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Review

Multicellularity, stem cells, and the neoblasts of the planarian *Schmidtea mediterranea*

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Abstract

All multicellular organisms depend on stem cells for their survival and perpetuation. Their central role in reproductive, embryonic, and post-embryonic processes, combined with their wide phylogenetic distribution in both the plant and animal kingdoms intimates that the emergence of stem cells may have been a prerequisite in the evolution of multicellular organisms. We present an evolutionary perspective on stem cells and extend this view to ascertain the value of current comparative studies on various invertebrate and vertebrate somatic and germ line stem cells. We suggest that somatic stem cells may be ancestral, with germ line stem cells being derived later in the evolution of multicellular organisms. We also propose that current studies of stem cell biology are likely to benefit from studying the somatic stem cells of simple metazoans. Here, we present the merits of neoblasts, a largely unexplored, yet experimentally accessible population of stem cells found in the planarian *Schmidtea mediterranea*. We introduce what we know about the neoblasts, and posit some of the questions that will need to be addressed in order to better resolve the relationship between planarian somatic stem cells and those found in other organisms, including humans.

Keywords: Planarians; Stem cells; Neoblasts; *Schmidtea mediterranea*

Contents

Multicellularity and stem cells

Natural selection operates at both the cellular and organismal levels [1]. In unicellular organisms, the fitness

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of the cell and the fitness of the organism are one and the same. This, however, cannot be said of multicellular beings, where cell-level and organismal-level fitness are quite frequently at odds with each other. Consider, for instance, the reduction in cellular fitness leading to the apoptosis of particular wing cells in order to increase the overall fitness of the entire *Drosophila melanogaster* wing during development [2]; or the increase in organismal longevity that results when the cells that give rise to the germ line are killed in *Caenorhabditis elegans* [3]. Clearly, conditions exist that can improve the fitness of the organism while being, in fact, deleterious to the fitness of its cells and vice versa. Thus, it has been suggested that in order for multicellularity to emerge, and for multicellular organisms to become subjects to natural selection, mechanisms must have been selected for to resolve conflicts between the fitness of the cell and the fitness of the multicellular individual [4–6].

One such "conflict-resolution" mechanism may be found in stem cells, in particular germ and toti- or pluripotent stem cells. Stem cells are undifferentiated cells capable of both, self-renewing and producing one or more different cell types. By sequestering multipotentiality (e.g., somatic stem cells) and/or immortality (e.g., germ stem cells) away from the differentiated soma and into highly specialized cells, the dynamics of variation and selection between the fitness of undifferentiated stem cells and that of their differentiated cohorts are effectively uncoupled. The physical separation of undifferentiated and differentiated cell functions generates conditions in which different selective pressures can act upon these two operationally opposite cell types to yield appropriate gains in their respective fitness. The result is an integration of key undifferentiated and differentiated cell functions such as inheritance and adaptation, respectively, that can now respond separately to natural selection. Thus, the emergence of stem cells could be viewed as a fundamental evolutionary adaptation that allows multicellular organisms to satisfy Darwin's conditions of heritability and variation in fitness [6,7].

Given the broad distribution of stem cells throughout the animal and plant kingdoms (Fig. 1), and that all known multicellular organisms (from plants to animals) rely on stem cells for their survival and perpetuation, the emergence of stem cells may have been a prerequisite for the evolution of multicellularity. This is underscored by the fact that for 3.5 billion years, the history of life in our planet was the history of unicellular organisms. Multicellularity, on the other hand, is quite recent and dates back to some 600 million years ago, right before the "cambrian explosion". In the case of animals, the fossil record indicates that the emergence of multicellularity occurred once during evolution, and that its appearance was likely accompanied by the invention of stem cells for either asexual or sexual reproduction (Fig. 1). If in fact, the emergence of the Metazoa required sequestering multipotency and immortality into stem cells, an ancestral origin of these cells would imply that the mechanisms regulating their biology may be found in all animals. Evidence for this is provided by the conserved function of the *wnt* pathway, for example, in the maintenance of *D. melanogaster* germ [8], mammalian hematopoietic [9], gut [10], and hair follicle [11] stem cells.

