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Chapter 2

Regenerative Medicine: Lessons from Planarians

Francesc Cebrià*, Teresa Adell and Emili Saló
Departament de Genètica i Institut de Biomedicina de la Universitat de Barcelona (IBUB), Av. Diagonal 645, edifici annex planta 1, 08028 Barcelona, Catalunya, Spain

Abstract

The emergence of regenerative medicine has raised the hope of treating an extraordinary range of diseases and serious injuries. However, to achieve such important goals, fundamental questions must first be answered, such as why regeneration occurs in some animals and not others. Thus, understanding the processes of cell proliferation, differentiation and pattern formation in regenerative organisms could help find ways to enhance the poor regenerative abilities shown by many other animals, including humans. In recent years, planarians have re-emerged as an attractive model in which to study regeneration. Remarkably, these animals possess a population of totipotent somatic stem cells (neoblasts) that differentiate into any cell type after amputation. Through the application of modern molecular and genomic tools, the planarian community has started to unravel the molecular mechanisms regulating stem cell biology and pattern formation during regeneration. Here, we summarize current understanding of neoblast biology, regeneration of the central nervous system and re-establishment of the anteroposterior and dorsoventral body axes during regeneration. We also discuss the relationship between regeneration and cancer.

1. Introduction

Interest in regenerative medicine has increased dramatically in recent years. Given its promise of curing diseases and injuries that affect millions of people worldwide, it is not surprising that information reaches us through the mass media almost weekly, or that the last

* E-mailfcebrias@ub.edu

three or four years has seen the establishment of a number of new specialized scientific journals, such as Journal of Tissue Engineering and Regenerative Medicine (2007), Regenerative Medicine (2006), Journal of Stem Cells and Regenerative Medicine (2006) and Cell Stem Cells (2007).

Research is mainly focused on identifying ways to manipulate stem cells in order to use them as therapeutic agents to cure traumatic injuries and various human diseases (such as neurodegenerative disease, diabetes, and heart disease). However, much remains to be understood about the fundamental mechanisms underlying regeneration or its absence in different organisms. While most metazoan phyla include species that are able to regenerate, the efficiency with which this occurs may vary (Sánchez-Alvarado, 2000; Sánchez-Alvarado and Tsonis, 2006; Tanaka, 2003; Tsonis, 2000).

In addition, even closely related species may show very different regenerative capacities. Thus, for example, amphibians and zebrafish are able to regenerate, among others, their limbs, tail, fins and heart (reviewed in Stoick-Cooper et al., 2007), whereas other vertebrates (e.g. humans) have lost the capacity to regenerate such complex structures. Regeneration requires the missing organ or structure to be sensed, proliferation and differentiation of new cells induced to re-build those missing parts, and then specific signals and instructions provided to properly patterns the newly regenerated tissues and organs.

Finally, those de novo created tissues, organs or structures must integrate within the old tissues to restore normal functionality. While current research efforts largely focus on controlling the differentiation of stem cells, either embryonic stem cells (ES) or induced-pluripotent stem cells (iPS) in vitro, use of those in vitro-differentiated cells faces a number of significant hurdles. In order to ensure that, once implanted, the cells go where they are required, integrate within the pre-existing tissues or organs, and ultimately restore normal function; much more may need to be understood about how this occurs in systems with a high regenerative capacity.

Thus, for example, Zebrafish has become an attractive model in which to study heart regeneration (Poss et al., 2002; Raya et al., 2003) and in the near future knowledge obtained in this system may be applied to the treatment of heart disease in humans. Zebrafish are also being used to study the regeneration of the mechanosensory hair cells in the lateral line (López-Schier and Hudspeth, 2006; Behra et al., 2009), and those studies might provide some insight into why mammals have lost the capacity to regenerate their inner ear hair cells, as well guiding the development of therapeutic approaches to solve hearing loss. In those cases, jumping from Zebrafish to humans may not be so complicated or far away. In other situations, however, the distance to cover appears longer and full of obstacles. Being able to induce the complete regeneration of an amputated arm or leg in humans as normally happens in amphibian limbs, for instance, appears a very distant goal.

One of the primary materials required for regeneration is a population of undifferentiated or progenitor cells that can proliferate and differentiate into the cell type(s) required in each different context. The origin and type of those primary cells vary depending on the animal model and the kind of regenerative process required. In amphibians, after limb or tail amputation, differentiated cells go through a process of dedifferentiation, re-enter the cell cycle, proliferate, and differentiate again into the missing cell type(s).

Recent studies addressing the question of how far the dedifferentiation process goes during axolotl limb regeneration have shown that most cell types do not actually reach a multipotent undifferentiated stage but instead seem mainly to re-differentiate into the same

cell type (Kragl et al. 2009). The mechanism by which these cells remember their origin is of obvious relevance for the field of regenerative medicine. In many vertebrates, some tissues contain specialized cells (e.g. the satellite cells of the muscle) that can be induced to differentiate after damage or injury (Lagha et al., 2008; Montarras et al., 2005). In other systems, such as planarians, a population of totipotent stem cells is found in adults. After amputation or damage, those specialized cells proliferate and differentiate into the missing structures. Given that controlling the differentiation of undifferentiated stem or progenitor cells is a prerequisite for future therapies, planarians thus offer us an important opportunity to study the behavior of such totipotent stem cells *in vivo.*

Planarians became a classical model in which to study regeneration because of their great plasticity (Brøndsted, 1969; Newmark and Sánchez-Alvarado 2000). For instance, if a planarian is cut into multiple pieces, each tiny piece will regenerate a whole animal within around two weeks. In recent years, however, the power of molecular biology has begun to be harnessed in planarians to gain insights into the molecular control of these regeneration processes (Agata, 2003; Reddien and Sánchez-Alvarado, A., 2004; Saló, 2006). What makes planarians particularly special and interesting as a model for research into regenerative medicine is that they maintain a population of adult stem cells throughout their lives. These stem cells are totipotent and are the source of all the differentiated cell types required during normal homeostasis, as well as after traumatic amputation (Baguñà et al., 1989). Planarians can reproduce both sexually and asexually.

In those species with asexual reproduction, a high regenerative capability is obviously correlated with their mode of reproduction. Asexual planarians reproduce by fission (Figure 1), and that means that after reaching a certain size, or depending on culture or environmental conditions, animals attach their tails to the substrate and stretch themselves until they break into two or more pieces (depending on the species). Subsequently, each piece will regenerate the missing part through proliferation and differentiation of their stem cells.

These unique features together make planarians an excellent model in which to study stem cell biology in vivo, and offer us a paradigm for investigating not only the regulation of stem-cell proliferation and differentiation but also how these cells subsequently integrate into pre-existing tissues or become properly organized into de novo generated tissues and organs.

Figure 1. Fissioning in *Schmidtea mediterranea*. Both resulting pieces will regenerate the missing parts. Scale bar: 1 mm.

In this chapter, we will begin by reviewing current understanding of the biology of planarian stem cells. We will then discuss the regeneration of the planarian central nervous system (CNS) before considering the role of two conserved signalling pathways in the control of tissue patterning during planarian regeneration. Finally, we will discuss some data suggesting that planarians may be also a good model in which to study the relationship between regeneration and cancer.

2. Planarian Stem Cells: The Neoblasts

2.1. Planarian Anatomy and Biology

Planarians are freshwater flatworms from the phylum Platyhelminthes (Order Tricladida, Class Turbellaria) belonging to the Lophotrocozoan clade of the Protostomes. They are triploblastic acoelomates and unsegmented, although some of their organs, like the gut and the central nervous system, do display iterations (Figure 2 A, B).

They lack circulatory, respiratory, and skeletal structures, but have well-defined bilateral symmetry, as well as anteroposterior (AP) and dorsoventral (DV) axes. They also show clear cephalisation, with a pair of cephalic ganglia and two ventral longitudinal nerve cords that are interconnected by commissural neurons, and sensory organs (photoreceptors, chemoreceptors and rheoreceptors), which are mostly located in the head area (Figure 2 A).

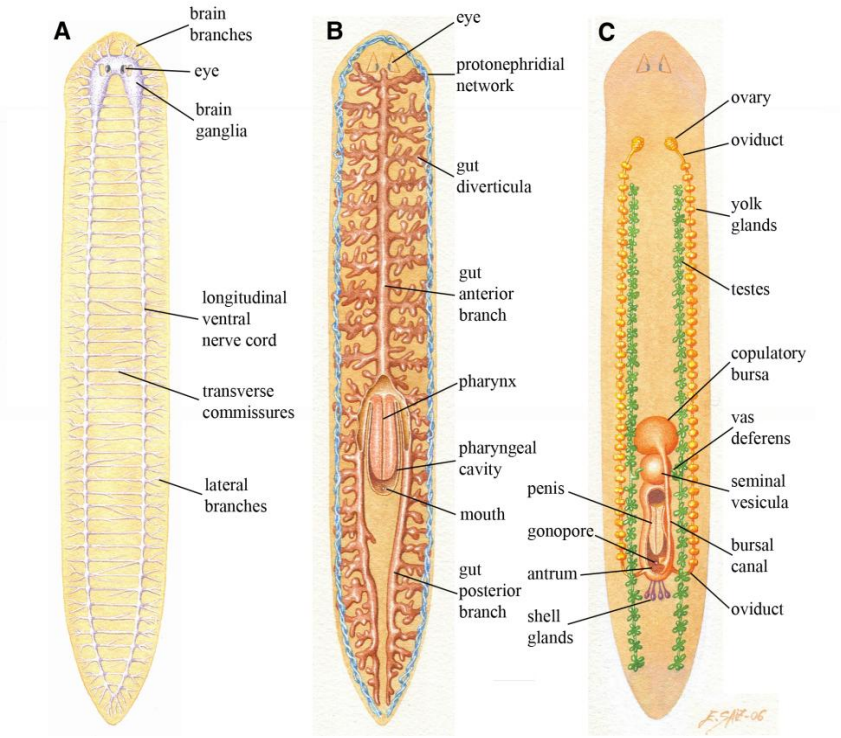

Figure 2. Diagram of the major organ systems in the freshwater planarian Schmidtea mediterranea. (A) Central nervous system with a bilobed cephalic ganglion with lateral branches and two ventral longitudinal nerve cords interconnected by transverse commissures. The eyes are located dorsal to the brain (B) The digestive system comprises a blind diverticulated gut with one anterior and two posterior branches and a single pharynx in the centre. The excretory system is a dorsal network of canals in which the flame cells reside. (C) The hermaphroditic S. mediterranea strain has a complex reproductive system with two ventrally located ovaries, two dorsolateral rows of testes and vitelline glands. The copulatory apparatus contains a copulatory bursa (where the sperm interchanged during copulation is stored), a penis and seminal vesicle (where the mature sperm is stored), and shell glands that connect to the atrium to produce the polyembrionic eggs or cocoons. Adapted from Saló, 2006.

The digestive system consists of a blind three-branched diverticulated gut with no anus and a pharynx in the central region, which evaginates through a ventral mouth opening (Figure 2 B) (Hyman, 1951; Rieger et al. 1991). The body wall musculature comprises four layers of muscle fibers (outside to inside): circular, longitudinal, diagonal and longitudinal (Cebrià et al., 1997). The excretory system is a dorsal network of canals containing specialized flame cells with continuously beating cilia (Figure 2 B). The parenchyma fills the space between the monostratified ciliated epidermis and the gut and contains diverse cell types such as epidermal gland cells, fixed parenchymal cells, which connect different cell types and tissues, pigment cells that give the planarians a brownish color, and the neoblasts (planarian stem cells).

In the anterior region, a pair of eyespots, consisting of rhabdomeric photoreceptor cells and pigment cells, can be distinguished on the dorsal side of the planarian (Figure 2 A). In the ventral epidermis, a large number of cilia facilitate movement—planarians are the largest organisms to employ ciliary locomotion. The animals also move through a combination of muscle contraction and slime secretion.

Planarians are usually hermaphroditic and most often possess the ability to reproduce both asexually and sexually. The sexual strains have a complex reproductive system with two ovaries situated anteroventrally and a large number of testes and vitelline glands located in long lateral and parallel lines in the dorsal part of the body (Figure 2 C). Polyembryonic eggs or cocoons are laid (embryogenesis is highly derived), and five to eight juveniles hatch after 10 days to several weeks, depending on the species and temperature.

2.2. Planarian Morphological Plasticity

Planarians show a high degree of plasticity in the control of morphology and cell turnover. Tissue homeostasis takes place continuously to renew all cell types, and in addition, planarians shrink by reducing their total body cell number in the event that food availability is restricted (González-Estévez et al., 2007; Baguñà and Romero, 1981).

When food becomes available again, the planarian will re-grow by increasing cell numbers (Baguñà and Romero, 1981). Growth and degrowth represents a dynamic equilibrium between cell proliferation and cell death (Bowen et al., 1976).

During growth there is more cell proliferation than cell death, leading to an increase in the total number of cells in the body. In contrast, during degrowth the equilibrium shifts towards cell death, so that the total number of cells in the planarian body will decrease and the planarian will get smaller, while remaining functional at any size (Baguñà and Romero, 1981; Baguñà, 1976) (Figure 3 A).

It has been suggested that a process of re-juvenilization occurs during the degrowth–growth process (Child, 1914; Hyman, 1951), and this possibility has been suggested for the regeneration process and for asexual reproduction (Child, 1914; Hyman, 1951), suggesting that planarians may be able to "escape" the aging process in many different ways. The lifespan of turbellarians is mostly known from laboratory cultures (Hyman, 1951), and several labs have maintained a clonal line from a single asexual *Schmidtea mediterranea* animal for years. In such lines, hundreds of fissions have already taken place, giving rise to thousands of individuals that are literally identical at a genomic level.

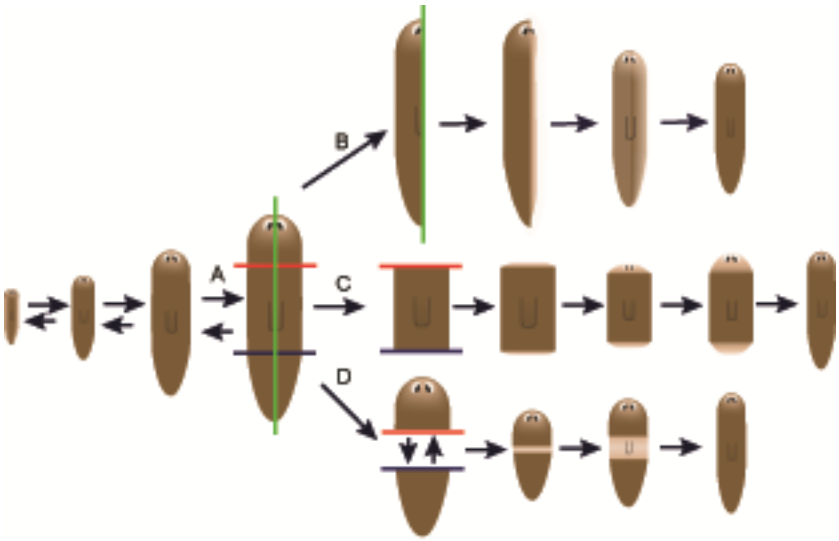

Figure 3.Planarian homeostatic and regenerative capacities. (A) Growth–degrowth. Planarians are very plastic, and upon starvation there is a reduction in the total body cell number (degrowth) caused by an increase in the rate of cell death compared with that of cell proliferation. After feeding, the planarian will grow in size and cell number will increase again (growth) through a shift in the equilibrium to favour mitosis over cell death. (B-D) Regeneration. Practically any imaginable amputation of the planarian can give rise to two individual animals. (B) A sagittal cut induces the formation of a lateral non-pigmented blastema along the length of the planarian body. The missing eye differentiates and the remaining organs are remodeled, giving rise to an entirely regenerated planarian that is smaller than the original animal. (C) Following transverse cuts, the middle fragment forms both an anterior and a posterior blastema. The head, including the eyes and the brain, is regenerated in the anterior blastema and the tail in the posterior blastema. A process of remodeling takes place in the middle fragment, adjusting the organs to the new smaller size of the planarian. (D) When two fragments with different positional values are joined together, intercalary regeneration reforms the missing tissues between the two regions and gives rise to a single planarian. Modified from Handberg-Thorsager et al, 2008.

