Disease Models & Mechanisms 4, 12-19 (2011) doi:10.1242/dmm.006692

The planarian flatworm: an in vivo model for stem cell biology and nervous system regeneration

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Planarian flatworms are an exception among bilaterians in that they possess a large pool of adult stem cells that enables them to promptly regenerate any part of their body, including the brain. Although known for two centuries for their remarkable regenerative capabilities, planarians have only recently emerged as an attractive model for studying regeneration and stem cell biology. This revival is due in part to the availability of a sequenced genome and the development of new technologies, such as RNA interference and next-generation sequencing, which facilitate studies of planarian regeneration at the molecular level. Here, we highlight why planarians are an exciting tool in the study of regeneration and its underlying stem cell biology in vivo, and discuss the potential promises and current limitations of this model organism for stem cell research and regenerative medicine.

Introduction

Neurodegenerative and cardiovascular diseases, as well as stroke, infection and injury, require therapies that aim to replace lost, damaged or inoperative tissues. Regenerative medicine is therefore a major focus of medical research. Whereas regeneration in humans is limited, several vertebrates, such as salamanders and fish, can regenerate amputated body parts with high efficiency (reviewed in Stoick-Cooper et al., 2007). The master of regeneration is, however, the planarian flatworm.

Planarians are free-living Platyhelminthes that can regenerate any part of the body, including the central nervous system (CNS). In addition to *Dugesia japonica* and *Girardia tigrina*, *Schmidtea mediterranea* is one of the most commonly used species in planarian research. This freshwater planarian is small in size (0.1-2 cm), has a diploid genome of about 800 Mb distributed on four chromosomes, which accounts for about 30,000 predicted genes (Cantarel et al., 2008), and can reproduce sexually as well as asexually by fission. The regenerative abilities of planarians depend on a large population of somatic stem cells (reviewed in Handberg-Thorsager et al., 2008). This feature, which, among bilaterians, is unique to planarian flatworms, means that planarians can serve as an in vivo Petri dish for the study and manipulation of stem cells in their natural environment.

In recent years, the unique properties of planarians, combined with the development of new technologies and the genome sequencing of *S. mediterranea* (http://genome.wustl.edu/genomes), have sparked planarian research. The application of RNA interference (RNAi) for gene-specific knockdown in planarians (Sanchez Alvarado and Newmark, 1999; Newmark et al., 2003) allowed identification of several genes and signal transduction pathways that regulate different aspects of regeneration, such as polarity and patterning, and stem cell proliferation, maintenance and differentiation (Guo et al., 2006; Oviedo et al., 2008; Adell et al., 2009; Rink et al., 2009; Felix and Aboobaker, 2010; Fernandez-Taboada et al., 2010; Scimone et al., 2010). The amenability to efficient RNAi treatments, rapid development of clear phenotypes and established cell biological readouts, combined with new post-genomic technologies, make planarians an outstanding tool for gene discovery and can reveal unidentified functions of known and unknown genes involved in human regeneration, development and disease. Table 1 summarises several planarian genomic regions that have significant similarity to human disease-related genes.

In this Primer article, we review the state-of-the-art of planarian research, focusing on stem cells, neural regeneration and re-establishment of polarity, and discuss how the knowledge gained from planarian research might be translated to higher organisms. We aim to bring the attention of the broader scientific community to these amazingly plastic animals as a promising model organism for the rapidly progressing fields of regenerative medicine and bioengineering.

Studying planarian regeneration: insights into how polarity is re-established

Freshwater planarians can perform all manner of amazing tricks when it comes to regeneration. Thomas Hunt Morgan was one of the first people to systematically study planarian regeneration in the late 19th century. Inspired by the observations of Harriet Randolph, he defined the minimal size of a fragment capable of regeneration as 1/279th of the intact animal's volume (Morgan, 1901). Morgan and others were well aware of the 'problem' of polarity during animal regeneration: if an animal capable of regenerating is transversely amputated, a

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Table 1. Planarian genes related to human disease

