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Old questions, new models: unraveling complex organ regeneration with new experimental approaches

Marco Grillo[1], Nikolaos Konstantinides[2] and Michalis Averof[1,3]

How do some animals like crabs, flatworms and salamanders regenerate entire body parts after a severe injury? Which are the mechanisms and how did that regenerative ability evolve over time? The ability to regenerate complex organs is widespread in the animal kingdom, but fundamental, centuries-old questions remain unanswered. Forward genetics approaches that were so successful in probing embryonic development are lacking in most regenerative models, and candidate gene approaches can be biased and misleading. We summarize recent progress in establishing new genetic tools and approaches to study regeneration and provide a personal perspective on the feasibility and value of establishing such tools, based on our experience with a new experimental model, the crustacean *Parhyale hawaiensis*.

Addresses
[1] Institut de Génomique Fonctionnelle de Lyon (IGFL), École Normale Supérieure de Lyon, 32 Avenue Tony Garnier, 69007 Lyon, France
[2] Department of Biology, New York University, 100 Washington Square East, New York, NY 10003-6688, USA
[3] Centre National de la Recherche Scientifique (CNRS), France

Corresponding authors: Grillo, Marco (marco.grillo@ens-lyon.fr), Konstantinides, Nikolaos (nkonst4@gmail.com) and Averof, Michalis (michalis.averof@ens-lyon.fr)

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Introduction

Several animals (hydra, planarians, fish and salamanders) can regenerate body parts that are injured or amputated. This ability extends far beyond the homeostatic/repair mechanisms present in most organisms, such as wound healing or the physiological renewal of epithelia and blood. Highly regenerative species are able to restore organs with complex architectures and multiple differentiated cell types, such as external appendages (limb, tails, fins), internal organs (heart, liver, brain) or, in some cases, the entire body from small fragments of tissue. Regeneration can restore both the number and the diversity of cells present in the lost tissue, by mobilizing specific populations of progenitor cells. Regeneration also restores pattern, giving rise to well-proportioned, functional organs that can be virtually indistinguishable from those of unharmed animals.

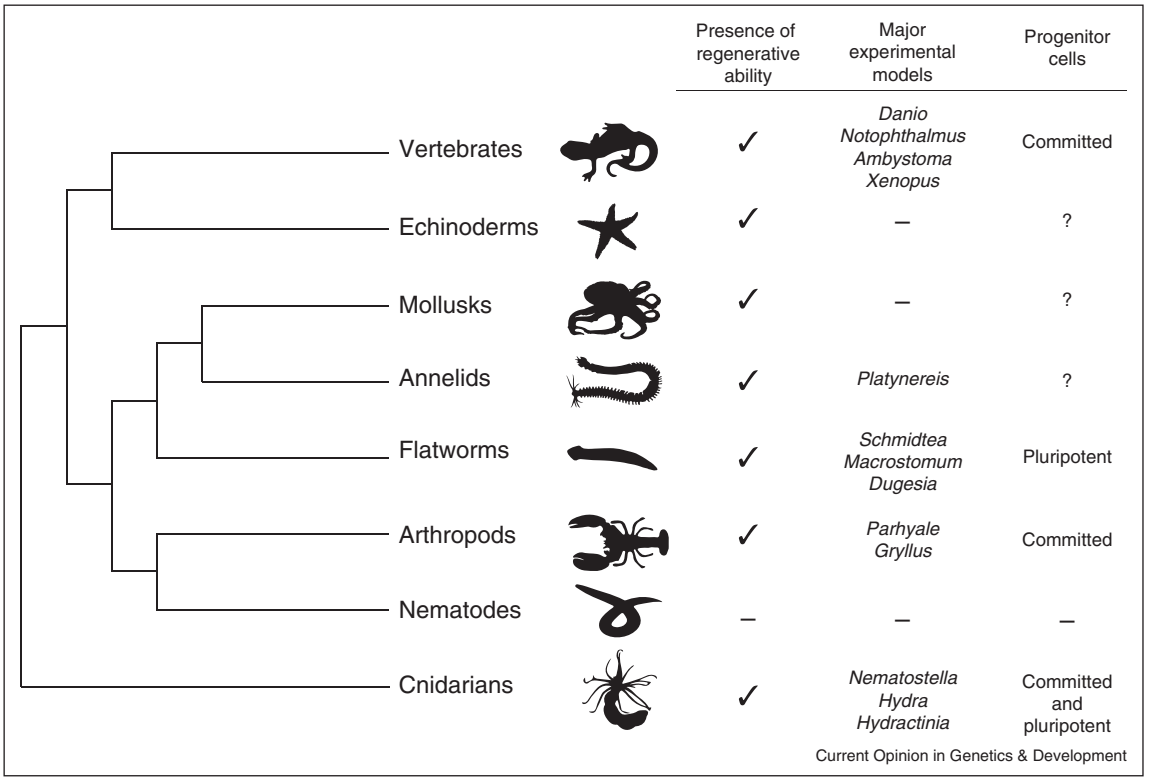
The ability to regenerate is widespread in the animal kingdom; complex organ regeneration is observed in a wide range of animals, including cnidarians, flatworms, molluscs, annelids, arthropods, echinoderms and vertebrates [1–3] (Figure 1). This ability is often coupled with asexual reproduction [4,5] and is common in basal metazoan lineages (sponges, cnidarians, placozoans), raising the possibility that early metazoans had powerful regenerative abilities. However, the ability to regenerate does not correlate well with phylogeny — animals with poor and extensive regenerative abilities can often be found in closely related groups [3,4,6,7,8•,9–11] — suggesting that regenerative ability has been lost and/or gained repeatedly during animal evolution.

There has been much speculation about the reasons behind this variation. Since regenerative ability is considered to be an advantageous trait, its loss seems puzzling. One explanation is that it results from evolutionary trade-offs with other adaptive characters: 'other advantages of greater importance [must be] gained in the bargain' [2]. For example, the ability to rapidly heal wounds by scarring has been proposed to be incompatible with regeneration, and perhaps the reason why mammals have largely lost the ability to regenerate (for discussion see [4,12]). Different abilities to regenerate could also reflect different selective pressures and life strategies adopted by each species [9]. Animals with a very rapid lifecycle may not gain sufficient benefits or have sufficient time to regenerate.

Different tissues and different life stages of the same organism may also regenerate to a different extent. Humans regenerate the endometrium and liver, but their regenerative ability is otherwise very limited. Some lizards can partly regenerate their tail but are not able to regenerate limbs. Most insects are able to regenerate their limbs during larval stages, but lose this ability once they reach the adult stage.