The capacity to regenerate is especially pronounced in some triclads such as *S. mediterranea* and *Dugesia japonica*, species used extensively in planarian research. These planarians can regenerate along any body axis, and small fragments, except the pharynx and the headpiece anterior to the eyes, are able to regenerate a complete organism.

Thus, a transverse or sagittal cut can lead to the formation of two animals in two weeks by producing a new region of undifferentiated tissue, or blastema, and remodeling the old tissue to the new smaller proportions (Figure 3 B, C).

Experimental manipulation of these organisms has shown that intercalary regeneration is also possible when bringing an anterior fragment together with a posterior fragment while leaving out the middle fragment (Brøndsted, 1942) (Figure 3 D).

Grafting experiments involving inversion of the DV axis of the donor tissue with respect to the host planarian induced new organizing centers in each of the confronted pieces, leading to the generation of multiple new planarian axes (Santos, 1929; Schilt, 1970; Agata and Watanabe, 1999).

These observations indicate that DV interaction and tissue confrontation with different positional values are two distinct scenarios in which regeneration is activated.

2.3. The Cell Biology and Dynamics of Neoblasts

The synapomorphy of Platyhelminthes is the presence of a unique totipotent and proliferative stem cell population in adult flatworms (reviewed in Handberg-Thorsager et al., 2008). This unique characteristic underlies the tremendous plasticity and regenerative capacity of the phylum. Neoblasts are small cells of 6-10 microns diameter with a high nucleus-to-cytoplasm ratio and, depending on the adult size, they account for 20-30% of the total cell number. This percentage corresponds to the true stem cells and the postmitotic cells that are already committed but retain neoblast-like features. The cytoplasm is strongly basophilic, rich in free ribosomes and with few mitochondria, and contains chromatoid bodies close to the nucleus (reviewed in Coward, 1974 and Gremigni, 1988). The chromatoid bodies are RNA processing centers that decrease in number during differentiation until they disappear (Coward, 1974; Higuchi et al., 2007). Theyare also present in the planarian germ cells, which, together with the neoblasts, are the only mitotic cells described in the planarian (Sato et al., 2006). The neoblasts are totipotent cells that differentiate to form all cell types, including the germ line (Baguña et al., 1989). At least 20 types of differentiated planarian cells have been described (Baguñà and Romero, 1981), although the differentiation pathway of the neoblasts remains an open question, and their cell lineages have not been fully established. Cytological studies divide the neoblasts into subpopulations based on ultrastructural features and cell cycle distribution. By looking at electron microscopy images of neoblasts, Higuchi and co-workers (2007) defined type A and type B neoblasts based on cell size, chromatin structure and number of chromatoid bodies. Type A neoblasts are larger (9.6 ± 2.9 µm) with more chromatoid bodies (4.4 ± 2.1) than type B neoblasts (size, 6.2 ± 1.4 µm; number of chromatoid bodies, 2.1 ± 0.9); type A neoblasts are also rich in euchromatin, whereas type B neoblasts are rich in heterochromatin. The nucleus-to-cell ratio of type A neoblasts is lower than that of type B. Fluorescence-activated cell sorting (FACS) has shown that type A cells belong to irradiation-sensitive dividing cells, whereas type B cells are irradiation-sensitive non-dividing cells (Hayashi et al., 2006; Higuchi et al., 2007). The same group also described a population of differentiating cells with chromatoid bodies and few organelles that continued to divide, suggesting that committed or progenitor cells may maintain proliferative ability (Higuchi et al., 2007). Feeding and regeneration are two physiological processes that increase the rate of neoblast division. Two peaks have been described in the time course of mitosis after feeding or amputation of the planarian, with the first maximum at 4-12 hours and a second higher maximum occurring after 2-4 days at 17ºC (Baguñà, 1974, 1976; Saló and Baguñà, 1984). Cell markers and karyotypes have been used to measure the cell kinetics in the blastema, the number of cells produced on the stump area and the rate of neoblast movement. These data showed that a small area of 300 µm below the blastema was enough to produce the cells necessary for the new tissue (Saló and Baguñà, 1985). In the same study, the ability of the chimeric animal to undergo intercalary regeneration was shown to be equal from both sides. A headpiece from a sexual animal was placed together witha tailpiece from an asexual one (with a Robertsonian translocation in their karyotype) and it was observed that the contribution to blastema formation was equal from both pieces. Local X-ray irradiation, which partially destroys the neoblast population, leads to repopulation of the irradiated area by healthy neoblasts and recovery of regenerative capacity (Dubois, 1949). This was interpreted as a demonstration of neoblast migration through the irradiated area. In contrast, tissue transplantation experiments comparing

chromosomal markers in an irradiated host compared with a non-irradiated one have shown that wounding does not trigger neoblast migration towards the damaged area when the graft is far from the wound (Saló and Baguña 1985). Finally, the cell turnover observed in areas of the planarian that are known not to have mitotic activity, mainly the area in front of the eyes and the pharynx, suggest that neoblasts are able to undergo directed migration (Newmark and Sánchez-Alvarado, 2000). Taken together, these findings indicate that neoblasts can migrate locally, depending on the tissue context, but regeneration appears not to require long-distance neoblast migration. Although neoblasts have generally been considered responsible for forming the blastema, the evidence is indirect, and in many models of regeneration the blastema cells, or the cells that participate in regeneration, arise through dedifferentiation of somatic cells. The main experiments in support of dedifferentiation in planarians were done by Gremigni and co-workers (1980, 1982) using a species in which chromosome numbers differ between somatic and germ cells. By analyzing cell ploidy, they observed that germ cells could contribute to blastema formation and differentiate into new structures by dedifferentiating and forming different cell types. However, these results could also be interpreted as transdetermination of partially determined stem cells, namely the germ line. Several classical observations, along with the results of more recent experiments, suggest that the blastema is formed by neoblasts. Thus, total irradiation causes loss of cell renewal and regenerative capabilities, and ultimately leads to death. This lethal phenotype can be rescued by injection of a neoblast-enriched cell suspension (Baguñà et al., 1989). In contrast, injection of a cell suspension enriched in differentiated cells did not rescue the phenotype and no mitosis was observed. Using different strains as host and donor, it was shown that the neoblasts were the sole source of regeneration and that they behaved as a totipotent cell, since an asexual irradiated host became sexual after injection of neoblasts from a sexual strain (Baguñà et al., 1989). We can thus conclude that neoblasts are capable of ‘‘transforming’’ one planarian strain into another, which strongly suggests that neoblasts can give rise to all differentiated cell types, including the germline.

2.4. Neoblast Molecular Markers and First Lineage Descriptions

Neoblasts are not homogeneously distributed along the parenchyma, and an anteroposterior gradient in the number of neoblasts undergoing mitosis has been described, with more dividing cells in the pre-pharyngeal area than in the posterior part of the planarian (Baguñà, 1976). Recent molecular studies analyzing histone H3 (phosphorylated -S10), a conserved marker of mitosis, showed a similar distribution of mitotic cells in S. mediterranea (Newmark and Sánchez-Alvarado, 2000). A number of neoblast markers have been described based on analysis of the molecular fingerprint of planarian cells (Table 1). These markers have been used to examine the distribution of the neoblasts in the planarian body and show that they are found throughout the mesenchyme except for the pharynx and the area in front of the eyes (Baguñà, 1973; Newmark and Sánchez-Alvarado, 2000; Orii et al, 2005).

Various experiments have now shown that they can be divided into subpopulations according to their distribution along the AP, DV, and mediolateral axes:

Table 1. Summary of genes and proteins characterized in planarian (Order Tricladida) neoblast cells (Species abbreviations: Dj, *Dugesia japonica*; Gd, *Girardia dorotocephala*; Ph, *Phagocata sp.*; Smed, *Schmidtea mediterranea)*

Gene (g)/ Protein (p)/ Stain (l)/ array(a)	Gene Product	Gene (Species)	Expression	Functional Features	References
BrdU-incorporation (l)	Incorporation of the thymidine analogue bromodeoxyuridine (BrdU) during DNA synthesis detected with the antibody anti-BrdU	(Ph, Gd, Smed)	Incorporation in proliferative cells, the neoblasts		Newmark and Sanchez Alvarado, 2000
Bruno (g/p)	RNA-binding protein (inhibiting translation)	*bruli*/Brul i (Smed)	Neoblasts and central nervous system (CNS)	Neoblast maintenance	Guo et al., 2006
α-H3Pser10 (p)	Anti-histone H3 phosphorylated serine 10		Mitotic cells, the neoblasts		Newmark and Sanchez Alvarado, 2000
Innexin (g)	Proteins forming gap junctions	*Smedinx-2* and *-11* (Smed)	Neoblasts	*smedinx-11* required for tissue regeneration, homeostasis and neoblast maintenance	Oviedo and Levin, 2007
MCM2	Minichromosom e maintenance protein, a DNA replication factor	*DjMCM2* (Dj)	Neoblasts		Salvetti et al., 2000
PCNA (g/p)	Proliferating Cell Nuclear Antigen, an auxiliary protein to DNA polymerase δ and associated with DNA replication	*Djpcna*/ DjPCNA (Dj)	Proliferative cells, neoblasts and germ cells		Orii et al., 2005
Piwi (g/p)	PAZ and PIWI protein, component of the RISC complex in the RNAinterference pathway	*Djpiwi-1* (Dj) *Smedwi-1 and -2* (Smed)	Neoblasts, germ cells and at the level of the cephalic ganglia	*Djpiwi-1* marks a neoblast subpopulation , *Smedwi-2* required for neoblast differentiation	Reddien et al., 2005a Rossi et al., 2006

Table 1. (Continued)

Gene (g)/ Protein (p)/ Stain (l)/ array(a)	Gene Product	Gene (Species)	Expression	Functional Features	References
PTEN (g)	Tumour suppressor phosphatase	*Smed-PTEN-1 and -2 (Smed)*	Neoblasts and differentiated cells	*Regulation of neoblast proliferation*	Oviedo et al., 2008
Pumilio (g)	PUF protein, RNA-binding protein (regulating translation)	*DjPum (Dj)*	Neoblasts and at the level of the cephalic ganglia	*Neoblast maintenance*	Salvetti et al., 2005
Vasa (g)	An ATP-dependent RNA helicase with DEAD box, component of the chromatoid body	*DjvlgA, Djvlg-B (Dj)*	Both in germ cells. DjvlgA also in neoblasts and in CNS		Shibata et al., 1999
DjCBC-1 (g)	RNA Helicase	*DjCBC-1* (Dj)	Chromatoid bodies		Yoshida-Kashikawa et al., 2007
RNAi-based screen	240 phenotypes	(Smed)		Genes involved in regeneration	Reddien et al., 2005b
Macroarray of neoblasts (a)	Neoblast genes activated at low X-ray doses	(Smed)		Neoblast cytoprotection	Rossi et al., 2007
Microarray Genes down-regulated after irradiation(a)	30 irradiation-sensitive genes	(Smed)	Neoblasts and their postmitotic descendants	Neoblast lineage	Eisenhoffer et al., 2008

Knockdown of smedinx-11, which encodes an innexin gap-junctional protein expressed in neoblasts, revealed a striking loss of mitotic activity in neoblasts that occurred in an anterior to posterior direction (Oviedo and Levin, 2007). The authors of that study suggested that smedinx-11 gap junctions regulate the movement of small molecules that control the maintenance, migration, and differentiation of the progeny of proliferative neoblasts. Along the DV axis, whereas S-phase neoblasts are scattered throughout the mensenchyme, mitotic neoblasts are mainly concentrated in external dorsal and ventral domains (although mitotic cells are also found in more internal regions of the mesenchyme), suggesting that the neoblasts migrate to these areas before division (Newmark and Sánchez-Alvarado, 2000). Two planarian PTEN homologues have been described in *S. mediterranea*, Smed-PTEN-1 and -2, which are capable of regulating neoblast proliferation and differentiation. PTEN protein is involved in the control of (PI3K)-AKT signaling, which is crucial for regulation of proliferation. RNAi for Smed-PTEN-1 and -2 leads to hyperproliferation of neoblasts with overgrowth of postmitotic cells, incapable of differentiation, followed by lysis of the

planarians (Oviedo et al., 2008). In the planarian species*D. japonica*, three neoblast markers DjMCM2, Djpcna and Dj-Piwi-1 (Table 1), have been used to define two spatially distinct neoblast populations with a mediolateral distribution in the dorsal mesenchyme: two lateral DjMCM-2 and Djpcna-positive domains and a domain containing DjMCM-2, Djpcna and Dj-Piwi-1-positive neoblasts along the midline (Orii et al., 2005; Rossi et al., 2006; Salvetti et al., 2000). Also, most of the genes described as expressed in neoblasts, such as bruno, Djcbc-1, piwi, pumilio and vasa, are found to be expressed around the brain (Guo et al., 2006; Salvetti et al., 2005; Shibata et al., 1999; Yoshida-Kashikawa et al., 2007) (see Table 1). Interestingly, this expression domain seems to be irradiation tolerant and could be corresponding to either non-proliferating, committed nerve cells or already differentiated neurons (Guo et al., 2006; Salvetti et al., 2005; Yoshida-Kashikawa et al., 2007). Even though no actual stem cell niche has been described in planarians, these findings indicate that, in addition to distribution throughout the parenchyma, neoblasts tendto accumulate in domains along the midline and lateral regions in D. japonica.

Several high-throughput strategies have been used to characterize neoblast genes. An RNAi screen of 1065 putative genes defined 240 genes with multiple phenotypes (Reddien et al., 2005b). An oligo-macrochip with 600 putative planarian genes was analyzed under high and low X-ray doses to isolate genes related to neoblast cytoprotection (Rossi et al. 2007). Recently, microarray analysis between normal versus irradiated planarians has defined a set of genes expressed in neoblasts and their cell progeny (Eisenhoffer et al. 2008). The use of BrdU labeling and in situ hybridization with those genes defined a first partial cell lineage with three discrete subpopulations of cells in planarians. These results can be considered the first example of the potential of the planarian model system to perform cell lineage in vivo in adult wild type and RNAi-treated organisms. The exploitation of such a plastic stem cell system as that found in planarians could pave the way for future applications in human regenerative medicine.

