Published in final edited form as:
Wiley Interdiscip Rev Dev Biol. 2013 May ; 2(3): 301–326. doi:10.1002/wdev.82.

THE HISTORY AND ENDURING CONTRIBUTIONS OF PLANARIANS TO THE STUDY OF ANIMAL REGENERATION

Sarah A. Elliott[1,2] and **Alejandro Sánchez Alvarado**[1,2,3,4]
[1]Stowers Institute for Medical Research; Kansas City, MO USA
[2]Department of Neurobiology and Anatomy, University of Utah; Salt Lake City, UT USA
[3]Howard Hughes Medical Institute

Abstract

Having an almost unlimited capacity to regenerate tissues lost to age and injury, planarians have long fascinated naturalists. In the Western hemisphere alone, their documented history spans more than 200 years. Planarians were described in the early 19th century as being “immortal under the edge of the knife,” and initial investigation of these remarkable animals was significantly influenced by studies of regeneration in other organisms and from the flourishing field of experimental embryology in the late 19th and early 20th centuries. This review strives to place the study of planarian regeneration into a broader historical context by focusing on the significance and evolution of knowledge in this field. It also synthesizes our current molecular understanding of the mechanisms of planarian regeneration uncovered since this animal’s relatively recent entrance into the molecular-genetic age.

PLANARIANS AND THEIR HISTORICAL CONTEXT

The study of regeneration has a rich, intertwined history with experimental embryology. In the 17th century, naturalists contemplated two ancient paradigms for thinking about embryology: preformationism versus epigenesis. Preformationism contended that animals were already formed in miniature at the time of conception and simply expanded in size over the course of development. In contrast, epigenesis stated that animals were built piece by piece during development, guided by some intrinsic information housed in the undifferentiated embryonic cells. The rediscovery of animal regeneration at the end of the 1600s cast serious doubt upon the validity of preformationism. As naturalists began gathering more insights into both regeneration and embryonic development, epigenesis eventually took center stage as one of the most important principles of biology.[1]

The earliest known description of animal regeneration came from Aristotle around 350 B.C.E. Among other things, he described that the tails of lizards regenerate.[2] In 1686, Thévenot, Perrault, and Duverney revived this finding.[3] This rediscovery of regeneration created a wave of excitement, and 18th century naturalists began experimenting on any animals they could find to determine if this was a common phenomenon across the tree of

[4]Correspondence to: asa@stowers.org.

FURTHER READING
Schmidtea mediterranea Genome Database (SmedGD): http://smedgd.neuro.utah.edu/
Sánchez Alvarado lab website: http://planaria.stowers.org
HathiTrust, full text of *Yu-Yang Tsa-Tsu* by T’uan, 860:[14] http://catalog.hathitrust.org/Record/003326204
HathiTrust, full text of *Kinmô-Zui* by Tekisai Nakamura, 1666:[10] http://catalog.hathitrust.org/Record/002269510
HathiTrust, full text of *Wakan Sansai-Zue* by Ryōan Terajima, 1713:[11] http://catalog.hathitrust.org/Record/002269488

life. de Réaumur showed that arthropods could lose appendages such as limbs and subsequently regenerate them.[4] Trembley systematically demonstrated that hydra, a member of the cnidarian phylum, regenerates after transection.[5] Bonnet proved that annelid worms regenerate,[6] and Spallanzani described the regenerative abilities in a variety of invertebrates such as snails and vertebrates like salamanders and frog tadpoles.[7]

The birth of the study of planarians is most frequently associated with Pallas, who encountered them while exploring the Ural mountains in the late 18th century. There, he observed that these animals regenerate missing body parts after fissioning.[8] However, other early reports of planarians exist. Trembley described feeding pieces of planarians to hydra in his monograph in 1774.[5] Müller described a number of planarian species in 1773, but erroneously grouped them with the trematode genus *Fasciola*.[9] Woodcut prints of land planarians can be found in Japanese encyclopedias dating as far back as the 17th century (FIG 1),[10,11] while written descriptions of the animals long precede that.[12,13] In fact, the oldest known reference to planarians comes from the Chinese text *Yu-Yang Tsa-Tsu* written around 860 AD by T'uan. He describes the animal "T'u-K'u" (likely the land planarian *Bipalium*), and hints at its regenerative abilities by saying that it can "easily separate into several pieces" when touched.[14,12,13]

Over the course of the 19th century, more than a dozen different European[15,16,17,18,19,20,21,22,23,24,25] and American[26,27,28,29,30,31,32,33] biologists—including Darwin himself—continued to study these animals and demonstrated that the robustness of regeneration was common across planarian species. Indeed, in Dalyell's eloquent words, planarians appear be "almost immortal under the edge of the knife," making them tantalizing animals for study.[16]

Work continued on planarians for the rest of the 20th century, as it did in other animal models of regeneration.[34] However, in all fields, progress towards a mechanistic understanding of regeneration was hampered by an incomplete understanding of basic cell biology and genetics, in addition to a lack of tools for experimentation. Only recently have significant advances in molecular biology, genetics, and sequencing technologies reignited interest in planarians and other regenerative organisms. Today we are poised to tease apart the molecular mechanisms of regeneration, and unlock the mysteries of this biological phenomenon that have fascinated so many for so long.

WHY STUDY REGENERATION IN PLANARIANS?

Planarians are masters of regeneration

Today's popular model organisms were selected for a simple reason: they are biological exaggerations. T.H. Morgan selected the fruit fly and Sydney Brenner, the nematode worm because their exaggerated reproductive biology made them ideal for performing forward genetic screens. Likewise, planarians are ideal for regeneration studies because they undergo amazing feats of restorative and physiological regeneration.[35]

Planarians undergo restorative regeneration in response to almost any type of injury. An upregulation of cell proliferation forms a mass of unpigmented newly-differentiating cells, called a blastema. From this blastema emerges many of the tissues lost to injury, producing a fully restored worm in as little as 1–2 weeks (FIG 3A–B, *dpa: days post amputation*).[28,31] This restorative response involves rebuilding anatomy *de novo*—a process Morgan called "epimorphosis." It also involves remodeling the pre-existing tissues and integrating them with the newly made anatomy so that the animal regains its proper proportions and restores function to its organs. Morgan termed this type of remodeling "morphallaxis" (FIG 3C).[35]

In addition to restorative regeneration, planarians display physiological regeneration, repairing anatomy as it naturally ages. In the absence of an injury, these animals constantly undergo impressive levels of cell proliferation to replace old tissues. Like many cnidarians, annelids, echinoderms, and ascidians, planarians can maintain physiological regeneration for decades without losing the ability to regenerate or developing cancer.[36,37] This not only makes planarians useful for asking questions about regeneration. It also makes these long-lived animals tantalizing subjects for aging research, which has so far included studies of the mechanisms of planarian telomere maintenance[38] and the function of genes that affect longevity in other organisms.[39,40,41]

Planarians are sufficiently complex in anatomy and behavior

Planarians are protostomes and members of the Lophotrochozoan clade. They are triploblastic and thus have tissues derived from all three germ layers (ecto-, meso-, and endoderm). While they have simpler body plans than vertebrate model systems, they have long been recognized to have discrete organ systems and behaviors amenable for regeneration studies (FIG 2A).

Planarians eat (VIDEO 1) and defecate (VIDEO 2) through a muscular feeding tube, or pharynx, which connects to the gut (FIG 2B–C).[42,43,44,45] A blind gut with one anterior branch and two posterior branches occupies much of the body cavity (FIG 2C).[46,47,48] The animals possess an organized nervous system composed of two anterior cephalic ganglia and two parallel nerve cords that run ventrally along the length of the body (FIG 2C). A pair of dorsal photoreceptors is connected to the nervous system by axons that make up the optic chiasm (FIG 2D).[49,50] They possess motile cilia on their ventral epithelium that enables them to glide across surfaces (FIG 2E).[51,52,53,54,55] Their body plan is peppered with protonephridia, organs that facilitate osmoregulation and may ultimately prove to be homologous rather than analogous to the vertebrate kidney (FIG 2F).[56,57,58] While much work has focused on regeneration in asexual planarians, sexual strains also exist as cross-fertilizing hermaphrodites, regenerating ovaries and testes after amputation or starvation.[59,60,61,62,63] Planarian tissues also have intricate domains of molecularly discrete cell populations, yielding a plethora of markers to assess wound responses and general organization of the body plan (FIG 2G).[64] Finally, these animals display complex behaviors including negative phototaxis, fissioning in response to stimuli like changes in population density, and even cannibalism (VIDEO 3).[16,17,18,65,66,67,68] Thus, planarian anatomy and behavior provide a sufficiently complex palette for studying regeneration.

An expanding molecular toolkit

Planarian studies prior to the end of the 20th century were plagued by a lack of cellular resolution. Investigators relied principally upon basic histology, electron microscopy, and the visualization of gross anatomy under a transmitted light microscope to assess the regenerative response. They had little means of distinguishing cellular identity or tracing the lineage and movement of cells over time. They also had few ways to perturb the animals, and frequently resorted to treating planarians with pharmacological agents or toxins which had unknown mechanistic effects. Ultimately, the lack of experimental tools hampered the understanding of planarian biology.

VIDEO 1: Planarian feeding. *Schmidtea mediterranea* extrudes its pharynx to feed on calf liver. Sped up to approximately twice normal speed.
VIDEO 2: Planarian defecation. Through its newly-regenerated pharynx, an 8 dpa *Schmidtea mediterranea* head fragment defecates in response to light eposure. Sped up to approximately twice normal speed.
VIDEO 3: Planarian cannibalism. A planarian consumes a decapitated head from another planarian of the same species. Sped up to approximately twice normal speed.

Within the last two decades, our ability to visualize planarian tissues has improved drastically. Unlike Morgan and his inability to visualize the cellular and molecular events underpinning regeneration (FIG 3A–B, *dpa: days post amputation*), we can now assess each step of the regenerative response with extensive panels of markers. We can detect changes in gene expression and protein function, yielding a much sharper picture of the unfolding morphological and cellular dynamics of regeneration.[69,70,71] For instance, it is possible to visualize the regeneration of endodermally and ectodermally derived organ systems like the gut and brain, respectively (FIG 3C). Cellular activities that are not necessarily associated with organogenesis can also be assessed, such as the reestablishment of anterior domain identities after amputation (FIG 3D, *hpa: hours post amputation*).

In addition, the genome of the species *Schmidtea mediterranea* has been sequenced,[72] to which EST, transcriptome, proteome, and small RNA datasets can be mapped.[73,74,75,76,77,78,79,80,81,82,83,84,85] Genome microarrays have been generated to identify genes important for regeneration.[86,87,88,89,58] High-throughput RNAi screens can be performed to characterize gene function.[90,91,92] Fluorescence Activated Cell Sorting (FACS) is used to identify and isolate discrete cell populations, which subsequently can be used for such purposes as single-cell gene profiling or functional transplantation studies.[93,94,95,96,97,98,99] All of these tools, coupled with the ability to perform lineage tracing using BrdU, have opened the door for rigorous study of the molecular mechanisms underlying planarian regeneration.[100]

PATTERNING THE PLANARIAN BODY AXIS

Polarity is maintained during regeneration

One of the earliest uses of the term “polarity” in reference to body plan regeneration can be found in the work of Allman to describe the tubularian’s propensity to regrow a head from anterior-facing wounds and not posterior ones.[101] The mechanisms that establish and maintain polarity are fundamental questions shared by the fields of regeneration and embryology. How do cells know they are different from other cells? How are these differences translated into proper specification of the body axes and subsequent organogenesis? How is polarity re-established in the face of unexpected perturbations—whether that perturbation is a blastomere ablation in an embryo or an appendage amputation in an adult?

During regeneration, adult planarians maintain the polarity of their body axes. A small piece of tissue removed from the flank of the animal conserves the original orientation of the anterior-posterior (A/P), dorsal-ventral (D/V), and medial-lateral (M/L) axes (FIG 4A).[28] Additional experiments from the earlier part of the 20th century demonstrated that the juxtaposition of tissues from different regions of the animal—such as the transplantation of anterior tissue to posterior regions—triggers abnormal regeneration, including the formation of an ectopic body axis (FIG 4B).[102,103] These results suggest that planarian tissues possess some type of intrinsic positional and polarity information. During regeneration, this starting information must be read and interpreted correctly such that the proper structures are made in the right location. Determining how polarity is re-specified and maintained is critical for understanding the mechanisms of animal regeneration, and we are now discovering that some of the genetic toolkit used to establish polarity in an embryo may also play similar roles in maintaining polarity during regeneration.

Historical views of regeneration polarity

Over the centuries, investigators have proposed diverse mechanisms to explain the phenomenon of polarity. Some of these models had distinctly preformationist undertones. Bonnet hypothesized that “germs” exist in the earthworm that contain a fully-formed miniature head or tail. Upon amputation, fluid flow transports "head germs" anteriorly and "tail germs" posteriorly so that a head and tail sprout at the proper locations.[6,104] Weismann, a declared preformationist, extended his theory of the germ-plasm to explain regeneration. He proposed that preformed cells containing an "idioplasm" facilitate the reconstitution of the limbs of salamanders and newts. "Idioplasm" is organic material that predetermines the reconstitution of the limb, regardless of cues from the environment or the regenerating appendage. As cells divide, portions of the nuclear "idioplasm" are lost, and the division progeny are left with only enough "ids" to produce the next most distal cells in the limb. Thus, this pre-determined regeneration program ensures that distal structures are never regenerated before more proximal ones.[105]

Other hypotheses regarding regeneration polarity were more grounded in the ideals of epigenesis, proposing that polarity came not from preformed germs or "ids," but instead developed progressively out of some instructive cues intrinsic to the cells and tissues. Bardeen argued that the pre-existing anatomy of a planarian exerts mechanical forces that constrain the location in which new anatomy can physically fit. He also argued that the nervous system was key in dictating polarity.[65,106] Pflüger proposed that the chemical composition of the pre-existing tissue's cut surface establishes polarity. Each tissue laid down during regeneration provides a chemical signal that instructs the fate of the next layer laid down on top of it, and regeneration thus proceeds in a proximal to distal direction.[107] Child thought that gradients of metabolic activity guide regeneration. He believed that anterior tissues have higher metabolic rates and, thus, display "physiological dominance" over more posterior tissues, establishing anterior-posterior polarity early on in regeneration.[108] Brøndsted, heavily influenced by Child, proposed that unknown effectors establish A/P polarity through a time-graded regeneration field. This field exposes "high points" in a planarian blastema where regeneration of the head occurs faster and more vigorously than in other regions, subsequently releasing factors that inhibit head formation in more posterior areas.[109,110] (*For additional examples of regeneration polarity theories, see.*[4,111,112,113,114,115])

While most of these hypotheses have been proven insufficient to fully explain regeneration polarity or the defects resulting from experimental manipulation, Morgan's theory has best withstood the test of time. Based upon meticulously-documented regeneration experiments performed in a wide variety of animals, Morgan observed that "something in the piece itself determines that a head shall develop at the anterior cut surface and a tail at the posterior cut surface. This 'something' is what we call polarity."[116] He hypothesized that polarity results from some type of physical and/or chemical gradient along the body axes.[117,118,119]

Anterior-posterior polarity in planarians

Early attempts to better understand axial polarity in planarians centered around perturbing regeneration through surgical means. The abnormal, surgically-produced regenerates were referred to as heteromorphoses. Before the use of chemicals, irradiation, electric fields or RNAi, heteromorphoses provided key insights from which hypotheses could be made about the mechanisms underpinning regeneration. Heteromorphoses of the A/P axis were described early on in studies of planarian regeneration, since the head was an easily recognized structure. Most notably, Morgan observed that transverse amputations producing short cross-pieces frequently regenerated bipolar heads or tails (FIG 4C).[31,33,120] Coupled with similar regeneration defects from experiments on earthworms and tubularians, Morgan suggested that a regenerate might interpret a gradient of chemical or physical information along the body axis to maintain proper axial polarity. Very thin slices of tissue could have too shallow of a gradient to be deciphered, causing the production of a head from posterior wounds by default.

RNAi screens have uncovered phenotypes recapitulating Morgan's double head and double tail defects, lending support to his gradient hypothesis (FIG 4D). The Wnt/β-catenin pathway, which is involved in many developmental processes across metazoa including establishing polarity along the primary axis,[121,122] is required for A/P polarity in planarians.[123,124] Knockdown of the pathway's core transcription factor *Smed-β-catenin-1* results in anteriorization of the body axis, causing a head to regenerate from a posterior wound instead of a tail (FIG 4E). This anteriorized phenotype is also produced by knockdown of upstream ligands *Dj/Smed-wnt1* and *Smed-wnt11-5,* receptor-associated agonists *Smed-dvl-1* and *Smed-dvl-2*, and the transmembrane protein required for secretion of WNTs *Smed-evi/wntless.* In contrast, upregulation of β-CATENIN-1 activity by knockdown of such inhibitors as *Smed-APC-1*, *Smed-notum, Smed-axinA,* and *Smed-axinB* elicits the opposite phenotype in which tails regenerate from anterior wounds (FIG 4E).[123,124,125,126,127,128,129, 130] These results suggest that during regeneration, graded levels of β-CATENIN activity along the body plan regulate the anterior-versus-posterior fate choice. β-CATENIN activity must be sufficiently high in posterior blastemas to facilitate tail regeneration and sufficiently low in anterior blastemas to produce a head. Likewise, this signaling system must be acting during physiological regeneration, as knockdown of *Smed-β-catenin-1* anteriorizes uninjured animals too.[123,124,125] Whether or not there is a posterior-to-anterior gradient of β-CATENIN nuclear localization is unknown. However, numerous posteriorly-expressed Wnt ligands and anteriorly-expressed Wnt inhibitors suggest that there may indeed be such an activity gradient.[123,124,126,131,129,127]

The Hedgehog pathway, well characterized during the development of many animals,[132] is also important for A/P polarity in planarians. RNAi of pathway activators *Dj/Smed-hh*, Dj/*Smed-gli-1*, and *Smed-smo* decreases Hh signaling and results in loss of posterior regeneration. In contrast to this "tailless" phenotype, increased Hh signaling through RNAi of pathway inhibitors *Dj/Smed-ptc* and *Dj/Smed-sufu* causes defects in anterior regeneration. In these animals, a tail regenerates instead of a head at anterior wounds. Thus, high levels of Hh signaling are required to properly specify posterior tissues, while lower levels are required for specifying anterior tissues. Furthermore, the Hh pathway may act upstream of the Wnt/β-catenin pathway by modulating the expression of *Dj/Smed-wnt1*, which likely signals through β-CATENIN to specify posterior fates.[126,133,51,134,128]

Additional parallel or convergent pathways are known to participate in the establishment and maintenance of A/P polarity. Simultaneous knockdown of putative gap junctions *Smed-innexin-5*, *-12*, and *-13* produces double heads.[135] RNAi of the LIM homeobox transcription factor *Djislet* causes a tailless phenotype.[129] Knockdown of the TALE class homeobox transcription factor *Smed-prep* causes cyclopic and headless phenotypes.[136] Graded membrane voltage, based at least in part on high intracellular calcium levels in anterior wounds, also plays a role in establishing A/P polarity in planarians.[137,138,139,140,141] At this point, however, it is unclear how all these collective signals are integrated to properly reestablish the A/P axis.

