

Model organisms and developmental biology

仲寒冰

zhonghb@sustc.edu.cn

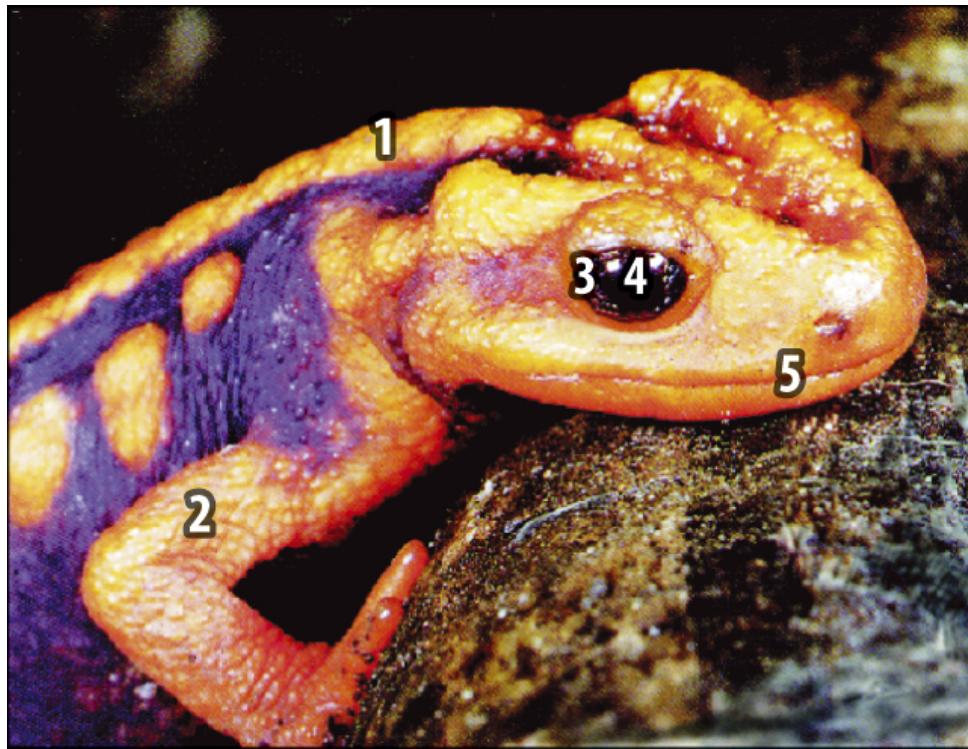
Regeneration

Regeneration is a homeostatic process that maintains or restores the original architecture and function of a tissue after damage in adult animal.

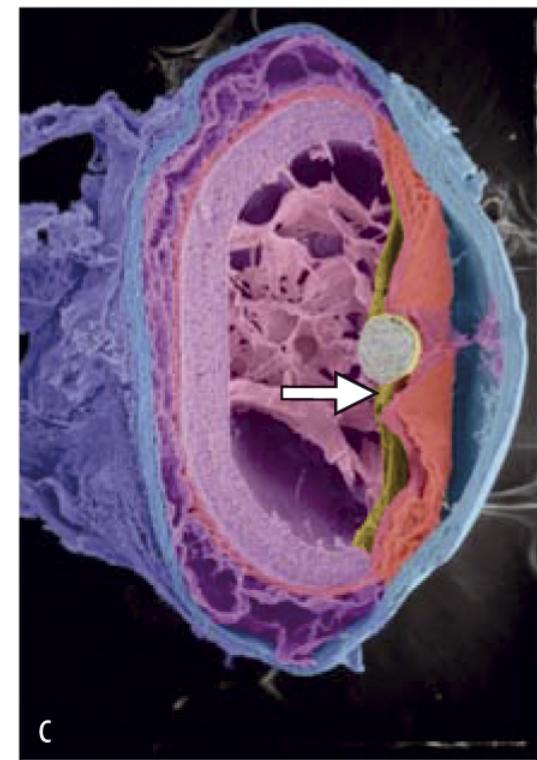
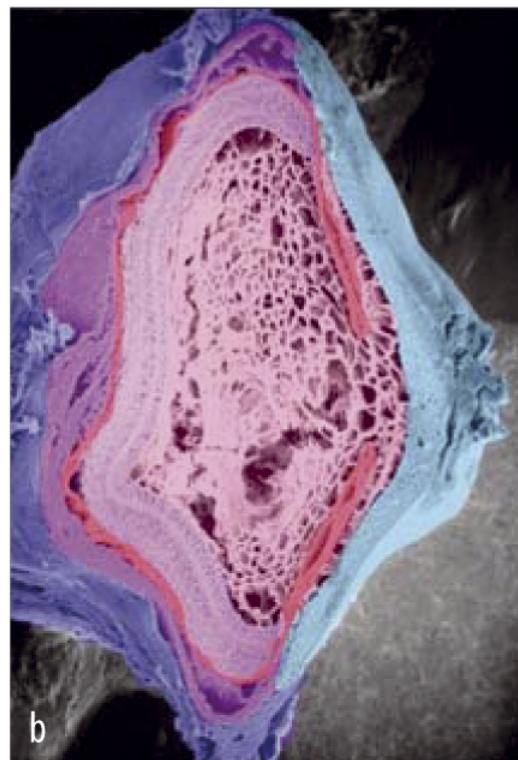
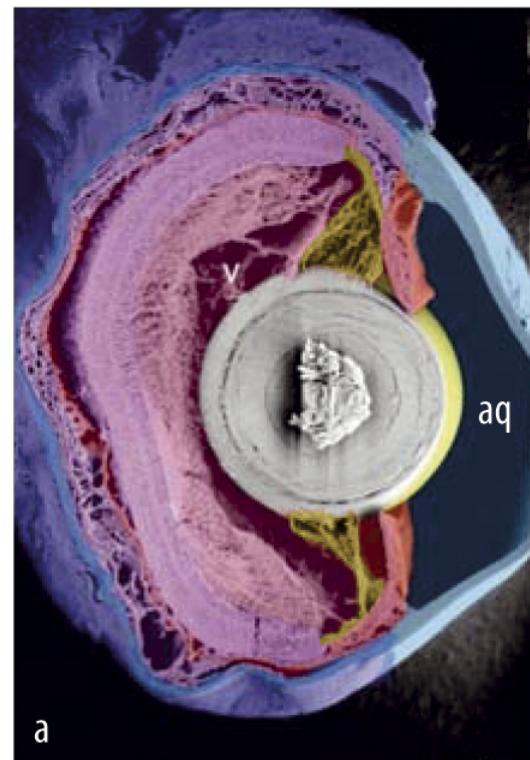
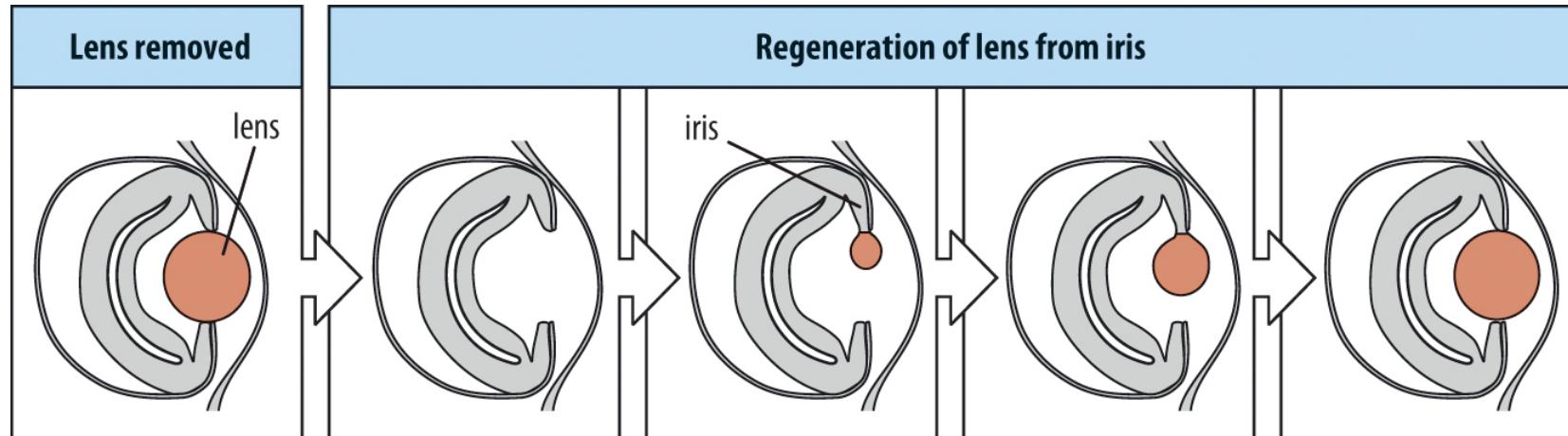
Surface epithelia and epithelia of many simple glands belong to the category of continuously renewing cell populations. The rate of cell turnover (i.e., the replacement rate) is characteristic of a specific epithelium. For example, the cells lining the small intestine are renewed every 4 to 6 days in humans.

Usually **cell renewal** is not considered as regeneration.

The capacity for regeneration in urodele amphibians



1, dorsal crest. 2, limbs. 3 and 4, retina and lens. 5, jaw.



Tylototriton verrucoosus Anderson
红瘰疣螈



Baidu 百科



Notophthalmus viridescens



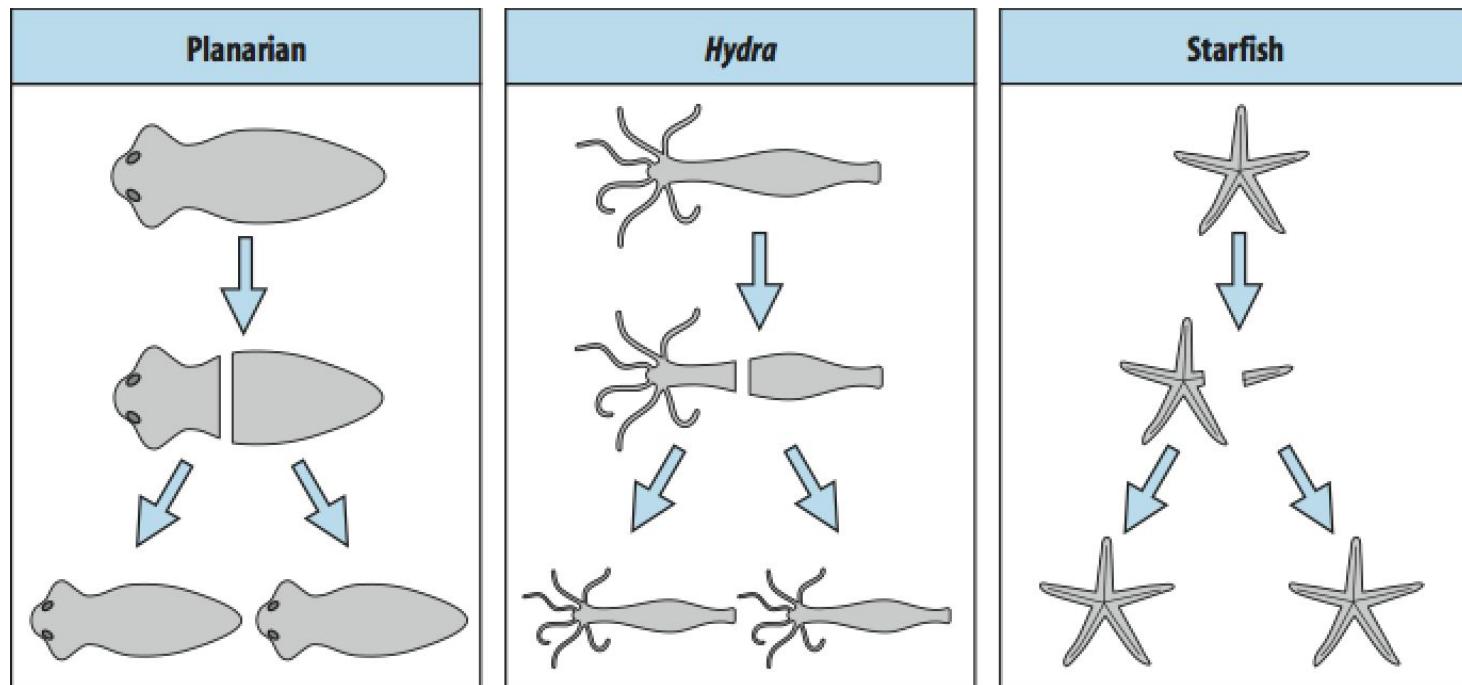
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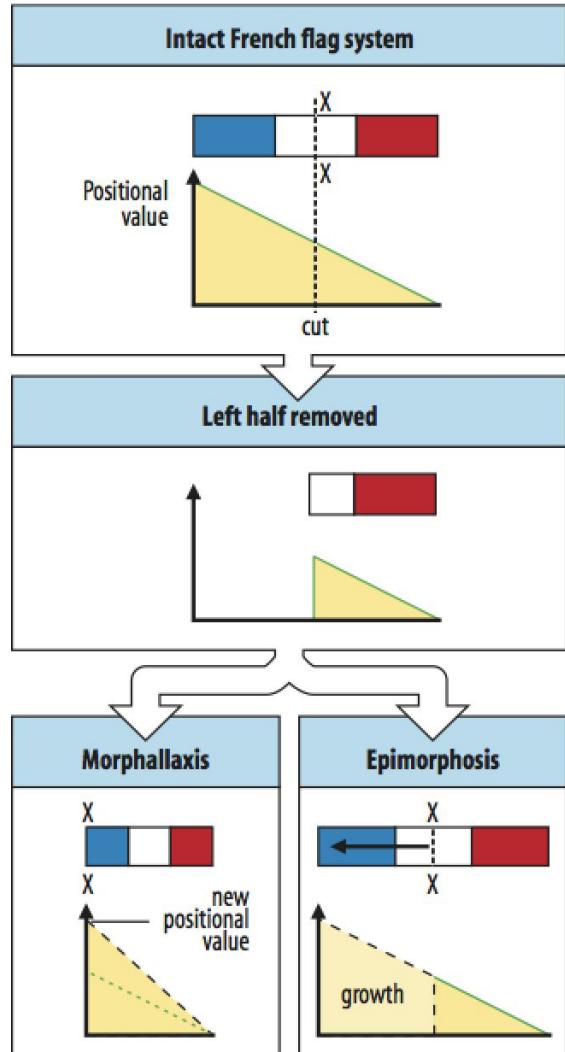
Axolotl, Mexican salamander (*Ambystoma mexicanum*)