3. CNS Regeneration and Complete Recovery of Neural Function in Planarians

One of the most impressive observations during planarian regeneration is how fast a tiny amputated body piece can regenerate a whole CNS de novo (reviewed in Cebrià, 2007). This newly regenerated CNS, which forms in just a few days, will integrate with the pre-existing one and take immediate control of normal behaviors, such as directed movement, negative phototaxis, and feeding behavior. How this is controlled at the molecular level remains largely unknown. Our understanding of the planarian CNS and its regeneration is increasing and, so far, the expression of dozens of neural-specific genes has been characterized (Table 2; Mineta et al., 2003; Nakazawa et al., 2003). Many distinct neuronal subpopulations have also been characterized based on the specific neurotransmitters they produce (Table 3). In addition, several functional analyses have started to unravel how the planarian CNS is regenerated (see below). However, some important questions remain unsolved, such as how neoblasts become committed to a neural lineage. In addition, unlike in vertebrates, very little is known about the molecular and cellular events occurring immediately after amputation of the planarian CNS that might be important to trigger successful regeneration. In vertebrates,

some of the important factors that block the regeneration of the CNS are the formation of a glial scar together with the presence of several inhibitory molecules that block the growth of the truncated axonal projections (reviewed in Yiu and He, 2006 and Busch and Silver, 2007). In planarians, however, it is not clear what happens during those first stages of neuronal regeneration: Are stimulating factors upregulated? Are inhibitory factors downregulated? What is the role of the extracellular matrix? Answering these and other related questions will help not only to understand how planarians are able to regenerate their CNS but may also provide critical information to enhance the poor neural regenerative capacity of higher animals, including humans. This becomes relevant if we consider the number of people affected by neurodegenerative diseases and spinal cord injuries, for example, for which there is currently no successful treatment. Thus, in 2006 the worldwide prevalence of Alzheimer disease was 26.6 million and it could reach more than 100 million by 2050 (Brookmeyer et al., 2007). In addition, data from the National Institute of Neurological Disorders and Stroke (NIH) in 2004 reported that, in the United States, at least 500,000 people were believed to suffer from Parkinson disease, with about 50,000 new cases every year. Finally, in 2005, the National Spinal Cord Association reported that as many as 450,000 people in the United States were living with a spinal cord injury (SCI), with an estimated 11,000 new SCIs occurring every year. In all these cases, the patients would require the differentiation of new neural cells to repair or replace the damaged and dying cells. Clearly, insights into what allows the complex planarian CNS to be regenerated might help to identify potential therapeutic targets in other systems.

Table 2. List of some genes expressed within the planarian central nervous system

Gene	Homology	Expression pattern	Species	Reference
Djotp	Orthopedia	Brain lateral branches	Dj	Umesono et al. 1997
PC2	Pro-hormone convertase	General	Dj	Agata et al. 1998
DjotxA	Orthodenticle	Cephalic ganglia (medial region)	Dj	Umesono et al. 1999
DjotxB	Orthodenticle	Cephalic ganglia	Dj	Umesono et al. 1999
GtPax6a	Pax6	General	Gt	Pineda et al. 2002
Djnlg	Noggin-like	Brain lateral branches	Dj	Ogawa et al. 2002a
nou-darake	Fibroblast growth factor receptor-like	Cephalic ganglia	Dj	Cebrià et al. 2002c
DjFGFR1 and 2	Fibroblast growth factor receptor	Cephalic ganglia	Dj	Ogawa et al. 2002b
1008HH	Glutamate receptor	Brain lateral branches	Dj	Cebrià et al. 2002a
4307HH	Nicotinic acetylcholine receptor	General	Dj	Cebrià et al. 2002b
Gtsix3	Six/so	Brain lateral branches	Gt	Pineda and Saló 2002

Gene	Homology	Expression pattern	Species	Reference
Gtwnt-5 *Smed-wnt5*	Wnt	External side of the central nervous system	Gt, Sm	Marsal et al. 2003 Adell et al. 2009
DjXnp	SNF2-like	Cephalic ganglia	Dj	Rossi et al. 2003
DjFoxG	Brain factor-1	Cephalic ganglia	Dj	Koinuma et al. 2003
Djeya	Eye absent	Cephalic ganglia	Dj	Mannini et al. 2004
DjPHM	Peptidylglycine α.hydroxilating monooxygenase	General	Dj	Asada et al. 2005
Inx3 and 4	Innexins	General	Dj	Nogi and Levin 2005
Smed-netR	Netrin receptor	General	Sm	Cebrià and Newmark 2005
DjCAM	N-CAM	General	Dj	Fusaoka et al. 2006
DjDSCAM	DSCAM	Cephalic ganglia	Dj	Fusaoka et al. 2006
Smed-roboA	Roundabout	General	Sm	Cebrià and Newmark 2007
DjCHC	Clathrin heavy chain	General	Sm	Inoue et al. 2007
Smed-bruli	Bruno	General	Sm	Guo et al. 2006
DjwntA	Wnt	Posterior half of the brain	Dj	Kobayashi et al. 2007
DjfzA	frizzled	Anterior half of the brain	Dj	Kobayashi et al. 2007
Smed-Smad1	Smad1	General	Sm	Molina et al. 2007
Smed-noggin1	Noggin	General	Sm	Molina et al. 2007
Djsnap-25	Snap-25	General	Dj	Takano et al. 2007
DjCBC-1	Me31B protein	Cephalic ganglia	Dj	Yoshida-Kashikawa et al. 2007
Smed-GSK3.2	Glycogen synthase kinase 3	General	Sm	Adell et al. 2008
DjmlgA, B and C	Musashi	General	Dj	Higuchi et al. 2008
Smed-dvl1, 2	Dishevelled	General	Sm	Gurley et al. 2008
Smed-βcatenin1	βcatenin	General	Sm	Iglesias et al. 2008 Gurley et al. 2008 Petersen and Reddien 2008
Smed-evi	Evi/Wntless	General	Sm	Adell et al. 2009
Smed-nlg8	Noggin-like	Cephalic ganglia	Sm	Molina et al. 2009

The term "general" is used for those genes that are broadly expressed in both the brain (=cephalic ganglia) and the VNCs, independently of the number or subpopulations of neurons that they label. Dj, *Dugesia japonica*; Gt, *Girardia tigrina*; Sm, *Schmidtea mediterranea*. Modified from Cebrià 2007.

Table 3. Distinct neuronal populations in planarians based on neurotransmitter gene expression and protein localization

Neuronal cell type	Marker	In situ / Immuno	Localization	Reference
Serotonergic neurons	Anti-5HT	Immuno	CNS, PNS	Cebrià, 2008; Reuter et al., 1995, 1996
Serotonergic neurons	Tryptophan hydroxylase	In situ / Immuno	CNS, eye pigment cells	Nishimura et al., 2007a
Allatostatin-positive neurons	Anti-allatostatin	Immuno	CNS	Cebrià, 2008
Neuropeptide F-positive neurons	Anti-NPF	Immuno	CNS, PNS	Cebrià, 2008
GYRFamide-positive neurons	Anti-GYRFamide	Immuno	CNS, PNS	Cebrià, 2008
Dopaminergic neurons	Tyrosine hydroxylase	In situ / Immuno	CNS, PNS	Nishimura et al., 2007b
GABAergic neurons	Glutamic acid decarboxylase	In situ / Immuno	CNS, PNS	Nishimura et al., 2008a
Octopaminergic neurons	Tyramine-β-hydroxylase	In situ / Immuno	CNS, PNS	Nishimura et al., 2008b

CNS, central nervous system; PNS, peripheral nervous system.

3.1. Planarian CNS: Structure and Regeneration

The CNS of freshwater planarians comprises a brain or pair of cephalic ganglia in the anterior region and a pair of ventral nerve cords that extend along the length of the animal (Figure 4). In the model species*S. mediterranea* and *D. japonica*, the two lobes of the cephalic ganglia are connected by a single anterior commissure, although additional brain commissures may be present in other species (Agata et al., 1998; Cebrià et al., 2002a; Hyman, 1951; Lentz, 1968; Reuter et al., 1995, 1996; Rieger et al., 1991). The cephalic ganglia have a spongy texture as they are traversed by dorsoventral muscle fibers and secretory cell processes (Baguñà and Ballester, 1978). These ganglia are organized as a central neuropil formed by the processes of the surrounding neural cell bodies (Fig. 4). In addition, lateral branches project from the brain to the body margins where they connect to the sensory organs of the head (Hyman, 1951; Rieger et al., 1991). Along the ventral nerve cords, neurons are organized in small knot-like ganglia that appear more or less regularly spaced along their length (Agata et al., 1998; Cebrià et al., 2002a). Similar to their distribution in the brain, the cell bodies of those neurons are mainly peripheral to the central thick track of neuronal processes that form the nerve cords (Figure 4). The ganglia from the left and right nerve cords are connected by transverse commissures and sends lateral projections to the lateral margins of the body. Although intimately connected, the brain and the ventral nerve cords can be considered as two independent structures at both a structural and a molecular level (Agata et al., 1998; Cebrià et al., 2002a).

Despite this apparent morphological simplicity, the planarian CNS has a striking molecular complexity. Thus, the cephalic ganglia can be subdivided into several molecular domains based on the expression patterns of specific genes. Planarian Otx gene homologues, for instance, define three distinct mediolateral domains (Umesono et al., 1999). These molecular domains can be further subdivided by the expression of additional neural-related genes (Cebrià et al., 2002b; Mineta et al., 2003; Nakazawa et al., 2003).

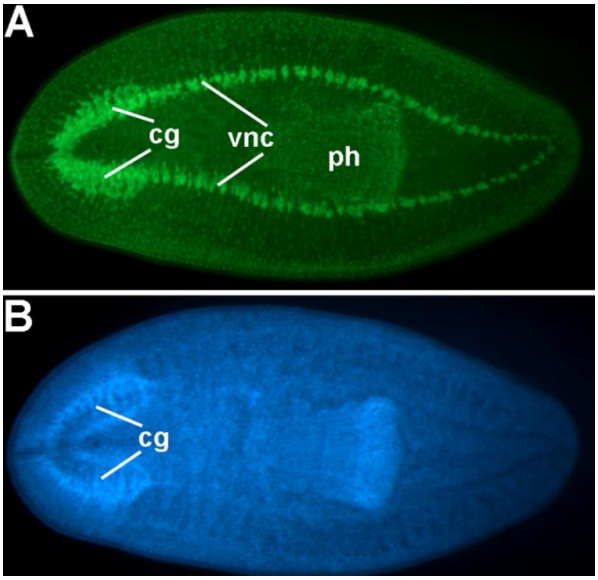

Figure 4. (A) Central nervous system of *Schmidtea mediterranea* after immunostaining with an anti-synapsin antibody (Developmental Studies Hybridoma Bank). (B) Nuclear counterstaining with DAPI. cg, cephalic ganglia; vnc, ventral nerve cords; ph, pharynx. Anterior to the left.

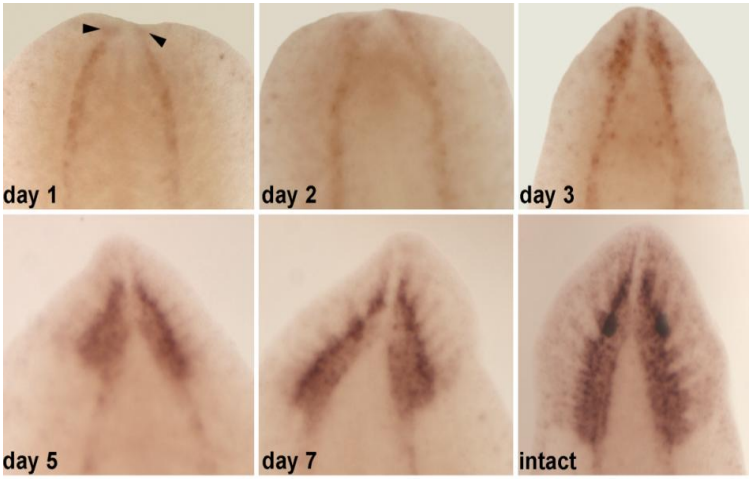

Figure 5. Whole mount in situ hybridizations with clone 953_HH (very low-density lipoprotein receptor homologue; Cebrià et al. 2002a) allows visualization of the process of CNS regeneration in the planarian *Dugesia japonica*. Arrowheads indicate the differentiating brain primordia.

Remarkably, planarian neural genes show a high degree of conservation with their homologes in vertebrates and they are functionally linked to processes such as neurotransmission, axon guidance, brain morphogenesis, neural differentiation and sensory systems (Mineta et al., 2003). That means that we can use planarian genes to study their function during regeneration and try to correlate the data obtained with the predicted role for those genes during vertebrate neural development.

After decapitation, planarians will regenerate a new cephalic region including a complete functional brain in 7-10 days (Figure 5). From a conceptual point of view, planarian CNS regeneration can be divided into three main stages: 1) brain rudiment formation, 2) growth of the brain and the truncated nerve cords with the subsequent formation of proper neural networks, and 3) functional recovery (Cebrià 2007; Cebrià et al., 2002a; Agata and Umesono, 2008). At each of these three stages, different neural genes are upregulated, suggesting a correlation between the expression of those genes and the cellular and molecular processes taking place (Cebrià et al., 2002a; Agata and Umesono, 2008).

As described above, planarian regeneration depends upon the proliferation and differentiation of totipotent stem cells called neoblasts. During regeneration, neoblasts will give rise to all cell types, including neurons.

Neoblasts are probably a highly heterogeneous cell population (Higuchi et al., 2007), comprising both true totipotent stem cells and already committed cells, and no molecular markers have so far been reported for progenitor cells such as neural precursor cells, . As a result, it has been difficult to study how neoblasts become committed to the neural lineage or to determine the origin of the brain rudiment (or brain primordium).

At day 1 of regeneration, many markers of differentiated neural cells are already expressed in the two small clusters of cells within the blastema that give rise to the brain primordia (arrowheads in Figure 5; Cebrià et al., 2002a). In addition, genes that are expressed in neural progenitor cells in other systems are also expressed within the blastema at those early stages (1-2 days of regeneration). For instance, three homologues of the musashi family have been identified in *D. japonica* (Higuchi et al., 2008). Musashi encodes a conserved RNA binding protein, which is expressed in the neural lineage, including neural stem cells, in many animals (reviewed in Okano et al., 2005).

In planarians, three musashi-like genes are expressed throughout the mature nervous system (Higuchi et al., 2008). These genes are expressed in post-mitotic differentiated cells and very little if any expression has been detected in proliferative cells, raising the question of whether true neural stem cells exist in planarians (Higuchi et al., 2008). Moreover, RNAi-based functional analyses have shown that even planarians in which all three musashi-like genes have been silenced are able to regenerate a normal brain (Higuchi et al., 2008). In that study, the only effect observed on the nervous system was a reduction in the expression of the neural markers choline acetyl transferase and glutaminase after RNAi for one of the musashi genes.

These unexpected results led the authors to suggest that a subset of lineage-restricted neural stem cells may not be present in planarians, or alternatively, if such neural stem cells existed yet unidentified genes would define them. Thus, more molecular markers are needed to better understand the initial stage of brain regeneration during which neoblasts are committed to give rise to the brain primordia.

3.2. Genes Required for the Reconstruction of Neural Networks

The second main step in planarian CNS regeneration is the growth of the initial brain primordia together with the outgrowth of the truncated ventral nerve cords. During this stage, new neural networks need to be re-built and the regenerating nervous system will be re-wired (Cebrià et al., 2002a; Agata and Umesono, 2008). Several studies indicate that conserved axon guidance cues play an important role at this stage of planarian regeneration. In his neurotropic theory, Santiago Ramón y Cajal suggested that unknown forces guided axons to their proper targets (reviewed in Ramón y Cajal, 1928). Nowadays there is a fair knowledge of the molecular mechanisms that guide axons to their targets. The main families of cues involved in axon guidance are netrins, slits, ephrins and semaphorins (Aráujo and Tear, 2003; Guan and Rao, 2003; O'Donnell et al., 2009). Some of these act as attractive cues for the axons, guiding them towards permissive areas, whereas others repel axons from non-permissive regions. Moreover, the same guidance cue may function as an attractive or repulsive signal depending on the context. These cues have a pivotal role in the wiring of the CNS during embryogenesis in a large variety of animals, from *Drosophila* and *C. elegans* to vertebrates. However, much less is known about the function they might have during regeneration. In most animals, axonal projections in the CNS do not usually regenerate because of intrinsic and environmental factors. However, the regenerative capacity of the planarian CNS makes it a good model in which to study the function of such cues during regeneration.