Dorsal-ventral polarity in planarians

As in other regenerative animals, an amputation in a planarian brings dorsal and ventral tissues into close contact at the wound site.[142] Grafting experiments in animals including newts,[143,144] arthropods,[145] and annelids[146] have suggested that signaling between tissues from different regions of the dorsoventral (D/V) axis—such as the interaction induced by wound closure—might be an early trigger for regeneration. Classical planarian experiments have also supported this idea. In particular, Santos grafted plugs of planarian tissue into a host in either normal D/V orientation or inverted orientation (FIG 5A). In the former case, the tissue healed and the animal appeared normal. In the latter case, a blastema formed at the interface between the graft and host tissues and large cup-shaped protrusions emerged at the graft site. In at least one case, Santos even observed an ectopic planarian developing from the graft with inverted D/V orientation to the host's body axis (FIG 5A). This suggested that the graft not only retained its original D/V polarity after transplantation, but the juxtaposition of dorsal and ventral tissues somehow triggered the formation of a new body axis.[102,103]

Seventy years after Santos' initial observations, investigators are revisiting his experiments with modern tools. Histological analyses and expression studies show that Santos' inverted transplants indeed maintain their original D/V polarity after grafting. In addition, the boundary between the host and graft tissues of inverted transplants ectopically expresses a body edge marker, while non-inverted control transplants do not.[147] While future studies are needed to determine whether an ectopic body axis is truly forming from these protrusions and how this is accomplished, these results do suggest that the closer positioning of dorsal and ventral tissues after an injury and wound healing might be an important aspect of the regenerative response of planarians, helping promote blastema formation and specification of a new body edge.[148]

Recent RNAi screens have uncovered numerous genes important in establishing and maintaining D/V polarity in planarians. So far, all genes identified in these screens are components of the BMP pathway, a branch of the TGF-β signal transduction cascade, which has a conserved role in organizing the D/V axis in diverse metazoans.[122] Reduction of BMP signaling by RNAi of *DjBMP/Smed-bmp4*, *Smed-smad1*, *Smed-smad4*, *Smed-admp*, and *Smed-noggin-like-8* ventralizes animals during both restorative and physiological regeneration (FIG 5B). Collectively, these ventralized defects include a dorsal duplication of the brain and ventral nerve cords, ectopic dorsal expression of ventral markers, and growth of dorsal cilia that enable the animals to swim in an inverted fashion on their dorsal side.[92,149,150,151,152,153] Likewise, increasing BMP signaling through knockdown of putative inhibitors *Smed-noggin-1* and *Smed-noggin-2* causes the opposite dorsalized phenotype in which animals ectopically express dorsal markers on their ventral side.[152,153]

In addition to identifying a molecular foothold for studying the regulation of the D/V axis during planarian regeneration, these defects hint that an interaction between dorsal and ventral tissues juxtaposed during wound closure may indeed be important for regeneration, as Santos proposed from his grafting experiments (FIG 5A).[102,103] All ventralized RNAi phenotypes examined thus far display reduced or absent blastemas (FIG 5B–C) and a loss of expression of body edge markers at the wound site. Perhaps critical signaling events between properly-specified dorsal and ventral tissues organizes or permits downstream events in regeneration. While such an interaction could explain the BMP pathway blastema phenotype and the formation of a second body axis observed by Santos, these spatially-introduced signaling events have yet to be confirmed.

Medial-lateral polarity in planarians

Randolph and Morgan both described a variety of perturbations in medial-lateral (M/L) regeneration.[28,31,33,59] One of the most striking experiments involved a simple midline incision in either the anterior or posterior region of the animal. This incision did not fully cut the animals in half, and the wounds were allowed to heal back together. While some animals simply healed, this midline incision triggered a duplication of the M/L axis in others. These animals became wider and sprouted ectopic pharynges and photoreceptors lateral to the pre-existing ones (FIG 6A–B). While this experiment has yet to be revisited with molecular markers, their results suggest that the M/L axis is tightly regulated during regeneration. Simple wounding may cause the animal to reassess the integrity of the M/L axis, and trigger cells to take on different positional identities as if the animal had been cut through and through.

RNAi screens have identified numerous genes important for regulating anatomical patterning with respect to the M/L axis in planarians. This includes the Slit/Netrin repulsion-attraction signaling system. Among other developmental processes, SLIT and NETRIN ligands cooperatively regulate the migration of axonal projections across the midline, with SLIT repulsing axons as NETRIN attracts them.[154,155] Similarly, *Dj/Smed-slit-1* is required to maintain the planarian midline during restorative and physiological regeneration. RNAi knockdown causes a collapse of lateral tissues towards the midline, including the cephalic ganglia, nerve cords, photoreceptors, optic chiasm, and posterior gut branches (FIG 6C).[156,157] While it is curious that knockdown of the SLIT receptor, *Dj/Smed-roboA*, does not fully recapitulate these midline defects, it does cause aberrant crossing and fasciculation of the axons of the optic chiasm, in addition to a lateral displacement of the ganglia and reduction of the anterior commissure. This suggests a defect in M/L patterning.[158,157] In contrast to the *slit-1(RNAi)* phenotype, the most penetrant defects associated with knockdown of Netrin signaling via *Smed-netR(RNAi)* and *Smed-netrin2(RNAi)* are a lateral expansion of the cephalic ganglia, reduction in anterior commissure, and disorganization of the axons of the ventral nerve cords.[159] These contrasting defects in M/L patterning, coupled with simultaneous double RNAi for *Dugesia japonica* Slit-Netrin signaling components,[157] suggest a Slit-Netrin signaling synergy may help direct various events in M/L patterning.

The Wnt pathway is also involved in midline patterning in planarians. *Smed-wnt11-2(RNAi)* causes a failed extension of *Smed-slit-1* cells at the tip of the regenerated tail, resulting in abnormal looping of the ventral nerve cords and inappropriate midline crossing of the posterior gut branches.[126,131] In contrast, knockdown of *Smed-wnt5* yields a phenotype very similar to the defects observed by Randolph and Morgan after midline incisions: a lateral expansion of medial structures towards the body periphery, in addition to the growth of lateral, ectopic pharynges (FIG 6D).[126,131,55] In light of these phenotypes, the M/L axis must be as tightly regulated during planarian regeneration and homeostasis as the A/P and D/V axes are.

Finally, the TGF-β pathway is important not just for D/V axis organization, but also M/L organization. *Smed-bmp4* (which is expressed along the dorsal midline of the adult planarian) is upregulated at the wound edge during lateral regeneration. RNAi of some components of this pathway (*Smed-bmp4, Smedolloid-1,* and *Smed-smad4*) completely abolishes regeneration from amputations that bisect the animals down the midline.[151] RNAi of TGF-β pathway members that cause ventralization results in conspicuous midline indentations in anterior and posterior blastemas, suggesting that the midline does not regenerate properly (FIG 5C).[92,149,150,151,152,153] Furthermore, collapse of the nervous system and regeneration of supernumerary photoreceptors at the midline, as well as lateral ectopic pharynges are among other midline abnormalities reported thus far.[150] The perturbation of the M/L axis was molecularly verified in *Smed-admp(RNAi)* animals, which ectopically express the midline marker *Smed-slit-1* at the body periphery,[152] as well as in *Smed-smad4(RNAi)* regenerates (FIG 5C). *(See TABLE 1 for summary of all polarity phenotypes described.)*

These results leave us with many questions. Does regeneration require establishment of one body axis before another can be specified? Or can an axis be specified independently of the other two, as in the case of zebrafish development?[160] Which cells provide polarity information and which cells interpret these cues? Are there organizing centers for body axis polarity analogous to those identified during embryogenesis? And how are all three axes integrated during regeneration? In order to understand how these animals regenerate in three dimensions, studies must focus on the timing of axis specification and the manner in which these axis decisions affect signaling cascades required for subsequent organogenesis.[161,63,48, 162,57,58]

NEOBLASTS: CELLULAR AGENTS OF PLANARIAN REGENERATION

A century-long debate regarding the cellular agents of regeneration

After the amazing regenerative abilities of planarians were discovered, the search for the cellular source of this phenomenon ensued. Surprisingly, many key insights predate the use of the molecular-genetic tools recently applied to study planarians.

The histological analysis of planarian tissues under the light microscope in the late 19th century allowed biologists to identify a subset of parenchymal cells that undergo cell division, as evidenced by the presence of mitotic figures. This proliferating population of 6–12 micron ovoid-shaped cells possesses large decondensed nuclei and scant, basophilic cytoplasm (FIG 7A). These cells were correctly identified as the main source of new tissues.[23,24,25,30] Over the years, many names were ascribed to these cells, including *verästelten bindegewebszellen* (branching connective tissue cells),[163] *bildungszellen* (forming cells),[23] *stammzellen* (stem cells),[24] *stoffträger* (support material),[164] *ersatzzellen* (replacement cells),[30] *cellules libres du parenchyme* (free cells of the parenchyma),[165] *regenerationszellen* (regeneration cells),[166] and *wanderzellen* (migratory cells).[167] Eventually, the term *neoblast* permanently designated these cells, a name applied by Randolph to describe the cells responsible for regeneration in the earthworm *Lumbriculus*.[168,169,170]

Biologists initially tried to integrate what they knew about embryogenesis with what they were learning about neoblast-based regeneration. At first, these cells were referred to as an "embryonic stock," likened to blastomeres that persisted into adulthood to replenish injured or aging tissues.[171,113] Keller even suggested that neoblasts comprise a previously-unidentified fourth germ layer.[24] Soon, however, some biologists challenged the idea that neoblasts were a persistent undifferentiated pool of cells, and suggested instead that they were derived from differentiated tissues that had dedifferentiated or transdifferentiated—a phenomenon termed metaplasia.[172,173,174,175] Still others thought that planarian regeneration and tissue homeostasis might involve a combination of these two phenomena.[176,106,177,178,179]

For much of the 20th century, the source of a planarian's regenerative abilities created a heated debate, especially as new scientific tools facilitated more sophisticated analyses. Some groups tried to specifically label differentiated cells versus neoblasts to determine what tissues they contributed to. Attempts were also made to visualize regenerated tissues using improved histochemical techniques. These experiments led a few researchers to believe they had observed various cell types dedifferentiate into neoblasts.[180,181] Others took advantage of differences in ploidy between the somatic and germline tissues, and reported that the germline could dedifferentiate or transdifferentiate into tissues such as muscle.[182] Some even argued that since dedifferentiation was the mechanism of regeneration identified in most other animals studied thus far, planarian biology must work the same way.[183]

However, one tool proved key to properly addressing this question. In the first half of the 20th century, it was demonstrated that ionizing radiation primarily kills neoblasts, causing the animals to lose the ability to undergo physiological and restorative regeneration (FIG 7C–E, *dpi: days post irradiation*).[184,185] This simple manipulation suggested that metaplasia might not play a major role in regeneration, as the animals died even though the differentiated tissues appeared to be intact. To further test this, Wolff and Dubois used a lead block to shield portions of planarians at different positions along the A/P axis, resulting in the destruction of all neoblasts not covered by the shield. Subsequently, they amputated the animals and demonstrated that the length of time required for blastema formation was proportional to the distance of the lead shield from the wound.[185,170] These results suggested that the surviving neoblasts migrated to the wound to facilitate regeneration, as opposed to the dedifferentiation of local tissues.

Fueled by improvements in cell labeling methods, cell culture, grafting techniques and microscopy, additional evidence mounted in support of neoblasts being collectively totipotent migratory stem cells.[186,187,188,189,190,191,192] Of note, Baguñà and colleagues took advantage of two different strains of *Schmidtea mediterranea* to identify the source of regenerated tissues. One of these strains is sexual while the other is asexual, and they can be distinguished at the cellular level by a distinct chromosomal translocation. Cell fractions enriched for either neoblasts or differentiated cells were isolated by serial filtration from sexual animals and injected into irradiated asexuals and vice versa. In both cases, only the neoblast-enriched fraction rescued irradiated animals. Furthermore, the host took on the sexuality and karyotype of the animal from which the cell fractions were isolated.[193] Coupled with the observation that dividing cells migrate out of unirradiated tissue grafts into irradiated host tissues,[194,195,196] these results strongly suggested that neoblasts are a collectively totipotent, migratory stem cell population.

Modern tools demonstrate that neoblasts are collectively totipotent stem cells

In an effort to pinpoint the cellular source of regenerated tissues using modern molecular tools, early efforts focused on identifying genetic markers for neoblasts (FIG 7B). These efforts have included cloning candidate stem cell and proliferation-dependent genes, generating EST libraries of regenerating animals, and testing antibodies against conserved proliferation-dependent histone modifications and cell cycle regulators.[197,198,44,199,70,200,94,201,202,203] Adaptation of FACS protocols enabled profiling and isolation of two side populations of cells mainly composed of neoblasts (termed “X1s”) and a mixture of neoblasts and their recent division progeny (termed “X2s”).[95] Also, after 35 years of attempts to incorporate modified thymidine analogs into proliferating neoblasts, BrdU was successfully optimized for use in planarians, facilitating the tracing of neoblasts and their division progeny.[204,205,100] Furthermore, the sequencing of the planarian genome[72] led to the development of microarrays to examine global gene expression changes after ablation of neoblasts by irradiation. These microarrays not only identified genes that define a molecular “signature” for neoblasts.[87] They also revealed a number of categories of genes that disappear at different timepoints after irradiation and have distinct distributions of expression in the planarian body plan. By performing BrdU tracing and co-localization studies with neoblast markers, it was shown that these genes are actually markers for lineages of differentiating neoblasts.[88]

The ability to sort, label, and trace neoblasts and their division progeny made possible an impressive series of experiments that have put the debate about neoblasts’ contribution to regeneration to rest. A single transplanted neoblast—termed a clonogenic neoblast (cNeoblast) for its ability to generate colonies of cells—has been shown sufficient to rescue a lethally irradiated planarian. cNeoblasts display extensive pluripotency, and can differentiate into all of the cell types in the animal, except possibly the germline. Furthermore, BrdU labeling and strain-specific SNPs used to discern tissues derived from the transplanted cNeoblast versus those of the host suggest that dedifferentiation is unlikely to contribute significantly to planarian regeneration.[99]

Neoblasts and their division progeny are a heterogeneous population

Historically, neoblasts were characterized by their morphology alone (FIG 7A). As a result, all neoblasts seemed roughly equivalent, with the exception that slight differences in cell shape could be observed.[206] However, modern studies demonstrate significant molecular heterogeneity amongst neoblasts and neoblast progeny. This suggests that while some neoblasts may be pluripotent, there could be subsets of neoblasts that are lineage-restricted and able to differentiate into only certain cell types.

Dj/Smed-nanos provided the first molecular hints of neoblast heterogeneity. In asexual planarians, it is expressed in only a subset of neoblasts.[61,62,207,63,98] Subsequently, single-cell PCR, immuno-EM, and *in situ* hybridization studies showed that neoblasts express various combinations of the canonical neoblast markers, in addition to genes normally associated with tissue-specific differentiation.[207,98,162,58,99,208] Even a number of the neoblast progeny markers identified by microarray do not colocalize extensively, suggesting a diversity of progeny lineages.[99]

Finally, the classical observation that neoblasts display subtle diversity in morphology has been confirmed with improved means of isolating these cells.[209] Recent functional data suggest that neoblast morphology might indeed be indicative of heterogeneity in the population. Single cNeoblasts possessing a distinct membrane protrusion produced 75% of all rescue events. However, since the rate of rescue was quite low, with only 7 out of 130 injections successfully grafting, it is possible that many cells expressing the pan-neoblast marker *Smed-piwi-1* might actually be a diverse population of multipotent cells.[99] It is currently unclear whether this molecular and morphological heterogeneity simply results from lineage restriction as pluripotent neoblasts differentiate, or whether there are permanent subpopulations of neoblasts that are restricted in potential.