Two types of regeneration

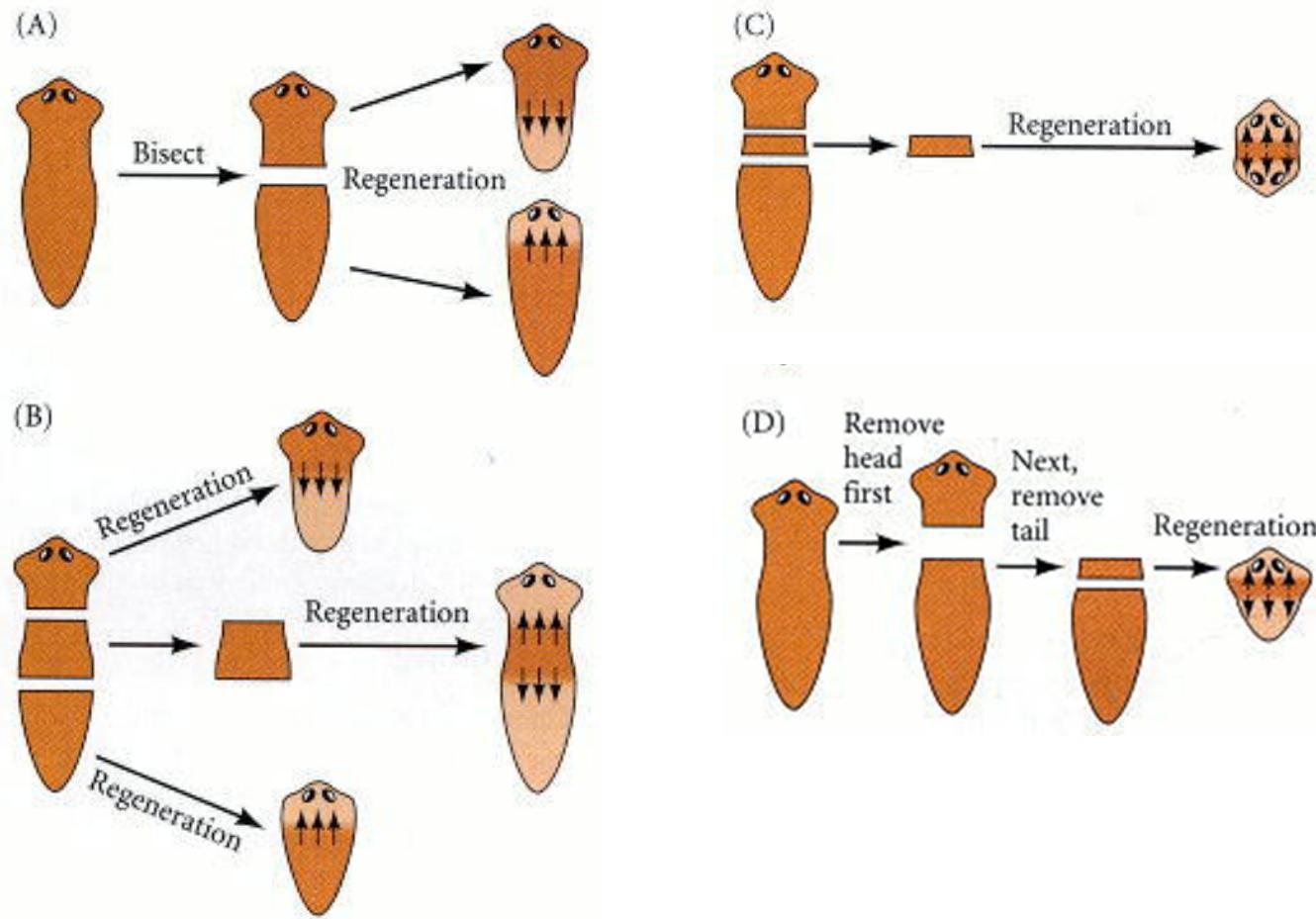


Morphallaxis (变形再生) and epimorphosis (割处再生)

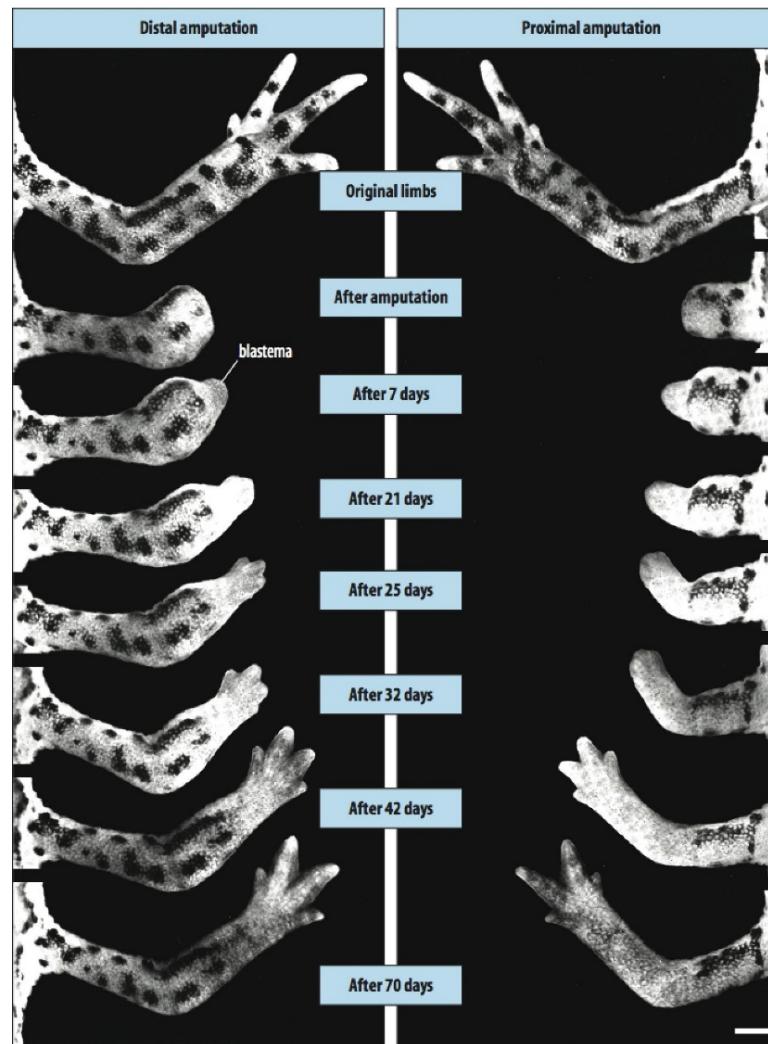


- Morphallaxis, there is little new cell division and growth, and regeneration of structure occurs mainly by the repatterning of existing tissue and the re-establishment of boundaries. Hydra.
- Epimorphosis, the growth of completely new, correctly patterned structures. A newt limb.

Flatworm regeneration and its limits



Regeneration of the forelimb in the red-spotted newt *Notophthalmus viridescens*



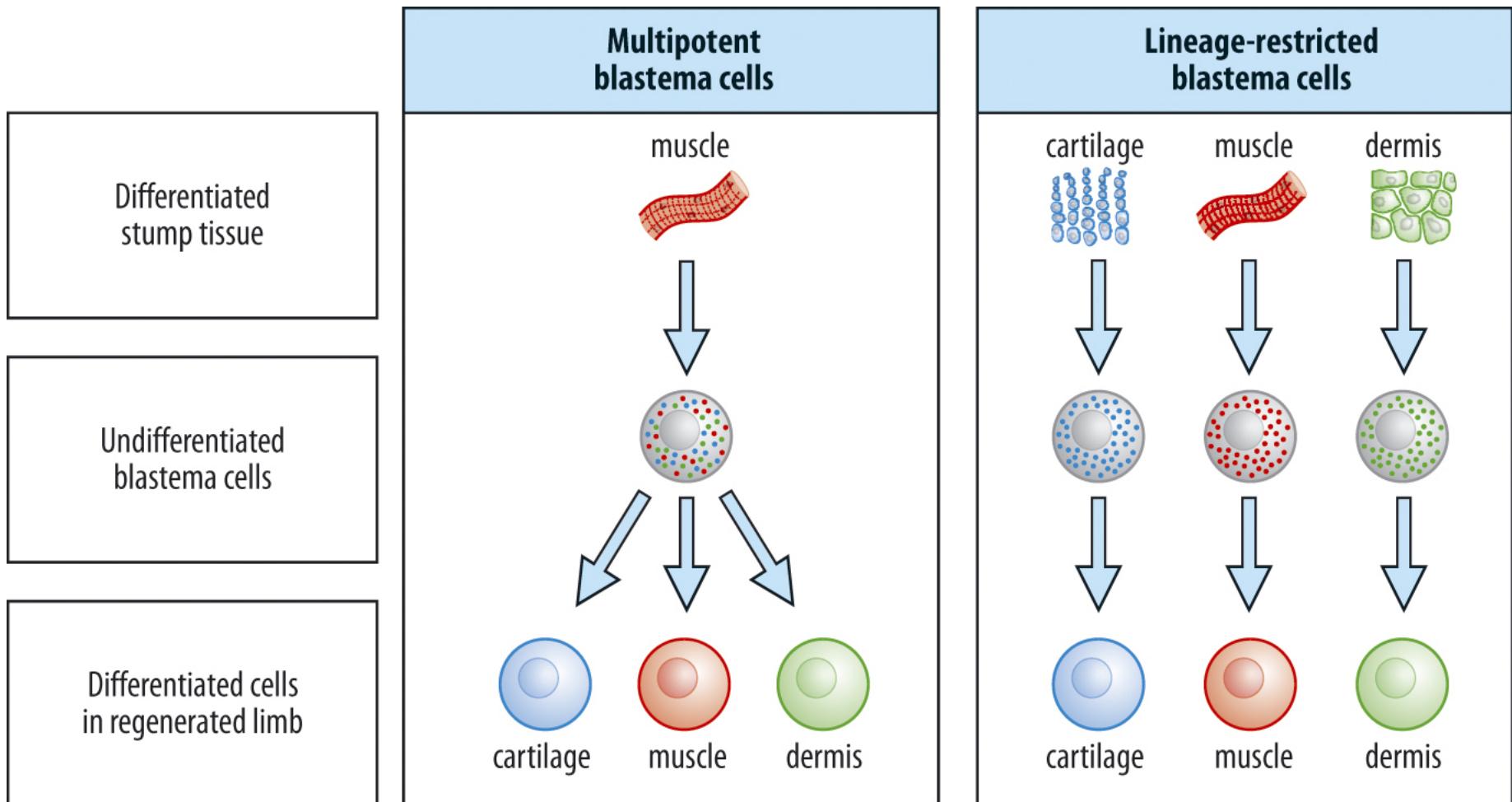
Notophthalmus viridescens



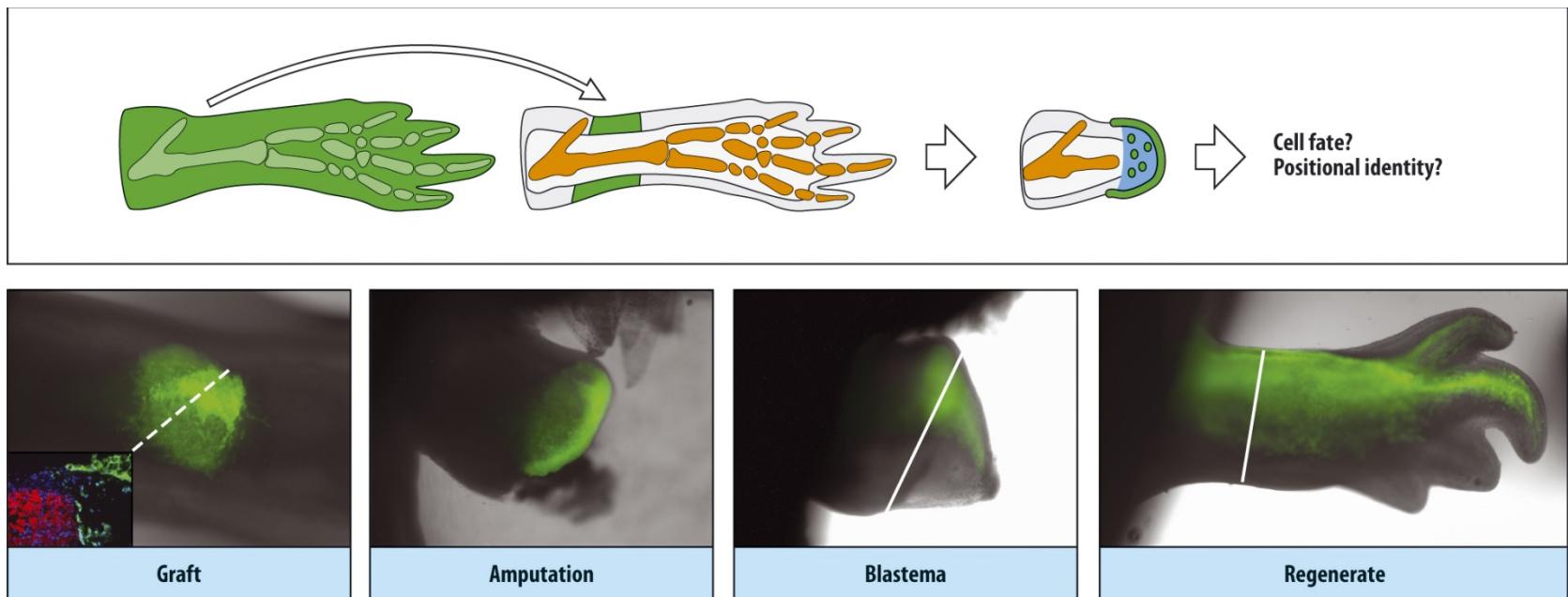
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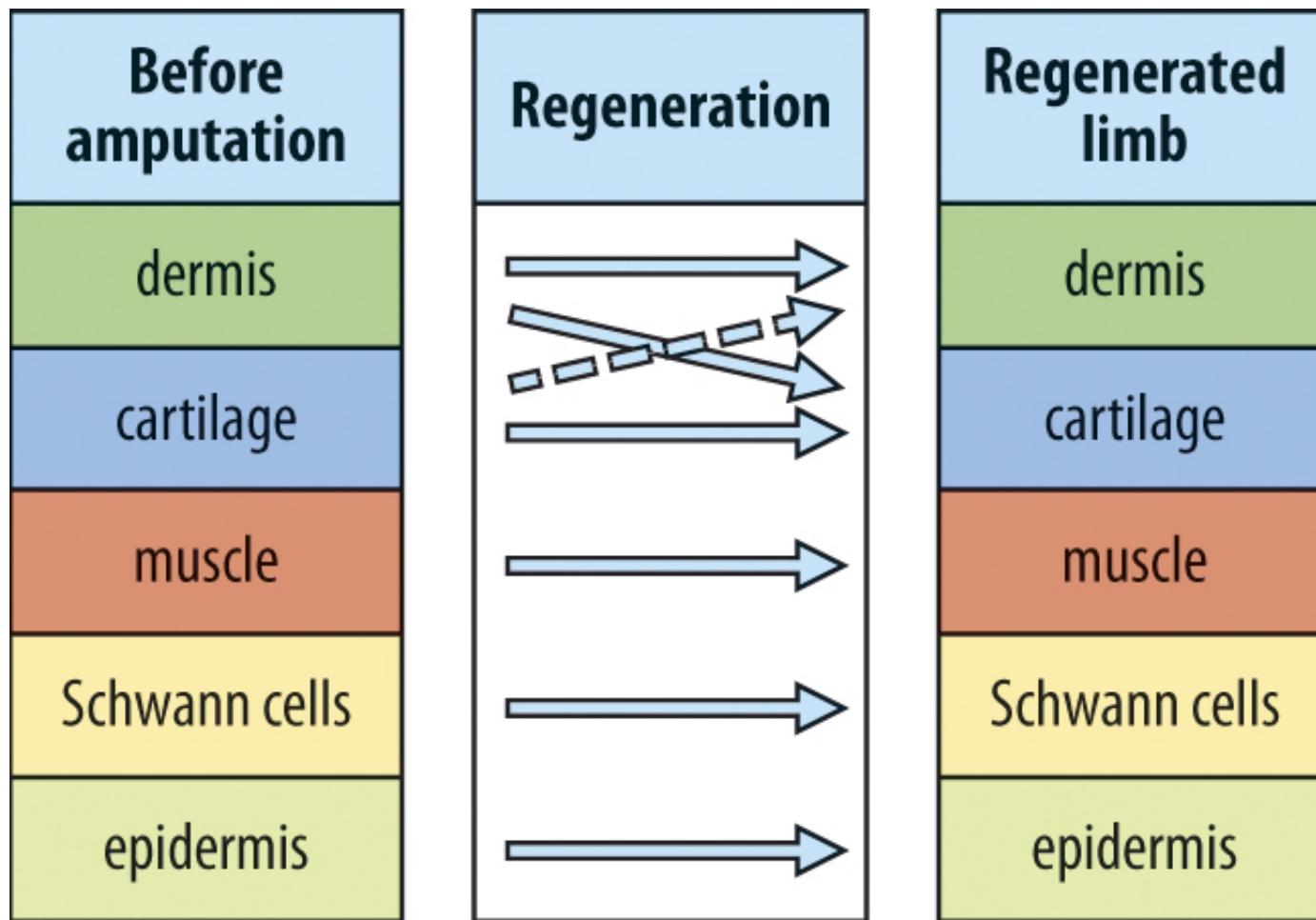
Are blastema cells multipotent or are they lineage restricted?



