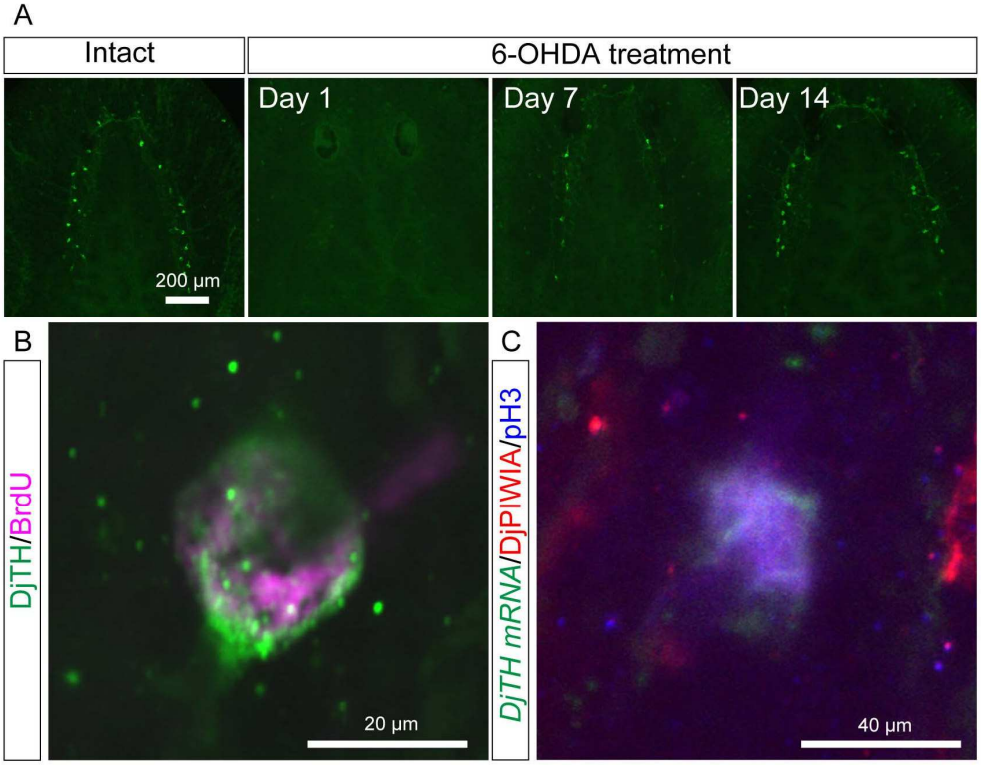


Fig. 4. Process of dopaminergic neurogenesis in the brain after 6-OHDA-induced-lesioning. Immunostaining of brain dopaminergic neurons in intact planarian and 1 day, 7 days, and 14 days after 6-OHDA-administration (A). BrdU-signal can be detected in newly generated dopaminergic neurons 10 days after 6-OHDA-administration (B). Newly generated dopaminergic neurons are produced from stem cells via cell division (C).

6-OHDA-administration. Then, newly generated dopaminergic neurons began to be detected in the brain 4 days after the 6-OHDA-induced lesion. Thereafter, the numbers and axons of dopaminergic neurons gradually recovered over a period of several days. Finally, dopaminergic neurons were completely recovered within 14 days after the 6-OHDA-induced lesion. We confirmed that in this process (1) X-ray-irradiated planarians never regenerate dopaminergic neurons after the 6-OHDA-induced lesion, (2) newly generated dopaminergic neurons are derived from pluripotent stem cells, as demonstrated by long-term trace experiments using BrdU. The dopaminergic neurogenesis after selective degeneration can be divided into three steps: (i) selective dopaminergic neurodegeneration (~24 hr after 6-OHDA-induced lesion), (ii) a transition period (24~72 hr), (iii) dopaminergic neurogenesis and dopaminergic neural network regeneration (96 hr~).

### 5.1 Recruitment of new dopaminergic neurons from pluripotent stem cells

Long-term chase experiments after BrdU-labeling clearly demonstrated that newly generated dopaminergic neurons are derived from proliferative stem cells. However, a BrdU-pulse chase analysis revealed that BrdU-incorporating cells were detected only in the trunk region but not around the brain region at all. In addition, immunohistochemical analysis using anti-proliferating cell nuclear antigen (PCNA) antibody revealed that PCNA-positive cells were never observed around the brain region (Orii et al., 2005). These results support the notion that essentially no proliferating stem cells that enter S-phase exist around the brain region. Thus, BrdU-positive cells detected in the brain by long term-chase experiments may migrate from the trunk region after proliferation (Newmark & Sánchez Alvarado 1999) (Fig. 4B). Therefore, we carefully investigated when proliferating stem cells are committed to differentiate into dopaminergic neurons during regeneration. Finally, we found that G2 phase stem cells are committed around the brain area to differentiate into dopaminergic neurons after lesioning. The most critical result was obtained by triple staining experiments immunostaining with anti-DjPIWIA antibody and anti-pH3 antibody and *in situ* hybridization using *a planarian tyrosine hydroxylase homologue (DjTH)* riboprobe. We detected *DjTH* mRNA/DjPIWIA protein/pH3-triple positive cells around the brain (Fig. 4C), suggesting that G2 phase stem cells may be accumulated in the head region and that these cells may participate in both regeneration and homeostatic events of the brain. It has already been suggested that the pluripotent stem cells may be committed at G2 phase into appropriate cell types (Hayashi et al., 2010), consistent with dividing stem cells immediately starting to differentiate to dopaminergic neurons. Based on these observations, we speculate that after proliferating in the trunk region, stem cells may migrate into the head region at G2 phase and then some of them might become committed to producing dopaminergic neurons (Nishimura et al., 2011).