Somatic and germ stem cells

While comparative studies of germ and somatic stem cells tend to highlight their similarities, their different ontogenies and functions suggest that key differences in their respective evolutionary origins, maintenance, and regulation must also exist. Whereas germ line stem cells are restricted to sexual reproduction by gamete production, somatic stem cells are known to participate in multiple biological functions such as the asexual reproduction of plants and animals, the replacement of cells lost to tissue homeostasis, and, in some cases, in the production of germ stem cells. In fact, recent phylogenetic analyses of character states that are dependent on stem cells (such as clonality and mode of germ line development) indicate that stem-cell-based clonality appears to be a shared, primitive character of metazoans, while the germ line appears to have been derived later at or near the origin of the first bilaterians [5]. As such, functional comparisons between somatic and germ cells are likely to be of limited value in understanding somatic stem cell biology.

Some solutions to balance cell and organismal fitness are shared by the various phyla, while others are not. One example encompassing both of these conserved and convergent mechanisms is provided by the way in which the germ line is formed in the Metazoa. In *Drosophila*, *C. elegans*, *Xenopus*, and zebrafish, the germ line is determined by maternally inherited factors that are segregated early during embryogenesis. In mammals, on the other hand, segregation of the germ line does not occur; rather, the mammalian germ line arises late in embryogenesis from a population of somatic stem cells through inductive interactions with surrounding tissues [12]. By sequestering the germ line early during embryogenesis, competition among stem cells [13] to produce such lineage is essentially eliminated, thus exposing somatic stem cells to a different selection landscape altogether. Clearly, the same principle applies to those organisms that do not segregate their germ line. As such, the parameters of variation and selection within organisms that form their germ line early in embryogenesis, and those that use stem cells to produce both somatic and germ cells later in their life history are bound to be different [14]. Such differences will, consequently, be manifested in the specific molecular, cellular, and tissue homeostatic mechanisms regulating the maintenance and function of stem cells, be they germ line or somatic stem cells. While some of these mechanisms may be shared (e.g., *wnt* signaling), some are just as likely to be exclusive to either germ or somatic stem cell functions. As such, specific and even fundamental mechanisms regulating the function of somatic stem cells are unlikely to be