Neoblasts cycle rapidly, migrate, and proliferate in response to injury

Classical descriptions of the behavior of neoblasts as a cell population are being re-examined with improved resolution and accuracy. For many decades, analysis of cell division in planarians was based on scoring mitotic figures in serial histological sections.[210] With the demonstration that BrdU could be incorporated by neoblasts in 2000,[100] the door opened for detailed analyses of the planarian cell cycle. We now know from continuous labeling with BrdU that around 20% of all planarian cells are cycling neoblasts, coming in at the lower end of classical estimates based on cell macerations.[211,97] Nearly all neoblasts enter S-phase and can be labeled with BrdU within 2–3 days of continuous exposure, suggesting that a large population of slow-cycling or G2-arrested neoblasts is unlikely to exist, as originally proposed.[212,100,97] The length of G2 has been estimated at approximately 6 hours and the average cell cycle length is around 21 hours.[100,97] In addition, changes in proliferation due to nutritional state were described classically, and it has been confirmed that a large proliferative burst 12–72 hours after feeding indeed corresponds with animal growth.[212,213,97] The basis of degrowth during starvation, however, is still debated. It may result from a decrease in neoblast proliferation, an increase in cell death of neoblast division progeny, or some combination of the two.[213,214,215]

Recent work has confirmed and expanded upon older descriptions of the temporospatial dynamics of neoblast proliferation.[216] It now seems that the regenerative response can be divided into two distinct mitotic phases. During the first phase, neoblasts initiate a global burst in proliferation within 6 hours of any type of amputation or wound. The second burst requires the removal of tissue and is concentrated near the blastema, peaking around 48–72 hpa (FIG 7F).[217] Neoblasts can migrate as they differentiate and, in accordance with classical observations, they stop dividing before entering the blastema.[218,100,88,217] Finally, there is evidence supporting classical hypotheses that a signal emanating from the wound may trigger proliferation, as the initial mitotic increase seems to progress away from the wound in a wave-like fashion.[219,217]

With this expanding toolkit and an improved understanding of the dynamics of neoblasts, we can now begin to identify the genes that regulate neoblast self-renewal and differentiation. While it is possible that conserved cell cycle regulators and pluripotency genes play similar roles in neoblasts as they do in other stem cell systems, it is equally likely that novel mechanisms for regulation of neoblasts may be discovered. Already, we are learning that even the most fundamental aspects of planarian cell division are surprisingly unique. Planarians, for instance, are the first animals identified that do not seem to require a centrosome for cell division at any point in their life history.[220] It is possible that such fundamental differences in regulation of cell division might be key to understanding why planarians have exceptionally robust regenerative abilities.

Many genes required for neoblast function have been identified

RNAi of genes from microarray experiments, expression profiling, EST libraries, and candidate ortholog searches of the planarian genome have already identified close to 200 genes whose phenotypes suggest defects in neoblast self-renewal and/or differentiation (*see Table 2 for references*). These phenotypes include reduced or absent blastemas, ventral curling, tissue regression, and lysis. Around thirty of these genes have been characterized in detail (TABLE 2).

While most of the phenotypes examined ultimately abolish proliferation and deplete neoblasts, an examination of the earlier stages of the phenotype progression reveals that neoblasts can be perturbed in numerous ways. First, neoblast self-renewal can be abrogated, as evidenced by a variety of phenotypes that display a decrease in proliferation and number of neoblasts soon after RNAi administration. Second, neoblasts can be disrupted at the level of differentiation. For instance, *Smed-piwi-2(RNAi)* does not affect neoblast numbers, their migratory ability, or their proliferative response after injury. Instead, differentiation is perturbed, as evidenced by the lack of regenerated tissues and the abnormal morphology of neoblast progeny that incorporate into the epithelium during homeostasis.[94,221] Additional examples of differentiation defects include *Smed-p53(RNAi)*, *Smed-CHD4(RNAi)*, and *Smed-PTEN-1/2(RNAi)*. Among other abnormalities, RNAi of these genes seems to stall differentiation, causing an accumulation of neoblasts at the expense of postmitotic progeny.[222,223,224] Lastly, the spatial distribution of proliferating neoblasts can be disrupted, as it is after RNAi of *Smed-egfr-3* or administration of a putative ERK inhibitor.[225,226]

THE EMERGING ROLE OF DIFFERENTIATED TISSUES IN REGENERATION

The regeneration field has focused much of its efforts on the study of stem cells proper. The role of differentiated tissues has been appreciated mostly within the context of a cellular microenvironment known as a niche, which protects and maintains stem cells.[227] Considering that planarian regeneration requires not only local restoration of missing tissues, but also a simultaneous reproportioning of the entire body plan, it stands to reason that differentiated tissues may play important roles in regeneration on scales larger than what has been previously described for a stem cell niche.

Historically, the function of differentiated tissues during planarian regeneration has been largely dismissed as secondary to the action of neoblasts. Brøndsted, for instance, argued that neoblasts and the blastema they generate provide inductive cues to establish axial polarity in the rest of the pre-existing tissues.[228] Likewise, Betchaku's cell culture experiments led him to view the fixed parenchymal cells as merely a vehicle for transporting neoblasts to the wound site so they can mount a regenerative response.[229] In the 1980s, the importance of differentiated tissues in directing neoblast differentiation was proposed.[195] However, only recently have the molecular tools become available to test this idea in a background completely devoid of neoblasts. These experiments have revealed a striking plasticity of the differentiated tissues during regeneration that occurs on a body-wide scale.

After irradiation ablates neoblasts and depletes their recent division progeny, the animals cannot regenerate new tissues. However, they can still undergo normal transcriptional responses after amputation. For example, in the complete absence of neoblasts, the differentiated tissues upregulate expression of early wound-response genes, in addition to re-specifying the A/P axis within 1 dpa.[230,151,133,131,226] Further examination of cell death dynamics reveals that two distinct waves of apoptosis occur within 4 hpa and 3 dpa. Surprisingly, the amount of apoptosis measured by TUNEL positive nuclei is normal in the absence of neoblasts, meaning that the cell death required for proper tissue remodeling occurs independently of stem cells.[231] Finally, it has been shown that the differentiated tissues in irradiated animals can dynamically modify body-wide transcriptional output for at least four days after an amputation, as evidenced by the oscillation of *Smed-wnt11-5* expression across the A/P axis in irradiated tail fragments. It is only after 4 dpa that obvious defects in the expression of this gene become apparent, suggesting that the differentiated tissues may eventually need to integrate their new positional identity with the regenerated anatomy or neoblasts after a certain point in time.[133,131] While it is still unknown whether a niche for neoblasts exists, neoblasts and their local microenvironment are likely not the only elements required to understand planarian regeneration. Something about the nature of the global pre-existing differentiated tissues could be an important factor in determining to what extent an animal—whether it be a planarian or a human—can regenerate.

LOOKING TO THE FUTURE

After centuries of fascination with planarians, their regenerative abilities have transformed from a curiosity ultimately deemed intractable for detailed study by Morgan to an established animal model of regeneration. If classical biologists could have peered into the future, they would probably have been impressed by the amount of knowledge generated in just the past 15 years. Topping this list of accomplishments, it was confirmed that a pool of pluripotent neoblasts act as stem cells to replenish missing tissues. Numerous genes important for neoblast self-renewal and differentiation have been identified, and the first markers of neoblast lineages have been described. The molecular principles underlying axial polarity and organogenesis are already being teased out. In addition, the surprisingly dynamic behavior of whole tissues devoid of neoblasts is challenging us to reassess a stem cell-centric philosophy of regeneration. These results indicate that instead of acting only as a local niche, differentiated tissues may provide a macroenvironment capable of initiating wound responses, specifying axial polarity, and integrating global positional information that direct the subsequent differentiation of neoblasts. Of course, much still remains to be learned about these fascinating organisms.

From the top down, there are many facets of planarian regeneration biology to be elucidated. At the highest level, the rapid changes in transcriptional output observed after amputation or wounding suggests that the chromatin landscape and access to genomic promoters must be tightly coordinated. Chromatin dynamics will likely play key roles in these global transcriptional responses as the cells of the animal reassess positional information and facilitate the proper differentiation of neoblast progeny. Transcription factors and the targets they regulate in response to injury should also be studied so that gene regulatory networks for regeneration of specific tissues can be made to integrate the large body of functional data that will undoubtedly be generated. Additionally, increased cellular resolution will be required to study the effects of genes that undoubtedly have diverse temporospatial roles during physiological and restorative regeneration. Tools must be developed for indelibly labeling cells *in vivo* for fate mapping and live imaging studies. Single-cell transcriptional profiling, in addition to analysis of post-transcriptional and post-translational modifications occurring shortly after injury will also be key to teasing apart the molecular tapestry underlying regeneration.

By using this knowledge base, we can begin exploring how the mechanisms of regeneration formally compare to planarian embryogenesis. Such a comparison would begin to address the long-standing question of whether regeneration is simply a recapitulation of development or whether it is made possible by independent mechanistic innovations. How, for instance, are embryonic stem cells functionally different from neoblasts? How and when are neoblasts specified during embryogenesis? Is the same genetic toolkit required during embryogenesis to organize the body axes and facilitate organogenesis as it is during regeneration? This comparison may provide vital insights into a particularly curious phenomenon. Specifically, a variety of organisms display an impressive ability to undergo regulative embryonic development. Animals like the mouse, fruit fly, and frog can recover from ablation of numerous blastomeres or substantial injury to embryonic organs. However, these animals display limited regenerative capacities as adults. Understanding whether regulative development happens in planarian embryos and how it might differ from these other organisms may help us identify key differences crucial to preserving regenerative abilities into adulthood.

Finally, one of the ultimate goals of studying planarian regeneration is to understand why some animals regenerate robustly while others—such as humans—do not. Comparing the mechanisms of adult regeneration in diverse animals that have varying abilities to regenerate may be another way of pinpointing the core requirements for regeneration. Such an approach may also provide insights into the permutations that evolution has enacted upon this biological process over time. The first step in this endeavor should be to compare different planarian species, some of which regenerate robustly, while others display more limited abilities depending upon the plane of amputation. Elegant irradiation and grafting experiments performed on *Procotyla fluviatilis*, which does not regenerate robustly after post-pharyngeal amputation, suggest that variation in regenerative ability may result from the signals provided by the differentiated tissues,[232,233,187,234] and may not be explained simply by total numbers of neoblasts, as first thought.[206] Revisiting these classical studies with modern tools may help us identify a core set of molecular and physical principles guiding regeneration, which could then be examined in more distantly-related animal species.

With a rich history and giant leaps forward in recent years, the future of the planarian field is bright. Our mechanistic view of regeneration will undoubtedly come into much greater focus as more techniques are developed and more investigators pursue questions in this classical model system. It will be exciting to see what biological insights these animals will reveal to us next.

Acknowledgments

The authors acknowledge Dr. Kiyokazu Agata, Dr. Kyle Gurley, Dr. Jochen Rink, Dr. Tamaki Suganuma, Nobuo Ueda, Hanh Thi-Kim Vu, and Shigeki Watanabe for contributions indicated in the figure legends, in addition to Longhua Guo for translation assistance with T'uan's *Yu-Yang Tsa-Tsu*. A.S.A is supported by NIH grant R37GM057260. A.S.A is a Howard Hughes Medical Institute and a Stowers Institute for Medical Research Investigator.

REFERENCES

1. Dinsmore CE. Urodele limb and tail regeneration in early biological thought: an essay on scientific controversy and social change. Int. J. Dev. Biol. 1996; 40:621–627. [PubMed: 8877433]

2. Aristotle; Balme, DM.; Gotthelf, A.; Peck, AL. Historia animalium. London; Cambridge, Mass: Heinemann; Harvard University Press; 1965.
3. Anonymous. Histoire de l'Académie Royale des Sciences depuis 1686 jusqu'à son renouvellement en 1699. Vol. Vol. 2. Paris: Bibliothèque De M. J.-A. Barral; 1773.
4. de Réaumur M. Sur les diverses reproductions qui se font dans les Ecrevisse, les Omars, les Crabes, etc. et entr'autres sur celles de leurs Jambes et de leurs Ecailles. Memoirs of the Royal Academie of Sciences. 1712:223–242.
5. Lenhoff, SG.; Lenhoff, HM.; Trembley, A. Hydra and the Birth of Experimental Biology, 1744: Abraham Trembley's Mémoires Concerning the Polyps. Pacific Grove, CA: Boxwood Press; 1986.
6. Bonnet, C. Traité d'Insectologie ou observations sur les Pucerons. Paris: Durand; 1745.
7. Spallanzani, L. Prodromo di un'opera da imprimersi sopra le riproduzioni animali dato in luce dall'abate Spallanzani. Modena: Nella stamperia di Giovanni Montanari; 1768.
8. Pallas, PS. Spicilegia zoologica : quibus novae imprimis et obscurae animalium species iconibus, descriptionibus atque commentariis illustrantur. Berolini: Prostant apud Gottl. August. Lange; 1774.
9. Müller, OF.; Stiles, CW. Academy of Natural Sciences of P. *Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum, succincta historia*. Havniae et Lipsiae: Apud Heineck et Faber; 1773.
10. Nakamura, T. Kinmō-Zui. Vol. 50. Tokyo: Waseda daigaku shuppanbu, Shōwa; 1666.
11. Terajima R. Wakan-Sansai-Zue. 1713
12. Kawakatsu M. A list of publications on Japanese Turbellarians (1968)--Including titles of publications on foreign Turbellarians written by the Japanese authors. Part II: On some old records of Turbellarians found in the Japanese books printed in wood-block. Bull. Fuji Women's College. 1969; 7:30–43. (plates 31–37).
13. Lue K, Kawakatsu M. History of the study of Turbellaria in China. Part 1: Ages of Materia Medica and of early expeditions by westerners. Hydrobiologia. 1986; 132:317–322.
14. T'uan, Cê-S. Yu-Yang Tsa-Tsu. Taiwan: 860.
15. Draparnaud, J. Tableau des mollusques terrestres et fluviatiles de la France. Montpellier: Renaud; 1801.
16. Dalyell, JG. Observations on some interesting phenomena in animal physiology, exhibited by several species of Planariae. Illustrated by coloured figures of living animals. Edinburgh: Archibald Constable; 1814.
17. Johnson J. Observations on the genus Planaria. Philosophical Transactions of the Royal Society of London. 1822; 112:437–447.
18. Johnson J. Further observations on Planariae. Philosophical Transactions of the Royal Society of London. 1825; 115:247–256.
19. Dugés A. Recherches sur l'organisation et les moeurs des Planariées. Ann. Sci. Nat. Zool. 1828; 1:139–183.
20. Faraday M. On the Planariae. Edinburgh New Philosophical Journal. 1833; 14:183–189.
21. Darwin C. XXIX.— Brief descriptions of several terrestrial Planariæ, and of some remarkable marine species, with an account of their habits. Annals and Magazine of Natural History. 1844; 14:241–251.
22. Harvey, W. The Sea-Side Book; Being an Introduction to the Natural History of the British Coasts. London: Bangor House, Shoe Lane; 1849.
23. Wagner F. Zur Kenntnis der ungeschlechtlichen Fortpflanzung von Microstoma nebst allgemeinen Bemerkungen über Teilung und Knospung im Tierreich. Z. Jahrb. Abth. f. Anat. u Ontog. d Thiere. 1890; 4:349–423.
24. Keller J. Die ungeschlechtliche Fortpflanzung der Süsswasserturbellarien. Jen. Zeit. Naturw. 1894; 94:3823–3827.
25. Van Duyne J. Uber Heteromorphose bei Planarien. Arch. f. d. ges. Physiol. 1896; 64:569.
26. Leidy J. Descriptions and anatomy of a new and curious sub-genus of planaria. Proceedings of the Academy of Natural Sciences of Philadelphia. 1847; 3:248–252.
27. Wyman J. An account of some experiments on planaria showing their power of repairing injuries. Proc. Bost. Soc. Nat. Hist., 1862–63. 1865; 9:157.

