nally, the smallest network protects 40% of the habitat, which is in agreement with theoretical work on the minimum fraction of coastline posited for persistence of populations (*21*).

The use of explicit socioeconomic variables in addition to biodiversity data is particularly important because in marine systems, where fishing is a major threat, ecological criteria and socioeconomic measures are not independent (*28*). Moreover, portfolios of solutions can be presented to decision-makers (*29, 30*), who can then evaluate the costs and benefits of different management options within socioeconomic constraints. Prioritization of the reserves can be carried out with this model, using a stepwise selection that evaluates the contribution of each reserve to the preservation of total biodiversity. In the future, new conservation models that account for soft bottoms, pelagic habitats, marine mammals, sea turtles, coastal lagoons, and additional social factors, including future threats, should be developed to obtain networks of reserves to preserve all marine biodiversity. Meanwhile, this procedure can be applied to any coastal region and offers a constructive approach to integrating the economic, social, and biological concerns of marine biodiversity preservation.

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16. Our goal was to protect enough critical habitats to allow the long-term persistence of commercial species, although this is difficult to predict when heavy fishing occurs outside the reserves (*31*). A reserve network must include all known spawning aggregations of vulnerable reef fishes (*15*). Mangroves are the only vulnerable habitats that provide nurseries for vulnerable species (goliath grouper, broomtail grouper, and most snappers) (*17*). Juveniles of these species, however, are not threatened directly by fishing. We set a goal of protecting at least 50% of mangroves.
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Supporting Online Material

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Materials and Methods
Figs. S1 and S2

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Ectoderm to Mesoderm Lineage Switching During Axolotl Tail Regeneration

Karen Echeverri and Elly M. Tanaka*

Foreign environments may induce adult stem cells to switch lineages and populate multiple tissue types, but whether this mechanism is used for tissue repair remains uncertain. Urodele amphibians can regenerate fully functional, multitissue structures including the limb and tail. To determine whether lineage switching is an integral feature of this regeneration, we followed individual spinal cord cells live during tail regeneration in the axolotl. Spinal cord cells frequently migrate into surrounding tissue to form regenerating muscle and cartilage. Thus, in axolotls, cells switch lineage during a real example of regeneration.

Lineage restriction into ectodermal, mesodermal, and endodermal germ layers that occurs during development has been thought to be a process that is not reversed. However, recent data indicate that adult cells from various sources, including brain, skin, and bone marrow, can form cell types of other lineages when exposed to novel or foreign environments (*1–5*). Whether such examples represent true cases of cell-type switching and whether lineage switching represents a rare or frequent event are still being debated (*6, 7*).

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, D-01307 Dresden, Germany.

*To whom correspondence should be addressed. E-mail: tanaka@mpi-cbg.de

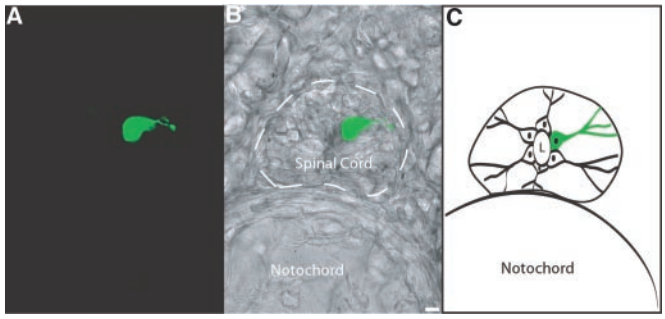

Fig. 1. Transverse cryosection of the axolotl tail containing a transfected radial glial cell. (**A**) Fluorescent image of a GFP-expressing radial cell. (**B**) The DIC image overlaid with the fluorescence image shows that the cell lies in the spinal cord and extends a process toward the pial layer of the spinal cord. (**C**) Spinal cord morphology. Radial glial cells of the ependymal layer lie closest to the lumen (L) and extend processes to the pial edge; a labeled cell is illustrated in green. Scale bar, 50 μm.