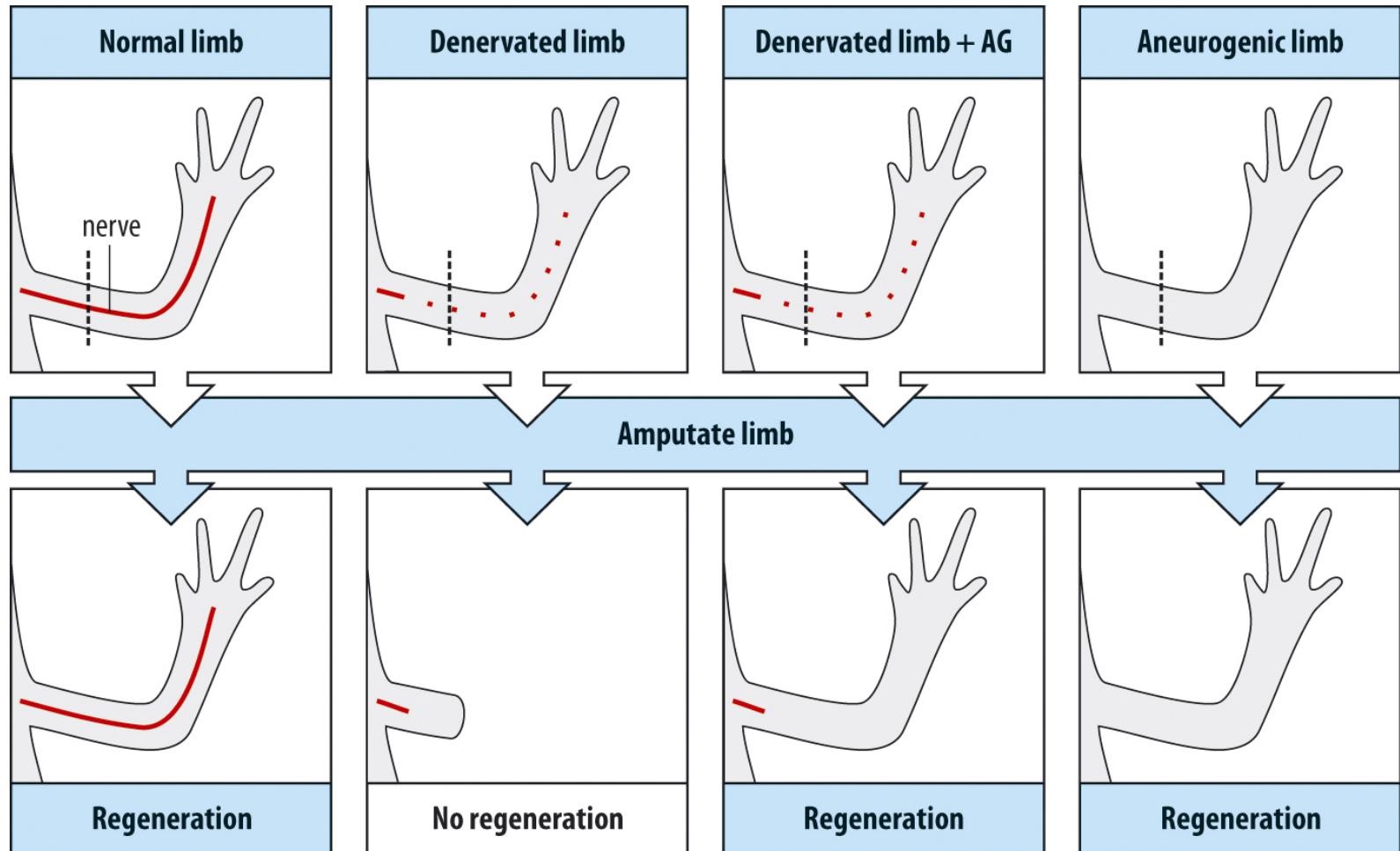
Cells that regenerate the **axolotl** limb have restricted developmental potential



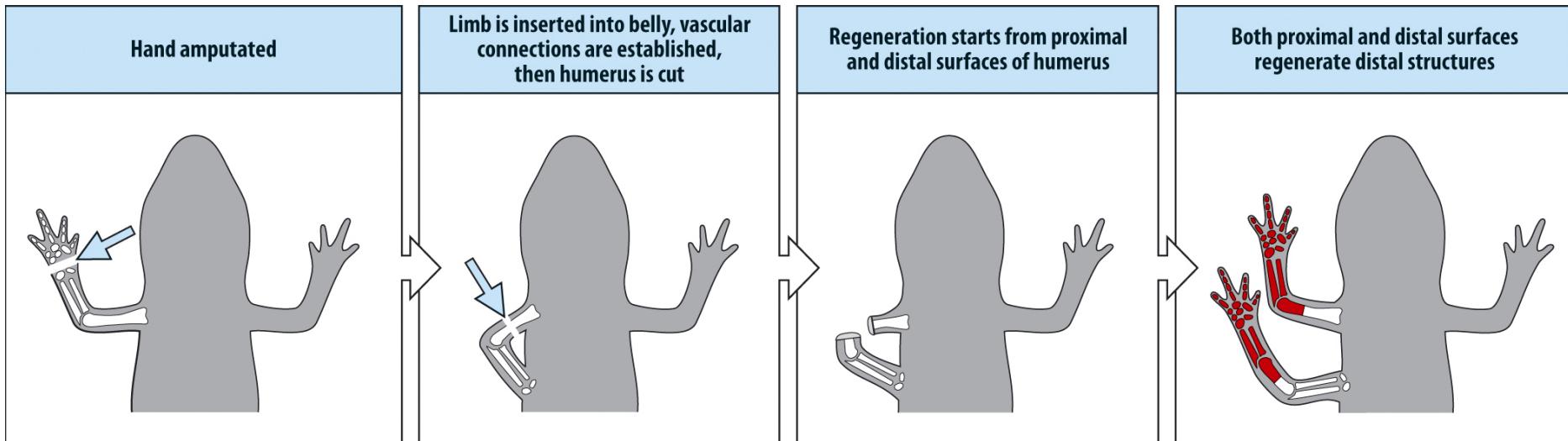
Lineage restriction of cells in the axolotl blastema



Innervation and limb regeneration



Limb regeneration is always in the distal direction



UC Irvine Limb Regeneration

Dr. David M. Gardiner and Dr. Susan V. Bryant

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University of California, Irvine:
Department of Developmental and Cell
Biology
Natural Sciences II
Irvine, California



Brains On! Science Podcast for Kids features Dr. David Gardiner

Dr. David Gardiner was featured on Brains On! — a science podcast for kids from Minnesota Public Radio News and Southern California Public Radio. "Alexander, 11 years old kid, question how axolotls can regrow their limbs." On the topic of regeneration, kids and many adults tend to gear towards an idea similar to Wolverine's ability to regenerate ...

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UC Irvine Limb Regeneration

Dr. David M. Gardiner and Dr. Susan V. Bryant

Welcome

News

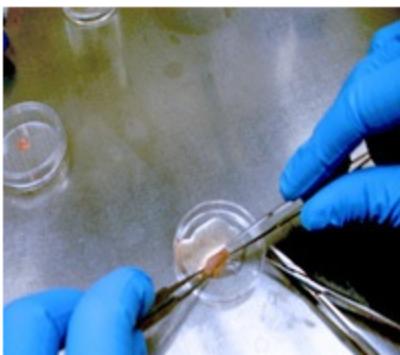
Regeneration

Lab Publications

Lab Members

FAQ

Support Regeneration Research



Dr. David M. Gardiner and Dr. Susan V. Bryant



Dr. DAVID M. GARDINER

Principle Investigator

Professor, Department of Developmental and
Cell Biology
and Developmental Biology Center

Research Programs and Expertise



Dr. SUSAN V. BRYANT

Principal Investigator

Research Professor, Department of
Developmental and Cell Biology and
Developmental Biology Center

Research

Accessory Limb Model

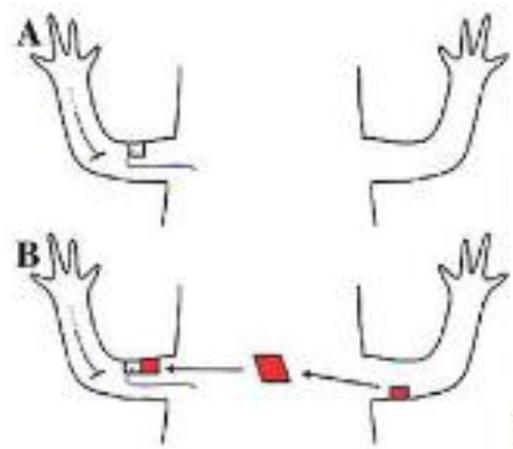


Fig. 1 (A-B)

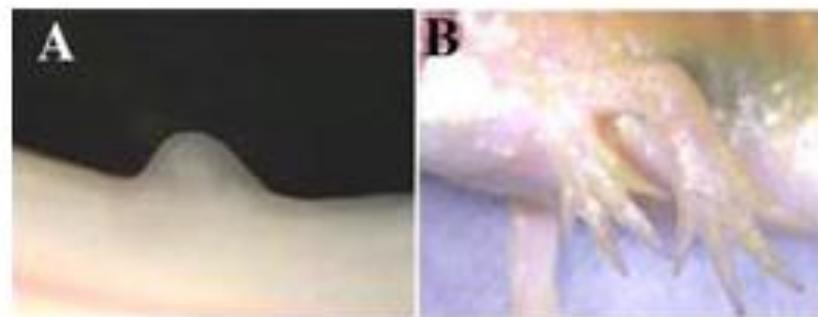
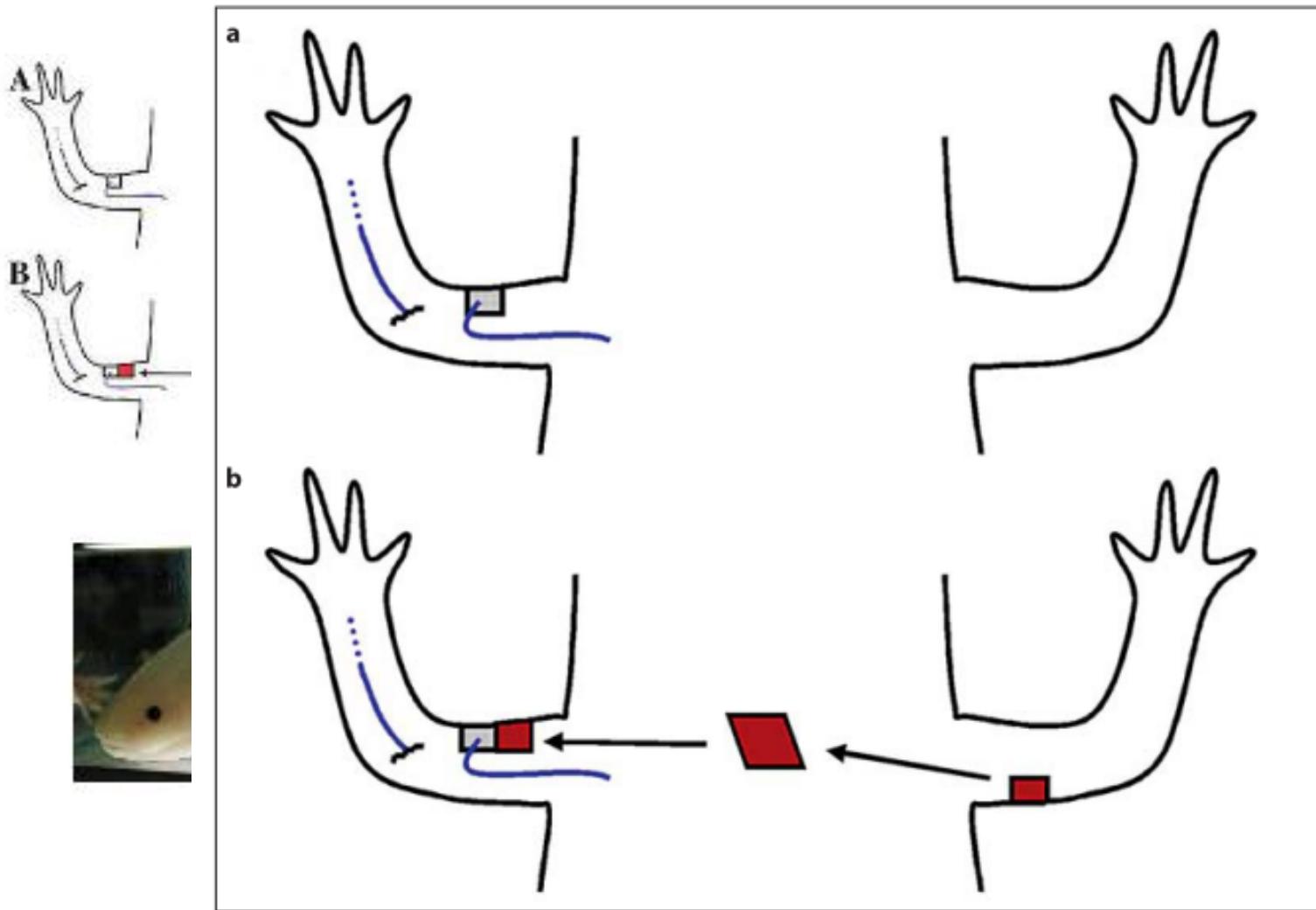


Fig. 2(A-B)



Fig. 3

Accessory Limb Model



Prometheus and liver regeneration



Liver regeneration

- In humans, as much as 70% of the liver can be removed, and the rest of liver will grow back to a normal size.
- The “critical mass” is 75%-80%.
- Hepatocytes start to divide a day after surgery.
- In zebrafish larva model, the liver can regenerate *de novo*.