Netrins were initially identified as secreted chemoattractant molecules for growing axons in *C. elegans, Drosophila* and vertebrates, but subsequent observations revealed that they can also act as chemorepellents (Ishii et al., 1992; Kennedy et al., 1994; Serafini et al., 1994; Colamarino and Tessier-Lavigne, 1995; Harris et al., 1996; Mitchell et al., 1996; Serafini et al., 1996). Netrins are recognized by two families of receptors—Deleted in Colorectal Cancer (DCC) and UNC5—that mediate the attractive or repulsive response of the growing axons (Chan et al., 1996; Keino-Masu et al., 1996; Hong et al., 1999; Keleman and Dickson, 2001). In planarians, Smed-netrin1, netrin2, and Smed-netrin receptor are necessary for normal regeneration of the CNS; also, these genes are required for the maintenance of nervous system architecture in adult planarians (Cebrià and Newmark, 2005).

Thus, after RNAi of either Smed-netrin 2 or Smed-netrin receptor planarians regenerate abnormal cephalic ganglia, which are shorter, wider and connected by a thicker anterior commissure. In addition, instead of the two parallel ventral nerve cords normally regenerated in control animals, RNAi-treated animals regenerate a completely disorganized meshwork of axonal projections (Cebrià and Newmark, 2005).

Outside the brain and nerve cords, these molecules also play an important role in the proper targeting of the axonal projections of the photosensitive cells. Planarian photosensitive cells express Smed-netrin receptor, whereas Smed-netrin2 is expressed in the brain region where those axons project (Cebrià et al., 2002a; Cebrià and Newmark, 2005). RNAi of any of these genes results in a failure of the visual axons to project to the proper brain region, indicating that Smed-netrin2 acts as an attractive cue for the proper targeting of those axons (Cebrià and Newmark, 2005).

Roundabout (robo) codes for a transmembrane receptor that binds the secreted protein SLIT (Brose et al., 1999; Kidd et al., 1999). Slit was first identified as a repulsive cue for commissural axons in Drosophila (Rothberg et al., 1988, 1990; Kidd et al., 1999), and the

Slit/Robo system has an evolutionarily conserved function in axon guidance (Long et al., 2004; Dickson and Gillestro, 2006).

In planarians, Smed-roboA is expressed throughout the CNS (Cebrià and Newmark, 2007). RNAi analyses indicate that Smed-roboA is required for normal regeneration of the planarian CNS. Silencing of Smed-roboA results in the disruption of neuronal connectivity during regeneration (Cebrià and Newmark, 2007). The cephalic ganglia are either become connected by a thinner anterior commissure or, in some cases, remain unconnected. While the truncated ventral nerve cords fail to properly regenerates and the connection between them and the newly formed cephalic ganglia is absent. In addition, the axons of the photosensitive cells have abnormal projections after RNAi experiments.

Other genes that have an important role in this stage of planarian CNS regeneration are homologues of cell adhesion molecules, such as DjCAM and DjDSCAM, as well as a homologue of a clathrin heavy chain (CHC) gene. After RNAi of DjCAM, the axonal bundles that project from the cephalic ganglia to the body margin appear defasciculated (Fusaoka et al., 2006). Silencing of DjDSCAM results in a clear disorganization of the neuropil that translates into a morphologically aberrant brain; in addition, the number of lateral branches of the brain is reduced and the branches appear disorganized (Fusaoka et al., 2006). Recently, Inoue and colleagues (2007) reported that planarian DjCHC is necessary for neurite growth, as neurons isolated from RNAi-treated animals and cultured in vitro extended significantly fewer and shorter neurites compared to controls. These results could explain the morphological defects observed in the regenerated brain after DjCHC RNAi.

In summary, several genes with important roles in the wiring of the nervous system of other organisms show a conserved function during planarian CNS regeneration. By comparing their expression dynamics and function in the CNS of planarians with that seen in animals with poor regenerative capacity , new strategies or approaches could be identified to enhance regeneration in other systems.

3.3. *Nou-Darake* Restricts Brain Tissues in the Cephalic Region

Regeneration of new tissues and organs must involve accurate regulation of their size and spatial position. The nou-darake (ndk) gene exemplifies how this spatial restriction is regulated during planarian CNS regeneration (Cebrià et al., 2002c). After decapitation, neoblasts proliferate and differentiate into neurons that give rise to a brain rudiment within the blastema. This brain rudiment will grow and form a definitive brain that will build new neural networks with the regenerating ventral nerve cords as well as with the peripheral nervous system. The regenerating brain grows within a new anterior region that is also being formed at the same time. Somehow, the growth of both the brain and the anterior region must be tightly coordinated so that the new brain ultimately occupies most of this anterior region. After ndk silencing by RNAi, planarians regenerate an apparently normal brain rudiment, which will grow to form a complete brain.

However, instead of staying restricted to the new anterior region, the newly formed brain expands posteriorly (Cebrià et al., 2002c). Ndk is specifically expressed in the anterior region of planarians, including the brain, and codes for a transmembrane receptor that displays similarity to the Fibroblast Growth Factor (FGF) receptor family; however, it lacks the cytoplasmic tyrosine kinase domain (Cebrià et al., 2002c).

Based on its expression pattern, domain structure and the results of overexpression in Xenopus embryos, it has been proposed that ndk could act as a negative regulator of FGF receptor signaling (Cebrià et al., 2002c; Agata and Umesono, 2008). Because the differentiation of ectopic brain tissues in the posterior regions of ndk-RNAi animals depends on the presence of FGFRs in those regions, it was proposed that ndk could restrict the diffusion of an FGF-like ligand that would work as a brain activator in planarians (Cebrià et al., 2002c; Agata and Umesono, 2008).

According to this model, ndk would trap excess ligand in the head region and prevent it diffusing to more posterior regions. In ndk-RNAi animals, this brain activator would diffuse outside the head region, interact with FGFRs, and trigger ectopic brain differentiation (Cebrià et al., 2002c; Agata and Umesono, 2008). Even though these results suggest the existence of an FGF-type signaling pathway to control brain differentiation in planarians, no FGF-like molecule has been identified in these animals yet.

Ndk shows sequence similarity to FGFRL1 (Fibroblast growth factor receptor-like 1), a gene identified in humans that also lacks the tyrosine kinase domain (Wiedemann and Trueb, 2000). The available functional data on this gene in vertebrates suggest that FGFRL1 has a negative effect on FGF signaling (Hall et al., 2006; Rieckmann et al., 2009). This would be in agreement with the proposed function for ndk in planarians. Recently, orthologues of FGFRL1 have been identified in a wide variety of bilateria, from the cnidarian Nematostella to amphioxus, echinoderms, Xenopus, tunicates, nematodes, crustaceans molluscs and insects (Bertrand et al., 2009; Beyeler and Trueb, 2006; Hayashi et al., 2004).

Interestingly, it has been found that FGFRL1 genes are in the vicinity of other genes of the FGF pathway (FGF8/17/18 and/or FGFR) in the genome of many bilaterians, forming a syntenic block that could have been conserved in Urbilateria (Bertrand et al., 2009). In addition, FGFRL1 shows some overlapping expression patterns with those of FGF8 in amphioxus and Xenopus (Bertrand et al., 2009; Hayashi et al., 2004). Further analyses should help to elucidate the exact function of FGFRL1/nou-darake genes in the regulation of the FGF signaling pathway.

3.4. miRNAs and Neural Regeneration and Repair

MicroRNAs (miRNAs) are short (~22 nucleotides) noncoding RNAs with a key role in regulating gene expression. They bind to complementary sequences in the 3' untranslated region of target mRNAs, inducing their degradation or inhibiting translation of the bound mRNAs (reviewed in Carthew and Sontheimer, 2009 and Flynt and Lai, 2008).

Hundreds of miRNAs have been identified in plants and animals, where they play important regulatory roles in many developmental and disease processes. Thus, miRNAs have been shown to be involved in regulating, among others, developmental timing (Lee et al., 1993; Reinhardt et al., 2000), Hox gene expression (Naguibneva et al., 2006), regeneration (Yin et al., 2008; Crist et al.; 2009), apoptosis (Cimmino et al., 2005), and cell proliferation (Brennecke et al., 2003). In addition, they also have important roles in stem cell division and differentiation (Forstemman et al., 2005; Hatfield et al., 2005; Houbaviy et al., 2003).

A large number of miRNAs are expressed in the CNS (Kapsimali et al., 2007) and, recently, miRNAs have been shown to function in neural stem cells and progenitors (Cheng et al., 2009; Schwamborn et al., 2009; Zhao et al., 2009). In addition, a role for miRNAs has

been reported in many neurodegenerative diseases (Hutchinson et al., 2009). Thus, alterations in the expression of miRNAs have been reported in Alzheimer (Cogswell et al., 2008; Hébert et al., 2008), Parkinson (Wang et al., 2008) and Hungtinton (Packer et al., 2008) diseases. Some recent studies have uncovered changes in the expression of multiple miRNAs after traumatic brain injuries (Lei et al., 2009; Redell et al, 2009), which may help us to better understand the regulation of events occurring after injury. Being able to control the expression of miRNAs could thus become a potent therapeutic target for the treatment of neurodegenerative disease and injury (Hutchison et al., 2009). Numerous miRNAs have now been identified in planarians (Palakodetti et al., 2006; Lu et al., 2009; Friedländer et al., 2009).

Remarkably, several have been shown to be downregulated after irradiation, an indication that they could be specific to neoblasts (Friedländer et al., 2009; Lu et al., 2009). Whole-mount in situ hybridizations have uncovered a variety of expression patterns (González-Estévez et al., 2009). Some miRNAs are specifically expressed in the digestive system, epidermis, parenchyma, or nervous system, whereas others show broader expression patterns (Gonzàlez-Estèvez et al., 2009).

Several planarian miRNAs are expressed in the CNS, including the miR-124 family, which has three members in these animals (Palakodeti et al., 2006). Planarian miR-124c is expressed throughout the CNS, whereas miR-124a and -124b are expressed in the parenchyma and in the cephalic ganglia. The expression of miR-124a and -124b in the cephalic ganglia and parenchyma (González-Estévez et al., 2009; Pearson et al., 2009) appears to be downregulated after irradiation (González-Estévez et al., 2009). MiR-124 is the most abundant miRNA in the adult mouse brain (Lagos-Quintana et al., 2002).

A recent report suggests that miR-124 controls neurogenesis in the adult subventricular zone (SVZ) niche (Cheng et al., 2009). The expression of miR-124 increases as the neuroblasts exit the cell cycle. Overexpressing miR-124 promotes neuronal differentiation whereas blocking it retains SVZ cells in an active proliferating state. Thus, miR-124 appears to regulate the number of progenitors and the timing of neuronal differentiation (Cheng et al., 2009). As no convincing molecular marker has been reported yet for planarian neural stem cells or progenitors, the miR-124 family may be an attractive target for further studies.

4. Pattern Formation during Planarian Regeneration

4.1. Pattern Formation and Development

Pattern formation in living organisms refers to the process by which cell fate is controlled in space and time to generate complex tissues and organs. Metazoans have highly diverse body patterns, ranging from the apparently asymmetric organization of sponges to the bilaterally symmetric body plan of vertebrates and most invertebrates. Three main perpendicular body axes, the anteroposterior (AP), the dorsoventral (DV) and the left-right, or mediolateral axis defines the symmetry of bilateral animals. Research into the gene network that controls cell growth, differentiation and morphogenesis—the main processes that must be coordinated for correct patterning of the organism—has led to a deep understanding of the

molecular mechanisms governing these events. For example, a detailed genetic cascade has been identified that controls patterning of the AP axis during fly embryogenesis (reviewed in Mann and Morata, 2000). However, most current data is derived from studies of embryogenesis, while few data exist on regeneration. The regenerative capacity of animals varies widely across phyla and even between species. While some species, such as humans, are only able to regenerate small parts of just a few organs, others, such as Hydra or planarians, can regenerate a complete individual from any small piece of the body. In all cases, however, the replacement of missing structures does not only require proliferation and growth but also correct differentiation and spatial organization of cells to re-establish the correct pattern. For historical and practical reasons, most research into patterning mechanisms has been done in only a few animal models, mainly fly, frog, and mouse. Even though several other models are now emerging, substantial efforts in the lophotrocozoa clade of protostomes, to which planarians belong, are still missing. Fortunately, the 'molecular age' is heralding the rebirth of planarians as an essential model in which to understand the mechanisms underlying pattern formation during regeneration.

4.2. The BMP and WNT/B-Catenin Pathways Direct Body Axis Establishment during Planarian Regeneration

Both the BMP and the Wnt/β-catenin pathways are evolutionarily conserved mechanisms used for cell-cell communication. Their essential role in cell growth and differentiation means that they are involved in almost every biological event during embryogenesis and regeneration, as well as in related pathological processes such as degenerative disease or cancer. BMPs (a subgroup of the larger TGF-beta superfamily) and Wnts, the secreted elements of these pathways, are classical morphogens, as they can confer different cell identities on receiving cells according to their concentration and, therefore, the distance from the source (reviewed in De Robertis and Kuroda, 2004; Ashe and Briscoe, 2006 and Ibañes and Izpisúa Belmonte, 2008). Activation of the BMP pathway by binding of BMPs to a complex of type 1 and 2 transmembrane serine threonine kinase receptors triggers the phosphorylation and activation of the type 1 receptor by the type 2 receptor kinase and the phosphorylation of a receptor-associated SMAD, which subsequently complexes with SMAD4 and translocates to the nucleus to regulate gene transcription (reviewed in Feng and Derynck, 2005). Similarly, the receptors of the Wnt pathway, the Frizzled proteins, bind Wnt ligands together with LRP co-receptors, leading to the disruption of the β-catenin 'degradation complex', composed of Axin, GSK3, CKI, and APC. Therefore, β-catenin accumulates in the cytoplasm, enters the nucleus and activates TCF transcription factors, which are able to regulate the expression of multiple genes (reviewed in Fuerer et al., 2008). During development, the BMP pathway is essential for the establishment of the DV axis (reviewed in De Robertis and Kuroda, 2004; Little and Mullins, 2006; Lowe et al., 2006 and Yu et al., 2007), whereas the most conserved role of the Wnt pathway is in establishing the AP axis (Kiecker and Niehrs, 2001; Hollad, 2002). Recently, several studies have been published reporting the role of the main elements of both pathways during planarian regeneration (Kobayashi et al., 2007; Gurley et al., 2008; Petersen and Reddien, 2008; Iglesias et al., 2008, Orii and Watanabe, 2007; Molina et al., 2007; Reddien et al., 2007). Those studies clearly demonstrate the essential role of the BMP and Wnt/β-catenin pathways

in establishment of the DV and AP body axis, respectively, during planarian regeneration. The results of these studies support the idea that the mechanisms involved in patterning during embryogenesis are essentially conserved during regeneration in adult organisms.

4.3. The BMP Pathway Is Essential For Establishing the DV Axis in Planarians

In both vertebrates and invertebrates, the BMP pathway has been shown to play an essential role in the establishment of the DV axis during development. However, it is interesting to note that many genes of the BMP pathway that are conserved between vertebrates and invertebrates show expression patterns that are inverted with respect the DV axis and to each other. For instances, whereas BMP4 is expressed ventrally in vertebrates, the Drosophila orthologue dpp, is expressed dorsally, and they exert ventralizing and dorsalizing functions, respectively (reviewed in Hogan et al., 1994).

These observations have lead to the suggestion that the DV axis and its patterning mechanisms were inverted during evolution (Arendt and Nubler-Jung, 1994). Several elements of the BMP pathway have been identified and functionally characterized in planarians, and it has been shown that this pathway is necessary for the re-establishment of a proper DV axis in regenerating animals (Orii and Watanabe, 2007; Molina et al., 2007; Reddien et al., 2007).

