A primer on regeneration

Centuries of observation have uncovered a diverse range of organisms capable of overcoming loss of tissue. The act of restoring lost anatomy and function is known as *regeneration*, and it is broadly represented in both plant and animal kingdoms. Cumulative studies have identified a series of events that take place during regeneration of complex animal structures. First, the organism recognizes damage and undergoes wound healing. Then, programmed cell death in the vicinity of the damaged tissue precedes proliferation and migration of cells that foster the development of replacement tissue. Finally, rearrangement of pre-existing tissue and integration with newly differentiated cells take place to restore the function and proportionality displayed previous to damage . Although the ability to regenerate is believed to be ancestrally common and lost throughout evolution, there is significant heterogeneity of some basic mechanisms displayed during regeneration in different animal species. Perhaps one of the most noticeable differences is the cellular source contributing to formation of the new tissue during regeneration. Organisms such as planarians and *Hydra* rely on active reservoirs of somatic pluripotent stem cells abundantly distributed throughout their bodies and maintained throughout their life. On the other hand, vertebrates rely mostly on progenitor cell activation and dedifferentiation, to regenerate cells with limited potential to regenerate specific structures. However, not all regenerative events rely on cellular replacement. Leading edge research has begun to uncover mechanisms involved in autonomous repair and functional regeneration of single cells – be it neurons or ciliated protozoa. The fact that organisms can achieve regeneration through diverse cellular sources is remarkable, but just as remarkable is the possibility that conserved molecular pathways could be activated to achieve regeneration in different species. Analysis of these pathways will contribute to understanding human development and potential avenues for regenerative medicine.

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A PRIMER ON REGENERATION

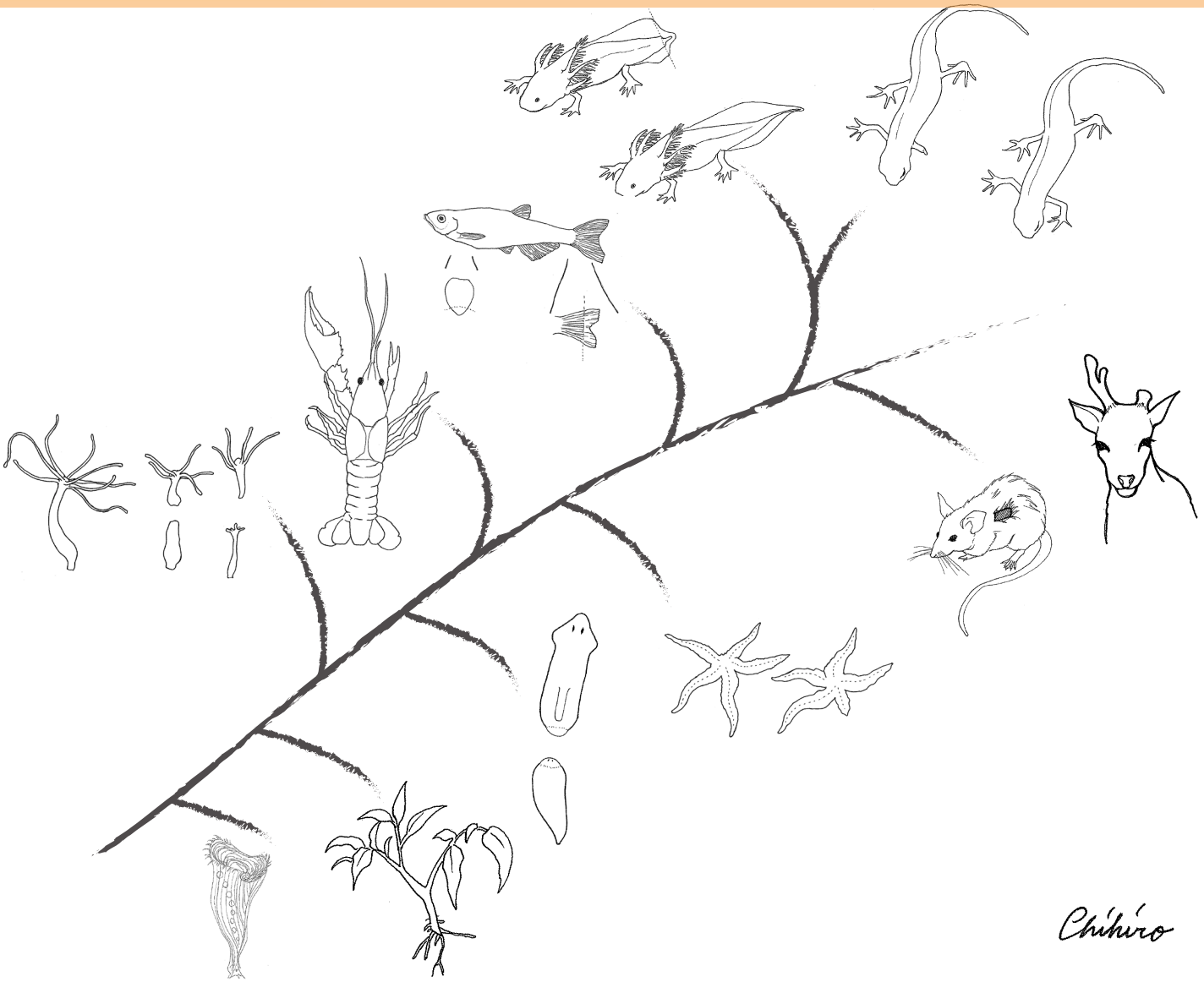
Labib Rouhana and Junichi Tasaki
Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435-0001