Children VS adults

- 1. Digit (figure) tip regeneration.
- 2. Regeneration VS scar formation (fibrosis).
- 3. It is believed that human embryos younger than 10-week have the same capacity of regeneration as urodele amphibians.

Figure regeneration

LETTER

doi:10.1038/nature12214

Wnt activation in nail epithelium couples nail growth to digit regeneration

Makoto Takeo¹, Wei Chin Chou¹, Qi Sun¹, Wendy Lee¹, Piul Rabbani¹, Cynthia Loomis¹, M. Mark Taketo² & Mayumi Ito¹

The tips of mammalian digits can regenerate after amputation^{1,2}, like those of amphibians. It is unknown why this capacity is limited to the area associated with the nail^{2–4}. Here we show that nail stem cells (NSCs) reside in the proximal nail matrix and that the mechanisms governing NSC differentiation are coupled directly with their ability to orchestrate digit regeneration. Early nail progenitors undergo Wnt-dependent differentiation into the nail. After amputation, this Wnt activation is required for nail regeneration and also for attracting nerves that promote mesenchymal blastema growth, leading to the regeneration of the digit. Amputations proximal to the Wnt-active nail progenitors result in failure to regenerate the nail or digit. Nevertheless, β -catenin stabilization in the NSC region induced their regeneration. These results establish a link between NSC differentiation and digit regeneration, and suggest that NSCs may have the potential to contribute to the development of novel treatments for amputees.

results show that the proximal matrix contains self-renewing NSCs that sustain nail growth. LacZ⁺ colonies in the nail fold, the epithelium surrounding the nail, were discontinuous from the streaks that produced the nail plate, suggesting that the nail fold did not contribute to the cells for nail growth (Supplementary Fig. 3).

Histological analyses revealed that proximal matrix cells possessed less interdigitations, characteristic of undifferentiated epidermal cells (Supplementary Fig. 4). Immunohistochemistry with proliferation and epidermal differentiation markers¹¹ found that proximal matrix cells containing NSCs were highly proliferative (Ki67^{high}) and expressed K17 in addition to K14 (Supplementary Fig. 4). Isolated proximal matrix cells, enriched with K14⁺K17⁺ expression (Fig. 1f, g), showed the highest colony-forming ability *in vitro*, a general characteristic of epithelial stem cells (Fig. 1h–j).

To understand the molecular mechanisms underlying NSC differentiation, we generated a microarray of proximal matrix versus distal

Figure regeneration

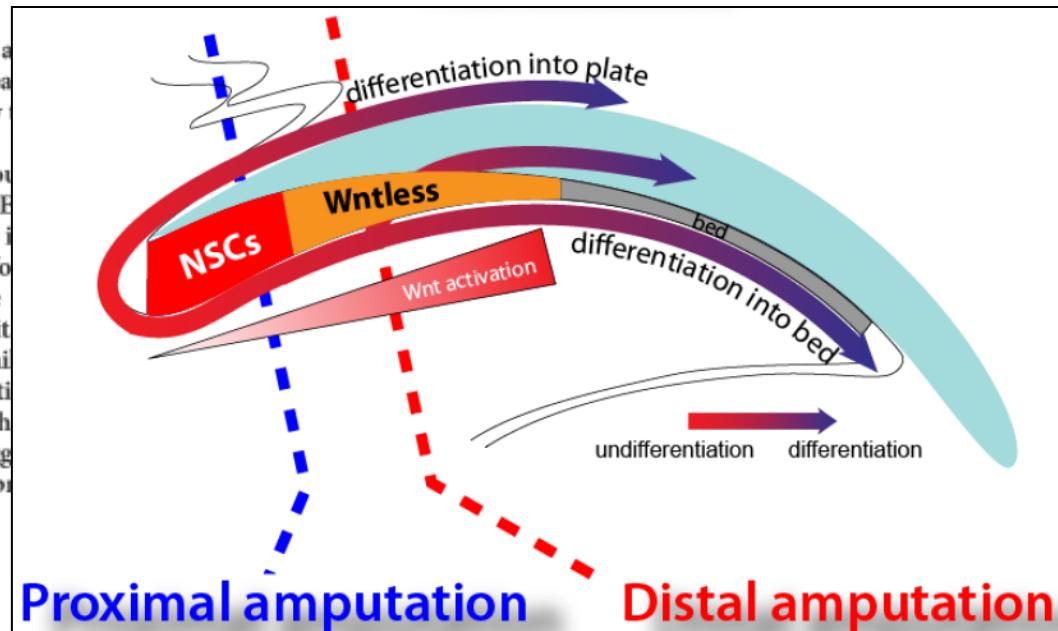
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Tentative principles

- 1. The ability of regeneration decreases with age.
- 2. Asexual reproduction animals show greater ability of regeneration than sexual reproduction animals.

Regenerative medicine

Regenerative medicine is a branch of translational research in Tissue Engineering and Molecular Biology which deals with the "process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function". This field holds the promise of engineering damaged tissues and organs via stimulating the body's own repair mechanisms to functionally heal previously irreparable tissues or organs.

Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implant them when the body cannot heal itself.

From wikipedia

Clinical needs

- Digits, figures and toes (War victims).
- Cardiac muscle (Heart attack).
- Pancreatic β -cell (Type I diabetes).
- Neurons in CNS (Spinal cord injury and neurodegenerative disease).

Center/Institute of Regenerative Medicine

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California Institute for Regenerative Medicine: California's ...

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Makes grants and provides loans for stem cell research, facilities and other opportunities, managed from San Francisco.

Wake Forest Institute for Regenerative Medicine (WFIRM) - ...

www.wakehealth.edu/WFIRM/ - Similar

What may seem like science fiction is happening right here at the Wake Forest **Institute for Regenerative Medicine**.

Penn Institute for Regenerative Medicine: Welcome

www.irm.upenn.edu/

2014-04-11 - The **Institute for Regenerative Medicine** hosted a Symposium on Cellular Reprogramming honoring Dr. John B. Gurdon, the 2012 Nobel Laureate ...

Institute for Stem Cell Biology and Regenerative Medicine ...

stemcell.stanford.edu/ - Similar

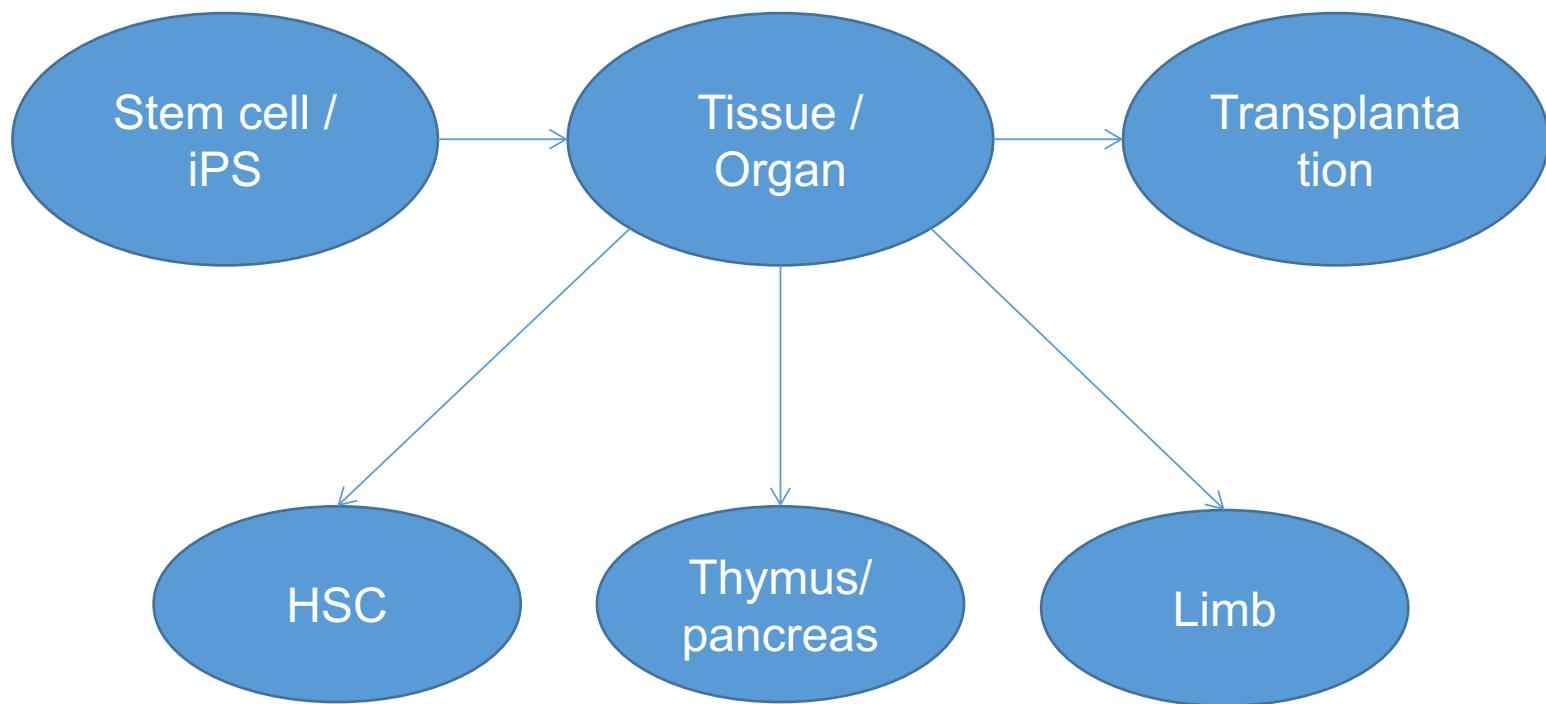
Researchers at the **institute** have devised an accurate way to find and analyze minute amounts of DNA that has been shed by cancer cells into the patient's ...

Institute for Regenerative Medicine

medicine.tamhsc.edu/irm/ - Similar

The Texas A&M Health Science Center College of Medicine **Institute for Regenerative Medicine (IRM)** at Scott & White Hospital was established in August 2008 ...

A simplified flowchart of regeneration medicine



The Vacanti mouse

The Vacanti mouse was a laboratory mouse that had what looked like a human ear grown on its back. The "ear" was actually an ear-shaped cartilage structure grown by seeding cow cartilage cells into a biodegradable ear-shaped mold and then implanted under the skin of the mouse (1997).





Engineered composite tissue as a bioartificial limb graft



Bernhard J. Jank ^{b,c}, Linjie Xiong ^b, Philipp T. Moser ^{b,c}, Jacques P. Guyette ^{b,c}, Xi Ren ^{b,c}, Curtis L. Cetrulo ^{c,d}, David A. Leonard ^{c,d}, Leopoldo Fernandez ^b, Shawn P. Fagan ^e, Harald C. Ott ^{a,c,*}

^a Division of Thoracic Surgery, Department of Surgery, Massachusetts General Hospital, USA

^b Center for Regenerative Medicine, Massachusetts General Hospital, USA

^c Harvard Medical School, Boston, MA, USA

^d Transplantation Biology Research Center, Department of Surgery, Massachusetts General Hospital, USA

^e Massachusetts General Hospital, Division of Burn Surgery, Harvard Medical School, USA

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ABSTRACT

The loss of an extremity is a disastrous injury with tremendous impact on a patient's life. Current mechanical prostheses are technically highly sophisticated, but only partially replace physiologic function and aesthetic appearance. As a biologic alternative, approximately 70 patients have undergone allogeneic hand transplantation to date worldwide. While outcomes are favorable, risks and side effects of transplantation and long-term immunosuppression pose a significant ethical dilemma. An autologous, bioartificial graft based on native extracellular matrix and patient derived cells could be produced on demand and would not require immunosuppression after transplantation. To create such a graft, we decellularized rat and primate forearms by detergent perfusion and yielded acellular scaffolds with preserved composite architecture. We then repopulated muscle and vasculature with cells of appropriate phenotypes, and matured the composite tissue in a perfusion bioreactor under electrical stimulation *in vitro*. After confirmation of composite tissue formation, we transplanted the resulting bio-composite grafts to confirm perfusion *in vivo*.



