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# The Case for Comparative Regeneration: Learning from Simpler Organisms How to Make New Parts from Old

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## ABSTRACT

**Regeneration is a primordial attribute of all living organisms, and as a biological problem it has attracted the attention of generations of experimental biologists for almost 300 years. Yet regeneration still awaits a satisfactory mechanistic explanation. In this brief review, I will argue for the reinstatement of planarians, a classical and currently understudied experimental organism, as a viable, molecular model system in which to functionally dissect the molecular basis of animal regeneration. The developmental plasticity and phylogenetic position of planarians, coupled with the technical ability to specifically silence gene expression via RNA interference should help usher these organisms to the forefront of regeneration research. Planarians are currently in a unique position to provide us with the fundamental tools required to begin the identification and functional characterization of the genetic interactions operating behind the regenerative abilities found in the metazoans.**

## OVERVIEW

Planarians are discussed as a viable system in which molecular studies of regeneration can be performed. Their plasticity and the molecular homologies known to exist between planarians and well-established animal models indicate that molecular studies of regeneration in planarians will have far reaching consequences in our understanding of the general problem of metazoan regeneration.

## INTRODUCTION

"The regenerative process is one of the fundamental attributes of living things..." In 1901, Thomas Hunt Morgan chose to conclude his book "Regeneration" in such unambiguous terms (1). Not too long ago, such a remark would have been considered incorrect by many, since regeneration is generally associated with a few organisms, notably hydra, planarians and salamanders. However, the discovery of liver regeneration in rats and humans (2–4), and the more recent demonstrations of regenerative events taking place in organs traditionally believed to be unable to regenerate such as the human central nervous system (5–8) underscore the validity of Morgan's reasoning. In fact, comparative studies on the regenerative properties of plants and animals led not only Morgan but others after him (9, 10) to maintain that regeneration is an at-

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tribute indissolubly associated with life. This is evidenced by the fact that the replacement of body parts (whether it be the mere replacement of a population of cells in a given organ, or the complete regeneration of missing appendages) is a physiological task performed by all known organisms.

In the Metazoa, regenerative powers are manifested in varying degrees of complexity, yet generally fall under two main categories: morphallactic and epimorphic. As defined by Thomas Hunt Morgan in 1901, morphallactic regeneration is the restoration of missing or damaged structures directed not by cellular proliferation, but by the remodeling of the remaining body parts. Epimorphic regeneration, on the other hand, requires cellular proliferation and sometimes depends on the formation of a complex stump referred to as the regeneration blastema. The cells that proliferate during this process are either toti- or pluripotential stem cells whose determination and differentiation result in the restoration of the missing body part(s).

Of particular interest is blastemal-based regeneration which shares striking similarities between very distant phyla (11), and it is generally associated with the regeneration of very complex structures such as limbs, tails and even heads. Considering how many different phyla share the formation of a regeneration blastema after injury or amputation, it is very likely that the mesenchymal-epithelial interactions taking place during the ontogeny of these specialized structures may share a common evolutionary origin and thus a common genetic complement (12). For instance, regeneration in the simple platyhelminth planarian, as in vertebrates such as the salamanders, begins with the formation of a blastema which subsequently grows and differentiates into the missing parts.

## PLANARIANS AS AN ANIMAL MODEL

A better understanding of regeneration and its importance in the evolution of the Metazoa would require the identification of organisms in which regeneration plays a prominent role in their life cycle. Furthermore, such animals would also have to be amenable to molecular manipulations. The recent identification of a mouse strain capable of deploying blastemal-based regeneration after their ears are punched through-and-through will go a long way in the identification of some of the genes which when activated will allow regeneration to take place in mammals (13, 14). However, given the functional redundancy known to exist in all vertebrate systems in which genetics is feasible (mouse and zebrafish) distilling the function of these genes at the molecular and then cellular level will not be trivial. One way to overcome this difficulty would be to study simpler organisms in which such functional redundancy would not be expected. One such animal is the triploblastic flatworm or planaria, long considered to be one of the most spectacular model systems in which to study blastema-mediated regeneration.

