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Abstract

Planarians are flatworms that constitutively maintain adult tissues through cell turnover and can regenerate entire organisms from tiny body fragments. In addition to requiring new cells (from neoblasts), these feats require mechanisms that specify tissue identity in the adult. Critical roles for Wnt and BMP signaling in regeneration and maintenance of the body axes have been uncovered, among other regulatory factors. Available data indicate that genes involved in positional identity regulation at key embryonic stages in other animals display persisting regionalized expression in adult planarians. These expression patterns suggest that a constitutively active gene expression map exists for maintenance of the planarian body. Planarians therefore present a fertile ground for identification of factors regulating regionalization of the metazoan body plan and for study of the attributes of these factors that can lead to maintenance and regeneration of adult tissues.

Adult developmental biology

Tissue turnover and regeneration are widespread features of adult biology. Many questions can be asked about these adult processes that are conceptually similar to important questions for embryonic development. Where and what is the information for polarizing and regionalizing newly forming tissues? What guides differentiation paths? What is the source of cells for particular differentiated cell types? How are tissues formed in the right scale and proportions? For embryonic development we can hope to connect the dots from gametogenesis and fertilization to the formation of the body plan in a temporal series of events. For tissue maintenance, a different situation is encountered in which new cells are continuously integrated into an already developed body plan (or regionalized tissue) to replace aged differentiated cells. During this process tissue pattern must be maintained rather than developed *de novo*. How pattern is maintained long-term and propagated through the generations of cells involved in tissue turnover is poorly understood. In addition to tissue maintenance, missing tissues can be restored following injury in many animals [1]. In these cases, the body plan or any patterned tissue/organ, already exists at the time of injury. Programs must exist for re-development of the missing regions/parts following a limitless number of injury types and, unlike the case for embryonic development, with very different physical starting points for the process. How regeneration tailors new tissue production to match the identity and scale of missing tissues is largely mysterious.

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In this review I focus on emerging data related to tissue identity determination in tissue maintenance and regeneration from planarians. A hypothesis that emerges from available data is that constitutive regional expression of developmental control genes provides a blueprint for maintenance -and perhaps in part, regeneration- of body parts.

Planarians as a model for study of tissue identity specification in adult biology

Planarians are famous for the ability to regenerate missing body parts [2] and can regenerate from tiny fragments containing roughly 10,000 or more cells [3]. Planarians are freshwater triclad (possessing an intestine with three main branches) flatworm species [4]. These animals have a complex internal anatomy with dozens of cell types organized in patterns along body axes (Figure 1A). Planarians have eyes, a brain with two cephalic ganglia, a diverse array of regionalized neuron types, musculature for turning, epidermis with ventral motile cilia for locomotion, a multibranched intestine (the gastrovascular system), protonephridia for waste removal, a centrally localized pharynx for feeding and defecation, and many other cell types [5]. Planarians maintain and regenerate, with exquisite control of proportions, all of these cell types, organs, and tissue patterns [2].

As members of the phylum Platyhelminthes, planarians are representatives of the Lophotrochozoa, a sister clade to the Ecdysozoa, which include arthropods (such as *Drosophila melanogaster*) and nematodes (such as *Caenorhabditis elegans*) [6]. Most animals with long life require mechanisms for extensive cell replacement and in some cases regrowth of missing body parts. In adult *C. elegans* and *Drosophila*, which are animals with short generation times, these aspects of adult life are either absent or limited to a particular tissue [7–9]. By contrast, the planarian *Schmidtea mediterranea* is highly suited for study of repair and replenishment of many tissues, processes fundamental to adult life in many animals [10].

Planarian regeneration involves new tissue outgrowth at the wound site (the blastema) [2]. However, small fragments do not produce all missing tissues, at original scale, by outgrowth at the wound. Indeed this could not be possible, because a fragment without mouth or brain must accomplish regeneration with existing materials and energy. Planarian regeneration, instead, is accomplished by a combination of blastema formation and changes in pre-existing tissues (often referred to as morphallaxis) [2, 11–13]. A critical source of new planarian tissues is a proliferative somatic cell population called neoblasts, which are of interest for molecular study of stem cell biology. Uninjured planarians undergo constant turnover of tissues, with aged differentiated cells replaced by neoblast progeny [2, 13–15]. Planarians take tissue turnover to the extreme, as they can grow and shrink depending upon nutrient status, involving cell number changes [16, 17]. The term adult could be deemed confusing for such an animal. In this review, the adult processes under discussion occur in animals that have developed the final and full complement of differentiated cell types, in proper proportions, and that are capable of reproduction.

Recent developments with molecular methods have helped make planarians a powerful molecular genetic system for studying tissue turnover and regeneration. For example, RNA interference (RNAi) methods work remarkably well for inhibiting planarian gene expression [18, 19] and can be used in screens [20]; labeling sites of gene expression with *in situ* hybridizations is robust [21, 22]; and the neoblasts can be labeled with BrdU, antibodies, or RNA probes [14, 20, 23], isolated using flow cytometry [24], and their differentiation can be assessed [14, 23, 25–27]. These emerging attributes of planarians as an experimental system have been reviewed elsewhere [10, 28, 29]. I also direct readers to other reviews that focus

on the potential of planarians for stem cell research (not discussed here) [2, 30–32], as well as other reviews on patterning and regeneration [33, 34] as resources.