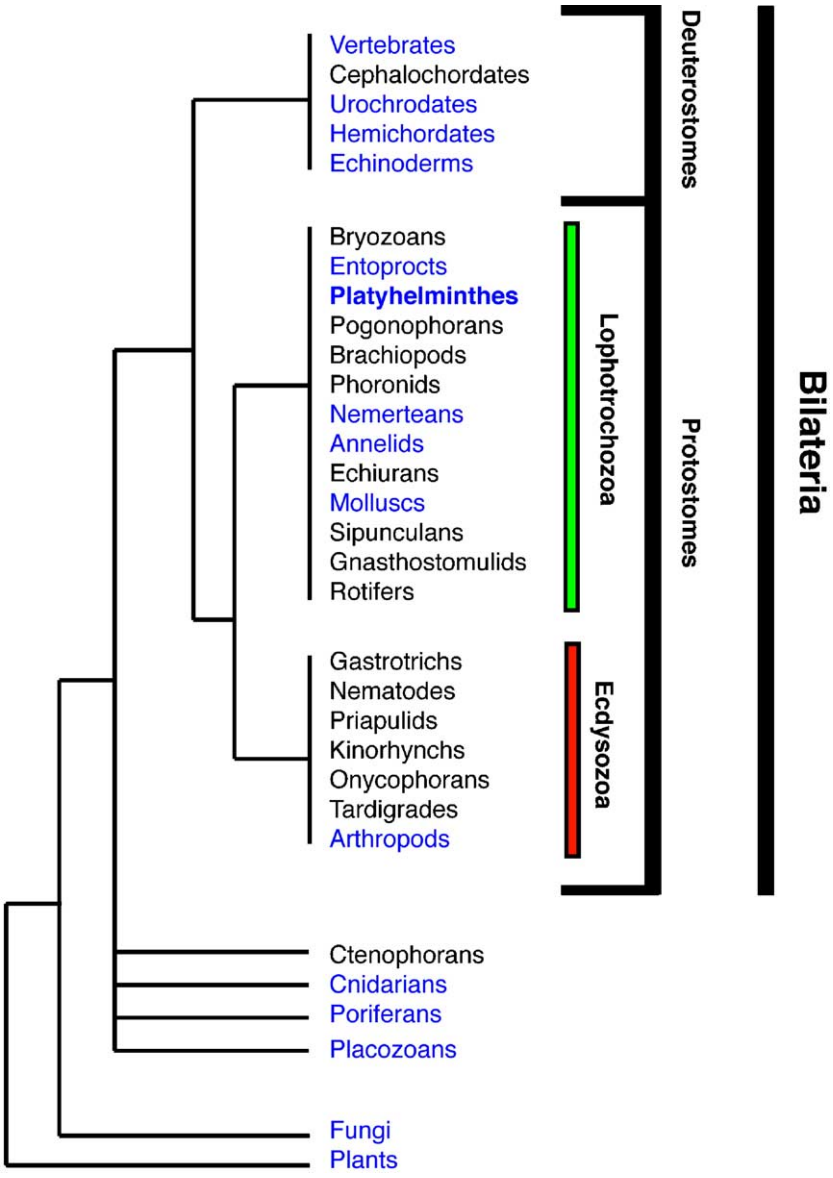

Fig. 1. Phylogenetic distribution of somatic stem cells in multicellular organisms. Only those taxa (in blue) for which unambiguous evidence is available about the existence of adult somatic stem cells are shown. For the remaining taxa (in black), either the absence of adult somatic stem cells has been reported, or their presence is unknown. The information utilized to score the different taxa was compiled from various sources [18,67–73]. The tree is modified from Adoutte et al. [74] to include the Placozoa, the taxon containing the simplest known animal and represented by the species *Trichoplax adhaerens* [75].

uncovered by studying germ line stem cells. Therefore, the distinction between germ cells and multipotent stem cell lineages is crucial to evolutionary discussions of stem cells in general [4,14], and to stem cell biology in particular. This is a generally underappreciated fact in current discussions of stem cells that is, nevertheless, of great biological and experimental significance.

In order to better understand stem cell functions, it would, therefore, be more appropriate to directly study somatic stem cells; however, these cells are not present in very large numbers and are rather difficult to visualize and access experimentally in vivo in higher organisms. Moreover, ubiquitous somatic stem cells have not been identified in the invertebrate genetic systems of choice (i.e., *D. melanogaster* and the nematode *C. elegans*). Consequently, little to no work has been carried out on invertebrate somatic stem cells to ascertain the extent of evolutionarily conserved attributes that may exist among metazoan stem cells. Hence, the ability to perform large numbers of experiments rapidly and systematically in vivo (a salient experimental trait of invertebrate model systems) has not been brought to bear on the study of somatic stem cells in animals.

The planarian *Schmidtea mediterranea*

One organism with the potential to inform somatic stem cell biology and overcome current experimental limitations

is the freshwater planarian *S. mediterranea*. This bilaterally symmetric, triploblastic animal possesses an abundant, largely unexplored, yet experimentally accessible population of somatic stem cells known as neoblasts. Neoblasts are capable of driving asexual reproduction through transverse fission. These cells can also generate germ line stem cells in sexual hermaphrodites through inductive processes, since segregation of the germ line does not occur during embryogenesis. Hence, the biology and evolutionary history of planarians, combined with the pronounced absence of somatic tissue turnover and regenerative properties in current invertebrate models, along with the difficulty of studying vertebrate somatic stem cells in vivo, provide compelling reasons to examine the functions and test the suitability of neoblasts to inform somatic stem cell biology at large.

Planarians belong to the phylum Platyhelminthes, and are phylogenetically placed in the Lophotrochozoa (Fig. 1), a sister group to both the Ecdysozoa (which include *D. melanogaster* and *C. elegans*) and the Deuterostomes (to which vertebrates belong). The Lophotrochozoa comprise animals displaying the largest collection of body plans on the planet (squids, mollusks, annelids, ribbonworms, etc.), yet have remained underrepresented in current molecular and cellular investigations. Planarians provide access to biological attributes not saliently manifested in traditional model systems. In particular, these acoelomate organisms with complex organ systems have the capacity to regenerate complete individuals from minuscule body parts [15,16]. In fact, over 100 years of scientific literature exists reporting on planarian experimentation [17]. Still, in order to fully exploit the biological potential of planarians, a suitable species had to be identified. There are thousands of different known species [18], but only several dozen have been characterized in some detail. Of these, the free-living, freshwater hermaphrodite *S. mediterranea* emerged as a likely candidate because it displays robust regenerative properties and, unlike most other planarians, it is a stable diploid ($2n = 8$) with a genome size of $\sim 4.8 \times 10^8$ bp (nearly half that of other common planarians).