### 5.2 System for recognition of the ablation of dopaminergic neurons

In planarians, it is known that older differentiated cells are constantly eliminated by apoptosis, and are then replaced by new cells by proliferation of stem cells under physiological conditions in planarians (Inoue et al., 2007; Pellettieri & Sánchez Alvarado, 2007). In our observation, a few BrdU-positive dopaminergic neurons were detected in vehicle-control-injected planarians, indicating that dopaminergic neurons could be replaced by stem cell proliferation in physiological conditions via homeostasis. Importantly, 6-

OHDA-induced lesioning accelerated the number and rate of the brain dopaminergic neurogenesis compared to that under physiological conditions in planarians. These results suggest that the number of dopaminergic neurons might be monitored by their surrounding environment. In the case of newts, a lower vertebrate, neurogenic potential for the repair of lost dopaminergic neurons is maintained even in adults (Parish et al., 2007), and this potential may work under conditions of injury-responsive cell-replacement that are induced by dopaminergic signals mediated by the DA receptor, but not under homeostatic conditions (Berg et al., 2010, 2011). In contrast, rodents have neural stem cells in restrict regions. It is known that the activity (proliferation and migration) of endogenous neural stem cells is enhanced in response to acute brain lesions caused by insults such as stroke and neurotoxin-exposure in the adult state (Arvidsson et al., 2002, Höglinger et al., 2004), suggesting that neural stem cells present in the adult brain can be responsive to alterations of the surrounding environment. In the future, it will be possible to identify the cellular and molecular systems that contribute to the recognition of dopaminergic ablation and the recruitment of new dopaminergic neurons, and it will become possible to use RNAi-mediated gene-knockdown and pharmacological drugs to further clarify the regulatory system of dopaminergic neurogenesis/regeneration.

## 6. Characterization of stem cell participation in brain regeneration

In both types of regeneration processes (*i.e.*, dopaminergic neurogenesis during brain regeneration and after selective degeneration of dopaminergic neurons), we have never observed the neural stem cell-like cells in planarians. Although commitment occurs at G2 phase, one committed stem cell produces only two differentiated cells. Committed stem cells can never enter into S phase after mitosis. Thus, we speculate that planarians have not yet invented a neural stem cell system. Histological analysis during regeneration supported the notion that pluripotent stem cells may directly give rise to fully differentiated neurons. First, we never observed proliferating cells in the brain rudiment during brain regeneration or in the intact brain. Second, the expression of the planarian *musashi* family genes supports the above hypothesis. *Musashi*, an RNA binding protein, is expressed in neural stem cells and/or progenitor cells in various animals (Okano et al., 2002). We isolated three *musashi*-like genes (*DjmlgA*, *DjmlgB* and *DjmlgC*) from planarians (Higuchi et al., 2008). Although they were expressed in the planarian CNS, their expression was not eliminated by X-ray irradiation, indicating that these genes were expressed after cells entered the differentiated state, not in the proliferative stem cells. Based on these observations, we hypothesized that the neural stem cell system probably evolved at a later stage of evolution independently in higher animals such as insects and vertebrates (Agata *et al.* 2006).

In the case of brain regeneration after decapitation, the brain rudiment is formed inside of the anterior blastema. The cells participating into blastema formation have already existed the proliferative state (Tasaki et al., 2011a, 2011b). A part of these cells then start to form the brain rudiment. Thus, commitment of dopaminergic neurons may occur after pattern formation of the brain. And then the neurons forming the primary brain might start to recruit G2 phase stem cells into brain neurons during enlargement of the brain and homeostasis (Takeda et al., 2009). In the case of dopaminergic neurogenesis after 6-OHDA-induced lesioning, G2 phase stem cells located around the brain may be recruited into dopaminergic neurons. The remaining neurons in the brain after 6-OHDA-induced

lesioning may have an important role for sensing loss of dopaminergic neurons and recruiting G2 phase stem cells into dopaminergic neurons. Planarians thus have two different ways to regenerate dopaminergic neurons, although pluripotent stem cells become the source of regeneration in both cases. The latter case may provide a unique system for considering how to recruit dopaminergic neuron-committed cells into the lesioned regions (Nishimura et al., 2011). One of the important findings is that commitment occurs at the G2 phase of stem cells. We should consider to what extent committed cells can be incorporated into the lesioned regions, and whether the location of commitment is an important factor for future incorporation of committed stem cells into appropriate positions. As our future work, we will make an attempt to answer several important questions. "How do the remaining cells recognize the loss of dopaminergic neurons?" "How are surrounding stem cells recruited into dopaminergic neurons?" "What kind of signaling pathway(s) are activated in the G2 phase stem cells to differentiate dopaminergic neurons" "How do the committed cells find the pathways to the lesion points?" Answers to the above questions may provide hints about how to realize cell-transplantation therapy in the future.