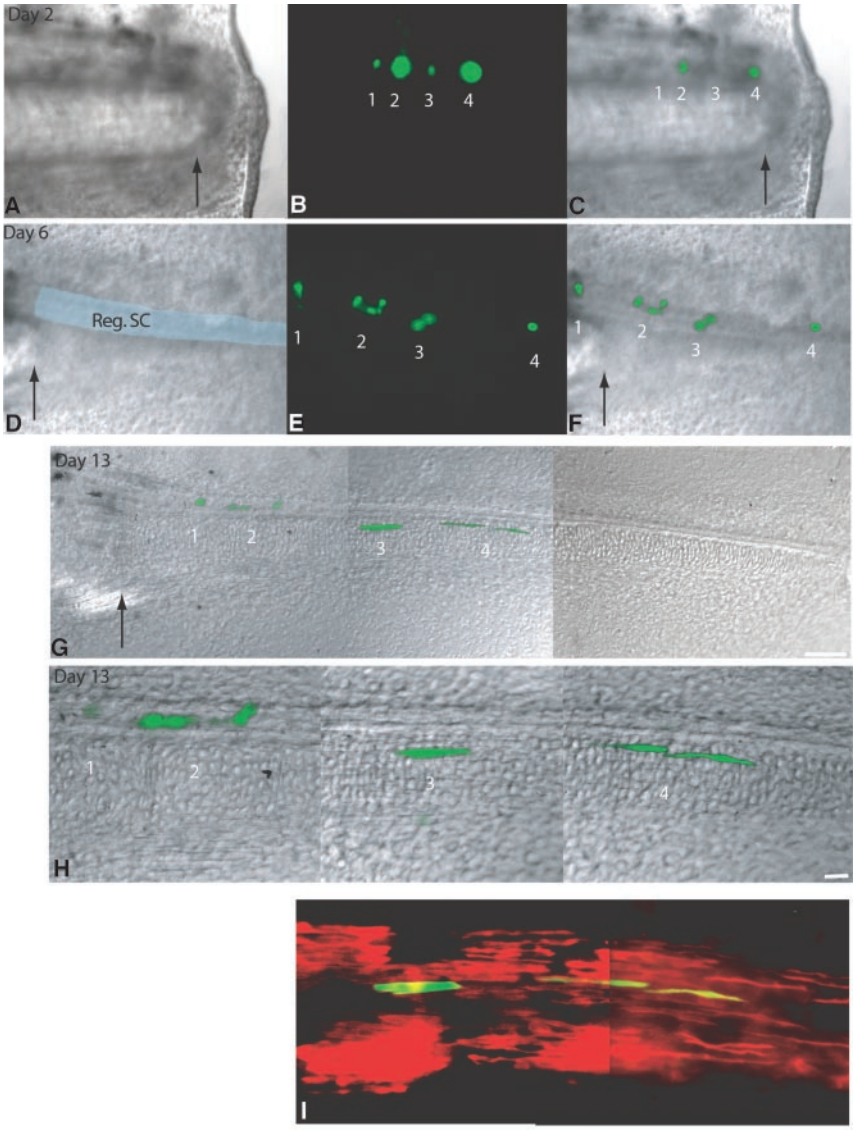

Fig. 2. Radial glial cells exit the spinal cord and contribute to muscle. (**A** to **C**) Day 2; (**D** to **F**) day 6; (**G** and **H**) day 13. Two days postamputation, four cells labeled by GFAP-GFP are visible in the spinal cord (B and C). In (B), the brightness of the fluorescent image has been increased to allow visualization of cells 1 and 3. Arrows in (A), (D), and (G), indicate the plane of amputation; the regenerating spinal cord (Reg. SC) is highlighted in blue in (D). By day 6, the cells have begun to proliferate (E and F). By day 13, the cells of cluster 4 have also exited the spinal cord, while cells from clusters 1 and 2 have migrated into the regenerate but remain within the spinal cord (G). Bar = 100 μm. At higher magnification (H), it is clear that the exited cells display an elongated shape resembling nascent muscle fibers. (**I**) The exited cells (green) are positive for the differentiated muscle marker, myosin heavy chain (red). Scale bar, 50 μm.