Interestingly, a Robertsonian translocation between chromosomes 1 and 3 produced an exclusively asexual biotype [19]. This naturally occurring alteration of the genome effectively allows us to investigate the roles of stem cells in both asexual and sexual reproduction, as both of these biotypes have proven easy to rear in the laboratory. Their ease of maintenance and manipulation has allowed us and others [20] to develop the requisite molecular tools to dissect the remarkable biology of these animals. We have established loss-of-function assays [21,22], large collections of cDNAs [23], have recently completed a large-scale RNAi-based screen [24], and have begun sequencing the genome of *S. mediterranea* at the Washington University Genome Sequencing Center with funding from the National Human Genome Research Institute [25]. These advances have permitted us to commence systematic cellular and molecular genetic studies on animal regeneration, tissue homeostasis, and the attendant stem cells driving these phenomena.

What are neoblasts and what do we know about them?

We know that neoblasts can self-renew and can give rise to all cell types in planarians, including the germ line. As alluded to earlier, such type of totipotent cells may have been the ancestral form of animal stem cells. Therefore, neoblasts and their ability to make the germ line late during development or indefinitely throughout adulthood provide us with a better model to study multipotency mechanisms than any other currently available stem cell system. Although over 100 years have passed since the initial description of neoblasts in flatworms [26], much remains to be learned about their cellular and molecular biology. Here, we summarize what is known about neoblasts and what has been learned about these cells in the past few years, particularly with regards to their cell cycle.

Morphology and known functions of neoblasts

The extraordinary tissue plasticity of planarians is in direct contrast to the lack of pliancy displayed by the somatic tissues of adult *C. elegans* and *D. melanogaster*. The difference lies in a population of adult somatic stem cells called neoblasts that are distributed throughout the planarian body. Morphologically, neoblasts share many attributes with the stem cells of other organisms, such as large nuclei with extensively decondensed DNA, and largely undifferentiated, highly basophilic cytosols (Fig. 2A). Neoblasts are the only mitotically active cells in planarians [27], and their division progeny generate the ~40 different cell types found in the adult organism (see Figs. 2C–I for examples). In intact planarians, neoblasts replace cells lost to normal physiological turnover [27], while giving rise in amputated animals to the regeneration blastema, the structure in which missing tissues are regenerated [17]. Because neoblasts are generally small in size (~6–8 μm), they can be enriched from whole animal cell suspensions by serial filtration [28]. Evidence for the stem cell nature of neoblasts comes from electron microscopy studies, BrdU labeling (see below) and injecting neoblast-enriched cell suspensions into planarians devoid of mitotic activity (lethally irradiated), which resulted in the restoration of both regenerative abilities and long-term viability of the recipient animals [28].

Besides playing central roles in the regenerative and tissue homeostatic capacities of planarians, neoblasts play a key function in the reproductive fitness of these animals. We alluded to the fact that *S. mediterranea* can, and does, reproduce by either sexual or asexual means. This is possible because the division progeny of self-renewing