The mechanisms of regeneration can also vary widely among animals. For example, planarians rely on pluripotent stem cells to regenerate missing body parts, whereas vertebrates and arthropods rely on lineage-committed

Figure 1



Wide distribution of regenerative capabilities in animals. The simplified phylogeny depicts 8 major animal phyla and the distribution of regenerative abilities among them. All the shown phyla except nematodes have some members that are able to regenerate complex organs or entire body regions. The major experimental models from each phylum and the type of progenitors found are indicated.

progenitors (Figure 1). Even closely related animals, such as newts and axolotls, can use different mechanisms to regenerate the same tissue [13••]. This complex picture raises questions on which aspects of regeneration are likely to be comparable and which unique to each group.

The challenge of studying regeneration

Unlike embryonic development, where the molecular and cellular mechanisms that underpin patterning and morphogenesis are described in increasing detail, the mechanisms of regeneration are still poorly understood. Fundamental questions, such as what triggers the regenerative programme, the identity and commitment of progenitor cells, how progenitor cells are regulated to compensate precisely for the missing parts, and how the new tissues are patterned to regenerate functional organs, remain largely unanswered in most species [14]. It is also unclear to what extent regeneration re-utilises mechanisms that operate during embryonic development and whether common principles/mechanisms of regeneration will emerge by comparing distant species [15].

We think the reasons for this failure largely boil down to the technical means at our disposal, namely the lack of powerful genetic approaches for studying regeneration. First, some of the best genetic models available — *C. elegans*, *Drosophila* and mice — have very limited regenerative abilities (with the notable exception of zebrafish, see below). These models were chosen for traits that favor genetic screens (a short generation time, fast development, r-oriented reproductive strategy) but are unlikely to be associated with regenerative ability. Second, regeneration occurs in adults, which complicates loss-of-function genetic studies. The powerful genetic screens carried out in *Drosophila*, *C. elegans* and zebrafish were effective in capturing mutations that influence early development, but probing adult traits is more challenging because many mutations are lethal at earlier stages. Forward genetic screens for adult traits are possible, but they are more demanding and often require advanced genetic tools [16]. Third, regeneration is not as amenable to direct observation. It is a lengthy process — extending over weeks or months — and occurs in adults that are often highly mobile, rendering continuous live imaging very challenging.

The classic experimental models used to study regeneration — hydrozoans, planarians, salamanders and *Xenopus* tadpoles — are species with impressive regenerative

abilities. The initial choice of these models was driven by their biology and by experimental approaches such as cell transplantation and grafting from which we learnt much in the past (see [1,2,17]). Attempts to establish genetic approaches in these organisms have had variable success: RNAi/antisense approaches and transgenesis have been successful in some species (Figure 2), but forward genetic screens have not been possible in any of these classic models. Zebrafish is a recent exception to this rule [18–20,21•,22–24].

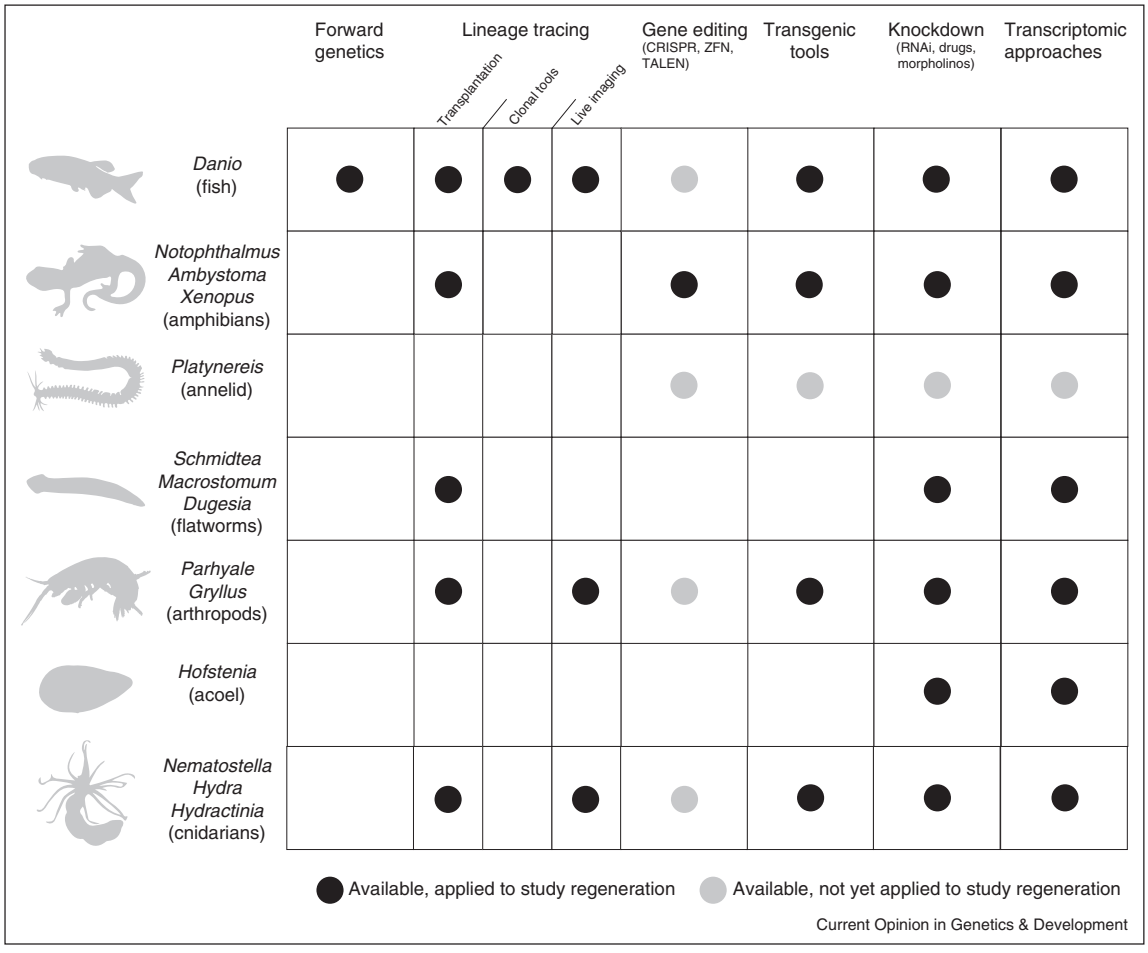
Given the genetic tools available, studies of regeneration have often taken a 'candidate gene' approach, using RNAi-, antisense- or drug-mediated knockdowns to assess the role of specific genes. Since this approach relies on knowledge (candidate genes) coming from models that lack regenerative abilities, it is biased towards common, conserved mechanisms and likely to miss novel mechanisms that are unique to regeneration. Also, while this approach can succeed in identifying essential factors, in the absence of advanced genetic tools it usually fails to probe mechanisms in depth.

We focus here on efforts to overcome the candidate gene approach by the introduction of new experimental approaches and new models to study regeneration.

