ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

Beyond Casual Resemblances: Rigorous Frameworks for Comparing Regeneration Across Species

Mansi Srivastava

Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, USA; email: mansi@oeb.harvard.edu

Annu. Rev. Cell Dev. Biol. 2021. 37:15.1–15.26

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-120319-114716

Keywords

regeneration, evolution, stem cells, homology, homoplasy, Wnt signaling

Abstract

The majority of animal phyla have species that can regenerate. Comparing regeneration across animals can reconstruct the molecular and cellular evolutionary history of this process. Recent studies have revealed some similarity in regeneration mechanisms, but rigorous comparative methods are needed to assess whether these resemblances are ancestral pathways (homology) or are the result of convergent evolution (homoplasy). This review aims to provide a framework for comparing regeneration across animals, focusing on gene regulatory networks (GRNs), which are substrates for assessing process homology. The homology of the wound-induced activation of Wnt signaling and of adult stem cells are discussed as examples of ongoing studies of regeneration that enable comparisons in a GRN framework. Expanding the study of regeneration GRNs in currently studied species and broadening taxonomic sampling for these approaches will identify processes that are unifying principles of regeneration biology across animals. These insights are important both for evolutionary studies of regeneration and for human regenerative medicine.

Contents

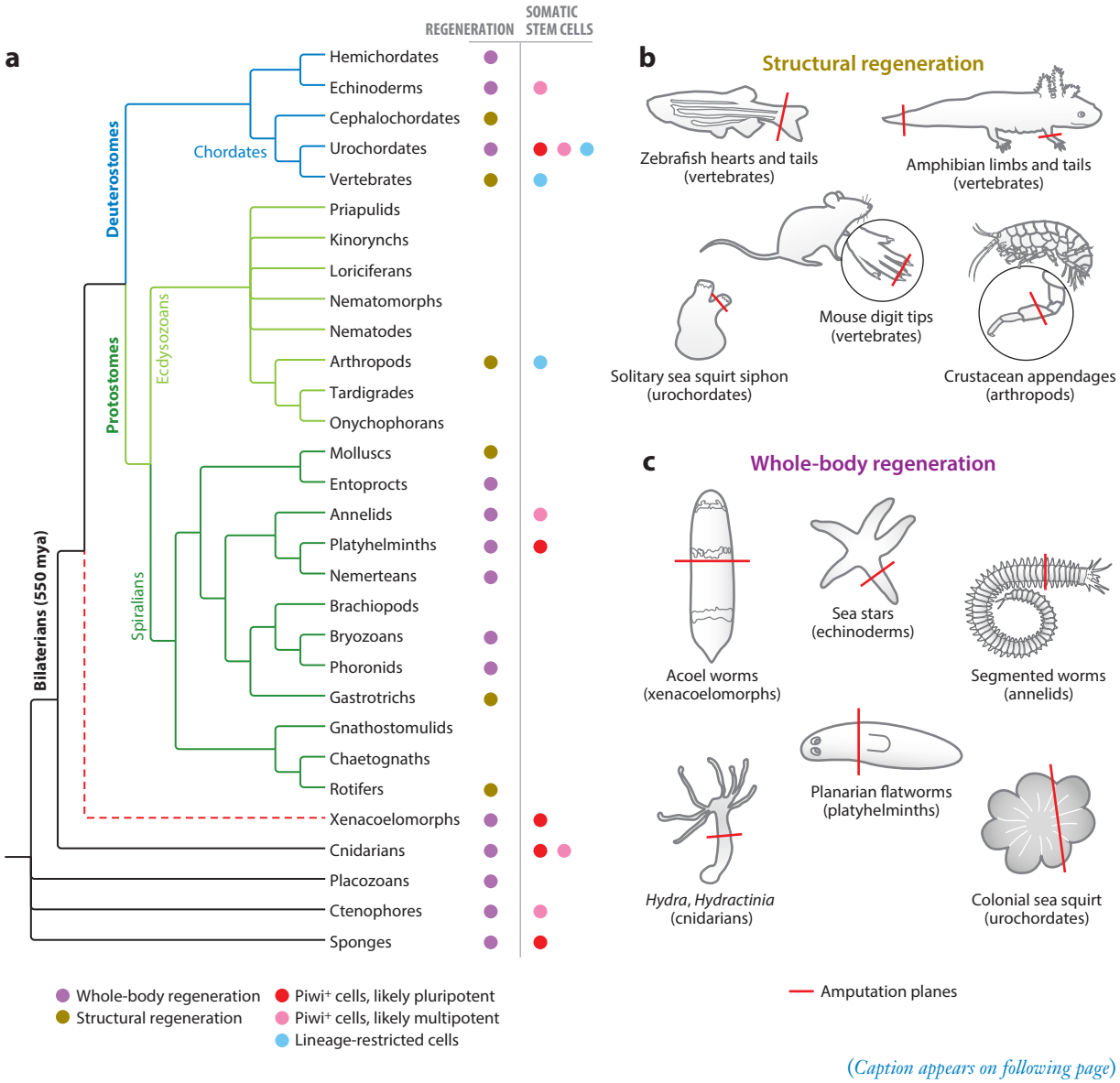
1. INTRODUCTION: THE NEED FOR COMPARATIVE STUDIES OF REGENERATION

All metazoans are multicellular and therefore face the challenge of cell loss during homeostatic tissue turnover or due to injury or disease. Regeneration refers to recovery that results in the restoration of lost or injured tissues and organs. Most animals regenerate, albeit to different degrees. The broad distribution of regenerative capacity raises the question of how mechanisms underlying regeneration compare across animals (**Figure 1**). Regeneration and comparative thinking are inseparable: The beginnings of scientific discourse on regeneration, in the eighteenth century, involved natural philosophers who studied more than one species and who communicated about and popularized each other's work (Benson 1991, Dinsmore 1991, Lenhoff & Lenhoff 1991, Skinner & Cook 1991). This comparative interest has continued into modern times, as the molecular and cellular mechanisms of regeneration have been investigated in a handful of model systems over the past few decades, revealing a diversity of processes but also some resemblances between species. For example, functional studies in animals that can regenerate entire body axes, such as planarians, acoels, and cnidarians, showed that Wnt signaling is required for the regeneration of correctly patterned tissues along the primary axis in all three species (Gurley et al. 2008, 2010; Lengfeld et al. 2009; Petersen & Reddien 2008, 2009b, 2011; Ramirez et al. 2020; Slack 2017; Srivastava et al. 2014) (Section 4.1).

Metazoa: the formal name for the clade (group) consisting of all animals

Assessing whether these resemblances in mechanism have significant similarities is important both to evolutionary biologists studying regeneration and to biomedical researchers. Formally, similarity can be caused by two alternative evolutionary histories: (*a*) homology, meaning that the mechanisms were present in the common ancestor and similarity is caused by descent (e.g., Wnt signaling could have an ancestral role in regeneration), or (*b*) homoplasy, meaning that the mechanisms were not used for regeneration in the common ancestor and the similarity is caused by convergent evolution (e.g., Wnt signaling could have been independently co-opted into regeneration many times). Distinguishing between these two scenarios is essential for inferring the evolutionary history of the process of regeneration. Furthermore, homologous mechanisms, and

Homology: characters (morphological traits, genes, or genetic pathways) in two or more species are homologous if they descended from the same ancestral character



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

The phylogenetic distribution of regenerative capacity. (*a*) A schematic phylogenetic tree shows the relationships of major animal lineages [spiralian relationships shown based on Marletaz et al. (2019)]. The red dashed line indicates uncertainty in the placement of xenacoelomorphs, as some studies suggest placement in the clade with echinoderms and hemichordates (Cannon et al. 2016, Kapli & Telford 2020, Philippe et al. 2019). Purple and gold circles next to the tree indicate the presence of at least one species capable of whole-body regeneration or of structural regeneration, respectively. Most annotations are based on Bely & Nyberg (2010); however, cephalochordates are annotated based on Somorjai et al. (2012). All lineages include species that cannot regenerate; lineages with whole-body regeneration also have species capable of structural regeneration. The presence of pluripotent/multipotent Piwi-expressing stem cells associated with whole-body regeneration (*red circles*) and structural regeneration (*pink circles*) as well as utilization of lineage-restricted progenitors during regeneration (*blue circles*) is also indicated. Panels *b* and *c* show schematics of examples of species and contexts used to study (*b*) structural and (*c*) whole-body regeneration. Red lines indicate amputation planes in regeneration assays. Abbreviation: mya, million years ago. Figure credits for PhyloPic schematics are as follows: Mali'o Kodis, photograph by Wildcat Dunny (sea star) (CC BY 3.0), Jake Warner (zebrafish), Mali'o Kodis, photograph by Melissa Frey (solitary sea squirt) (CC BY NC 3.0), and Daniel Jaron (mouse). Other schematics obtained with permission from Alyson Ramirez (acoel worm, planarian flatworm, and *Hydra*), Jessica Whited (axolotl), Jessica Lehoczky (mouse paw), Duygu Özpolat (segmented worm and colonial sea squirt), and Michalis Averof (crustacean).

even pathways that have evolved convergently many times in diverse regenerative species, reveal the molecular and cellular principles upon which animal regeneration operates. The identification of these fundamental pathways that underlie regeneration across metazoans is important for progress in human regenerative medicine.

Frameworks for assessing homology and homoplasy developed in the field of evolutionary developmental biology can provide rigor in comparative studies of regeneration. Explaining these frameworks (Section 3) and illustrating how they can be applied to ongoing studies of regeneration (Sections 4 and 5) is the main focus of this review.

2. THE PROCESS OF REGENERATION ACROSS METAZOANS

2.1. Defining Regeneration

Historically, the term regeneration has been applied to the formation of new tissue upon injury (referred to by some as restorative, accidental, or pathological regeneration) or upon homeostatic cell loss (referred to by some as physiological regeneration) (Morgan 1901). Upon injury, some animals can rebuild whole organisms from small fragments of the original animal (e.g., planarian worms, the cnidarian *Hydra*, and sea stars). This capacity, often referred to as whole-body regeneration, enables an animal to reestablish its body plan, to remake all missing cell types, and to integrate new and old tissues to reboot all organismal functions (Bely & Nyberg 2010) (**Figure 1**). In contrast, other animals are able to regenerate some but not all organs, tissues, and cell types, a capacity referred to as structural regeneration (e.g., fish and salamanders can regenerate fins or limbs, respectively, but not brains). Regardless of the extent of restoration, most instances of regeneration are epimorphic, that is, they involve cell proliferation that results in the formation of a blastema, an outgrowth of new tissue (Morgan 1901, Reddien & Sanchez Alvarado 2004). In this review, most instances of the word regeneration refer to restorative, epimorphic regeneration.

Homoplasy: similarity in characters without shared ancestry; homoplesious characters can evolve because of independent, convergent evolution

Co-option: the use of a gene or gene network for a new process or function relative to the ancestral function

2.2. The Distribution of Regenerative Capacity Across the Animal Tree

A phylogenetic tree, which depicts the evolutionary relationships between organisms, provides context for understanding the distribution of whole-body and structural regeneration across animal lineages. **Figure 1*a*** shows the relationships of major lineages or groups of animals, most at the phylum level. Annotating each lineage with the presence of at least one species that is capable of regeneration illustrates that the majority of animal lineages have regenerative species

(Bely & Nyberg 2010). However, note that most phyla that have species with whole-body regeneration also include species with more limited or absent capacities to regenerate.

Vertebrates can regenerate many structures through a blastema-based mechanism, ranging from organs such as hearts and lenses to complex, multi-tissue structures such as fins, limbs, and digit tips (Tsonis 2000). Although vertebrates only display structural regeneration, whole-body regeneration is widespread in invertebrates. Strikingly, this more complete type is found even in the phylum (Chordata) that vertebrates belong to, as urochordates (sea squirts), the closest invertebrate cousins of vertebrates, include colonial species that can remake whole animals from amputated fragments (Carnevali & Burighel 2010). Solitary sea squirts, such as *Ciona intestinalis*, can regenerate certain structures such as the siphon and neural tissues (Carnevali & Burighel 2010). The third chordate lineage, cephalochordates, also includes species with extensive, but not whole-body, regeneration (Somorjai et al. 2012). Chordates are deuterostomes, a major grouping of animals with bilateral symmetry (bilaterians). The two other major deuterostome lineages, hemichordates (acorn worms) and echinoderms (e.g., sea stars), also have regenerative species showing both structural and whole-body regeneration (Carnevali & Burighel 2010, Rychel & Swalla 2009).