RNAi phenotypes and the specification of adult tissue identity

With complete genome sequence and well-developed RNAi methods in hand, it has become possible to identify numerous planarian phenotypes. Genes described in this review were identified using RNAi screening and study of genes homologous to developmental control genes from other organisms. Numerous RNAi phenotypes affect the nuts and bolts of the regeneration process, such as the action of neoblasts [20, 23, 26, 27, 32, 35–39]. Here, by contrast, I highlight genes that are not required for regeneration *per se* (regeneration in general or for regeneration of individual cell types), but that are required for specification of regional tissue identity in regeneration. These genes are associated with dramatic RNAi phenotypes including homeotic-like changes (replacement of one body part/region with that of another), aberrations in regionalized regeneration of a tissue type with respect to others, or failed regeneration of only certain body regions (Figure 1B). Many of these genes also have roles in maintenance, in addition to regeneration, of regional tissue identity that can be revealed under long-term RNAi conditions (in some cases following months of tissue turnover). Analysis of these genes reveals constitutive regional gene expression domains described below that provide spatial information guiding tissue replenishment.

Regeneration and maintenance of the anteroposterior axis

The primary body axis is polarized from head-to-tail and is commonly referred to as the anteroposterior (AP) axis. Planarians can regenerate an entire AP axis from nearly any body fragment, providing a robust assay for genes required for primary body axis regeneration and regionalization.

Head-versus-tail planarian tissue identity

A head or a tail can be regenerated at any transverse amputation plane, depending upon whether the wound site is anterior- or posterior-facing. How is the correct regeneration decision made (head versus tail)? This process, referred to as regeneration polarity, has been a classic problem for over a century [11, 40]. RNAi of the Wnt-signaling component *β-catenin-1*, followed by head and tail amputation, results in the dramatic regeneration phenotype of two-headed animals [41–43] (Figure 1B left). Similarly, RNAi of the Wnt gene *wnt1* (previously named *wntP-1*, see Figure 2 legend) results in regeneration of two-headed animals [44, 45], a defect enhanced by inhibition of *wntP-2* [45] (with proposed name *wnt11-5* [46], see Figure 2 legend). Consistent with these data, RNAi of *wntless*, which encodes a protein required for Wnt secretion [47–49], results in regeneration of two-headed planarians [44]. By contrast, inhibition of the *APC* gene, which encodes a β-catenin inhibitor, results in the opposite phenotype of regeneration of two tails [42] (Figure 1B). *β-catenin-1* inhibition in intact planarians undergoing tissue turnover results in a striking body plan transformation with heads appearing around the body periphery [41–43] (Figure 1C). These RNAi animals lose a body axis, nearing radial symmetry about an intact dorsal-ventral axis. These data suggest that a switch-like process occurs at wounds, with Wnt signaling promoting tail regeneration and absence of Wnt signaling leading to head regeneration, and that Wnt signaling also promotes maintenance of the polarized primary axis during tissue turnover.

Numerous other genes must be involved in AP axis regeneration, but few additional phenotypes have been described. The planarian *prep* gene is required for normal head regeneration, providing one example [50]. *prep* encodes a TALE-class homeodomain protein; these proteins can act as co-factors for Hox proteins and have other roles [51]. *prep*

RNAi anterior blastemas do not produce cephalic ganglia and fail to express normal anterior makers (but do not become tails). This phenotype suggests that Prep has a role in promoting anterior tissue identity during head regeneration.

Establishment of axis pattern by restriction of a tissue domain within the AP axis

Study of the planarian *nou darake* gene revealed another phenotype class that implicates the gene in adult tissue/body-plan regulation. In this case, the regionalization of only particular tissues is affected. *nou darake* (*ndk* - "brains everywhere" in japanese) encodes an FGF-receptor-like protein homologous to vertebrate FGFRL proteins [52]. Unlike normal FGF receptors, Nou Darake and FGFRL proteins lack a cytoplasmic kinase domain [53]; available data suggest Nou Darake inhibits FGF signaling [52]. *nou darake* RNAi causes the striking phenotype of posterior brain expansion during regeneration and homeostatic tissue turnover in the adult (ectopic brain cells and eyes extend from the head into more posterior regions) [52] (Figure 1B–C). Not all head cells were expanded in these RNAi animals, indicating a tissue-specific effect. How *nou darake* acts mechanistically, within the pattern of the AP axis, to accomplish proper brain regionalization awaits elucidation. As was the case for Wnt signaling, study of *nou darake* reveals a process acting in regeneration and during tissue maintenance.

Regeneration and maintenance of the dorsal-ventral and mediolateral axes

The back-to-belly, or dorsal-ventral (DV), axis is orthogonal to the AP axis and present in animals with bilateral symmetry. Establishment of pattern along this axis frequently involves BMP signaling [54]. Medial-to-lateral tissue polarization (often referred to as the mediolateral (ML) axis) is also present in animals with bilateral symmetry.