The *S. mediterranea* BMP homolog Smed-BMP is expressed in small clusters of cells all along the dorsal midline, as in other invertebrates, and silencing by RNAi leads to a ventralized phenotype (Figure 6 A,B). Moreover, planarian homologues of Smad1 and Smad4, the cytoplasmic transducers of the signal, have been described (Molina et al., 2007; Reddien et al., 2007).

According to the dorsalizing role of BMP, silencing of Smed-Smads also results in animals in which the dorsal region is ventralized. This ventralization is clearly observed by the disappearance of dorsal molecular markers together with the ectopic expression of ventral ones on the dorsal sides of the RNAi-treated planarians (Molina et al., 2007; Reddien et al., 2007). In the most severe transformations, a duplication of the eyes or an almost complete dorsal ectopic central nervous system can be observed (Figure 6B) (Molina et al., 2007). Because Smed-BMP is expressed at the midline, it has also been suggested that the BMP pathway regulates midline patterning during planarian regeneration (Reddien et al., 2007).

In planarians amputated parasagittally along their anteroposterior axis, the BMP pathway instructs the formation of proper blastemas in bilaterally asymmetric fragments (Reddien et al., 2007). Recently, the existence of a large family of BMP antagonists, the noggins, has been reported in S. mediterranea (Molina et al. 2009).

In contrast to most animals, in which between two and four noggin genes are present, planarians have an expanded noggin family with up to ten members. Planarian noggins have been subdivided into noggin genes (2), with a canonical noggin domain, and noggin-like genes (8) with an insertion of 50-60 amino acids between the fifth and sixth conserved cysteine residues (Molina et al., 2009).

In planarians, these genes show diverse and complex expression patterns throughout the planarian body (Molina et al., 2009). Future functional analysis will determine the role, if any, of these genes in the re-establishment of the DV axis.

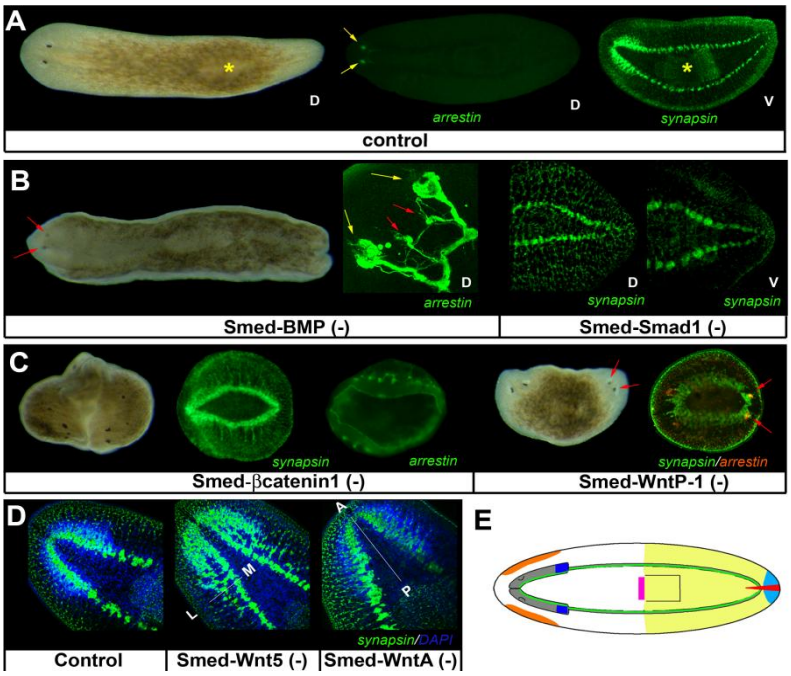

Figure 6. Phenotypes generated after silencing of BMP and Wnt signalling pathway elements. (A) Control planarians. (B) *Smed-BMP* and *Smed-Smad1*-silenced planarians show a ventralized phenotype: ectopic differentiation of eyes deep in the mesenchyma and ectopic differentiation of nerve cords in the dorsal region. (C) *Smed-bcat1* and *Smed-WntP-1*-silenced planarians show an anteriorized phenotype. Multiple eyes appear around a large circular cephalic ganglion in *Smed-bcat1*-silenced planarians, and a posterior head appears in *Smed-WntP1*-RNAi animals. (D) *Smed-Wnt5* and *Smed-WntA*-silenced planarians show defects in the mediolateral and anteroposterior patterning of the brain, respectively. The axis affected in each case is indicated. (E) Schematic drawing of a planarian showing the expression patterns of *Smed-Wnts* (light blue, *Smed-Wnt11-1/2*; red, *Smed-WntP-1*; yellow, *Smed-WntP-2*; pink, *Smed-WntP-2*; dark blue, *Smed-WntA*; green, *Smed-Wnt5*; orange, *Smed-Wnt2-1*). The antibody used for immunostaining is indicated in each image (anti-synapsin, which labels the CNS, and anti-arrestin, which labels the visual axons). Yellow asterisks indicate the pharynx and yellow arrows indicate the eyes. Red arrows point to ectopic eyes. Anterior to the left.

4.4. The WNT/β-Catenin Pathway Is Essential for Establishing the AP Axis in Planarians

The main element of the canonical Wnt signaling pathway, β-catenin, is required for DV polarity in early vertebrate embryos (De Robertis and Kuroda 2004). However, the most conserved role of the pathway in animals is in establishing the AP axis during development of several species, such as mouse (Marikawa, 2006), chick (Nordstrom et al., 2002), zebrafish (Schier and Talbot, 2005), Xenopus (Kiecker and Niehrs, 2001), amphioxus (Holland, 2002), C. elegans (Huang et al., 2007) and Platynereis (Schneider and Bowerman, 2007). In cnidarians, diploblastic animals with only one body axis, it specifies the oral-aboral embryonic axis (Lee et al., 2006).

Several elements of the Wnt/β-catenin pathway have been characterized in the genome of *S. mediterranea* and functional studies have been reported (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). The most extreme phenotype is obtained after silencing

of Smed-βcatenin1, the *S. mediterranea* homolog of β-catenin. Thus, Smed-βcatenin1-silenced planarians appear as 'radial-like hypercephalized' animals, which have a large circular cephalic ganglion that induces the differentiation of several ectopic eyes all around the planarian body (Iglesias et al., 2008) (Figure 6C). Analysis of the phenotypes of live trunk pieces from regenerating Smed-βcatenin1-silenced animals along with expression of neuronal and positional markers (HoxD, AbdBA) has shown that those animals are fully anteriorized and that medial and posterior identities are lost (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Moreover, although the AP axis is completely abolished in those animals, the DV axis is normal; demonstrating that disruption of the Wnt/β-catenin pathway exclusively affects the establishment of the AP axis during planarian regeneration (Iglesias et al., 2008). The role of the Wnt/β-catenin pathway in AP axis patterning in planarians has also been demonstrated by silencing other elements of the pathway. For example, two-headed animals are obtained following silencing of Smed-dsh, the planarian homolog of dishevelled, an intracellular protein required Wnt signal transduction, or Smed-evi/wls, the homolog of evi/wntless, which is required for Wnt secretion (Adell et al., 2009a; Gurley et al., 2008). Accordingly, it has been reported that when APC-1, a component of the β-catenin 'degradation complex' is silenced, the opposite phenotype is obtained: a tail instead of a head appears in regenerating planarians (Gurley et al., 2008). Taken together, these data demonstrate a conserved role for the Wnt/β-catenin pathway in AP axis establishment in planarians.

Based on currently available data, it has been proposed that there is a gradient of β-catenin activity along the length of the planarian body, with its maximum concentration in the posterior region (Adell et al., 2009b). According to this hypothesis, inhibition of Smed-βcatenin1 activity would lead all planarian cells to adopt an anterior fate. The morphogens of the pathway, namely the *S. mediterranea* Wnt family, have been the subject of recent studies. Nine Wnts have been found in the S. mediterranea genome, and they have region-specific expression patterns (Figure 6E). The mRNA of three of them (Smed-wntP-1, Smed-wnt11-1 and Smed-wnt11-2) is only found in a very small number of cells in the posterior region of the animals (Petersen and Reddien 2008; Adell et al 2009a), and therefore they are candidates to have a role in AP patterning in planarians through Smed-βcatenin1 regulation. Recent RNAi experiments have also demonstrated the involvement of two planarian Wnts in AP axis establishment. Silencing of Smed-Wnt11-2 leads to a tailless phenotype, while RNAi for Smed-WntP-1, the clearest effector of the canonical Wnt signal, generates two-headed planarians (Figure 6C) (Adell et al., 2009; Petersen and Reddien, 2009). It has also been demonstrated that although silencing of Smed-WntP-2, which is expressed in the posterior half of the planarian body, does not generate any apparent phenotype alone, it leads to an increased number of two-headed planarians when silenced together with Smed-WntP-1 (Petersen and Reddien 2009).

Based on currently available data, the only Wnt, which clearly transduces the signal through Smed-βcatenin1, would be Smed-WntP-1, because it is the only one for which silencing leads to regeneration of a head instead of a tail. However, it should be noted that Smed-WntP-1 is detected not only in the posterior blastema but in any blastema during the first few hours of regeneration (Petersen and Reddien 2009), and that Smed-βcatenin1 mRNA is also detected in any blastema, as well as broadly all around the planarian body (Gurley at al., 2008; Iglesias et al., 2008). Therefore, although the role of the Wnt/β-catenin pathway in AP patterning is clear and consistent with the expression patterns of several Wnt ligands, the

precise mechanism of cell-cell communication, which leads to AP patterning during planarian regeneration, is still unknown. Moreover, although the activity of Smed-βcatenin1 itself seems to be sufficient to instruct posterior identity, its expression may not be exclusively regulated through the Wnt pathway. For instance, it has been recently reported that silencing of patched (ptc), an element of the Hedgehog (Hh) pathway, induces the same posteriorized phenotype as APC, and that its effect depends on wntP-1 expression (Rink et al., 2009, Yazawa et al., 2009).

4.5. Differential and Restricted Planarian WNT Domains

As discussed, four Wnts are expressed in the posterior part of the planarian body: Smed-wntP-1, Smed-wnt11-1, and Smed-wnt11-2 are expressed in only a few cells at the most posterior end of the animal, whereas Smed-wntP-2 is expressed in the posterior half. The remaining planarian Wnts also show regionally restricted expression patterns: Smed-wntP-3 is expressed in a few cells in the proximal part of the pharynx; Smed-wnt2-1 is expressed in both lateral regions of the head; Smed-wnt5 is expressed in the most external part of the cephalic ganglia; and Smed-wntA is enriched in the posterior part of the cephalic ganglia (Figure 6E). Interestingly, and consistent with their expression patterns, inhibition of wntA by RNAi induces posterior expansion of the anterior part of the brain (Kobayashi et al., 2007; Adell et al., 2009) (Figure 6D), and silencing of Wnt5 induces lateral expansion of the cephalic ganglia (Adell et al., 2009) (Figure 6D). Thus, most *S. mediterranea* Wnts seem to be involved in AP axis patterning at different levels of the planarian body, whereas Smed-Wnt5 could have a role in patterning along the mediolateral body axis. These data suggest that the different wnts could pattern the different regions of the planarian body and act together to achieve the correct global patterning, as previously proposed for cnidarians (Guder et al., 2006).

4.6. Planarians Represent a Unique Model for Patterning Studies

The Wnt and BMP gradients act together in the developing embryo to determine the AP and DV body axis, respectively. However, as they are universal mechanisms of cell-cell communication, both pathways also have an essential role in the control of cell proliferation, differentiation and tissue morphogenesis in adult organisms (Reya and Clevers, 2005; Kitisin et al., 2009). All the data presented from planarians are consistent with the hypothesis that those pathways represent an evolutionarily conserved mechanism to confer cell identity and subsequent tissue polarity not only during embryonic development but also in adult organisms, during regeneration processes. Furthermore, essentially the same phenotypes are obtained after silencing most of the described BMP and Wnt elements in intact planarians. This means that the same mechanisms governing regeneration also govern normal homeostasis in adult planarians. Of course, this is not very surprising considering that planarians are in continuous growth and degrowth and remodeling, but it is nevertheless an important observation, because it supports the general idea that developmental genes and functional networks are also active in adults.

Although the same developmental networks appear to be functional many times in adults, the Wnt pathway has only been found to be involved in patterning during regeneration of cnidarians, animals with plasticity comparable to planarians (Lee et al., 2006). With the exception of planarians, the Wnt and BMP pathways only have demonstrated roles in regenerative capacity or extent of regeneration through their essential activity as mitotic signals. Nevertheless, this appears to be more down to the limitations of the experimental systems used, and their potential role in patterning remains clear. For instance, Wnt/β-catenin signaling, together with FGF, is required for wound healing and blastema formation during limb regeneration in urodeles and fin regeneration in zebrafish, two of the main vertebrate models of regeneration currently employed. However, its role in patterning cannot be assessed, because regeneration is impaired when the Wnt pathway is up- or downregulated (reviewed in Stoick-Cooper et al., 2007). Another example is seen with limb regeneration in crickets. These animals can regenerate their limbs when amputated, and it has been demonstrated with classical graft experiments that they can not only regenerate terminal structures but also are also able to intercalate the missing structures after different kinds of grafts. While β-catenin silencing in planarians leads to clear anteriorization of regenerating structures, in case of crickets β-catenin silencing leads to inhibition of the regeneration (Nakamura et al., 2007). Thus, the plasticity of planarians allows visualization of the essential patterning role of β-catenin, while in the cricket it is not possible to determine whether β-catenin would have a patterning role besides its function as a proliferative signal. The striking phenotypes obtained in adult planarians after silencing of developmental/patterning genes, even in intact organisms, appear to be unique among current models, making planarians in an essential tool for the functional analysis of these genes during regeneration processes in adult organisms.

An increasing volume of literature describes the involvement of cell-cell communication pathways, such as the BMP and Wnt pathways, in common human diseases such as cancer and degenerative illnesses (Hardwick, 2008; Kim and Kim 2006; Massagué, 1998; Chen et al., 2004). For instance, the common sporadic form of colorectal cancer, familial adenomatous polyposis, results from mutations in adenomatous polyposis coli, which leads to aberrant Wnt pathway activity, and mutations in BMP pathway elements have been also found in several polyposis syndromes that predispose to colorectal cancer (Hardwick et al., 2008). Planarians may therefore also become a model of choice for addressing the molecular mechanisms by which these pathways could govern cell proliferation and why misregulation leads to the development of so many tumors.

5. Regeneration, Tumor Formation and Cancer

Tumors occur because of misregulation of the proliferative properties of cells leading to abnormal tissue growths. In cancers (malignant neoplasms), these uncontrolled proliferating cells acquire invasive properties and in the worst cases metastasize to other body locations. Since tumor and cancer development depend upon an initial uncontrolled cell proliferation, regenerative processes need to be tightly regulated to avoid tissue overgrowths and tumor formation. Interestingly, high regenerative capacity has been associated with striking resistance to the development of tumors (Brockes 1998).

Recently, Oviedo and Beane (2009) reviewed the relationship between regeneration and cancer in amphibians and planarians. In their excellent review, the authors discuss the apparently paradoxical situation in which regeneration may represent a source of abnormal growth while also preventing and correcting growth abnormalities.

Two hypotheses have been proposed to explain the relationship between regeneration and tumor formation: 1) tumors occur through impaired or incomplete regeneration, and 2) regeneration processes may bring the independent growth of tumors under control (Seilern-Aspang and Kratochwil, 1965). A good example of the resistance to tumor formation in regenerating urodele amphibians is found in Triturus cristatus (Seilern-Aspang and Kratochwil, 1965). These animals are able to regenerate their tail if amputated posterior to the last sacral vertebra; however, if amputation is done anterior to this point regeneration is absent. After injection of a mixture of the carcinogens benzpyrene and dibenzanthracene in the regenerative area of the tail (posterior to the last sacral vertebra), 9% of the animals developed tumors, this percentage increased to 15% when carcinogens were injected in the non-regenerative region (anterior to the last sacral vertebra).