Among protostomes, the second major grouping of bilaterians, ecdysozoan lineages appear to lack species with whole-body regeneration, but structural regeneration is well known among arthropods. Cockroaches, crickets, and some crustaceans are capable of replacing amputated limbs and other structures via epimorphic regeneration (Khan et al. 2016, Maruzzo & Bortolin 2013). Imaginal discs in the fruit fly *Drosophila melanogaster* have been developed as a new system for mechanistic studies of blastema-based regeneration (Khan et al. 2016). Many spiralian lineages, however, have species that are capable of extensive regeneration (Bely & Nyberg 2010). Better-studied examples include planarian flatworms and some segmented worms (annelids), which can reestablish major body axes and all organs even from small fragments, and molluscs, such as snails, which can regenerate specific structures and organs. Some species of acoel worms, which belong to the likely sister lineage (xenacoelomorphs) to all other animals with bilateral symmetry, are also capable of whole-body regeneration. Extensive regeneration is observed among non-bilaterians, which include lineages that diverged early during animal evolution, such as cnidarians and sponges.

2.3. A Schema for the Process of Regeneration

Regeneration is a complex process that, as a whole, cannot easily be compared across species. However, different aspects of regeneration can be defined to facilitate comparison. Many regeneration biologists use a simplified schema consisting of three major aspects to probe the mechanisms of this process (Tiozzo & Copley 2015): (*a*) wound signaling: injury rapidly elicits molecular changes in cells at the wound site, which send signals to other tissues to begin a coordinated regeneration program; (*b*) generation of new cells: whereas some examples of regeneration in the absence of proliferation are known, most instances of regeneration are associated with an increase in cell proliferation, which is the source of new tissue; and (*c*) morphogenesis: newly formed cells must then interpret patterning information to acquire the correct cell fates and integrate with each other and existing tissue to form a functional organ or organism. Furthermore, underlying the three nodes of this schema are many biological processes, including programmed cell death, cell migration, and cell differentiation. This schema and the processes it summarizes are substrates for comparative studies of regeneration. Section 4 focuses on comparing the connection between wound signaling and morphogenesis, and Section 5 considers how the sources of new cells can be compared across species.

3. COMPARATIVE THINKING APPLIED TO REGENERATION

3.1. Assessing the Homology of Regeneration

Gene regulatory network (GRN): a collection of genes and gene products that interact with each other to determine gene expression at the mRNA level

The study of the evolution of regeneration has been central to the field of regeneration biology since its beginning (Goss 1991). Many have attempted to address the question of whether regeneration evolved once, that is, all extant species that regenerate descended from a common regenerative ancestor, or many times, that is, regeneration has appeared independently in regenerative lineages (Sanchez Alvarado 2000, Slack 2017). This question is the equivalent of asking whether the instances of regeneration observed in different species are homologous or not, as homology is the hypothesis of shared ancestry (Panchen 1999). However, although regeneration is widely distributed across animal phylogeny, each lineage that has regenerative species also has species that do not regenerate. Thus, the mere presence or absence of the capacity to regenerate cannot be used to reconstruct the ancestral state.

Instead of assessing homology at the level of regenerative capacity as a character, homology can be assessed at the level of genetic pathways that operate in regeneration, much as developmental pathways have been considered to be characters (Gilbert & Bolker 2001). Comparisons of genetic pathways may reveal similarity across species, but rigor is needed in making inferences based on this similarity. Consider the case of the Wnt signaling pathway, which has been implicated in numerous regeneration contexts, including in the regeneration of the primary axis of planarians, acoels, and cnidarians. This is a provocative similarity that hints at homology. However, the shared use of Wnt signaling in regeneration can be explained as either a homology or a homoplasy. Developmental pathways, such as Wnt signaling, are modular (Gilbert et al. 1996, Raff 1996), that is, they operate as units that can be used in different contexts within the same animal. Thus, the Wnt pathway, which is an ancestral metazoan developmental pathway, could have been independently co-opted from developmental roles into regenerative processes.

If regenerative processes might appear similar across species because they have been co-opted from developmental processes (Goss 1991), what can then be learned by studying regeneration from a comparative perspective? The correspondence between regeneration and development has been noted in many systems (Soubigou et al. 2020, Warner et al. 2019) and examined extensively in vertebrates (Muneoka & Bryant 1982, Tickle 1981), in which similar mechanisms are known to operate in both processes (Avaron et al. 2006, Han et al. 2003, Imokawa & Yoshizato 1997, McCusker et al. 2015, Muneoka & Bryant 1984). A key question, which was explicitly formulated by researchers even before specific genes and pathways were discovered, is about how these developmental mechanisms are called to action during regeneration (Muneoka & Bryant 1982). Discovering genetic modules that activate developmental programs during regeneration, particularly those that are shared across species, is an exciting goal for comparative studies for regeneration. Comparing these activating pathways, which would be unique to the process of regeneration, will be key to understanding the evolution of regeneration. Section 4 focuses on the activation of the Wnt pathway during regeneration.

3.2. Gene Regulatory Networks and the Homology of Process

The field of evolutionary developmental biology has developed frameworks for comparing genetic pathways that underlie embryonic development (Abouheif 1997; Gilbert & Bolker 2001; Shubin et al. 1997, 2009), which, like regeneration, is a process. Coordinated assemblies of genes and the proteins they encode into functional modules that are shared across species would be considered homologies of process (Gilbert & Bolker 2001). Inferring gene regulatory networks (GRNs) is an important approach for identifying such assemblies of genes and has been applied to studies

of development (Arnone & Davidson 1997). Diagrams depicting GRNs summarize *cis*-regulatory relationships between genes as they progress over developmental time and across different tissue types (Levine & Davidson 2005). Comparisons of these networks have revealed subcircuits of the GRNs (called kernels) that appear to be conserved over long evolutionary distance (Davidson & Erwin 2006). GRN kernels correspond to homologies of process, whereas so-called plug-ins correspond to modules that are found to be co-opted into many processes (Erwin & Davidson 2009). In addition to revealing conserved features, these studies (Erkenbrack et al. 2018, Halfon 2017, Hinman & Davidson 2007) have also revealed plasticity in GRNs that allows different networks to achieve the same function in different species. Thus, GRNs are proving to be effective frameworks for depicting process and for comparing processes across species (Abouheif 1999, Cary et al. 2020, Hinman et al. 2003, Peter & Davidson 2011) (**Figure 2**).

The construction of GRNs for regeneration, which is already underway in some species (Goldman & Poss 2020), is therefore an important step toward making inferences about the evolution of underlying processes (**Figure 2**). The finding of a similar GRN for the same aspect of regeneration in two distantly related species could occur for one of three reasons: (*a*) The shared network could be a kernel in that it was present in the common ancestor and thereby is a homology of process; (*b*) the network is a plug-in that was present in the common ancestor but was independently co-opted into regeneration in the two species, with the similarity reflecting homoplasy; or (*c*) the network was assembled independently in both species from genes that were present in the genome of the common ancestor, which is also indicative of homoplasy (**Figure 2*a–c***). The finding of different GRNs playing the same roles in regeneration can be explained by two alternative scenarios (Wagner 2007): (*a*) An ancestral GRN may become modified in one species via developmental system drift (Halfon 2017, True & Haag 2001), or (*b*) both species independently arrived at novel solutions to the challenge of regeneration (**Figure 2*d*,*e***).

The key to distinguishing between these scenarios lies in broadening the study of regeneration to include more species and in expanding functional studies to consider both upstream and downstream pathways. If more species are found to have identical networks, or the network is expanded to include many connections that are unlikely to be assembled by chance, the inference of homology would be supported. Finding different upstream regulators that activate the same network would be suggestive of homoplasy. Broader taxonomic sampling could also provide support for drift (if a subset of the species studied is found to have all or portions of the same network) or independent evolution (if no shared networks are identified). Section 4 of this review examines known GRNs for Wnt pathway activation during regeneration to determine if currently available data can disentangle these scenarios (**Figure 3**).

3.3. The Homology of Cell Types

Cell types themselves could serve as characters to study homology (Wagner 2014). Cell identity is a complex concept (Daley 2015, Morris 2019), and this review relies on a recently proposed evolutionary definition of cell type (Arendt et al. 2016, Wagner 2014). This framework has emerged from studies of cell fate specification during development that revealed that cellular identity is often controlled by a core regulatory network. These networks, sometimes referred to as cell type–identity networks, consist of transcription factors that often form core regulatory complexes and activate downstream effector genes to enable cell type–specific functions (Arendt et al. 2016). In this framework, cells in different species would be recognizable as homologous cells if they are specified by similar cell type–identity networks. Homologous cell types could have lineage-specific features and functions but would show strong evolutionary conservation of

Kernel: a GRN subcircuit that is shared across distantly related species, likely reflecting a homologous process

Plug-in: a GRN subcircuit that is shared across species because it is easily co-opted into many functions

Developmental system drift: change that can cause developmental processes, such as gene regulatory networks, to be different across species despite having shared ancestry

Taxonomic sampling: the number of species from distinct lineages or groups that are under study

Cell type–identity network: a GRN that causes and maintains a cell type–specific phenotype; network genes often encode transcription factors that form a core regulatory complex

Core regulatory complex: a complex of transcription factors that activates cell type–effector genes and suppresses genes for alternative cell fates