New experimental approaches

In the last decade significant progress was made in introducing new genetic tools in classic models, such as hydra, planarians and salamanders (summarized in Figure 2). RNAi, morpholinos and other knockdown approaches were mostly applied to test the role of candidate genes, but on some occasions they have been used to conduct large screens, searching for factors that influence regeneration in a more unbiased fashion [25•,26]. Transgenesis was established in a few regenerative models, namely in zebrafish, axolotl, newt, *Xenopus*, *Hydra* and in

Figure 2



Genetic approaches available in different regenerative models. References: forward genetics [18–20,21•,22–24], transplantation of genetically marked cells [29,34•,36,37,38•,39••,41,42••,78••,116], clonal genetic tools [43–49,117], live imaging [81–85], gene editing techniques [51–60], transgenesis [27–33,34•,35,40,118,119], RNAi and morpholino [25•,73,92•,103,107,109,120–122], transcriptional profiling approaches [7,62–71,73,123–125].

the emerging cnidarian and arthropod models *Nematostella*, *Hydractinia*, *Gryllus* and *Parhyale* [27–33,34•,35]. In some species it has been used to home in on precursor cells and to study their degree of commitment [29,34•,36,37,38•,39••,40,41,42••]. The most advanced transgenic tools have been established in zebrafish, where the Cre/lox recombinase system is now commonly used to investigate cell lineage (e.g. [43–49]).

Recently, gene editing techniques based on engineered nucleases (zinc-finger nucleases, TALENs and CRISPR) have brought in a much higher degree of precision in gene manipulation. CRISPR in particular seems to be robust, accessible and widely applicable in diverse organisms, blurring the boundary between model and non-model species [50]. Among regenerative models, TALENs and CRISPR have been successfully applied in zebrafish, axolotl, newt, *Xenopus*, *Platynereis*, *Nematostella*, *Gryllus* and *Parhyale* [51–61].

Finally, among the new technologies, high-throughput sequencing has been widely applied to sequence the genomes and transcriptomes of several regenerative models. RNA-Seq is increasingly used to survey the transcriptional profiles and responses of cells during the course of regeneration (e.g. [62–69]). Single-cell sequencing is starting to be used to determine the cellular heterogeneity and differential transcriptional responses of individual cell types [70,71].

These technologies have often been used to support 'gene-oriented' approaches, for example using transcriptional profiling to identify differentially expressed genes and knockdowns to test the role of new candidates (e.g. [7,69,72–74]). In other cases they have been combined with classic experimental techniques, such as X-irradiation and cell transplantation, to address questions on the role and fate of particular cell types during regeneration. Here, we wish to highlight the latter 'cell-oriented' approaches.

Cell-oriented approaches exploit new ways of labelling specific populations of cells to track their fates and their influence on neighbouring cells during regeneration. Classical studies achieved this by cell transplantation/grafting between donors and hosts with visible differences in their cells [75], for example between *Hydra* with white-pigmented or green-pigmented cells [76]. In some cases cells could be labelled by incorporation of a nucleotide analogue in the donor cell's DNA, or by transplanting cells from genetically distinct donors; the former approach was recently used to show that cells circulating in the blood can serve as regenerative progenitors of neurons in crayfish [77•] and the latter to demonstrate the regenerative potential of neoblast cells in planarians [78••]. Transgenesis can play an important role in developing such approaches by generating donor strains whose cells are labelled via fluorescent protein expression; this approach has been used to determine the regenerative potential of different populations of cells in fish, *Xenopus*, axolotls, crustaceans and hydrozoans [29,34•,36,37,38•,39••,41,42••].

Transgenesis also allows us to generate tools for clonal analysis (using the Cre/lox, Flp/FRT or phiC31 recombinases, [75,79]) and genetically-controlled cell ablation [80]. Such tools are now commonly used in zebrafish [43–49], but they also seem applicable to other regenerative models.

Finally, transgenic lines that express fluorescent proteins allow us to study regeneration through live imaging, to directly trace cell behaviours and cell fates. Continuous live imaging is challenging because of the long duration of the process and the mobility of adult animals, which is often necessary for their survival. Nevertheless, significant progress on this front has been made in zebrafish [81–83], hydrozoans [84,85] and *Parhyale* (Alwes and Averof, unpublished).

Altogether, a variety of new genetic approaches are becoming accessible in classic regenerative models. Some of these are applicable to a wide range of animals and may contribute to the emergence of new models for regenerative studies.

New model organisms

The value of genetically tractable models is best illustrated by the recent introduction of zebrafish as a model for studying regeneration of different organs, including fins, heart and brain (reviewed in [86]). Zebrafish is currently the only experimental model where we can study complex organ regeneration using advanced lineage-tracing tools, gene editing and forward genetic screens. These approaches have been used to make very substantial discoveries on the mechanisms that initiate regeneration, progenitor cells and key signalling events (e.g. [46,64,87–89,90•,91]).

Given the rich history of classic regenerative models, the impact of new technologies and the experimental power of zebrafish, it is reasonable to ask whether new models are needed in the field. What could new models offer?

There are at least two ways in which additional models can contribute to regenerative studies. First, each model comes with different opportunities for performing experiments. Different organisms are best suited for genetics, microsurgical experiments, biochemistry or live imaging; each brings traits that aid some types of experimentation and hamper others. Vertebrate developmental biology provides a nice example of this complementarity, with microsurgical approaches in *Xenopus* and chick having played a complementary role to genetics in mouse and

zebrafish. Second, it is unclear whether the ability to regenerate has a common evolutionary origin or evolved independently in different branches of the animal phylogeny. Its patchy distribution and evolutionary plasticity [3,4,6,7,10,11,13••] suggest that, even if regeneration is an ancestral trait, the mechanisms by which it occurs change during evolution, producing significant diversity among species. Only comparative studies sampling different species can reveal whether common principles/mechanisms of regeneration — due to shared ancestry or convergent evolution — exist among diverse animals. Common mechanisms might relate to the properties of progenitor cells, the re-use of mechanisms that operate during embryonic development, triggering signals following injury, de-differentiation, etc.

With this rationale in mind, several research groups have started to explore new experimental models, bringing new facets of regeneration, wider phylogenetic sampling and new tools into the field (see Figure 2). The new models include *Hofstenia*, a member of the acoels, which share some striking similarities with planarians but belong to a separate phylum that may represent one of the earliest bilaterian branches [92•], *Hydractinia* and *Nematostella*, representing two widely divergent clades of the cnidarians [34•,85,93–96], and the spiny mouse *Acomys*, a mammal with an unusual ability to shed and regenerate its skin [8•]. Our own efforts have focused on the crustacean *Parhyale hawaiensis*, a system that combines regenerative ability with genetic tractability and live imaging [42••].

A new arthropod model and what we have learned

In this section we give an account of what we have learnt after 8 years of regenerative work with *Parhyale*. This account is meant to encourage the exploration of new experimental models.