The issue of neural cell plasticity has been particularly controversial. Cells derived from the ventricles of the adult brain have been documented to form muscle in transplantation and coculture experiments and bone marrow when infused into irradiated mice (*1, 4, 8, 9*). Furthermore, when transplanted into chicken embryos, these neural progenitors populated tissues arising from all three embryonic germ layers (*3*). The following questions have been raised: (i) whether the cell that gave rise to other cell types was truly of neural origin rather than a contaminating cell type, (ii) whether long-term culture may have selected cell characteristics not present in the original neural cell itself (*7*), and (iii) whether the cells merely fused with other cells and their fate was thereby determined by fusion to a predetermined cell type (*10, 11*). Such ambiguities are largely due to the cell assays that involve placing populations of cultured cells into unnatural environments and examining the outcome after numerous cell doublings have occurred. Understanding cell plasticity is important because of its potential for creating progenitor pools for tissue regeneration, but because most mammalian tissues do not naturally regenerate, it has been difficult to assess the relevance of this phenomenon for tissue repair.

To determine whether lineage switching is an integral part of tissue regeneration when it occurs in a natural context, we developed a method to follow individual neural precursor cells live during axolotl tail regeneration. In amphibian axolotls, tail amputation results in perfect regeneration of all tail structures, including the spinal cord, muscle, cartilage, dermis, and skin, in several weeks (*12–14*). The entire process of tail regeneration can be visualized with a light microscope with single-cell resolution in 2-cm-long animals (*15*). Immediately after amputation, epidermal cells migrate over the cut end to form the wound epidermis. Over the next few days, a zone of proliferating progenitor cells called the blastema forms. Blastema cells largely derive from muscle and dermis dedifferentiation and in the late stages of regeneration redifferentiate into muscle, cartilage, and dermis (*15, 16*). The regenerating spinal cord constitutes a tube of neuroepithelial cells separate from the blastema (see fig. S1). As regeneration proceeds the tube elongates, and spinal cord neurons differentiate.

Histological studies indicated that lineage restrictions were likely maintained during regeneration; the wound epidermis derived from mature epidermis, the regenerating spinal cord and neural crest formed from the spinal cord, and mesodermal tissues such as muscle and cartilage formed from dedifferentiation of tissues originating from mesoderm (*17–20*). However, these observations depended on reconstructing events from static

images of cell populations within regenerating tissue. Therefore, whether cells crossed lineage boundaries during regeneration remained an open question.

To follow spinal cord cells during regeneration, we used single-cell electroporation to express the green fluorescent protein (GFP) in the neural precursor cells of the spinal cord. These precursor cells are radial glial cells similar to radial glial cells that act as neural precursors in the mammalian brain (*21*). We targeted GFP expression to radial glial cells by inserting the DNA-filled microelectrode into the lumen of the spinal cord through the cut end of the tail (*22*) and by driving GFP expression with the glial acidic fibrillary protein (GFAP) promoter (*23*) via the GAL4-VP16 enhancer (*24, 25*). We chose the GFAP promoter because GFAP is expressed in the radial glial cells of the mature axolotl spinal cord and expression is maintained in the regenerating spinal cord cells (*26*). Expression of GFP was reliably visible 48 hours postelectroporation, the starting point of our tracking experiments. By this time one to four cells were labeled in the spinal cord, depending on the animal. Whether the profile of cell labeling is due to division of a single cell or to electroporation of multiple cells is not known. We confirmed the specificity of the labeling method in cryosections of the transfected tails ($n = 10$) where the labeled cell was always found in the cell layer lining the neural canal (Fig. 1). Figure 1A shows a transfected cell in a cross section of the spinal cord. The cell has the characteristic fine radial processes of a GFAP-positive spinal cord cell, and the overlay with a differential interference contrast (DIC) microscopy image of the cross section confirms that the cell is in the layer lining the spinal cord lumen (*27*). Figure 1C illustrates the overall layout of the cross section and the positions of the GFAP-positive radial glial cells.