28. Randolph H. Observations and experiments on regeneration in planarians. Arch. Entw. Mech. Org. 1897; 5:352–372.
29. Lillie FR, Knowlton FP. On the effect of temperature on the development of animals. Zoological Bulletin. 1897; 1:179–193.
30. Flexner S. The regeneration of the nervous system of *Planaria torva* and the anatomy of the nervous system of double-headed forms. Journal of Morphology. 1898; 14:337–346.
31. Morgan TH. Experimental studies of the regeneration of *Planaria maculata.* Arch. Entw. Mech. Org. 1898; 7:364–397.
32. Lillie FR. Some notes on regeneration and regulation in planarians. The American Naturalist. 1900; 34:173–177.
33. Morgan TH. Regeneration in planarians. Archiv. Entwick. Mech. 1900; 10:58–119.
34. Tanaka EM, Reddien PW. The cellular basis for animal regeneration. Developmental Cell. 2011; 21:172–185. [PubMed: 21763617]
35. Morgan, TH. Regeneration. New York: Macmillan; 1901.
36. Brøndsted, HV. Planarian Regeneration. London: Pergamon Press; 1969.
37. Nilsson Sköld H, Obst M. Potential for clonal animals in longevity and ageing studies. Biogerontology. 2011; 12:387–396. [PubMed: 21479578]
38. Tan TC, Rahman R, Jaber-Hijazi F, Felix DA, Chen C, Louis EJ, Aboobaker A. Telomere maintenance and telomerase activity are differentially regulated in asexual and sexual worms. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:4209–4214. [PubMed: 22371573]
39. González-Estévez C, Felix DA, Smith MD, Paps J, Morley SJ, James V, Sharp TV, Aboobaker AA. SMG-1 and mTORC1 act antagonistically to regulate response to injury and growth in planarians. PLoS Genetics. 2012; 8 e1002619.
40. Peiris TH, Weckerle F, Ozamoto E, Ramirez D, Davidian D, Garcia-Ojeda ME, Oviedo NJ. TOR signaling regulates planarian stem cells and controls localized and organismal growth. Journal of Cell Science. 2012 *In press*.
41. Tu KC, Pearson BJ, Sánchez Alvarado A. TORC1 is required to balance cell proliferation and cell death in planarians. Developmental Biology. 2012 *In press*.
42. Ishii S. Electron microscopic observations on the planarian tissues. I. A survey of the pharynx. Fukishima J. Med. Sci. 1962/1963; 9/10:51–73.
43. Kobayashi C, Watanabe K, Agata K. The process of pharynx regeneration in planarians. Developmental Biology. 1999; 211:27–38. [PubMed: 10373302]
44. Ito H, Saito Y, Watanabe K, Orii H. Epimorphic regeneration of the distal part of the planarian pharynx. Development, Genes & Evolution. 2001; 211:2–9. [PubMed: 11277401]
45. Sakai T, Kato K, Watanabe K, Orii H. Planarian pharynx regeneration revealed by the expression of myosin heavy chain-A. The International Journal of Developmental Biology. 2002; 46:329–332. [PubMed: 12068956]
46. Metschnikoff VE II. Wissenschaftliche Mittheilungen: Über die Verdauungsorgane einiger Süsswasserturbellarien. Zoologischer Anzeiger. 1878; 1:387–390.
47. Arnold G. Intra-cellular and general digestive processes in planariæ. Quarterly Journal of Microscopical Science. 1909; 54:207–221.
48. Forsthoefel DJ, Park AE, Newmark PA. Stem cell-based growth, regeneration, and remodeling of the planarian intestine. Developmental Biology. 2011; 356:445–459. [PubMed: 21664348]
49. Okamoto K, Takeuchi K, Agata K. Neural projections in planarian brain revealed by fluorescent dye tracing. Zoological Science. 2005; 22:535–546. [PubMed: 15930826]
50. Umesono Y, Agata K. Evolution and regeneration of the planarian central nervous system. Development, Growth & Differentiation. 2009; 51:185–195.
51. Rink JC, Gurley KA, Elliott SA, Sánchez Alvarado A. Planarian Hh signaling regulates regeneration polarity and links Hh pathway evolution to cilia. Science. 2009; 326:1406–1410. [PubMed: 19933103]
52. Rompolas P, Patel-King RS, King SM. *Schmidtea mediterranea*: a model system for analysis of motile cilia. Methods in Cell Biology. 2009; 93:81–98. [PubMed: 20409812]

53. Glazer AM, Wilkinson AW, Backer CB, Lapan SW, Gutzman JH, Cheeseman IM, Reddien PW. The Zn finger protein Iguana impacts Hedgehog signaling by promoting ciliogenesis. Developmental Biology. 2010; 337:148–156. [PubMed: 19852954]
54. Rompolas P, Patel-King RS, King SM. An outer arm Dynein conformational switch is required for metachronal synchrony of motile cilia in planaria. Molecular Biology of the Cell. 2010; 21:3669–3679. [PubMed: 20844081]
55. Almuedo-Castillo M, Saló E, Adell T. Dishevelled is essential for neural connectivity and planar cell polarity in planarians. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:2813–2818. [PubMed: 21282632]
56. Oviedo NJ, Levin M. *smedinx-11* is a planarian stem cell gap junction gene required for regeneration and homeostasis. Development. 2007; 134:3121–3131. [PubMed: 17670787]
57. Rink JC, Vu HT, Sánchez Alvarado A. The maintenance and regeneration of the planarian excretory system are regulated by EGFR signaling. Development. 2011; 138:3769–3780. [PubMed: 21828097]
58. Scimone ML, Srivastava M, Bell GW, Reddien PW. A regulatory program for excretory system regeneration in planarians. Development. 2011; 138:4387–4398. [PubMed: 21937596]
59. Morgan TH. Growth and regeneration in *Planaria lugubris*. Arch. Ent. Mech. Org. 1901; 13:179–212.
60. Schultz E. Über reduktionen. I. Über hungererscheinungen bei *Planaria lactea*. Arch. Entwm. 1904; 18:555–577.
61. Sato K, Shibata N, Orii H, Amikura R, Sakurai T, Agata K, Kobayashi S, Watanabe K. Identification and origin of the germline stem cells as revealed by the expression of *nanos*-related gene in planarians. Development, Growth & Differentiation. 2006; 48:615–628.
62. Handberg-Thorsager M, Saló E. The planarian nanos-like gene *Smednos* is expressed in germline and eye precursor cells during development and regeneration. Development Genes and Evolution. 2007; 217:403–411. [PubMed: 17390146]
63. Wang Y, Zayas RM, Guo T, Newmark PA. *nanos* function is essential for development and regeneration of planarian germ cells. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:5901–5906. [PubMed: 17376870]
64. Reddien PW. Constitutive gene expression and the specification of tissue identity in adult planarian biology. Trends in Genetics. 2011; 27:277–285. [PubMed: 21680047]
65. Bardeen CR. The function of the brain in *Planaria maculata*. Am. J. Physiol. 1901; 5:175–179.
66. Taliaferro W. Reactions to light in *Planaria maculata*, with special reference to the function and structure of the eyes. Journal of Experimental Zoölogy. 1920; 31:59–117.
67. Hull FM. Observations on cannibalism in planarians. Transactions of the American Microscopical Society. 1947; 66:96–98. [PubMed: 20242307]
68. Inoue T, Kumamoto H, Okamoto K, Umesono Y, Sakai M, Sánchez Alvarado A, Agata K. Morphological and functional recovery of the planarian photosensing system during head regeneration. Zoological Science. 2004; 21:275–283. [PubMed: 15056922]
69. Bueno D, Baguñà J, Romero R. Cell-, tissue-, and position-specific monoclonal antibodies against the planarian *Dugesia (Girardia) tigrina*. Histochemistry and Cell Biology. 1997; 107:139–149. [PubMed: 9062799]
70. Robb SM, Sánchez Alvarado A. Identification of immunological reagents for use in the study of freshwater planarians by means of whole-mount immunofluorescence and confocal microscopy. Genesis. 2002; 32:293–298. [PubMed: 11948917]
71. Pearson BJ, Eisenhoffer GT, Gurley KA, Rink JC, Miller DE, Sánchez Alvarado A. Formaldehyde-based whole-mount in situ hybridization method for planarians. Developmental Dynamics. 2009; 238:443–450. [PubMed: 19161223]
72. Robb SM, Ross E, Sánchez Alvarado A. SmedGD: The *Schmidtea mediterranea* Genome Database. Nucleic Acids Research. 2008; 36:D599–D606. [PubMed: 17881371]
73. Sánchez Alvarado A, Newmark PA, Robb SM, Juste R. The *Schmidtea mediterranea* database as a molecular resource for studying platyhelminthes, stem cells and regeneration. Development. 2002; 129:5659–5665. [PubMed: 12421706]

74. Zayas RM, Hernandez A, Habermann B, Wang Y, Stary JM, Newmark PA. The planarian *Schmidtea mediterranea* as a model for epigenetic germ cell specification: analysis of ESTs from the hermaphroditic strain. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:18491–18496. [PubMed: 16344473]

75. Friedländer MR, Adamidi C, Han T, Lebedeva S, Isenbarger TA, Hirst M, Marra M, Nusbaum C, Lee WL, Jenkin JC, et al. High-resolution profiling and discovery of planarian small RNAs. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:11546–11551. [PubMed: 19564616]

76. Lu YC, Smielewska M, Palakodeti D, Lovci MT, Aigner S, Yeo GW, Graveley BR. Deep sequencing identifies new and regulated microRNAs in *Schmidtea mediterranea*. RNA. 2009; 15:1483–1491. [PubMed: 19553344]

77. Abril JF, Cebrià F, Rodríguez-Esteban G, Horn T, Fraguas S, Calvo B, Bartscherer K, Saló E. Smed454 dataset: unravelling the transcriptome of *Schmidtea mediterranea*. BMC Genomics. 2010; 11:731. [PubMed: 21194483]

78. Blythe MJ, Kao D, Malla S, Rowsell J, Wilson R, Evans D, Jowett J, Hall A, Lemay V, Lam S, et al. A dual platform approach to transcript discovery for the planarian *Schmidtea mediterranea* to establish RNAseq for stem cell and regeneration biology. PLoS ONE. 2010; 5:e15617. [PubMed: 21179477]

79. Collins JJ 3rd, Hou X, Romanova EV, Lambrus BG, Miller CM, Saberi A, Sweedler JV, Newmark PA. Genome-wide analyses reveal a role for peptide hormones in planarian germline development. PLoS Biology. 2010; 8 e1000509.

80. Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak SD, Gogol-Doering A, Oenal P, et al. De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics. Genome research. 2011; 21:1193–1200. [PubMed: 21536722]

81. Sandmann T, Vogg MC, Owlarn S, Boutros M, Bartscherer K. The head-regeneration transcriptome of the planarian *Schmidtea mediterranea*. Genome Biology. 2011; 12:R76. [PubMed: 21846378]

82. Fernández-Taboada E, Rodríguez-Esteban G, Saló E, Abril JF. A proteomics approach to decipher the molecular nature of planarian stem cells. BMC Genomics. 2011; 12:1–13.

83. Galloni M. Global irradiation effects, stem cell genes and rare transcripts in the planarian transcriptome. The International Journal of Developmental Biology. 2012; 56:103–116. [PubMed: 22450998]

84. Resch AM, Palakodeti D, Lu YC, Horowitz M, Graveley BR. Transcriptome analysis reveals strain-specific and conserved stemness genes in *Schmidtea mediterranea*. PLoS ONE. 2012; 7:e34447. [PubMed: 22496805]

85. Solana J, Kao D, Mihaylova Y, Jaber-Hijazi F, Malla S, Wilson R, Aboobaker A. Defining the molecular profile of planarian pluripotent stem cells using a combinatorial RNA-seq, RNAi and irradiation approach. Genome Biology. 2012; 13:R19. [PubMed: 22439894]

86. Nakazawa M, Cebrià F, Mineta K, Ikeo K, Agata K, Gojobori T. Search for the evolutionary origin of a brain: planarian brain characterized by microarray. Molecular Biology and Evolution. 2003; 20:784–791. [PubMed: 12679532]

87. Rossi L, Salvetti A, Marincola FM, Lena A, Deri P, Mannini L, Batistoni R, Wang E, Gremigni V. Deciphering the molecular machinery of stem cells: a look at the neoblast gene expression profile. Genome Biology. 2007; 8:R62. [PubMed: 17445279]

88. Eisenhoffer GT, Kang H, Sánchez Alvarado A. Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian *Schmidtea mediterranea*. Cell Stem Cell. 2008; 3:327–339. [PubMed: 18786419]

89. Wang Y, Stary JM, Wilhelm JE, Newmark PA. A functional genomic screen in planarians identifies novel regulators of germ cell development. Genes & Development. 2010; 24:2081–2092. [PubMed: 20844018]

90. Sánchez Alvarado A, Newmark PA. Double-stranded RNA specifically disrupts gene expression during planarian regeneration. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:5049–5054. [PubMed: 10220416]

91. Newmark PA, Reddien PW, Cebrià F, Sánchez Alvarado A. Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:11861–11865. [PubMed: 12917490]

92. Reddien PW, Bermange AL, Murfitt KJ, Jennings JR, Sánchez Alvarado A. Identification of genes needed for regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in planaria. Developmental Cell. 2005; 8:635–649. [PubMed: 15866156]

93. Asami M, Nakatsuka T, Hayashi T, Kou K, Kagawa H, Agata K. Cultivation and characterization of planarian neuronal cells isolated by fluorescence activated cell sorting (FACS). Zoological Science. 2002; 19:1257–1265. [PubMed: 12499670]

94. Reddien PW, Oviedo NJ, Jennings JR, Jenkin JC, Sánchez Alvarado A. SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. Science. 2005; 310:1327–1330. [PubMed: 16311336]

95. Hayashi T, Asami M, Higuchi S, Shibata N, Agata K. Isolation of planarian X-ray-sensitive stem cells by fluorescence-activated cell sorting. Development, Growth & Differentiation. 2006; 48:371–380.

96. Inoue T, Hayashi T, Takechi K, Agata K. Clathrin-mediated endocytic signals are required for the regeneration of, as well as homeostasis in, the planarian CNS. Development. 2007; 134:1679–1689. [PubMed: 17376807]

97. Kang H, Sánchez Alvarado A. Flow cytometry methods for the study of cell-cycle parameters of planarian stem cells. Developmental Dynamics. 2009; 238:1111–1117. [PubMed: 19322765]

98. Hayashi T, Shibata N, Okumura R, Kudome T, Nishimura O, Tarui H, Agata K. Single-cell gene profiling of planarian stem cells using fluorescent activated cell sorting and its "index sorting" function for stem cell research. Development, Growth & Differentiation. 2010; 52:131–144.

99. Wagner DE, Wang IE, Reddien PW. Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. Science. 2011; 332:811–816. [PubMed: 21566185]

100. Newmark PA, Sánchez Alvarado A. Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. Developmental Biology. 2000; 220:142–153. [PubMed: 10753506]

101. Allman GJ. Report on the present state of our knowledge of the reproductive system in the Hydroida. Report of the Thirty-Third Meeting of the British Association for the Advancement of Science; Held at Newcastle-Upon-Tyne in August and September 1863. 1864:351–426.

102. Santos F. Studies on transplantation in planaria. Biological Bulletin. 1929; 57:188–197.

103. Santos F. Studies on transplantation in planaria. Physiological Zoology. 1931; 4:111–164.

104. Bonnet, C. Contemplation de la nature. Vol. Vol. 1. Amsterdam: Chez Marc-Michel Rey; 1764.

105. Weissman, A. The Germ-Plasm. A Theory of Heredity. New York: Charles Scribner's Sons; 1893.

106. Bardeen CR. Embryonic and regenerative development in planarians. Biological Bulletin. 1902; 3:262–288.

107. Pflüger E. Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen und auf die Entwicklung des Embryo. Zweite abhandlung. Archiv für die gesamte Physiologie des Menschen und der Thiere. 1883; 32:1–79.

108. Child, CM. Patterns and Problems of Development. Chicago: The University of Chicago Press; 1941.

109. Brøndsted HV. Experiments on the time-graded regeneration field in planarians. Biol. Medd. Dan. Vid. Selsk. 1956; 23:1–39.

110. Brøndsted HV. Influence of temperature on rate of regeneration in the time-graded regeneration field in planarians. Journal of Embryology and Experimental Morphology. 1961; 9:159–166.

111. Spencer, H. The Principles of Biology. Vol. Vol. 2. New York: D. Appleton and Company; 1871.

112. von Sachs J. Stoff und Form der Pflanzenorgane. Arb. Bot. Inst. Würzburg. 1880; 2:452–488.

113. Lillie FR. Notes on regeneration and regulation in planarians (continued). American Journal of Physiology. 1901; 6:129–141.

114. Wolpert L. Positional information and the spatial pattern of cellular differentiation. Journal of Theoretical Biology. 1969; 25:1–47. [PubMed: 4390734]

115. Chandebois R. The dynamics of wound closure and its role in the programming of planarian regeneration. II - Distalization. Development, Growth & Differentiation. 1980; 22:693–704.
116. Morgan TH. Polarity and axial heteromorphosis. Amer. Nat. 1904; 38:502–505.
117. Morgan T. Regeneration: Old and new interpretations. Biological Lectures. 1900:185–208.
118. Morgan TH. "Polarity" considered as a phenomenon of gradation of materials. Journal of Experimental Zoölogy. 1905; 2:495–506.
119. Morgan, T. Experimental Zoölogy. New York: The Macmillan Company; 1907.
120. Morgan TH. Regeneration of heteromorphic tails in posterior pieces of *Planaria simplicissima*. The Journal of Experimental Zoölogy. 1904; 1:385–393.
121. Petersen CP, Reddien PW. Wnt signaling and the polarity of the primary body axis. Cell. 2009; 139:1056–1068. [PubMed: 20005801]
122. Niehrs C. On growth and form: a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. Development. 2010; 137:845–857. [PubMed: 20179091]
123. Gurley KA, Rink JC, Sánchez Alvarado A. β-catenin defines head versus tail identity during planarian regeneration and homeostasis. Science. 2008; 319:323–327. [PubMed: 18063757]
124. Petersen CP, Reddien PW. *Smed*-β*catenin-1* is required for anteroposterior blastema polarity in planarian regeneration. Science. 2008; 319:327–330. [PubMed: 18063755]
125. Iglesias M, Gómez-Skarmeta JL, Saló E, Adell T. Silencing of *Smed*-β *catenin1* generates radial-like hypercephalized planarians. Development. 2008; 135:1215–1221. [PubMed: 18287199]
126. Adell T, Saló E, Boutros M, Bartscherer K. *Smed-Evi/Wntless* is required for β-catenin-dependent and -independent processes during planarian regeneration. Development. 2009; 136:905–910. [PubMed: 19211673]
127. Petersen CP, Reddien PW. Polarized notum activation at wounds inhibits Wnt function to promote planarian head regeneration. Science. 2011; 332:852–855. [PubMed: 21566195]
128. Evans DJ, Owlarn S, Tejada Romero B, Chen C, Aboobaker AA. Combining classical and molecular approaches elaborates on the complexity of mechanisms underpinning anterior regeneration. PLoS ONE. 2011; 6:e27927. [PubMed: 22125640]
129. Hayashi T, Motoishi M, Yazawa S, Itomi K, Tanegashima C, Nishimura O, Agata K, Tarui H. A LIM-homeobox gene is required for differentiation of Wnt-expressing cells at the posterior end of the planarian body. Development. 2011; 138:3679–3688. [PubMed: 21828095]
130. Iglesias M, Almuedo-Castillo M, Aboobaker AA, Saló E. Early planarian brain regeneration is independent of blastema polarity mediated by the Wnt/β-catenin pathway. Developmental Biology. 2011; 358:68–78. [PubMed: 21806978]
131. Gurley KA, Elliott SA, Simakov O, Schmidt HA, Holstein TW, Sánchez Alvarado A. Expression of secreted Wnt pathway components reveals unexpected complexity of the planarian amputation response. Developmental Biology. 2010; 347:24–39. [PubMed: 20707997]
132. Ingham PW, Nakano Y, Seger C. Mechanisms and functions of Hedgehog signalling across the metazoa. Nature Reviews. Genetics. 2011; 12:393–406.
133. Petersen CP, Reddien PW. A wound-induced Wnt expression program controls planarian regeneration polarity. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:17061–17066. [PubMed: 19805089]
134. Yazawa S, Umesono Y, Hayashi T, Tarui H, Agata K. Planarian Hedgehog/Patched establishes anterior-posterior polarity by regulating Wnt signaling. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:22329–22334. [PubMed: 20018728]
135. Oviedo NJ, Morokuma J, Walentek P, Kema IP, Gu MB, Ahn JM, Hwang JS, Gojobori T, Levin M. Long-range neural and gap junction protein-mediated cues control polarity during planarian regeneration. Developmental Biology. 2010; 339:188–199. [PubMed: 20026026]
136. Felix DA, Aboobaker AA. The TALE class homeobox gene *Smed-prep* defines the anterior compartment for head regeneration. PLoS Genetics. 2010; 6 e1000915.
137. Marsh G, Beams HW. Electrical control of morphogenesis in regenerating *Dugesia tigrina*. I. Relation of axial polarity to field strength. Journal of Cellular and Comparative Physiology. 1952; 39:191–213.