Genomic region in *S. mediterranea*	Protein homologue related to human disease
v31.001379:47968..64091	gi|28395045|ref|NP_078925.3| Bardet-Biedl syndrome 1
v31.000220:86311..86494	Bromodomain adjacent to zinc finger domain protein 1B (Williams-Beuren syndrome chromosome region 9 protein; Williams syndrome transcription factor; hWALP2)
v31.000545:52712..53003	Shwachman-Bodian-Diamond syndrome protein
v31.000507:70017..70139	Meckelin (Meckel syndrome type 3 protein homolog; Transmembrane protein 67)
v31.013121:13617..13682	Neural Wiskott-Aldrich syndrome protein (N-WASP)
v31.005885:14010..15569	Folliculin (Birt-Hogg-Dube syndrome protein; BHD skin lesion fibrofolliculoma protein)
v31.005162:18616..18792	Werner syndrome helicase
v31.000681:135349..135484	Non-imprinted in Prader-Willi/Angelman syndrome region protein 2 homolog
v31.055418:228..377	Anosmin-1 precursor (Kallmann syndrome protein homolog)
v31.006713:16756..17292	gi|12667790|ref|NP_075356.1| Down syndrome critical region gene 1-like 2
v31.015203:13445..13467	Sjoegren syndrome nuclear autoantigen 1 homolog
v31.004490:9815..10453	Inositol polyphosphate 5-phosphatase OCRL-1 (Lowe oculocerebrorenal syndrome protein)
v31.009851:9552..9944	Fragile X mental retardation syndrome-related protein 1 (hFXR1p)
v31.007987:16693..17886	Alstrom syndrome protein, putative
v31.001291:49513..50505	gi|94383167|ref|XP_992416.1| PREDICTED: Wolf-Hirschhorn syndrome candidate 1-like 1 isoform 10
v31.000842:97603..97731	gi|29789014|ref|NP_034177.1| gi|29789014|ref|NP_034177.1| DiGeorge syndrome critical region gene 6
v31.023451:6607..9888	Dysbindin (Dystrobrevin-binding protein 1; Hermansky-Pudlak syndrome 7 protein homolog; Hps7-like protein)
v31.000649:93991..96267	gi|28849903|ref|NP_789817.1| Usher syndrome 1G homolog
v31.007716:6307..6672	gi|82885666|ref|XP_930720.1| PREDICTED: similar to Unc-112-related protein 1 (Kindlin-1; Kindlerin; Kindlin syndrome protein) isoform 9
v31.001595:115581..117152	Bloom syndrome protein (RecQ protein-like 3; DNA helicase, RecQ-like type 2)
v31.000242:176063..176271	DNA excision repair protein ERCC-6 (ATP-dependent helicase ERCC6; Cockayne syndrome protein CSB)
v31.000644:72418..72533	Maspardin (Spastic paraplegia 21 autosomal recessive Mast syndrome protein homolog)
v31.001256:57266..57779	Cat eye syndrome critical region protein 5 homolog precursor
v31.000089:50070..50263	AMME syndrome candidate gene 1 protein homolog
v31.001829:29299..31869	gi|45827744|ref|NP_079350.3| Fraser syndrome 1 isoform 1
v31.000655:128469..129764	gi|25914754|ref|NP_740754.1| McKusick-Kaufman syndrome protein
v31.001941:98295..98622	Vacuolar protein sorting-associated protein 13B (Cohen syndrome protein 1)
v31.003493:42636..42856	Nibrin (Nijmegen breakage syndrome protein 1 homolog)
v31.011614:5530..6895	Oral-facial-digital syndrome 1 protein (Protein 71-7A)

Data was obtained from the *S. mediterranea* Genome Database (http://smedgd.neuro.utah.edu/index.html) (Robb et al., 2008). A search of the database reveals that many planarian genes are homologous to genes associated with human disease. Alternative protein names are listed in parentheses.