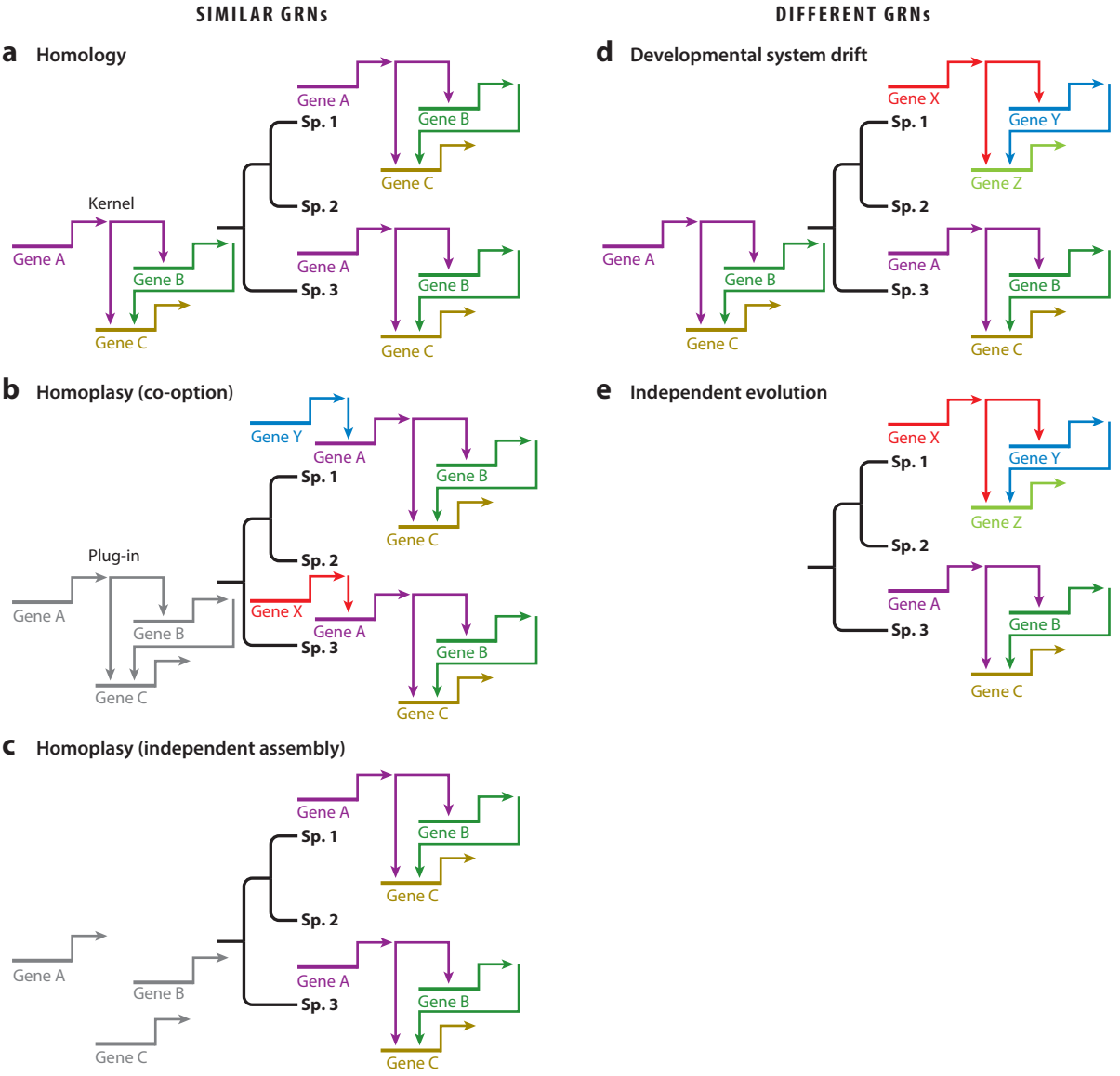
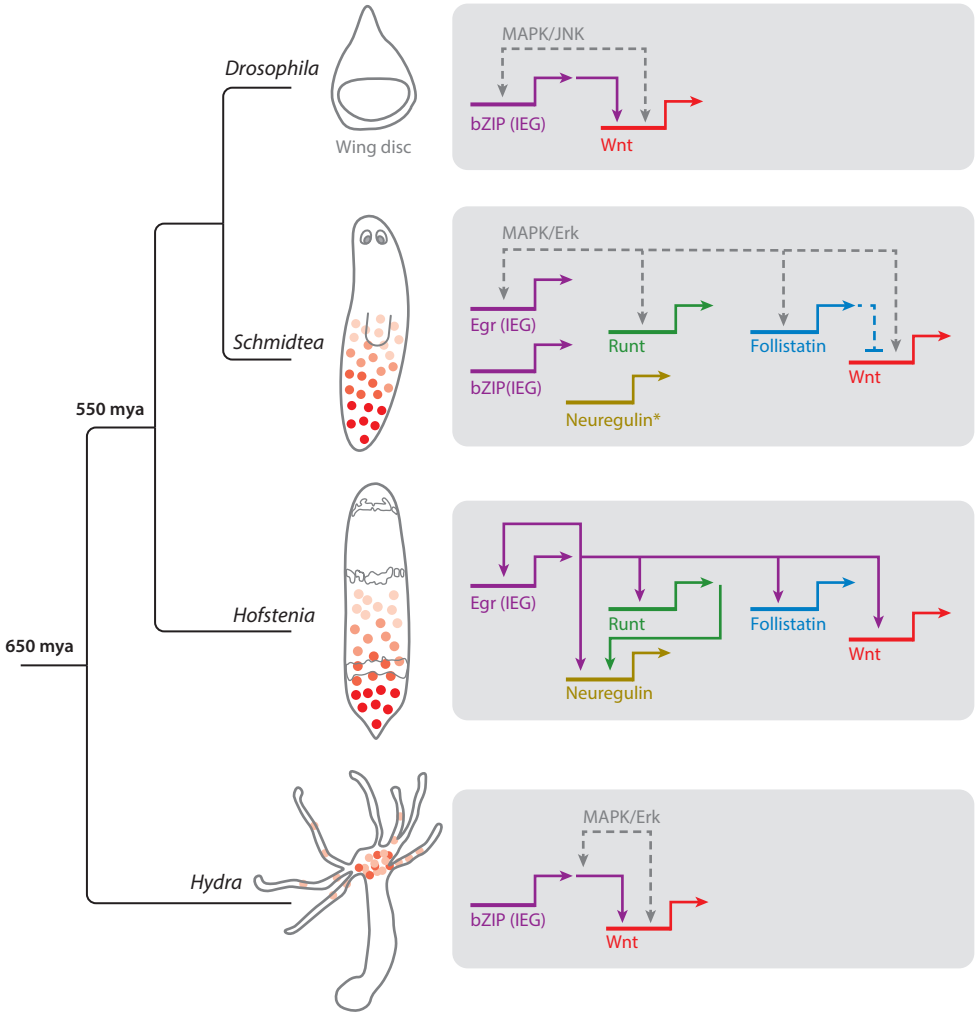


Figure 2

Gene regulatory network (GRN) evolution. The observation of similar GRNs for an aspect of regeneration in two species (Sp.) can have (*left panels*) three alternative explanations. (*a*) Under homology, the GRN is a kernel that was present in the common ancestor and was used in regeneration. Similarity could be caused by homoplasy, in which the network became (*b*) independently co-opted in regeneration in the two Sp. (i.e., the GRN is a plug-in that is often co-opted as a module) or (*c*) independently assembled using ancestral genes. Gray color indicates the presence of genes and GRNs in the ancestor that did not play roles in regeneration. The observation of different GRNs for an aspect of regeneration in two Sp. can have (*right panels*) two alternative explanations. (*d*) One of the GRNs could be ancestral, but one may have diverged because of developmental system drift. (*e*) Alternatively, both networks could have evolved independently. GRNs are shown for only two of three Sp. depicted to reflect the reality that data are usually only available from a few lineages. Broader Sp. Sampling and/or studies to expand the GRNs could distinguish between these scenarios.

their underlying regulatory mechanisms (Arendt et al. 2016). This cellular perspective could also apply to regeneration (Section 5).

Strikingly, many invertebrate species that are highly regenerative harbor putatively pluripotent stem cells that are marked by a small set of homologous genes. For example, regeneration-associated stem cells in sponges, cnidarians, acoels, planarians, and sea squirts express Piwi family proteins (Lai & Aboobaker 2018, van Wolfswinkel 2014) (**Figures 1** and **4*b***). This raises the question of whether many instances of regeneration might involve a homologous cell type. The identity of mammalian embryonic stem cells (ESCs) has been shown to be regulated by a core regulatory network consisting of transcription factors (Boyer et al. 2005, Liu et al. 2008, Young 2011) that displays properties similar to identity-determining networks in differentiated cell types (Wagner 2014). This suggests that such networks could be identified for regeneration-associated pluripotent adult stem cells (**Figure 4*a***).



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Gene regulatory networks for wound-induced activation of Wnt ligand expression. Immediate early genes (IEGs) and Wnt ligands are upregulated during whole-body regeneration and have been studied functionally in planarians, acoels, and the cnidarian *Hydra*. In planarians, extracellular signal–regulated kinase (Erk) signaling is needed for the basic leucine zipper (bZIP) and early growth response (Egr) IEGs and Wnt expression. In *Hydra*, Erk signaling is needed for bZIP IEG activation (phosphorylation) and Wnt expression. In acoels, the Egr IEG appears to be a direct transcriptional regulator of a wound-induced Wnt ligand; in *Hydra*, binding sites for bZIP IEGs are present in multiple wound-induced Wnt ligands. Homologs of the other direct targets of Egr in acoels are also upregulated in planarian regeneration (the asterisk on neuregulin indicates that it is not known to be wound-induced in planarians but is known to function during the process). While there are some missing data from each species, these networks indicate the use of the mitogen-activated protein kinase (MAPK)/Erk-IEG-Wnt pathway in the cnidarian–bilaterian ancestor [650 mya (million years ago)]. This hypothesis should be tested in more regenerative species. Notably, a MAPK/c-Jun N-terminal kinase (JNK)-IEG-Wnt pathway operates during blastema-based wing disc regeneration in fruit flies. Multiple Wnt ligands are involved in some of the species shown, and the exact orthology of Wnts has not been determined for all of these species. Schematics of the animals and wing discs are shown; pink dots depict the expression of Wnt homologs along the primary axis for species with whole-body regeneration. Solid lines indicate evidence of direct regulation, whereas dashed lines are used when evidence of direct regulation is lacking. Figure schematics adapted with permission from Alyson Ramirez (acoel worm, planarian flatworm, and *Hydra*).

4. THE HOMOLOGY OF PROCESS: INTEGRATING WOUND RESPONSE AND PATTERNING PATHWAYS

Wnt signaling is arguably the most well-studied molecular process in the context of regeneration, particularly in systems with whole-body regeneration. Given the wealth of data relative to other regeneration processes, this pathway serves as a focal point to illustrate the value of a GRN perspective in understanding the evolution of regeneration mechanisms.

4.1. Pathways for Patterning in Regeneration

A striking aspect of regeneration in many animals is that the newly formed part is faithful to the original in form and function. Biologists have long considered the particular question of how some animals are able to correctly regenerate along the head–tail axis (Wolpert 1991). Functional genetic studies over the last two decades (Broun et al. 2005; Chen et al. 2020; Gehrke & Srivastava 2016; Gurley et al. 2008, 2010; Lengfeld et al. 2009; Petersen & Reddien 2008, 2009b, 2011; Ramirez et al. 2020; Slack 2017; Srivastava et al. 2014) have clarified how patterning information is maintained and used during the regeneration of the head–tail axis, converging upon Wnt signaling as a key player in many invertebrate species.

In bilaterians, the head–tail axis corresponds to the anterior-posterior (AP) axis. In planarians, perturbing Wnt signaling by inhibiting positive regulators of the pathway results in the regeneration of heads in the place of tails, whereas inhibition of negative regulators results in tail formation where heads would normally have formed (Gurley et al. 2008, 2010; Petersen & Reddien 2008, 2009b, 2011). Notably, components of the Wnt pathway show graded expression along the head–tail axis, even in intact animals (**Figure 3**). Wnt ligands tend to be more highly expressed in the tail, whereas Wnt antagonists are highly expressed in head tissue. Upon amputation, this pattern is reestablished, with Wnt ligand expression becoming stabilized at posterior wound sites and Wnt antagonist expression at anterior wound sites, enabling the regeneration of heads and tails in the correct places. Similarly, Wnt pathway components are expressed along the AP axis, and Wnt signaling is required for correct regeneration of heads and tails in *Hofstenia miamia*, an acoel worm, which is a xenacoelomorph and therefore represents a bilaterian lineage distantly related to planarians (Srivastava et al. 2014) (**Figure 1*a***, **Figure 3**).

In cnidarians, Wnt ligands are highly expressed in the headlike region, which corresponds to the oral end of the oral–aboral (OA) axis (Hobmayer et al. 2000, Lengfeld et al. 2009, Schaffer et al. 2016) (**Figure 3**). Upon amputation of this region in *Hydra*, the ligand *Wnt3* becomes highly expressed at oral wound sites, and the ectopic activation of this protein results in the formation of supernumerary heads (Chera et al. 2009, Vogg et al. 2019). Thus, Wnt signaling is required for correct patterning of regenerating tissues along the primary axes (AP in planarians and acoels and OA in *Hydra*) in both cnidarians and bilaterians. This finding could be suggestive of a striking hypothesis: At least one process underlying patterning during regeneration is homologous between species that shared a common ancestor 650 million years ago. However, a rigorous assessment of process homology is warranted to clarify the precise inferences that can be made.

The Wnt signaling pathway is an extremely versatile process. Within a single species, the pathway can be deployed in many spatially and temporally segregated modules to achieve many biological functions (Logan & Nusse 2004, Nusse & Varmus 1992). The most well-studied role for Wnt signaling in model organisms is its role in patterning the primary axis of animal embryos, including both the AP axis of bilaterians and the OA axis of cnidarians (Petersen & Reddien 2009a). This role, together with the modularity of the pathway, suggests that the use of Wnt signaling during regeneration could have been co-opted from a developmental process. The mechanisms of this co-option, that is, what lies upstream, are an important focus for comparing the role of the Wnt pathway during regeneration in different animals (Brockes & Kumar 2008). If the activation of the Wnt pathway during regeneration is similar across species, it would lend support to process homology; if it is regulated by unrelated mechanisms, then there would be no evidence for an ancestral process for using Wnt signaling during regeneration (**Figure 2**). The mechanisms of how the Wnt pathway is activated during regeneration are only beginning to be uncovered in planarians, acoels, and cnidarians and are discussed in Section 4.3.

4.2. Wound Response Across 650 Million Years of Evolution

Genes upregulated early upon wounding could serve as direct or indirect activators of Wnt signaling activation. Immediate early genes (IEGs) were identified as the transcriptional first responders in many contexts ranging from stimulation by growth factors, synaptic transmission, and injury in mammals (Martin & Nobes 1992, Sheng & Greenberg 1990, Verrier et al. 1986). In general, three results have been found about IEGs: (*a*) they encode transcription factors; (*b*) their messenger RNA (mRNA) expression levels are low before injury or delivery of the activating stimulus; and (*c*) their transcription is rapidly induced within minutes, occurring independently of new protein synthesis. Studies of gene expression in many systems with whole-body regeneration revealed that homologs of IEGs, including early growth response (Egr), Jun, Fos, and Creb, are activated upon wounding in distantly related invertebrate systems (Cary et al. 2019, Cazet & Juliano 2020, Gehrke et al. 2019, Kaloulis et al. 2004, Wenemoser et al. 2012, Wurtzel et al. 2015). These observations make the wound response a candidate for further assessment of process homology in regeneration.