Engineered composite tissue as a bioartificial limb graft

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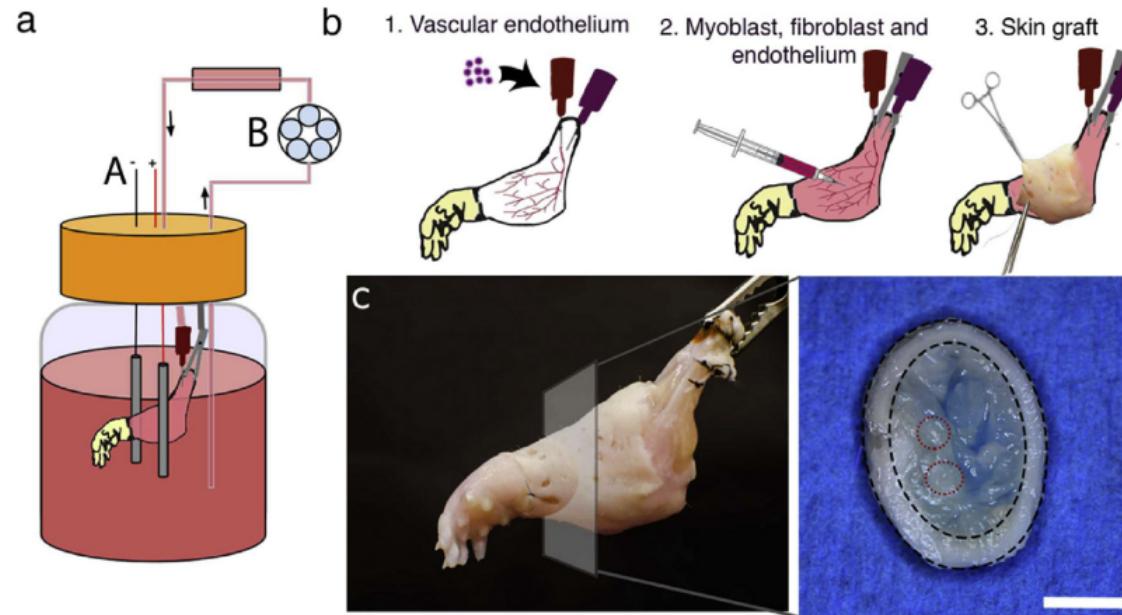
^c Harvard

^d Translational

^e Massachusetts General Hospital

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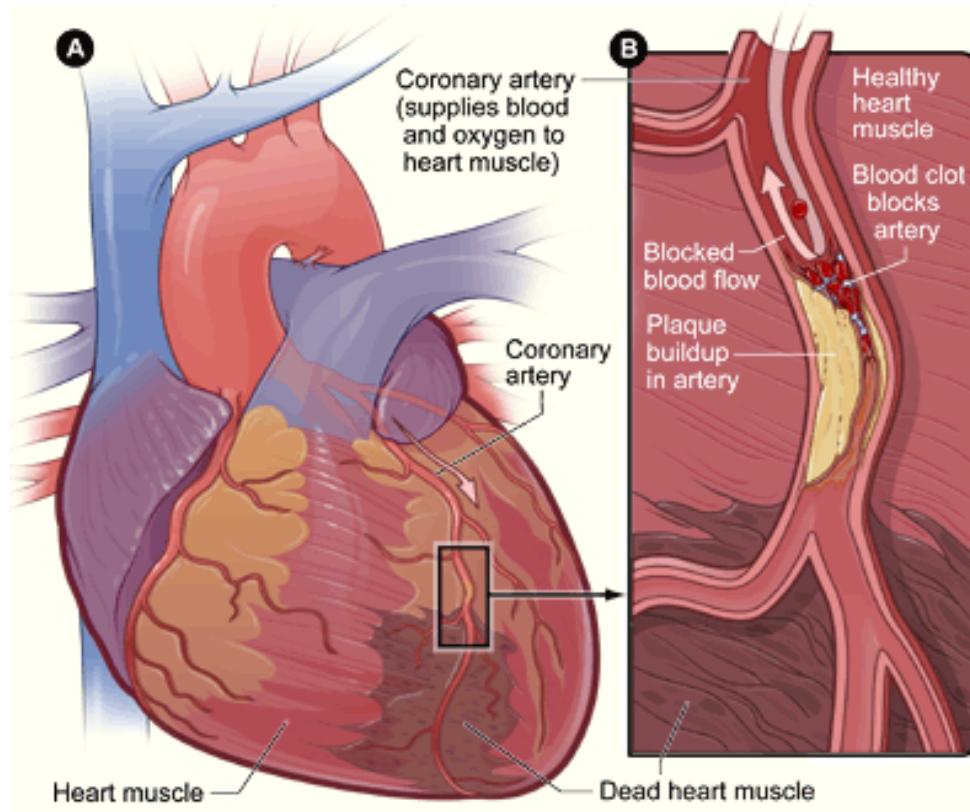
Bioprinting

Mechanobiology

Muscle

Bone

Myocardial infarction (heart attack, 心肌梗死)



Evidence for Cardiomyocyte Renewal in Humans

Olaf Bergmann,^{1*} Ratan D. Bhardwaj,^{1*} Samuel Bernard,² Sofia Zdunek,¹ Fanie Barnabé-Heider,¹ Stuart Walsh,³ Joel Zupicich,¹ Kanar Alkass,⁴ Bruce A. Buchholz,⁵ Henrik Druid,⁴ Stefan Jovinge,^{3,6} Jonas Frisén^{1†}

It has been difficult to establish whether we are limited to the heart muscle cells we are born with or if cardiomyocytes are generated also later in life. We have taken advantage of the integration of carbon-14, generated by nuclear bomb tests during the Cold War, into DNA to establish the age of cardiomyocytes in humans. We report that cardiomyocytes renew, with a gradual decrease from 1% turning over annually at the age of 25 to 0.45% at the age of 75. Fewer than 50% of cardiomyocytes are exchanged during a normal life span. The capacity to generate cardiomyocytes in the adult human heart suggests that it may be rational to work toward the development of therapeutic strategies aimed at stimulating this process in cardiac pathologies.

Myocardial damage often results in chronic heart failure due to loss and insufficient regeneration of cardiomyocytes. This has prompted efforts to devise cardiomyocyte replacement therapies by cell transplantation or by the promotion of endogenous regenerative processes. The development of cell transplantation strategies is advancing rapidly, and some are currently being evaluated in clinical trials (*1, 2*). Stimulating endogenous regenerative processes is attractive as it potentially could provide a non-invasive therapy and circumvent the immunosuppression required for allografts. However, it is unclear whether such regenerative strategies are realistic because it has been difficult to establish whether cardiomyocytes can be generated after the perinatal period in humans.

Stem/progenitor cells with the potential to generate cardiomyocytes *in vitro* remain in the adult rodent and human myocardium (*3, 4*). Moreover, mature cardiomyocytes have been suggested to be able to reenter the cell cycle and duplicate (*5*). However, studies over several decades in rodents with labeled nucleotide analogs have led to conflicting results, ranging from no to substantial generation of cardiomyocytes postnatally (*6*). A recent genetic labeling study, which enabled detection of cardiomyocyte generation by stem/progenitor cells (but not by cardiomyocyte duplication), demonstrated cardiomyocyte renewal after myocardial injury, but not during 1 year in the healthy mouse (*7*).

It is possible that humans, who live much longer than rodents, may have a different requirement for cardiomyocyte replacement. Cell turnover has been difficult to study in humans because the use of labeled nucleotide analogs and other strategies commonly used in experimental animals cannot readily be adapted for studies in humans

duce the future fate of a potentially dividing cell in terms of differentiation and long-term survival.

We have measured carbon-14 (^{14}C) from nuclear bomb tests in genomic DNA of human myocardial cells, which allows retrospective birth dating (*9–11*). ^{14}C concentrations in the atmosphere remained relatively stable until the Cold War, when aboveground nuclear bomb tests caused a sharp increase (*12, 13*). Even though the detonations were conducted at a limited number of locations, the elevated amounts of ^{14}C in the atmosphere rapidly equalized around the world as $^{14}\text{CO}_2$. After the Limited Nuclear Test Ban Treaty in 1963, the ^{14}C concentrations dropped exponentially, not primarily because of radioactive decay (half-life of 5730 years), but by diffusion from the atmosphere (*14*). Newly created atmospheric ^{14}C reacts with oxygen to form $^{14}\text{CO}_2$, which is incorporated by plants through photosynthesis. Humans eat plants, and animals that live off plants, so the ^{14}C concentration in the human body mirrors that in the atmosphere at any given time (*15–18*). Because DNA is stable after a cell has gone through its last cell division, the concentration of ^{14}C in DNA serves as a date mark for when a cell was born and can be used to retrospectively birth date cells in humans (*9–11*).

We first carbon-dated left ventricle myocardial cells, including cardiomyocytes and other cell types, to determine the extent of postnatal DNA synthesis in the human heart. DNA was extracted, and ^{14}C concentrations were measured by accelerator mass spectrometry (see tables S1 and S2 for ^{14}C values and associated data). The cellular birth dates can be inferred by determining the time at which the sample's ^{14}C concentration corresponded to the atmospheric concentration (Fig. 1A). ^{14}C concentrations from all individuals born around or after the nuclear bomb tests corresponded to atmospheric concentrations several years after the subjects' birth (Fig. 1B), indicating

¹Department of Cell and Molecular Biology, Karolinska Institutet, SE-171 77 Stockholm, Sweden. ²CNRS UMR5208, Institut Camille Jordan, Université Claude Bernard Lyon 1, 69622 Villeurbanne cedex, France. ³Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund Uni-

Heart Regeneration in Zebrafish

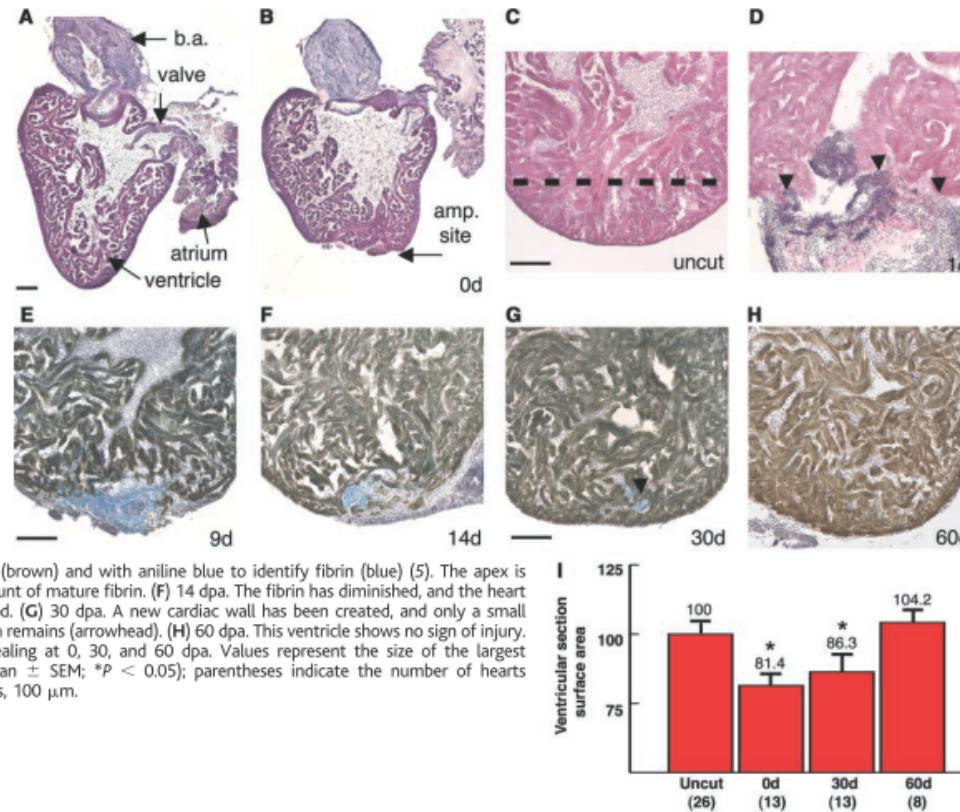
Kenneth D. Poss,* Lindsay G. Wilson, Mark T. Keating*

Cardiac injury in mammals and amphibians typically leads to scarring, with minimal regeneration of heart muscle. Here, we demonstrate histologically that zebrafish fully regenerate hearts within 2 months of 20% ventricular resection. Regeneration occurs through robust proliferation of cardiomyocytes localized at the leading epicardial edge of the new myocardium. The hearts of zebrafish with mutations in the Mps1 mitotic checkpoint kinase, a critical cell cycle regulator, failed to regenerate and formed scars. Thus, injury-induced cardiomyocyte proliferation in zebrafish can overcome scar formation, allowing cardiac muscle regeneration. These findings indicate that zebrafish will be useful for genetically dissecting the molecular mechanisms of cardiac regeneration.

Injured human hearts do not regenerate. Instead, damaged myocardium is replaced by fibrotic scar tissue. Cardiomyocytes, the major

structural cells of the heart, may undergo hypertrophy in the wound area to increase muscular mass. Although recent findings suggest

Fig. 1. Regeneration of ventricular myocardium in the resected zebrafish heart. Hematoxylin and eosin stain of the intact zebrafish heart before (A) and after about 20% ventricular resection (B) (5). b.a., bulbous arteriosus. (C) An intact ventricular apex at higher magnification, indicating the approximate amputation plane (dashed line). All images in this and subsequent figures display longitudinal ventricular sections of the amputation plane. (D) 1 dpa. The large clot is filled with nucleated erythrocytes (arrowheads). (E) 9 dpa. The heart section is stained for the presence of myosin heavy chain to identify cardiac muscle (brown) and with aniline blue to identify fibrin (blue) (5). The apex is sealed with a large amount of mature fibrin. (F) 14 dpa. The fibrin has diminished, and the heart muscle has reconstituted. (G) 30 dpa. A new cardiac wall has been created, and only a small amount of internal fibrin remains (arrowhead). (H) 60 dpa. This ventricle shows no sign of injury. (I) Quantification of healing at 0, 30, and 60 dpa. Values represent the size of the largest ventricular section (mean \pm SEM; * $P < 0.05$); parentheses indicate the number of hearts examined (5). Scale bars, 100 μ m.