The ability to regenerate limbs is widespread in arthropods [97,98]. In spite of abundant (and fascinating) classic literature on regeneration in cockroaches, crabs and other arthropods (e.g. [99,100]), genetic approaches to arthropod limb regeneration have been limited. The prime genetic model, *Drosophila*, can only regenerate tissues with a low degree of complexity and differentiation (e.g. imaginal discs [101]). Hemimetabolous insects can regenerate their limbs during nymphal stages, but lose this ability when they reach the adult stage; important studies have been carried out in cockroaches and more recently in the cricket *Gryllus* [73,102,103]. Apterygote insects and crustaceans (including *Parhyale*) are able to regenerate limbs during the entire course of their life [97,98].

Parhyale is a small amphipod crustacean, similar to the common sand-hopper, that lives in shallow-water marine habitats in the tropics. It is easy to culture in the laboratory, breeds throughout the year and is accessible at all developmental stages, from single-cell embryo to adult. The adults continue to molt and grow throughout their lifetime, and can fully regenerate their appendages (antennae, mouthparts, thoracic and abdominal limbs) at all ages. Limbs can be seen regenerating, enclosed within the exoskeleton of the amputated stump, within 1 week after amputation. They are released from the old exoskeleton and become functional after molting.

Parhyale was first introduced in the laboratory in 1997 [104] and has since been used for a range of developmental studies (e.g. [60,61,105–112]). Our laboratory has used transgenesis as the basis for bringing a range of genetic tools to *Parhyale*, including conditional gene expression, gene trapping, transposon-based mosaic analysis and fluorescent reporters for live imaging [33,42••,108,113]. Efforts in other labs have established gene knockdown approaches via RNAi and morpholinos [107,109], CRISPR-mediated gene editing [60,61] and generated transcriptome and genome resources.

These tools have thus far allowed us to explore what type of progenitor cells are used to regenerate limbs in *Parhyale*. Taking advantage of the stereotypic fate of each blastomere at the 8-cell *Parhyale* embryo [105] and microinjections that allow us to label the cells that derive from each blastomere by stably inserting transposable elements in its genome [33], we have been able to visualize the contributions that each cell lineage makes to regenerating limbs using a fluorescent marker [42••]. Our study showed that some of these lineages contribute to regenerated ectoderm (epidermis and neurons) and others contribute to regenerated mesoderm (muscles), but none contribute to both ectoderm and mesoderm, indicating that *Parhyale* uses different progenitors for regenerating ectodermal and mesodermal tissues. These progenitors appear to be committed to their germ-layer of origin and to reside locally in the amputated limb stump [42••]. This is reminiscent of the strategy used by vertebrates, where distinct progenitors give rise to ectodermal and mesodermal cell types [36,38•,39••,114], and differs from that of planarians, which relies on pluripotent stem cells [78••,115].

Exploiting a transgenic line that expresses a fluorescent marker in mesodermal cells, we identified a specific population of mesodermal cells that are closely associated with muscle fibers and resemble the satellite cells of vertebrates [42••]. Similar to vertebrate satellite cells, these cells also express *Pax3/7* family genes. By transplanting these cells from transgenically-marked donors to unmarked recipients, we could demonstrate that these cells can serve as progenitors for regenerating muscle and are thus also functionally equivalent to satellite cells [42••].

This result has some interesting evolutionary implications: if crustacean and vertebrate satellite cells are homologous, their origins must date back to the last common ancestors of protostomes and deuterostomes, at the base of the bilaterians. This raises interesting questions about the likely function of those satellite-like cells in early bilaterian animals (in the maintenance/repair of muscles, complete muscle regeneration, or both) and about their fate and possible functions in other extant bilaterian phyla. New experimental models will be required to answer these questions.

Conclusions

It is an exciting time to be studying regeneration. Until recently regenerative research was constrained by a lack of genetic approaches, but now widely applicable methods (RNAi, transcriptional profiling, CRISPR) can be used to approach century-old questions. The identity and commitment of progenitor cells can be probed by rigorous lineaging approaches, including the transplantation of genetically-marked cells, clonal analysis and live imaging. Molecular regulators acting on these cells are becoming accessible by studying transcriptional responses in single cells. Which mechanisms are conserved or change during evolution can be answered by comparative studies on diverse species. One day we may be able to recount how regenerative capacities evolved, and assess why some animals have such striking regenerative abilities while others do not.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Morgan TH: *Regeneration*. The Macmillan Company; 1901.

2. Goss RJ: *Principles of Regeneration*. Academic Press; 1969.

3. Bely AE, Nyberg KG: **Evolution of animal regeneration: re-emergence of a field**. *Trends Ecol Evol (Amst)* 2010, **25**:161-170.

4. Brockes JP, Kumar A, Velloso CP: **Regeneration as an evolutionary variable**. *J Anat* 2001, **199**:3-11.

5. Watanabe H, Hoang VT, Mättner R, Holstein TW: **Immortality and the base of multicellular life: lessons from cnidarian stem cells**. *Seminars Cell Dev Biol* 2009, **20**:1114-1125.

6. Bely AE, Sikes JM: **Latent regeneration abilities persist following recent evolutionary loss in asexual annelids**. *Proc Natl Acad Sci U S A* 2010, **107**:1464-1469.

7. Liu SY, Selck C, Friedrich B, Lutz R, Vila-Farré M, Dahl A, Brandl H, Lakshmanaperumal N, Henry I, Rink JC: **Reactivating head regrowth in a regeneration-deficient planarian species**. *Nature* 2013, **500**:81-84.

8. • Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer TM, Maden M: **Skin shedding and tissue regeneration in African spiny mice (*Acomys*)**. *Nature* 2012, **489**:561-565.
The paper demonstrates that, unlike many other mammals, spiny mice (*Acomys*) have the ability to regenerate skin over a large surface of their body. This establishes *Acomys* as a new regenerative model within the mammals.

9. Seifert AW, Monaghan JR, Smith MD, Pasch B, Stier AC, Michonneau F, Maden M: **The influence of fundamental traits on mechanisms controlling appendage regeneration**. *Biol Rev Camb Philos Soc* 2012, **87**:330-345.

10. Sikes JM, Newmark PA: **Restoration of anterior regeneration in a planarian with limited regenerative ability**. *Nature* 2013, **500**:77-80.

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

12. Seifert AW, Maden M: **New insights into vertebrate skin regeneration**. *Int Rev Cell Mol Biol* 2014, **310**:129-169.

13. •• Sandoval-Guzmán T, Wang H, Khattak S, Schuez M, Roensch K, Nacu E, Tazaki A, Joven A, Tanaka EM, Simon A: **Fundamental differences in dedifferentiation and stem cell recruitment during skeletal muscle regeneration in two salamander species**. *Cell Stem Cell* 2014, **14**:174-187.
The paper performs lineage tracing on satellite cells and myofibres in axolotls and newts using the *Cre/lox* system, revealing that regenerated muscle derives from different progenitors in each species. A striking example of differences in regeneration among closely related animals.

