Ca²⁺ Signaling and Regeneration

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Regeneration is the process by which lost or damaged tissue is replaced in adult organisms. Some organisms exhibit robust regenerative capabilities, while others, including humans, do not. Understanding the molecular principles governing the regenerative malleability of different organisms is of fundamental biological interest. Further, this problem has clear impact for the field of “regenerative medicine,” which aspires to understand how human cells, tissues, and organs may be restored to normal function in scenarios of disease, damage, or age-related decline. This review will focus on the planarian flatworm as a powerful model system for studying the role of Ca²⁺ signals in regeneration. These invertebrate animals display an astounding innate regenerative capacity capable of regenerating complete organisms from tiny, excised fragments. New knowledge and methodological capabilities in this system highlight the potential for studying the role of Ca²⁺ signaling at multiple stages of the regenerative blueprint that controls stem cell behavior in vivo.

Ca²⁺ signals are potent effectors of cellular responses (Berridge et al. 2000; Clapham 2007; Boulware and Marchant 2008). Acutely, Ca²⁺ transients transduce information through contemporaneous regulation of protein activity. More chronically, Ca²⁺ transients shape cell properties and differentiative fate through execution of specific transcriptional programs. These attributes underpin the fundamental contribution made by Ca²⁺-signaling events to reparative physiology, encompassing responses to wounding, repair, and tissue regeneration that are essential for life.

Despite the importance of studying the role of Ca²⁺ in “regeneration,” this area has traditionally received less attention than studies of aberrant Ca²⁺ signaling in cell “degeneration.” However, a growing body of literature under-

scores how endogenous Ca²⁺ signals help coordinate physiological responses to injury, cell turnover, and tissue replacement. Contributions to date have largely derived from well-established model systems that are tractable for probing and manipulating Ca²⁺ signals within an intact tissue architecture. These encompass in vivo studies in *Caenorhabditis elegans* (Ghosh-Roy et al. 2010; Xu and Chisholm 2011; Yan and Jin 2012; Sun et al. 2014), *Drosophila* (Restrepo and Basler 2016), *Xenopus* (Stanisstreet 1982; Tu and Borodinsky 2014), and zebrafish (Yoo et al. 2012; Kujawski et al. 2014). Perhaps the strongest mechanistic implication derives from experiments in *C. elegans*. This system is highly manipulable, combining powerful genetic tractability and compatibility for small-molecule screening with great optical transparency en-

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abling, for example, accessibility of the *C. elegans* neuronal network (a total of 302 neurons) to axotomy. Surgical ablation of neuronal processes using femtosecond laser pulses creates spatially defined lesions at which repair and regrowth can be studied and manipulated (Ghosh-Roy and Chisholm 2010). Studies on specific mechanosensory touch neurons using this method have shown that injury-evoked Ca^{2+} transients are required for axonal regrowth (Ghosh-Roy et al. 2010; Sun et al. 2014). One conserved Ca^{2+} -dependent effector has been identified as the dual leucine zipper bearing kinase-1 (DLK-1), a target of the regulator of presynaptic morphology 1 (RPM-1) complex (Yan and Jin 2012). Similarly, in assays studying epidermal wounding in *C. elegans*, localized Ca^{2+} signals empower cytoskeletal changes required for wound closure (Xu and Chisholm 2011). In both these examples, the repair-coordinating Ca^{2+} signals are initiated by Ca^{2+} entry (via voltage-operated Ca^{2+} channels or transient receptor potential [TRP] channels) and, subsequently, amplified through intracellular Ca^{2+} release (both inositol 1,4,5-trisphosphate receptors [IP₃Rs] and ryanodine receptors [RyRs] have been implicated) (Xu and Chisholm 2011; Sun et al. 2014).