More importantly, however, only 7% of the tumors that developed in the regenerative region shifted to infiltrative growth; in contrast, 90% of the tumors induced in the non-regenerative region gave rise to metastases (Seilern-Aspang and Kratochwil, 1965). Another example in which there is a clear relationship between regenerative potential and decreased tumorigenesis is the case of amphibian lens regeneration. During lens regeneration, differentiated dorsal cells of the iris dedifferentiate and provide a cellular source for regeneration. Application of carcinogenic molecules during lens regeneration results in normal regeneration that sometimes occurs alongside the development of tumors from the ventral iris, which does not have regenerative potential (reviewed in Oviedo and Beane, 2009).

Compared to amphibians, planarians do not appear to be quite so resistant to tumor development after treatment with carcinogens (Best and Morita, 1982; Schaeffer, 1993). Histology reveals the formation of a variety of different growths in planarians exposed to mammalian carcinogens, ranging from mammalian-like neoplasms to increased proliferation with some degree of differentiation and cellular organization (Schaeffer, 1993). A special case comes from the study of the planarian Dendrocoelum lacteum.

If amputated anterior to the pharynx, the trunk piece is able to regenerate a new head; however, if the amputation plane is posterior to the pharynx, the resulting tailpiece is unable to regenerate a head (Brondsted 1969). This is similar to the situation described above for tail regeneration in Triturus. In a study involving implantation of the carcinogenic compound Scarlet red in the regeneration-competent anterior part of Dendrocoelum, hyperplasia of different tissues was only observed in 2 out of 200 animals (Seilern-Aspang and Kratochwil, 1965). However, if Scarlet red was applied to the posterior region, which is not able to regenerate, 15 out of 200 animals developed infiltrating tumors. Those results suggest that the regeneration-competent region is somehow able to control the cell proliferation induced by the carcinogen, whereas such a regulatory mechanism may not be present in the non-regenerative part of the animal, thus leading to malignant growth. Remarkably, if an animal with a posterior tumor is then decapitated and forced to regenerate a new head, then those tumors either are assimilated or differentiate into several structures (Seilern-Aspang and Kratochwil, 1965).

As neoblasts are the only proliferative cells in planarians, and dedifferentiation of somatic cells has not been convincingly shown to occur in planarians, it seems plausible that transformed neoblasts are the source of planarian tumors. Recently, genes related to cancer have been identified in planarians, thus opening a door to the analysis of neoblast behavior and regulation in regeneration and tumor formation.

Several tumor suppressor genes, such as p53, Rb (retinoblastoma) and PTEN, have been identified in planarians (Oviedo et al., 2008; Pearson and Sánchez Alvarado, 2009). These genes are frequently mutated in a variety of human cancers. Rb also plays an important role during regeneration in amphibians. Following amputation in those animals, muscle cells dedifferentiate and re-enter the cell cycle (a key event for successful regeneration) in response to thrombin in culture by inhibiting Rb (Tanaka et al., 1997, 1999, Brockes, 1998).

In contrast, mouse myotubes are not able to phosphorylate Rb or re-enter the cell cycle after serum stimulation (Tam et al., 1995). A simple interpretation of this finding would be that the ability to regulate Rb could partially explain the plasticity and regenerative capacity of amphibian muscle cells. Interestingly, silencing of planarian PTEN homologues results in hyperproliferation of neoblasts, tissue disorganization, disruption of the basement membrane, tissue regression in front of the photoreceptors, and development of abnormal outgrowths, that resulting in the death of the animals (Oviedo et al., 2008).

These results indicate that planarian PTEN genes function as tumor suppressor gene in these animals. Further functional analyses of these and other cancer-related genes could help to clarify the relationship between regeneration and cancer. Planarians, therefore, can be used to study how regenerative tissues are able to regulate growth and control potential malignancies (Oviedo and Beane, 2009).

Conclusion

It is obvious that the study of stem cell biology and regeneration has direct applications in the field of regenerative medicine, as one of its main goals is to control the ‘in vitro’ differentiation of stem cells to the desired fate. In addition to controlling the fate of undifferentiated stem cells, the newly differentiated cells must be properly organized to form a complete tissue that is correctly patterned. The future success of regenerative medicine will therefore depend not only on a deeper understanding of the biology of stem cells but also on the study of pattern formation. Planarians are already an organism of choice for molecular studies of patterning and regeneration —the coming years are likely to see them rapidly gain ground as a model system for research into regenerative medicine.

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References

Adell, T.; Marsal, T. and Saló, E. (2008). Planarian GSK3s are involved in neural regeneration. *Dev. Genes Evol. 218*: 89-103.

Adell, T.; Saló, E.; Boutros, M. and Bartscherer, K. (2009a). Smed-Evi/Wntless is required for beta-catenin-dependent and –independent processes during planarian regeneration. *Development 136*: 905-910.

Adell, T.; Cebrià, F. and Saló, E. (2009b). Gradients in planarian regeneration and homeostasis. Cold Spring Harb. Perspect. Biol. doi: 10.1101/cshperspect.a000505.

Agata, K. and Watanabe, K. (1999). Molecular and cellular aspects of planarian regeneration. *Semin. Cell. Dev. Biol. 10:* 377-383.

Agata, K. and Umesono, Y. (2008). Brain regeneration from pluripotent stem cells in planarian. *Philos. Trans. R. Soc. Lond. B Biol. Sci. 363*: 2071-2078.

Agata, K.; Soejima, Y.; Kato, K.; Kobayashi, C.; Umesono, Y. and Watanabe, K. (1998). Structure of the planarian central nervous system (CNS) revealed by neuronal cell markers. *Zoolog. Sci. 15*: 433-440.

Agata, K.; Tanaka, C.; Kobayashi, C.; Kato, K. and Saitoh, Y. (2003). Intercalary regeneration in planarians. *Dev. Dyn. 226*: 308-316.

Araújo, S.J. and Tear, G. (2003). Axon guidance mechanisms and molecules: lessons from invertebrates. *Nat. Rev. Neurosci. 4*: 910-922.

Arendt D. and Nübler-Jung K. (1994). Inversion of dorsoventral axis?. *Nature. 371:*26.

Asada, A.; Orii, H.; Watanabe, K. and Tsubaki, M. (2005). Planarian peptidylglycinehydroxylating monooxygenase, a neuropeptide processing enzyme, colocalizes with cytochrome b561 along the central nervous system. *FEBS J. 272*: 942-955.

Ashe H.L. and Briscoe J. (2006). The interpretation of morphogen gradients. *Development. 133:*385-394.

Baguñà, J. and Ballester, R. (1978). The nervous system in planarians: peripheral and gastrodermal plexuses, pharynx innervation, and the relationship between central nervous system structure and the acoelomate organization. *J. Morph. 155*: 237-252.

Baguñà, J. and Romero, R. (1981). Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians Dugesia mediterranea and Dugesia tigrina. *Hydrobiologia, 84*: 181-194.

Baguñà, J. (1973). Estudios citotaxonomicos, ecologicos, e histofisiologia de la regulacion morfogenetica durante el crecimiento y la regeneracion de la raza asexuada de la planaria Dugesia mediterranea n. sp. (Turbellaria, Trioladida, Paludicola). *PhD Thesis*, University of Barcelona.

Baguñà, J. (1974). Dramatic mitotic response in planarians after feeding, and a hypothesis for the control mechanism. *J. Exp. Zool*. *190*: 117-22.

Baguñà, J. (1976). Mitosis in the intact and regenerating planarian Dugesia mediterranea n. sp. I. Mitotic studies during growth, feeding and starvation. *J. Exp .Zool.195*: 65-80.

Baguñà, J.; Saló, E. and Auladell, C. (1989). Regeneration and pattern formation in planarians III. Evidence that neoblasts. Are totipotent stem cells and the source of blastema cells. *Development 107*: 77-86.

Behra, M.; Bradsher, J.; Sougrat, R.; Gallardo, V.; Allende, M.L. and Burgess, S.M. (2009). Phoenix is required fro mechanosensory hair cell regeneration in the zebrafish lateral line. *PLoS Genet. 5*: e1000455.

Bertrand, S.; Somorjai, I.; Garcia-Fernandez, J.; Lamoneire, T. and Escriva, H. (2009). FGFRL1 is a neglected putative actor of the FGF signalling pathway present in all major metazoan phyla. *BMC Evolutionary Biology 9*: 226.

Best, J.B. and Morita, M. (1982). Planarians as a model system for in vitro teratogenesis studies. *Teratogenesis, Carcinogenesis and Mutagenesis 2*: 277-291.

Beyeler, M. and Trueb, B. (2006). Fgfrl1, a fibroblast growth factor receptor-like gene, is found in the cephalochordate Branchiostoma floridae but not in the urochordate Ciona intestinalis. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 145*: 43-49.

Bowen, E.D.; Ryder, T.A. and Dark, C. (1976). The effects of starvation on the planarian worm Polycelis tenuis Iijima. *Cell Tissue Res. 169*: 193-209.

Brennecke, J.; Hipfner, D.R.; Stark, A., Russell, R.B. and Cohen, S.M. (2003). Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulate the proapoptotic gene hid in Drosophila. *Cell 113*: 25-36.

Brickes, J.P. (1998). Regeneration and cancer. Biochimica et Biophysica Acta 1377: M1-M11.

Brøndsted, H.V. (1942). Further experiments on regeneration - problems in planarians. *Det Kgl Danske Videnskabernes Selskab, Biol. Medd.14*: 1-27.

Brøndsted, H.V. (1969). *Planarian regeneration*. Oxford Pergamon Press.

Brookmeyer, R.; Johnson, E.; Ziegler-Graham, K. and Arrighi H.M.. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement. 3*: 186-191.

Brose, K.; Bland, K.S:; Wang, K.H.; Arnott, D.; Henzel, W.; Goodman, C.S.; Tessier-Lavigne, M. and Kidd, T. (1999). Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell 96*: 795-806.

Busch, S.A. and Silver, J. (2007). The role of extracellular matrix in CNS regeneration. *Curr. Op. Neurobiol. 17*: 120-127.

Guder C.; Philipp I.; Lengfeld T.; Watanabe H.; Hobmayer B. and Holstein T.W. (2006). The Wnt code: cnidarians signal the way. *Oncogene 25*: 7450–7460.

Carthew, R.W. and Sontheimer, E.J. (2009). Origins and mechanisms of miRNAs and siRNAs. *Cell 136*: 642-655.

Cebrià, F. (2007). Regenerating the central nervous system: how easy for planarians! *Dev. Genes Evol. 217*: 733-748.

Cebrià, F. (2008). Organization of the nervous system in the model planarian Schmidtea mediterranea: an immunocytochemical study. *Neurosci. Res. 61*: 375-384.

Cebrià, F. and 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 132*: 3691-3703.

Cebrià, F. and Newmark, P.A. (2007). Morphogenesis defects are associated with abnormal nervous system regeneration after roboA RNAi in planarians. *Development 134*: 833-837.

Cebrià, F.; Kobayashi, C.; Umesono, Y.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T.; Itoh, M.; Taira, M.; Sánchez-Alvarado, A. and Agata, K. (2002c). FGFR-related gene nou-darake restricts brain tissues to the head region of planarians. *Nature 419*: 620-624.

Cebrià, F.; Kudome, T.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T. and Agata, K. (2002b). The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system. *Mech. Dev. 116*: 199-204.

Cebrià, F.; Nakazawa, M.; Mineta, K.; Ikeo, K.; Gojobori, T. and Agata, K. (2002a). Dissecting planarian central nervous system regeneration by the expression of neural-specific genes. *Dev. Growth Differ. 44*: 135-146.

Cebrià, F.; Vispo, M.; Newmark, P.A:; Bueno, D. and Romero, R. (1997). Myocyte differentiation and body wall musculatura in the planarian Girardia tigrina. *Dev. Genes Evol. 207*: 306-316.

Chan, S.S.; Zheng, H.; Su, M.W.; Wilk, R.; Killeen, M.T.; Hedgecock, E.M. and Culotti, J.G. (1996). UNC-40, a C.elegans homolog of DCC (Deleted in Colorectal Cancer), is required in motile cells responding to UNC-6 netrin cues. *Cell 87*: 187-195.

Chen G.; Shukeir N.; Potti A.; Sircar K.; Aprikian A.; Goltzman D. and Rabbani S.A.(2004). Up-regulation of Wnt-1 and beta-catenin production in patients with advanced metastatic prostate carcinoma: potential pathogenetic and prognostic implications. *Cancer 101:*1345-1356.

Cheng, L.C.; Pastrana, E.; Tavazoie, M. and Doetsch, F. (2009). miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat. Neurosci. 12*: 399-408.

Child, C.M. (1914). Asexual breeding and prevention of senescence in Planaria velata. *Biol. Bull.*, 26: 286-293.

Cimmino, A.; Calin, G.A.; Fabbri, M.; Iorio, M.V.; Ferracin, M.; Shimizu, M.; Wojcik, S.E.; Aqeilan, R.I.; Zupo, S.; Dono, M.; Rassenti, L.; Alder, H.; Volinia, S.; Liu, C.G.; Kipps, T.J.; Negrini, M. and Croce, CM. (2005). miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl. Acad. Sci. USA 102*: 13944-13949.

Cogswell, J.P.; Ward, J.; Taylor, I.A.; Waters, M.; Shi, Y.; Cannon, B.; Kelnar, K.; Kemppainen, J.; Brown, D.; Chen, C:; Prinjha, R.K.; Richardson, J.C.; Saunders, A.M.; Roses, A.D. and Richards, C.A. (2008). Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *J. Alzheimers Dis. 14*: 27-41.

Colamarino, S.A. and Tessier-Lavigne, M. (1995). The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. *Cell 81*: 621-629.

Coward, S. J. (1974). Chromatoid Bodies in Somatic Cells of the Planarian: Observations on Their Behavior during Mitosis. *The Anatomical Record*, 180: 533-546.

Crist, C.G.; Montarras, D.; Pallafacchina, G.; Rocancourt, D.; Cumano, A.; Conway, S.J. and Buckingham, M. (2009). Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. *Proc. Natl. Acad. Sci. USA 106*: 13383-13387.

De Robertis E.M. and Kuroda H. (2004). Dorsal–ventral patterning and neural induction in Xenopus embryos. *Annu. Rev. Cell. Dev. Biol. 20*:285-308.

Dickson, B.J. and Gilestro, G.F. (2006). Regulation of commissural pathfinding by slit and its Robo receptors. *Annu. Rev. Cell Dev. Biol. 22*: 651-675.

Dubois, F. (1949). Contribution á l'ètude de la migration des cellules de règènèration chez les Planaires dulcicoles. *Bull. Biol. Fr. Belg. 83*: 213-283.

Eisenhoffer, G.T.; Kang, H. and Sánchez-Alvarado, A. (2008). Molecular analysis of stem cells and their descendents during cell turnover and regeneration in the planarian Schmidtea mediterranea. *Cell Stem Cell 3*: 327-339.

Feng X.H. and Derynck R. (2005). Specificity and versatility in TGF-beta signaling through Smads. *Annu. Rev. Cell Dev. Biol. 21*: 659–693.

Flynt, A.S. and Lai, E.C. (2008). Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nat. Rev. Genet. 9*: 831-842.

Forstemman, K.; Tomari, Y.; Du, T.; Vagin, V.V.; Denli, A.M.; Bratu, D.P.; Klattenhoff, C.; Theurkauf, W.E. and Zamore, P.D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires loquacious, a double-stranded rna-binding domain protein. *PLoS Biol 3*: e236.