14. Tanaka EM, Reddien PW: **The cellular basis for animal regeneration**. *Dev Cell* 2011, **21**:172-185.

15. Brockes JP, Kumar A: **Comparative aspects of animal regeneration**. *Annu Rev Cell Dev Biol* 2008, **24**:525-549.

16. St Johnston D: **The art and design of genetic screens: *Drosophila melanogaster***. *Nat Rev Genet* 2002, **3**:176-188.

17. Sanchez Alvarado A, Yamanaka S: **Rethinking differentiation: stem cells, regeneration, and plasticity**. *Cell* 2014, **157**:110-119.

18. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP *et al.*: **The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio***. *Development* 1996, **123**:1-36.

19. Rojas-Muñoz A, Rajadhyksha S, Gilmour D, van Bebber F, Antos C, Rodríguez Esteban C, Nüsslein-Volhard C, Izpisua Belmonte JC: **ErbB2 and ErbB3 regulate amputation-induced proliferation and migration during vertebrate regeneration**. *Dev Biol* 2009, **327**:177-190.

20. Chen C-H, Merriman AF, Savage J, Willer J, Wahlig T, Katsanis N, Yin VP, Poss KD: **Transient laminin beta 1a induction defines the wound epidermis during zebrafish fin regeneration**. *PLoS Genet* 2015, **11**:e1005437.

21. • Poss KD, Nechiporuk A, Hillam AM, Johnson SL, Keating MT: **Mps1 defines a proximal blastemal proliferative compartment essential for zebrafish fin regeneration**. *Development* 2002, **129**:5141-5149.
The paper describes a forward genetic screen for temperature-sensitive mutations affecting fin regeneration in zebrafish. The authors identify a gene that would have been missed in conventional screens due to lethality at embryonic stages, highlighting the challenge of recovering regenerative mutants (also see [22–24]).

22. Nechiporuk A, Poss KD, Johnson SL, Keating MT: **Positional cloning of a temperature-sensitive mutant emmental reveals a role for sly1 during cell proliferation in zebrafish fin regeneration**. *Dev Biol* 2003, **258**:291-306.

23. Whitehead GG, Makino S, Lien C-L, Keating MT: **fgf20 is essential for initiating zebrafish fin regeneration**. *Science* 2005, **310**:1957-1960.

24. Makino S, Whitehead GG, Lien C-L, Kim S, Jhawar P, Kono A, Kawata Y, Keating MT: **Heat-shock protein 60 is required for blastema formation and maintenance during regeneration**. *Proc Natl Acad Sci U S A* 2005, **102**:14599-14604.

25. • Reddien PW, Bermange AL, Murfitt KJ, Jennings JR, Sanchez Alvarado A: **Identification of genes needed for regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in planaria**. *Dev Cell* 2005, **8**:635-649.

The paper describes a large RNAi screen to identify genes that influence regeneration in the planarian *Schmidtea*. One of the most comprehensive screens for genes that regulate tissue homeostasis and regeneration in planarians.

26. Rink JC, Vu HT-K, Sánchez Alvarado A: **The maintenance and regeneration of the planarian excretory system are regulated by EGFR signaling**. *Development* 2011, **138**:3769-3780.

27. Kroll KL, Amaya E: **Transgenic *Xenopus* embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation**. *Development* 1996, **122**:3173-3183.

28. Sobkow L, Epperlein HH, Herklotz S, Straube WL, Tanaka EM: **A germline GFP transgenic axolotl and its use to track cell fate: dual origin of the fin mesenchyme during development and the fate of blood cells during regeneration**. *Dev Biol* 2006, **290**:386-397.

29. Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TCG: **Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis**. *Proc Natl Acad Sci U S A* 2006, **103**:6208-6211.

30. Suster ML, Kikuta H, Urasaki A, Asakawa K, Kawakami K: **Transgenesis in zebrafish with the tol2 transposon system**. *Methods Mol Biol* 2009, **561**:41-63.

31. Casco-Robles MM, Yamada S, Miura T, Nakamura K, Haynes T, Maki N, Del Rio-Tsonis K, Tsonis PA, Chiba C: **Expressing exogenous genes in newts by transgenesis**. *Nat Protoc* 2011, **6**:600-608.

32. Shinmyo Y, Mito T, Matsushita T, Sarashina I, Miyawaki K, Ohuchi H, Noji S: **piggyBac-mediated somatic transformation of the two-spotted cricket, *Gryllus bimaculatus***. *Dev Growth Differ* 2004, **46**:343-349.

33. Pavlopoulos A, Averof M: **Establishing genetic transformation for comparative developmental studies in the crustacean *Parhyale hawaiensis***. *Proc Natl Acad Sci U S A* 2005, **102**:7888-7893.

34. • Künzel T, Heiermann R, Frank U, Müller W, Tilmann W, Bause M, Nonn A, Helling M, Schwarz RS, Plickert G: **Migration and differentiation potential of stem cells in the cnidarian *Hydractinia* analysed in eGFP-transgenic animals and chimeras**. *Dev Biol* 2010, **348**:120-129.

The paper employs transplantation from transgenic, EGFP-labeled donors to demonstrate that migratory interstitial stem cells of *Hydractinia* are totipotent.

35. Renfer E, Amon-Hassenzahl A, Steinmetz PR, Technau U: **A muscle-specific transgenic reporter line of the sea anemone, *Nematostella vectensis***. *Proc Natl Acad Sci U S A* 2010, **107**:104-108.

36. Gargioli C, Slack JM: **Cell lineage tracing during Xenopus tail regeneration**. *Development* 2004, **131**:2669-2679.

37. Tu S, Johnson SL: **Clonal analyses reveal roles of organ founding stem cells, melanocyte stem cells and melanoblasts in establishment, growth and regeneration of the adult zebrafish fin**. *Development* 2010, **137**:3931-3939.

38. • Tu S, Johnson SL: **Fate restriction in the growing and regenerating zebrafish fin**. *Dev Cell* 2011, **20**:725-732.

The paper uses transposon insertions as clonal markers, to determine the regenerative potential of different cell populations in the zebrafish fin.

39. •• Kragl M, Knapp D, Nacu E, Khattak S, Maden M, Epperlein HH, Tanaka EM: **Cells keep a memory of their tissue origin during axolotl limb regeneration**. *Nature* 2009, **460**:60-65.

The paper uses transplantation experiments from GFP-marked donors to assess the commitment of progenitors during axolotl limb regeneration, demonstrating the power of combining transplantation with basic transgenic tools. The authors conclude that axolotl regeneration relies on lineage-restricted progenitors.