new head or anterior region develops from the anterior-facing wound, whereas a new tail or posterior region regenerates from the posterior-facing wound. As cited by Morgan (Morgan, 1901), Allman was the first to give the name of 'polarity' to this phenomenon (Allman, 1864). It was known that polarity reversal (two-headed or Janus head) in planarians could occur following amputation either just behind the eyes or after dissection of short cross-pieces (more wide than long) (Morgan, 1904). However, re-establishment of anteroposterior (AP) polarity during planarian regeneration perplexed researchers until 2008, when three studies on the role of the Wnt/β-catenin pathway provided a glimpse into the underlying mechanisms (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008). Upon silencing of β-*catenin-1* in *S. mediterranea* (*Smed-*β-*catenin-1*), any regeneration blastema (the unpigmented tissue at the wound site) differentiates into a head, regardless of the polarity of the original tissues. In trunk fragments, RNAi of *Smed-*β-*catenin-1* not only induces the regeneration of two heads but also, after a given time, brain and photoreceptors expand posteriorly, turning the animals into fully anteriorized planarians (Iglesias et al., 2008). Amazingly, this anteriorization also occurs in intact non-regenerating animals (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008), beginning with the conversion of the tail into a head. Conversely, over-activation of the Wnt/β-catenin pathway results in tail regeneration from the anterior blastema (Gurley et al., 2008). Recently, it has been proposed that the Hedgehog (Hh) pathway acts upstream of Wnt/β-catenin signalling in controlling posterior fate specification (Rink et al., 2009; Yazawa et al., 2009). Consistent with Morgan's thoughts more than 100 years ago, these observations indicate that head identity is the developmental and homeostatic default setting, which needs to be repressed by Wnt/β-catenin at the posterior-facing wound in order to allow tail formation: "…there is always a stronger tendency in the material that develops over a cut surface to produce a head than to produce a tail, and that a head will appear unless the polarity of the piece is sufficiently strong to overcome this tendency, and cause a tail to regenerate" (Morgan, 1904).

Planarians also possess dorsoventral (DV) polarity. Recently, the bone morphogenetic protein (BMP) pathway was shown to be essential for the re-establishment of the DV axis. Inactivation of this pathway leads to a dorsal-to-ventral conversion, accompanied by the duplication of the CNS (Molina et al., 2007; Orii and Watanabe, 2007; Reddien et al., 2007). In some cases, a second planarian forms out of the dorsal side of the treated animals, similar to a Siamese twin (Molina

et al., 2007). Thus, similarly to other invertebrates, the BMP pathway specifies the dorsal identity in planarians.

Recent research is helping to answer long-standing questions regarding planarian polarity (Meinhardt, 2009; De Robertis, 2010). For example, additional elements related to the Wnt/β-catenin and Hh pathways involved in AP polarity have been characterized (Adell et al., 2009; Petersen and Reddien, 2009; Rink et al., 2009; Yazawa et al., 2009; Gurley et al., 2010). Moreover, the block of gap-junction-mediated communications, coupled or uncoupled with the disruption of the nervous system continuity, results in the alteration of the AP polarity of regenerating planarian fragments (Nogi and Levin, 2005; Oviedo and Levin, 2007; Oviedo et al., 2010). Thus, one of the next challenges in the field is to integrate the functions of the signalling pathways with long-range neural cues and gap-junction-mediated patterning signals in order to understand how the AP and DV axes are determined during regeneration (Forsthoefel and Newmark, 2009; Adell et al., 2010). A second important issue to be addressed is to determine how stem cells respond to the activation of the different signalling pathways and how they interpret them to generate their progeny in the correct spatial and temporal manner.

The functional conservation of signal transduction pathways – exemplified by the roles of Wnt/β-catenin and BMP pathways in axis polarity in both planarians and vertebrates – offers the opportunity to study mammalian development and disease using planarian regeneration as a model.

Planarians: performing artists of nervous system regeneration

Owing to the presence of chemo-, mechano- and light-sensory neurons that are assembled with others into a CNS, planarians react rapidly to environmental conditions, contact and light (reviewed in Cebrià, 2007; Agata and Umesono, 2008). Two cephalic ganglia constitute a bi-lobed brain, which is connected to a pair of nerve cords that extends ventrally from the head to the tip of the tail and that sends projections to nearly all regions of the body (Fig. 1A). Although relatively simple at the morphological level, the planarian CNS displays a much higher complexity when its molecular compartmentalization (Umesono et al., 1999; Cebrià et al., 2002b) and the degree of conservation with vertebrate CNS genes is considered (Mineta et al., 2003). Amazingly, whereas the mammalian CNS is highly limited in its regenerative abilities, planarians structurally and functionally recover damages to their nervous system in only a short time. Even more spectacularly, they can regenerate a whole brain de novo within a few days.