In mammals, the activation of IEGs is detectable within minutes to hours upon injury (Grose et al. 2002). For example, *egr-1* is upregulated within 30 min of skin excision and a Fos homolog is upregulated in muscle satellite cells within 1.5–3 h upon trauma. An AP-1 complex with cFos and JunB is upregulated as part of the proregenerative response of glial cells during spinal cord regeneration in axolotls (Sabin et al. 2019). In planarians, Egr homologs were identified as being upregulated at the wound site within 30 min of amputation (Wenemoser et al. 2012). Notably, this upregulation occurs in many injury contexts, including those in which tissue is removed and those in which it is not removed (for example, in incisions or stab wounds). Homologs of Jun

and Fos are also detectable with similar temporal and spatial dynamics in planarians. The basic leucine zipper (bZIP) transcription factors Jun, Fos, and Creb, all IEGS, are upregulated upon amputation in *Hydra* (Cazet & Juliano 2020, Kaloulis et al. 2004). Egr and Creb are also detected early upon amputation in the larva of the sea star *Patiria miniata*, an emerging system for the study of regeneration in echinoderms (Cary et al. 2019). Egr mRNA is significantly upregulated at many types of wound sites in the acoel *H. miamia* (Gehrke et al. 2019). To assess if these IEGs participate in homologous processes, we should consider how they are activated and which targets are downstream of their activation.

In mammals, studies of IEG upregulation in response to stimuli in cell culture systems have revealed many inputs, prominent among which is extracellular signal–regulated kinase (Erk) signaling (Fowler et al. 2011). Erk is activated within minutes of amputation in planarians, and the inhibition of Erk signaling prevents the wound-induced expression of an Egr homolog (Owlarn et al. 2017). Erk signaling also plays important roles in regeneration in cnidarians (Arvizu et al. 2006, Chera et al. 2011, DuBuc et al. 2014, Fabila et al. 2002, Manuel et al. 2006), including the activation of Creb in *Hydra* (Kaloulis et al. 2004). Mechanisms upstream of IEGs are not known in acoel regeneration.

The functions and downstream targets of IEGs in the context of whole-body regeneration have been identified in acoels. In *Hofstenia*, the only known member of the Egr family is required for regeneration (Gehrke et al. 2019). Upon *egr* knockdown, other wound-induced genes fail to be activated. Chromatin-profiling studies comparing control and *egr* knockdown animals suggest that Egr is likely a direct transcriptional regulator of itself and other wound-induced genes, revealing an injury-induced GRN that is necessary for regeneration (Gehrke et al. 2019). The Egr-controlled GRN that has been inferred for the launch of regeneration in acoels provides a framework for comparison to regeneration processes in other species (**Figure 3**).

Notably, as observed in acoels, chromatin containing the EGR-binding motif, the binding site where Egr proteins are expected to bind to DNA, is the most dynamic within 6 h of amputation in planarians (Gehrke et al. 2019). Whereas some Egr homologs have been shown to be required for the formation of differentiated cell types (Fraguas et al. 2014, Tu et al. 2015), the relationship between wound-induced Egr family members and other wound-induced genes is not known. The EGR-binding motif is also enriched in dynamic, injury-responsive chromatin in *Hydra* (Cazet & Juliano 2020). However, the expression dynamics and functions of Egr homologs are unknown in *Hydra*.

Homologs of three Egr target genes in this GRN, *runt* (Wenemoser et al. 2012), *follistatin* (Gavino et al. 2013, Roberts-Galbraith & Newmark 2013, Tewari et al. 2018), and *neuregulin-1* (Lei et al. 2016), have been shown to have important roles during planarian regeneration (**Figure 3**). However, their regulatory relationship to Egr or to each other is not known. Runt expression is also upregulated in sea star larval regeneration (Cary et al. 2019). Furthermore, homologs of these genes have also been implicated in regeneration in varied contexts in vertebrates. Runx transcription factors are involved in repair and regeneration processes in many tissues, including upregulation upon myopathic damage (Mevel et al. 2019), and have been shown to cooperate with AP-1/cJun to drive the transcriptional program of muscle regeneration (Umansky et al. 2015). Follistatin-like-1 is upregulated upon myocardial infarction in mammals and can be administered in epicardial patches to promote repair (Cao & Poss 2018, Oshima et al. 2008, Wei et al. 2015). Neuregulin-1 signaling is upregulated during and is required for regeneration of axolotl limbs (Farkas et al. 2016) and zebrafish hearts (Gemberling et al. 2015) and fins (Rojas-Muñoz et al. 2009). To assess whether these instances of resemblance to the acoel GRN are meaningful similarities, the regulatory relationships of these genes need to be determined in these other species.

4.3. Connecting the Wound Response to Patterning During Regeneration

With some evidence of homologous genes being used in both the wound response and in patterning along the primary axis in planarians, acoels, and cnidarians, can we compare the process of Wnt pathway activation in these distantly related species? Upon transverse amputation, Wnt ligand expression is reestablished at posterior wound sites in acoels and planarians and in oral wound sites in *Hydra* (Cazet & Juliano 2020, Gurley et al. 2010, Lengfeld et al. 2009, Petersen & Reddien 2009b, Ramirez et al. 2020, Vogg et al. 2019). The specific localization of this expression to the wound site that regenerates tissue with high levels of Wnt signaling is indicative of pattern reestablishment, and the failure to achieve this pattern results in incorrect regeneration along the axis. Given that this pattern is reestablished within hours of amputation, how does the wound response connect to the Wnt pathway? Importantly, can we reconstruct GRNs with *cis*-regulatory linkages leading from the wound response to Wnt pathway reestablishment (**Figure 3**)?

Wnt ligand upregulation, which occurs at both oral and aboral wound sites, requires Erk signaling in *Hydra* (Tursch et al. 2020). As mentioned in Section 4.2, Erk signaling also connects to Creb activation (Kaloulis et al. 2004). Further, transcription factor–binding site motifs for Jun/Fos/Creb are found to be present in injury-responsive chromatin in the loci of Wnt ligands, suggesting direct control of Wnt ligands by bZIPs in this species (Cazet & Juliano 2020, Tursch et al. 2020). Together, these data lend support to a mitogen-activated protein kinase (MAPK)/Erk-bZIP(IEG)-Wnt pathway during regeneration in *Hydra* (**Figure 3**). In acoels, the locus for *wnt-3*, the wound-induced Wnt ligand, contains injury-responsive chromatin containing an EGR-binding site. Upon *egr* knockdown, this chromatin fails to open, the binding site appears to be unbound, and *wnt-3* fails to be expressed. This suggests that *wnt-3* is likely a direct target of Egr (Ramirez et al. 2020). These data support an Egr(IEG)-Wnt regulatory linkage in acoel regeneration (**Figure 3**). In planarians, the Wnt ligand *wnt1* is upregulated at both anterior and posterior wound sites but is subsequently eliminated from anterior wound sites (Petersen & Reddien 2009b). Erk signaling is required for the wound-induced expression of *wnt1* (Owlarn et al. 2017); however, the transcriptional decision-making underlying the upregulation of *wnt1* is not known. These data, together with the effect of Erk inhibitors on wound-induced genes, suggest the presence of MAPK/Erk-Wnt and MAPK/Erk-Egr(IEG) pathways in planarians (**Figure 3**). Although a full understanding of the wound-induced activation of Wnt ligands is lacking in any one species, this work raises the possibility of a homologous MAPK/Erk-IEG-Wnt process during regeneration.

One important approach to test this hypothesis of homology would be to aim for a more complete elucidation of the GRNs in these species. For example, the bZIP-binding motif is also associated with highly dynamic chromatin in acoels (Gehrke et al. 2019); however, direct evidence of the activation of the associated transcription factors or their relationship to the Wnt pathway needs to be investigated. Similarly, the EGR-binding motif is enriched in injury-responsive chromatin in *Hydra*, but the functions of the associated transcription factors are yet to be determined. Transcription factor–binding sites in Wnt loci need to be identified in planarians. The finding of large GRNs that show extensive similarity across the three species would support the hypothesis of process homology.

A second approach, which should be implemented in parallel, is to broaden the taxonomic sampling by studying this process in more species. Wnt signaling is involved in insect leg (Nakamura et al. 2007) and wing disc (Smith-Bolton et al. 2009) regeneration and also plays important roles, such as regulating the proliferation of blastema cells, during vertebrate regeneration in, for example, amphibian limbs (Kawakami et al. 2006, Yokoyama et al. 2007) and tails (Lin & Slack 2008), fish fins (Stewart et al. 2014, Stoick-Cooper et al. 2007, Wehner et al. 2014), and mammal digit tips (Lehoczky & Tabin 2015; Takeo et al. 2013, 2016). Studies in the *Drosophila* wing disc, which has emerged as a powerful system for studying blastema-based regeneration

Orthologs: homologous genes in different species that descended from a common ancestral gene as a result of speciation

(Smith-Bolton et al. 2009), are revealing interesting parallels to the MAPK/Erk-IEG-Wnt process in whole-body regeneration (Harris et al. 2016) (**Figure 3**). Wnt ligands are upregulated upon damage in *Drosophila* wing discs and are required for regenerative growth (Harris et al. 2016, Smith-Bolton et al. 2009). This upregulation is mediated via AP-1 complex (Fos/Jun)–binding sites, under the control of a different MAPK pathway, c-Jun N-terminal kinase (JNK) signaling, revealing a MAPK/JNK-bZIP(IEG)-Wnt pathway (**Figure 3**). Interestingly, JNK signaling is required for the wound-induced expression of an Egr homolog and Runt (Almuedo-Castillo et al. 2014) and for late-phase Wnt expression but not for early wound-induced Wnt ligand expression in planarians (Tejada-Romero et al. 2015). JNK signaling plays a complex role during *Hydra* regeneration, with positive regulation of some Wnt ligands and negative regulation of others (Tursch et al. 2020). Erk signaling has been implicated in vertebrate regeneration contexts in, for example, retinal regeneration in newts (Yasumuro et al. 2017) and cardiac regeneration in zebrafish (Liu & Zhong 2017). In addition to drawing attention to the need for studying both Erk and JNK signaling, these findings more broadly illustrate the need for thorough investigations of regeneration processes both in species that have been studied thus far and in understudied regenerative species.

Although the general theme of an Erk/JNK-IEG-Wnt pathway is emerging, thus far there is no evidence of exact process homology. Jun, Fos, and Creb (bZIP transcription factors) are not homologous to Egr (a C2H2 zinc finger transcription factor) and the Wnt ligands upregulated in these systems are not exact orthologs. Further study might reveal that multiple homologous processes operate in these systems; for example, Erk-bZIP-Wnt and Erk-Egr-Wnt pathways may be found in acoels, planarians, and cnidarians. Alternatively, we may find that a MAPK-IEG-Wnt process is a unifying principle of animal regeneration but that there is plasticity in exactly which MAPK pathway, IEG, or Wnt ligand is used.