Friedländer, M.R.; Adamidi, C.; Han, T.; Lebedeva, S.; Isenbarger, T.A.; Hirst, M.; Marra, M.; Nusbaum, C.; Lee, W.L.; Jenkin, J.C.; Sánchez-Alvarado, A.; Kim, J.K. and Rajewsky, N. (2009). High-resolution profiling and discovery of planarian small RNAs. Proc. Natl. Acad. Sci. USA 106: 11546-11551.

Fuerer C.; Nusse R. and Ten Berge D. (2008). Wnt signalling in development and disease. *EMBO Rep. 9:*134-8.

Fusaoka, E.; Inoue, T.; Mineta, K.; Agata, K. and Takeuchi, K. (2006). Structure and function of primitive immunoglobulin superfamily neural cell adhesión molecules: a lesson from studies on planarian.*Genes Cells 11*: 541-555.

González-Estévez, C., Felix, D.A.; Aboobaker, A.A. and Saló, E. (2007). Gtdap-1 promotes autophagy and is required for planarian remodeling during regeneration and starvation. *Pro.c Natl. Acad. Sci. U S A104*: 13373-13378.

González-estévez, C.; Arseni, V.; Thambyrajah, R.S.; Felix, D.A. and Aboobaker, A.A. (2009). Diverse miRNA spatial expression patterns suggest important roles in homeostasis and regeneration in planarians. *Int. J. Dev. Biol. 53*: 493-505.

Gremigni V.; Miceli, C. and Puccinelli, I. (1980). On the role of germ cells in planarian regeneration. A karyological investigation. *J. Embryol. Exp. Morph*. *55*: 53-63.

Gremigni V.; Nigro, M. and Puccinelli, I. (1982). Evidence of male germ cell redifferentiation into female germ cells in planarian regeneration. *J. Embryol. Exp. Morphol*. *70*: 29-36.

Gremigni, M. (1988). Planarian Regeneration: An Overview of Some Cellular Mechanisms. *Zool. Sci.5*: 1153-1163.

Guan, K.L. and Rao, Y. (2003). Signalling mechanisms mediating neuronal responses to guidance cues. *Nat. Rev. Neurosci. 4*: 941-956.

Guo, T.; Peters, A.H. and Newmark, P.A. (2006). A Bruno-like gene is required for stem cell maintenance in planarians. *Dev. Cell11*: 159-69.

Gurley, K.A.; Rink, J.C. and Sánchez-Alvarado, A. (2008). Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science 319*: 323-327.

Hall, C.; Flores, M.V.; Murison, G.; Crosier, K. and Crosier, P. (2006). An essential role for zebrafish Fgfrl1 during gill cartilage development. *Mech. Dev. 123*: 925-940.

Handberg-Thorsager, M.; Fernández-Taboada, E. and Saló, E. (2008). Stem cells and regeneration in planarians. *Front. Biosci*. 13: 6374-6394.

Harris, R.; Sabatelli, L.M. and Seeger, M.A. (1996). Guidance cues at the Drosophila CNS midline: identification and characterization of two Drosophila Netrin/UNC-6 homologs. *Neuron 17*: 217-228.

Hatfield, S.D.; Shcherbata, H.R.; Fischer, K.A.; Nakahara, K.; Carthew, R.W. and Ruohola-Baker, H. (2005). Stem cell division is regulated by the microRNA pathway. *Nature 435*: 974-978.

Hayashi, S.; Itoh, M.; Taira, S.; Agata, K. and Taira, M. (2004). Expression patterns of Xenopus FGF receptor-like 1/nou-darake in early Xenopus development resemble those of planarian nou-darake and Xenopus FGF8. *Dev. Dyn. 230*: 700-707.

Hayashi, T.; Asami, M.; Higuchi, S.; Shibata, N. and Agata, K. (2006). Isolation of planarian X-ray-sensitive stem cells by fluorescence-activated cell sorting. *Dev. Growth Differ*. *48*: 371-80.

Hébert, S.S.; Horré, K.; Nicolaï, L.; Papadopoulou, A.S:; Mandemakers, W.; Silahtaroglu, A.N.; Kauppinen, S.; Delacourte, A. and De Strooper B. (2008). Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta –secretase expression. *Proc. Natl. Acad. Sci. USA* 105: 6415-6420.

Higuchi, S.; Hayashi, T.; Hori, I.; Shibata, N.; Sakamoto, H. and Agata, K. (2007). Characterization and categorization of fluoresecence activated cell sorted planarian stem cells by ultrastructural analysis. *Dev Growth Differ. 49*: 571-581.

Higuchi, S.; Hayashi, T.; Tarui, H.; Nishimura, O.; Nishimura, K.; Shibata, N.; Sakamoto, H. and Agata, K. (2008). Expression and functional analysis of musashi-like genes in planarian CNS regeneration. *Mech. Dev. 125*: 631-645.

Hogan B.L.; Blessing M.; Winnier G.E.; Suzuki N. and Jones C.M. (1994). Growth factors in development: the role of TGF-beta related polypeptide signalling molecules in embryogenesis. *Dev Suppl.* 53-60.

Holland L.Z. (2002). Heads or tails? Amphioxus and the evolution of anterior-posterior patterning in deuterostomes. *Dev. Biol.241*:209-28.

Hong, K.; Hinck, L.; Nishiyama, M.; Poo, M.M.; Tessier-Lavigne, M. and 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 97*: 927-941.

Houbaviy, H.B.; Murray, M.F. and Sharp, P.A. (2003). Embryonic stem cell-specific microRNAs. *Dev. Cell 5*: 351-358.

Huang S.; Shetty P.; Robertson S.M. and Lin R. (2007). Binary cell fate specification during C. elegans embryogenesis driven by reiterated reciprocal asymmetry of TCF POP-1 and its coactivator beta-catenin SYS-1. *Development 134*:2685-2695.

Hutchison, E.R.; Okun, E. and Mattson, M.P. (2009). The therapeutic potential of microRNAs in nervous system damage, degeneration and repair. *Neuromolecular Med. 11*: 153-161.

Hyman, L.H. (1951). *The invertebrates: Platyhelminthes and Rhynchocoela*. McGraw-Hill, New York.

Ibañes M. and Izpisúa Belmonte J.C. (2008). Theoretical and experimental approaches to understand morphogen gradients. *Mol. Syst. Biol. 4*:176.

Iglesias, M.; Gómez-Skarmeta, J.L.; Saló, E. and Adell, T. (2008). Silencing of Smed-betacatenin1 generates radial-like hypercephalized planarians. *Development 135*: 1215-1221.

Inoue, T.; Hayashi, T.; Takechi, K. and Agata, K. (2007). Clathrin-mediated endocytic signals are required for the regeneration of, as well as homeostasis, in the planarian CNS. *Development 134*: 1679-1689.

Ishii, N.; Wadsworth, W.G.; Stern, B.D.; Culotti, J.G. and Hedgecock, E.M. (1992). UNC-6, a laminin related protein, guides cell and pioneer axon migrations in C. elegans. *Neuron 9*: 873-881.

Hardwick J.C.; Kodach L.L.; Offerhaus G.J. and van den Brink G.R.(2008). Bone morphogenetic protein signalling in colorectal cancer. *Nat. Rev. Cancer 8*: 806-812.

Kapsimali, M.; Kloosterman, W:P.; de Bruijn, E.; Rosa, F.; Plasterk, R.H. and Wilson, S.W. MicroRNAs show a wide diversity of expresión profiles in the developing and mature central nervous system. *Genome Biol. 8*: R173.

Keino-Masu, K.; Masu, M.; Hinck, L.; Leonardo, E.D.; Chan, S.S.; Culotti, J.G. and Tessier-Lavigne, M. (1996). Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. *Cell 87*: 175-185.

Keleman, K. and Dickson, B.J. (2001). Short- and long-range repulsion by the Drosophila Unc5 netrin receptor. *Neuron 32*: 605-617.

Kennedy, T.E.; Serafini, T.; de la Torre, J.R. and Tessier-Lavigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell 78*: 425-435.

Kidd, T.; Bland, K.S. and Goodman, C.S. (1999). Slit is the midline repellent for the Robo receptor in Drosophila. *Cell 96*: 785-794.

Kiecker C. and Niehrs C. (2001). A morphogen gradient of Wnt/beta-catenin signaling regulates anteroposterior neural patterning in Xenopus. *Development 128*:4189-4201.

Kim I.Y.and Kim S.J. (2006). Role of bone morphogenetic proteins in transitional cell carcinoma cells. *Cancer Lett. 241*:118-23.

Kitisin K.; Saha T.; Blake T.; Golestaneh N.; Deng M.; Kim C.; Tang Y.; Shetty K.; Mishra B. and Mishra L. (2007). Tgf-Beta signaling in development. *Sci STKE.399:*cm1.

Kobayashi, C.; Saito, Y.; Ogawa, K. and Agata, K. (2007). Wnt signaling is required for antero-posterior patterning of the planarian brain. *Dev. Biol. 306*: 714-724.

Koinuma, S.; Umesono, Y.; Watanabe, K. and Agata, K. (2003). The expression of planarian brain factor homologs, DjFoxG and DjFoxD. *Gene Expr. Patterns 3*: 21-27.

Kragl, M.; Knapp, D.; Nacu, E.; Khattak, S.; Maden, M.; Epperlein, H.H. and Tanaka, E.M. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature 460*: 60-65.

Lagha, M.; Sato, T.; Bajard, L.; Daubas, P.; Esner, M.; Montarras, D.; Relaix, F. and Buckingham, M. (2008). Regulation of skeletal muscle ítem cell behavior by Pax3 and Pax7. *Cold Spring Harb. Symp. Quant. Biol. 73*: 307-315.

Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W. and Tuschl, T. (2002). Identification of tissue-specific microRNAs from mouse. *Curr. Biol. 12*: 735-739.

Lee P.N.; Pang K.; Matus D.Q. and Martindale M.Q. (2006). A WNT of things to come: evolution of Wnt signaling and polarity in cnidarians. *Semin. Cell. Dev. Biol. 17:*157-167.

Lee, R.C.; Feinbaum, R.L. and Ambros, V. (1993). The C.elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell 75*: 843-854.

Lei, P.; Li, Y.; Chen, X.; Yang, S. and Zhang, J. (2009). Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury. *Brain Res. 1284*: 191-201.

Lentz, T.L. (1968). Primitive nervous sytem. Yale University Press, New Have, CT.

Little S.C. and Mullins M.C. (2006). Extracellular modulation of BMP activity in patterning the dorsoventral axis. *Birth Defects Res. C. Embryo Today. 78*:224-242.

Long, H.; Sabatier, C.; Ma, L.; Plump, A.; Yuan, W.; Ornitz, D.M.; Tamada, A.; Murakami, F.; Goodman, C.S. and tessier-Lavigne, M. (2004). Conserved roles for slit and robo proteins in midleine commissural axon guidance. *Neuron 42*: 213-223.

López-Schier, H. and Hudspeth, A.J. (2006). A two-step mechanism underlies the planar polarization of regenerating sensory hair cells. *Proc. Natl. Acad. Sci. USA 103*: 18615-18620.

Lu, Y.C.; Smielewska, M.; Palakodeti, D.; Lovci, M.T.; Aigner, S.; Yeo, G.W. and Graveley, B.R. (2009). Deep sequencing identifies new and regulated microRNAs in Schmidtea mediterranea. *RNA 15*: 1483-1491.

Mann R.S. and Morata G. (2000). The developmental and molecular biology of genes that subdivide the body of Drosophila. *Annu. Rev. Cell. Dev. Biol.16:*243–271.

Mannini, L.; Rossi, L.; Deri, P.; Gremigni, V.; Salvetti, A.; Saló, E. and Batistoni, R. (2004). *Djeyes absent (Djeya)* controls prototypic planarian eye regeneration by cooperating with the transcription factor *Djsix-1*. *Dev. Biol. 269*: 346-359.

Marsal, M.; Pineda, D. and Saló, E. (2003). Gtwnt-5 a member of the wnt family expressed in a subpopulation of the nervous system of the planarian *Girardia tigrina*. *Gene Expr. Patterns 3*: 489-495.

Massagué J.(1998). TGF-beta signal transduction. *Annu. Rev. Biochem. 67:* 753-791.

Mineta, K.; Nakazawa, M.; Cebrià, F.; Ikeo, K.; Agata, K. and Gojobori, T. (2003). Origin and evolutionary process of the CNS elucidated by comparative genomics analysis of planarian ESTs. *Proc. Natl. Acad. Sci. USA 100*: 7666-7671.

Mitchell, K.J.; Doyle, J.L.; Serafini, T.; Kennedy, T.E.; Tessier-Lavigne, M.; Goodman, C.S: and Dickson, B.J. (1996). Genetic analysis of netrin genes in Drosophila: Netrins guide CNS commissural axons and peripheral motor axons. *Neuron 17*: 203-215.

Molina, M.D.; Saló, E. and Cebrià, F. (2007). The BMP pathway is essential for re-specification and maintenance of the dorsoventral axis in regenerating and intact planarians. *Dev. Biol. 311*: 79-94.

Molina, M.D.; Saló, E. and Cebrià, F. (2009). Expresión pattern of the expanded noggin gene family in the planarian Schmidtea mediterranea. *Gene Expr. Patterns* 9: 246-253.

Montarras, D.; Morgan, J.; Collins, C.; Relaix, F.; Zaffran, S.; Cumano, A.; Partridge, T. and Buckingham, M. (2005). Direct isolation of satellite cells for skeletal muscle regeneration. *Science 309*: 2064-2067.

Naguibneva, I.; Ameyar-Zazoua, M.; Polesskaya, A.; Ait-Si-Ali, S.; Groisman, R.; Souidi, M.; Cuvellier, S. and Harel-Bellan, A. (2006). The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. *Nat. Cell Biol. 8*: 278-284.

Nakamura T.; Mito T.; Tanaka Y.; Bando T.; Ohuchi H. and Noji S. (2007). Involvement of canonical Wnt/Wingless signaling in the determination of the positional values within the leg segment of the cricket *Gryllus bimaculatus. Develop. Growth Differ. 49:* 79-88.

Nakazawa, M.; Cebrià, F.; Mineta, K.; Ikeo, K.; Agata, K. and Gojobori, T. (2003). Search for the evolutionary origin of the brain: planarian brain characterized by microarray. *Mol. Biol. Evol. 20*: 784-791.

Newmark, P.A. and Sánchez-Alvarado, A. (2000). Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. *Dev. Biol. 220*: 142-53.

Newmark, P.A. and Sánchez-Alvarado, A. (2000). Not your father's planarian: a classic model enters the era of functional genomics. *Nat. Rev. Genet. 3*: 210-219.

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Sano, S.; Yoshimoto, K.; Inden, M.; Takata, K.; Taniguchi, T.; Shimohama, S. and Agata,K. (2007b). Reconstruction of dopaminergic neural network and locomotion function in planarian regenerates. *Dev. Neurobiol. 67*: 1059-1078.

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Yoshimoto, K.; Takeuchi, K.; Taniguchi, T. and Agata, K. (2007a). Identification and distribution of tryptophan hydroxylase (TPH)-positive neurons in the planarian *Dugesia japonica*. *Neurosci. Res. 59*: 101-106.

Nishimura, K.; Kitamura, Y.; Inoue, T.; Umesono, Y.; Yoshimoto, K.; Taniguchi, T. and Agata, K. (2008). Characterization of tyramine beta-hydroxylase in planarian Dugesia japonica: cloning and expression. Neurchem. Int. 53: 184-192.