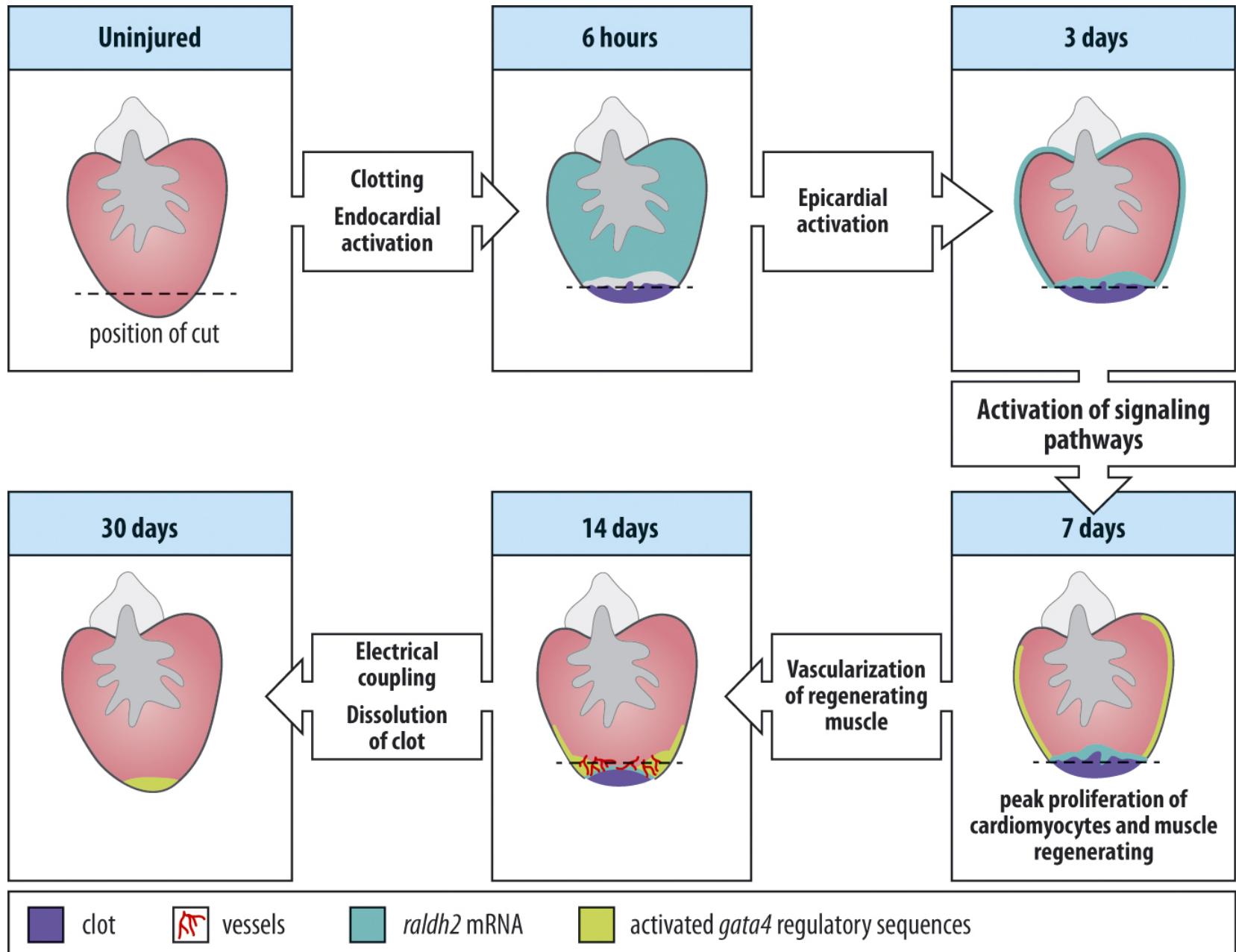
that cardiomyocytes within the diseased human heart can proliferate (1), most evidence to date indicates that myocyte proliferation is not a significant component of the mammalian response to cardiac injury (2).

Teleost fish, including zebrafish, can regenerate spinal cord, retina, and fins (3, 4). To determine whether zebrafish can also regenerate heart muscle, we surgically removed ~20% of the ventricular myocardium from 1- to 2-year-old adults (Fig. 1, A and B) and

Department of Cell Biology, Department of Cardiology, Howard Hughes Medical Institute, Harvard Medical School, Children's Hospital, 320 Longwood Avenue, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: kposs@enders.tch.harvard.edu (K.D.P.); mkeating@enders.tch.harvard.edu (M.T.K.)

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Major questions

- 1. What is the origin of the cells that give rise to the regenerated structures?
- 2. What mechanisms pattern the regenerated tissue?
- 3. How are these related to the patterning processes that occur in embryogenesis?
- 4. What are the factors that regulate these activities?

Approaches to study regeneration

- Animal Models
- Determining the Cellular Origins of Regenerated Tissues
- Analyzing the Niche Regulation of Cell Activities
- Cell Imaging and Identification (lineage tracing)
- Comparative Analysis of Regeneration and Fibrosis

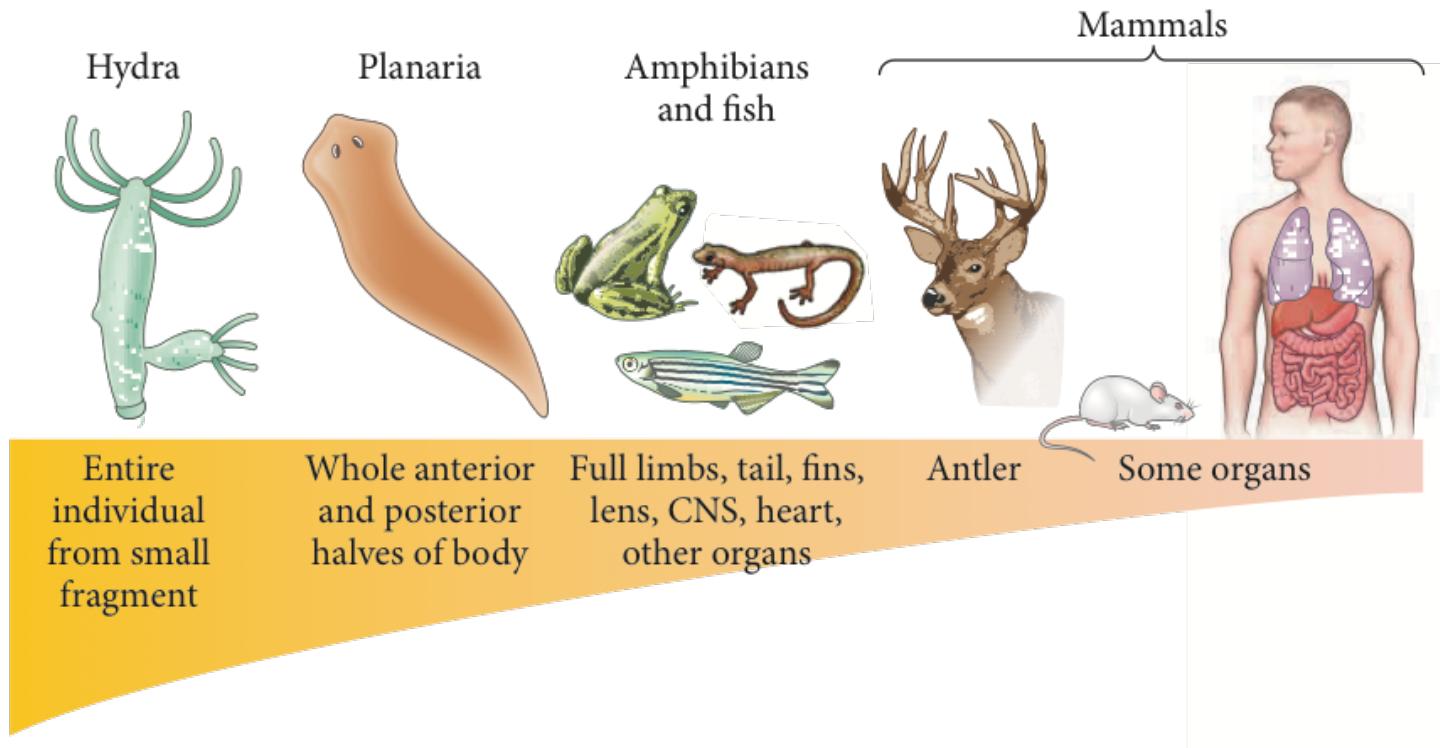
Questions

- Why human adults have very weak capacity to regeneration?
- Can present regenerative models mimic the situation of human?

What inhibits regeneration? Take limb as an example

- Maturation of the Immune System Suppresses the Ability to Regenerate Limbs
- A Relationship Between the Cell Cycle and Dedifferentiation is Deficient in Frogs, Birds and Mammals

Representative organisms and their comparative regenerative capabilities.



Maturation of the Immune System (adaptive) Suppresses the Ability to Regenerate Limbs

- Urodeles do have a more primitive immune system than *Xenopus*.
- The immune system of *Xenopus* changes profoundly during tadpole development, coincident with loss of limb regenerative capacity (Skin taken from a regeneration-competent early tadpole and cold preserved was rejected when autografted to the donor after its metamorphosis).
- Mouse fetal limb buds have a limited capacity for regeneration, and mouse fetal skin regenerates until late in gestation, then it shifts to the adult scarring response to wounding, a shift that is correlated with the maturation of the immune system, but which can take also place autonomously *in vitro* in the absence of the immune system.

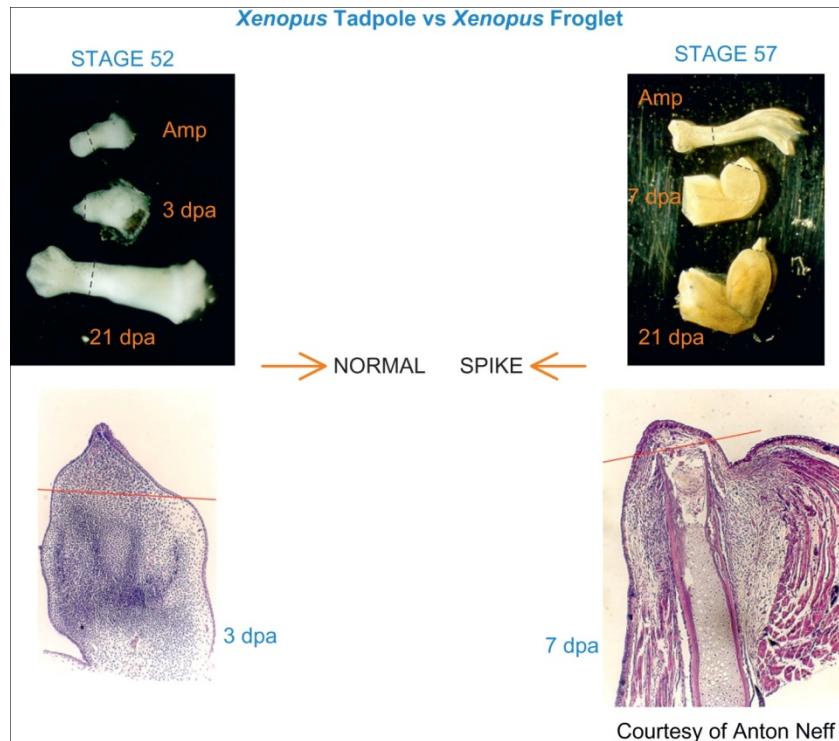
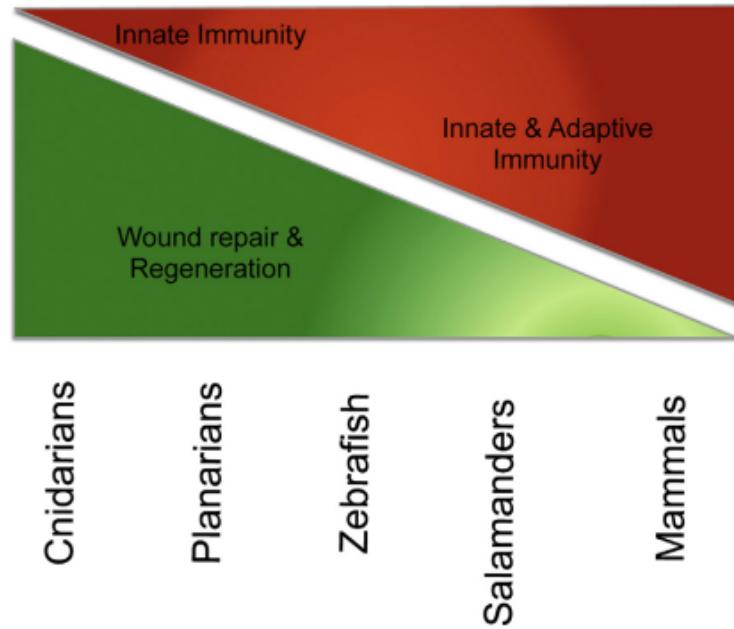
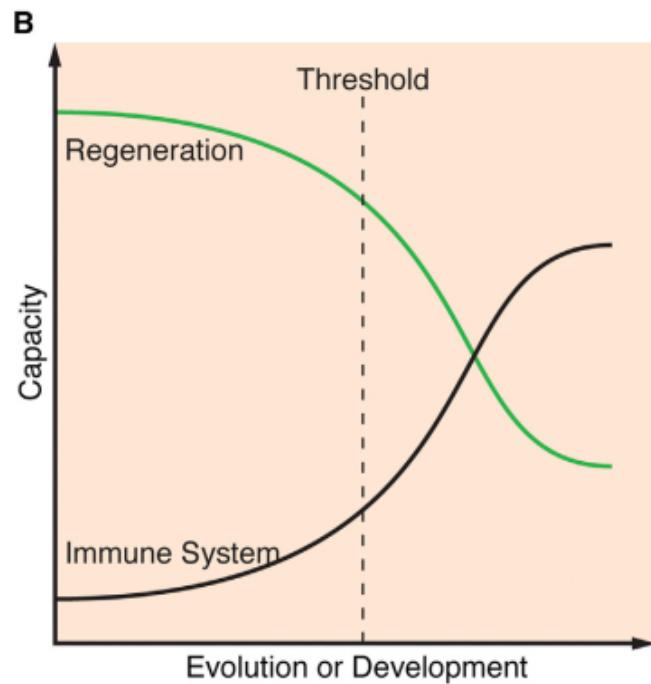


Figure 8.32 Loss of regenerative capacity with progressive development of the *Xenopus* tadpole hindlimb. **Left:** Upper panel shows normal regeneration over a three week period after amputation through the tarsal region (dashed line) at stage 52. Lower panel illustrates a longitudinal section of a well-formed, growing blastema at 3 days post-amputation. **Right:** Upper panel shows hypomorphic regeneration over a three week period after amputation through the distal femur (dashed line) at stage 57. Lower panel is a longitudinal section showing a poorly formed fibroblastema 7 days post-amputation. Orange lines on longitudinal sections indicate amputation level. *Courtesy of Anton Neff.*

Inverse relationship between the capacity to regenerate and the strength and intricacy of the immune system



A Relationship Between the Cell Cycle and Dedifferentiation is Deficient in Frogs, Birds and Mammals

- First, a bioinformatic analysis failed to find homologues of Prod1 in other vertebrate taxa, suggesting that this crucial regeneration gene is special to urodeles.
- Second, newt and mammalian myotube nuclei appear to differ in their ability to re-enter the cell cycle in response to serum stimulation.
- Birds and mammals have evolved an additional cell cycle checkpoint gene not present in urodeles whose protein product synergizes with pRb to suppress cell cycle re-entry upon muscle cell differentiation.