However, despite such progress, it is important to remember that many commonly employed model organisms are actually highly restricted in regenerative potential, lacking populations of adult stem cells that empower regenerative responses to replace damaged or missing tissue. For example, neuronal “regeneration” in *C. elegans* really represents an example of cellular repair rather than bona fide regeneration; *C. elegans* does not replace damaged neurons as these worms do not harbor adult stem cells. As such, the robustness of neuronal repair in *C. elegans* is highly contextual: dependent on the type of neuron, the site of injury and even then restoration of connectivity is often achieved in only a minority of trials (Ghosh-Roy and Chisholm 2010; Ghosh-Roy et al. 2010). Therefore, there is clearly a place for models capable of tissue-/organism-level regeneration, where the role of Ca^{2+} signaling processes in shaping the in vivo behavior of stem cells that empower re-

generation can be assessed. As regeneration is an innate trait exhibited by many animal phyla, a variety of models have been prioritized as systems for studying the process of regeneration (Sanchez Alvarado and Tsonis 2006; King and Newmark 2012; Grillo et al. 2016).

The purpose of this review is to introduce one such model—the planarian flatworm—to the Ca^{2+} signaling community. The growing methodological capabilities and resources available in this system are enabling the study of Ca^{2+} signaling in a fascinating organism capable of wholesale regeneration and tissue rejuvenation. Here, I will outline key features of planarian biology (first section) and review methodological tools and newly developed resources in this system (second section). Finally, I will summarize data demonstrating how components of the Ca^{2+} signaling toolkit impact regenerative outcomes and highlight future research topics in this area (third section).

THE PLANARIAN FLATWORM—THE BASICS

Planarians are flatworms, free-living (“turbellarian”) members of the phylum *Platyhelminthes*. While considerable diversity exists in planarian size and appearance, in terms of outward morphology, flatworms show bilateral symmetry with a distinct head-to-tail (anterior-to-posterior) polarity (Fig. 1A). Systematically, planarians are classified as Lophotrochozoans (Fig. 1B; Laumer et al. 2015), a fascinating group of organisms that arguably exhibits broader gene conservation of human disease orthologs than the well-established *C. elegans* and *Drosophila* invertebrate models (Reddien et al. 2005; Fredlander et al. 2009).

While many characteristics of these animals provide compelling reasons for their study, it is their robust regenerative and rejuvenative capabilities that has long tweaked biologists’ curiosity (Morgan 1898; Newmark and Sánchez-Alvarado 2002). Indeed, the ability of planarians to regenerate likely reflects an extreme of regenerative prowess; tiny body fragments excised from the worm have the capability to regenerate the entire organism over a timeframe of days to weeks depending on the species. During asexual

Figure 1. Planarian morphology and classification. (A, Top), morphology of the planarian *Dugesia japonica*. Flatworms are bilaterally symmetrical and dorsoventrally flattened, displaying a clear head (anterior, “a”)–to–tail (posterior, “p”) polarity. (Bottom) Examples of *D. japonica* polarity defects after regeneration of trunk fragments (region illustrated by box in A) following RNAi of key patterning genes β -catenin-1 (yielding two-headed animals, eyespots shown by red arrows, and pharynx in green) or APC-1 (yielding two-tailed animals). (B) Systematic classification of free-living planaria and parasitic worms within the phylum Platyhelminthes.

reproduction by fission, planarians split transversely into two, with the posterior fragment regenerating the missing head structures and the anterior fragment regenerating the tail structure. Injuries and amputations are also easily repaired, with missing structures reformed. Although, through an anthropomorphic lens, there may be a bias to view these invertebrates as “simple,” deconstruction of the challenges inherent to these robust regenerative responses illuminate the scale of the biological feat that underpins this remarkable plasticity (Reddien 2018). The regenerative blueprint must coordinate the initial wounding response that leads to formation of the regenerative blastema, with positional signaling processes that instruct a diverse population of planarian stem cells (historically called “neoblasts,” see below) to migrate, differentiate, and reform the missing structures with appropriate polarity, size, and functional integration with the remaining tissue.