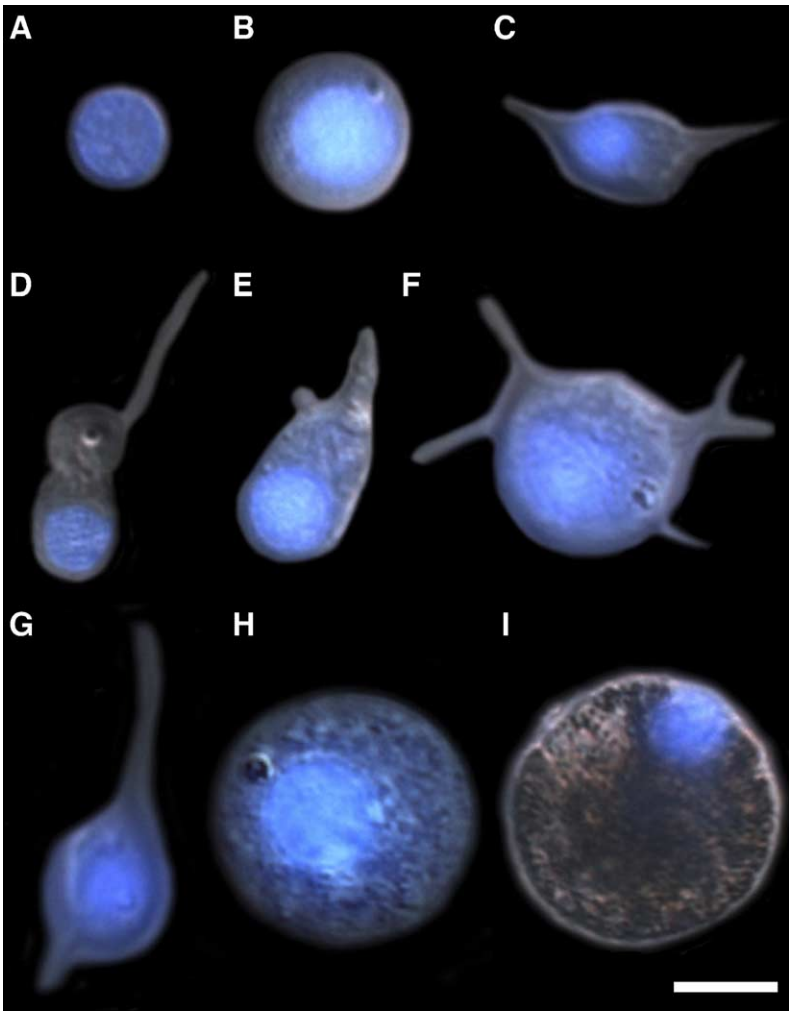

Fig. 2. The planarian neoblasts and their division progeny. Dissociated cells of the planarian *S. mediterranea* were stained with Hoechst 33342 and photographed using Nomarski optics. Three types of neoblasts are shown: (A) The canonical neoblast, 6–10 μm in diameter with a large nucleus (blue). (B) A neoblast progressing through the cell cycle (twice as large as the one pictured in panel A and usually containing 4n DNA). (C) A "Betchaku" neoblast [76] with polar elongated processes. Remaining figures show various morphologies of the neoblast division progeny. The identities of these cells are based on morphological features alone [69] and remain ultimately tentative. (D) Monociliated cell, likely a sensory neuron of the anterior cephalic margin. (E) Epithelial cell. (F) Parenchymal cell displaying multiple cytoplasmic processes. (G) Neuron. (H) Digestive cell of the gastrovascular system. (I) Pigment cell. Scale bar is 10 μm.

neoblasts not only give rise to somatic tissues, but also to the germ line. A clear demonstration that adult somatic stem cells can give rise to a functional germ line and the reproductive structures associated with this cell population was provided by Thomas Hunt Morgan in 1902 [29]. Morgan demonstrated that a planarian head fragment devoid of germ line structures could regenerate functional gonads from the remaining somatic tissue [29]. This is an important attribute of planarians, i.e., the presence of a multipotent, adult somatic stem cell lineage able to produce functional germ cells. In sexually reproducing planarians, the germ cell lineage does not appear to be segregated during embryogenesis. Instead, the gonads and the copulatory apparatus are formed de novo in the appropriate regions of the worm once the planarian reaches an appropriate size [30].

Neoblasts and the cell cycle

We know little about how the cell cycle in adult somatic stem cells may be regulated [31]. This is in stark contrast to our extensive knowledge of cell cycle parameters in differentiated cells [32]. Only recently, for example, has it been shown that self-renewing mammary epithelial stem cells selectively retain their tritiated thymidine-labeled template DNA strands, and pass newly synthesized bromodeoxyuridine (BrdU)-labeled DNA to their progeny during asymmetric divisions [33]. Such findings suggest that tight regulatory mechanisms of cell proliferation have been selected during evolution to promote the longevity of stem cell populations. Moreover, the ability of stem cells to respond to changes in tissue homeostasis caused by

physiological turnover or injury is also poorly understood. Thus, in order to understand how changes in homeostasis trigger stem cell proliferation, it is necessary to know if the cell cycle itself is regulated in response to these changes, and which phases of the cycle are being affected.