Molecular studies of planarian biology have been increasing steadily in the past few years. Hox gene homologues and some of their expression patterns have been identified in these organisms (15), as well as a large number of developmentally important molecules such as bone morphogenetic protein (BMP) (16), and the homeodomain transcription factors otxA, otxB and otp (17, 18). The temporal and spatial data obtained from these genes intimate that several key molecular cascades regulating morphogenesis are conserved between planarians and other triploblastic organisms. Therefore, it appears likely that the delineation of the molecular events underpinning regeneration in planarians will also shed light on those regenerative events that occur in higher organisms.

## FUNCTIONAL STUDIES

Nevertheless, functional analyses of these and other genes in planarians have not been possible, hampering the determination of the roles these genes may play during planarian regeneration. However, the recent introduction of RNA interference methodologies devised originally in *C. elegans* into the study of planarian regeneration overcomes this difficulty. Through mechanisms which are not entirely clear, but which may involve viral response pathways in eukaryotic cells (19), double-stranded RNA specifically abrogates expression of the gene from which that RNA was derived. Taking advantage of this methodology my laboratory has demonstrated the potency and specificity of these molecules to silence gene expression in pla-

narians (20). An example is shown in Figure 1 using the planarian *Dugesia gonodocephala* in which elimination of the body wall musculature is accomplished by the introduction of dsRNA made from myosin heavy chain transcripts. Currently, we are analyzing the effects of eliminating in planarians many developmentally- and regeneration-modulated genes (singly and in combination) in order to elucidate their respective roles during planarian regeneration. We hope that this approach will help us assemble a series of genetic interactions which may help us understand why some animals are capable of regenerating complex structures while others are either unable or severely restricted in their ability to do so.

## PLANARIAN STEM CELLS: THE NEOBLASTS

A key biological trait of planarians is the presence of a relatively large and stable population of stem cells as part of their general bauplan (20–30% of the cell population in an adult planaria). These cells, known as neoblasts, are the only mitotically active cells in planarians and give rise to the 20 to 30 different cell types known to exist in these organisms, including the germ lineage (21, 22). Neoblasts are small (5–10 $\mu$m in diameter), undifferentiated cells randomly scattered throughout the planarian mesenchyme (Figure 2). After amputation, these cells are locally signaled to proliferate, and their concomitant increase in numbers gives rise to the regeneration blastema. In planarians as in salamanders, the regeneration blastema will eventually differentiate with the appropriate dorsoventral, anteroposterior and proximodistal polarities to regenerate the missing body parts.

Neoblasts, therefore, play a key role in the regenerative powers planarians are so well-known for. However, little is known about the true nature of these cells. Historically, neoblasts have been considered to be