Tylototriton verrucoosus Anderson
红瘰疣螈



Baidu 百科



Notophthalmus viridescens



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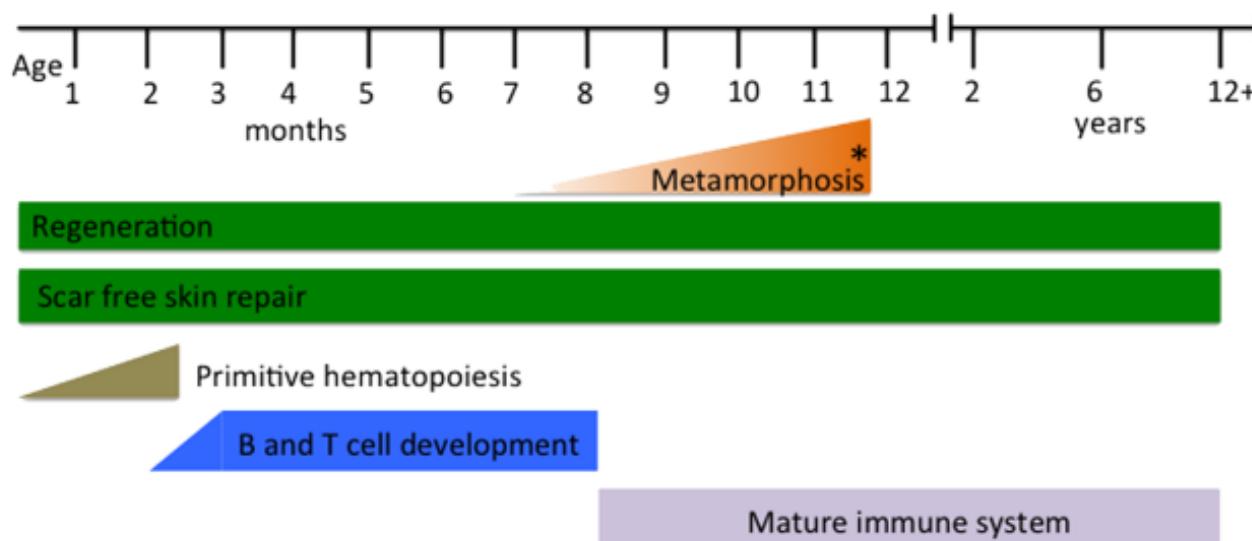
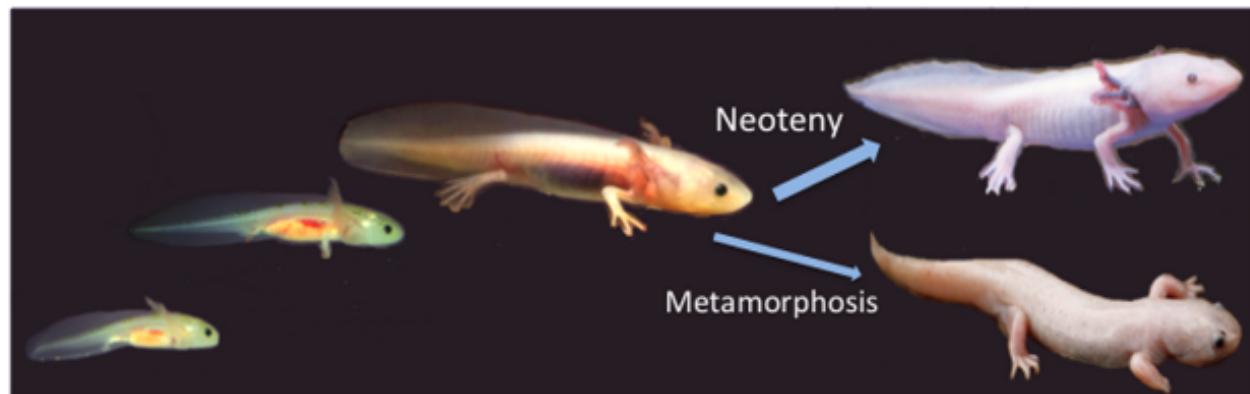


Axolotl, Mexican salamander (*Ambystoma mexicanum*)



Some species like the axolotl have neotenic life cycles

B





Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes

David C Zebrowski^{1,2*}, Silvia Vergarajauregui¹, Chi-Chung Wu³, Tanja Piatkowski², Robert Becker¹, Marina Leone^{1,2}, Sofia Hirth⁴, Filomena Ricciardi⁵, Nathalie Falk⁶, Andreas Giessl⁶, Steffen Just⁴, Thomas Braun², Gilbert Weidinger³, Felix B Engel^{1,2*}

¹Experimental Renal and Cardiovascular Research, Department of Nephropathology, Institute of Pathology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; ²Department of Cardiac Development and Remodeling, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; ³Institute for Biochemistry and Molecular Biology, University of Ulm, Ulm, Germany; ⁴Department of Medicine II, University of Ulm, Ulm, Germany; ⁵Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; ⁶Department of Biology, Animal Physiology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Abstract Mammalian cardiomyocytes become post-mitotic shortly after birth. Understanding how this occurs is highly relevant to cardiac regenerative therapy. Yet, how cardiomyocytes achieve and maintain a post-mitotic state is unknown. Here, we show that cardiomyocyte centrosome integrity is lost shortly after birth. This is coupled with relocalization of various centrosome proteins to the nuclear envelope. Consequently, postnatal cardiomyocytes are unable to undergo ciliogenesis and the nuclear envelope adopts the function as cellular microtubule organizing center. Loss of centrosome integrity is associated with, and can promote, cardiomyocyte G0/G1 cell cycle arrest suggesting that centrosome disassembly is developmentally utilized to achieve the post-mitotic state in mammalian cardiomyocytes. Adult cardiomyocytes of zebrafish and newt, which are able to proliferate, maintain centrosome integrity. Collectively, our data provide a novel mechanism underlying the post-mitotic state of mammalian cardiomyocytes as well as a potential explanation for why zebrafish and newts, but not mammals, can regenerate their heart.

*For correspondence: david.zebrowski@gmail.com (DCZ); felix.engel@uk-erlangen.de (FBE)

Competing interests: The authors declare that no competing interests exist.

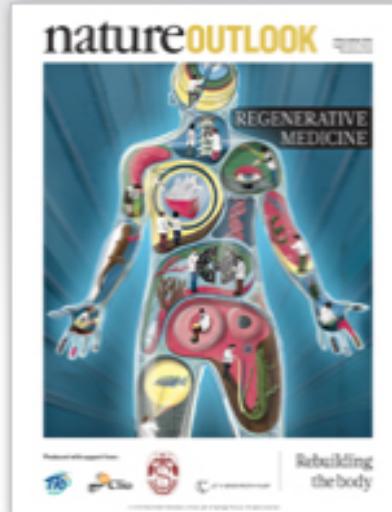
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SUPPLEMENT

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Regenerative medicine

Vol. 540 No. 7632_supp ppS49-S91



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Our bodies aren't forever: parts wear out, trauma breaks things and organs stop functioning. Sometimes, a drug can remedy a chemical imbalance or surgery can repair a structural failure, but there are times when there is no substitute for replacing a part with human tissue or even an entire organ. Rapid advances in the field of regenerative medicine are bringing that possibility closer to reality.

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Bodies wear out. Tissue thins and tears. Organs stop functioning. Cells lose their biological way. Trauma breaks things. And as a result, we become ill or disabled. This has always been our fate.

Regenerative medicine is the bold collection of techniques and technologies that aim to restore our physiology to something that resembles its original condition. Its roots trace back to antiquity (see page S50) but it has, in recent years, become much more effective. For example, 3D printers can construct tissue and organs that in some cases can function as well as the originals (S56). The central nervous system, however, has proved stubbornly difficult to repair. Scientists hope that stem-cell advances might finally restore mobility to those with spinal-cord injuries (S52). Excitement is already building over the potential of these intriguing cells to create drug-free treatments for chronic diseases such as type 1 diabetes (S60). Regeneration researchers are also taking cues from the animal world: species such as salamanders have the power to regrow limbs. Understanding the cellular mechanisms behind this ability might lead to techniques that can work in humans (S58). Indeed, one scientist argues that for progress to continue, researchers will need to do a better job of emulating and working alongside natural systems (S55).

The creation of these therapies brings with it questions of how to regulate them. Clinics are sprouting up to offer dubious stem-cell-based treatments for dire conditions, and policymakers are crafting rules to accelerate the availability of effective treatments without endangering desperate patients (S64).

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Herb Brody

Chief supplements editor

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RESEARCH ARTICLE

TISSUE REGENERATION

Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration

Yongyou Zhang,^{1,*} Amar Desai,^{1,*} Sung Yeun Yang,^{1,2*} Ki Beom Bae,^{1,3*} Monika I. Antczak,^{4,*} Stephen P. Fink,^{1,*} Shruti Tiwari,^{1,5*} Joseph E. Willis,^{6,7,5} Noelle S. Williams,⁴ Dawn M. Dawson,^{6,7} David Wald,^{6,7,5} Wei-Dong Chen,^{1,†} Zhenghe Wang,^{6,8} Lakshmi Kasturi,¹ Gretchen A. Larusch,¹ Lucy He,^{1,‡} Fabio Cominelli,^{1,5} Luca Di Martino,¹ Zora Djuric,⁹ Ginger L. Milne,¹⁰ Mark Chance,¹¹ Juan Sanabria,^{12,5} Chris Dealwis,¹³ Debra Mikkola,¹ Jacinth Naidoo,⁴ Shuguang Wei,⁴ Hsin-Hsiung Tai,^{14§} Stanton L. Gerson,^{1,6,5||} Joseph M. Ready,^{4,15§||} Bruce Posner,^{4,15§||} James K. V. Willson,^{15§||} Sanford D. Markowitz^{1,6,5§||}

Agents that promote tissue regeneration could be beneficial in a variety of clinical settings, such as stimulating recovery of the hematopoietic system after bone marrow transplantation. Prostaglandin PGE2, a lipid signaling molecule that supports expansion of several types of tissue stem cells, is a candidate therapeutic target for promoting tissue regeneration *in vivo*. Here, we show that inhibition of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a prostaglandin-degrading enzyme, potentiates tissue regeneration in multiple organs in mice. In a chemical screen, we identify a small-molecule inhibitor of 15-PGDH (SW033291) that increases prostaglandin PGE2 levels in bone marrow and other tissues. SW033291 accelerates hematopoietic recovery in mice receiving a bone marrow transplant. The same compound also promotes tissue regeneration in mouse models of colon and liver injury. Tissues from 15-PGDH knockout mice demonstrate similar increased regenerative capacity. Thus, 15-PGDH inhibition may be a valuable therapeutic strategy for tissue regeneration in diverse clinical contexts.

Tissue regeneration is often required during recovery from injury, disease, and certain medical treatments. For example, hematopoietic stem cell (HSC) transplantation, which includes bone marrow transplantation, is a potentially curative therapy for many hematologic malignancies (1). However, after HSC transplantation, individuals are at high risk of potentially lethal infections while awaiting regen-

Prostaglandin PGE2 is a candidate molecule for potentiating regeneration in multiple tissues. PGE2 is a lipid signaling molecule produced by the enzyme activity of cyclooxygenase-1 or cyclooxygenase-2 (COX-1 or COX-2) followed sequentially by that of prostaglandin E synthase (9). PGE2 augments Wnt signaling (10, 11), a pathway that is involved in the maintenance of several types of tissue stem cells, including hematopoie-

in vivo degradation of PGE2. The enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which acts *in vivo* as a negative regulator of prostaglandin levels and activity (20–22), provides a candidate target. Specifically, 15-PGDH catalyzes the first step in the degradation of prostanoid family molecules, oxidizing the prostanoid 15-hydroxyl group to a ketone and thereby abrogating binding to prostaglandin receptors (20). Here, we explore whether pharmacological inhibition of 15-PGDH can potentiate tissue repair in several mouse models of injury and disease.

Results

Genetic deletion or pharmacologic inhibition of 15-PGDH increases tissue PGE2 levels

To confirm that 15-PGDH broadly regulates PGE2 *in vivo*, we compared PGE2 levels in 15-PGDH knockout (21) and wild-type mice, retesting lung (27) and colon (22) and newly interrogating bone marrow and liver. Although basal PGE2 levels varied by a factor of 5 across these four tissues, the 15-PGDH knockout mice exhibited a consistent twofold increase in PGE2 levels (Fig. 1A). We hypothesized that a chemical inhibitor of 15-PGDH would have a similar effect and, further, would provide a tool to explore 15-PGDH as a therapeutic target for potentiating tissue regeneration.