40. Khattak S, Schuez M, Richter T, Knapp D, Haigo SL, Sandoval-Guzmán T, Hradlikova K, Duemmler A, Kerney R, Tanaka EM: **Germline transgenic methods for tracking cells and testing gene function during regeneration in the axolotl**. *Stem Cell Rep* 2013, **1**:90-103.

41. Siebert S, Anton-Erxleben F, Bosch TCG: **Cell type complexity in the basal metazoan *Hydra* is maintained by both stem cell based mechanisms and transdifferentiation**. *Dev Biol* 2008, **313**:13-24.

42. •• Konstantinides N, Averof M: **A common cellular basis for muscle regeneration in arthropods and vertebrates**. *Science* 2014, **343**:788-791.

The paper establishes the crustacean *Parhyale* as a genetically tractable model for regenerative studies. Demonstrates that *Parhyale* limb regeneration relies on lineage-restricted progenitors and identifies a muscle progenitor that resembles the vertebrate satellite cells.

43. Jopling C, Sleep E, Raya M, Martí M, Raya A, Belmonte JCI: **Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation**. *Nature* 2010, **464**:606-609.

44. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, Evans T, MacRae CA, Stainier DYR, Poss KD: **Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes**. *Nature* 2010, **464**:601-605.

45. Kroehne V, Freudenreich D, Hans S, Kaslin J, Brand M: **Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors**. *Development* 2011, **138**:4831-4841.

46. Knopf F, Hammond C, Chekuru A, Kurth T, Hans S, Weber CW, Mahatma G, Fisher S, Brand M, Schulte-Merker S *et al.*: **Bone regenerates via dedifferentiation of osteoblasts in the zebrafish fin**. *Dev Cell* 2011, **20**:713-724.

47. Singh SP, Holdway JE, Poss KD: **Regeneration of amputated zebrafish fin rays from de novo osteoblasts**. *Dev Cell* 2012, **22**:879-886.

48. Zhang R, Han P, Yang H, Ouyang K, Lee D, Lin Y-F, Ocorr K, Kang G, Chen J, Stainier DYR *et al.*: **In vivo cardiac reprogramming contributes to zebrafish heart regeneration**. *Nature* 2013, **498**:497-501.

49. Gupta V, Poss KD: **Clonally dominant cardiomyocytes direct heart morphogenesis**. *Nature* 2013, **484**:479-484.

50. Gilles AF, Averof M: **Functional genetics for all: engineered nucleases, CRISPR and the gene editing revolution**. *EvoDevo* 2014, **5**:43.

51. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh J-RJ, Joung JK: **Efficient genome editing in zebrafish using a CRISPR-Cas system**. *Nat Biotechnol* 2013, **31**:227-229.

52. Blitz IL, Biesinger J, Xie X, Cho KWY: **Biallelic genome modification in F0 *Xenopus tropicalis* embryos using the CRISPR/Cas system**. *Genesis* 2013, **51**:827-834.

53. Flowers GP, Timberlake AT, Mclean KC, Monaghan JR, Crews CM: **Highly efficient targeted mutagenesis in axolotl using Cas9 RNA-guided nuclease**. *Development* 2014, **141**:2165-2171.

54. Fei J-F, Schuez M, Tazaki A, Taniguchi Y, Roensch K, Tanaka EM: **CRISPR-mediated genomic deletion of Sox2 in the axolotl shows a requirement in spinal cord neural stem cell amplification during tail regeneration**. *Stem Cell Rep* 2014, **3**:444-459.

55. **Mutagenesis in newts: protocol for iberian ribbed newts**. *Methods in Molecular Biology*. Springer; 2016:: 119-126.

56. Bannister S, Antonova O, Polo A, Lohs C, Hallay N, Valinciute A, Raible F, Tessmar-Raible K: **TALENs mediate efficient and heritable mutation of endogenous genes in the marine annelid *Platynereis dumerilii***. *Genetics* 2014, **197**:77-89.

57. Ikmi A, McKinney SA, Delventhal KM, Gibson MC: **TALEN and CRISPR/Cas9-mediated genome editing in the early-branching metazoan *Nematostella vectensis***. *Nat Commun* 2014, **5**:5486.

58. Watanabe T, Ochiai H, Sakuma T, Horch HW, Hamaguchi N, Nakamura T, Bando T, Ohuchi H, Yamamoto T, Noji S *et al.*: **Non-transgenic genome modifications in a hemimetabolous insect**

using zinc-finger and TAL effector nucleases. *Nat Commun* 2012, **3**:1017-1018.

59. Awata H, Watanabe T, Hamanaka Y, Mito T, Noji S, Mizunami M: **Knockout crickets for the study of learning and memory: dopamine receptor Dop1 mediates aversive but not appetitive reinforcement in crickets**. *Sci Rep* 2015, **5**:15885.

60. Martin A, Serano JM, Jarvis E, Bruce HS, Wang J, Ray S, Barker CA, O'Connell LC, Patel NH: **CRISPR/Cas9 mutagenesis reveals versatile roles of Hox genes in crustacean limb specification and evolution**. *Curr Biol* 2016, **26**:14-26.

61. Serano JM, Martin A, Liubicich DM, Jarvis E, Bruce HS, La K, Browne WE, Grimwood J, Patel NH: **Comprehensive analysis of Hox gene expression in the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2016, **409**:297-309.

62. Eisenhoffer GT, Kang H, Sánchez Alvarado A: **Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian *Schmidtea mediterranea***. *Cell Stem Cell* 2008, **3**:327-339.

63. Hemmrich G, Khalturin K, Boehm A-M, Puchert M, Anton-Erxleben F, Wittlieb J, Klostermeier UC, Rosenstiel P, Oberg H-H, Domazet-Lošo T *et al.*: **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* 2012, **29**:3267-3280.

64. Nachtrab G, Kikuchi K, Tornini VA, Poss KD: **Transcriptional components of anteroposterior positional information during zebrafish fin regeneration**. *Development* 2013, **140**:3754-3764.

65. Sousounis K, Looso M, Maki N, Ivester CJ, Braun T, Tsonis PA: **Transcriptome analysis of newt lens regeneration reveals distinct gradients in gene expression patterns**. *PLoS ONE* 2013, **8**:e61445.

66. Stewart R, Rascón CA, Tian S, Nie J, Barry C, Chu L-F, Ardalani H, Wagner RJ, Probasco MD, Bolin JM *et al.*: **Comparative RNA-seq analysis in the unsequenced axolotl: the oncogene burst highlights early gene expression in the blastema**. *PLoS Comp Biol* 2013, **9**:e1002936.

67. Lee-Liu D, Moreno M, Almonacid LI, Tapia VS, Muñoz R, Marées von J, Gaete M, Melo F, Larraín J: **Genome-wide expression profile of the response to spinal cord injury in *Xenopus laevis* reveals extensive differences between regenerative and non-regenerative stages**. *Neural Dev* 2014, **9**:12.