138. Oviedo NJ, Nicolas CL, Adams DS, Levin M. Live imaging of planarian membrane potential using DiBAC4(3). CSH Protocols. 2008; 2008 pdb prot5055.

139. Nogi T, Zhang D, Chan JD, Marchant JS. A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel beta subunits: subversion of flatworm regenerative polarity. PLoS Neglected Tropical Diseases. 2009; 3:e464. [PubMed: 19554083]

140. Beane WS, Morokuma J, Adams DS, Levin M. A chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration. Chemistry & Biology. 2011; 18:77–89. [PubMed: 21276941]

141. Zhang D, Chan JD, Nogi T, Marchant JS. Opposing roles of voltage-gated Ca2+ channels in neuronal control of regenerative patterning. The Journal of Neuroscience. 2011; 31:15983–15995. [PubMed: 22049441]

142. Pedersen K. Scanning electron microscopical observations on epidermal wound healing in the planarian *Dugesia tigrina*. Wilhelm Roux's Archives. 1976; 179:251–273.

143. Bryant S, Iten L. Supernumerary limbs in amphibians: Experimental production in *Notophthalmus viridescens* and a new interpretation of their formation. Developmental Biology. 1976; 50:212–234. [PubMed: 1269829]

144. L'heureux E. Importance des associations de tissus du membre dans le développement des membres surnuméraires induits par déviation de nerf chez le Triton *Pleurodeles waltlii* Michah. Journal of Embryology and Experimental Morphology. 1977; 38:151–173. [PubMed: 886241]

145. French V. Leg regeneration in the cockroach, *Blatella germanica*. II. Regeneration from a non-congruent tibial graft/host junction. Journal of Embryology and Experimental Morphology. 1976; 35:267–301. [PubMed: 939940]

146. Boilly-Marer Y. Néoformation de parapodes surnuméraires par greffe hétérologue de paroi de corps chez *Nereis pelagica* L. (Annélide Polychète). C.R. Acad. Sc. Paris. 1971; 272:79–82.

147. Kato K, Orii H, Watanabe K, Agata K. The role of dorsoventral interaction in the onset of planarian regeneration. Development. 1999; 126:1031–1040. [PubMed: 9927603]

148. Agata K, Tanaka T, Kobayashi C, Kato K, Saitoh Y. Intercalary regeneration in planarians. Developmental Dynamics. 2003; 226:308–316. [PubMed: 12557208]

149. Molina MD, Saló E, Cebrià F. The BMP pathway is essential for re-specification and maintenance of the dorsoventral axis in regenerating and intact planarians. Developmental Biology. 2007; 311:79–94. [PubMed: 17905225]

150. Orii H, Watanabe K. Bone morphogenetic protein is required for dorso-ventral patterning in the planarian *Dugesia japonica*. Development, Growth & Differentiation. 2007; 49:345–349.

151. Reddien PW, Bermange AL, Kicza AM, Sánchez Alvarado A. BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. Development. 2007; 134:4043–4051. [PubMed: 17942485]

152. Gaviño MA, Reddien PW. A Bmp/Admp regulatory circuit controls maintenance and regeneration of dorsal-ventral polarity in planarians. Current Biology. 2011; 21:294–299. [PubMed: 21295483]

153. Molina MD, Neto A, Maeso I, Gómez-Skarmeta JL, Saló E, Cebrià F. Noggin and noggin-like genes control dorsoventral axis regeneration in planarians. Current Biology. 2011; 21:300–305. [PubMed: 21295481]

154. Kaprielian Z, Runko E, Imondi R. Axon guidance at the midline choice point. Developmental Dynamics. 2001; 221:154–181. [PubMed: 11376484]

155. Ypsilanti AR, Zagar Y, Chedotal A. Moving away from the midline: new developments for Slit and Robo. Development. 2010; 137:1939–1952. [PubMed: 20501589]

156. Cebrià F, Guo T, Jopek J, Newmark PA. Regeneration and maintenance of the planarian midline is regulated by a slit orthologue. Developmental Biology. 2007; 307:394–406. [PubMed: 17553481]

157. Yamamoto H, Agata K. Optic chiasm formation in planarian I: Cooperative netrin- and robo-mediated signals are required for the early stage of optic chiasm formation. Development, Growth & Differentiation. 2011; 53:300–311.

158. Cebrià F, Newmark PA. Morphogenesis defects are associated with abnormal nervous system regeneration following roboA RNAi in planarians. Development. 2007; 134:833–837. [PubMed: 17251262]

159. Cebrià F, Newmark PA. Planarian homologs of netrin and netrin receptor are required for proper regeneration of the central nervous system and the maintenance of nervous system architecture. Development. 2005; 132:3691–3703. [PubMed: 16033796]

160. Varga M, Maegawa S, Weinberg ES. Correct anteroposterior patterning of the zebrafish neurectoderm in the absence of the early dorsal organizer. BMC Developmental Biology. 2011; 11:1–16. [PubMed: 21194500]

161. Cebrià F, Kobayashi C, Umesono Y, Nakazawa M, Mineta K, Ikeo K, Gojobori T, Itoh M, Taira M, Sánchez Alvarado A, et al. FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians. Nature. 2002; 419:620–624. [PubMed: 12374980]

162. Lapan SW, Reddien PW. *dlx* and *sp6–9* control optic cup regeneration in a prototypic eye. PLoS Genetics. 2011; 7:1–13.

163. Iijima I. Untersuchungen über den Bau und die Entwicklungsgeschichte der Süswasser-Dendrocoelen (Tricladen). Zeitschrift f. wissensch. Zoologie. 1884; 40:359–464.

164. Lehnert GH. Beobachtung an landplanarien. Arch. Naturgech. 1891; 1:306–350.

165. Prenant, M. Recherches sur le parenchyme des plathelminthes, Essai D'histologie comparee. Paris: Librairie Octave Doln; 1922.

166. Bartsch O. Die Histiogenese der Planarienregenerate. ARCHIV FUR MIKROSKOPISCHE ANATOMIE UND ENTWICKLUNGSGESCHICHTE. 1923; 99:187–221.

167. Steinmann P. Das Verhalten der Zellen und Gewebe im regenerierenden Tricladenkörper. Verh. Naturforsch. Ges. Basel. 1925; 36:133–162.

168. Randolph H. Regeneration of the tail in *Lumbriculus*. Zoologischer Anzeiger. 1891; 14:154–156.

169. Buchanan J. Regeneration in *Phagocata gracilis* (Leidy). Physiological Zoology. 1933; 6:185–204.

170. Wolff E, Dubois F. Sur la migration des cellules de régénération chez les Planaires. Revue Suisse de Zoologie. 1948; 55:218–227.

171. Randolph H. The regeneration of the tail in *Lumbriculus*. Journal of Morphology. 1892; 7:317–344.

172. Schultz E. Aus dem gebiete der regeneration, II., Über die regeneration bei Turbellarien. Zeitsch. Wiss. Zool. 1902; 72:1–30.

173. Steinmann P. Untersuchungen über das Verhalten des Verdauungssystems bei der Regeneration der Tricladen. Arch. Ent. Mech. Org. 1908; 25:523–568.

174. Lang P. Über Regeneration bei Planarien. Archiv für mikroskopische Anatomie. 1912; 79:361–426.

175. Child CM, Watanabe Y. The head frequency gradient in *Euplanaria dorotocephala*. Physiological Zoology. 1935; 8:1–40.

176. Stevens NM. Notes on regeneration in *Planaria lugubris*. Archiv. Für. Entwick. 1901; 13:396–409.

177. Bandier J. Histologische untersuchungen über die regeneration von landplanarien. Wilhelm Roux's Archives of Developmental Biology. 1936; 135:316–348.

178. Chandebois R. The respective roles of mitotic activity and of cell differentiation in planarian regeneration. Journal of Embryology and Experimental Morphology. 1968; 20:175–188. [PubMed: 5699958]

179. Hay ED, Coward SJ. Fine structure studies on the planarian, *Dugesia*. I. Nature of the "neoblast" and other cell types in noninjured worms. Journal of Ultrastructure Research. 1975; 50:1–21. [PubMed: 1113364]

180. Woodruff LS, Burnett AL. The origin of the blastemal cells in *Dugesia tigrina*. Experimental Cell Research. 1965; 38:295–305. [PubMed: 14284510]

181. Rose C, Shostak S. The transformation of gastrodermal cells to neoblasts in regenerating *Phagocata gracilis* (Leidy). Experimental Cell Research. 1968; 50:553–561. [PubMed: 5663067]

182. Gremigni V, Miceli C. Cytophotometric evidence for cell 'transdifferentiation' in planarian regeneration. Wilhelm Roux's Archives. 1980; 188:107–113.

183. Coward S. Regeneration in planarians: Some unresolved problems and questions. Journal of Biological Psychology. 1969; 11:15–19.

184. Bardeen CR, Baetjer FH. The inhibitive action of the Roentgen rays on regeneration in planarians. Journal of Experimental Zoölogy. 1904; 1:191–195.

185. Dubois F, Wolff E. Sur une méthode d'irradiation localisée permettant de mettre en évidence la migration des cellules de régénération chez les planaires. Société de Biologie Strasbourg. 1947; 141:903–906.

186. Pedersen KJ. Morphogenetic activities during planarian regeneration as influenced by triethylene melamine. Journal of Embryology and Experimental Morphology. 1958; 6:308–334. [PubMed: 13575644]

187. Stéphan-Dubois F. Les cellules de régénération chez la planaire *Dendrocoleum lacteum*. Bulletin de la Société Zooologique de France. 1961; 86:172–185.

188. Wolff E. Migrations et contacts cellulaires dans la régénération. Experimental Cell Research. 1961; 8:246–259. [PubMed: 13786329]

189. Fedecka-Bruner B, Kiortsis V, Trampusch HAL. Régénération des testicules des planaires après destruction par les rayons X. Regeneration in Animals and Related Problems. 1965:185–192.

190. Lender T, Gabriel A. Neoblasts labelled with tritiated uridine migrate and construct the regeneration blastema in fresh-water planaria. Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences. 1965; 260:4095–4097.

191. Bowen ID, den Hollander JE, Lewis GH. Cell death and acid phosphatase activity in the regenerating planarian *Polycelis tenuis* (Iijima). Differentiation. 1982; 21:160–167. [PubMed: 7106450]

192. Morita M, Best J. Electron microscopic studies of planarian regeneration. III. Degeneration and differentiation of muscles. The Journal of Experimental Zoology. 1984; 229:413–424.

193. Baguñà J, Saló E, Auladell C. Regeneration and pattern formation in planarians. III. Evidence that neoblasts are totipotent stem cells and the source of blastema cells. Development. 1989; 107:77–86.

194. Teshirogi W. Fusion of different body segments, using X-ray-irradiated animals and non-irradiated ones in a freshwater planarian *Bdellocephala brunnea*. Sci. Rep. Hirosaki Univ. 1963; 10:22–33.

195. Saló E, Baguñà J. Proximal and distal transformation during intercalary regeneration in the planarian *Dugesia (S) mediterranea*. Roux's Arch. Dev. Biol. 1985; 194:364–368.

196. Saló E, Baguñà J. Cell movement in intact and regenerating planarians. Quantitation using chromosomal, nuclear and cytoplasmic markers. Journal of Embryology and Experimental Morphology. 1985; 89:57–70. [PubMed: 3867725]

197. Shibata N, Umesono Y, Orii H, Sakurai T, Watanabe K, Agata K. Expression of *vasa(vas)*-related genes in germline cells and totipotent somatic stem cells of planarians. Developmental Biology. 1999; 206:73–87. [PubMed: 9918696]

198. Salvetti A, Rossi L, Deri P, Batistoni R. An MCM2-related gene is expressed in proliferating cells of intact and regenerating planarians. Developmental Dynamics. 2000; 218:603–614. [PubMed: 10906779]

199. Ogawa K, Kobayashi C, Hayashi T, Orii H, Watanabe K, Agata K. Planarian fibroblast growth factor receptor homologs expressed in stem cells and cephalic ganglions. Development, Growth & Differentiation. 2002; 44:191–204.

200. Orii H, Sakurai T, Watanabe K. Distribution of the stem cells (neoblasts) in the planarian *Dugesia japonica*. Development, Genes & Evolution. 2005; 215:143–157. [PubMed: 15657737]

201. Salvetti A, Rossi L, Lena A, Batistoni R, Deri P, Rainaldi G, Locci MT, Evangelista M, Gremigni V. *DjPum*, a homologue of *Drosophila Pumilio*, is essential to planarian stem cell maintenance. Development. 2005; 132:1863–1874. [PubMed: 15772127]

202. Rossi L, Salvetti A, Lena A, Batistoni R, Deri P, Pugliesi C, Loreti E, Gremigni V. *DjPiwi-1*, a member of the PAZ-Piwi gene family, defines a subpopulation of planarian stem cells. Development, Genes & Evolution. 2006; 216:335–346. [PubMed: 16532341]

203. Guo T, Peters AH, Newmark PA. A *bruno-like* gene is required for stem cell maintenance in planarians. Developmental Cell. 2006; 11:159–169. [PubMed: 16890156]
204. Best JB, Rosenvold R, Souders J, Wade C. Studies on the incorporation of isotopically labeled nucleotides and amino acids in planaria. The Journal of Experimental Zoology. 1965; 159:397–403. [PubMed: 5883956]
205. Coward S. Thymidine kinase activity during regeneration in the planarian *Dugesia dorotocephala*. The Journal of Experimental Zoology. 1970; 173:269–278.
206. Curtis WC, Schulze LM. Studies upon regeneration. I. The contrasting powers of regeneration in planaria and procotyla. Journal of Morphology. 1934; 55:477–513.
207. Yoshida-Kashikawa M, Shibata N, Takechi K, Agata K. *DjCBC-1*, a conserved DEAD box RNA helicase of the RCK/p54/Me31B family, is a component of RNA-protein complexes in planarian stem cells and neurons. Developmental Dynamics. 2007; 236:3436–3450. [PubMed: 17994545]
208. Shibata N, Hayashi T, Fukumura R, Fujii J, Kudome-Takamatsu T, Nishimura O, Sano S, Son F, Suzuki N, Araki R, et al. Comprehensive gene expression analyses in pluripotent stem cells of a planarian, *Dugesia japonica*. The International Journal of Developmental Biology. 2012; 56:93–102. [PubMed: 22450997]
209. Higuchi S, Hayashi T, Hori I, Shibata N, Sakamoto H, Agata K. Characterization and categorization of fluorescence activated cell sorted planarian stem cells by ultrastructural analysis. Development, Growth & Differentiation. 2007; 49:571–581.
210. Dubois F. Contribution á l 'ètude de la migration des cellules de règènèration chez les *Planaires dulcicoles*. Bull. Biol. Fr. Belg. 1949; 83:213–283.
211. Baguñà J, Romero R. Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians *Dugesia mediterranea* and *Dugesia tigrina*. Hydrobiologia. 1981; 84:181–194.
212. Baguñà J. Dramatic mitotic response in planarians after feeding, and a hypothesis for the control mechanism. The Journal of Experimental Zoology. 1974; 190:117–122. [PubMed: 4436618]
213. Baguñà J. Mitosis in the intact and regenerating planarian *Dugesia mediterranea* n.sp. I. Mitotic studies during growth, feeding and starvation. Journal of Experimental Zoölogy. 1975; 195:53–64.
214. González-Estévez C, Felix DA, Rodríguez-Esteban G, Aboobaker AA. Decreased neoblast progeny and increased cell death during starvation-induced planarian degrowth. The International Journal of Developmental Biology. 2012 E-pub ahead of print.
215. Miller CM, Newmark PA. An insulin-like peptide regulates size and adult stem cells in planarians. The International Journal of Developmental Biology. 2012 Epub ahead of print.
216. Baguñà J. Mitosis in the intact and regenerating planarian *Dugesia mediterranea*n.sp. II. Mitotic studies during regeneration, and a possible mechanism of blastema formation. Journal of Experimental Zoölogy. 1975; 195:65–80.
217. Wenemoser D, Reddien PW. Planarian regeneration involves distinct stem cell responses to wounds and tissue absence. Developmental Biology. 2010; 344:979–991. [PubMed: 20599901]
218. Spiegelman M, Dudley P. Morphological stages of regeneration in the planarian *Dugesia tigrina*: A light and electron microscopic study. The Journal of Morphology. 1973; 139:155–184.
219. Stéphan-Dubois F, Lender T. Corrélations humorales dans la régénération de planaires paludicoles. Ann. des Sc. Nat., Zool. 1956; 18:223–230.
220. Azimzadeh J, Wong ML, Downhour DM, Sánchez Alvarado A, Marshall WF. Centrosome loss in the evolution of planarians. Science. 2012; 335:461–463. [PubMed: 22223737]
221. Palakodeti D, Smielewska M, Lu YC, Yeo GW, Graveley BR. The PIWI proteins SMEDWI-2 and SMEDWI-3 are required for stem cell function and piRNA expression in planarians. RNA. 2008; 14:1174–1186. [PubMed: 18456843]
222. Oviedo NJ, Pearson BJ, Levin M, Sánchez Alvarado A. Planarian PTEN homologs regulate stem cells and regeneration through TOR signaling. Disease Models & Mechanisms. 2008; 1:131–143. [PubMed: 19048075]
223. Pearson BJ, Sánchez Alvarado A. A planarian p53 homolog regulates proliferation and self-renewal in adult stem cell lineages. Development. 2010; 137:213–221. [PubMed: 20040488]