Aristotle was captivated by the observation that lizards were capable of re-growing a tail after having it cut (Aristotle, *Historia animalium*, chap. 17). Regeneration – the ability to redevelop lost body parts – has been displayed in myths and folktales for centuries. Today, accumulating evidence shows that regenerative events that may seem fictitious are a reality in a wide range of organisms, from unicellular ciliates to large plants and animals. The regenerative capacities of different organisms vary immensely, as some are restricted to specific tissues or periods of time during development, while others are capable of regenerating their entirety over uncountable occasions. The mechanisms involved in regeneration have mystified observers throughout history and left them wondering whether a cellular permit forgiving the loss of a limb or an eye could be uncovered and shared with us, the unlucky humans who seem obligated to get through life with only one set of body parts.

Over 300 years ago, the famous French entomologist René-Antoine Ferchault de Réaumur reported detailed observations of crayfish claw regeneration (Réaumur, 1712). Previous accounts existed regarding the regenerative capacity of lizard tails (described by Claude Perrault and Melchisedech Thevenot in 1688), human fingertips (reviewed by Roger, 1963), and the aforementioned crayfish limbs (Du Tertre, 1654). It was Réaumur's detailed accounting of the regenerative process that is often credited for the increased interest in the topic amongst the scientific community. Since, descriptions of regeneration events in vertebrates have been reported widely, ranging from limbs, tails and retinas of Urodele amphibians [i.e. newts, salamanders] (Spallanzani, 1768; Todd, 1823; Colluci, 1891; Wolff, 1895; Thorton, 1938; Oberpriller and Oberpriller, 1974) to hearts and fins of fish (Morgan, 1900; Poss et al., 2002), deer antlers (Goss, 1961), and skin of spiny mice (Seifert et al., 2012).

Even though the study of vertebrates and crustaceans has uncovered regenerative capabilities that surpass the expectations of past and present scientists, their capacity for regeneration remains relatively modest when compared to a collection of invertebrates that rely (at least partially) on asexual reproduction. Freshwater organisms belonging to the genus *Hydra* (named after the mythological multi-headed monster futilely decapitated by Hercules) and planarian flatworms can reproduce asexually through budding (or fission) followed by regeneration of missing structures. These organisms are not only able to re-grow lost body parts, but also their entire anatomy from a small piece of tissue. Thus, slicing a planarian into 20 different fragments could result in the formation of 20 completely functional descendents. Early reports describing the regenerative potential of these organisms (Trembley, 1744; Pallas, 1774) were followed by decades of experimental investigation based on amputations, dissections, transplantations and microscopic analyses. Ultimately, these studies were the foundation of current investigations using modern molecular techniques to identify the genes and cellular processes that drive regeneration (reviewed by Galliot, 2012; Elliot and Sanchez Alvarado, 2012). The revival of regeneration research in the era of molecular genomics, RNA-interference (a technique used to specifically inhibit gene function amenable to the study of most eukaryotes), and modern microscopy, has resulted in detailed experimental accounts of the regenerative processes in a wide range of organisms. Altogether, these studies have illustrated a few mechanistic commonalities and differences involved in regeneration of complex structures. These are detailed below:

1) **Distalization followed by intercalation -** Agata et al. (2007) proposed that a common phenomenon shared amongst complex regenerative events, be it a newt limb or the entire head of a polyp or a planarian, was the initiation of regenerative deployment by establishment of the most distal structure first (distalization) followed by a subsequent expansion of the structures in between (intercalation). This view contrasts from previous models in which the regenerative process was thought to take place as a progression from proximal to distal, akin to a mason laying bricks to build a wall. Normally, complex tissue regeneration establishes the identity of the furthest end of the missing tissue, and gradually develops the regions in between. Although perplexing at first sight, distalization and intercalation seems logical if one considers that embryonic development constitutes a continuously morphing and moving mass of cells that follow signaling gradients, and not a linear progression from one end to the organism to the other. Regeneration does not reinvent development; it applies pre-existing mechanisms observed through embryogenesis.



Phylogenetic distribution of regenerative organisms. Evolution of regenerative ability tends to decline as complexity increases through evolution. For instance, *Hydra* and planarians can regenerate their whole bodies, whereas regeneration in deer or spiny mouse is limited to certain parts of their body such as antlers or skin, respectably. Representatives from different phyla are illustrated in clockwise direction from top-left: *Hydra* (Cnidaria), crayfish (Crustacea), fish, axolotl and newt (Urodela), deer and spiny mouse (Mammalia), starfish (Echinodermata), planarian (Platyhelminthes), Plants, *Stentor* (Ciliophora). Phylogenetic distances and organisms are not drawn to scale. Illustration by Chihiro Uchiyama Tasaki.