When we followed individual radial glial cells during regeneration, we found that their descendants were not all restricted to an ectodermal cell fate. Although many labeled cells formed the expected neural cell types (see fig. S2), at high frequency the descendants of labeled spinal cord cells migrated out of the regenerating spinal cord and contributed to the regenerating mesodermal structures: muscle and cartilage (Table 1). Two of the major classes of cell fates are illustrated in Figs. 2 and 3.

The most frequent cases of cell type switching were to muscle (Fig. 2). Two days posttransfection, four cells were labeled in the mature spinal cord (Fig. 2, A to C). By day 6 the cells had divided and spread into four clusters in the regenerating spinal cord (Fig. 2, D to F). By day 13, cells of clusters 3 and 4 (three cells) had moved outside the spinal cord and displayed an elongated shape resembling nascent muscle fibers (Fig. 2, G and H). In contrast, the cells from clusters 1 and 2 remained in the spinal cord (Fig. 2, G and H). To confirm that the cells of clusters 3 and 4 had transdifferentiated into muscle, we fixed the regenerating tail and processed it for whole-mount immunohistochemistry with muscle-specific myosin heavy chain. Figure 2I shows that the cells were positive for the myosin marker and were lying within the area of the tail undergoing extensive muscle differentiation. This illustrates that radial glial cells originally derived from the ectoderm can switch lineage during tail regeneration and contribute to muscle, a cell type originally made from mesoderm.

Whether these radial glial cells were predetermined to form muscle before exiting the spinal cord and fusing with nearby cells is not yet known. However, we also observed labeled cells contributing to the cartilage rod that forms ventral to the regenerating spinal cord (Fig. 3). Differentiation into cartilage does not involve cell fusion. In this example, two cells were initially transfected in the mature spinal cord (Fig. 3, A to C). By day 8, cell 2 had divided within the regenerating spinal cord (Fig. 3, D to F), but then these cells exited out of the spinal cord, rapidly changed shape (day 9) (Fig. 3, G to I), and became morphologically distinct cartilage cells by day 11 (Fig. 3, J to L, arrowheads). Cartilage cells are mononucleate, and their fate could not be determined by fusion with other predetermined cells.

Table 1. Different cell types deriving from GFAP-positive cells during tail regeneration. Frequency is calculated based on the number of animals followed (*25*). Animals contained multiple labeled cells, and some labeled cells gave rise to descendants that had different fates, so the total frequency is greater than 100%.

Cell type	Frequency (%)
Muscle	24 (6/25)
Cartilage	12 (3/25)
Neurons	16 (4/25)
Neural crest, side*	28 (7/25)
Neural crest, end†	24 (6/25)
Melanocytes	8 (2/25)
Glial cells	28 (7/25)

*Neural crest cells that exited through the wall of the regenerating spinal cord. †Neural crest cells that exited out of the end of the regenerating spinal cord.