Planarian brain regeneration can be divided into three major processes (Cebrià et al., 2002c). Inside the newly formed blastema, a brain rudiment can be detected within 1 day after head amputation (step 1; Fig. 1B). This rudiment grows and develops into the properly patterned brain (step 2). In the third step, old and new nervous tissues connect, and function is recovered after 4-7 days (step 3). As regeneration proceeds, different sets of genes are expressed that promote proper re-growth, patterning and functional recovery of the planarian brain. One of the early proteins that is activated during brain regeneration is the conserved fibroblast growth factor receptor (FGFR)-related protein Nou-darake (Ndk). *ndk* is expressed in the head region of the animal and is pivotal for positional identity of the brain. RNAi against *ndk* leads to FGFR-dependent ectopic brain formation throughout the animal, suggesting that Ndk determines the location of brain tissue development through capturing a putative FGF-like molecule, thereby restricting brain formation to the head region (Cebrià et al., 2002a). However, whether an FGF-like molecule is required for brain formation remains to be determined.

Another conserved family of proteins involved in brain regeneration is the netrin family. These proteins act as chemoattractants or chemorepellents and are essential for neuronal network formation. RNAi against planarian netrins and netrin receptors results in a disorganized meshwork of axonal projections and interrupted growth of visual axons, suggesting that these proteins act as axon guidance signals during regeneration (Cebrià and Newmark, 2005). Slit, a member of another conserved family of axon guidance cues, is also crucial for axon guidance in

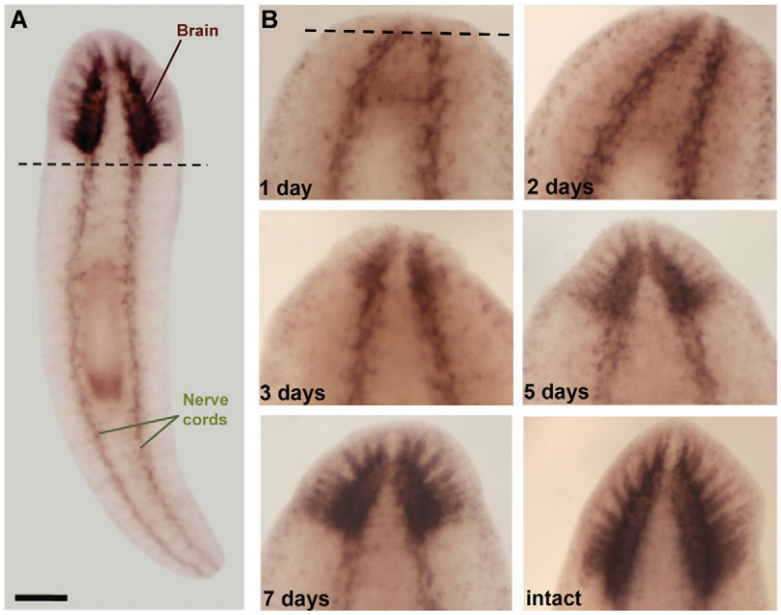

Fig. 1. Planarian brain regeneration. The CNS of the planarian *D. japonica*, visualized by whole-mount in situ hybridization against a pan-neural marker (a receptor protein tyrosine phosphatase) (Cebrià et al., 2002c) in intact (A) and regenerating (B) animals. Planarians were decapitated (dashed line in A) and allowed to regenerate for the indicated time points (as indicated in B). Note that de novo brain regeneration is complete after less than 7 days. Dashed line in B corresponds to the original amputation site marked in A. Scale bar: 0.5 mm. Panel A is adapted from Mineta et al. (Mineta et al., 2003), ©2003 National Academy of Sciences, USA; and panels B (1-3 days) are adapted from Cebrià et al. (Cebrià et al., 2002c).

planarians (Cebrià et al., 2007), as previously shown in other organisms (Long et al., 2004; Dickson and Gilestro, 2006).

Recent studies have also uncovered the role of *Smed-Wnt5* in planarian neural regeneration. Released from cells along the CNS and body edge, SMED-WNT5 protein seems to restrict axonal growth and assist integration of new and existing neuronal networks, because RNAi against *Smed-Wnt5* leads to the expansion of the brain and disconnections along the ventral nerve cords (Adell et al., 2009; Gurley et al., 2010). How the different axon guidance cues are regulated, and how they act together, possibly in combination with uncharacterized proteins and microRNAs (Friedlander et al., 2009; Lu et al., 2009), is currently unclear.