BMP signaling and dorsal-ventral polarity

Bmp proteins are secreted signaling ligands that activate Smad proteins, which mediate transcriptional pathway output [55]. RNAi of BMP pathway genes *bmp4*, *smad1*, or *smad4* leads to similar phenotypes involving ventralized, medially indented blastemas (ventral cell types such as neurons and motile cilia regenerate dorsally) [56–58] (Figure 1B right). The Bmp-family gene *Admp* promotes BMP signaling in vertebrates [59, 60], and a planarian *admp* gene is required for regeneration of cells at the midpoint between DV poles, and promotes *bmp4* activity [61, 62]. BMP signaling can be inhibited by a variety of signaling antagonists, such as Noggin proteins. Planarian *noggin*-related genes [63, 64] are categorized as "*noggin*" (*nog*) or "*noggin-like*" (*nlg*) (*nlg* genes possess an insertion in the *noggin* domain-encoding region) [64]. Simultaneous inhibition of *nog1* and *nog2* results in a dorsalized phenotype [61]. Together, these results indicate BMP signaling promotes dorsal and inhibits ventral tissue regeneration.

RNAi of BMP signaling components in intact animals leads to a dramatic transformation of the body plan with ventral cell types (such as neurons and motile cilia) steadily appearing dorsally [56, 57] (Figure 1C). These animals are able ultimately to glide on their backs or bellies and have a near radial symmetry, with loss of the dorsoventral axis and maintenance of an anteroposterior axis. These data indicate that polarity of the two orthogonal body axes (AP and DV) are constitutively maintained by conserved signaling pathways (Wnt for AP and BMP for DV) and that regeneration of axis polarity requires these axis-maintenance pathways.

Maintenance and regeneration of mediolateral tissue pattern

When BMP pathway components are inhibited and animals are amputated sagittally, regeneration completely fails [56, 57]. Therefore, genes involved in DV tissue polarization

are also required for lateral regeneration for poorly understood reasons. RNAi studies have also implicated additional genes (*slit, wnt5,* and *admp*) in regeneration and maintenance of ML tissue pattern. Slit is an extracellular, leucine-rich-repeat protein that mediates midline repulsion of axons during nervous system development in many animals [43, 65]. In planarians, *slit* RNAi results in medial collapse of nervous system and intestine during regeneration [66]; *slit* maintains existing ML pattern as well [66]. RNAi of planarian *wnt5* (homologous to Wnts that mediate non-canonical Wnt signaling) results in nervous system thickening [44] and can cause appearance of ectopic pharynges laterally [46]. *wnt5* RNAi [46] and *admp* RNAi [62] cause ectopic lateral expression of *slit*, and *slit* RNAi causes expansion of *wnt5* expression into medial regions [46]. Together these observations indicate roles for BMP signaling, Wnt5, and Slit in maintenance and regeneration of ML pattern. The AP, DV, and ML phenotypes described indicate that signaling pathways affect very broad body axis regions in planarians to provide regulatory control of regeneration and tissue turnover.

A regionalized expression map for the body plan persists in adult planarians

The phenotypes described above identify key factors that guide regeneration and tissue turnover. Many of these genes are expressed during normal adult life (Figure 2). A central thesis of this review is that these expression patterns reveal a molecular blueprint for the body plan that persists in the adult, described below (Figure 3). The genes described are expressed in discrete body-axis regions, and in some cases mRNA-expression levels are graded with position [34]. Many of these expression patterns are regional in dispersed subepidermal cells as opposed to being regional simply by occurring within a known localized organ system. For some genes, regional expression corresponds to very large body domains whereas other genes are restricted in expression to a few cells. A number of these genes have not been directly implicated in tissue-pattern maintenance with a homeostasis RNAi phenotype, but are presented here because of homology and expression pattern.

Regionalized expression domains for the anteroposterior axis

Location of active Wnt signaling can be approximated by the location of expression of Wnt ligands or pathway antagonists. Assessment of nuclear β-catenin protein location provides a more direct pathway read-out, but has not yet been accomplished in planarians. There are nine identified planarian Wnt genes. The two Wnt genes implicated in regeneration polarity, *wnt1* and *wntP-2 (wnt11-5)* are expressed in the posterior of intact planarians, with *wnt1* expressed in only a few cells at the tail tip and *wntP-2* (*wnt11-5*) expressed in a posterior-to-anterior gradient extending for the majority of the head-to-tail length of the animal [41] (Figure 2A).