The differentiative trajectories of planarian neoblasts are numerous as the planarian body plan is constituted from multiple cell types. This complexity has been illuminated by single-cell transcriptomic studies coalescing data from tens of thousands of individual planarian cells. Recent studies identify numerous differen-

tiated cell types (between 20 and ~100 in asexual worms) (Fincher et al. 2018; Plass et al. 2018) that comprise the major planarian tissues. These tissues include the epidermis, intestine, multiple types of muscle, a specialized pharyngeal tube for eating food and expelling waste, an excretory and fluid regulatory system (protonephridia), a functionally enigmatic class of cells (termed *cathepsin*⁺, which envelops two previously identified cell types, glia [Wang et al. 2016] and pigment cells [Stubenhaus et al. 2016]), the surrounding parenchyma, and, finally, the most diverse groupings of cell type and state, cells of the nervous system, and the stem cell population itself.

The planarian nervous system is a complex network of thousands of cells, exhibiting a high degree of specialization and expressing a broad neurotransmitter portfolio (Collins et al. 2010; Fincher et al. 2018; Plass et al. 2018). The anterior end of the animal contains a mass of cephalic ganglia organized into a brain-like structure (Agata et al. 1998). Dual ventral nerve cords, interconnected by transverse fibers, run the length of the animal. Anatomically, the bilobed planarian brain and ventral nerve cord anatomy are considered a key ancestral stage in the evolution of the vertebrate central nervous

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system (CNS), with features of this basal bilaterian nervous system more closely resembling vertebrate neuronal properties than more commonly used invertebrate models (Agata et al. 1998; Sarnat and Netsky 2002; Mineta et al. 2003). The expansion of transcriptomic datasets has enabled bioinformatic prospecting for the presence, and now organismal distribution (Fincher et al. 2018; Plass et al. 2018), of genes of interest within the planarian model. For example, analysis of the *Dugesia japonica* transcriptome revealed that planarians express a broad voltage-gated channel-like portfolio to support their excitable cell physiology (Fig. 2; Chan et al. 2016, 2017b). The number of predicted voltage-gated channels is close to the size of the gene set found in humans (114 vs. 130 VGL sequences) with planarians lacking only inward rectifying K^+ channels compared with humans. Contrasting with *C. elegans*, planarians express voltage-gated sodium channels, hyperpolarization-activated cyclic nucleotide-gated

channels, and two-pore channels. Flatworms are also endowed with a diverse Ca^{2+} signaling toolbox, containing homologs for intracellular Ca^{2+} release channels and organellar Ca^{2+} pumps, components supporting store-operated Ca^{2+} entry (STIM, Orai), as well as cell-surface Ca^{2+} transporters and numerous Ca^{2+} -binding proteins (Prole and Taylor 2011; Zhang et al. 2013; Marechal et al. 2018). In terms of neurophysiology, intact worms are clearly receptive to a broad range of environmental stimuli, likely mediated through sensory neurons expressing a broad portfolio of planarian TRP channels (≥ 30 sequences) that reside within the epidermis and submuscular nervous plexus (Inoue et al. 2014, 2015; Arenas et al. 2017). A planarian TRPA1 isoform has been recently demonstrated to respond to reactive oxygen species as a mechanism for sensing noxious heat (Arenas et al. 2017). The convergence of long-standing anatomical knowledge with the recent single-cell transcriptomic data (Fincher et al. 2018; Plass et al. 2018; Zeng et al. 2018) should herald considerable new insight into the functional properties and biological roles of the multiplicity of Ca^{2+} -handling proteins in different subtypes of planarian neurons.