## 7. Conclusion

It is difficult to analyze whether dopaminergic neurogenesis/neuroregeneration occurs in the adult mammalian midbrain (Zhao et al., 2003; Frielingsdorf et al., 2004), although it has been demonstrated that neurogenesis occurs in the restricted regions of the adult mammalian brain (Doetsch et al., 1997; Eriksson et al., 1998). However, it is still controversial whether dopaminergic neurogenesis/neuroregeneration potential is "lost" or "quiescent" in the adult mammalian midbrain. In any case, the potential for dopaminergic neurogenesis/neuroregeneration is not sufficient to recover the missing dopaminergic neurons in mammals. Our findings in planarians provide unique opportunities to consider how pluripotent stem cells respond to their surrounding environment, and how new dopaminergic neurons are recruited after the degeneration of dopaminergic neurons.

Cell-transplantation therapy is one possible way to compensate the missing dopaminergic neurons in Parkinson's disease patients. One of the important issues for cell-transplantation therapy is what state of dopaminergic neural precursor cells can be accepted into the host brain environment. For clinical application, non-regulated proliferative ability of donor cells may cause abnormal conditions such as tumor formation after grafting, and therefore, proliferative cells, including undifferentiated cells, should be eliminated as donor cells (Fukuda et al., 2006). Another approach would be to block proliferative ability artificially before grafting. Recently, it was demonstrated that N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT)-mediated Notch inhibition delays G1/S-phase transition of human ESC-derived neural stem cells, and promotes the onset of neuronal differentiation. However, the outcome of striatal transplantation of DAPT-treated neural stem cells was not different from that of non-DAPT-treated neural stem cells at a late period after grafting (Borghese et al., 2010). Consequently, inhibition of the G1/S-phase transition of donor cells to block proliferation may not enhance the efficiency of transplantation. Our findings from planarian studies suggest that G2-phase stem cells may be in a suitable cell state for harmonization with the host brain environment. Planarians are suitable model animals for analyzing the system that recognizes the ablation of dopaminergic signals and the system for recruitment of new dopaminergic neurons. Thus, our findings give useful

suggestions about which state and type(s) of cells would be suitable for cell-replacement therapy with integration into the host brain environment using ESCs and/or iPSC-derived neural precursor cells to treat diseases such as Parkinson's disease.

## 8. Acknowledgments

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## 9. References

Agata, K.; Soejima, Y.; Kato, K.; Kobayashi, C.; Umesono, Y. & Watanabe K. (1998) Structure of the planarian central nervous system (CNS) revealed by neuronal cell markers. *Zoological Science,* Vol.15, No.3, (Jun 1998), pp. 433-440, ISSN 0289-0003

Agata, K.; Nakajima, E.; Funayama, N; Shibata, N; Saito, Y. & Umesono Y. (2006) Two different evolutionary origins of stem cell systems and their molecular basis. *Seminars in Cell & Developmental Biology*, Vol.17, No.4, (August 2006), pp. 503-509, ISSN 1084-9521

Agata, K. & Umesono, Y. (2008) Brain regeneration from pluripotent stem cells in planarian. *Philosophical Transactions The Royal Society B Biological Sciences,* Vol.363, No.1500, (Jun 2008), pp. 2071-2078, ISSN 1471-2970

Arvidsson, A.; Collin, T.; Kirik, D.; Kokaia, Z. & Lindvall, O. (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Medicine*, Vol.8, No.9, (September 2002), pp. 963-970, ISSN 1078-8956

Borghese, L.; Dolezalova, D.; Opitz, T.; Haupt, S.; Leinhaas, A.; Steinfarz, B.; Koch, P.; Edenhofer, F.; Hampl, A. & Brüstle, O. (2010) Inhibition of notch signaling in human embryonic stem cell-derived neural stem cells delays G1/S phase transition and accelerates neuronal differentiation *in vitro* and *in vivo*. *Stem Cells*, Vol.28, No.5, (May 2010), pp. 955-964, ISSN 1066-5099

Berg, D.A.; Kirkham, M.; Beljajeva, A.; Knapp, D.; Habermann, B.; Ryge, J.; Tanaka, E.M. & Simon, A. (2010) Efficient regeneration by activation of neurogenesis in homeostatically quiescent regions of the adult vertebrate brain. *Development,* Vol.137, No.24, (December 2010), pp. 4127-4134, ISSN 0950-1991

Berg, D.A.; Kirkham, M.; Wang, H.; Frisén, J. & Simon, A. (2011) Dopamine controls neurogenesis in the adult salamander midbrain in homeostasis and during regeneration of dopamine neurons. *Cell Stem Cell,* Vol.8, No.4, (April 2011), pp. 426-433, ISSN 1934-5909