Other Wnt genes have intriguing AP-regionalized expression domains as well (*wnt2:* laterally in the anterior; *wntA/wnt11-6*: posterior end of the cephalic ganglia; *wnt11-1* and *wnt11-2*: at the posterior pole) [41, 46] (Figure 2A). Frizzled proteins are Wnt receptors, and the *frizzled-4* (*fz4*) gene is also expressed at the posterior pole (Figure 2A). There are three candidate Wnt signaling antagonists described, *sFRP-1, sFRP-2,* and *sFRP-3* [41, 42, 46]. *sFRP-1* is expressed at the anterior planarian pole [41, 42]. *sFRP-2*, while absent from the anterior-most region, is expressed in an anterior-to-posterior gradient that extends for half or more of the length of the animal [46] (Figure 2A). *sFRP-3* is expressed in the head and broadly along the primary axis [46]. Overall, these expression patterns are most consistent with varying degrees of Wnt signaling activity existing along the AP axis, with posterior activity and anterior inhibition (Figure 3A).

Additional regional expression domains along the AP axis have been revealed from examination of multiple *nou darake*-like genes, Hox genes, and the *prep* gene (Figure 2A). *nou darake* expression domains suggest anterior restriction of FGF signaling [52, 67]. *prep* is expressed in animal heads, with weaker expression elsewhere [50]. Finally, at least some Hox genes, which are famous for regional AP expression across the animal kingdom [68], are expressed regionally in adult planarians, emanating from the posterior [69, 70]. Taken together, described expression patterns reveal a complex, spatially overlapping set of gene expression domains, defining possible regulatory regions of the AP axis (Figures 2A, 3A).

Expression domains for the dorsoventral and mediolateral axes

The BMP-pathway signaling ligand *bmp4* is expressed on the dorsal side of animals, strongest medially and extending laterally in a graded fashion, which should approximate the site of pathway activity [71] (Figure 2B). Other BMP pathway components, such as *admp*, *tolloid*, *noggin*, and *noggin-like* genes, are expressed in unique DV-polarized domains [56, 61, 62, 64] (Figure 2B). Many expression domains that are DV-polarized also occur in different ML positions and additional ML-polarized gene expression domains have also been identified (Figure 2B, C). *slit* is expressed medially, throughout the dorsal-ventral axis, consistent with its role in mediolateral patterning [66] (Figure 2C). *admp* displays a medial expression domain on the ventral side [61, 62] and the *nlg7* gene is expressed in two ventral domains flanking the ventral medial-most zone [72] (Figure 2B). The genes *wnt2, wnt5*, *admp*, and *nog1* have lateral expression domains (in addition to other expression locations) [44, 61, 62, 72] (Figure 2A–C). Overall, a diversity of broad DV and ML-polarized, constitutively active, expression domains have been revealed (Figures 2B, C and 3B), with BMP signaling appearing maximally active at the dorsal midline.

Comparison of the adult planarian gene expression map to embryogenesis in other organisms

Embryos pass through key stages during which regionalized gene expression controls development of differentiated cells in proper positions [73–76]. Establishment of the body plan during embryogenesis occurs in many stages and study of body plan regulation can therefore be challenging. For example, some genes have early roles in an embryo that precede an axial patterning role; gene perturbation experiments can thus result in early defects that obscure later roles. *Xenopus laevis* β-catenin, for instance, promotes establishment of Spemann's organizer, which impacts DV-axis development, but later, β-*catenin* is important for AP-axis patterning [77, 78]. Furthermore, early embryonic events can vary dramatically in animals. However, despite numerous differences between early embryos in the Bilateria, it is clear that some prominent patterning similarities exist, most famously illustrated by the regional action of Hox genes [68]. Planarians, with constant, easy to visualize gene expression in the adult therefore present a straightforward and attractive venue for identification and study of conserved factors that regulate metazoan body plans.

The constitutive expression domains of position-regulatory genes in planarians bear striking resemblance to expression domains of homologous genes at key embryonic stages in other organisms [75, 79, 80]. Wnt signaling in the posterior and Wnt inhibition in the anterior has emerged as a prominent and widespread feature of primary body axis patterning in animals [79] (Figure 3A). For example, posterior Wnt-pathway activity and/or anterior pathway inhibition occurs during AP patterning of *Xenopus* neuroectoderm [75, 78, 81–83], in mouse embryos [82, 84], and in *C. elegans* [85–87], among numerous other examples. Although further investigations are needed, Hox genes are regionally expressed on the AP axis in planarians [69, 70], as is the case in most bilaterians. Finally, a *Xenopus* homolog of *nou*

darake, XFGFRL1/Xndk is expressed at the early neurula stage in an anterior region across multiple germ layers, similar to the head expression of planarian *nou darake* (Figure 3A) [88].

Polarization of the dorsoventral axis by BMP signaling, as is observed in planarians, occurs in frogs, fish, flies, and other animals, and has emerged as one of the central tenets of animal development (Figure 3B) [75, 89–91]. The opposite orientation of Bmp activity (Bmp active on the dorsal side in *Drosophila* and planarians, or on the ventral side in vertebrates) reflects a probable dorsal-ventral axis inversion that occurred during the evolution of the deuterostomes [54]. Furthermore, the expression of planarian *noggin* and *admp* genes, spatially opposing the location of *bmp* and *tolloid* expression, is reminiscent of vertebrate axis molecular architecture (Figure 3B) [59, 60, 77]. The similarities in gene expression in adult planarians and during embryogenesis in other organisms identify candidate gene expression domains underlying body plans in a large number of animals; similar roles for these genes in body plan development were possibly present in the ancestor of the Bilateria [75, 79, 80]. Prominent in this proposal is the BMP system for the DV axis and Wnt signaling for the AP axis (Figure 3A, B).