68. Petersen HO, Höger SK, Looso M, Lengfeld T, Kuhn A, Warnken U, Nishimiya-Fujisawa C, Schnölzer M, Krüger M, Özbek S *et al.*: **A comprehensive transcriptomic and proteomic analysis of hydra head regeneration**. *Mol Biol Evol* 2015, **32**:1928-1947.

69. Wu C-C, Kruse F, Vasudevarao MD, Junker JP, Zebrowski DC, Fischer K, Noël ES, Grün D, Berezikov E, Engel FB *et al.*: **Spatially resolved genome-wide transcriptional profiling identifies BMP signaling as essential regulator of zebrafish cardiomyocyte regeneration**. *Dev Cell* 2016, **36**:36-49.

70. van Wolfswinkel JC, Wagner DE, Reddien PW: **Single-cell analysis reveals functionally distinct classes within the planarian stem cell compartment**. *Cell Stem Cell* 2014, **15**:326-339.

71. Wurtzel O, Cote LE, Poirier A, Satija R, Regev A, Reddien PW: **A generic and cell-type-specific wound response precedes regeneration in planarians**. *Dev Cell* 2015, **35**:632-645.

72. Forsthoefel DJ, James NP, Escobar DJ, Stary JM, Vieira AP, Waters FA, Newmark PA: **An RNAi screen reveals intestinal regulators of branching morphogenesis, differentiation, and stem cell proliferation in planarians**. *Dev Cell* 2012, **23**:691-704.

73. Bando T, Ishimaru Y, Kida T, Hamada Y, Matsuoka Y, Nakamura T, Ohuchi H, Noji S, Mito T: **Analysis of RNA-Seq data reveals involvement of JAK/STAT signalling during leg regeneration in the cricket *Gryllus bimaculatus***. *Development* 2013, **140**:959-964.

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

75. Kretzschmar K, Watt FM: **Lineage tracing**. *Cell* 2012, **148**:33-45.

76. Browne EN: **The production of new hydranths in Hydra by the insertion of small grafts**. *J Exp Zool* 1909, **7** 1-U13.

77. • Benton JL, Kery R, Li J, Noonin C, Söderhäll I, Beltz BS: **Cells from the immune system generate adult-born neurons in crayfish**. *Dev Cell* 2014, **30**:322-333.
The paper uses EdU labelling and transplantation to show that cells circulating in the blood can serve as progenitors of neurons in adult crayfish. Illustrates how classic approaches in new experimental models can yield novel and unexpected insights.

78. •• Wagner DE, Wang IE, Reddien PW: **Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration**. *Science* 2011, **332**:811-816.
The paper provides the long-sought proof that planarian neoblasts are totipotent progenitors. Using elegant transplantation experiments between genetically distinct donors and recipients, the authors prove that at least some neoblasts (c-neoblasts) are totipotent.

79. Richier B, Salecker I: **Versatile genetic paintbrushes: brainbow technologies**. *WIREs Dev Biol* 2014, **4**:161-180.

80. Curado S, Stainier DY, Anderson RM: **Nitroreductase-mediated cell/tissue ablation in zebrafish: a spatially and temporally controlled ablation method with applications in developmental and regeneration studies**. *Nat Protoc* 2008, **3**:948-954.

81. Mateus R, Pereira T, Sousa S, de Lima JE, Pascoal S, Saúde L, Jacinto A: **In vivo cell and tissue dynamics underlying zebrafish fin fold regeneration**. *PLoS ONE* 2012, **7**:e51766.

82. Chen C-H, Durand E, Wang J, Zon LI, Poss KD: **Zebrafish transgenic lines for in vivo bioluminescence imaging of stem cells and regeneration in adult zebrafish**. *Development* 2013, **140**:4988-4997.

83. Barbosa JS, Sanchez-Gonzalez R, Di Giaimo R, Baumgart EV, Theis FJ, Götz M, Ninkovic J: **Neurodevelopment. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain**. *Science* 2015, **348**:789-793.

84. Khalturin K, Anton-Erxleben F, Milde S, Plötz C, Wittlieb J, Hemmrich G, Bosch TCG: **Transgenic stem cells in *Hydra* reveal an early evolutionary origin for key elements controlling self-renewal and differentiation**. *Dev Biol* 2007, **309**:32-44.

85. Bradshaw B, Thompson K, Frank U: **Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian *Hydractinia echinata***. *Elife* 2015, **4**:e05506.

86. Gemberling M, Bailey TJ, Hyde DR, Poss KD: **The zebrafish as a model for complex tissue regeneration**. *Trends Genet* 2013, **29**:611-620.

87. Kikuchi K, Holdway JE, Major RJ, Blum N, Dahn RD, Begemann G, Poss KD: **Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration**. *Dev Cell* 2011, **20**:397-404.

88. Kyritsis N, Kizil C, Zocher S, Kroehne V, Kaslin J, Freudenreich D, Iltzsche A, Brand M: **Acute inflammation initiates the regenerative response in the adult zebrafish brain**. *Science* 2012, **338**:1353-1356.

89. Blum N, Begemann G: **Retinoic acid signaling controls the formation, proliferation and survival of the blastema during adult zebrafish fin regeneration**. *Development* 2012, **139**:107-116.

90. • Wehner D, Cizelsky W, Vasudevaro MD, Ozhan G, Haase C, Kagermeier-Schenk B, Röder A, Dorsky RI, Moro E, Argenton F *et al.*: **Wnt/β-catenin signaling defines organizing centers that orchestrate growth and differentiation of the regenerating zebrafish caudal fin**. *CellReports* 2014, **6**:467-481.
The paper explores the role of Wnt signaling in zebrafish fin regeneration, illustrating the wide range of approaches that can be used to study regeneration in this model, including transgenic reporters of signaling, conditional and tissue-specific genetic manipulation, expression profiling and functional rescue experiments.

91. Mahmoud AI, O'Meara CC, Gemberling M, Zhao L, Bryant DM, Zheng R, Gannon JB, Cai L, Choi W-Y, Egnaczyk GF *et al.*: **Nerves**

regulate cardiomyocyte proliferation and heart regeneration. *Dev Cell* 2015, **34**:387-399.

92. • Srivastava M, Mazza-Curll KL, van Wolfswinkel JC, Reddien PW: **Whole-body acoel regeneration is controlled by Wnt and Bmp-Admp signaling**. *Curr Biol* 2014, **24**:1107-1113.
The paper establishes the acoel *Hostenia* as an experimental model for studying regeneration, establishing transcriptome-based and RNAi-based approaches. The phylogenetic position of acoels makes them an interesting group for comparative studies.

93. Plickert G, Frank U, Müller WA: ***Hydractinia*, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency**. *Int J Dev Biol* 2012, **56**:519-534.