Björklund, L.M.; Sánchez-Pernaute, R.; Chung, S.; Andersson, T.; Chen, I.Y.; McNaught, K.S.; Brownell, A.L.; Jenkins, B.G.; Wahlestedt, C.; Kim, K.S. & Isacson, O. (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *National Academy of Sciences of the United States of America,* Vol.99, No.4, (February 2002), pp. 2344-2349. ISSN 0027-8424

Brose, K.; Bland, K.S.; Wang, K.H.; Arnott, D.; Henzel, W.; Goodman, C.S.; Tessier-Lavigne, M. & Kidd, T. (1999) Slit proteins bind Robo receptors and have an evolutionarily

conserved role in repulsive axon guidance. *Cell*, Vol.96, No.6, (March 1999), pp. 795-806, ISSN 0092-8674

Chambers, S.M.; Fasano, C.A.; Papapetrou, E.P.; Tomishima, M.; Sadelain, M. & Studer, L. (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nature Biotechnology*, Vol.27, No.3, (May 2009), pp. 275-280, ISSN 1087-0156

Cebrià, F.; Kudome, T.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T. & Agata, K. (2002a) The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system. *Mechanisms of Development*, Vol.116, No.1-2, (August 2002), pp. 199-204, ISSN 0925-4773

Cebrià, F.; Kobayashi, C.; Umesono, Y.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T.; Itoh, M.; Taira, M.; Sánchez Alvarado, A. & Agata, K. (2002b) FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians. *Nature,* Vol.419, No.6907, (October 2002), pp. 620-624, ISSN 0028-0836

Cebrià, F.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T. & Agata, K. (2002c) Dissecting planarian central nervous system regeneration by the expression of neural-specific genes. *Development Growth & Differentiation*, Vol.44, No.2, (April 2002), pp. 135-146, ISSN 0012-1592

Cebrià, F. & Newmark, P.A. (2005) 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*, Vol.132, No.16, (August 2005), pp. 3691-3703, ISSN 0950-1991

Cebrià, F, Guo, T, Jopek, J. & Newmark, P.A. (2007) Regeneration and maintenance of the planarian midline is regulated by a *slit* orthologue. *Developmental Biology,* Vol.307, No.2, (July 2007), pp. 394-406, ISSN 0012-1606

Cebrià, F. & Newmark, P.A. (2007) Morphogenesis defects are associated with abnormal nervous system regeneration following *roboA* RNAi in planarians. *Development*, Vol.134, No.5, (March 2007), pp. 833-837, ISSN 0950-1991

Cebrià, F. (2008) Organization of the nervous system in the model planarian *Schmidtea mediterranea*: an immunocytochemical study. *Neuroscience Resersch,* Vol.61, No.4, (August 2008), pp. 375-384, ISSN 0168-0102

Couch, J.A.; Chen, J.; Rieff, H.I.; Uri, E.M. & Condron, B.G. (2004) *robo2* and *robo3* interact with eagle to regulate serotonergic neuron differentiation. *Development,* Vol.131, No.5, (March 2004) pp. 997-1006, ISSN 0950-1991

Doetsch, F.; García-Verdugo, J.M. & Alvarez-Buylla, A. (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *The Journal of Neuroscience*, Vol.17, No.13, (July 1997), pp. 5046-5061, ISSN 270-6474

Eisenhoffer, G.T.; Kang, H. & Sánchez Alvarado, A. (2008) Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian *Schmidtea mediterranea*. *Cell Stem Cell,* Vol.3, No.3, (September 2008) pp. 327-339, ISSN 1934-5909

Eriksson, P.S.; Perfilieva, E.; Björk-Eriksson, T.; Alborn, A.M.; Nordborg, C.; Peterson, D.A. & Gage, F.H. (1998) Neurogenesis in the adult human hippocampus. *Nature Medicine,* Vol.4, No.11, (November 1998), pp. 1313-1317, ISSN 1078-8956

Fukuda, H.; Takahashi, J.; Watanabe, K.; Hayashi, H.; Morizane, A.; Koyanagi, M.; Sasai, Y. & Hashimoto, N. (2006) Fluorescence-activated cell sorting-based purification of

embryonic stem cell-derived neural precursors averts tumor formation after transplantation. *Stem Cells,* Vol.24, No.3, (March 2006), pp. 763-771, ISSN 1066-5099.

Gaviño, M.A. & Reddien, P.W. (2011) A Bmp/Admp regulatory circuit controls maintenance and regeneration of dorsal-ventral polarity in planarians. *Current Biology*, Vol.21, No.4, (February 2011), pp.294-299, ISSN 0960-9822

Gurley, K.A.; Rink, J.C. & Sánchez Alvarado, A. (2008) *ß-catenin* defines head versus tail identity during planarian regeneration and homeostasis. *Science*, Vol.319, No.5861, (January 2008), pp. 323-327, ISSN 0036-8075

Hayashi, T.; Asami, M.; Higuchi, S.; Shibata, N. & Agata K. (2006) Isolation of planarian X-ray-sensitive stem cells by fluorescence-activated cell sorting. *Development Growth & Differentiation*, Vol.48, No.6, (August 2006), pp. 371-380. ISSN 0012-1592