Regeneration: specification of tissue identity and restoration of tissue pattern

Planarian regeneration is confronted with essentially unlimited potential "starting points. " A reasonable hypothesis is that common regeneration programs exist that can handle the diversity of injuries that occur. Identifying and understanding such programs is a major challenge of regeneration research. Neoblasts in the tail can regenerate head cells and vice versa, consistent with regional information for regeneration being housed outside of the neoblasts. Regenerative cells (neoblasts and neoblast progeny cells) therefore probably interpret surroundings, such as the regionally expressed patternregulatory factors described here, for regeneration decisions.

Does regeneration act by simply re-scaling existing activity gradients to fit the dimensions of a now smaller tissue fragment, by regeneration-specific programs, or by a combination of both types of processes [45, 92, 93]? One example that has conceptual similarities to adult regeneration involves study of embryonic regulation in *Xenopus*, in which dorsal halves of bisected embryos produce a normal frog. This process is proposed to involve re-scaling of BMP-signaling expression domains involving inherent BMP-signaling circuitry attributes, without need to invoke any regeneration-specific components [60, 77, 93–95]. Unexpectedly, however, the *wnt1* gene (normally expressed at the posterior pole) is induced at essentially all planarian wound types [45]. *wnt1* expression is required for initiation of a posterior-specific gene expression program [45]. These observations suggest that wounds have input into re-setting the AP axis and that the programs that result in body plan regeneration may involve regeneration-specific steps.

Additional conditions cause regeneration of two heads or tails and should prove to be important for understanding axis regeneration. Hedgehog (Hh) signaling promotes planarian tail regeneration [96–98] (Figure 1B), and can impact *wnt1*-expression levels at wounds [96, 97]. No homeostatic AP axis regionalization phenotype has been reported for Hh pathway perturbation. It will therefore be intriguing to investigate a candidate regeneration-specific role for Hh signaling. Simultaneous inhibition of three *innexin* genes in the planarian *Dugesia japonica* results in the regeneration of heads in place of tails, suggesting a gap junction-mediated process is important in regeneration polarity [99]. Finally, modulation of Ca^{2+} influx also can disrupt regeneration polarity [100].

Numerous other genes display expression changes during regeneration that, in contrast to the wound-site expression for *wnt1*, roughly reflect their expression domains in intact animals. For example, *wntP-2*, *sFRP-1,* and *Hox* genes all turn on with time at only posterior-facing or anterior-facing wounds during regeneration, similar (in orientation) to their normal polarized expression in homeostasis [41, 42, 44–46, 69, 70]. Aspects of the gene expression program occurring at posterior-facing wounds can run, at least in part, in the absence of neoblasts, such as *wnt1* wound-site expression [45], *wntP-2* (*wnt11-5*) expression at posterior-facing wounds [45], and *sFRP-1*, *wnt2,* and *sFRP-2* expression at anterior-facing wounds [46]. This indicates that wound-site expression changes occur in differentiated tissues and, in part, independently from blastema formation. Following injury, it is not always the case that regional expression for a gene is missing. By contrast, some expression domains remain but out of proportion for the size of the new fragment and rapid re-scaling occurs. For example, *wntP-2* expression in amputated tail fragments rapidly shrinks to the tail tip [45], with differentiated tissues becoming present in new signaling environments as a consequence of amputation [46] being a potential factor for morphallaxis.

BMP signaling provides a second case study for examination of regional expression domain regeneration. Following parasagittal amputation, lateral fragments induce *bmp4* expression, which ultimately resolves to a new midline in pre-existing tissue [56, 71]. Induction of *bmp4* expression in these fragments occurs even in the absence of neoblasts, indicating (as is the case for early AP regeneration) that changes in expression domains of DV regulatory factors can occur independently from blastema formation [56]. How *bmp4* expression is induced and resolves to a new midline remains unknown. Additional mysteries abound; for example, *admp* expression initially abates near wounds and later returns [62].

These data represent the beginning of investigation into programs of sequential gene expression acting at wounds for regeneration. The re-establishment of proper expression domain scale and position during regeneration, for the myriad of factors with normal regionalized expression (Figure 2), will be a fascinating process to investigate. It will also be important to determine whether genes exist that are expressed only transiently during regeneration with important roles in formation of pattern, similar to transient steps in embryogenesis. In addition, some genes could prove to have one role during homeostatic maintenance of the body plan and a different role during regeneration.

Concluding remarks and future perspectives

Identifying and understanding the functions of regulatory factors that specify position in the metazoan body plan is one of the most fundamental goals of developmental biology. Regionalized gene expression domains in adult planarians present a static snapshot of the body plan, with striking similarities to the expression positions of important regulatory factors in many animals (Figure 3A, B). Because of this attribute, key factors that regulate positional tissue identity in the metazoan body plan could continue to be identified by studying planarian tissue maintenance and regeneration.