2) **Programmed Cell Death and cellular proliferation –** Analyses of the initial events which follow tissue loss and wound healing in flies, planarians, frogs and mice have revealed that signals released by dying cells induce a proliferative response in progenitor cells of regenerating tissue (reviewed by Bergmann and Steller, 2010). There are two major modes by which cells die: Necrosis, which occurs when cells are exposed to unusual conditions or ruptured; and Apoptosis, in which the cell actively participates in its own demise. It is still unclear whether necrotic cells that arise from tissue damage release any molecules that specifically induce downstream regenerative events. On the other hand, studies in varied regenerative contexts support that a burst in apoptosis occurs following tissue amputation (reviewed by Bergmann and Steller, 2010). Apoptotic cells near the wound site release signaling factors that induce the increased proliferation of progenitor cells that are needed to support the redevelopment of missing tissue. Apoptosis also plays a role at later steps of the regenerative process, during which preexisting tissue rearrangement guides the functional connection and proportionality of new and old parts of the organism.

3) **"Stemness" and cellular sources for regeneration –** The presence of regenerative abilities in a wide range of organisms distributed throughout the animal kingdom suggests the evolutionary conservation of mechanisms involved in regeneration (Sanchez Alvarado and Tsonis, 2006; Baly, 2010). A difference that

has become apparent amongst the mechanisms that drive regeneration in different organisms regards the source of cells used when re-development of missing tissue is needed. "Simpler" organisms such as *Hydra*, planarians, acoels, sponges, and plants, rely on reservoirs of somatic stem cells classified as pluripotent (able to differentiate into any cell type) or highly multipotent (able to differentiate into many cell types), which continuously proliferate and differentiate to provide for growth and homeostatic maintenance. On the other hand, regenerative events in more complex organisms, such as regeneration of a vertebrate limb, heart, or retina, depends on cells with limited lineage differentiation potential, which often arise from dormant or dedifferentiated cells (reviewed by Jopling et al., 2011). Humans continuously repair their intestinal epithelium through use of small reservoirs of intestinal stem cells that continuously proliferate and differentiate into a handful of epithelial cell types (reviewed by Barker, 2014). These cells, however, would be expected to fail at restoring damaged tissue outside the intestine under normal conditions, due to their limited potency.

4) **The search for the "gene" in regeneration –** It may seem unsatisfactory to propose that cellular events that occur during regeneration are not exclusive to this phenomenon. Wound healing is a common process that occurs in regenerative and non-regenerative tissue. Growing limbs, retinas or heads are events that take place during normal embryonic development. The surprise is that so many organisms are capable of replicating embryonic processes as adults by reactivating developmental genetic pathways within the context of differentiated, previously grown tissue. So what then is the secret to regeneration? One key component is the availability of proliferative cells with the potential to differentiate into the cellular makeup of the missing tissue, whether these are obtained from reservoirs of stem cells or reactivation and reprogramming of partially differentiated cells that respond to injury. However, the wealthiest accumulation of stem cells does not ensure that regeneration will take place. This concept is beautifully demonstrated by three separate studies of planarians with decreased regenerative capabilities (Sikes and Newmark, 2013; Umesono et al., 2013; Liu et al., 2013). These studies showed that the evolutionary loss of head regeneration observed in posterior fragments of some planarian species was not due to insufficient populations of stem cells, but by differences in expression levels of components in the conserved wnt/β-catenin developmental pathway. More importantly, the researchers were able to turn back years of evolution and restore regeneration in normally non-regenerative planarian fragments by decreasing expression of the gene encoding for β-catenin. The influence of the wnt/β-catenin pathway on regeneration is not unique to planarians; this pathway also controls digit regeneration in mice (Takeo et al., 2013). Another developmental signaling circuit that can dictate mammalian digit regeneration outcomes is the noggin/Bone Morphogenic Protein (BMP) pathway. Yu et al. (2010) demonstrated that noggin inhibits capable digit regeneration, whereas the fate of non-regenerating amputation wounds was reversed by BMP treatments that re-initiate digit tip development at the wound. These amazing feats are encouraging to efforts in regenerative medicine, as they suggest that tinkering with the expression of single genetic units may activate regenerative capabilities.

Closing statements

The field of regenerative biology has been reinvigorated by advances in genomic and stem cell biology. Now more than ever we are able to learn about the different ways in which a multitude of organisms overcome loss of tissue. Even repair and regeneration of single cells, whether free-living (e.g. protozoan *Stentor*) or as part of complex structures (axonal regeneration in spinal cord neurons of lamprey), are being analyzed with a molecular lens (Slabodnick and Marshall, 2014; Smith et al., 2011). The study of regeneration not only reveals the secrets of this fascinating phenomenon, it uncovers developmental pathways of differentiation, molecules that influence the longevity of cells, roles of programmed cell death, and the control of cellular proliferation.

Acknowledgments

We thank Laura V. Rouhana for revising our manuscript and Chihiro Tasaki for her artistic talents.

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