Hayashi, T.; Shibata, N.; Okumura, R.; Kudome, T.; Nishimura, O.; Tarui, H. & Agata, K. (2010) Single-cell gene profiling of planarian stem cells using fluorescent activated cell sorting and its "index sorting" function for stem cell research. *Development Growth & Differentiation*, Vol.52, No.1, (January 2010), pp. 131-144, ISSN 0012-1592

Hendzel, M.J.; Wei, Y.; Mancini, M.A.; Van Hooser, A.; Ranalli, T.; Brinkley, B.R.; Bazett-Jones, D.P. & Allis, C.D. (1997) Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during $G_2$ and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma,* Vol.106, No.6, (August 2006), pp. 348-360, ISSN 0009-5915

Heikkila, R.E. & Nicklas, W.J. (1971) Inhibition of biogenic amine uptake by hydrogen peroxide: mechanism for toxic effects of 6-hydroxydopamine. *Science,* Vol.172, No.989, (Jun 1971), pp. 1257-1258, ISSN 0036-8075

Higuchi, S.; Hayashi, T.; Hori, I.; Shibata, N.; Sakamoto, H. & Agata K. (2007) Characterization and categorization of fluorescence activated cell sorted planarian stem cells by ultrastructural analysis. *Development Growth & Differentiation*, Vol.49, No.7, (September 2007), pp. 571-581, ISSN 0012-1592

Higuchi, S.; Hayashi, T.; Tarui, H.; Nishimura, O.; Nishimura, K.; Shibata, N.; Sakamoto, H. & Agata, K. (2008) Expression and functional analysis of *musashi*-like genes in planarian CNS regeneration. *Mechanisms of Development,* Vol.125, No.7, (July 2008), pp. 631-645, ISSN 0925-4773

Höglinger, G.U.; Rizk, P.; Muriel, M.P.; Duyckaerts, C.; Oertel, W.H.; Caille, I. & Hirsch, E.C. (2004) Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nature Neuroscience*, Vol.7, No.7, (July 2004), pp. 726-735, ISSN 1097-6256

Hong, K.; Hinck, L.; Nishiyama, M.; Poo, M.M.; Tessier-Lavigne, M. & Stein, E. (1999) A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion. *Cell*, Vol.97, No.7, (Jun 1999), pp. 927-941, ISSN 0092-8674

Inoue, T.; Kumamoto, H.; Okamoto, K.; Umesono, Y.; Sakai, M., Sánchez Alvarado, A. & Agata, K. (2004) Morphological and functional recovery of the planarian photosensing system during head regeneration. *Zoological Science,* Vol.21, No.3, (March 2004), pp. 275-283, ISSN 0289-0003

Inoue, T.; Hayashi, T.; Takechi, K. & Agata, K. (2007) Clathrin-mediated endocytic signals are required for the regeneration of, as well as homeostasis in, the planarian CNS. *Development,* Vol.134, No.9, (May 2007), pp. 1679-1689, ISSN 0950-1991

Kato, K.; Orii, H.; Watanabe, K. & Agata, K. (1999) The role of dorsoventral interaction in the onset of planarian regeneration. *Development,* Vol.126, No.5, (February 1999), pp. 1031-1040, ISSN 0950-1991

Kawasaki, H.; Mizuseki, K.; Nishikawa, S.; Kaneko, S.; Kuwana, Y.; Nakanishi, S.; Nishikawa, S.I. & Sasai, Y. (2000) Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron,* Vol.28, No.1, (October, 2000), pp. 31-40, ISSN 0896-6273

Kobayashi, C.; Saito, Y.; Ogawa K. & Agata K. (2007) *Wnt* signaling is required for antero-posterior patterning of the planarian brain. *Developmental Biology*, Vol.306, No.2, (Jun 2007), pp. 714-724, ISSN 0012-1606

Mineta, K.; Nakazawa, M.; Cebrià, F.; Ikeo, K.; Agata, K. & Gojobori, T. (2003) Origin and evolutionary process of the CNS elucidated by comparative genomics analysis of planarian ESTs. *National Academy of Sciences of the United States of America,* Vol.100, No.13, (Jun 2003), pp. 7666-7671, ISSN 0027-8424

Molina, M.D.; Neto, A.; Maeso, I.; Gómez-Skarmeta, J.L.; Saló, E. & Cebrià, F. (2011) Noggin and noggin-like genes control dorsoventral axis regeneration in planarians. *Current Biology,* Vol.21, No.4, (February 2011), pp. 300-305, ISSN 0960-9822

Morgan, T.H. (1989) Experimental Studies of the regeneration of *Planaria maculata*. *Arch Entwicklungsmech Org*., Vol.7, pp. 364-397.

