It's No Fluke: The Planarian as a Model for Understanding Schistosomes

James J. Collins, III, Phillip A. Newmark*

Howard Hughes Medical Institute and Department of Cell and Developmental Biology, Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, Illinois, United States of America

Introduction

Schistosomiasis is among the most prevalent human parasitic diseases, affecting more than 200 million people in the developing world [1]. Indeed, some estimates suggest the global disease-associated disability resulting from schistosomiasis may reach levels on par with global killers including malaria, tuberculosis, or HIV/AIDS [2,3]. The causative agents of this disease are flatworms (Schistosoma or blood flukes) that feed on blood in the veins surrounding the host’s intestine or bladder. These worms produce hundreds to thousands of eggs per day, many of which lodge in host tissues and cause diverse pathologies, including hepatic fibrosis, splenomegaly, and in some cases, perhaps cancer. Despite the grim statistics, only a single therapeutic agent (praziquantel) is currently used to treat schistosome infection. With the looming possibility that praziquantel-resistant Schistosoma strains could arise, an urgent need exists to identify novel therapeutic agents to combat these parasites.

Schistosomes Have Complicated Life Cycles

Like all digenetic trematodes, schistosomes have complex life cycles that alternate between phases of obligate parasitism of snail intermediate and vertebrate definitive hosts [4] (Figure 1). This life cycle initiates when schistosome eggs, passed via the urine or feces of a vertebrate host, come into contact with freshwater and release a short-lived, ciliated stage called the miracidium. These miracidia employ a sophisticated set of sensory and penetration organs to seek out and ultimately infect a suitable snail host. Following penetration, miracidia undergo a striking developmental transformation whereby major organ systems degenerate and pluripotent cells called germinal cells begin to proliferate. This stage of the life cycle, the sporocyst, is key to the successful propagation of schistosomes. Simply put, stem cells in the sporocysts have the capacity to undergo an asexual embryogenesis, producing either additional sporocysts or thousands of infective cercariae, which are released from the snail. Attracted by fatty acids from vertebrate skin, cercariae burrow through the host’s epidermis, enter the circulatory system, and eventually migrate to the mesenteric veins (S. mansoni and S. japonicum) or the venous plexus of the bladder (S. haematobium). The parasites then feed on blood, develop to adulthood as either male or female worms (which then pair together), and begin to reproduce, thus completing the life cycle.

Over the past decade, a wealth of modern molecular tools have been made available to study schistosomes, including: genome sequences for the three major human schistosome species [5–7], procedures for genetic manipulation using RNA interference [8,9], robust protocols for whole-mount in situ hybridization [10] and immunofluorescence [11], and breakthroughs in the development of transgenic approaches [12]. However, the complexity of the schistosome life cycle, coupled with a dearth of methods for in vitro propagation, has continued to hinder progress toward developing novel therapeutics. Therefore, new approaches are needed to address this important challenge.

Lessons from Roundworms

Studies of model organisms (e.g., yeast, ciliated protozoans, nematode worms, and fruit flies) have shaped our understanding of central questions in biology (e.g., cell-cycle control, telomerase function, programmed cell death, and embryonic patterning). Similarly, free-living invertebrates have provided meaningful contributions toward our understanding of their parasitic relatives. For example, studies of the free-living nematode Caenorhabditis elegans have provided novel insight into nematocidal drug action [13–15], parasite genome evolution [16], and parasite gene function [17–19]. Procedures developed for C. elegans have even guided the establishment of transgenic approaches for parasitic nematodes [20]. Furthermore, studies of C. elegans have revealed how exploiting “eccentricities” shared between free-living and parasitic nematodes could lead to novel ways to combat disease. For example, a nuclear hormone receptor (DAF-12) that regulates a C. elegans alternative third-larval stage (L3), called Dauer, similarly regulates the analogous infective L3 (IL3) stage of parasitic nematodes [21]. Of note, administration of the C. elegans DAF-12 ligand is capable of inducing precocious IL3 recovery of Strongyloides stercoralis or Ascocystoma caninum and causing S. stercoralis to aberrantly enter iL3. Thus, the modulation of DAF-12 activity represents a novel means to disrupt the life cycle of parasitic nematodes.

Although work on C. elegans has contributed to our understanding of nematode parasites, no equivalent free-living models have become widely accepted for studies of the phylogenetically distinct parasitic flatworms (e.g., Schistosoma and Fasciola). Indeed, not all worms are created equal: nematodes are members of a group of molting animals called Ecdysozoa, whereas flatworms are Lophotrochozoans, a clade of animals that includes mollusks and annelids [22]. Thus, in evolutionary terms, schistosomes share...
Planarians as a Free-Living Model for Schistosomes?

Planarians are free-living platyhelminths and have been a valued experimental model for over a century due to their nearly unlimited capacity to regenerate damaged tissues [24]. Indeed, the more in common with snails and squid than they do with hookworms and whipworms! Despite their differing lifestyles, free-living flatworms and their parasitic relatives share numerous evolutionarily conserved features that are central to their physiology and reproduction (a few examples are shown in Figure 2A and are discussed below). Furthermore, in contrast to the nematodes, in which parasitism has arisen multiple times independently [23], all parasitic flatworms (trematodes, cestodes, and monogeneans) form a derived monophyletic group known as the “neodermata” (Figure 2B). Thus, applying lessons learned from free-living relatives has the potential to directly aid our understanding of not just schistosomes, but the more than 6,000 species of parasitic flatworms.