Nishimura, K.; Kitamura, Y.; Umesono, Y.; Takeuchi, K.; Takata, K.; Taniguchi, T. and Agata, K. (2008a). Identification of glutamic acid decarboxylase gene and distribution of GABAergic nervous system in the planarian *Dugesia japonica*. Neuroscience 153: 1103-1114.

Nogi, T. and Levin, M. (2005). Characterization of innexin gene expression and functional roles of gap-junctional communication in planarian regeneration. *Dev. Biol. 287*: 314-335.

O'Donnell, M.; Chance, R.K. and Bashaw, G.J. (2009). Axon growth and guidance: receptor regulation and signal transduction. *Ann. Rev. Neurosci. 32*: 383-412.

Ogawa, K.; Ishihara, S.; Saito, Y.; Mineta, K.; Nakazawa, M.; Ikeo, K.; Gojobori, T.; Watanabe, K. and Agata, K. (2002a). Induction of a noggin-like gene by ectopic DV interaction during planarian regeneration. *Dev. Biol. 250*: 59-70.

Ogawa, K.; Kobayashi, C.; Hayashi, T.; Orii, H.; Watanabe, K. and Agata, K. (2002b). Planarian fibroblast growth factor receptor homologs expressed in stem cells and cephalic ganglions. *Dev. Growth Differ. 44*: 191-204.

Okano, H.; Kawahara, H.; Toriya, M.; Nakao, K.; Shibata, S. and Imai, T. (2005). Function of RNA-binding protein Musashi-1 in stem cells. *Exp. Cell Res. 306*: 349-356.

Orii H. and Watanabe K. (2007). Bone morphogenetic protein is required for dorso-ventral patterning in the planarian Dugesia japonica. *Dev. Growth Differ. 49:* 345-349.

Orii, H.; Sakurai, T. and Watanabe, K. (2005). Distribution of the stem cells (neoblasts) in the planarian Dugesia japonica. *Dev. Genes Evol. 215*: 143-57.

Oviedo, N.J. and Beane, W.S. (2009). Regeneration: the origin of cancer or a possible cure? *Sem. Cell Dev. Biol. 20*: 557-564.

Oviedo, N.J. and Levin, M. (2007). Smedinx-11 is a planarian ítem cell gap junction required for regeneration and homeostasis. *Development 134*: 3121-3131.

Oviedo, N.J.; Pearson, B.J.; Levin, M. and Sánchez-Alvarado, A. (2008). Planarian PTEN homologs regulate stem cells and regeneration through TOR signaling. *Dis. Model Mech. 1*: 131-143.

Packer, A.N.; Xing, Y.; Harper, S.Q.; Jones, L. and Davidson, B.L. (2008). The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *J. Neurosci. 28*: 14341-14346.

Palakodeti, D.; Smielewska, M. and Graveley, B.R. (2006). MicroRNAs from the planarian *Schmidtea mediterranea*: A model system for stem cell biology. *RNA 12*: 1640-1649.

Pearson, B.J. and Sánchez-Alvarado, A. (2008). Regeneration, ítem cells and the evolution of tumor suppressión. *Cold Spring Harb. Symp. Quant. Biol. 73*: 565-572.

Petersen C.P. and Reddien P.W. (2009). A wound-induced Wnt expression program controls planarian regeneration polarity. *Proc. Natl. Acad. Sci. U S A 106:*17061-17066.

Petersen, C.P. and Reddien, P.W. (2008). Smed-betacatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. *Science 319*: 327-330.

Pineda, D. and Saló, E. (2002). Planarian Gtsix3, a member of the Six/so gene family, is expressed in brain branches but not in eye cells. *Gene Expr. Patterns 2*: 169-173.

Pineda, D.; Rossi, L.; Batistoni, R.; Salvetti, A.; Marsal, M.; Gremigni, V.; Fallen, A.; González-Linares, J.; Deri, P. and Saló, E. (2002). The genetic network of prototypic planarian eye regeneration is Pax6 independent. Development 129: 1423-1434.

Poss, K.D.; Wilson, L.G. and Keating, M.T. (2002). Heart regeneration in zebrafish. *Science 298*: 2188-2190.

Ramón y Cajal, S. (1928). *Degeneration and regeneration of the nervous system*. Oxford University Press, London.

Raya,A.; Koth, C.M.; Büscher, D.; Kawakami, Y.; Itoh, T.; Raya, R.M.; Sternik, G.; Tsai, H.J.; Rodríguez-Esteban, C. and Izpisúa-Belmonte, J.C. (2003). Activation of Notch signaling pathway precedes heart regeneration in zebrafish. *Proc. Natl. Acad. Sci. USA Suppl1*: 11889-11895.

Reddien P.W.; Bermange A.L.; Kicza A.M. and Sánchez Alvarado A. (2007). BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. *Development 134*: 4043-4051.

Reddien, P.W. and Sánchez-Alvarado, A. (2004). Fundamentals of planarian regeneration. *Annu. Rev. Cell Dev. Biol. 20*: 725-757.

Reddien, P.W.; Bermange, A.L.; Murfitt, K.J.; Jennings, J.R. and Sánchez-Alvarado, A. (2005b). Identification of genes needed for regeneration, stem cell function and tissue homeostasis by systematic gene perturbation in planaria. *Dev. Cell 8*: 635-649.

Reddien, P.W.; Oviedo, N.J.; Jennings, J.R.; Jenkin. J.C. and Sánchez-Alvarado, A. (2005a). SMEDWI-2 is a PIWI-like protein that regulates planarian ítem cells. *Science 310*: 1327-1330.

Redell, J.B.; Liu, Y. and Dash, PK. (2009). Traumatic brain injury alters expression of hippocampal microRNAs potential regulators of multiple pathophysiological processes. *J. Neurosc. Res. 87*: 1435-1448.

Reinhart, B.J.; Slack, F.J.; Basson, M.; Pasquinelli, A.E.; Bettinger, J.C.; Rougvie, A.E., Horvitz, H.R. and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature 403*: 901-906.

Reuter, M.; Gustafsson, M.K.S.; Mäntylä, K. and Grimmelikhuijzen, C.J.P. (1996). The nervous system of Tricladida. III. Neuroanatomy of DEndrocoelum lacteum and Polycelis tenuis (Plathelminthes, Paludicola): an immunocytochemical study. *Zoomorphology 116*: 111-122.

Reuter, M.; Gustafsson, MKS.; Sheiman, I.M.; Terenina, N.; Halton, D.W.; Maule, A.G. and Shaw, C. (1995). The nervous system of Tricladida. II. Neuroanatomy of Dugesia tigrina (Paludicola, Dugesiidae): an immunocytochemical study. *Invert. Neurosc. 1*: 133-143.

Reya T. and Clevers H. (2005). Wnt signalling in stem cells and cancer.*Nature 434:* 843-50.

Rieckmann, T.; Zhuang, L.; Flück, C.E. and Trueb, B. (2009). Characterization of the first FGFRL1 mutation identified in a craniosynostosis patient, *Biochim. Biophys. Acta 1792*: 112-121.

Rieger, R.M.; Tyler, S.; Smith, J.P.S. III and Rieger, G.E. (1991). Platyhelminthes: Turbellaria. In: Harrison F.W., Bogitsh B.J. (eds*) Microscopic anatomy of invertebrates*, vol. 3. Wiley-Liss, New York.

Rink J.C.; Kyle A.; Gurley K.A.; Elliott S.A. and Sánchez Alvarado A. (2009). Planarian Hh Signaling Regulates Regeneration Polarity and Links Hh Pathway Evolution to Cilia. *Science* 22 October 2009: 1178712v1.

Rossi, L.; Deri, P.; Andreoli, I.; Gremigni, V.; Salvetti, A. and Batistoni, R. (2003). Expresión of DjXnp, a novel member of the SNF2-like ATP-dependent chromatin remodelling genes, in intact and regenerating planarians. *Int. J. Dev. Biol.* 47: 293-298.

Rossi, L.; Salvetti, A.; Lena, A.; Batistoni, R.; Deri, P.; Pugliesi, C.; Loreti, E. and Gremigni, V. (2006). DjPiwi-1, a member of the PAZ-Piwi gene family, defines a subpopulation of planarian stem cells. *Dev. Genes Evol. 216*: 335-346.

Rossi, L.; Salvetti, A.; Marincola, F.M.; Lena, A.; Deri, P.; Mannini, L.; Batistoni, R.; Wang, E. and Gremigni, V. (2007). Deciphering the molecular machinery of stem cells: a look at the neoblast gene expression profile. *Genome Biol. 8*: R62.

Rothberg, J.M.; Hartley, D.A.; Walther, Z. and Artavanis-Tsakonas, S. (1988). Slit: an EGF-homologous locus of D.melanogaster involved in the development of the embryonic central nervous system. *Cell 55*: 1047-1059.

Rothberg, J.M.; Jacobs, J.R.; Gooman, C.S. and Artavanis-Tsakonas, S. (1990). Slit: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. *Genes Dev. 4*: 2169-2187.

Saló E. and Baguñà, J. (1985). Proximal and distal transformation during intercalary regeneration in the planarian Dugesia(S)mediterranea. *Roux. Arch. Dev. Biol.194* : 364-368.

Saló, E. and Baguñà, J. (1984). Regeneration and pattern formation in planarians. I. The pattern of mitosis in anterior and posterior regeneration in Dugesia (G) tigrina, and a new proposal for blastema formation. *J. Embryol. Exp. Morphol. 83*: 63-80.

Saló, E. (2006). The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). *BioEssays 28*: 546-559.

Salvetti, A.; Rossi, L.; Deri, P. and Batistoni, R. (2000). An MCM2-related gene is expressed in proliferating cells of intact and regenerating planarians. *Dev. Dyn. 218*: 603-614.

Salvetti, A.; Rossi, L.; Lena, A.; Batistoni, R.; Deri, P.; Rainaldi, G.; Locci, M.T.; Evangelista, M. and Gremigni, V. (2005). DjPum, a homologue of Drosophila Pumilio, is essential to planarian stem cell maintenance. *Development132*: 1863-1874.

Sánchez-Alvarado, A. and Tsonis, P.A. (2006). Bridging the regeneration gap: genetic insights from diverse animal models. *Nature Reviews Genetics 7*: 873-884.

Sánchez-Alvarado, A. (2000). Regeneration in the metazoans: why does it happen? *BioEssays 22*: 578-590.

Santos, F.V. (1929). Studies on transplantation in planarian. *Biol. Bull.57*: 188-197.

Sato, K.; Shibata, N.; Orii, H.; Amikura, R.; Sakurai, T.; Agata, K.; Kobayashi, S. and Watanabe, K. (2006). Identification and origin of the germline stem cells as revealed by the expression of nanos-related gene in planarians. *De.v Growth Differ. 48*: 615-628.

Schaeffer, D.J. (1993). Planarians as a model system for in vivo tumorigenesis studies. *Ecotoxicology and Environmental Studies 25*: 1-8.

Schier A.F. and Talbot W.S. (2005). Molecular genetics of axis formation in zebrafish. *Annu. Rev. Genet. 39*: 561-613.

Schilt J.(1970). Induction expèrimentale d'excroissances par des greffes hétéropolaires chez la planaire Dugesia lugubris O. Schimdt. *Ann Embryol Morphogenet. 3:* 93-106.

Schneider S.Q. and Bowerman B. (2007). beta-Catenin asymmetries after all animal/vegetal-oriented cell divisions in *Platynereis dumerilii* embryos mediate binary cell-fate specification. *Dev. Cell. 13*: 73-86.

Schwamborn, J.C:; Berezikov, E. and Knoblich, J.A. (2009). The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. *Cell 136*: 913-925.

Seilern-Aspang, F. and Kratochwil, K. (1965). Relation between regeneration and tumor growth. In *Regeneration in animals and related problems*, V. Kiortsis, Trampusch, H., ed. (north-Holland Publishing Company, Amsterdam, pp 452-473.

Serafini, T.; Colamarino, S.A.; Leonardo, E.D.; Wang, H.; Beddington, R.; Skarnes, W.C. and Tessier-Lavigne, M. (1996). Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell 87*: 1001-1014.

Serafini, T.; Kennedy, T.E.; Galko, M.J.; Mirzayan, C.; Jessell, T.M. and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to C.elegans UNC-6. *Cell 78*: 409-424.

Shibata, N.; Umesono, Y.; Orii, H.; Sakurai, T.; Watanabe, K. and Agata, K. (1999). Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians. *Dev. Biol*. *206*: 73-87.

Stoick-Cooper, C.L; Moon, R.T. and Weidinger, G. (2007). Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. *Genes Dev. 21*: 1292-1315.

Takano, T.; Pulvers, J.N.; Inoue, T.; Tarui, H.; Sakamoto, H.; Agata, K. and 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. *Dev. Growth Differ. 49*: 383-394.

Tam, S.K.; Gu, W.; Mahdavi, V. and Nadal-Ginard, B. (1995). Cardiac myocyte terminal differentiation. Potential for cardiac regeneration. *Ann. N.Y. Acad. Sci. 725*: 72-79.

Tanaka, E.M. (2003). Regeneration: if they can do it, why can't we? *Cell113*: 559-562.

Tanaka, E.M., Drechel, D.N., and Brockes, J.P. (1999). Thrombin regulates S-phase re-entry by cultured newt myotubes. Curr. Biol. 9: 792-799.

Tanaka, E.M.; Gann, A.A.F.; Gates, P.B. and Brockes, J.P. (1997). Newt myotubes reenter the cell cycle by phosphorilation of the retinoblastoma protein. *J. Cell Biol. 136*: 155-165.

Tsonis, P.A. (2000). Regeneration in vertebrates. *Dev. Biol. 221*: 273-284.

Umesono, Y.; Watanabe, K. and Agata, K. (1997). A planarian orthopedia homolog is specifically expressed in the branch region of both the mature and regenerating brain. *Dev. Growth Differ*. *39*: 723-727.

Umesono, Y.; Watanabe, K. and Agata, K. (1999). Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Dev. Genes Evol*. *209*: 31-39.

Wang, G.; van der Walt, J.M.; Mayhew, G.; Li, Y.J.; Züchner, S.; Scott, W.K.; Martin, E.R. and Vance, J.M. (2008). Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. *Am. J. Hum. Genet. 82*: 283-289.

Wiedemann, M. and Trueb, B. (2000). Characterization of a novel protein (FGFRL1) from human cartilage to FGF receptors. *Genomics 69*: 275-279.

Yazawa, S.; Umesono, Y.; Hayashi, T.; Tarui, H. and Agata, K. (2009). Planarian Hedgehog/Patched establishes anterior–posterior polarity by regulating Wnt signaling. *Proc. Natl. Acad. Sci. USA 106*, 22329-22334

Yin, V.P.; Thomson, J.M.; Thummel, R.; Hyde, D.R.; Hammond, S.M. and Poss, K.D. (2008). Fgf-dependent depletion of microRNA-133 promotes appendage regeneration in zebrafish. *Genes Dev. 22*: 728-733.

Yiu, G. and He, Z. (2006). Glial inhibition of CNS axon regeneration. Nat. Rev. Neurosci. 7: 617-627.

Yoshida-Kashikawa, M.; Shibata, N.; Takechi, K. and Agata, K. (2007). DjCBC-1, a conserved DEAD box RNA helicase of the RCK/p54/Me31B family, is a component of RNA-protein complexes in planarian stem cells and neurons. *Dev. Dyn*. 236: 3436-3450.

Yu J.K.; Satou Y.; Holland N.D.; Shin-I T.; Kohara Y.; Satoh N.; Bronner-Fraser M. and Holland LZ. (2007). Axial patterning in cephalochordates and the evolution of the organizer. *Nature 445:* 613-617.

Zhao, C.; Sun, G.; Li, S. and Shi, Y. (2009). A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nat. Struct. Mol. Biol. 16*: 365-371.