The reasons why planarians can regenerate an entire brain whereas humans struggle to repair a single axon is still obscure. Data on intrinsic and extrinsic inhibitors of axonal regeneration in humans is still minimal (for a review, see Sun and He, 2010), and just as little is known about the factors that facilitate this process in planarians. However, owing to the relatively high degree of conservation between neural proteins in planarians and vertebrates (Mineta et al., 2003), planarian research should help to better understand the regulatory programmes that control axon regeneration and circuitry re-formation, and might lead to the ability to activate the competence of mammalian neuronal networks to regenerate after damage- or disease-caused neurodegeneration.

Pluripotent stem cells: planarians as a Petri dish

The classical definition of a stem cell – a cell that can indefinitely self-renew and maintain

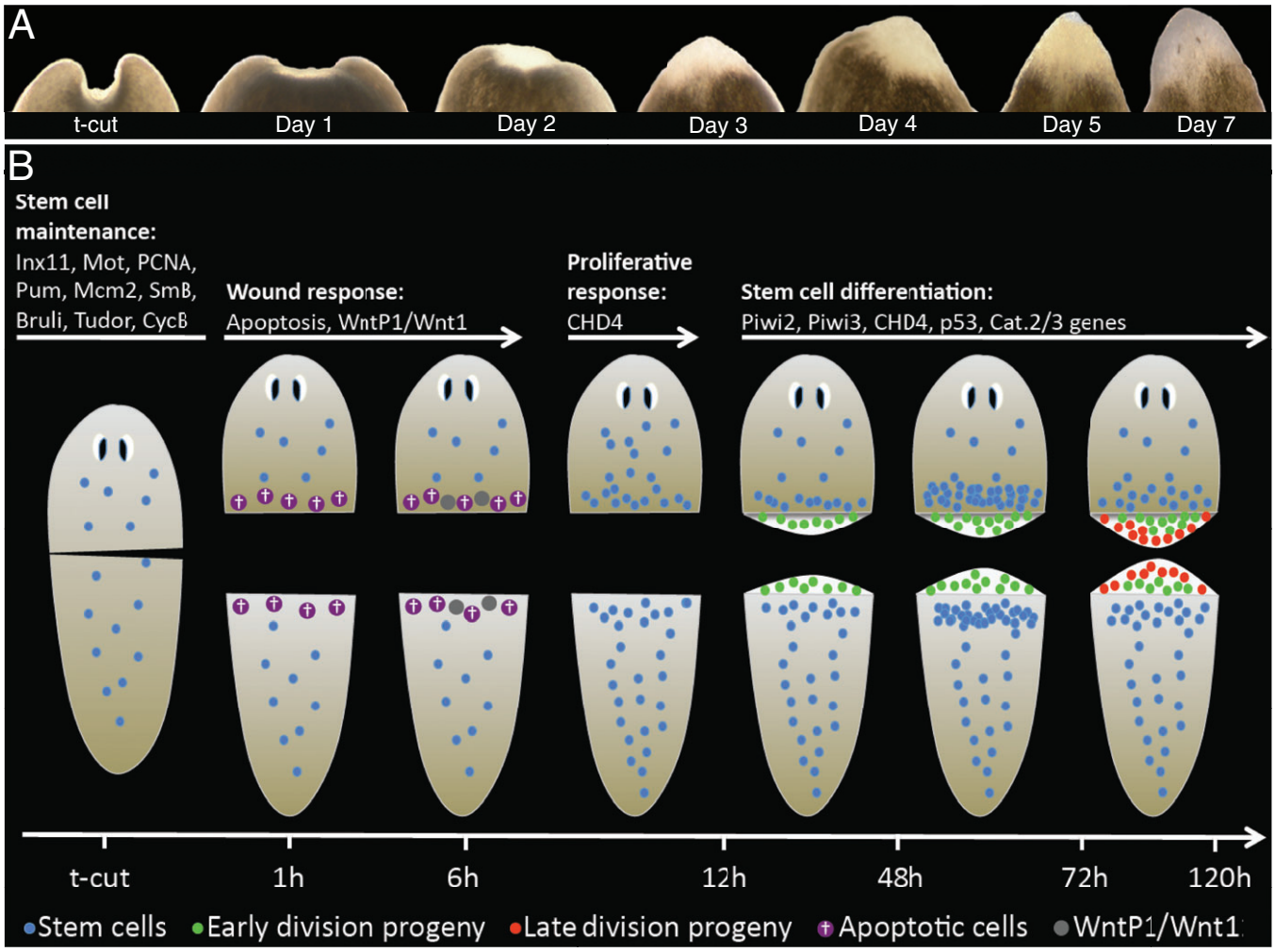

Fig. 2. Stem cells and regeneration in planaria. (A) Head regeneration in *S. mediterranea* after transverse amputation (t-cut). During the first 7 days following amputation, a blastema forms and expands, in which missing tissues are differentiated de novo. (B) RNAi data produced in the last decade have contributed to the partial dissection of the molecular features of planarian stem cells. Neoblast-specific genes can now be classified on the basis of their role in the regeneration process. After the wound response, which initiates the pre-patterning (WntP1/Wnt1 expression) and probably recruits stem cells to the wound, the stem cells begin to proliferate, reaching their proliferation peak between 48 and 72 hours. Meanwhile, stem cells also begin to differentiate. Although many neoblast-specific genes are involved in the maintenance of the stem cell pool, others seem to trigger differentiation, such as the planarian argonaute proteins Piwi2 and Piwi3 (probably involved in silencing active transposons) and the ATP-dependent helicase CHD4. The expression of early neoblast progeny markers [Category 2 genes (Eisenhoffer et al., 2008)] becomes detectable in the blastema 36 hours after amputation, whereas Category 3 genes (late neoblast progeny markers) are expressed from 96 hours onwards.

the ability to differentiate into a diverse range of specialized cell types – encompasses the important concept of 'potency', which describes the capacity of a stem cell to differentiate into specialized progeny (Schöler, 2007). Foetal and adult (or somatic) stem cells are usually multipotent (can give rise to a limited number of cell types), whereas inner blastomeres of blastocysts and their in vitro counterparts [cultured embryonic stem (ES) cells] are pluripotent and can differentiate into all three germ layers (Tiedemann et al., 2001). Pluripotency is maintained in mammalian ES cells by what is referred to as the 'pluripotency network', a transcriptional regulatory circuitry, integrated by external signalling pathways, that specifies an ES-cell-specific gene expression (Boyer et al., 2005; Mathur et al., 2008).