5. THE HOMOLOGY OF CELL TYPE: COMPARING THE SOURCE OF NEW TISSUE

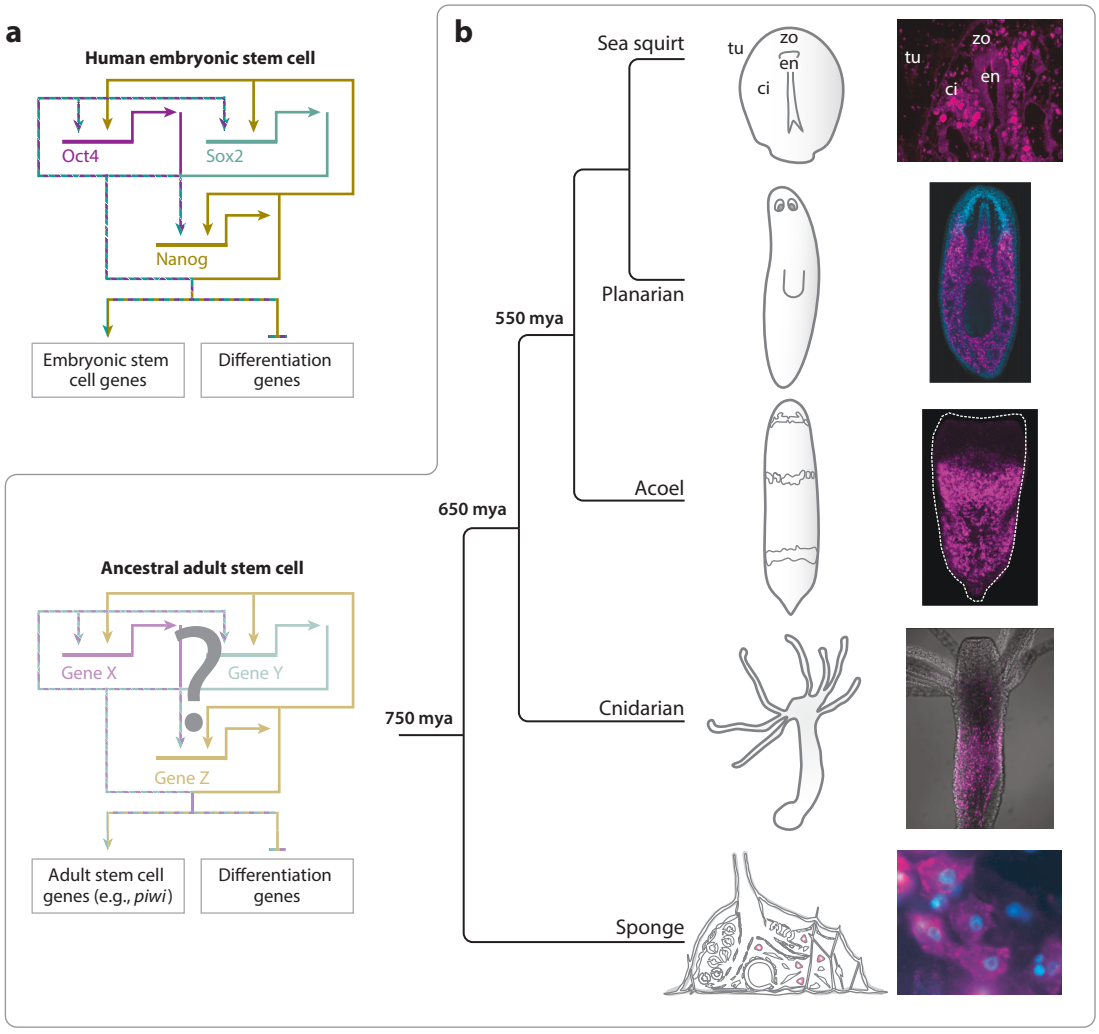
Extensive regeneration requires the formation of new tissue, with the regulation of this process representing the third major aspect of regeneration biology (**Figure 1*d***). The source of new cells has been investigated in a wide array of regenerative species (Lai & Aboobaker 2018, Miller et al. 2019, Tanaka & Reddien 2011), leading to two distinct mechanisms: one involving lineage-restricted progenitors (derived from resident stem cells or dedifferentiated cells) and another featuring putatively pluripotent stem cells (**Figure 1*a***). Similarity of lineage-restricted stem cells across bilaterians has been reported; for example, satellite-like cells expressing Pax3/7 contribute to muscle regeneration in crustaceans (Konstantinides & Averof 2014) and cephalochordates (Somorjai et al. 2012), reminiscent of the well-known role of satellite cells in vertebrate muscle regeneration (Fei et al. 2017, Sandoval-Guzman et al. 2014). This review focuses on assessing the homology of pluripotent adult stem cells as source cells for regenerated tissues.

5.1. Piwi-Expressing Cells in Regenerative Invertebrates

Many regenerative animals capable of whole-body regeneration harbor a population of potentially pluripotent stem cells that all express homologs of the Piwi family and, in some cases, other well-known germline genes [together referred to as the GMP (germline multipotency program)] (Juliano et al. 2010, van Wolfswinkel 2014) (**Figures 1** and **4*b***). Piwi is the most commonly reported GMP gene in animals in which regeneration-associated stem cells have been investigated; therefore, this review refers to these cells as Piwi-expressing or Piwi$^+$ cells.

Among bilaterians, in some species such as platyhelminths (flatworms) and acoels, these cells, which are referred to as neoblasts, are the only proliferative cells and have been shown to be required for regeneration and homeostatic tissue turnover (De Mulder et al. 2009a, 2009b; Newmark & Sanchez Alvarado 2000; Reddien et al. 2005; Srivastava et al. 2014). In the planarian *Schmidtea mediterranea*, double in situ hybridization, functional studies via RNA interference, and inference of differentiation trajectories via single-cell RNA-sequencing have provided evidence that differentiated cell types are derived from neoblasts (Adler et al. 2014, Fincher et al. 2018, Plass et al. 2018, Reddien 2013). In colonial sea squirts, a circulating cell type that expresses Piwi is observed (Kassmer et al. 2019), Piwi is required for regeneration (Rinkevich et al. 2010), and, in one species, Piwi$^+$ cells have been shown to be the source of new tissue during whole-body regeneration (Kassmer et al. 2020).

Pluripotent Piwi$^+$ stem cells are also found in nonbilaterian species. The Piwi-expressing stem cells of cnidarians, called i-cells, show slightly different potency in different species (Plickert et al.



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Assessing the homology of Piwi-expressing adult stem cells in regenerative invertebrates. (*a*) The cell type–identity network for maintaining human embryonic stem cell state is shown. Oct4 and Sox2 proteins form a complex that upregulates Nanog expression; the Oct4/Sox2 complex and Nanog regulate the loci of all three genes. The three proteins then regulate many downstream effector genes to maintain an undifferentiated stem cell state (the tricolor line does not indicate complex formation). Panel *a* network schematic based on Boyer et al. (2005) and Wagner (2014). (*b*) A phylogenetic tree showing the relationships among sponges, cnidarians, acoels, planarians, and urochordates (sea squirts), which all have Piwi/GMP-expressing putatively pluripotent stem cells. Images to the right show the expression of Piwi homologs (*magenta*) in *Ephydatia fluviatilis* (a sponge), *Hydractinia symbiolongicarpus* (a cnidarian), *Hofstenia miamia* (an acoel), and *Schmidtea mediterranea* (a planarian), as well as in an adult zooid of *Botryllus schlosseri* (a sea squirt). The white dashed line in the acoel image marks the boundary of the specimen, while the blue color in the sponge and planarian images indicates nuclear staining. Transcription factor core regulatory networks for Piwi-expressing cells have not been identified from any species. The identification of these networks and their comparisons will provide evidence to assess whether these cells are a homologous type. Abbreviations: ci, cell islands; en, endostyle; GMP, germline multipotency program; mya, million years ago; tu, tunic; zo, zooid. Figure schematics adapted and images reproduced with permission: Alyson Ramirez (acoel, planarian, and cnidarian schematics), Noriko Funayama [*EflPiwiA* mRNA expression in the sponge *Ephydatia* (Funayama 2010) and schematic (Funayama 2018)], Timothy DuBuc and Uri Frank [*Hydractinia* Piwi1 transgenic reporter (DuBuc et al. 2020)], Diana Marcela Bolanos Rodriguez [*piwi-1* mRNA expression in the acoel *Hofstenia* (Srivastava et al. 2014)], Kutay Deniz Atabay and Peter Reddien [*smedwi-1* messenger RNA (mRNA) expression in the planarian *Schmidtea* (Reddien et al. 2005)], Amalia Rosner and Buki Rinkevich [*Botryllus* piwi mRNA expression (Rinkevich et al. 2010) and schematic (Rinkevich et al. 2013)].

2012). In *Hydra*, i-cells produce neurons, cnidocytes (stinging cells), gland cells, and germ cells, but epithelial cells are derived from lineage-restricted ectodermal and endodermal cells (Juliano et al. 2014, Siebert et al. 2019). In contrast, in the colonial hydrozoan *Hydractinia*, i-cells can produce all differentiated cell types of the animal (Gahan et al. 2016, Rebscher et al. 2008). Studies of sponges, an early-diverging animal lineage, have revealed the presence of a stem cell, called an archeocyte, that can differentiate into other cell types of the animal. Molecular studies have revealed that this cell type also expresses Piwi homologs (Funayama et al. 2010).

In several invertebrate regeneration contexts, Piwi expression is observed in stem cells with restricted fate potential. Blastema cells that can be labeled with an anti-Piwi antibody, considered to be multipotent progenitors, are observed during siphon regeneration in a solitary sea squirt (Jeffery 2015). Piwi expression has also been reported in cells that contribute to the formation of cells of the nervous system during growth and regeneration in echinoderms (Mashanov et al. 2013, 2015). In annelids, GMP-expressing cells are found at the wound site in some species, and a $Piwi^+$ population of putative stem cells is observed in the posterior growth zone (Ozpolat & Bely 2016). Piwi expression is also observed in mitotic cells in lineage-restricted stem cells in ctenophores (Alie et al. 2011) and the jellyfish *Clytia* (Denker et al. 2008).

Based on their roles in regeneration and shared expression of the GMP, it has been proposed that $Piwi^+$ stem cells in adult animals are homologous, that is, that the last common ancestor of all animals possessed a Piwi-expressing stem cell that operated postembryonically (Alie et al. 2015, Hemmrich et al. 2012, Solana 2013). The next section considers how these hypotheses can be tested rigorously.

5.2. Assessing Cell Type Homology

Piwi proteins are prominent in germ cell biology in many animals. The molecular function of these proteins in Piwi-interacting RNA biology was deciphered through studies in model systems (van Wolfswinkel 2014). Transposon suppression is often discussed as a major role for Piwi in germ cells, as transposon suppression may limit mutations that could be passed on to the next generation, given that the germline represents an immortal cell lineage in sexually reproducing animals. Piwi and other germline genes likely represent an ancient module that functioned in the germline of the ancestral animal. Was this module also used in a pluripotent adult stem cell population in the

ancestor, or was it independently co-opted in extant lineages to enable the functions of stem cells in adults? To study this question, we need to broadly compare gene expression in these cells, to ask whether there are other shared features of these cells beyond the expression of GMP genes. Further, we need data on how Piwi or other GMP components are regulated in stem cells in these distantly related species.

Mechanisms for the regulation of GMP expression are not known in any of these systems. Upstream of Piwi, transcriptional regulators might be identified that hold a neoblast or an i-cell in a pluripotent state. For example, only a handful of transcription factors are needed to maintain a mammalian cell in a pluripotent state. The expression of Yamanaka, or OSKM (Oct4, Sox2, Klf, c-Myc), factors in differentiated cells causes these cells to revert to a pluripotent state [induced pluripotent stem cells (iPSCs)], from which they can be redifferentiated to various cell types (Takahashi & Yamanaka 2006, 2016). Whereas iPSCs are made in cell culture, ESCs are the only operationally pluripotent cells found in vivo in mammals. The pluripotent state of ESCs is regulated by Oct4, Sox2, and Nanog (Boyer et al. 2005). As with differentiated cell types, in which cell type–identity networks consisting of a handful of transcription factors have been shown to maintain cell type identity (Arendt et al. 2016), Oct4, Sox2, and Nanog are considered to make a core regulatory network, in which Oct4 and Sox2 form a regulatory complex, for ESC identity (Wagner 2014) (**Figure 4*a***). Could such a cell type–identity network underlie the pluripotency of neoblasts or i-cells (**Figure 4*b***)? The identification and comparison of core cell type–identity networks for Piwi-expressing cells from various regenerative species would provide evidence for or against the homology of these cell types.

The core regulatory networks known for mammalian embryonic stem cells are unlikely to underlie invertebrate stem cells (Hemmrich et al. 2012). However, transcriptomic comparisons of planarian and mammalian stem cells have revealed some shared components, which need to be studied systematically and functionally in more species (Labbe et al. 2012). Homologs of the transcription factor FoxO play roles in stem cell maintenance in *Hydra* and mammals (Boehm et al. 2012, Salih & Brunet 2008), and more studies are needed to assess if this is a homologous process. Beyond comparisons to mammalian pluripotent cells, it is important to ask if any core regulatory networks can be identified for Piwi-expressing cells in invertebrates. Comparisons of sponge, planarian, and *Hydra* stem cells have also revealed a shared set of genes with a surprising lack of transcription factors (Alie et al. 2015). New studies with deeper sequencing may address this issue. For example, recent transcriptome sequencing of archaeocytes in a sponge revealed expression of the bHLHZip proteins Myc and Max (Sogabe et al. 2019), which are also key regulators of mammalian pluripotent stem cells (Fagnocchi & Zippo 2017). Large-scale transcriptomic data sets in an increasing number of animals will allow us to determine the expression of orthologous genes within these pluripotent populations, but to assess homology additional work must be done to assemble the networks in which these genes are acting and to identify their functions.

The complexity of Piwi-expressing stem cells may pose a challenge to the goal of cross-species comparisons. For example, the neoblasts of planarians are known to be a heterogeneous population that is composed of a mix of lineage-committed progenitors and, possibly, a subset that is truly undifferentiated, that is, cells that have not begun their journey toward differentiation (van Wolfswinkel et al. 2014, Zeng et al. 2018). Thus, in this case, the expression of Piwi and other GMP proteins does not define just one cell type or state. The goal of determining cell type–identity networks for pluripotent cells then also requires a better understanding of the dynamics of differentiation in adult stem cell populations in many species. Detailed studies of these cells may reveal many lineage-specific features, ranging from gene expression to differentiation dynamics, which will have to be reconciled with any shared cell type–identity networks that are identified.