224. Scimone ML, Meisel J, Reddien PW. The Mi-2-like *Smed-CHD4* gene is required for stem cell differentiation in the planarian *Schmidtea mediterranea*. Development. 2010; 137:1231–1241. [PubMed: 20223763]
225. Fraguas S, Barberán S, Cebrià F. EGFR signaling regulates cell proliferation, differentiation and morphogenesis during planarian regeneration and homeostasis. Developmental Biology. 2011; 354:87–101. [PubMed: 21458439]
226. Tasaki J, Shibata N, Nishimura O, Itomi K, Tabata Y, Son F, Suzuki N, Araki R, Abe M, Agata K, et al. ERK signaling controls blastema cell differentiation during planarian regeneration. Development. 2011; 138:2417–2427. [PubMed: 21610023]
227. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008; 132:598–611. [PubMed: 18295578]
228. Brøndsted HV. Regeneration in planarians investigated with a new transplantation technique. Biol. Medd. Dan. Vid. Selsk. 1939; 15:1–39.
229. Betchaku T. Isolation of planarian neoblasts and their behavior *in vitro* with some aspects of the mechanism of the formation of regeneration blastema. The Journal of Experimental Zoology. 1967; 164:407–433. [PubMed: 6035672]
230. Ogawa K, Ishihara S, Saito Y, Mineta K, Nakazawa M, Ikeo K, Gojobori T, Watanabe K, Agata K. Induction of a *noggin*-like gene by ectopic DV interaction during planarian regeneration. Developmental Biology. 2002; 250:59–70. [PubMed: 12297096]
231. Pellettieri J, Fitzgerald P, Watanabe S, Mancuso J, Green DR, Sánchez Alvarado A. Cell death and tissue remodeling in planarian regeneration. Developmental Biology. 2010; 338:76–85. [PubMed: 19766622]
232. Stéphan-Dubois F, Kolmayer S. La migration et la différenciation des cellules de régénération chez la planaire *Dendrocoelum lacteum*. C R Seances Soc Biol Fil. 1959; 153:1856–1858. [PubMed: 13834359]
233. Stéphan-Dubois F, Gilgenkrantz F. Régénération après transplantation chez la Planaire Dendrocoelum lacteum. Soc. Biol. Strasbourg. 1961; 160:115–118.
234. Stéphan-Dubois F, Gilgenkrantz F. Transplantation et régénération ches la planaire *Dendrocoelum lacteum*. Journal of Embryology and Experimental Morphology. 1961; 9:642–649. [PubMed: 13916900]
235. Ijima, I. Copyrights Holder: Marine Biological Laboratory Archives; Leuckart Chart, Series I, Chart 28: Vermes; Classe: Platodes; Ordung: Turbellaria; Planaria, Dendrocoelum, etc. Circa 1890.
236. Stéphan-Dubois. Les néoblasts dans la régénération chez les planaires. Amsterdam: North-Holland Publishing Company; 1965.
237. Solana J, Lasko P, Romero R. *Spoltud-1* is a chromatoid body component required for planarian long-term stem cell self-renewal. Developmental Biology. 2009; 328:410–421. [PubMed: 19389344]
238. Conte M, Deri P, Isolani ME, Mannini L, Batistoni R. A *mortalin-like* gene is crucial for planarian stem cell viability. Developmental Biology. 2009; 334:109–118. [PubMed: 19616535]
239. Bonuccelli L, Rossi L, Lena A, Scarcelli V, Rainaldi G, Evangelista M, Iacopetti P, Gremigni V, Salvetti A. An RbAp48-like gene regulates adult stem cells in planarians. Journal of Cell Science. 2010; 123:690–698. [PubMed: 20124416]
240. Fernández-Taboada E, Moritz S, Zeuschner D, Stehling M, Scholer HR, Saló E, Gentile L. Smed-SmB, a member of the LSm protein superfamily, is essential for chromatoid body organization and planarian stem cell proliferation. Development. 2010; 137:1055–1065. [PubMed: 20215344]
241. Rouhana L, Shibata N, Nishimura O, Agata K. Different requirements for conserved post-transcriptional regulators in planarian regeneration and stem cell maintenance. Developmental Biology. 2010; 341:429–443. [PubMed: 20230812]
242. Tasaki J, Shibata N, Sakurai T, Agata K, Umesono Y. Role of c-Jun N-terminal kinase activation in blastema formation during planarian regeneration. Development, Growth & Differentiation. 2011; 53:389–400.

243. Li YQ, Zeng A, Han XS, Wang C, Li G, Zhang ZC, Wang JY, Qin YW, Jing Q. Argonaute-2 regulates the proliferation of adult stem cells in planarian. Cell Research. 2011; 21:1750–1754. [PubMed: 21894189]
244. Cowles MW, Hubert A, Zayas RM. A Lissencephaly-1 homologue is essential for mitotic progression in the planarian *Schmidtea mediterranea*. Developmental Dynamics. 2012; 241:901–910. [PubMed: 22411224]
245. Isolani ME, Conte M, Deri P, Batistoni R. Stem cell protection mechanisms in planarians: The role of some heat shock genes. The International Journal of Developmental Biology. 2012; 56:127–133. [PubMed: 22451000]
246. Rouhana L, Vieira AP, Roberts-Galbraith RH, Newmark PA. PRMT5 and the role of symmetrical dimethylarginine in chromatoid bodies of planarian stem cells. Development. 2012; 139:1083–1094. [PubMed: 22318224]
247. Sakurai T, Lee H, Kashima M, Saito Y, Hayashi T, Kudome-Takamatsu T, Nishimura O, Agata K, Shibata N. The planarian P2X homolog in the regulation of asexual reproduction. The International Journal of Developmental Biology. 2012; 56:173–182. [PubMed: 22451005]
248. Wagner DE, Ho JJ, Reddien PW. Genetic regulators of a pluripotent adult stem cell system in planarians identified by RNAi and clonal analysis. Cell Stem Cell. 2012; 10:299–311. [PubMed: 22385657]
249. Conte M, Isolani ME, Deri P, Mannini L, Batistoni R. Expression of hsp90 mediates cytoprotective effects in the gastrodermis of planarians. Cell Stress & Chaperones. 2011; 16:33–39. [PubMed: 20706815]
250. Conte M, Deri P, Isolani M, Mannini L, Batistoni R. Characterization of hsp genes in planarian stem cells. Belg. J. Zool. 2010; 140:137–143.

1A

1B

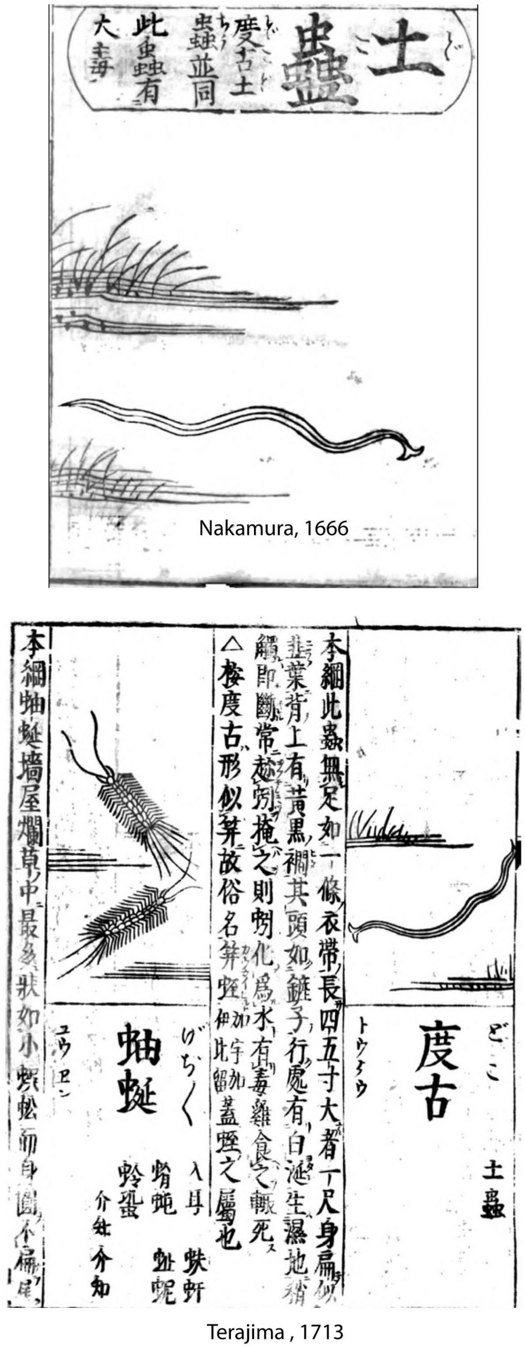

FIGURE 1. Ancient Japanese texts describe planarians and their ability to fission

1A: Wood-print adapted from the Japanese encyclopedia *Kinmô-Zui* by Tekisai Nakamura, 1666.[10] Image depicts a striped land planarian (likely *Bipalium*). The caption indicates that "it is very poisonous and similar to another soil insect (nematode) previously described." *(Translation assistance provided by Dr. Tamaki Suganuma, Nobuo Ueda, and Shigeki Watanabe.)*

1B: Wood-print adapted from the illustrated Japanese encyclopedia *Wakan Sansai-Zue* by Ryōan Terajima, 1713.[11] Image depicts a striped land planarian (likely *Bipalium*) in the left column. The vertical text is translated to read:

"'Doko' or 'Toku'. The animal has the shape of a Japanese belt in general appearance and is without legs. It measures up to 12 to 15 cm in length; a large specimen attains about 30 cm. The body is flattish in shape as a leaf of leek. There are yellow and black folds on the dorsal surface. The animal has a head shaped like a Japanese forceps…**If the animal is touched, fission may occur…**"[13]

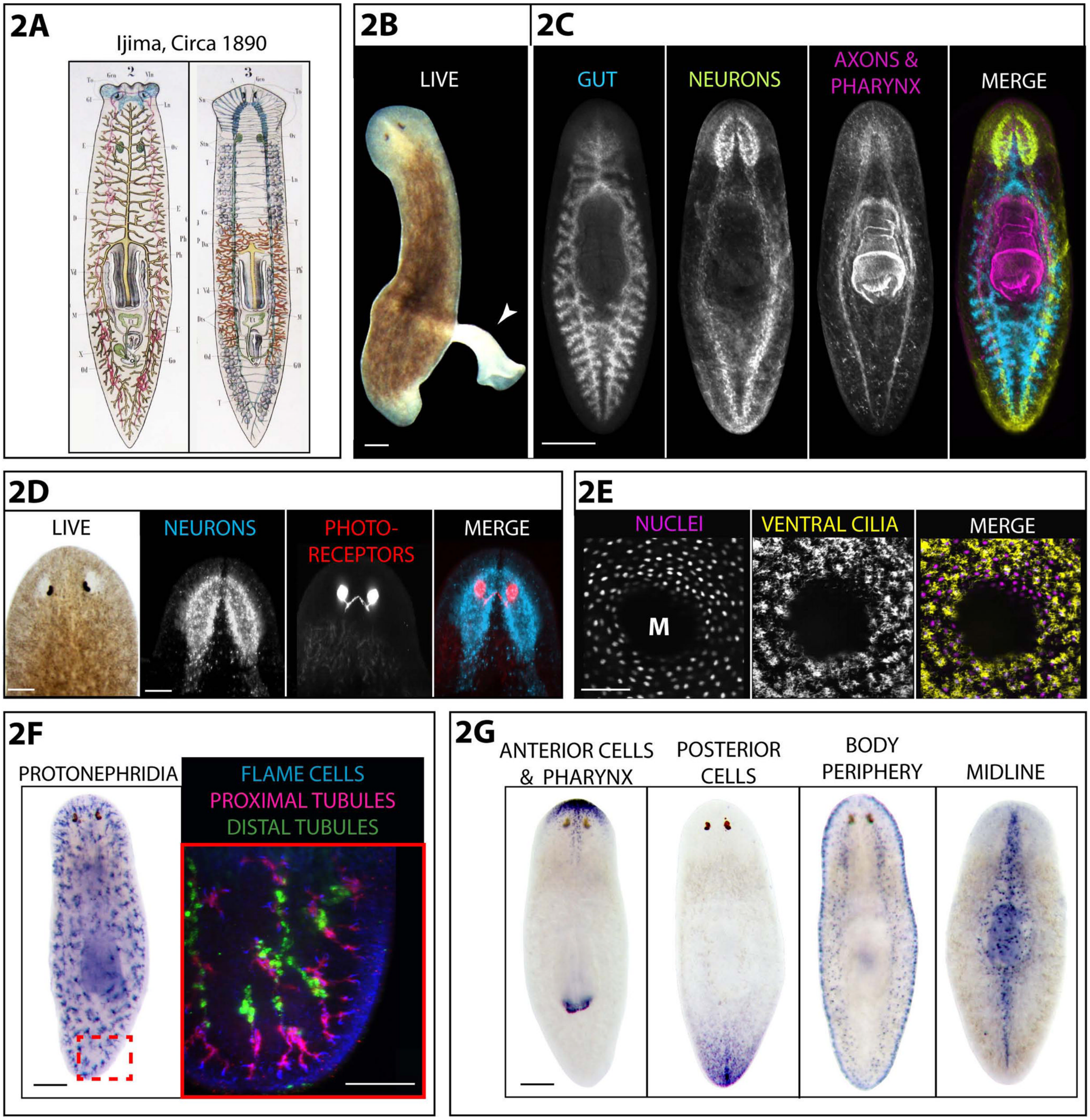

FIGURE 2. Planarian anatomy is sufficiently complex for regeneration studies

2A: Two depictions of planarian anatomy adapted from Leuckart's zoological wall chart series entitled "Vermes," circa 1890.[235] *(Image obtained from MBLWHOI Library, Rare Books Archive)*

2B: A live planarian extruding its pharynx (*arrowhead*). Scale bar 200 um.

(All animals depicted in Figures 2–7 are the asexual strain of Schmidtea mediterranea unless otherwise noted.)

2C: Overlay of gut (blue, *Smed-porcn-1*), neurons (yellow, *Smed-PC-2*), axons, and pharynx (magenta, anti-α-tubulin antibody). Scale bar 200 um.

2D: Left panel: Head of a live planarian. Photoreceptors are darkly pigmented. Right panels: A different specimen showing neurons of the cephalic ganglia (blue, *Smed-PC-2*), photoreceptors, and commissural visual axons (red, anti-arrestin antibody; a kind gift of Dr. Kiyokazu Agata). Scale bars 200 um.

2E: Tufts of ventral cilia (yellow, anti-acetylated-tubulin antibody) projecting from epithelial cells (nuclei: magenta, TOPRO-3) facilitate swimming. Image focused around opening to the pharynx cavity (M, mouth). Scale bar 50 um.

2F: Left panel: Protonephridia, which compose the excretory system (*Smed-innexin-10)*. Scale bar 200 um. Right panel: Close up of tail tip of a different specimen. Confocal maximum projection of protonephridial system, including flame cells (blue, anti-α-tubulin antibody), proximal tubules (magenta, *Smed-innexin-10*), and distal tubules (green, *Smed-CAVII-1)*. Scale bar 50 um. *(Images provided by Hanh Thi-Kim Vu.)*

2G: Markers labeling distinct body regions. Left to right: anterior cells and distal tip of pharynx (*Smed-sfrp-1)*, posterior cells (*Smed-wnt11-2)*, body periphery (*Smed-wnt5),* and midline (*Smed-slit-1)*.