As a first step in exploring the therapeutic potential of pharmacologically inhibiting 15-PGDH, we conducted a high-throughput screen to identify small-molecule modulators of 15-PGDH enzyme activity. We screened 230,000 synthetic compounds in the University of Texas Southwestern Medical Center chemical library using a cell-based assay designed to identify compounds

¹Department of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA. ²Department of Gastroenterology, Haeundae Paik Hospital, Inje University, Busan 612896, South Korea. ³Department of Surgery, Busan Paik Hospital, and Paik Institute of Clinical Research and Ocular Neovascular Research Center, Inje University, Busan, South Korea. ⁴Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. ⁵Case Medical Center, University Hospitals of Cleveland, Cleveland, OH 44106, USA. ⁶Case Comprehensive Cancer

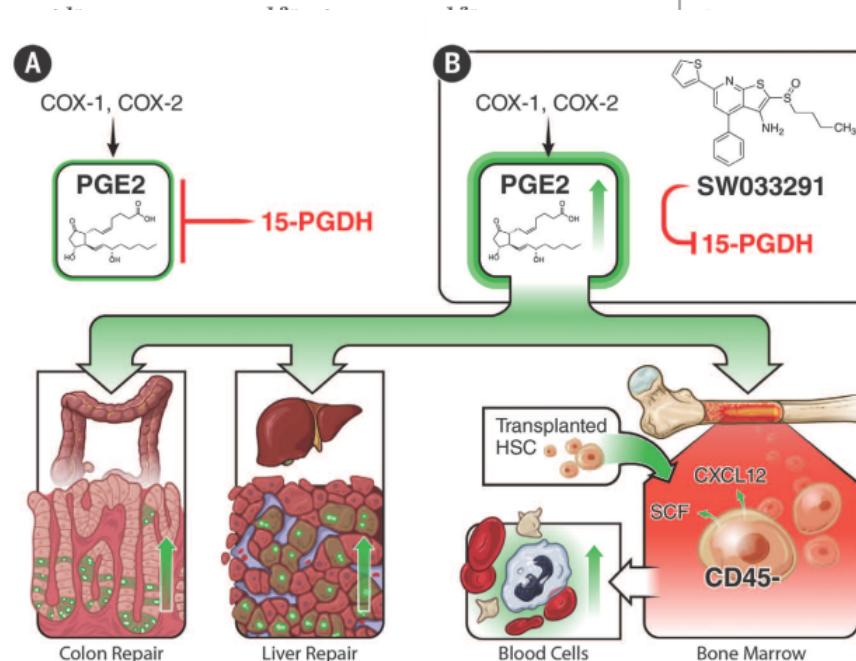
RESEARCH ARTICLE

TISSUE REGENERATION

Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration

Yongyou Zhang,^{1,*} Ami Monika I. Antczak,^{4,*} Noelle S. Williams,⁴ D Zhenghe Wang,^{6,8} Lak Luca Di Martino,¹ Zora Chris Dealwis,¹³ Debra Stanton L. Gerson,^{1,6,5} James K. V. Willson,¹⁵

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Results

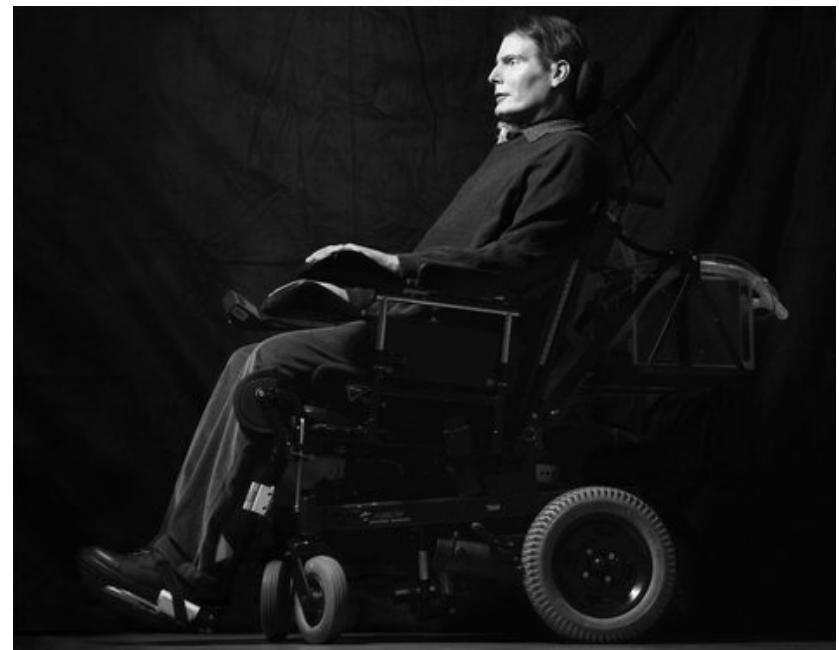
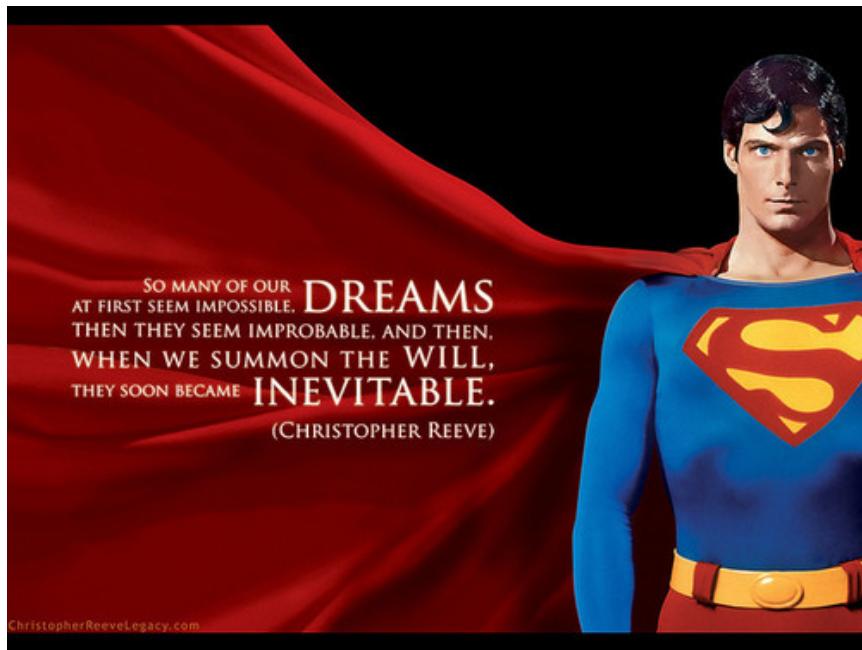
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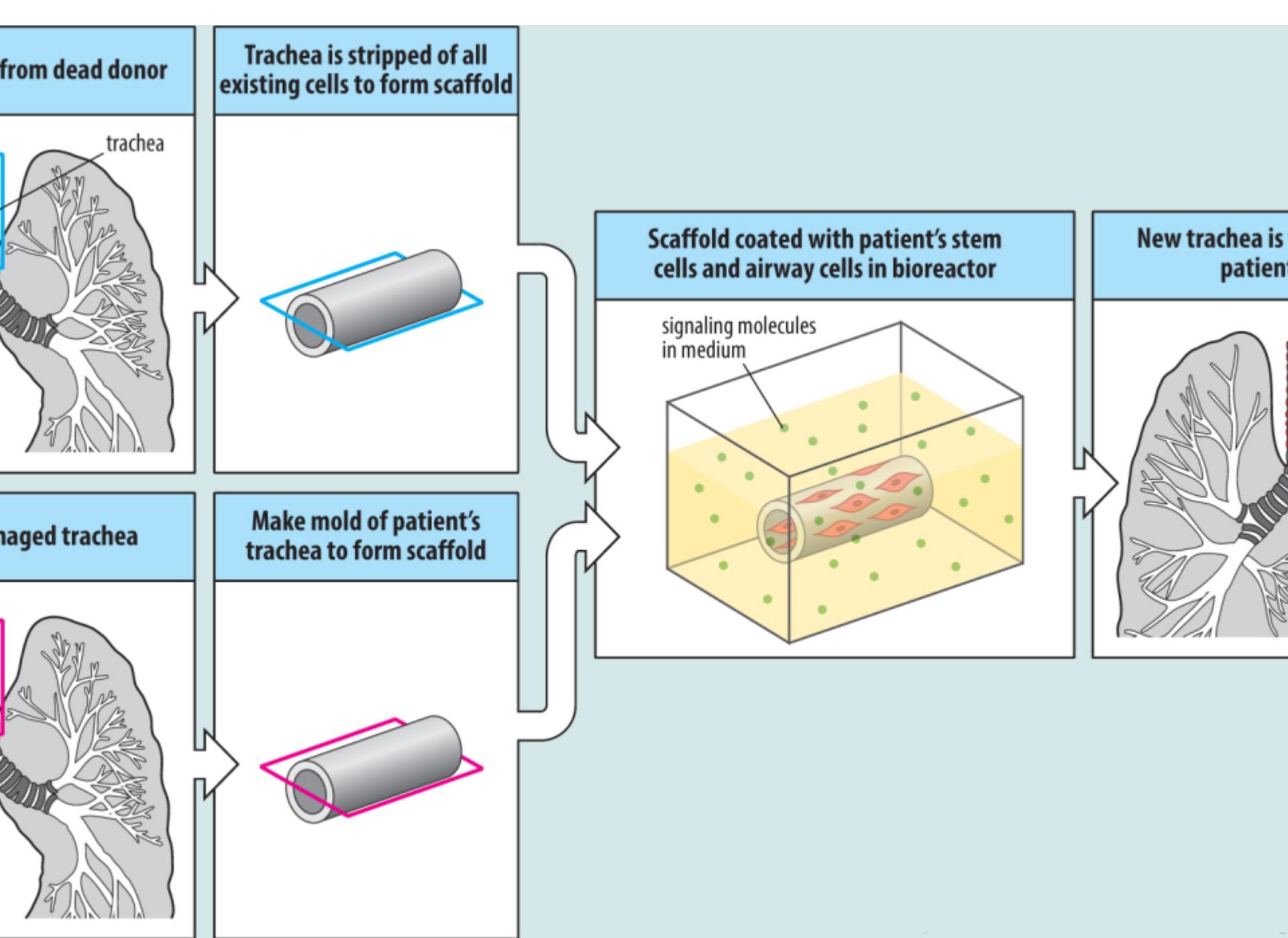
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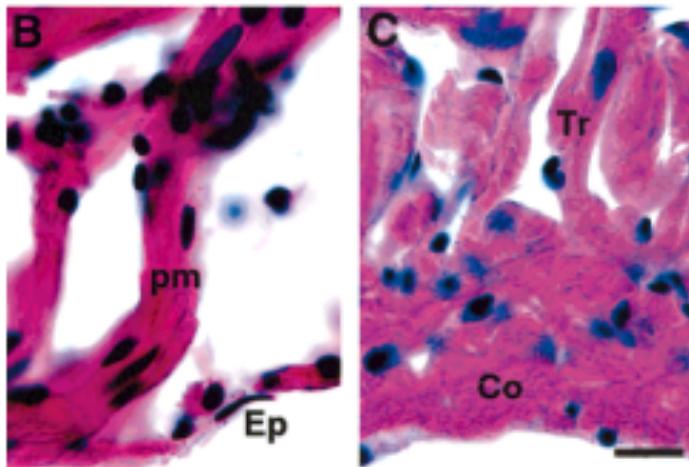
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Superman Christopher Reeve

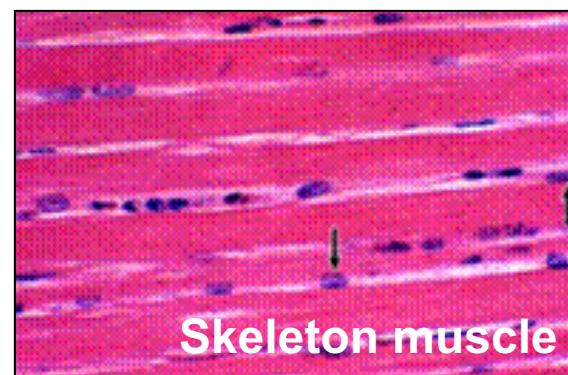
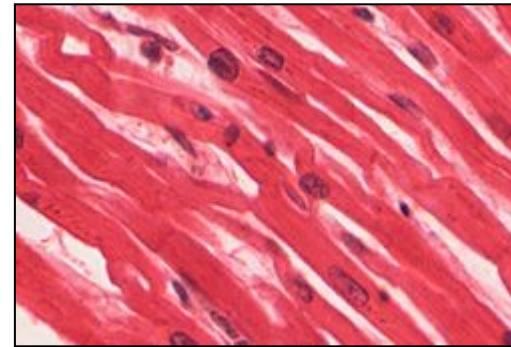




Zebrafish cardiomyocyte



Human cardiomyocyte



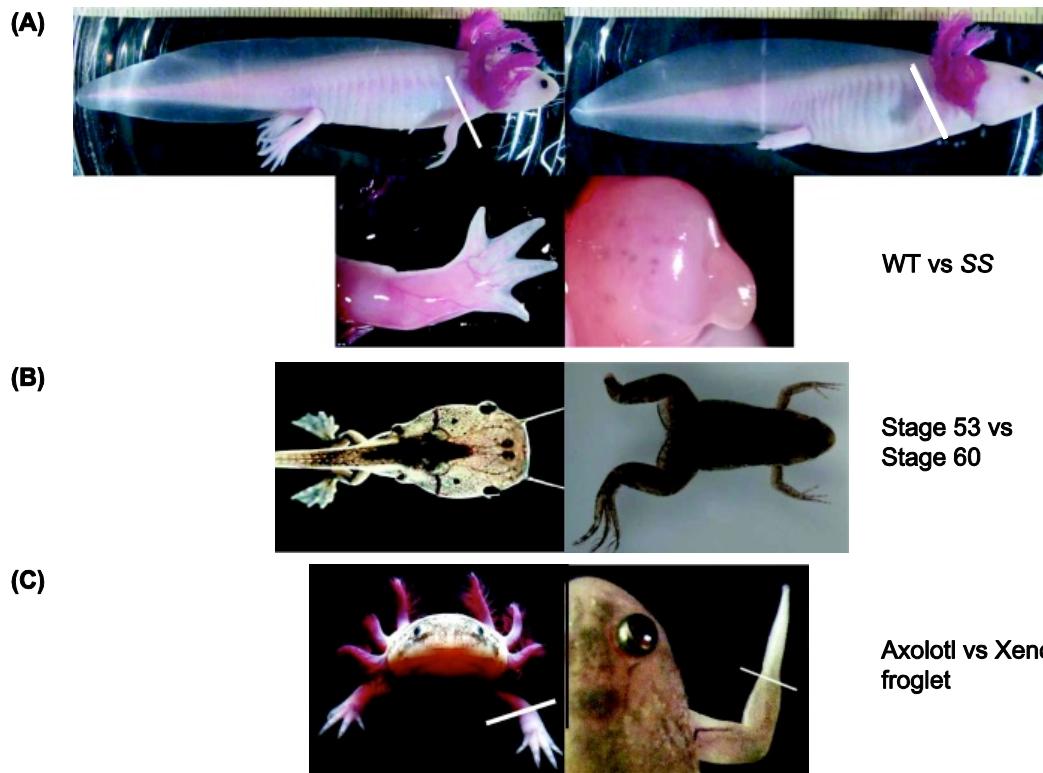


Figure 1.10 Comparative models of regeneration-competent tissues vs. regeneration-deficient tissues. Regenerating amphibian limbs have examples of all three models. **(A)** Wild type vs. mutant gain or loss of function. Left, regeneration-competent white axolotl which by 6 weeks post-amputation (white line) through the upper forelimb has regenerated the amputated parts. Right, by contrast the *short-toe* mutant, which is characterized by stubby digits and shortened long bones, has lost the ability to regenerate, as shown by the small blob of tissue that has formed at the amputation site by 6 weeks. **(B)** Change in regenerative competence with development. Left, a *Xenopus* tadpole hindlimb amputated through the tarsal region at stage 53 has regenerated perfectly at 60 days post-amputation. Right, a froglet amputated through the tarsus at stage 60 is regenerating a cartilage spike 60 days post-amputation. **(C)** Species differences in regenerative competence. Left, adult axolotl regenerates perfectly after amputation through the radius/ulna of the forelimb. Right, a *Xenopus* froglet amputated through the same region of the forelimb regenerates only a symmetrical cartilage spike.