Even less is known about the proliferation dynamics of planarian neoblasts. Traditionally, neoblast cell cycle activities were measured by counting mitotic figures in histological sections of planarians [17]. More recently, the distribution of neoblasts in whole-mount specimens of *S. mediterranea* has been revealed by BrdU labeling [27], as these cells are the only mitotically active cell population in planarians. In *S. mediterranea*, neoblasts are distributed throughout the parenchyma of the animal, with the exception of the area in front of the photoreceptor and the pharynx. Interestingly, both of these non-mitotic areas were shown by T. H. Morgan to be incapable of regenerating a complete worm [15]. Later, BrdU labeling was extended to other flatworm species in which the S-phase cells are found either throughout the parenchyma (*Convolutriloba longifissura*) [27,34], or along the lateral sides of the body as in the rhabdocoel *Macrostomum* sp. [35]. In all cases, however, the function of the neoblasts appears to be to migrate and differentiate to replace cells lost to aging or wounding.

During the initial 12 h after amputation or feeding, a mitotic burst in the neoblast population is observed in planarians [36,37]. Because of the rate at which this burst occurs, it was thought that a large population of neoblasts arrested in the G2 phase of the cell cycle existed in planarians [36,38]. If so, G2 checkpoint mechanisms would become primary targets of stem cell function regulation. However, we have shown that G2 arrests do not occur in *S. mediterranea*. This conclusion was reached after examining the results of both fraction of labeled mitoses (FLM) and continuous BrdU labeling experiments [27]. Double-labeling neoblasts with BrdU and a mitotic marker (an antibody against the phosphorylated form of histone H3) allowed us to directly test whether the initial burst of proliferation after feeding or amputation was due to G2-arrested cells. If cells that have already traversed S phase (e.g., G2-arrested cells) were responsible for the initial mitotic peak, then the mitotic cells observed in the initial 12 h after addition of BrdU should be devoid of this thymidine analog. We found that 96% of the mitotic cells were labeled with BrdU 12 h after BrdU feeding, suggesting that, in *S. mediterranea*, large populations of G2-arrested stem cells are unlikely to exist.

Continuous BrdU labeling experiments also corroborated the FLM experiments. Two potential outcomes from the application of this method to a cycling cell population were possible. First, that if a large number of G2-arrested, or slow cycling neoblasts exists, then the maximum percentage of cells with the morphological characteristics of neoblasts that could be labeled with BrdU within a defined period of time will not reach 100%. And second, if all of the neoblasts cycle, close to 100% of the cells would become BrdU labeled. We found that more than 99% of neoblasts incorporated BrdU after 3 days of continuous labeling, suggesting again that a large sub-population of stem cells did not remain quiescent for more than 3 days [27]. In fact, these experiments also demonstrated that at any given time, an average of 6% of neoblasts are labeled soon after a single BrdU bolus, suggesting that the planarian stem cells are entering S at a relatively rapid rate [27].

FLM studies and continuous BrdU labeling methods have also been extended to the rhabdocoel *Macrostomum* sp. [39]. Curiously, it was shown that the gonadal proliferating cells are slow cycling neoblasts in this species. While about 95% of mitotic cells are labeled with BrdU after 24 h, only 74% of germ line cells in the male gonads are double labeled with BrdU and anti-H3P staining after 24 h, suggesting that, in *Macrostomum* sp., there may be a slow cycling cell population. In addition, some of the cells arrested at the G1 to S-phase transition by hydroxyurea were labeled with BrdU after 24 h of recovery in the gonads, which the authors took to indicate that gonadal stem cells escape from G1 arrest faster than somatic stem cells [39]. These differences intimate the existence of different regulatory events acting on the cell cycle of somatic versus germ line stem cells in *Macrostomum* sp. Whether these observations can be applied to other planarian species, such as sexual *S. mediterranea* animals, remains to be tested.

What we do not know about neoblasts and their regulation

Even though some progress has been made in delineating aspects of the neoblast cell cycle, we know little about how neoblast functions are regulated in planarians. In fact, we do not even know the extent of heterogeneity and lineage specified sub-populations that may or may not exist in the otherwise morphologically similar neoblast population. Below, we have chosen to highlight a few areas that will have to be addressed in order to gain a better understanding of how the planarian adult somatic stem cells may be regulated in vivo.