Planarians present the opportunity to study the key properties of the regulatory circuitry involved in regional specification that make this particular circuitry so successful for allowing tissue turnover and regeneration. First, expression domains in adult planarian tissues must be stable, in contrast to embryos, where development-regulatory factors can be transiently expressed. Second, gene expression domains can change in scale, while roughly maintaining proportions, as is seen during growth or de-growth. Characterizing the mechanisms that allow the adult gene expression map to scale with body size could be relevant to understanding how similar genes act in embryogenesis. For example, some embryos have changed size during the evolution of closely related species, or, for some

species, embryos grow during development. Furthermore, regions of cells can expand or move in embryogenesis. In these cases, regulatory systems must be capable of scaling to accommodate changing dimensions [93, 101]. Some patterning mechanisms may prove to be adept at this task. Finally, there exists in planarians the capacity to regenerate the patterns of gene expression. Naturally, investigation of this process is of interest for understanding regeneration. In addition, understanding pattern regeneration in adult planarians may also prove relevant for understanding the remarkable ability of embryos to develop normally in the face of physical or environmental perturbation [102].

Adult tissue replenishment is important for life in most animals, and some animals have robust regenerative abilities. Whether constitutive expression of developmental control genes occurs and promotes tissue maintenance and regeneration in other adult animals is underexplored. Hox genes are continuously regionally expressed in vertebrate skin, presumably for maintaining skin positional information [103]. It will be important to more thoroughly investigate the adult expression domains of position-regulatory factors in regenerative and non-regenerative tissues in multiple organisms. Continued study of the identity and attributes of genes constitutively expressed in adult planarians should prove important to understanding in general how animals maintain and regenerate adult tissues.

Acknowledgments

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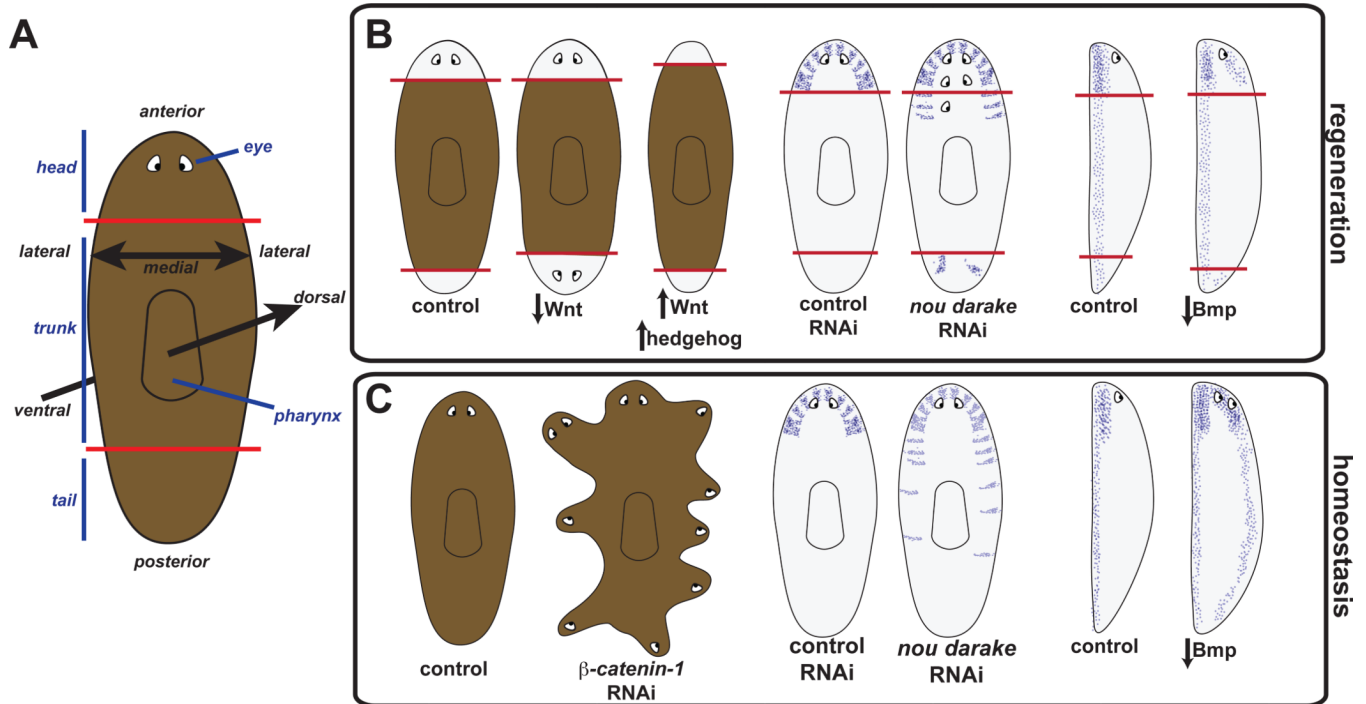
Figure 1. RNAi studies have identified genes required for guiding tissue identity in regeneration and tissue turnover in adult planarians