Owing to their ability to differentiate into various cell types, stem cells hold great potential for regenerative medicine. Although somatic stem cells are widely used in medical therapies (Stocum, 2001), the powerful pluripotent ES cells have not been of clinical use thus far owing to the associated high risks of teratoma formation, as well as legal and ethical concerns. The recently developed induced pluripotent stem (iPS) cells, which are derived from terminally differentiated cells by overexpressing a specific combination of transcription factors (Takahashi and Yamanaka, 2006), seem to bypass these controversies. These cells can be patient-specific, derived without the need for viral integration (Si-Tayeb et al., 2010) and are claimed to have the same potential as ES cells to treat a wide variety of diseases. iPS cells, however, also share the same drawbacks as ES cells: the differentiation process must take place in vitro (with several limitations, above all chromosomal and epigenetic instability) (Humpherys et al., 2001) and the transplanted cells must be devoid of pluripotent cells to prevent the formation of teratomas. A possible alternative to overcome these problems is to therapeutically stimulate resident progenitor cells to promote and enhance the endogenous regenerative process, as in the case of the TWEAK-dependent activation of oval cells in the liver (Jakubowski et al., 2005) or the Tβ4-induced restoration of multipotency in adult epicardial cells (Smart et al., 2007). In order to develop this strategy to its full potential for future clinical applications, it is pivotal to obtain a clearer understanding of the molecular mechanisms that underlie every aspect of stem cell biology in vivo. Because these studies are relatively limited in mammalian models, planarians and their pluripotent stem cells represent a suitable complementary system for dissecting relevant molecular details.

Planarian adult stem cells (pASCs), historically referred to as neoblasts, are small (5-10 μm diameter) undifferentiated cells that localize in the parenchyma, a loose tissue that lies beneath the muscle layers and surrounds the organs. Although definitive proof of their pluripotency has not yet been provided, planarian neoblasts are regarded as adult pluripotent stem cells (reviewed in Shibata et al., 2010) and are responsible for the remarkable regeneration ability shown by these animals (Saló and Baguñà, 1984; Rossi et al., 2008) (Fig. 2A).

