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Unconventional Functions of Muscles in Planarian Regeneration

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Abstract

Muscles are traditionally considered in the context of force generation. Scimone et al. (2017), reporting in *Nature*, now examine muscles in a developmental setting and find unexpected roles for distinct planarian muscle fibers. The authors show that muscles provide patterning signals to promote regeneration and guide tissue growth after injury.

Regeneration of the entire body from a miniature fragment is a remarkable trait of some animal species, including the freshwater planarian *Schmidtea mediter-ranea*. Pieces of planarian as small as 1/279th of the body can regenerate a whole animal (Morgan, 1898). This process depends on stem-cell-dependent proliferation and differentiation to replace lost cell types and patterning cues from positional control genes (PCGs). Recently, it was found that during planarian regeneration, various PCGs are expressed in different types of body wall muscle (BWM) (Witchley et al., 2013). However, the mechanisms that specify different muscle fibers and their particular contributions to the regenerative response are not well understood. Using genetic perturbation experiments, Scimone et al. (2017) reveal distinct regulatory roles of different muscle fibers in coordinating tissue regeneration after amputation in S. *mediterranea* (Figure 1B).

Neoblasts—undifferentiated stem cells—are the only mitotically active cells in adult planarians, present throughout the mesenchyme (Newmark and Sánchez Alvarado, 2000). In response to injury, mitotic activity first increases in neoblasts throughout the entire organism and then is maintained in progenitors proximal to the amputation site (Wenemoser and Reddien, 2010). Neoblasts are totipotent: a single transplanted neoblast can fully reconstitute all the cell types of a regeneration-capable adult planarian (Wagner et al., 2011).

In addition to neoblast proliferation, planarian regeneration also depends on the expression of PCGs to specify tissue identity during regeneration (Reddien, 2011). After injury, PCG expression is induced in BWMs (Witchley et al., 2013); however, it is important to consider that these tissues are not homogeneous. They consist of three distinct layers: a diagonal muscle fiber layer sandwiched between an outer circular muscle fiber layer and an inner longitudinal muscle fiber layer (Figure 1A; Scimone et al., 2017). Interestingly, Scimone et

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al. (2017) identified that expression of *myoD*—which encodes a helix-loop-helix transcription factor with evolutionarily conserved roles in myogenesis—is localized to just a fraction of *collagen*⁺ BWM cells. RNA interference (RNAi) against *myoD* led to significant loss of longitudinal muscle fibers without perturbing circular or diagonal fibers, resulting in an increased length-to-width ratio in planarian mutants. RNA sequencing (RNA-seq) of these uninjured *myoD(RNAi)* animals revealed a subset of PCGs, typically co-expressed with *myoD*, that were significantly downregulated, indicating their predominant expression in longitudinal muscle fibers.

Surprisingly, *myoD(RNAi)* animal fragments failed to initiate regeneration after amputation (Figure 1B), even though their neoblasts retained the ability to differentiate into various cell types (Scimone et al., 2017). Although *myoD(RNAi)* animals could repair small injuries like eye resection and tissue turnover through neoblast differentiation, these progenitors failed to undergo sustained proliferation, limiting repair of larger tissues. RNA-seq of injured *myoD(RNAi)* animals revealed significantly reduced expression of two wound-induced genes that are typically enriched in longitudinal fibers after injury: *notum* and *fst*. Both genes are critical in the regenerative response: *notum* encodes a WNT depalmitoylation enzyme and determines the planarian head- versus-tail decision after amputation by negatively regulating the Wnt signaling pathway (Zhang et al., 2015); *fst* encodes follistatin and enables sustained wound- induced gene expression for elevated neoblast proliferation during regeneration (Gaviño et al., 2013; Roberts-Galbraith and Newmark, 2013). Scimone et al. (2017) found that *fst* RNAi, like *myoD* RNAi, allowed tissue turnover but completely prevented regeneration. Tail fragments from *myoD(RNAi)* animals and *fst(RNAi)* animals failed to restrict posterior PCG expression and to initiate anterior PCG expression properly. Follistatin negatively regulates TGFβ ligand activins, and inhibition of *activin* rescues regeneration in *fst(RNAi)* animals (Gaviño et al., 2013; Roberts-Galbraith and Newmark, 2013). Therefore, the authors also investigated the contribution of aberrant *fst* expression to the regenerative defect in *myoD(RNAi)* animals. RNAi against the activin-1-encoding *act-1* rescued regeneration in most short-term *myoD(RNAi)* planarian fragments, although a fraction of animals became cycloptic. However, *act-1* inhibition did not reduce the length-to-width ratio in uninjured *myoD(RNAi)* animals; thus, these results suggested that longitudinal muscles themselves are critical regulators of the regenerative response, rather than being merely required to maintain contractility after a wound.