Figure 1. The schistosome life cycle. The various stages of the schistosome life cycle are depicted using fluorescence (sporocysts and cercariae in snail host labeled with DAPI (green), phalloidin (magenta), and peanut agglutinin (orange)), bright-field (egg), or scanning electron micrographs (remaining stages). Images are not shown to scale.
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Figure 2. Similarities between schistosomes and planarians. (A) Schistosomes (left column) and planarians (right column) possess common cell types and organ systems. Top row, (left) scanning electron micrograph of male and female *S. mansoni* and (right) dark-field image of *S. mediterranea*. Second row, eggs. Both organisms lay ectolecithal egg capsules with a polyphenolic shell. Only a subset of derived flatworm species (called neoophora) lay this unique type of egg. Third row, glands associated with the egg-laying apparatus. These glands (Mehlis’ gland in schistosomes and cement gland in planarians) labeled with the lectin, peanut agglutinin (green) [11,37]. Although these glands are found in both free-living and parasitic flatworms, their function is unclear. Fourth row, confocal images of protonephridia labeled with (left) anti-acetylated tubulin (green)/wheat germ agglutinin (magenta) and (right) anti-acetylated tubulin (green). Protonephridia are found in most platyhelminths and play
regenerative abilities of planarians are so robust that minuscule tissue fragments (<1/200th the size of the original animal) can fully regenerate a new animal. This regenerative prowess is made possible by a population of pluripotent stem cells, called neoblasts, which proliferate and serve as progenitors for regenerating tissues. In addition to their role in regeneration, neoblasts also drive long-term tissue homeostasis such that planarians deprived of neoblasts fail to maintain their tissues and die within a few weeks.

Over the past decade, *Schmidtea mediterranea* has become the preeminent model for understanding the biology of stem cells and regeneration in planarians. Its rise to prominence is due in part to the ease with which it can be maintained in the laboratory, its favorable genetic features (i.e., relatively small diploid genome), and its fully sequenced and assembled genome [24,25]. Furthermore, robust methods exist for analysis of gene function, including tissue screens and to perturb the function of hundreds to thousands of genes [26]. 

In addition to their role in regeneration, neoblasts also drive long-term tissue homeostasis. Neoblasts are responsible for the renewal of tissues lost during the normal wear and tear of daily life. These cells are described in various worms (e.g., *Caenorhabditis elegans*), in which they are found in the nervous system, and in flatworms (e.g., *Schistosoma mansoni*), in which they are found in the reproductive system. Neoblasts are also found in the liver and other organs in humans and other species. Neoblasts are thought to be responsible for the repair of damaged tissues following sublethal doses of praziquantel [35].

Like Planarians, Schistosomes Have Neoblasts

Although the eggs are the source of pathology in schistosomiasis, the chronic nature of this disease can be attributed in part to the extreme longevity of these parasites. In fact, reproductive activity of schistosomes has been observed in patients more than three decades after leaving endemic regions [4]. While the mechanisms responsible for this longevity remain a mystery, studies of planarians may provide new insights. As mentioned above, neoblasts not only fuel regeneration but they also promote the renewal of tissues lost during the normal wear and tear of daily life. Guided by this knowledge, and the observation that neoblast-like cells have been described in tapeworms [33], a recent study identified a novel population of neoblast-like stem cells in adult *S. mansoni* [34]. These cells were morphologically similar to neoblasts and also shared their ability to produce differentiated cell types, such as muscle and intestine. Furthermore, these cells expressed numerous genes known to regulate neoblasts in planarians. At present, it is not clear what role these cells play in the parasite; however, the observation that schistosomes can regenerate damaged tissues following sublethal doses of praziquantel [35] suggests that these stem cells may play a pivotal role, not only in longevity, but in numerous facets of the parasite’s biology.

Conclusions

This short pearl has only scratched the surface of the potential for using planarians to inform the biology of schistosomes. Indeed, many questions remain: Can planarians be used to identify new therapeutics? Can planarians help us characterize the mechanism of action of existing therapeutics [36]? Are any of the hundreds of
genes specific to flatworms functionally important? Do neoblast-like stem cells play a role in other stages of the schistosome life cycle?

Due to space constraints, this review has focused upon features shared between these flatworms; however, it is important to note that there are fundamental differences between them, too. Schistosomes must invade two different hosts, navigate their immune systems, and respond to (and in some cases modulate) host physiology. For all that we may learn about flatworm biology and response to (and in some cases modulate) host physiology. For all that we may learn about flatworm biology and immune systems, and respond to (and in some cases modulate) host physiology. For all that we may learn about flatworm biology and immune systems, and respond to (and in some cases modulate) host physiology. For all that we may learn about flatworm biology and immune systems, and respond to (and in some cases modulate) host physiology. For all that we may learn about flatworm biology and immune systems, and respond to (and in some cases modulate) host physiology.

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