Homology at the level of the cell may be best assessed based on the sharing of cell type–identity networks rather than on developmental origins or function (Wagner 2014). However, given the paucity of knowledge about how Piwi-expressing stem cells are made during embryogenesis or how they facilitate regeneration, investigations of these aspects will likely also be important for understanding the biology of these cells and for ultimately assessing their homology.

Paralogs: homologous genes in different species that evolved as a result of gene duplication

6. PROSPECTS

6.1. Challenges That Are Easy to Solve

This review has highlighted only some of the instances in which similarity in regeneration programs has been noted across large evolutionary distances. These examples reveal that there are gaps in our knowledge. Components could be missing because transcriptome data are not available for comparable time points or because certain pathways have not been investigated functionally in all species. This missing knowledge will be filled in over time, as a reemerging interest in diverse research organisms and technological advances such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9-mediated genome editing is enabling functional studies of regeneration in many animals. Furthermore, techniques such as the assay for transposase-accessible chromatin using sequencing (ATAC-seq) (Buenrostro et al. 2015) are easily adaptable to many systems and have enabled the identification of regulatory DNA important for regeneration (Kang et al. 2016, Mokalled & Poss 2018). These data are crucial for building GRNs with direct transcriptional regulatory events, which are particularly important in the search for kernels or plug-ins for regeneration and in pursuit of cell type–identity networks for adult pluripotent stem cells.

6.2. Challenges That Are Difficult to Solve with Available Methods

Comparing GRNs between two or more species can reveal the regulatory linkages that are shared, but whether there is a particular level of similarity that would be meaningful beyond casual resemblance is currently unknown. Approaches for quantifying similarities between GRNs are needed. This would require us to develop models for the likelihood of interactions between transcription factors and for the probabilities of change in these interactions. This goal is in line with the growing recognition that null hypotheses are needed in evolutionary developmental biology (Church & Extavour 2020).

Another challenge in comparing GRNs is the importance of considering true orthologs, that is, genes that have descended from a single common ancestral gene (Koonin 2005). However, finding exact orthologs across large evolutionary distances can be challenging. For example, one-to-one orthologs cannot be identified between Egr proteins in planarians and humans or between Wnt proteins in acoels and humans. If GRNs containing genes of the same family (but not direct orthologs) are found in two distantly related species, would this circuit be disqualified as representing process homology? For example, JunB is important for regeneration in axolotls, whereas its paralog c-Jun is activated in the mammalian glial cell response. Is this indicative of a conserved role for this IEG family or of developmental system drift?

The cellular and tissue contexts of how regeneration GRNs operate are important to consider. The different cellular contexts for the expression of Msx1 in the mouse digit tip and amphibian limb regeneration have been used to argue for the convergent evolution of regeneration in vertebrates (Han et al. 2003). However, developmental biologists have found that many structures and their underlying regulatory networks that are clearly homologous can have different cell/tissue origins in embryogenesis in different species (Wagner 2014). As data from more species become available, regeneration biologists will be able to assess whether cellular contexts for regeneration GRNs are static or plastic.

6.3. Coda

Regardless of their objectives in making comparisons, regeneration biologists can benefit from thinking about homology. Uncovering diversity in mechanisms and inferring ancestral regenerative processes is a major goal for evolutionary biologists who study regeneration, making the pursuit of evidence for homology versus homoplasy important. If a GRN subcircuit is consistently found to operate similarly during regeneration in distantly related animals, it might be immaterial to a biomedical researcher if it was used for the same functions in the ancestor (i.e., if it is a kernel) or if it was convergently co-opted into regeneration (i.e., if it is a plug-in). However, this type of consistently utilized GRN is precisely the kind of discovery that could be useful in regenerative medicine. In either scenario, that is, if this GRN is an ancestral regeneration program that fails to launch upon injury in humans or if it is a solution that evolution arrived at many times in regenerative species, this GRN would be relevant to human biology. This type of network, which represents a fundamental principle of regeneration biology, cannot be identified without an explicitly comparative approach. Thus, broadening studies of regeneration to include more species and applying concepts from evolutionary developmental biology can enhance our understanding of the diversity in mechanisms of regeneration and also enable new approaches in human regenerative medicine.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I apologize to those whose work I was not able to cite due to space constraints; I tried to cite reviews whenever possible. I am grateful for the valuable feedback given by many colleagues and trainees: Marcela Bolanos, Vikram Chandra, Andrew Gehrke, Iswar Hariharan, Ryan Hulett, Celina Juliano, Julian Kimura, Kristen Koenig, Katy Loubet-Senear, Kyle McCullogh, Alyson Ramirez, Amber Rock, Lucila Scimone, Elaine Seaver, Josien van Wolkswinkel, and Jessica Whited. Thank you also to those who generously shared schematic drawings and images: Michalis Averof, Marcela Bolanos, Uri Frank, Noriko Funayama, Jessica Lehoczky, Duygu Özpolat, Alyson Ramirez, Peter Reddien, Buki Rinkevich, and Jessica Whited. I am supported by grants from the National Institutes of Health (R35GM128817) and the National Science Foundation (IOS-1652104) and by the Smith Family Foundation Odyssey Award.

LITERATURE CITED

Abouheif E. 1997. Developmental genetics and homology: a hierarchical approach. *Trends Ecol. Evol.* 12:405–8

Abouheif E. 1999. Establishing homology criteria for regulatory gene networks: prospects and challenges. In *Novartis Foundation Symposium 222 - Homology*, ed. GK Bock, G Cardew, pp. 207–25. Chichester, UK: John Wiley & Sons

Adler CE, Seidel CW, McKinney SA, Sanchez Alvarado A. 2014. Selective amputation of the pharynx identifies a FoxA-dependent regeneration program in planaria. *eLife* 3:e02238

Alie A, Hayashi T, Sugimura I, Manuel M, Sugano W, et al. 2015. The ancestral gene repertoire of animal stem cells. *PNAS* 112: E7093–100

Alie A, Leclere L, Jager M, Dayraud C, Chang P, et al. 2011. Somatic stem cells express *Piwi* and *Vasa* genes in an adult ctenophore: ancient association of "germline genes" with stemness. *Dev. Biol.* 350:183–97

Almuedo-Castillo M, Crespo-Yanez X, Seebeck F, Bartscherer K, Salo E, Adell T. 2014. JNK controls the onset of mitosis in planarian stem cells and triggers apoptotic cell death required for regeneration and remodeling. *PLOS Genet.* 10:e1004400

This article provides examples of how cell-type identity networks inform the evolution of cell types.

Arendt D, Musser JM, Baker CVH, Bergman A, Cepko C, et al. 2016. The origin and evolution of cell types. *Nat. Rev. Genet.* 17:744–57

Arnone MI, Davidson EH. 1997. The hardwiring of development: organization and function of genomic regulatory systems. *Development* 124:1851–64

Arvizu F, Aguilera A, Salgado LM. 2006. Activities of the protein kinases STK, PI3K, MEK, and ERK are required for the development of the head organizer in *Hydra magnipapillata*. *Differentiation* 74:305–12

Avaron F, Smith A, Akimenko MA. 2006. Sonic hedgehog signalling in the developing and regenerating fins of zebrafish. In *Shh and Gli Signalling and Development*, ed. CE Fisher, SEM Howie, pp. 93–106. New York: Springer

Bely AE, Nyberg KG. 2010. Evolution of animal regeneration: re-emergence of a field. *Trends Ecol. Evol.* 25:161–70

Benson KR. 1991. Observation versus philosophical commitment in eighteenth-century ideas of regeneration and generation. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 91–100. Cambridge, UK: Cambridge Univ. Press

Boehm AM, Khalturin K, Anton-Erxleben F, Hemmrich G, Klostermeier UC, et al. 2012. FoxO is a critical regulator of stem cell maintenance in immortal *Hydra*. *PNAS* 109:19697–702

Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, et al. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 122:947–56

Brockes JP, Kumar A. 2008. Comparative aspects of animal regeneration. *Annu. Rev. Cell Dev. Biol.* 24:525–49

Broun M, Gee L, Reinhardt B, Bode HR. 2005. Formation of the head organizer in hydra involves the canonical Wnt pathway. *Development* 132:2907–16

Buenrostro JD, Wu B, Litzenburger UM, Ruff D, Gonzales ML, et al. 2015. Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature* 523:486–90

Cannon JT, Vellutini BC, Smith J, Ronquist F, Jondelius U, Hejnol A. 2016. Xenacoelomorpha is the sister group to Nephrozoa. *Nature* 530:89–93

Cao J, Poss KD. 2018. The epicardium as a hub for heart regeneration. *Nat. Rev. Cardiol.* 15:631–47

Carnevali MDC, Burighel P. 2010. Regeneration in echinoderms and ascidians. In *Encyclopedia of Life Sciences*. Chichester, UK: John Wiley & Sons

Cary GA, McCauley BS, Zueva O, Pattinato J, Longabaugh W, Hinman VF. 2020. Systematic comparison of sea urchin and sea star developmental gene regulatory networks explains how novelty is incorporated in early development. *Nat. Commun.* 11:6235

Cary GA, Wolff A, Zueva O, Pattinato J, Hinman VF. 2019. Analysis of sea star larval regeneration reveals conserved processes of whole-body regeneration across the metazoa. *BMC Biol.* 17:16

Cazet J, Juliano CE. 2020. Oral regeneration is the default pathway triggered by injury in *Hydra*. BioRxiv 189811. **https://doi.org/10.1101/2020.07.06.189811**

Chen C-Y, Yueh W-T, J-H. C. 2020. Canonical Wnt signaling is involved in anterior regeneration of the annelid *Aeolosoma viride*. BioRxiv 972448. **https://doi.org/10.1101/2020.03.01.972448**

Chera S, Ghila L, Dobretz K, Wenger Y, Bauer C, et al. 2009. Apoptotic cells provide an unexpected source of Wnt3 signaling to drive *Hydra* head regeneration. *Dev. Cell* 17:279–89

Chera S, Ghila L, Wenger Y, Galliot B. 2011. Injury-induced activation of the MAPK/CREB pathway triggers apoptosis-induced compensatory proliferation in hydra head regeneration. *Dev. Growth Differ.* 53:186–201

Church SH, Extavour CG. 2020. Null hypotheses for developmental evolution. *Development* 147:dev178004

Daley GQ. 2015. Stem cells and the evolving notion of cellular identity. *Philos. Trans. R. Soc. B* 370:20140376

Davidson EH, Erwin DH. 2006. Gene regulatory networks and the evolution of animal body plans. *Science* 311:796–800

De Mulder K, Kuales G, Pfister D, Willems M, Egger B, et al. 2009a. Characterization of the stem cell system of the acoel *Isodiametra pulchra*. *BMC Dev. Biol.* 9:69

De Mulder K, Pfister D, Kuales G, Egger B, Salvenmoser W, et al. 2009b. Stem cells are differentially regulated during development, regeneration and homeostasis in flatworms. *Dev. Biol.* 334:198–212

Denker E, Manuel M, Leclere L, Le Guyader H, Rabet N. 2008. Ordered progression of nematogenesis from stem cells through differentiation stages in the tentacle bulb of *Clytia hemisphaerica* (Hydrozoa, Cnidaria). *Dev. Biol.* 315:99–113

Dinsmore CE. 1991. Lazzaro Spallanzani: concepts of generation and regeneration. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 67–90. Cambridge, UK: Cambridge Univ. Press

DuBuc TQ, Schnitzler CE, Chrysostomou E, McMahon ET, Febrimarsa, et al. 2020. Transcription factor AP2 controls cnidarian germ cell induction. *Science* 367:757–62

DuBuc TQ, Traylor-Knowles N, Martindale MQ. 2014. Initiating a regenerative response; cellular and molecular features of wound healing in the cnidarian *Nematostella vectensis*. *BMC Biol.* 12:24

Erkenbrack EM, Davidson EH, Peter IS. 2018. Conserved regulatory state expression controlled by divergent developmental gene regulatory networks in echinoids. *Development* 145:dev167288

Erwin DH, Davidson EH. 2009. The evolution of hierarchical gene regulatory networks. *Nat. Rev. Genet.* 10:141–48