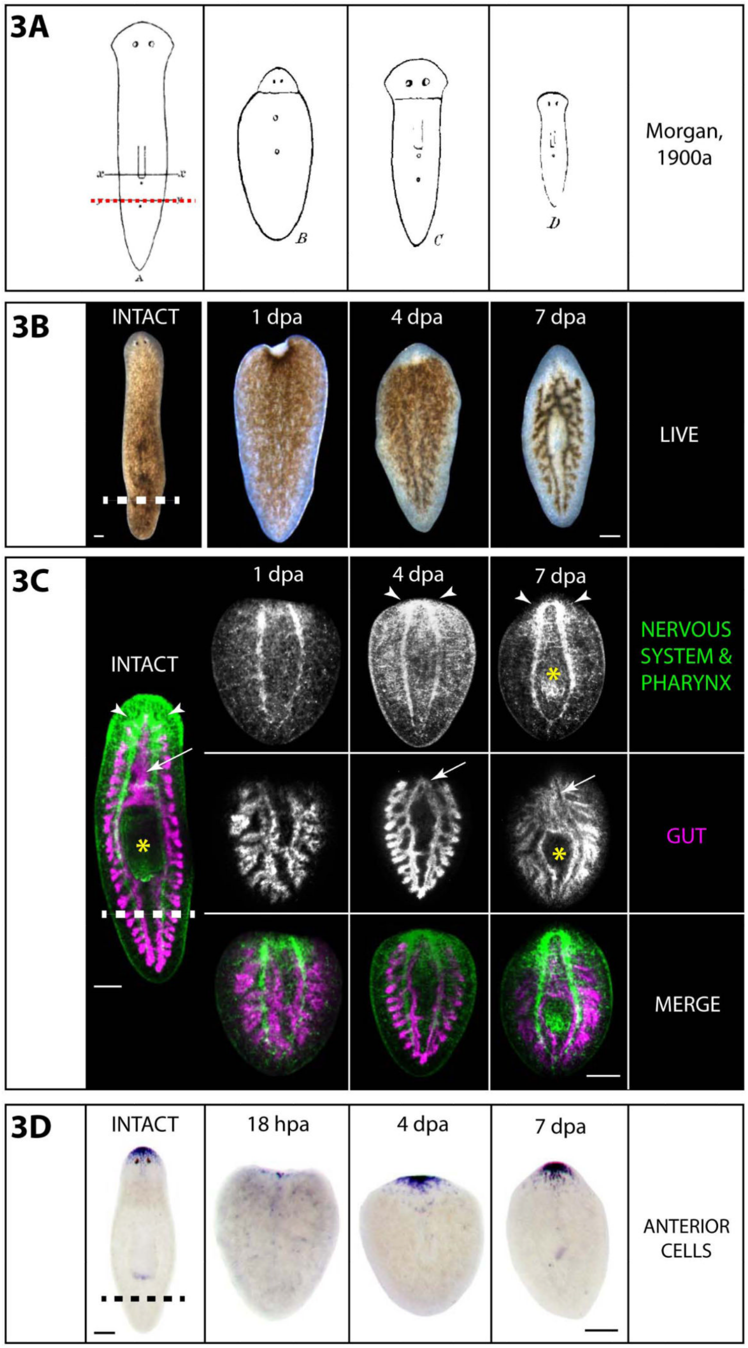

FIGURE 3. Upon injury, planarians regenerate lost tissues, re-establish scale and proportion, and maintain axial polarity

3A: T.H. Morgan amputated an adult planarian (red dashed line) and observed it regenerate missing anatomy ("epimorphosis") and re-establish proper body proportions ("morphallaxis"). (A*dapted from Morgan, 1900*[33])

3B: A live intact planarian was amputated (white dashed line), and the regenerating tail fragment is shown at 1, 4, and 7 dpa. Scale bar 200 um. *dpa: days post amputation*.

3C: The cephalic ganglia (arrowheads), pharynx (yellow asterisk), and anterior gut branch (arrow) regenerate by 7 dpa. An intact planarian (left) was amputated (white dashed line) and regenerating tail fragments were stained at timepoints indicated (right) for nervous system, pharynx (green, anti-α-tubulin antibody) and gut (*Smed-porcn-1)*. Scale bars 200 um. (*Adapted from Gurley, et al., 2010*[131])

3D: The A/P decision is made by 1 dpa, preceding tissue regeneration and anatomical remodeling. Regenerating tail fragments stained at timepoints indicated for marker of anterior cell identity (*Smed-sfrp-1)*. Scale bars 200 um. *hpa= hours post amputation* (*Adapted from Gurley, et al., 2010*[131])

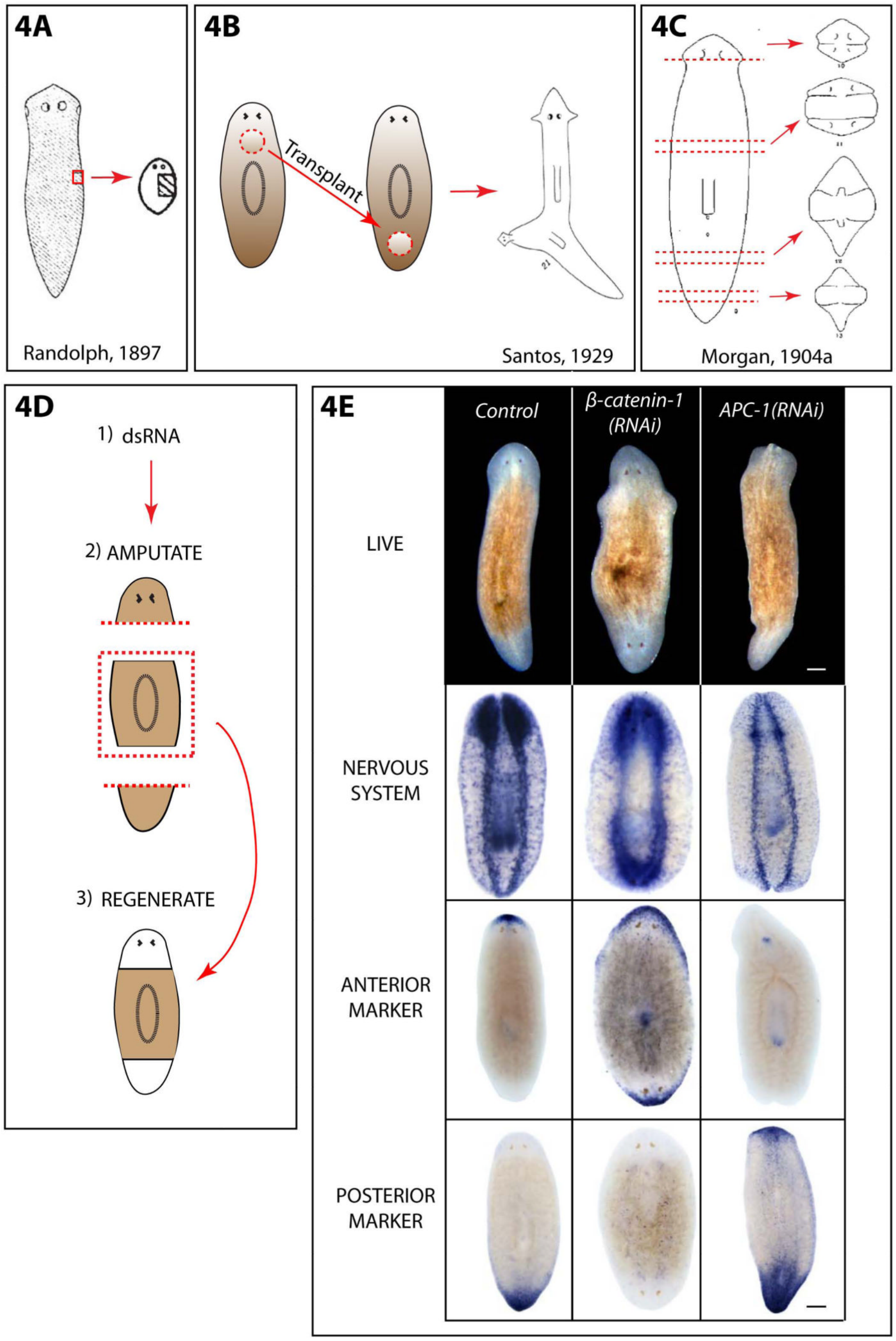

FIGURE 4. Anterior-posterior polarity

4A: Randolph showed that a small piece of tissue amputated from the flank of the body (left, red box) maintains axial polarity during regeneration (right). (*Adapted from Randolph, 1897*[28])

4B: Transplanting tissue from the anterior region of one planarian to a posterior region of another (left) results in outgrowth of a new body axis (right). (*Adapted from Santos, 1929*[102])

4C: Thin transverse amputations (left, red dashed lines) cause heteromorphic regeneration, resulting in double-headed (top) or double-tailed (bottom) regenerates. *(Adapted from Morgan, 1904*[120])

4D: RNAi strategy employed for Figures 4–6. Animals were 1) fed dsRNA to knockdown a gene of interest, 2) amputated, and 3) allowed to regenerate.

4E: Wnt/β-catenin signaling controls A/P polarity. Live images and fixed animals stained for the nervous system (*Smed-PC-2)*, anterior cell identity (*Smed-sfrp-1),* and posterior cell identities (*Smed-fz-4)*. Controls regenerate normally. *Smed-*β*-catenin-1(RNAi)* causes a head to regenerate from posterior blastemas. *Smed-APC-1(RNAi)* causes a tail to regenerate from anterior blastemas. Scale bars 200 um. (*Live images provided by Dr. Kyle A. Gurley and Dr. Jochen C. Rink.)*

NIH-PA Author Manuscript
NIH-PA Author Manuscript

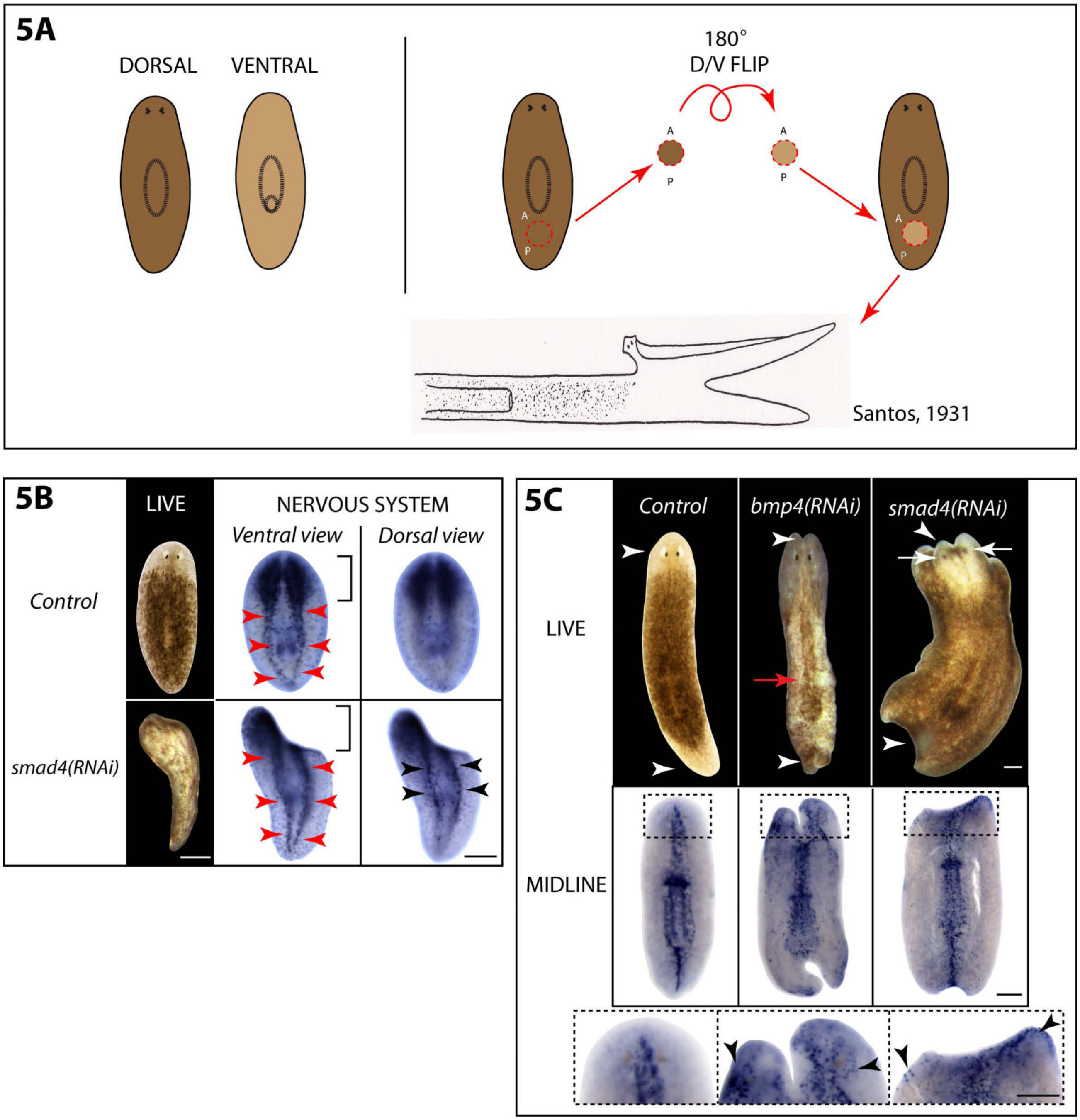

FIGURE 5. Dorsal-ventral polarity

5A: Santos showed that flipping the D/V orientation of a tissue plug without altering the A/P orientation results in the outgrowth of an ectopic body axis. This ectopic growth has inverted D/V polarity compared to the main body's D/V axis. Only the tail region is pictured in Santos' sketch; the main body's head is to the left, out of view.*(Adapted from Santos, 1931*[103])

5B: BMP signaling controls D/V polarity. Control tail fragments form a blastema (bracket) and regenerate nerve cords localized ventrally only (*Smed-PC-2,* red arrowheads; compare ventral vs. dorsal views). *Smed-smad4(RNAi)* causes a loss of blastema formation, regeneration of the cephalic ganglia (compare brackets), and growth of ectopic dorsal nerve cords (black arrowheads; compare ventral vs. dorsal views). Scale bars 200 um.

5C: BMP signaling is required for blastema formation and organization of the midline. Control animals form anterior and posterior blastemas (white arrowheads) and regenerate a midline (*Smed-slit-1*). *Smed-bmp4(RNAi)* causes midline indentations in the blastemas, dorsal ruffling (red arrow), and ectopic expression of a midline marker (black arrowheads). *Smed-smad4(RNAi)* causes a loss of blastema formation (white arrowheads), photoreceptor regeneration in old tissue (white arrows), and ectopic expression of a midline marker (black arrowheads, compare insets). Scale bars 200 um.

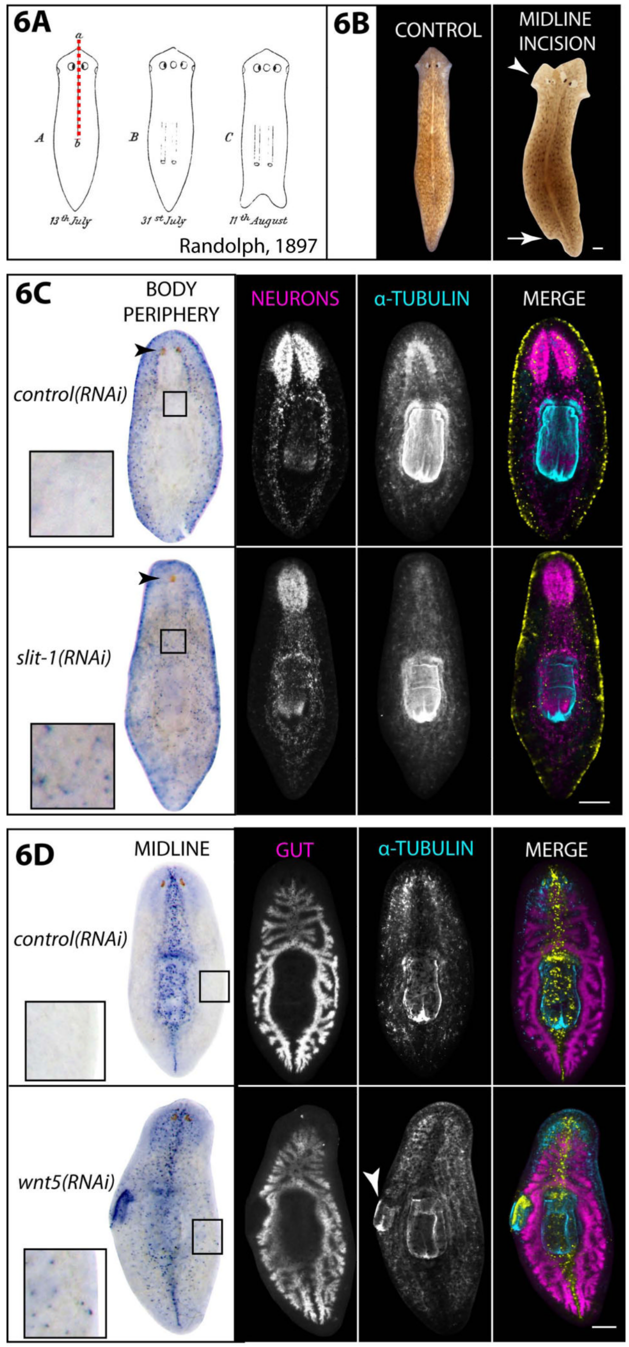

FIGURE 6. Medial-lateral polarity

6A: Randolph showed that a midline incision (red dashed line) that is allowed to heal together causes, with some frequency, a duplication of midline structures. *(Adapted from Randolph, 1897*[28])

6B: Live images 14 days after the midline incision depicted in 6A. Although the tissue was allowed to heal back together, the head is duplicated at the site of the incision (white arrowhead). The tail is forked (white arrow), even though the posterior was never injured. Species *Dugesia sanchezi*. Scale bar 200 um.