Neoblasts, a collective term for planarian stem cells, are the only somatic cell population with proliferative ability. These abundant, dividing cells (visualized by bromodeoxyuridine labeling) empower normal tissue turnover and regeneration. Neoblasts are sensitive to γ irradiation, with high doses causing lethality by destroying these cells to annihilate rejuvenative (and regenerative) competency. In terms of their properties, neoblasts are a heterogeneous cell population. One subpopulation of neoblasts is pluripotent (clonogenic neoblasts, “cNeoblasts”; Wagner et al. 2011) capable of generating any planarian cell type. The remaining subpopulations comprise fate-restricted neoblasts, which are able to generate only specific cell lineages/types (progenitor-like cells, christened “specialized neoblasts”). The pluripotency and clonal growth of cNeoblasts was demonstrated in elegant transplantation experiments, where injection of a single cell (isolated by flow cytometry from an unirradiated worm) into a

Figure 2. The diverse planarian ionotropic toolkit. Cladogram describing the *Dugesia japonica* voltage-gated-like ion channel superfamily. Dashed groupings indicated predicted gene loss in the parasitic flatworm *Schistosoma mansoni*. Channel topology is shown adjacent to the predicted groupings. Prediction of sequence number is intentionally conservative, encompassing only sequences with conservation of key motifs and architecture with human counterparts. Accessory ion channel subunits (nonpore forming) are not included in this figure. Sequence analysis supporting attribution and alignment is provided in Chan et al. (2017b), from which the figure has been modified.

lethally irradiated animal preserved viability and restored regenerative ability (Wagner et al. 2011). All types of neoblasts are broadly distributed in the animal, and the molecular properties of these cells are being rapidly elucidated using single-cell sequencing methods that capture cells across the continuum of differentiative states. Markers for cNeoblasts, as well as different classes of fate-specified neoblasts have recently been identified (Fincher et al. 2018; Plass et al. 2018; Zeng et al. 2018).

But how are neoblasts activated? Research over the last decade has discovered key players involved in positional signaling, the first step in choreographing neoblast behavior. Numerous planarian patterning genes, referred to as position-control genes (PCGs) (Witchley et al. 2013; Reddien 2018), have been identified through the discovery of clear polarity defects resulting from doubled stranded RNA-mediated genetic interference (RNAi) manipulations in intact and regenerating worms. Many of the polarity phenotypes are striking (Fig. 1A), for example, regeneration of multiheaded worms (Gurley et al. 2008; Iglesias et al. 2008; Petersen and Reddien 2008), two-tailed animals (Gurley et al. 2008; Petersen and Reddien 2011), or organs that are duplicated and/or misplaced (Lander and Petersen 2016; Scimone et al. 2016). Small-molecule screening has also yielded polarity phenotypes, although mechanistic definition of how these polarity mis-cuing drugs act in vivo is more challenging (Nogi and Levin 2005; Nogi et al. 2009; Beane et al. 2011; Chan et al. 2015). Collectively, these studies have demonstrated central roles for regionalized Wnt signaling in regulating the planarian anteroposterior (AP) axis, and Bmp signaling in regulating dorsoventral (DV) patterning. Both these signaling pathways are known to be regulated by Ca²⁺ signals (Kohn and Moon 2005; Swapna and Borodinsky 2012). Most surprising, and highly relevant to the role of Ca²⁺ signaling, are two further observations.

First, planarian PCGs, while individually expressed across unique spatial domains, are all constitutively expressed. Not only does this provide an endogenous spatial blueprint for maintaining the adult body plan during normal tissue

turnover (knockdown of many PCGs causes phenotypes in intact animals), it is critical for allowing rapid reconstitution of this endogenous blueprint after injury. Wound-evoked signals initiate a rapid and generic injury response, independent from any changes in neoblast behavior, which is central to resetting and reestablishing positional information in an amputated fragment. Ca²⁺ signals are part of the initial wounding response in many systems (Tseng et al. 2010; Cheng et al. 2014, 2015; Luxardi et al. 2014; Moe et al. 2015), and it has long been known that Ca²⁺ is essential for planarian regeneration (Franquinet 1979). How Ca²⁺ signals regulate the generic wounding program in planarians and whether Ca²⁺ transients help reboot normal PCG expression patterns are key areas for future study.

