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Opening a New Can of Worms: A Large-Scale RNAi Screen in Planarians

In this issue of *Developmental Cell*, Reddien et al. describe the first large-scale RNAi screen in freshwater planarians, classic models for regeneration studies. Their work paves the way for a detailed understanding of regeneration and tissue maintenance in these fascinating animals.

Freshwater planarians have been favorite subjects of regeneration experiments for over a century (Newmark and Sánchez Alvarado, 2002; Reddien and Sánchez Alvarado, 2004). These free-living members of the phylum Platyhelminthes (the flatworms) are capable of regenerating a complete animal from a tiny portion of the body. Planarian regeneration relies upon a stem cell population that is maintained throughout the life of the organism (Baguñà et al., 1989; Newmark and Sánchez Alvarado, 2000). These stem cells (the neoblasts) are the only proliferating cells in the flatworm; in uninjured worms, they serve as the source of new cells during physiological cell turnover. After injury, neoblasts proliferate to form the regeneration blastema in which most of the missing structures will be re-formed (Baguñà et al., 1989; Newmark and Sánchez Alvarado, 2000).

Research on planarians declined dramatically beginning in the 1970s; however, the study of these animals has been revitalized by the recent application of the tools of molecular and cellular biology (Newmark and Sánchez Alvarado, 2002). The susceptibility of planarians to RNA interference (RNAi) (Sánchez Alvarado and Newmark, 1999) has been critical for their reemergence as a model system. RNAi allows the characterization of gene function in organisms that are not amenable to traditional genetic analysis; in the case of planarians, for example, RNAi can be used to dissect their developmental plasticity. With the generation of thousands of expressed sequence tags (ESTs) from planarians (Sánchez Alvarado et al., 2002; Mineta et al., 2003) and the upcoming complete genome sequence of *Schmidtea mediterranea*, genomic-scale resources for studying planarians have become available. Furthermore, recent work has shown that, like *C. elegans*, planarians can be fed bacteria that express double-stranded RNA, resulting in specific gene inhibition (Newmark et al., 2003).

Thus, the stage has been set for the next logical step: large-scale, unbiased RNAi screens for genes involved in regeneration and tissue maintenance in planarians. In this issue of *Developmental Cell*, Reddien et al. (2005) describe the results of the first such screen in planarians. Their massive undertaking was made possible by improvements they made in the vector used for expressing dsRNA in bacteria as well as by extensive optimization experiments to maximize the efficiency of the bacterial feeding procedure. With these technical improvements in hand, Reddien et al. (2005) moved on to assess the consequences of RNAi knockdowns of 1,065 planarian genes obtained from cDNA libraries from planarian heads and neoblast-enriched cell populations. The animals were fed multiple times and taken through two rounds of regeneration to minimize the effects of protein perdurance and/or incomplete RNAi knockdowns. After RNAi treatment, gross morphological defects and behavioral phenotypes were characterized. In all, 54,300 amputations were performed (handily defeating the previous world record for largest number of planarians chopped in the course of a research project).

This tour de force resulted in the identification of 240 genes (22.5% of those screened) in which RNAi knockdowns yielded defects in regeneration, tissue maintenance, and/or behavior (see Figure 1). Given the large number of cellular mechanisms encompassed by the process of regeneration, this large percentage is not particularly surprising. Many of the genes yielding observable RNAi defects were rescreened by immunofluorescent staining of the photoreceptors and mitotic cells in knockdown animals to characterize further the RNAi phenotypes. Reddien et al. (2005) provide an exhaustive categorization of the phenotypes that they obtained. They use these categories to assign genes to seven stages in the regeneration process, from the initial events of wound healing and neoblast proliferation to patterning of the new structures, remodeling within the old tissues, as well as maintenance and function of the regenerated structure(s). For example, they show that knockdowns of 48 genes result in phenotypes similar to those observed after X-irradiation, which is known to destroy the planarian stem cells; genes in this category may be involved in maintaining the stem cell population. This work also provides the first descriptions of a large number of interesting planarian phenotypes including: pointy blastemas, indented blastemas, various types of regression and lysis, asymmetries, novel defects in photoreceptor axon projections, and many intriguing behavioral phenotypes, from flattening and sideways motility to tight substrate adherence and stretching.

As striking as many of these new phenotypes are, it is interesting also to consider the kinds of phenotypes that were not obtained in this screen. To take an example of perhaps the most impressive planarian phenotype to emerge from the earlier RNAi work, knockdowns of the gene *nou-darake* ("brains everywhere" in Japanese) result in the formation of ectopic brains and photoreceptors throughout the body (Cebrià et al., 2002). Likewise, one might anticipate identifying RNAi knockdowns that result in the formation of Janus heads (in which heads are observed at both ends of the animal) or tails, classic phenotypes observed after many experimental manipulations. Similarly, no RNAi knockdowns were identified that resulted in blastema overgrowth. Thus, the RNAi knockdowns that generated elevated numbers of phospho-histone H3 (ser10)-positive cells apparently did so by disrupting progression

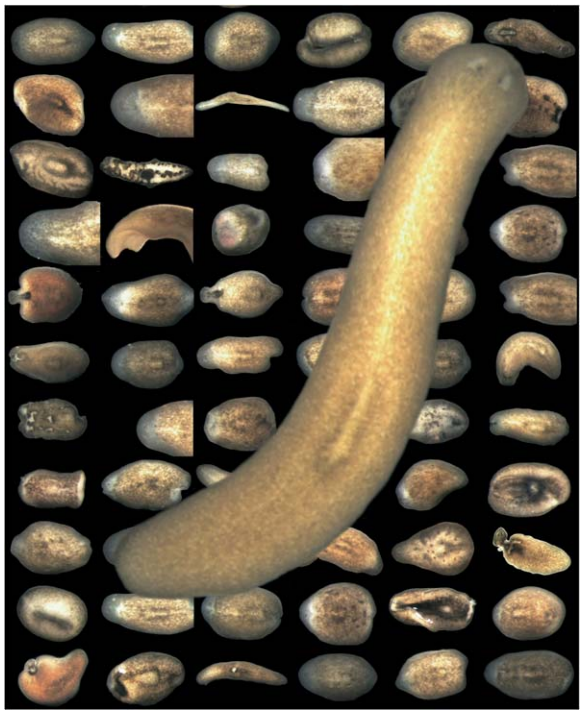

Figure 1. Large-Scale RNAi in Planarians

A wild-type planarian (*Schmidtea mediterranea*) set against a montage of different mutant phenotypes obtained from the RNAi screen. The image was kindly provided by Peter Reddien and Alejandro Sánchez Alvarado.

through mitosis, rather than leading to overproliferation. If one estimates that the planarian genome contains on the order of 15,000–20,000 genes, this screen would represent 5%–7% of the total genes in the organism; thus, there is good reason to suspect that many additional phenotypes will be obtained during the course of future screens.

The work of Reddien et al. (2005) represents a quantum leap in studies of planarians, truly ushering this organism into the age of functional genomics. Anybody who has observed a planarian and marveled at its regenerative potential should be able to appreciate the significance of this work. One of the next challenges will be to understand the molecular bases for the phenotypes described here. Using an expanded pool of markers to label specific differentiated cell types or different subsets of the neoblast population will be crucial for refining (and expanding) the categories of planarian phenotypes. The fruits of all of this labor ultimately will be an understanding of the cellular and molecular bases of the planarian's remarkable developmental plasticity. Everybody grab a razor blade!

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