Fabila Y, Navarro L, Fujisawa T, Bode HR, Salgado LM. 2002. Selective inhibition of protein kinases blocks the formation of a new axis, the beginning of budding, in *Hydra*. *Mech. Dev.* 119:157–64

Fagnocchi L, Zippo A. 2017. Multiple roles of MYC in integrating regulatory networks of pluripotent stem cells. *Front. Cell Dev. Biol.* 5:7

Farkas JE, Freitas PD, Bryant DM, Whited JL, Monaghan JR. 2016. Neuregulin-1 signaling is essential for nerve-dependent axolotl limb regeneration. *Development* 143:2724–31

Fei J-F, Schuez M, Knapp D, Taniguchi Y, Drechsel DN, Tanaka EM. 2017. Efficient gene knockin in axolotl and its use to test the role of satellite cells in limb regeneration. *PNAS* 114:12501–6

Fincher CT, Wurtzel O, de Hoog T, Kravarik KM, Reddien PW. 2018. Cell type transcriptome atlas for the planarian *Schmidtea mediterranea*. *Science* 360:eaaq1736

Fowler T, Sen R, Roy AL. 2011. Regulation of primary response genes. *Mol. Cell* 44:348–60

Fraguas S, Barberan S, Iglesias M, Rodriguez-Esteban G, Cebria F. 2014. *egr-4*, a target of EGFR signaling, is required for the formation of the brain primordia and head regeneration in planarians. *Development* 141:1835–47

Funayama N. 2010. The stem cell system in demosponges: insights into the origin of somatic stem cells. *Dev. Growth Differ.* 52:1–14

Funayama N. 2018. The cellular and molecular bases of the sponge stem cell systems underlying reproduction, homeostasis and regeneration. *Int. J. Dev. Biol.* 62:513–25

Funayama N, Nakatsukasa M, Mohri K, Masuda Y, Agata K. 2010. *Piwi* expression in archeocytes and choanocytes in demosponges: insights into the stem cell system in demosponges. *Evol. Dev.* 12:275–87

Gahan JM, Bradshaw B, Flici H, Frank U. 2016. The interstitial stem cells in *Hydractinia* and their role in regeneration. *Curr. Opin. Genet. Dev.* 40:65–73

Gavino MA, Wenemoser D, Wang IE, Reddien PW. 2013. Tissue absence initiates regeneration through follistatin-mediated inhibition of activin signaling. *eLife* 2:e00247

Gehrke AR, Neverett E, Luo Y-J, Brandt A, Ricci L, et al. 2019. Acoel genome reveals the regulatory landscape of whole-body regeneration. *Science* 363:eaau6173

Gehrke AR, Srivastava M. 2016. Neoblasts and the evolution of whole-body regeneration. *Curr. Opin. Genet. Dev.* 40:131–37

Gemberling M, Karra R, Dickson AL, Poss KD. 2015. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *eLife* 4:e05871

Gilbert SF, Bolker JA. 2001. Homologies of process and modular elements of embryonic construction. *J. Exp. Zool.* 291:1–12

Gilbert SF, Opitz JM, Raff RA. 1996. Resynthesizing evolutionary and developmental biology. *Dev. Biol.* 173:357–72

Goldman JA, Poss KD. 2020. Gene regulatory programmes of tissue regeneration. *Nat. Rev. Genet.* 21:511–25

Goss RJ. 1991. The natural history (and mystery) of regeneration. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 7–24. Cambridge, UK: Cambridge Univ. Press

Grose R, Harris BS, Cooper L, Topilko P, Martin P. 2002. Immediate early genes *krox-24* and *krox-20* are rapidly up-regulated after wounding in the embryonic and adult mouse. *Dev. Dyn.* 223:371–78

Gurley KA, Elliott SA, Simakov O, Schmidt HA, Holstein TW, Sanchez Alvarado A. 2010. Expression of secreted Wnt pathway components reveals unexpected complexity of the planarian amputation response. *Dev. Biol.* 347:24–39

Gurley KA, Rink JC, Sanchez Alvarado A. 2008. Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science* 319:323–27

Halfon MS. 2017. Perspectives on gene regulatory network evolution. *Trends Genet.* 33:436–47

Han M, Yang X, Farrington JE, Muneoka K. 2003. Digit regeneration is regulated by *Msx1* and *BMP4* in fetal mice. *Development* 130:5123–32

Harris RE, Setiawan L, Saul J, Hariharan IK. 2016. Localized epigenetic silencing of a damage-activated WNT enhancer limits regeneration in mature *Drosophila* imaginal discs. *eLife* 5:e11588

Hemmrich G, Khalturin K, Boehm AM, Puchert M, Anton-Erxleben F, et al. 2012. Molecular signatures of the three stem cell lineages in *Hydra* and the emergence of stem cell function at the base of multicellularity. *Mol. Biol. Evol.* 29:3267–80

Hinman VF, Davidson EH. 2007. Evolutionary plasticity of developmental gene regulatory network architecture. *PNAS* 104:19404–9

Hinman VF, Nguyen AT, Cameron RA, Davidson EH. 2003. Developmental gene regulatory network architecture across 500 million years of echinoderm evolution. *PNAS* 100:13356–61

Hobmayer B, Rentzsch F, Kuhn K, Happel CM, Cramer von Laue C, et al. 2000. WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra*. *Nature* 407:186–89

Imokawa Y, Yoshizato K. 1997. Expression of *Sonic hedgehog* gene in regenerating newt limb blastemas recapitulates that in developing limb buds. *PNAS* 94:9159–64

Jeffery WR. 2015. Distal regeneration involves the age dependent activity of branchial sac stem cells in the ascidian *Ciona intestinalis*. *Regeneration* 2:1–18

Juliano CE, Reich A, Liu N, Götzfried J, Zhong M, et al. 2014. PIWI proteins and PIWI-interacting RNAs function in *Hydra* somatic stem cells. *PNAS* 111:337–42

Juliano CE, Swartz SZ, Wessel GM. 2010. A conserved germline multipotency program. *Development* 137:4113–26

Kaloulis K, Chera S, Hassel M, Gauchat D, Galliot B. 2004. Reactivation of developmental programs: the cAMP-response element-binding protein pathway is involved in hydra head regeneration. *PNAS* 101:2363–68

Kang J, Hu J, Karra R, Dickson AL, Tornini VA, et al. 2016. Modulation of tissue repair by regeneration enhancer elements. *Nature* 532:201–6

Kapli P, Telford MJ. 2020. Topology-dependent asymmetry in systematic errors affects phylogenetic placement of Ctenophora and Xenacoelomorpha. *Sci. Adv.* 6:eabc5162

Kassmer SH, Langenbacher AD, De Tomaso AW. 2020. Integrin-alpha-6+ Candidate stem cells are responsible for whole body regeneration in the invertebrate chordate *Botrylloides diegensis*. *Nat. Commun.* 11:4435

Kassmer SH, Nourizadeh S, De Tomaso AW. 2019. Cellular and molecular mechanisms of regeneration in colonial and solitary Ascidians. *Dev. Biol.* 448:271–78

Kawakami Y, Rodriguez Esteban C, Raya M, Kawakami H, Martí M, et al. 2006. Wnt/β-catenin signaling regulates vertebrate limb regeneration. *Genes Dev.* 20:3232–37

Khan SJ, Schuster KJ, Smith-Bolton RK. 2016. Regeneration in crustaceans and insects. In *Encyclopedia of Life Sciences*. Chichester, UK: John Wiley & Sons

Konstantinides N, Averof M. 2014. A common cellular basis for muscle regeneration in arthropods and vertebrates. *Science* 343:788–91

Koonin EV. 2005. Orthologs, paralogs, and evolutionary genomics. *Annu. Rev. Genet.* 39:309–38

Labbe RM, Irimia M, Currie KW, Lin A, Zhu SJ, et al. 2012. A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals. *Stem Cells* 30:1734–45

Lai AG, Aboobaker AA. 2018. EvoRegen in animals: time to uncover deep conservation or convergence of adult stem cell evolution and regenerative processes. *Dev. Biol.* 433:118–31

Lehoczky JA, Tabin CJ. 2015. Lgr6 marks nail stem cells and is required for digit tip regeneration. *PNAS* 112:13249–54

Lei K, Thi-Kim Vu H, Mohan RD, McKinney SA, Seidel CW, et al. 2016. Egf signaling directs neoblast repopulation by regulating asymmetric cell division in planarians. *Dev. Cell* 38:413–29

Lengfeld T, Watanabe H, Simakov O, Lindgens D, Gee L, et al. 2009. Multiple Wnts are involved in *Hydra* organizer formation and regeneration. *Dev. Biol.* 330:186–99

This article provides a comprehensive review of Piwi$^+$ cells and their potency across animals.

Lenhoff HM, Lenhoff SG. 1991. Abraham Trembley and the origin of research on regeneration in animals. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 47–66. Cambridge, UK: Cambridge Univ. Press

Levine M, Davidson EH. 2005. Gene regulatory networks for development. *PNAS* 102:4936–42

Lin G, Slack JM. 2008. Requirement for Wnt and FGF signaling in *Xenopus* tadpole tail regeneration. *Dev. Biol.* 316:323–35

Liu P, Zhong TP. 2017. MAPK/ERK signalling is required for zebrafish cardiac regeneration. *Biotechnol. Lett.* 39:1069–77

Liu X, Huang J, Chen T, Wang Y, Xin S, et al. 2008. Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Res.* 18:1177–89

Logan CY, Nusse R. 2004. The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* 20:781–810

Manuel GC, Reynoso R, Gee L, Salgado LM, Bode HR. 2006. PI3K and ERK 1–2 regulate early stages during head regeneration in hydra. *Dev. Growth Differ.* 48:129–38

Marletaz F, Peijnenburg K, Goto T, Satoh N, Rokhsar DS. 2019. A new spiralian phylogeny places the enigmatic arrow worms among gnathiferans. *Curr. Biol.* 29:312–18.e3

Martin P, Nobes CD. 1992. An early molecular component of the wound healing response in rat embryos—induction of c-*fos* protein in cells at the epidermal wound margin. *Mech. Dev.* 38:209–15

Maruzzo D, Bortolin F. 2013. Arthropod regeneration. In *Arthropod Biology and Evolution*, ed. A Minelli, G Boxshall, G Fusco, pp. 149–69. Berlin: Springer

Mashanov VS, Zueva OR, Garcia-Arraras JE. 2013. Radial glial cells play a key role in echinoderm neural regeneration. *BMC Biol.* 11:49

Mashanov VS, Zueva OR, Garcia-Arraras JE. 2015. Heterogeneous generation of new cells in the adult echinoderm nervous system. *Front. Neuroanat.* 9:123

McCusker C, Bryant SV, Gardiner DM. 2015. The axolotl limb blastema: cellular and molecular mechanisms driving blastema formation and limb regeneration in tetrapods. *Regeneration* 2:54–71

Mevel R, Draper JE, Lie-a-Ling M, Kouskoff V, Lacaud G. 2019. RUNX transcription factors: orchestrators of development. *Development* 146:dev148296

Miller BM, Johnson K, Whited JL. 2019. Common themes in tetrapod appendage regeneration: a cellular perspective. *Evodevo* 10:11

Mokalled MH, Poss KD. 2018. A regeneration toolkit. *Dev. Cell* 47:267–80

Morgan TH. 1901. *Regeneration*. New York: MacMillan

Morris SA. 2019. The evolving concept of cell identity in the single cell era. *Development* 146:dev169748

Muneoka K, Bryant SV. 1982. Evidence that patterning mechanisms in developing and regenerating limbs are the same. *Nature* 298:369–71

Muneoka K, Bryant SV. 1984. Cellular contribution to supernumerary limbs in the axolotl, *Ambystoma mexicanum*. *Dev. Biol.* 105:166–78

Nakamura T, Mito T, Tanaka Y, Bando T, Ohuchi H, Noji S. 2007. Involvement of canonical Wnt/Wingless signaling in the determination of the positional values within the leg segment of the cricket *Gryllus bimaculatus*. *Dev. Growth Differ.* 49:79–88

Newmark PA, Sanchez Alvarado A. 2000. Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. *Dev. Biol.* 220:142–53

Nusse R, Varmus HE. 1992. Wnt genes. *Cell* 69:1073–87

Oshima Y, Ouchi N, Sato K, Izumiya Y, Pimentel DR, Walsh K. 2008. Follistatin-like 1 is an Akt-regulated cardioprotective factor that is secreted by the heart. *Circulation* 117:3099–108

Owlarn S, Klenner F, Schmidt D, Rabert F, Tomasso A, et al. 2017. Generic wound signals initiate regeneration in missing-tissue contexts. *Nat. Commun.* 8:2282