Second, the tissue site of PCG expression is body wall muscle (Witchley et al. 2013; Scimone et al. 2017). PCGs are expressed across different spatial domains but likely all within subepidermal muscle, and often unique expression within specific types of fiber. Knockdown of the transcription factor *myoD*, which specifies the longitudinal fibers in the body wall muscle, blocked regenerative responses (Scimone et al. 2017). *myoD(RNAi)* animals were unable to remodel PCG expression after amputation, inhibiting instructive signaling to neoblasts. Disruption of circular muscle fibers also revealed a role in mediolateral regeneration (Scimone et al. 2017). Therefore, in addition to the well-known contractile function of muscle, body wall muscle fibers clearly play a unique additional instructive role in coordinating regenerative responses. With hindsight, the ability of many polarity-miscuing drugs to perturb Ca²⁺ homeostasis in muscle seems highly relevant to the discovery of PCG localization to muscle (Nogi et al. 2009; Chan et al. 2015). Future research will address how muscle physiology engages neoblast behavior in vivo to illuminate pathways involved in normal tissue homeostasis, and those engaged by injury.

THE PLANARIAN EXPERIMENTAL TOOLBOX

This section addresses resources that enable researchers to study specific genes of interest in

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planaria. First, it is important to point out that thousands of planarian species exist worldwide. Optimization of methods and resources to date have predominantly focused on two freshwater planarian species, *Schmidtea mediterranea* and *D. japonica*, although resources for other species are growing (Brandl et al. 2016; Accorsi et al. 2017). These two species are now easily maintained in the laboratory using optimized protocols, such that husbandry is straightforward and inexpensive. Detailed protocols to aid establishment, maintenance, and expansion of planarian colonies are available (Oviedo et al. 2008a). Worms can be obtained from commercial vendors (e.g., Carolina Biological Supply) as well as through individual laboratories (e.g., Sánchez Laboratory, planosphere.stowers.org). The characteristics of these experimental models are described elsewhere (Newmark and Sánchez-Alvarado 2002; Oviedo et al. 2008b), as they display differences reflective of planarian diversity and their derivation into clonal lines optimized for the laboratory. In our laboratory, we use *D. japonica* owing to their marked drug sensitivity in regenerative assays that facilitates small-molecule screening (Nogi et al. 2009; Chan et al. 2014; Srougi et al. 2014). Workflows for basic regenerative assays (Chan and Marchant 2011) and protocols for immunostaining (Forsthoefel et al. 2014; Ross et al. 2015) and in situ hybridization (Pearson et al. 2009; King and Newmark 2013) are tailored for individual species.

Planarian gene function can be probed by loss-of-function analyses (Newmark et al. 2003; Reddien et al. 2005; Roberts-Galbraith et al. 2016), but not as yet with gain-of-function or transgenesis. Loss-of-function assays are executed using double-stranded RNAi, using multiple feeding or microinjection protocols. Readers are referred to a foundational RNAi screen in *S. mediterranea* (Reddien et al. 2005) that illustrates the breadth of phenotypes obtained (~1 in 5 targeted genes yielded phenotypes in this study; Reddien et al. 2005). Specific RNAi assays that yield clear and penetrant outcomes have also been described to help evidence protocols are optimized for laboratory staff learning these approaches (Chan and Marchant 2011; Accorsi et al. 2017).

Genomic and transcriptomic resources, including a long read PacBio SMRT assembly of the *S. mediterranea* genome (Grohme et al. 2018), can be accessed through online database portals such as PlanMine (Brandl et al. 2016), the *S. mediterranea* Genome Database (SmedGD [Robb et al. 2015] and www.planarian.jp [An et al. 2018]). These resources facilitate comparative bioinformatic analyses and design of sequence-specific tools for the growing number of species with transcriptomic data. Of particular interest from studies using different planarian species has been the demonstration of manipulations that confer regenerative capability in planarian species that have specific regeneration defects (Liu et al. 2013; Sikes and Newmark 2013), a triumph for demonstrating the experimental capacity to reverse engineer regenerative potential.