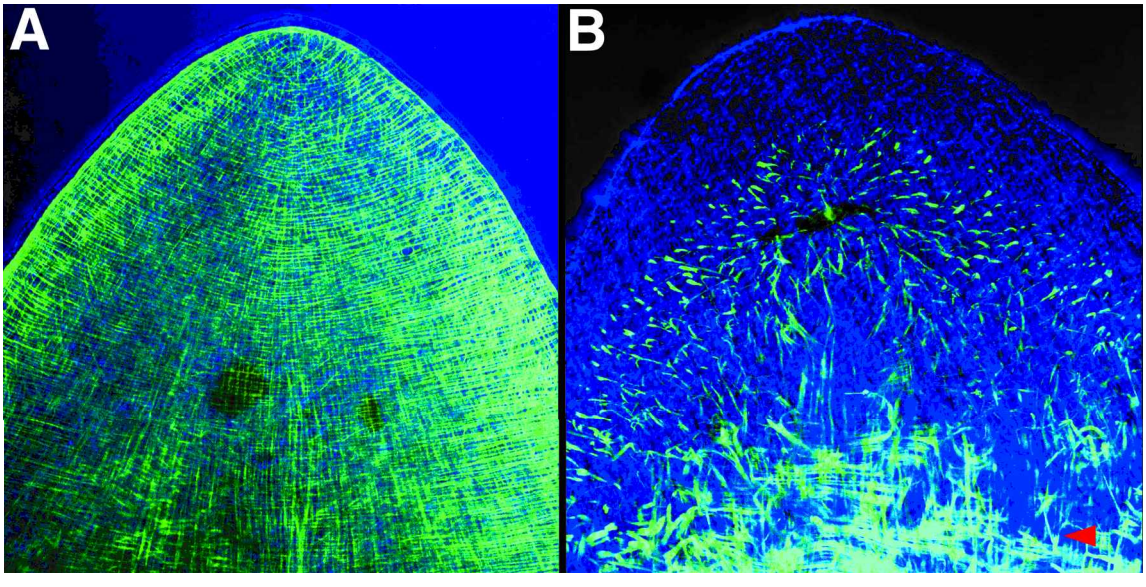


**FIG. 1.** Ablation of myosin heavy chain gene expression using double stranded RNA. The heads of both animals shown (*Dugesia gonodocephala*) are the result of seven days of regeneration at room temperature after amputation. The planarian in (**A**) was injected with water immediately after amputation. The planarian in (**B**) was injected with myosin heavy chain dsRNA. Fluorescent phalloidin was used to visualize the body wall musculature using a confocal microscope. In (**A**), the musculature regenerated normally as did the photoreceptors (dark round spots), and both structures are indistinguishable from wild type. In (**B**), the musculature failed to regenerate properly as evidenced by the fused photoreceptors, the lack of fluorescence in various regions of the regeneration blastema as well as the disorganization of the muscular fibers detected. In fact, even the pre-existing body wall musculature was affected by the injected dsRNA (red arrowhead).

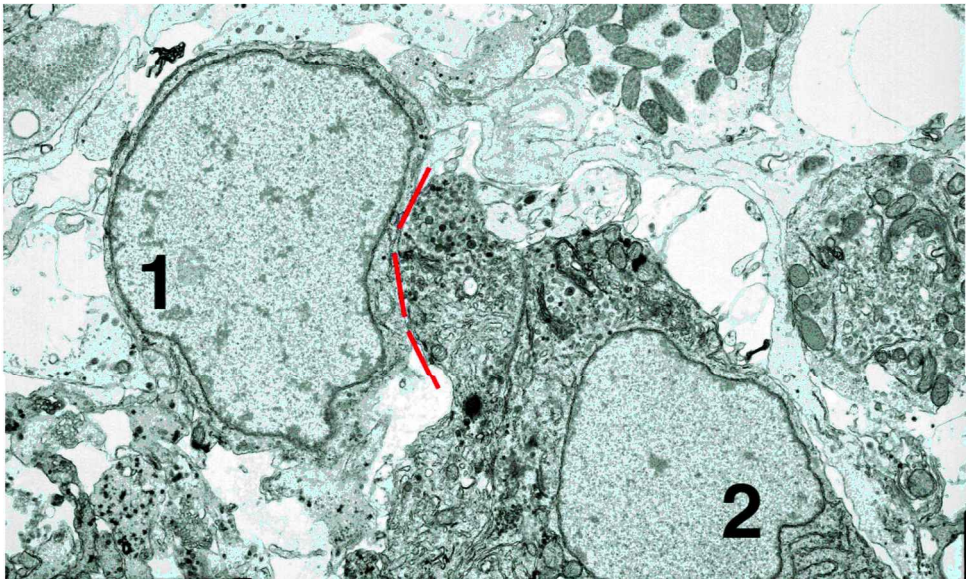


**FIG. 2.** The planarian neoblast. Electron micrographs of neoblasts near site of amputation in the planarian *Schmidtea mediterranea* . A typical neoblast 8–10 μm in diameter exhibiting characteristics of undifferentiated cells, such as a very large nucleus and a scant cytoplasm is shown (1). A neoblast in the process of differentiation is also shown (2). Cell membrane boundaries between these cells are denoted by the dashed red line.