As is the case for other metazoans, a typical pluripotency network has not been characterized for planarian stem cells. However, pASCs possess their own molecular signature, a set of irradiation-sensitive genes (Table 2) that have different roles in the regeneration process (Fig. 2B). Most of these genes are RNA-binding proteins (Rouhana et al., 2010); at least three of them [*D. japonica Cbc-1* (*DjCbc-1*), *Schmidtea polychroa Tud-1* (*Splotud-1*) and *Smed-SmB*] localize to a ribonucleoprotein granule called a chromatoid body that is typically found in stem cells, germ cells and neurons (Coward, 1974; Yoshida-Kashikawa et al., 2007). Remarkably, transcription factors that are specific to pASCs have not yet been identified, although genes involved in other aspects of neoblast biology were described, such as the chaperone *Djmot* and the chromatin-remodelling factors *Smed-CHD4* and *DjRbAp48*, which are needed for planarian stem cell viability and differentiation, respectively (Conte et al., 2009; Bonuccelli et al., 2010; Scimone et al., 2010).

There is also evidence to suggest the existence of different stem cell subpopulations in planarians. Although germline stem cells are morphologically indistinguishable from the neoblasts (Coward, 1974), they express the germ-cell marker *nanos*, which localizes to the gonads, or to the gonadal primordia in asexual strains (Sato et al., 2006; Handberg-Thorsager and Salo, 2007; Wang et al., 2007). Moreover, although previous reports indicated that all pASCs cycle regularly (Newmark and Sanchez Alvarado, 2000), it has recently been suggested that there is a subpopulation of G0-arrested stem cells in the planarian species *D. japonica* (Higuchi et al., 2007). In the same species, the argonaute family gene *DjPiwi-1* was found to be expressed by a subpopulation of neoblast-like cells that are preferentially located at the anterior dorsal midline (Rossi et al., 2006). Together with the different levels of radiotolerance displayed by pASCs exposed to low-dose irradiation (Salvetti et al., 2009), this suggests the existence of distinct stem cell subpopulations in *D. japonica*.

Although they differ in several respects from mammalian ES cells, pASCs offer a unique opportunity to study pluripotent stem cells and their contribution to the regenerative process in vivo in an intact adult animal.

Planarian research community: hopes and efforts

Although planarians are a promising model system for in vivo stem cell biology and nervous system regeneration, we are still in the fledgling stage in understanding the molecular principles governing the associated regulatory mechanisms. This is largely owing to the absence of important techniques, such as the generation of transgenic animals and maintenance of planarian stem cells in culture, but also to the lack of manpower. Until 5 years ago, few laboratories worldwide worked on planarian regeneration research. However, the exploitation of RNAi and sequencing technologies have now attracted many new researchers from different areas of biology, and the number of planarian laboratories has exploded within a short time. Thus, to generate a collaborative research community to drive planarian research forward, EuroPlanNet (www.europlannet.org) was launched during the 1st International Meeting on Planarian Biology (IMPB), held in May, 2010 in Münster, Germany. As with other scientific communities, the joint efforts of the network are likely to succeed in tackling current problems and overcoming existing technical limitations. Immediate goals of the network are to develop missing techniques and to create an open-access database to accommodate integrated genomic and transcriptomic datasets. The network will also provide the planarian community with common infrastructures and a student exchange programme. These innovative actions will be essential for