A key resource, mentioned earlier, has been the recent publication of cell-type-specific transcriptomic datasets for *S. mediterranea* (Fincher et al. 2018; Plass et al. 2018). Here, single-cell isolation and subsequent RNA sequencing (of up to 50,000 individual cells; Fincher et al. 2018) has generated a comprehensive atlas of the transcriptomes of likely every cell type present within a planarian. These resources encompass differentiated cells, as well as the continuum of transition states that are being continually populated through neoblast differentiation, and the traceable lineages interrelating these states (Fincher et al. 2018; Plass et al. 2018). Data underpinning this powerful approach have been made accessible in a queryable format (digiworm.wi.mit.edu, see also shiny.mdc-berlin.de/psca) to facilitate analysis of expression patterns of specific genes of interest.

Finally, planaria have been successfully used for drug-screening assays, encompassing intact worm behavioral studies (Shomrat and Levin 2013; Inoue et al. 2015) and drug effects on regeneration (Nogi et al. 2009; Oviedo et al. 2010; Beane et al. 2011; Chan et al. 2014, 2015; Srougi et al. 2014; Owlarn et al. 2017). Regenerative screens are of interest to understand how small molecules impact stem cell differentiation trajectories in vivo, as relevant for regenerative medicine. While in vitro screens in other systems have demonstrated how Ca^{2+} signaling

modulators regulate stem cell proliferation and coax differentiation toward particular cell fates (Diamandis et al. 2007; Schneider et al. 2008), it is of interest to ascertain whether the same principles hold in vivo where the efficacy of agents is modified by the local environment of the stem cell niche. A further area of impact is that study of drug action in free-living flatworms will prove illuminating for identifying druggable pathways in parasitic flatworms, most notably blood flukes (*Trematoda*) and tapeworms (*Cestoda*). These parasitic flatworms are responsible for a significant worldwide disease and economic burden. For example, the neglected tropical disease schistosomiasis infects over 200 million people worldwide, encumbering Third World economies with an annual loss of up to 70 million disability-adjusted life years (Hotez and Fenwick 2009). Considerable success has been achieved in targeting excitable cell physiology to develop anthelmintic agents, to the extent that Ca²⁺ channels and pumps will likely provide productive opportunities for antiparasitic drug discovery. Indeed, the effects of the main therapeutic for treating schistosomiasis, a drug called praziquantel (PZQ), on Ca²⁺ signaling in the parasite have been long appreciated (Chan et al. 2013). Recent studies have revealed the both individual enantiomers of PZQ also elicit Ca²⁺ signals in host tissues, that may contribute to the therapeutic efficacy of this drug (Chan et al. 2017a, 2018; Gunaratne et al. 2018). Finally, given the importance of stem cells for powering parasitic life cycle transitions, studies of planarian neoblasts will also help discovery of efficacious targets and structures that can disrupt parasite stem cell function.

Ca²⁺ SIGNALING AND THE REGENERATIVE BLUEPRINT

Changes in cellular Ca²⁺ levels during planarian regeneration were first reported several decades ago (Martelly et al. 1983; Martelly 1984; Moraczewski et al. 1986). Early changes in worm Ca²⁺ content were correlated with changes in neurotransmitter levels and DNA content over a window now known to overlap with the period of enhanced neoblast proliferation after injury.

The absence of calcium prevents regeneration (Franquinet 1979) and drugs that perturb Ca²⁺ homeostasis cause regenerative polarity defects if applied contemporaneously or shortly after tissue amputation (Nogi et al. 2009; Chan et al. 2017b). These observations merit analysis of the underlying mechanisms.