Morizane, A.; Doi, D.; Kikuchi, T.; Nishimura, K. & Takahashi, J. (2011) Small-molecule inhibitors of bone morphogenic protein and activin/nodal signals promote highly efficient neural induction from human pluripotent stem cells. *Journal of Neuroscience Research,* Vol.89, No.2, (February 2011), pp. 117-126, ISSN 0360-4012

Nakazawa, M.; Cebrià, F.; Mineta, K.; Ikeo, K.; Agata, K. & Gojobori T. (2003) Search for the evolutionary origin of a brain: planarian brain characterized by microarray. *Molecular Biology & Evolution,* Vol.20, No.5, (May 2003), pp. 784-791, ISSN 0737-4038

Nass, R.; Hall, D.H.; Miller, D.M. 3rd; & Blakely, R.D. (2002) Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans*. *National Academy of Sciences of the United States of America,* Vol.99, No.5, (March 2002), pp. 3264-3269, ISSN 0027-8424

Newmark, P.A. & Sánchez Alvarado, A. (2000) Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. *Developmental Biology,* Vol.220, No.2, (April 2000), pp. 142-153, ISSN 0012-1606

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Sano, S.; Yoshimoto, K.; Inden, M.; Takata, K.; Taniguchi, T.; Shimohama, S. & Agata, K. (2007a) Reconstruction of dopaminergic neural network and locomotion function in planarian regenerates. *Developmental Neurobiology,* Vol.67, No.8, (July 2007), pp. 1059-1078, ISSN 1932-8451

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Yoshimoto, K.; Takeuchi, K.; Taniguchi, T. & Agata, K. (2007b) Identification and distribution of tryptophan hydroxylase (TPH)-positive neurons in the planarian *Dugesia japonica*. *Neuroscience Research,* Vol.59, No.1, (September 2007), pp. 101-106, ISSN 0168-0102

Nishimura, K.; Kitamura, Y.; Umesono, Y.; Takeuchi, K.; Takata, K.; Taniguchi, T. & Agata, K. (2008a) Identification of glutamic acid decarboxylase gene and distribution of GABAergic nervous system in the planarian, *Dugesia japonica*. *Neuroscience,* Vol.153, No.4, (Jun, 2008), pp. 1103-1114, ISSN 0306-4522

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Taniguchi, T. & Agata, K. (2008b) Characterization of tyramine ß-hydroxylase in planarian *Dugesia japonica*: Cloning and expression. *Neurochemistry International,* Vol.53, No.6-8, (December 2008), pp. 184-192, ISSN 0197-0186

Nishimura, K.; Yamamoto, H.; Kitamura, Y.; & Agata, K. (2008c) Brain and Neural Network, In: *Planaria: A Model for Drug Action and Abuse*, (Raffa, R.B. & Rawls, S.M., (Ed.), 4-12 Landes Bioscience, ISBN: 978-1-58706-332-9, Texas, USA

Nishimura, K.; Kitamura, Y.; Taniguchi, T. & Agata, K. (2010) Analysis of motor function modulated by cholinergic neurons in planarian *Dugesia japonica. Neuroscience,* Vol.168, No.1, (Jun 2010), pp. 18-30, ISSN 0306-4522

Nishimura, K.; Inoue, T.; Yoshimoto, K.; Taniguchi, T.; Kitamura, Y. & Agata K. Regeneration of dopaminergic neurons after 6-hydroxydopamine-induced lesion in planarian brain. *Journal of Neurochemistry*, Vol.119, No.6, (December 2011), pp. 1219-1231 ISSN 0022-3042

Ogawa, K.; Ishihara, S.; Saito, Y.; Mineta, K.; Nakazawa, M.; Ikeo, K.; Gojobori, T.; Watanabe, K. & Agata K. (2002) Induction of a *noggin*-like gene by ectopic DV interaction during planarian regeneration. *Developmental Biology*, *Vol.*250, No.1, (October 2002), pp. 59-70, ISSN 0012-1606

Okamoto, K.; Takeuchi, K. & Agata, K. (2005) Neural projections in planarian brain revealed by fluorescent dye tracing. *Zoological S*cience, Vol.22, No.5, (May 2005), pp. 535-546. ISSN 0289-0003

Okano, H.; Imai, T. & Okabe, M. (2002) *Musashi*: a translational regulator of cell fate. *Journal of Cell Science,* 2002 Apr Vol.115, No.Pt 7, (April 2002), pp. 1355-1359, ISSN 0021-9533

Orii, H., Sakurai, T. & Watanabe, K. (2005) Distribution of the stem cells (neoblasts) in the planarian *Dugesia japonica*. *Development Genes & Evolution,* Vol.215, No.3, (March 2005), pp. 143-157. ISSN 0949-944X

Parish, C.L.; Beljajeva, A.; Arenas, E. and Simon, A. (2007) Midbrain dopaminergic neurogenesis and behavioural recovery in a salamander lesion-induced regeneration model. *Development,* Vol.134, No.15, (August 2007), pp. 2881-2887, ISSN 0950-1991

Pellettieri, J. & Sánchez Alvarado, A. (2007) Cell turnover and adult tissue homeostasis: from humans to planarians. *Annual Review of Genetics*, Vol.41, pp. 83-105, ISSN 0066-4197

Perrier, A.L.; Tabar, V.; Barberi, T.; Rubio, M.E.; Bruses, J.; Topf, N.; Harrison, N.L. & Studer, L. (2004) Derivation of midbrain dopamine neurons from human embryonic stem cells. *National Academy of Sciences of the United States of America,* Vol.101, No.34, (August 2004), pp. 12543-12548, ISSN 0027-8424