94. Passamaneck YJ, Martindale MQ: **Cell proliferation is necessary for the regeneration of oral structures in the anthozoan cnidarian *Nematostella vectensis***. *BMC Dev Biol* 2012, **12**:34.

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

96. Amiel AR, Johnston HT, Nedoncelle K, Warner JF, Ferreira S, Röttinger E: **Characterization of morphological and cellular events underlying oral regeneration in the sea anemone, *Nematostella vectensis***. *Int J Mol Sci* 2015, **16**:28449-28471.

97. Hopkins PM: **Regeneration in crustaceans and insects**. *eLS* 2001. [no volume].

98. Maruzzo D, Bortolin F: **Arthropod regeneration**. In *Arthropod Biology and Evolution.* Edited by Minelli A. Springer-Verlag; 2013: 149-169.

99. Emmel VE: **A study of the differentiation of tissues in the regenerating crustacean limb**. *Am J Anat* 1910, **10**:109-158.

100. French V, Bryant PJ, Bryant SV: **Pattern regulation in epimorphic fields**. *Science* 1976, **193**:969-981.

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

102. Nakamura T, Mito T, Bando T, Ohuchi H, Noji S: **Dissecting insect leg regeneration through RNA interference**. *Cell Mol Life Sci* 2008, **65**:64-72.

103. Ishimaru Y, Nakamura T, Bando T, Matsuoka Y, Ohuchi H, Noji S, Mito T: **Involvement of dachshund and Distal-less in distal pattern formation of the cricket leg during regeneration**. *Sci Rep* 2015, **5**:8387.

104. Browne WE, Price AL, Gerberding M, Patel NH: **Stages of embryonic development in the amphipod crustacean, *Parhyale hawaiensis***. *genesis* 2005, **42**:124-149.

105. Gerberding M, Browne WE, Patel NH: **Cell lineage analysis of the amphipod crustacean *Parhyale hawaiensis* reveals an early restriction of cell fates**. *Development* 2002, **129**:5789-5801.

106. Extavour CG: **The fate of isolated blastomeres with respect to germ cell formation in the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2005, **277**:387-402.

107. Liubicich DM, Serano JM, Pavlopoulos A, Kontarakis Z, Protas ME, Kwan E, Chatterjee S, Tran KD, Averof M, Patel NH: **Knockdown of *Parhyale* Ultrabithorax recapitulates evolutionary changes in crustacean appendage morphology**. *Proc Natl Acad Sci U S A* 2009, **106**:13892-13896.

108. Pavlopoulos A, Kontarakis Z, Liubicich DM, Serano JM, Akam M, Patel NH, Averof M: **Probing the evolution of appendage specialization by Hox gene misexpression in an emerging model crustacean**. *Proc Natl Acad Sci U S A* 2009, **106**:13897-13902.

109. Özhan-Kizil G, Havemann J, Gerberding M: **Germ cells in the crustacean *Parhyale hawaiensis* depend on Vasa protein for their maintenance but not for their formation**. *Dev Biol* 2009, **327**:230-239.

110. Price AL, Modrell MS, Hannibal RL, Patel NH: **Mesoderm and ectoderm lineages in the crustacean *Parhyale hawaiensis* display intra-germ layer compensation**. *Dev Biol* 2010, **341**:256-266.

111. Vargas-Vila MA, Hannibal RL, Parchem RJ, Liu PZ, Patel NH: **A prominent requirement for single-minded and the ventral midline in patterning the dorsoventral axis of the crustacean *Parhyale hawaiensis***. *Development* 2010, **137**:3469-3476.

112. Hannibal RL, Price AL, Patel NH: **The functional relationship between ectodermal and mesodermal segmentation in the crustacean, *Parhyale hawaiensis***. *Dev Biol* 2012, **361**:427-438.

113. Kontarakis Z, Pavlopoulos A, Kiupakis A, Konstantinides N, Douris V, Averof M: **A versatile strategy for gene trapping and trap conversion in emerging model organisms**. *Development* 2011, **138**:2625-2630.

114. Rinkevich Y, Lindau P, Ueno H, Longaker MT, Weissman IL: **Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip**. *Nature* 2012, **476**:409-413.

115. Reddien PW, Alvarado AS: **Fundamentals of planarian regeneration**. *Annu Rev Cell Dev Biol* 2004, **20**:725-757.

116. Kato K, Orii H, Watanabe K, Agata K: **The role of dorsoventral interaction in the onset of planarian regeneration**. *Development* 1999, **126**:1031-1040.

117. Pan YA, Freundlich T, Weissman TA, Schoppik D, Wang XC, Zimmerman S, Ciruna B, Sanes JR, Lichtman JW, Schier AF: **Zebrabow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish**. *Development* 2013, **140**:2835-2846.

118. Backfisch B, Rajan VBV, Fischer RM, Lohs C, Arboleda E, Tessmar-Raible K, Raible F: **Stable transgenesis in the marine annelid *Platynereis dumerilii* sheds new light on photoreceptor evolution**. *Proc Natl Acad Sci U S A* 2013, **110**:193-198.

119. Zantke J, Bannister S, Rajan VBV, Raible F, Tessmar-Raible K: **Genetic and genomic tools for the marine annelid *Platynereis dumerilii***. *Genetics* 2014, **197**:19-31.

120. Heasman J: **Morpholino oligos: making sense of antisense?** *Dev Biol* 2002, **243**:209-214.

121. Conzelmann M, Williams EA, Tunaru S, Randel N, Shahidi R, Asadulina A, Berger J, Offermanns S, Jékely G: **Conserved MIP receptor-ligand pair regulates *Platynereis* larval settlement**. *Proc Natl Acad Sci U S A* 2013, **110**:8224-8229.

122. Duffy DJ, Plickert G, Kuenzel T, Tilmann W, Frank U: **Wnt signaling promotes oral but suppresses aboral structures in *Hydractinia* metamorphosis and regeneration**. *Development* 2010, **137**:3057-3066.

123. Sousounis K, Qi F, Yadav MC, Millán JL, Toyama F, Chiba C, Eguchi Y, Eguchi G, Tsonis PA: **A robust transcriptional program in newts undergoing multiple events of lens regeneration throughout their lifespan**. *Elife* 2015:4.

124. Love NR, Chen Y, Bonev B, Gilchrist MJ, Fairclough L, Lea R, Mohun TJ, Paredes R, Zeef LA, Amaya E: **Genome-wide analysis of gene expression during *Xenopus tropicalis* tadpole tail regeneration**. *BMC Dev Biol* 2011, **11**:1.

125. Achim K, Pettit J-B, Saraiva LR, Gavriouchkina D, Larsson T, Arendt D, Marioni JC: **High-throughput spatial mapping of single-cell RNA-seq data to tissue of origin**. *Nat Biotechnol* 2015, **33**:503-509.