Table 2. Planarian genes involved in stem cell biology

Planarian gene	Homologous human protein (accession no.)	Expression pattern	RNAi phenotype (regeneration)	Proposed function in planarian stem cells	References
Argonaute/Piwi family					
Djpiwi-1	HIWI (Q96J94)	Dorsal midline	N.D.	Unknown	Rossi et al., 2006
Smedwi1	HIWI (Q96J94)	Parenchyma	No phenotype	Unknown	Reddien et al., 2005
Smedwi2	HIWI (Q96J94)	Parenchyma	Blastema formation, then regression; death in 2-3 weeks	piRNA expression; stem cell differentiation	Reddien et al., 2005; Palakodeti et al., 2008
Smedwi3	HILI (Q8TC59)	Parenchyma	Reduced blastema formation, then regression; death in 3 weeks (after three rounds of RNAi and amputation)	piRNA expression; stem cell differentiation	Palakodeti et al., 2008
Other RNA-binding proteins					
DjVlgA	DDX3X (O00571)	Germ cells, parenchyma, blastema	N.D.	Post-transcriptional regulation	Shibata et al., 1999
DjPum	PUM2 (Q8TB72)	Parenchyma (strongly expressed in the posterior dorsal stripe), brain	Reduced blastema formation; death in 3-4 weeks (after two rounds of RNAi and amputation)	Post-transcriptional regulation	Salvetti et al., 2005
Smed-Bruli	TNRC4 (Q9BTF3)	Parenchyma, brain	Reduced blastema formation, then regression; death in 3 weeks	Stem cell self-renewal	Guo et al., 2006
Smednos/ Djnos	NANOS1 (Q8WY41)	Primordial germ cells, spermatagonia, oogonia, eye precursor cells	No regeneration of gonads and copulatory apparatus	Zinc-finger-containing RNA-binding protein; germline specification	Sato et al., 2006; Handberg-Thorsager and Salo, 2007; Wang et al., 2007
Spoltud-1	TDRD gene family	Ovaries, testes, CNS, parenchyma	Regeneration defects; loss of stem cells (after 4 rounds of RNAi and amputation)	Chromatoid body component; stem cell long-term self-renewal	Solana et al., 2009
Djcbc-1	RCK (D17532)	Parenchyma, brain, germline stem cells	No phenotype	Chromatoid body component	Yoshida-Kashikawa et al., 2007
Smed-SmB	SMB (P14678)	Parenchyma, brain	No blastema formation; loss of stem cells; death in 2 weeks	Chromatoid body component; stem cell self-renewal	Fernandez-Taboada et al., 2010
DNA replication					
Djmcm2	MCM2 (P49736)	Parenchyma (strongly expressed in the dorsal midline)	N.D.	DNA replication	Salvetti et al., 2000
DjPCNA	DDBJ (M15796)	Parenchyma (strongly expressed in the dorsal midline and in the bilateral lines)	N.D.	DNA replication	Orii et al., 2005
Chromatin remodelling					
Smed-CHD4	CHD4 (Q14839)	Parenchyma, CNS	Reduced proliferation response; decreased number of neoblasts progeny cells; death in 3-4 weeks	ATP-dependent helicase; stem cell differentiation	Scimone et al., 2010
DjRbAp48	RBBP4 (Q09028)	Parenchyma, blastema	Reduced blastema formation; ventral curling; reduced motility; death in 6 weeks	Histone-binding protein; nucleosome remodelling	Bonuccelli et al., 2010
Other functions					
Smedinx-11	Not present	Parenchyma, pharynx, region surrounding the brain and region anterior to photoreceptors	No blastema formation; reversal of the neoblast gradient; death in 5 weeks	Gap junction protein (innexin)	Oviedo and Levin, 2007
Category 2 and Category 3 genes	Various	Early blastema, outer parenchyma of intact animals	N.D.	Early stem cell progeny differentiation	Eisenhoffer et al., 2008
Smed-p53	TP53 (P04637)	Parenchyma, stem cell progeny	Decreased number of neoblast progeny cells	Early stem cell progeny differentiation	Pearson and Sanchez Alvarado, 2010
Djmot	HSPA9 (Q8N1C8)	Parenchyma (strongly expressed in the dorsal midline), blastema	Reduced blastema of neoblast reduced regeneration; death in 5-7 weeks	Chaperone, sequestration of p53	Conte et al., 2009

N.D., not determined; piRNA, Piwi-interacting RNA.

Advantages of planarians as a model for stem cell biology and regeneration

- Planarians can regenerate any body part, even the CNS, from small pieces within a few days
- Planarians contain a large number of adult stem cells, some of which, if not all, are pluripotent
- Planarians are easy and cost-effective to maintain, and can be grown to large populations
- Many planarian proteins are significantly similar to human proteins
- RNA interference can be efficiently carried out in planarians by feeding, injecting or soaking with solutions of double-stranded RNA
- The genome of *Schmidtea mediterranea* has been sequenced; transcriptome datasets are being generated to facilitate understanding of the genetic regulation of planarian regeneration

promoting the use of the planarian model in the field of regenerative medicine, to which the planarian community aims to contribute by providing novel insights into the molecular machineries controlling stem cell behaviour and CNS regeneration.

ACKNOWLEDGEMENTS

We thank Sören Moritz, Suthira Owlarn and Matthias Vogg for critical comments on the manuscript. F.C. is a Ramon y Cajal researcher funded by grants BFU2008-00710 (MICINN, Spain) and 2009SGR1018 (AGAUR, Generalitat de Catalunya).

COMPETING INTERESTS

The authors declare no competing financial interests.

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