6C: *Smed-slit-1* maintains the M/L axis. Compared to *control(RNAi), Smed-slit-1(RNAi)* causes the cephalic ganglia, nerve cords (magenta, *Smed-PC-2;* blue, anti-α-tubulin antibody), photoreceptors (black arrowhead), and markers for the body periphery (*Smed-wnt5;* compare insets) to collapse towards the midline. Scale bar 200 um. *(Adapted from Gurley, et al., 2010*[131])

6D: *Smed-wnt5* maintains the M/L axis. Compared to *control(RNAi), Smed-wnt5(RNAi)* causes the lateral expansion of the axon tracts, and the formation of an ectopic lateral pharynx (blue, anti-α-tubulin antibody; white arrowhead) flanked by gut branches (magenta, *Smed-porcn-1)*. Expression of a midline marker expands laterally (*Smed-slit-1;* compare insets*)*. Scale bar 200 um. *(Adapted from Gurley, et al., 2010*[131])

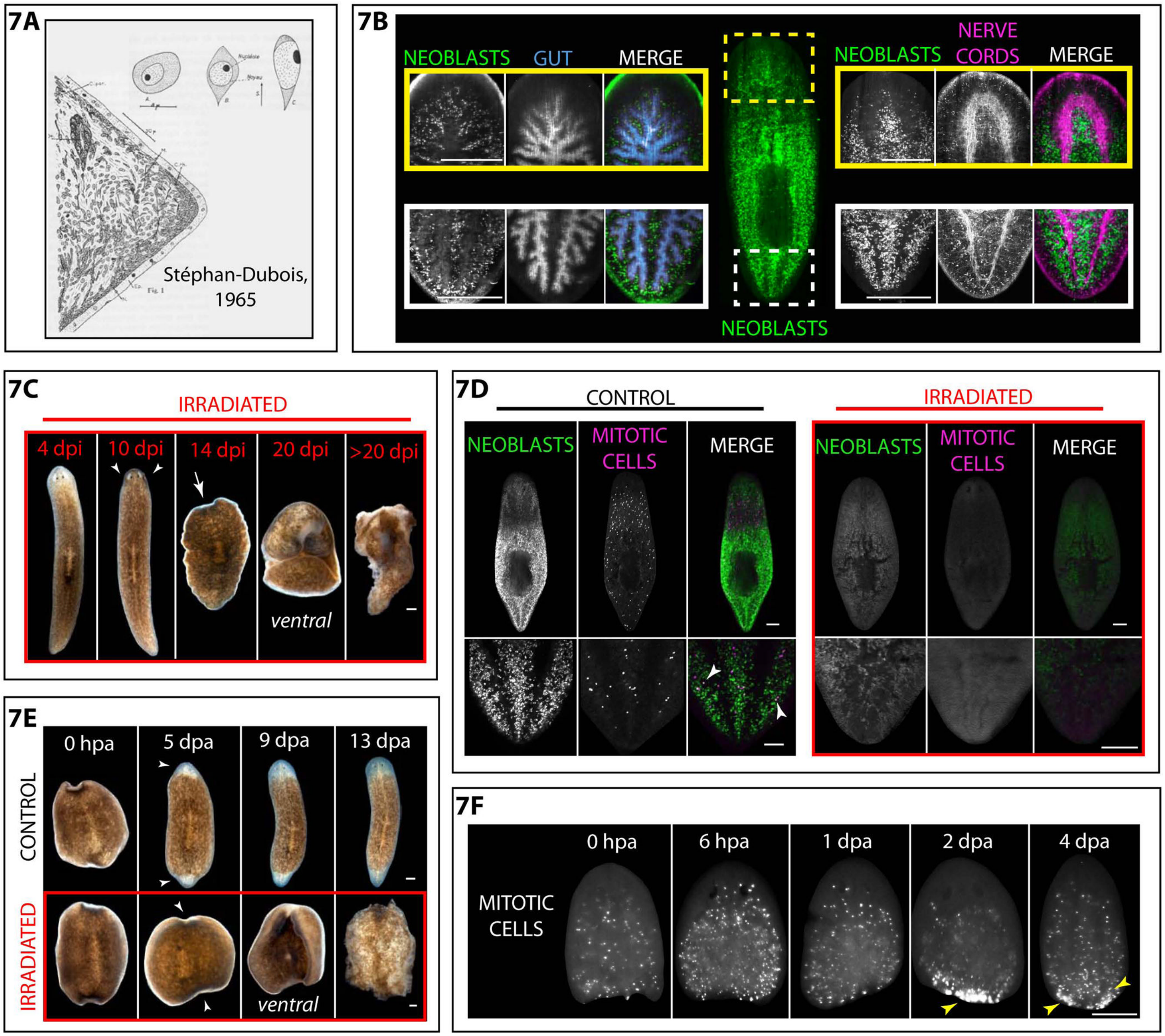

FIGURE 7. Neoblasts as agents for regeneration

7A: Classical depiction of neoblasts by histology. *(Adapted from Stéphan-Dubois, 1965*[236])

7B: Neoblasts (green, *Smed-piwi-1*) distributed throughout the parenchyma in between gut branches (blue, *Smed-porcn-1*) and in proximity to the nerve cords (magenta, anti-α-tubulin antibody). Images are confocal maximum projections. Scale bars 50 um.

7C: Irradiation disrupts physiological regeneration. Representative intact planarians at specified dpi, exposed to 10,000 rads from a cesium source. Head regression is observed by 10 dpi, followed by ventral curling around 20 dpi. Lysis generally occurs after 20 dpi. Scale bar 200 um. *dpi: days post irradiation*

7D: Irradiation eliminates neoblasts and proliferation. Left panels: Neoblasts (green, *Smed-piwi-1)* are the only mitotic cells (magenta, anti-H3P antibody). White arrowheads indicate examples of colocalization in the tail of a different animal. Right panels: Neoblasts and mitotic cells are eliminated after irradiation by 3dpi. Images are confocal maximum projections. Scale bars 200 um. *(Images provided by Dr. Kyle A. Gurley)*

7E: Irradiation disrupts restorative regeneration. Top panels: Representative control trunk fragments displaying unpigmented regeneration blastemas by 5 dpa (white arrowheads). Bottom panels: Representative irradiated trunk fragments do not form blastemas (white arrowheads) or regenerate new tissues. Fragments curl ventrally and eventually lyse around 13 dpi. Scale bars 200 um.

7F: Amputation induces two waves of cell proliferation. Mitotic cells (white, anti-H3P antibody) are visualized in regenerating head fragments. A global burst in proliferation is observed within 6 hpa. By 2 dpa, a second proliferative burst occurs at the wound site (yellow arrowheads). Scale bar 200 um.

TABLE 1

Summary of body axis patterning phenotypes described in text. Data is summarized for A/P, D/V, and M/L patterning defects. Dorsalization and ventralization refers to ectopic expression of axis markers or regeneration of ectopic anatomy. Midline defects refer to any ectopic or missing anatomy at the midline. These include misguidance of the visual axons that cross the midline; expansion or collapse of midline structures like the brain, nerve cords, photoreceptors, or pharynx; loss of neural connectivity at the midline; ectopic expression of midline or body periphery markers; reduction in lateral regeneration, or midline indentations in the blastema. *(See text for references and additional details for each phenotype.)*

		Anterior Regeneration Defects			Posterior Regeneration Defects			Dorsalization	Ventralization	Medical|Lateral Defects
PATHWAY	**RNAi Knockdown**	**Two tails**	**Headless**	**Cyclopia**	**Reduced Tail**	**Tailless**	**Two heads**			
Wnt	*Dj/Smed-6-catenin-1*						X			
	Smed/dvl-1/2						X			X
	Smed/evi/wntless						X			X
	Dj/Smed-wnt1						X			
	Smed-wnt5									X
	Smed-wnt11-2				X					X
	Smed-wnt11-5						X			
	Smed-notum	X								
	Dj/Smed-APC-1	X								
	Smed-axinA	X								
	Smed-axinB	X								
Hh	*Dj/Smed-ptc*	X	X	X						X
	Smed-smo				X	X				
	Dj/Smed-Hh				X	X				
	Dj/Smed-sufu	X	X	X						X
	Dj/Smed-gli-1				X	X				
Tgf-β	*Smed-smad1*								X	X
	Smed-smad4								X	X
	Smed-bmp-1 (smedolloid-1)								?	X
	DjBMP/Smed-bmp4								X	X
	Smed-admp								X	X
	Smwd-niggin-like-8								X	X
	Smed-noggin-1/2							X		

		Anterior Regeneration Defects			Posterior Regeneration Defects			Dorsalization	Ventralization	Medical|Lateral Defects
PATHWAY	**RNAi Knockdown**	**Two tails**	**Headless**	**Cyclopia**	**Reduced Tail**	**Tailless**	**Two heads**			
Slit/Netrin	*Dj/Smed-slit-1*			X						X
	Dj/Smed-roboA									X
	Smed-netrin-2									X
	Smed-netR									X
	Dj/Smed-roboA									X
	DjnetB									X (low penetrance)
	Djdcc									X (low penetrance)
	Djunc5A									X (low penetrance)
Miscellaneous	*Smed-innexin-5*						X			
	Smed-innexin-12						X			
	Smed-innexin-13						X			
	Smed-prep		X	X						X
	Djislet-1					X				

TABLE 2

Summary of neoblast dysfunction phenotypes. All phenotypes characterized that cause a loss or reduction in blastema formation (not associated with a known polarity defect) and/or ventral curling and lysis are listed in alphabetical order for each species. (201, 92, 94, 203, 56, 207, 221, 222, 88, 237, 238, 223, 239, 224, 240, 241, 226, 242, 225, 243, 215, 244, 39, 245, 40, 246, 247, 41, 248, 249, 82, 250, 85) Reported data regarding numbers of neoblasts, cell proliferation based on Phosphor-Histone H3 (Ser10) staining, expression of early and late neoblast progeny markers,[88] results from clonogenic expansion assays,[248] and miscellaneous phenotype data are included.

NEOBLAST DYSFUNCTION PHENOTYPES

SPECIES AND GENE	# OF STEM CELLS				# OF PROLIFERATIVE CELLS (H3P+)					DIFFERENTIATION (EARLY AND LATE PROGENY EXPRESSION)							CLONOGENIC EXPANSION ASSAY			NOTES	CITATION	SPECIES AND GENE
	Normal	Decreased	Increased	Not examined	Normal	Decreased	Increased	Ectopic	Not examined	Early & late progeny normal	Early and/or late progeny lost before neoblasts	Early and/or late progeny lost concurrently with or shortly after neoblasts	Early and/or late progeny ectopically distributed	Excess numbers of early and/or late progeny	Normal	Not examined	Failed colony expansion after sublethal irradiation	Failed colony differentiation after sublethal irradiation	Not examined			
Schmidtea mediterranea:																						*Schmidtea mediterranea:*
Smed-ago-2		X				X				X									X	Form small blastemas that subsequently regress; expression of several miRNAs is reduced	243	*Smed-ago-2*
Smed-bruli		X				X										X	X			Form small blastemas that subsequently regress	203, 248	*Smed-bruli*
Smed-CHD4			X			X					X							X		Faster loss of progeny markers and lethality after irradiation	224, 248	*Smed-CHD4*
Smed-cip29		X							X							X	X			Reduced blastema and impaired differentiation of photoreceptors	248	*Smed-cip29*
Smed-eed-1		X							X							X	X			Reduced blastema and impaired differentiation of photoreceptors	248	*Smed-eed-1*
Smed-egfr-1			X				X						X						X	Outgrowths develop that express various tissue markers; degrowth; overexpress p53	225	*Smed-egfr-1*
Smed-egfr-3	X				X			X		X									X	Loss of some markers of differentiated cell types	225	*Smed-egfr-3*
Smed-ezh		X							X							X	X			Reduced blastema and impaired differentiation of photoreceptors	248	*Smed-ezh*
Smed-fgfr-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-fgfr-1*
Smed-fhl-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-fhl-1*
Smed-H2B		X				X								X					X		85	*Smed-H2B*
Smed-Hsp40				X					X							X			X		82	*Smed-Hsp40*
Smed-Hsp60				X					X							X			X		82	*Smed-Hsp60*
Smed-Hsp70				X					X							X			X		82	*Smed-Hsp70*
Smed-ilp-1	X					X										X			X	Degrowth; reduced testes lobes and number of mature spermatids	215	*Smed-ilp-1*
Smed-innexin-11		X				X										X			X		56	*Smed-innexin-11*
Smed-inr-1	X					X										X			X	Degrowth; reduced testes lobes and number of mature spermatids	215	*Smed-inr-1*
Smed-junl-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-junl-1*
Smed-khd-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-khd-1*
Smed-lis-1		X					X									X				Mitotic spindles are misorganized, and many neoblasts may be stuck in metaphase/anaphase	244	*Smed-lis-1*
Smed-lst8				X					X							X			X		39	*Smed-lst8*
Smed-mrg-1									X							X	X			Regeneration and homeostasis appear normal	248	*Smed-mrg-1*
Smed-nudC	X								X							X					244	*Smed-nudC*
Smed-nudE		X					X									X				Mitotic spindles are misorganized, and many neoblasts may be stuck in metaphase/anaphase	244	*Smed-nudE*
Smed-p53			X				X				X							X		Outgrowths develop that express various tissue markers	223, 248	*Smed-p53*
Smed-piwi-2			X		X (regenerate)		X (intact)									X	X			Neoblast progeny in epithelium have abnormal morphology; decrease in putative piRNA expression	94, 221, 248	*Smed-piwi-2*
Smed-piwi-3		X							X							X	X			Decrease in putative piRNA expression	221, 248	*Smed-piwi-3*
Smed-PrkC				X					X							X			X		82	*Smed-PrkC*
Smed-PRMT5		X							X							X			X	Degrowth; fewer chromatoid bodies; increased transcription of transposons and repetitive DNA; disorganized blastema; may methylate SMEDWI-3	246	*Smed-PRMT5*
Smed-PTEN-1/PTEN-2			X				X									X			X	Tissue hyperplasia and disorganization; reduction in some differentiated cell types	222	*Smed-PTEN-1/PTEN-2*
Smed-Rab-11B				X					X							X			X		82	*Smed-Rab-11B*
Smed-Rac-1				X					X							X			X		82	*Smed-Rac-1*
Smed-raptor	X[39,41]	X[40]				X[39,40]					X[39]	X?[40]			X[41]				X	Regeneration of new anatomy in old tissue (39,40)	39, 40, 41	*Smed-raptor*
Smed-RbAP48	X					X										X			X		239	*Smed-RbAP48*
Smed-RRM2				X					X							X			X		88	*Smed-RRM2*
Smed-rtel-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-rtel-1*
Smed-setd8-1									X							X	X			Regeneration and homeostasis appear normal	248	*Smed-setd8-1*
Smed-SmB		X (regenerate)	X (intact)						X							X			X	Chromatoid bodies are lost; impaired splicing of cyclinB	82, 240	*Smed-SmB*
Smed-smg-1	X (intact)		X (regenerate)			X (fed)	X (regenerate & intact)	X					X	X					X	Regeneration of new anatomy in old tissue; outgrowths; epidermal hypertrophy; phenotype rescued by rapamycin	39	*Smed-smg-1*
Smed-soxP-1		X							X			X?					X				248	*Smed-soxP-1*
Smed-sz12-1		X							X							X	X			Reduced blastema and impaired differentiation of photoreceptors	248	*Smed-sz12-1*
Smed-TOR	X[39,41]	X[40]			X (intact[41]) (fed[40])	X (regenerates[39,40,41]) (intact[40])				X[39,41]		X?[40]							X	Regeneration of new anatomy in old tissue (39,40); increased TUNEL+ nuclei in intact (40,41) and regenerates (40); some anatomical remodeling occurs (41); decrease in cell size (40); faster degrowth during starvation (41); failure to grow when fed (40)	39, 40, 41	*Smed-TOR*
Smed-vasa-1		X							X			X?						X			248	*Smed-vasa-1*
Smed-vasa-2									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-vasa-2*
Smed-zf207-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-zf207-1*
Smed-zfp-1		X							X		X?							X			248	*Smed-zfp-1*
Smed-zmym-1									X							X	X			Regeneration and tissue homeostasis appear normal	248	*Smed-zmym-1*
~140 additional genes from first planarian RNAi screen				X	SOME	SOME	SOME									X	SOME			All show loss or reduction in blastemas, curling, and/or lysis; awaiting characterization	92, 248	~140 additional genes from first planarian RNAi screen
Dugesia japonica:																						*Dugesia japonica:*
Djago-2		X				X										X			X		241	*Djago-2*
Djbruli		X				X										X			X		241	*Djbruli*
Djcbc-1	X						X									X			X		207, 241	*Djcbc-1*
Djdicer-1				X					X							X			X		241	*Djdicer-1*
Djedc-4	X				X											X			X		241	*Djedc-4*
DjeIF-2A	X					X										X			X		241	*DjeIF-2A*
DjeIF-3A	X					X										X			X		241	*DjeIF-3A*
DjeIF-4a1				X					X							X			X		241	*DjeIF-4a1*
DjeIF-4a3				X					X							X			X		241	*DjeIF-4a3*
DjerkA / MEK inhibitor U0126	X				X			X								X			X	Reduction in differentiated cells labeled after 5 days of BrdU administration	226, 242	*DjerkA* / MEK inhibitor U0126
Djgeminin-5				X					X							X			X		241	*Djgeminin-5*
Djhsp60		X							X							X			X		245, 250	*Djhsp60*
Djhsp90				X					X							X			X	Degrowth	245, 249	*Djhsp90*
Djmot		X				X										X			X		238, 250	*Djmot*
DjP2XA				X		X (starved)	X (fed)									X			X	Increased frequency of fissioning, animals regenerate normally	247	*DjP2XA*
Djpabpc-1				X					X							X			X		241	*Djpabpc-1*
Djpumilio		X				X										X			X		201	*Djpumilio*
Djupf-1	X						X									X			X		241	*Dj-upf-1*
Djupf-2				X					X							X			X		241	*Dj-upf-2*
Djvas-1	X						X									X			X		241	*Djvas-1*
Djxrn-1	X						X									X			X		241	*Djxrn-1*
Djxrn-2	X				X											X			X		241	*Djxrn-2*
JNK inhibitors SP600125 & AS601245	X					X										X			X	Loss of expression of the early wound-response gene *DjnglA* and brain marker *Djndk*	242	JNK inhibitors SP600125 & AS601245
Schmidtea polychroa:																						*Schmidtea polychroa:*
Spoltud-1	X				X											X			X	Neoblasts function normally without SPOLTUD-1 for ~3 weeks, then neoblasts are lost	237	*Spoltud-1*