PZQ is one example of a drug that causes Ca²⁺ signals in isolated planarian cells (Fig. 3A; Zhang et al. 2011). Application of PZQ to regenerating trunk fragments of the planarian *D. japonica* causes an axis duplication, dependent on media Ca²⁺ concentration, yielding viable, two-headed worms (Fig. 3B; Nogi et al. 2009). At maximal doses of PZQ, all regenerating trunk fragments regenerate as bipolar animals. PZQ causes depolarization and muscle contraction and PZQ-evoked bipolarity can be

Figure 3. Praziquantel (PZQ) evoked bipolarity and Ca²⁺ signaling. (A) PZQ-evoked Ca²⁺ signals in dissociated planarian cells. (Inset) Structure of PZQ. Colored traces show fluo-4 fluorescence traces in dissociated single planarian cells on addition of vehicle (DMSO) and PZQ (100 μM). (Right) Images of fluorescence from cells at timepoints “1” and “2” during confocal imaging trace. (Figure modified from data in Zhang et al. 2011.) (B) Bipolar planarian produced by PZQ treatment of trunk fragment.

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prevented by knockdown of a specific voltage-operated Ca^{2+} channel (Ca_v1A , one of five Ca_v channels present in planarians; Zhang et al. 2011; Chan et al. 2017b) or accessory $\text{Ca}_v\beta$ subunits (Nogi et al. 2009). RNAi of Ca_v1A also prevented PZQ-stimulated Ca^{2+} uptake and PZQ-evoked decreases in posteriorizing Wnts (Zhang et al. 2011). These data are consistent with an instructive role for small molecule-evoked Ca^{2+} signaling in anteriorization (Nogi et al. 2009), likely as an effector of membrane voltage-mediated changes during normal regenerative physiology (Beane et al. 2011). In parallel, a second Ca_v (Ca_v1B) has been implicated to play a role in posteriorization, with Ca_v1B -mediated Ca^{2+} influx acting as an early posterior-determining cue (Chan et al. 2017b). One would speculate these discrete Ca_v channels are involved in opposing regulation of planarian muscle activity (Chan et al. 2015), where the PCGs are localized. However, the tropism of small-molecular action on muscle, and particular types of muscle fiber, require further knowledge of planarian muscle physiology.

In light of this emerging role for Ca^{2+} signaling in AP signaling, the planarian system also provides ample opportunity to interrogate the role of Ca^{2+} signals across other aspects of the regenerative blueprint: the initial wounding response, blastema formation, positional signaling, neoblast engagement, migration, and execution of specific differentiation trajectories. Ca^{2+} is a known effector of wounding and membrane repair (Tseng et al. 2010; Cheng et al. 2014, 2015; Luxardi et al. 2014; Moe et al. 2015), and an established regulator of ERK signaling, implicated as a key node between the planarian wounding response and engagement of neoblasts (Owlarn et al. 2017). ERK is activated within minutes of amputation in a stem cell-independent manner; inhibition of ERK signaling does not impair wound closure but blocks regeneration initiation (Owlarn et al. 2017). How Ca^{2+} signals in subepidermal muscle regulate instructive response to stem cells merits attention. Also, the role of Ca^{2+} in neoblast proliferation, migration, and differentiation remains completely unexplored. Ca^{2+} signaling is an established regulator of Wnt (Kohn

and Moon 2005), BMP (Swapna and Borodinsky 2012), and Hedgehog signaling (Belgacem and Borodinsky 2011), pathways that coordinate positional signaling and tissue specification during planarian regeneration. Given the recent pace of progress in dissecting the molecular choreography of planarian regenerative signaling, new insight into the regulation of these pathways by Ca^{2+} will likely be forthcoming.

CONCLUSION

Studying regenerative processes in planarians and other model systems will aid our understanding of Ca^{2+} signaling in human regenerative responses in both paradigms of continuous cell renewal (e.g., hematopoiesis) and in tissues (e.g., liver and muscle) capable of mounting regenerative responses after injury (Lam et al. 2011; Paredes-Gamero et al. 2012; Cheng et al. 2014; Taira et al. 2016; Tu et al. 2016). Currently, much remains to be understood about the role of Ca^{2+} signals in regulating both adult and tissue-specific stem cell behavior, as well as in regulating dedifferentiation or transdifferentiation of previously differentiated cells in reparative responses. It is likely that regulatory roles for endogenous Ca^{2+} signaling events will continue to emerge in many of these scenarios.

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