Cell cycle regulation

Above, we showed experimental evidence indicating that the neoblast cell cycle can be modulated. In eukaryotic cells, a group of evolutionarily conserved molecules is known to regulate the progression of cells through the cell cycle. These are known as cyclin-dependent kinases (Cdks) and play a key role in controlling cell cycle progression in response to cell cycle checkpoints. Generally speaking, DNA damage delays cell cycle progression by inhibition of Cdks, causing cell cycle arrest at the G1/S or G2/M transition [40]. However embryonic stem (ES) cells lack a G1 checkpoint and are hypersensitive to DNA damage, inducing apoptosis to get rid of damaged cells [41]. In

mouse ES cells, G1 arrest has been shown to be restored by ectopic expression of Chk2, a protein kinase that is activated following DNA damage and inhibits Cdk2 to induce G1 arrest at G1/S transition [42]. Whether Cdks are involved in the regulation of the neoblast cell cycle, and whether the G1 or G2 checkpoints are under the control of Cdks following amputation and feeding remain to be determined. Given the wide phylogenetic use of Cdks in the regulation of the eukaryotic cell cycle, it is likely that these molecules will play key roles in regulating neoblasts. Future investigations of neoblast cell cycle regulation will have to define if conservation of Cdk functions occurs, and determine whether or not Cdks are regulated by homeostatic changes defining basic planarian biological functions such as regeneration and tissue homeostasis. The availability of large collections of cDNAs, double-stranded RNA-mediated interference, and an ongoing genome sequencing project make these problems accessible to experimental manipulation, and should allow for a detailed mechanistic dissection of neoblast cell cycle activities in response to metabolic changes.

Cell signaling

There are a number of different mechanisms known to regulate stem cell maintenance and differentiation. Proliferation signals and cell cycle regulators may control cell kinetics or total number of cell divisions [43,44]. Loss of trophic support and cytokine receptor activation may contribute to the induction of cell death at specific stages of development [45,46]. Signaling from differentiated progeny or asymmetric distribution of specific molecules may alter the self-renewal characteristics of stem cells [47–49]. Thus, it appears as if the final decision of a cell to self-renew, differentiate, or remain undifferentiated is dependent on an integration of multiple signaling pathways which in turn depend on cell density, metabolic state, ligand availability, type and levels of receptor expression, and downstream cross-talk between distinct signaling pathways. Although planarian stem cells respond to environmental factors such as nutrient status by undergoing cell cycle arrest or activation [39], little is known about the molecular mechanisms. Even though several signaling pathways have been shown to operate in various stem cell populations from *Drosophila* to humans [50–52], it is not clear which signal pathways are involved in planarian stem cells specifically, and how they affect the regulation of the cell cycle. In fact, how multiple signal pathways cooperate or antagonize each other to regulate the functions of metazoan stem cells still remains an open question.

Chromatin dynamics

It is likely that factors controlling chromatin architecture may play key roles in defining the developmental fate of the division progeny of stem cells, including their multipotency and self-renewal capacities. In fact, it is believed that the higher order of chromatin organization [53] may be responsible for regulating the genomic output of cells, since specific and heritable attachments of interphase chromosomes to the nuclear membrane have been observed. The best evidence is provided by studies of GFP-tagged H2B in mammalian cells, in which experimentally induced photobleaching patterns remained unchanged from one cell generation to the next, arguing for a heritable transmission of chromatin positional information [54]. Thus, local changes in chromatin organization are likely to be required before changes in genomic output may occur in a given cell. Hence, the maintenance and/or restriction of cellular multipotentiality are likely to be reflected by the mobility and configuration of chromatin in their nuclei. One prediction of this hypothesis would be that distinct, specific, and heritable differences between the nuclear and chromatin architecture of somatic and germ cells may exist explaining, in part, their similar morphologies, but widely different functions.

It is also interesting to note that stem cells generally possess a large nucleus to cytosolic ratio, and that this ratio decreases as cells differentiate [55]. Moreover, the correlation between nucleus to cytosolic ratio also holds for chromatin, since decondensed chromatin appears to be much more abundant in stem cells [56], and the amounts of heterochromatin increase progressively as the cells become differentiated. Given that heterochromatic DNA is normally inaccessible to the transcription machinery, the pronounced absence of heterochromatin in somatic stem cells may in fact be related to the multipotency of these cells. For instance, hematopoietic stem cells express multiple non-hematopoietic genes as well as hematopoietic genes, but their developmental potential and genomic output is restricted progressively through differentiation [57]. In the case of neoblasts, it has been shown that these stem cells have large nuclei (Fig. 2A), with their chromatin evenly distributed throughout the large nuclear space [58]. Therefore, it seems plausible that throughout their cell cycle, chromatin remodeling may control the functions of planarian and other metazoan stem cells. Whether gene expression of planarian stem cells is controlled by chromatin remodeling, and whether such changes affect the fate of stem cells and their division progeny are questions that merit detailed and systematic experimental attention.