Petersen, C.P. & Reddien, P.W. (2008) Smed-*ßcatenin-1* is required for anteroposterior blastema polarity in planarian regeneration. *Science*, Vol.319, No.5861, (January 2008), pp. 327-330, ISSN 0036-8075

Petersen, C.P. & Reddien, P.W. (2009) Wnt signaling and the polarity of the primary body axis. *Cell,* Vol.139, No.6, (December 2009), pp. 1056-1068, ISSN 0092-8674

Reddien, P.W. & Sánchez Alvarado, A. (2004) Fundamentals of planarian regeneration. *Annual Review of Cell Developmental Biology,* Vol.20, pp. 725-757, ISSN 1081-0706

Reddien, P.W.; Oviedo, N.J.; Jenning, J.R.; Jenkin, J.C. & Sánchez Alvarado, A. (2005) SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. *Science,* Vol.310, No.5752, (November 2005), pp. 1327-1330, ISSN 0036-8075

Reddien, P.W. (2011) Constitutive gene expression and the specification of tissue identity in adult planarian biology. *Trends in Genetics,* Vol.27, No.7, (July 2011), pp. 277-285, ISSN 0168-9525

Rink, J.C.; Gurley, K.A.; Elliott, S.A. & Sánchez Alvarado, A. (2009) Planarian Hh signaling regulates regeneration polarity and links Hh pathway evolution to cilia. *Science*, Vol.326, No.5958, (December 2009), pp. 1406-1410, ISSN 0036-8075

Sachs, C. & Jonsson, G. (1975) Mechanisms of action of 6-hydroxydopamine. *Biochemical Pharmacology,* Vo.24, No.1, (January 1975), pp. 1-8, ISSN 0006-2952

Saito, Y.; Koinuma, S.; Watanabe, K. & Agata, K. (2003) Mediolateral intercalation in planarians revealed by grafting experiments. *Developmental Dynamics,* Vol.226, No.2, (February 2003), pp. 334-340, ISSN 1058-8388

Salvetti, A.; Rossi, L.; Deri, P. & Batistoni, R. (2000) An MCM2-related gene is expressed in proliferating cells of intact and regenerating planarians. *Developmental Dynamics*, Vol.218, No.4, (August 2000), pp. 603-614, ISSN 1058-8388

Salvetti, A.; Rossi, L.; Lena, A.; Batistoni, R.; Deri, P.; Rainaldi, G.; Locci.; M.T.; Evangelista, M. & Gremigni, V. (2005) *DjPum*, a homologue of *Drosophila Pumilio*, is essential to planarian stem cell maintenance. *Development*, Vol.132, No.8, (April 2005), pp. 1863-1874, ISSN 0950-1991

Sakai, F.; Agata, K.; Orii, H. & Watanabe, K. (2000) Organization and regeneration ability of spontaneous supernumerary eyes in planarians -eye regeneration field and pathway selection by optic nerves-. *Zoological Science,* Vol.17, No.3, (April 2000), pp. 375-81, ISSN 0289-0003

Sanchez-Pernaute, R.; Lee, H.; Patterson, M.; Reske-Nielsen, C.; Yoshizaki, T.; Sonntag, K.C.; Studer, L. & Isacson, O. (2008) Parthenogenetic dopamine neurons from primate embryonic stem cells restore function in experimental Parkinson's disease. *Brain*, Vol.131, No. Pt.8, (August 2008), pp. 2127-2139, ISSN 0006-8950

Schwarting, R.K. & Huston, J.P. (1996) The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. *Progress in Neurobiolgy,* Vol.50, No.2-3, (October 1996), pp. 275-331, ISSN 0301-0082

Shibata, N.; Umesono, Y.; Orii, H.; Sakurai, T.; Watanabe, K. & Agata, K. (1999) Expression of *vasa(vas)*-related genes in germline cells and totipotent somatic stem cells of planarians. *Developmental Biology,* Vol.206, No.1, (February 1999), pp. 73-87, ISSN 0012-1606

Shibata, N.; Rouhana, L. & Agata K. (2010) Cellular and molecular dissection of pluripotent adult somatic stem cells in planarians. *Development Growth & Differentiation,* Vol.52, No.1, (January 2010), pp. 27-41, ISSN 0012-1592

Tasaki, J.; Shibata, N.; Nishimura, O.; Itomi, K.; Tabata, Y.; Son, F.; Suzuki, N.; Araki, R.; Abe, M.; Agata, K. & Umesono, Y. (2011a) ERK signaling controls blastemacell differentiation during planarian regeneration. *Development*, Vol.138, No. 12, (Jun 2011), pp. 2417-2427, ISSN 0950-1991

Tasaki., J.; Shibata, N.; Sakurai, T.; Agata, K. & Umesono, Y. (2011b) Role of c-JunN-terminal kinase activity in blastema formation during planarian regeneration. *Development Growth & Differentiation,* Vol.53, No.3, (April 2011), 389-400, ISSN 0012-1592