(A) Schematic of planarian depicting visible body regions and animal axes. Red lines indicate amputation planes approximately utilized to generate animals cartooned in part B. (B) Left, decrease in Wnt signaling components, such as following RNAi of *β-catenin-1*, cause regeneration of two heads whereas increase in Wnt signaling (with *APC* RNAi) or increase in Hedgehog signaling (with *patched* RNAi) cause regeneration of two tails [41–44, 96, 97]. Middle and right, schematized phenotypes are assessed with anatomical markers using *in situ* hybridizations. Middle, *nou darake* RNAi causes regeneration of ectopic brain cells (purple) and eyes (adapted from [52]). Right, decrease in BMP signaling causes ventralization of regeneration blastemas (normally ventral neurons are depicted in purple) [56–58]. Side views are depicted with ventral to the left. Red lines indicate amputation planes, white areas depict regeneration blastemas and pigmented areas depict pre-existing tissues. (C) Left, *β-catenin-1* RNAi causes intact animals to develop ectopic heads around the periphery. Middle, ectopic brain cells appear progressively more posterior in intact *nou darake(RNAi)* animals. Right, inhibition of BMP signaling components with RNAi causes intact animals to develop normally ventral structures (such as brain and nerve cords, purple) on their dorsal sides (see part B for references).
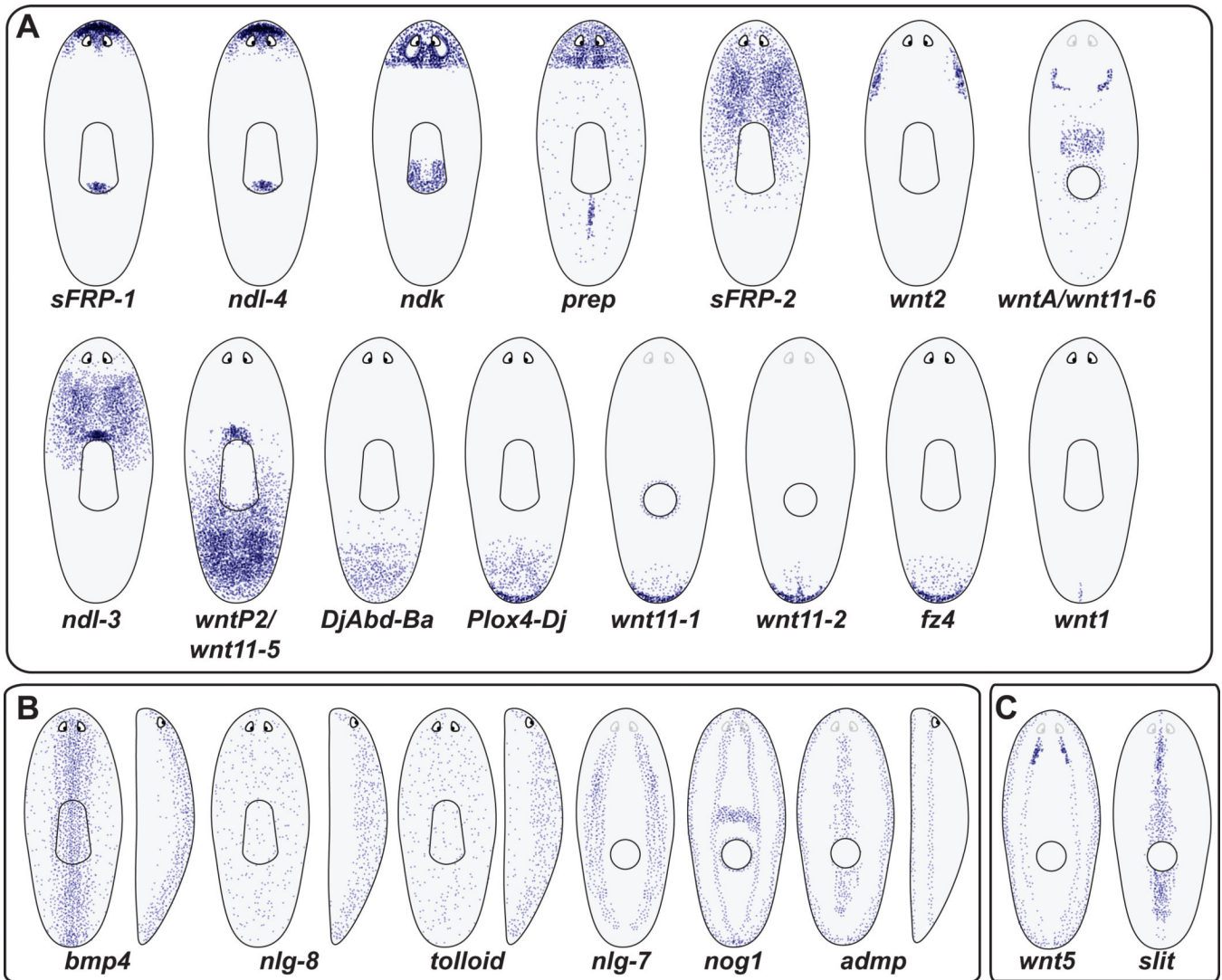
Figure 2. A persistent, adult gene expression map regulates maintenance and regeneration of the planarian body plan