Ozpolat BD, Bely AE. 2016. Developmental and molecular biology of annelid regeneration: a comparative review of recent studies. *Curr. Opin. Genet. Dev.* 40:144–53

Panchen AL. 1999. Homology – history of a concept. In *Novartis Foundation Symposium 222 - Homology*, ed. GK Bock, G Cardew, pp. 5–23. Chichester, UK: John Wiley & Sons

Peter IS, Davidson EH. 2011. Evolution of gene regulatory networks controlling body plan development. *Cell* 144:970–85

Petersen CP, Reddien PW. 2008. *Smed-βcatenin-1* is required for anteroposterior blastema polarity in planarian regeneration. *Science* 319:327–30

Petersen CP, Reddien PW. 2009a. Wnt signaling and the polarity of the primary body axis. *Cell* 139:1056–68

Petersen CP, Reddien PW. 2009b. A wound-induced Wnt expression program controls planarian regeneration polarity. *PNAS* 106:17061–66

Petersen CP, Reddien PW. 2011. Polarized *notum* activation at wounds inhibits Wnt function to promote planarian head regeneration. *Science* 332:852–55

Philippe H, Poustka AJ, Chiodin M, Hoff KJ, Dessimoz C, et al. 2019. Mitigating anticipated effects of systematic errors supports sister-group relationship between Xenacoelomorpha and Ambulacraria. *Curr. Biol.* 29:1818–26.e6

Plass M, Solana J, Wolf FA, Ayoub S, Misios A, et al. 2018. Cell type atlas and lineage tree of a whole complex animal by single-cell transcriptomics. *Science* 360:eaaq1723

Plickert G, Frank U, Muller WA. 2012. *Hydractinia*, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. *Int. J. Dev. Biol.* 56:519–34

Raff RA. 1996. *The Shape of Life*. Chicago: Chicago Univ. Press

Ramirez AN, Loubet-Senear K, Srivastava M. 2020. A regulatory program for initiation of Wnt signaling during posterior regeneration in acoels. *Cell Rep.* 32:108098

Rebscher N, Volk C, Teo R, Plickert G. 2008. The germ plasm component Vasa allows tracing of the interstitial stem cells in the cnidarian *Hydractinia echinata*. *Dev. Dyn.* 237:1736–45

Reddien PW. 2013. Specialized progenitors and regeneration. *Development* 140:951–57

Reddien PW, Oviedo NJ, Jennings JR, Jenkin JC, Sanchez Alvarado A. 2005. SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. *Science* 310:1327–30

Reddien PW, Sanchez Alvarado A. 2004. Fundamentals of planarian regeneration. *Annu. Rev. Cell Dev. Biol.* 20:725–57

Rinkevich Y, Rosner A, Rabinowitz C, Lapidot Z, Moiseeva E, Rinkevich B. 2010. *Piwi* positive cells that line the vasculature epithelium, underlie whole body regeneration in a basal chordate. *Dev. Biol.* 345:94–104

Rinkevich Y, Voskoboynik A, Rosner A, Rabinowitz C, Paz G, et al. 2013. Repeated, long-term cycling of putative stem cells between niches in a basal chordate. *Dev. Cell* 24:76–88

Roberts-Galbraith RH, Newmark PA. 2013. Follistatin antagonizes Activin signaling and acts with Notum to direct planarian head regeneration. *PNAS* 110:1363–68

Rojas-Muñoz A, Rajadhyksha S, Gilmour D, van Bebber F, Antos C, et al. 2009. ErbB2 and ErbB3 regulate amputation-induced proliferation and migration during vertebrate regeneration. *Dev. Biol.* 327:177–90

Rychel AL, Swalla BJ. 2009. Regeneration in hemichordates and echinoderms. In *Stem Cells in Marine Organisms*, ed. B Rinkevich, V Matranga, pp. 245–65. Dordrecht, Neth.: Springer

Sabin KZ, Jiang P, Gearhart MD, Stewart R, Echeverri K. 2019. AP-1$^{cFos/JunB}$/miR-200a regulate the pro-regenerative glial cell response during axolotl spinal cord regeneration. *Commun. Biol.* 2:91

Salih DA, Brunet A. 2008. FoxO transcription factors in the maintenance of cellular homeostasis during aging. *Curr. Opin. Cell Biol.* 20:126–36

Sanchez Alvarado A. 2000. Regeneration in the metazoans: why does it happen? *Bioessays* 22:578–90

Sandoval-Guzman T, Wang H, Khattak S, Schuez M, Roensch K, et al. 2014. Fundamental differences in dedifferentiation and stem cell recruitment during skeletal muscle regeneration in two salamander species. *Cell Stem Cell* 14:174–87

Schaffer AA, Bazarsky M, Levy K, Chalifa-Caspi V, Gat U. 2016. A transcriptional time-course analysis of oral vs. aboral whole-body regeneration in the Sea anemone *Nematostella vectensis*. *BMC Genomics* 17:718

Sheng M, Greenberg ME. 1990. The regulation and function of c-*fos* and other immediate early genes in the nervous system. *Neuron* 4:477–85

Shubin N, Tabin C, Carroll S. 1997. Fossils, genes and the evolution of animal limbs. *Nature* 388:639–48

Shubin N, Tabin C, Carroll S. 2009. Deep homology and the origins of evolutionary novelty. *Nature* 457:818–23

Siebert S, Farrell JA, Cazet JF, Abeykoon Y, Primack AS, et al. 2019. Stem cell differentiation trajectories in *Hydra* resolved at single-cell resolution. *Science* 365:eaav9314

Skinner DM, Cook JS. 1991. New limbs for old ones: some highlights in the history of regeneration in Crustacea. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 25–46. Cambridge, UK: Cambridge Univ. Press

Slack JM. 2017. Animal regeneration: ancestral character or evolutionary novelty? *EMBO Rep*. 18:1497–508

Smith-Bolton RK, Worley MI, Kanda H, Hariharan IK. 2009. Regenerative growth in *Drosophila* imaginal discs is regulated by Wingless and Myc. *Dev. Cell* 16:797–809

Sogabe S, Hatleberg WL, Kocot KM, Say TE, Stoupin D, et al. 2019. Pluripotency and the origin of animal multicellularity. *Nature* 570:519–22

Solana J. 2013. Closing the circle of germline and stem cells: the Primordial Stem Cell hypothesis. *Evodevo* 4:2

Somorjai IM, Somorjai RL, Garcia-Fernandez J, Escriva H. 2012. Vertebrate-like regeneration in the invertebrate chordate amphioxus. *PNAS* 109:517–22

Soubigou A, Ross EG, Touhami Y, Chrismas N, Modepalli V. 2020. Regeneration in the sponge *Sycon ciliatum* partly mimics postlarval development. *Development* 147:dev193714

Srivastava M, Mazza-Curll K, van Wolfswinkel J, Reddien PW. 2014. Whole-body acoel regeneration is controlled by Wnt and Bmp-Admp signaling. *Curr. Biol.* 24:1107–13

Stewart S, Gomez AW, Armstrong BE, Henner A, Stankunas K. 2014. Sequential and opposing activities of Wnt and BMP coordinate zebrafish bone regeneration. *Cell Rep*. 6:482–98

Stoick-Cooper CL, Weidinger G, Riehle KJ, Hubbert C, Major MB, et al. 2007. Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* 134:479–89

Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–76

Takahashi K, Yamanaka S. 2016. A decade of transcription factor-mediated reprogramming to pluripotency. *Nat. Rev. Mol. Cell Biol.* 17:183–93

Takeo M, Chou WC, Sun Q, Lee W, Rabbani P, et al. 2013. Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature* 499:228–32

Takeo M, Hale CS, Ito M. 2016. Epithelium-derived Wnt ligands are essential for maintenance of underlying digit bone. *J. Invest. Dermatol.* 136:1355–63

Tanaka EM, Reddien PW. 2011. The cellular basis for animal regeneration. *Dev. Cell* 21:172–85

Tejada-Romero B, Carter JM, Mihaylova Y, Neumann B, Aboobaker AA. 2015. JNK signalling is necessary for a Wnt- and stem cell-dependent regeneration programme. *Development* 142:2413–24

Tewari AG, Stern SR, Oderberg IM, Reddien PW. 2018. Cellular and molecular responses unique to major injury are dispensable for planarian regeneration. *Cell Rep*. 25:2577–90.e3

Tickle C. 1981. Limb regeneration. *Am. Sci.* 69:639–46

Tiozzo S, Copley RR. 2015. Reconsidering regeneration in metazoans: an evo-devo approach. *Front. Ecol. Evol.* 3:67

This article presents comparative thinking about regeneration from an evo-devo perspective.

True JR, Haag ES. 2001. Developmental system drift and flexibility in evolutionary trajectories. *Evol. Dev.* 3:109–19

Tsonis PA. 2000. Regeneration in vertebrates. *Dev. Biol.* 221:273–84

Tu KC, Cheng L-C, Vu HTK, Lange JJ, McKinney SA, et al. 2015. *Egr-5* is a post-mitotic regulator of planarian epidermal differentiation. *eLife* 4:e10501

Tursch A, Bartsch N, Holstein TW. 2020. MAPK signaling links the injury response to Wnt-regulated patterning in *Hydra* regeneration. BioRxiv 189795. **https://doi.org/10.1101/2020.07.06.189795**

Umansky KB, Gruenbaum-Cohen Y, Tsoory M, Feldmesser E, Goldenberg D, et al. 2015. Runx1 transcription factor is required for myoblasts proliferation during muscle regeneration. *PLOS Genet*. 11:e1005457

van Wolfswinkel JC. 2014. Piwi and potency: PIWI proteins in animal stem cells and regeneration. *Integr. Comp. Biol.* 54:700–13

van Wolfswinkel JC, Wagner DE, Reddien PW. 2014. Single-cell analysis reveals functionally distinct classes within the planarian stem cell compartment. *Cell Stem Cell* 15:326–39

Verrier B, Müller D, Bravo R, Müller R. 1986. Wounding a fibroblast monolayer results in the rapid induction of the c-fos proto-oncogene. *EMBO J.* 5:913–17

Vogg MC, Beccari L, Iglesias Olle L, Rampon C, Vriz S, et al. 2019. An evolutionarily-conserved Wnt3/β-catenin/Sp5 feedback loop restricts head organizer activity in *Hydra*. *Nat. Commun.* 10:312

This book provides a framework for assessing homology using core regulatory networks.

Wagner GP. 2007. The developmental genetics of homology. *Nat. Rev. Genet.* 8:473–79

Wagner GP. 2014. *Homology, Genes, and Evolutionary Innovation*. Princeton, NJ: Princeton Univ. Press

Warner JF, Amiel AR, Johnston H, Röttinger E. 2019. Regeneration is a partial redeployment of the embryonic gene network. BioRxiv 658930. **https://doi.org/10.1101/658930**

Wehner D, Cizelsky W, Vasudevaro MD, Ozhan G, Haase C, et al. 2014. Wnt/β-catenin signaling defines organizing centers that orchestrate growth and differentiation of the regenerating zebrafish caudal fin. *Cell Rep*. 6:467–81

Wei K, Serpooshan V, Hurtado C, Diez-Cuñado M, Zhao M, et al. 2015. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature* 525:479–85

Wenemoser D, Lapan SW, Wilkinson AW, Bell GW, Reddien PW. 2012. A molecular wound response program associated with regeneration initiation in planarians. *Genes Dev*. 26:988–1002

Wolpert L. 1991. Morgan's ambivalence: a history of gradients and regeneration. In *A History of Regeneration Research: Milestones in the Evolution of a Science*, ed. CE Dinsmore, pp. 201–18. Cambridge, UK: Cambridge Univ. Press

Wurtzel O, Cote LE, Poirier A, Satija R, Regev A, Reddien PW. 2015. A generic and cell-type-specific wound response precedes regeneration in planarians. *Dev. Cell* 35:632–45

Yasumuro H, Sakurai K, Toyama F, Maruo F, Chiba C. 2017. Implications of a multi-step trigger of retinal regeneration in the adult newt. *Biomedicines* 5:25

Yokoyama H, Ogino H, Stoick-Cooper CL, Grainger RM, Moon RT. 2007. Wnt/β-catenin signaling has an essential role in the initiation of limb regeneration. *Dev. Biol.* 306:170–78

Young RA. 2011. Control of the embryonic stem cell state. *Cell* 144:940–54

Zeng A, Li H, Guo L, Gao X, McKinney S, et al. 2018. Prospectively isolated tetraspanin$^+$ neoblasts are adult pluripotent stem cells underlying planaria regeneration. *Cell* 173:1593–608.e20