Non-coding RNAs

Recently, miRNAs have been shown to modulate the fates of the differentiating progeny of stem cells. This has been functionally demonstrated for miR-181which when overexpressed in hematopoietic stem cells led to an increased fraction of B-lineage cells in both tissue-culture differentiation assays and adult mice [59]. Specific miRNAs have also been isolated from mouse ES cells and their progeny, suggesting that the differential expression of these miRNAs may play a role in the fate restriction of these cells.

This hypothesis, however, has not been formally tested. Moreover, a connection between small non-coding RNAs, and chromatin regulation has been newly uncovered in yeast, where these molecules target complementary genomic sequences and promote Histone H3 lysine-9 methylation and silencing of gene expression [60]. Taken together, a picture is beginning to emerge indicating that small ncRNAs may play key roles in the maintenance and differentiation of stem cells. The cross-talk between ncRNAs and chromatin is interesting as it implies that mechanisms for the stabilization of the genomic output of cells must exist in both stem and differentiated cells. Since planarians reside in a phylogenetically separate position from *C. elegans* and *Drosophila* (Fig. 1), the study of miRNAs in the neoblasts and their division progeny in *S. mediterranea* will provide critical information on the evolution of these ncRNAs and new contexts in which to study and mechanistically dissect their function.

Conclusion

Much is unknown about the biology of somatic stem cells, particularly during the adult condition of multicellular organisms. This is reflected, by the limited understanding we possess in regards to the regulation of tissue homeostasis. The replacement of differentiated cells is a major challenge for all metazoans throughout their life span [61,62]. Humans, for example, must replace an estimated 10 billion cells every day [63], while other animals face the often frequent prospect of having to replace complete body parts lost to predators [64], or even their entire body as a result of continuous tissue homeostasis [19]. One way in which such turnover and body part replacement is regulated is through the maintenance, proliferation, and differentiation of somatic stem cells. Yet, despite the importance of tissue homeostatic processes to human biology and health, relatively little is known about the mechanisms controlling adult tissue homeostasis. Thus, many questions remain unanswered. For example, how are cells in intact organs specified to die during turnover? And how does the death of a cell trigger its subsequent replacement by somatic stem cells? Furthermore, how do organ systems maintain their form and function while in a state of cell flux?

Answering these questions will require that we understand the context in which somatic stem cells evolved and the evolutionary relationships of stem cells in the Metazoa, since these factors alone are strong determinants in the selection of the regulatory mechanisms responsible for preserving the fitness of these cells. If in fact somatic stem cells are subjected to similar selective pressures in organisms in which the germ line is not segregated early during embryogenesis (e.g., mammals and planarians), it should be feasible to inform human stem cell biology by studying simpler metazoans. *S. mediterranea*, for example, should allow for the in vivo identification and characterization of regulatory mechanisms controlling the functions of somatic stem cells. Such mechanisms are likely to be complex and multidimensional. For example, attempts at defining common transcription signatures of various in vitro cultured stem cells [65] have proven unreliable [66], and did not produce enough information to explain the multipotency of stem cells. Thus, it will be necessary not only to identify transcriptionally active genes in vivo, but also to understand the mechanics of chromatin regulation effecting such transcription, and how the interaction of somatic stem cells with their environment modulate their activities. As such, analyses of the cell cycle will help identify those phases upon which environmental changes may be exerting their regulation, while studies on the function of non-coding small RNAs may help explain how the full complement of genes present in a seemingly euchromatic (decondensed) genomic state is regulated. The evolutionary position of *S. mediterranea*, the ease with which it can be manipulated, its relative simplicity when compared to higher organisms, and the abundance and experimental accessibility of its stem cells provide us with unique opportunities to study in vivo these and other mechanisms involved in the regulation of somatic stem cells.

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