Cartoon depiction of gene expression domains (purple spots, individual cells) for genes expressed in a regional manner. These genes are selected candidate position-regulatory factors, for which the expression domain is simply regional, as opposed to matching the distribution of a known differentiated cell type. Patterns are approximations based upon primary data referenced within the body text (see text for details and references). (A) Gene expression domains distributed along the anteroposterior axis. Patterns are roughly arranged from most anterior (top, left) to most posterior (bottom, right). Some animals are displayed with a dorsal view (entire pharynx depicted as a central, oblong structure). Other animals are depicted from a ventral view, with the mouth visible as a circle and the eyes lighter. Wnt names are challenging because of difficulty with orthology assessment [41, 46, 104]: *wnt1* is former *wntP-1*; *wnt2* was previously named as *wnt2-1*; orthology for several wnt genes is still as yet unresolved*: wnt11-2, wntP-4* (*wnt11-3*), *wntP-2* (*wnt11-5*), and *wntA* (*wnt11-6*). (B) Gene expression domains that display strong dorsal-ventral asymmetry are depicted. In addition to dorsal and ventral views, some side views are provided. In these instances ventral is to the left and dorsal is to the right (recognized with an eye depicted anterior,

right). C. Gene expression domains that are polarized mediolaterally. *wnt5* expression is also largely ventral.
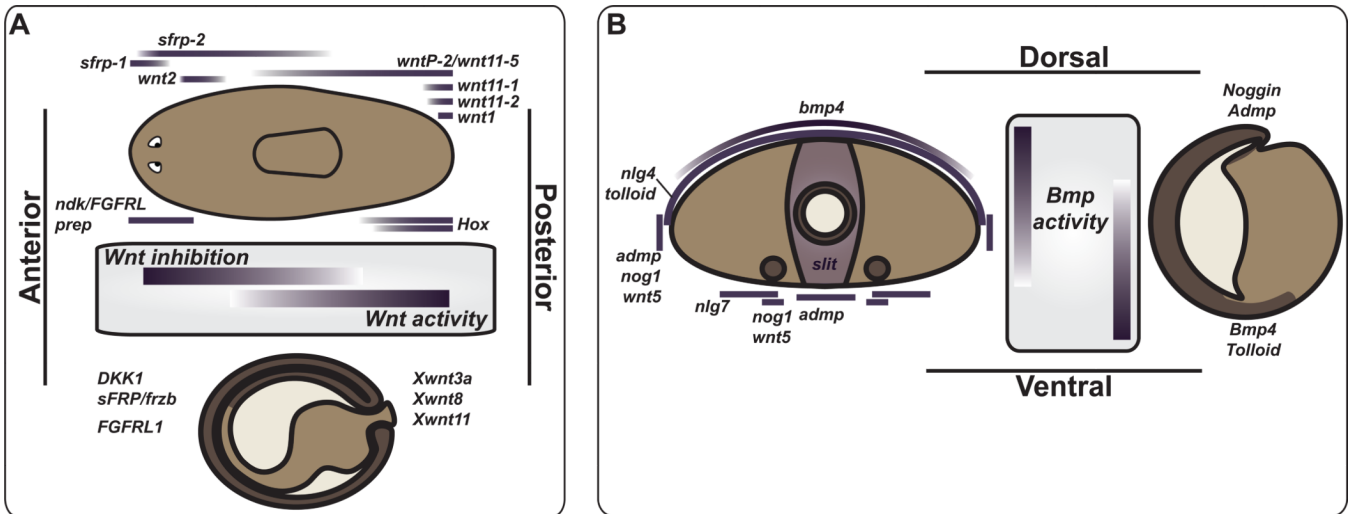
Figure 3. A regulatory map for metazoan body plans
Comparison of gene expression related to the planarian body plan to body plan development during embryogenesis in *Xenopus*. (A) Wnt signaling in control of the primary, anteroposterior, axis and (B) BMP signaling in control of the dorsoventral axis (see text for references). (A) AP axis comparison between an intact planarian and neurula-stage *Xenopus* embryo. The planarian is depicted with a dorsal view, the *Xenopus* embryo is a sagittal view with dorsal up. Wnt inhibition in the anterior and Wnt expression in the posterior are present in both cases. FGFRL expression in the head region is also observed, as is regional expression of Hox genes (see text). (B) BMP signaling is active on the dorsal side of planarians and on the prospective ventral side of *Xenopus* gastrula-stage embryos. A transverse section of a planarian is depicted with the central hole representing a gut branch and the ventral circles representing nerve cords. The *Xenopus* embryo is depicted as a sagittal section. *Admp* and *Noggin* expression spatially oppose the site of *Bmp* expression in both cases. A proposed axis inversion in the evolution of deuterostomes could explain the opposite orientation in protostomes and chordates.