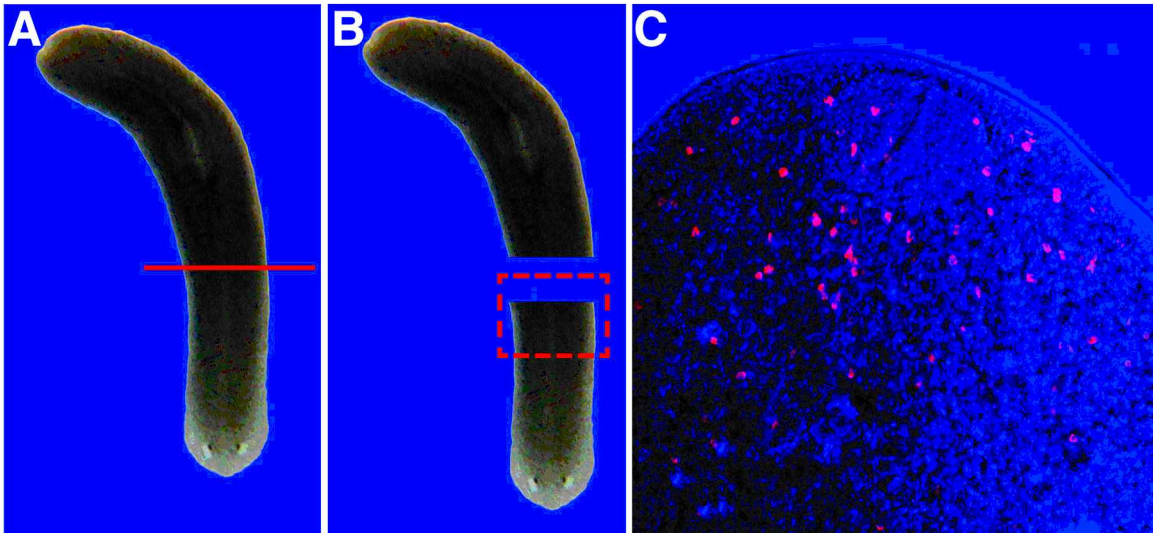


**FIG. 3.** Labeling the planarian neoblasts. A recently developed methodology in my laboratory by Dr. Phillip A. Newmark for the identification and lineage-tracing of neoblasts in planarians is illustrated. A *Schmidtea mediterranea* is shown in (**A**), with the red line demarcating the site of amputation. In (**B**) the types of fragments that would result from amputating a planarian at the level shown in (**A**) are illustrated. The dashed, red rectangle demarcates the area from which the posterior (caudal) blastema will emerge. (**C**) The distribution of neoblasts undergoing mitoses is detected by anti-phospho-histone H3 antibody (32) in the caudal regeneration blastema. In this panel, confocal projections of the fluorescent image (in red) were superimposed upon a Nomarski image of the flatworm (in blue).

impervious to labeling with DNA precursors (23–26) and this has forced many planarian biologists to rely on much less specific methods to measure mitotic activity in these organisms (27–29). Preliminary experiments in my laboratory indicate that neoblasts can, in fact, be labeled with DNA precursor analogs and that mitotic activity can be assayed using mitosis-specific antibodies (Figure 3). Using continuous labeling techniques and a fraction of labeled mitoses (FLM) methods (30), we have begun to delineate basic parameters of the neoblast cell cycle, and have found that (contrary to previous findings (29, 31)) a large subpopulation of slow cycling neoblasts does not exist in these organisms (Newmark and Sánchez Alvarado, manuscript submitted for publication).

Future studies will focus on further characterizing this remarkable cell population with the aim of elucidating the mechanisms regulating their maintenance, proliferation and differentiation. Better purification and culture methods of the planarian stem cells are being devised with the objective of developing gain-of-function assays. In principle, neoblasts can be used as vectors to introduce DNA into planarians and thus bring transgenesis as a tool to further explore regeneration in this fascinating metazoan.

In conclusion, the capacity to specifically silence gene expression by the use of double-stranded RNA-mediated genetic interference (20), combined with the ability to label the regenerative stem cells in planarians lay the necessary molecular foundations to exploit the unique biology of these organisms. Planarians present to the experimental biologist with a unique opportunity to significantly improve our understanding not only in the area of stem cell proliferation and maintenance, but also in the field of metazoan regeneration at large.

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