This discovery begged the question of whether other components of the BWM regulate planarian regeneration. By profiling the transcriptome of planarian muscles, Scimone et al. (2017) found that *nkx1–1*, a transcription factor-encoding gene, was predominantly expressed in a fraction of BWM cells separate from the subset expressing *myoD*. RNAi against *nkx-1* resulted in wider animals with depleted circular muscle fibers and normal diagonal and longitudinal fibers. RNA-seq of these animals revealed downregulation of muscle-specific gene expression, including two PCGs, *wnt11–1* and *act-2* (Figure 1B; Scimone et al., 2017). Transversely amputated fragments from *nkx1–1(RNAi)* planarians regenerated, albeit with numerous deformities in bilateral symmetry. Interestingly, some regenerated heads were bifurcated, occasionally bearing ectopic eyes and brain lobes.

Collectively, these data suggest that muscle tissue is not merely a contractile apparatus, but rather a critical regulator of the regenerative response, encoding positional information vital for proper regeneration. The questions answered — the way different muscle fibers are specified and the contribution of muscle fibers to planarian regeneration—are as insightful as the questions raised by these findings. What signals do *nkx1–1*⁺ muscles provide to restrict PCG expression in the anterior pole? Are *wnt11–1* and *act-2* (expressed in the *nkx1– 1*⁺ muscles) indispensable mediators? In addition, how is the third muscle type, diagonal muscle fibers, specified, and what is its contribution to regeneration, if any? How are different PCGs activated in distinct muscle fibers post-amputation? Furthermore, it would be interesting to explore whether the regulatory role of muscle in regeneration is conserved in other lineages, such as zebrafish and axolotls, and whether muscles provide other circulatory and local signals for tissue homeostasis and remodeling in physiological and pathological conditions. Altogether, the study revises the conventional thinking that the function of different muscle fibers is largely restricted to generating unique directional forces. Rather, Scimone et al. (2017) suggest the possibility that these individual fiber types may play distinct unappreciated signaling roles in development, regeneration, and disease.

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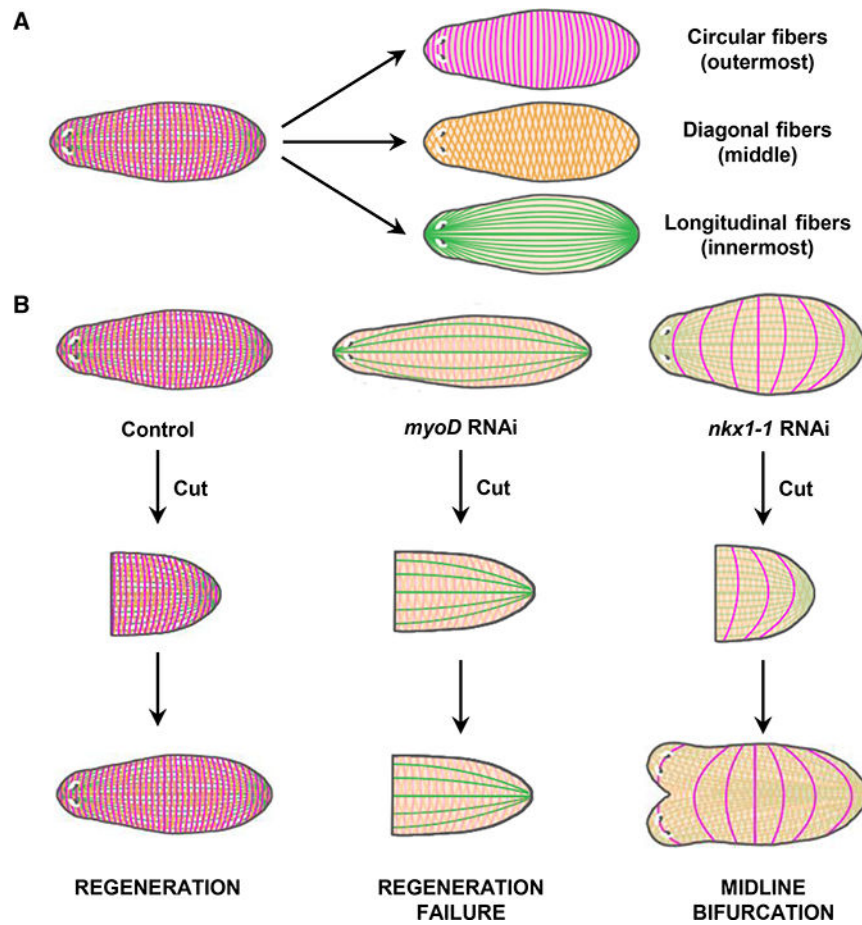
Figure 1. Planarian Body Wall Muscle Fibers and Their Critical Roles in Regeneration and Patterning.
(A) The body wall muscle of *S. mediterranea* consists of three primary fibers: circular (pink), diagonal (orange), and longitudinal (green). (B) Patterning defects and regeneration irregularities after genetic perturbation. RNAi- mediated knockdown of *myoD* depletes longitudinal fibers and inhibits regeneration, whereas knockdown of *nkx1–1* results in midline bifurcation.