Takagi, Y.; Takahashi, J.; Saiki, H.; Morizane, A.; Hayashi, T.; Kishi, Y.; Fukuda, H.; Okamoto, Y.; Koyanagi, M.; Ideguchi, M.; Hayashi, H.; Imazato, T.; Kawasaki, H.; Suemori, H.; Omachi, S.; Iida, H.; Itoh, N.; Nakatsuji, N.; Sasai, Y. & Hashimoto, N. (2005) Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. *The Journal of Clinical Investigation,* Vol.115, No.1, (January 2005), pp. 102-109. ISSN 0021-9738

Takano, T.; Pulvers, J.N.; Inoue, T.; Tarui, H.; Sakamoto, H.; Agata, K. & Umesono, Y. (2007) Regeneration-dependent conditional gene knockdown (Readyknock) in planarian: demonstration of requirement for Djsnap-25 expression in the brain for negative phototactic behavior. *Development Growth & Differentiation,* Vol.49, No.5, (Jun 2007), pp. 383-394, ISSN 0012-1592

Takeda, H.; Nishimura, K. & Agata K. (Jun 2008) Planaria nervous system, In: *Scholarpedia*, 3, 5558, 2008, Available from http://www.scholarpedia.org/article/Planaria_nervous_system

Takeda, H.; Nishimura, K. & Agata K. (2009) Planarians maintain a constant ratio of different cell types during changes in body size by using the stem cell system. *Zoological Science,* Vol.26, No.12, (December 2009), pp. 805-813, ISSN 0289-0003

Tazaki, A.; Gaudieri, S.; Ikeo, K.; Gojobori, T.; Watanabe, K. & Agata, K. (1999) Neural network in planarian revealed by an antibody against planarian synaptotagmin homologue. *Biochemical and Biophysical Research Communications* Vol.260, No.2, (July 1999), pp. 426-432. ISSN 0006-291X

Umesono, Y.; Watanabe, K. & Agata, K. (1997) A planarian *orthopedia* homolog is specifically expressed in the branch region of both the mature and regenerating brain. *Development Growth & Differentiation,* Vol.39, No.6, (December 1997), pp. 723-727, ISSN 0012-1592

Umesono, Y.; Watanabe, K. & Agata, K. (1999) Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Development Genes & Evolution,* Vol.209, No.1, (January 1999), pp. 31-39, ISSN 0949-944X

Umesono, Y.; & Agata, K. (2009) Evolution and regeneration of the planarian central nervous system. *Development Growth & Differentiation,* Vol.51, No.3, (April 2009), pp. 185-195, ISSN 0012-1592

Umesono, Y.; Tasaki, J.; Nishimura, K.; Inoue, T. & Agata, K. Regeneration in an evolutionarily primitive brain: the planarian *Dugesia japonica* model. *European Journal of Neuroscience*.,Vol.34, No.6, (September 2011), pp. 863-839. ISSN 0953-816X

Ungerstedt, U. & Arbuthnott, G.W. (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Research,* Vol.24, No.3, (Decmber 1970), pp. 485-493, ISSN 0006-8993

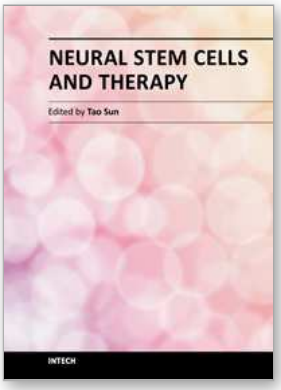
Yamamoto, H. & Agata, K. (2011) Optic chiasm formation in planarian I: Cooperative *netrin*- and *robo*-mediated signals are required for the early stage of optic chiasm formation. *Development Growth & Differentiation,* Vol.53, No.3, (April 2011), pp. 300-311, ISSN 0012-1592

Yazawa, S.; Umesono, Y.; Hayashi, T.; Tarui, H. & Agata, K. (2009) Planarian Hedgehog/Patched establishes anterior-posterior polarity by regulating Wnt signaling. *National Academy of Sciences of the United States of America,* Vol.106, No.52, (December 2009), pp. 22329-22334, ISSN 0027-8424

Wagner, D.E.; Wang, I.E., & Reddien, P.W. (2011) Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science*, Vol.332, No.6031, (May 2011), pp. 811-816, ISSN 0036-8075

Wenemoser, D. & Reddien, P.W. (2010) Planarian regeneration involves distinct stem cell responses to wounds and tissue absence. *Developmental Biology*, Vol.344, No.2, (August 2010), pp. 979-991, ISSN 0012-1606

Wernig, M.; Zhao, J.P.; Pruszak, J.; Hedlund, E.; Fu, D.; Soldner, F.; Broccoli, V.; Constantine-Paton, M.; Isacson, O. & Jaenisch, R. (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *National Academy of Sciences of the United States of America,* Vol.105, No.15, (April 2008), pp. 5856-5861, ISSN 0027-8424

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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most advanced discoveries in neural stem cells, and summarizes the mechanisms of neural stem cell development. In particular, this book provides strategies and discusses the challenges of utilizing neural stem cells for therapy of neurological disorders and brain and spinal cord injuries. It is suitable for general readers, students, doctors and researchers who are interested in understanding the principles of and new discoveries in neural stem cells and therapy.



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51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821