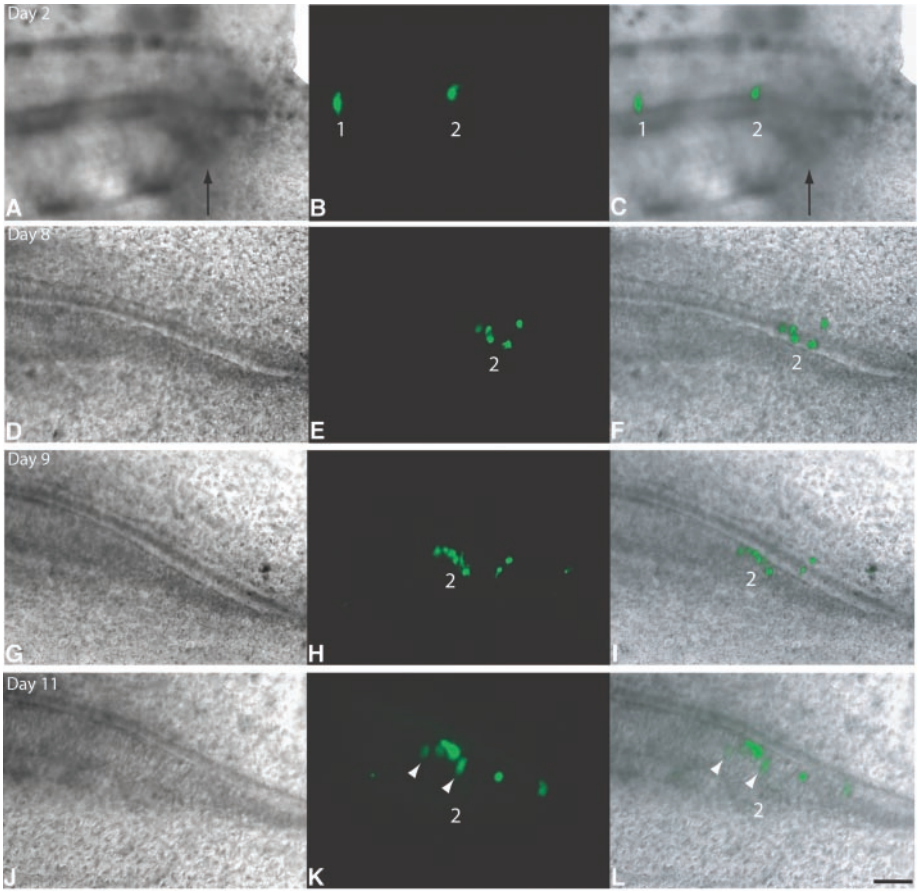

Fig. 3. GFAP-positive radial glial cells can also form cartilage. (**A** to **C**) Day 2. Two cells are initially labeled in the spinal cord (B and C). (**D** to **F**) Day 8. The cell closest to the plane of amputation, cell 2, migrates out into the regenerating spinal cord and begins to proliferate (E and F). Cell 1 is not visible in this image. It remained close to the plane of amputation and gave rise to new radial glial cells. (**G** to **I**) By day 9, cells from cell cluster 2 begin to exit the spinal cord (H and I). (**J** to **L**) By day 11, all labeled cells have left the spinal cord, rapidly changing shape to become morphologically distinct cartilage cells; see arrowheads (K and L). Scale bar, 100 μm.

In 24% of the animals, we observed glial cells that exited the spinal cord to form muscle; in 12% of the animals, cells exited to form cartilage (Table 1). Because each animal contained multiple labeled cells at the start point of our experiments, this represents 20% and 8%, respectively, of the starting number of labeled cells. Other radial glial cells formed the expected neural cell types, including glia, neurons, and neural crest derivatives such as fin mesenchyme and melanocytes. During formation of the neural crest, cells took two exit routes from the regenerating spinal cord: they exited through the side walls of the spinal cord, as expected from previous histological results, and also out of the end of the growing tube (Table 1).

Neural stem cells have been described as having the potential to differentiate into neurons, astrocytes, and oligodendrocytes and to undergo self-renewal through the process of asymmetric cell division. Although individual cells may harbor such potential, our observations suggest that, in practice, a single neural progenitor may generate a limited number of cell types and may not even undergo self-renewal. We have observed that some cells proliferate to replenish the pool of radial glial cells, whereas others produce progeny that all differentiate. All the visible progeny of a cell apparently exit the spinal cord, leaving no self-renewing cell behind in the spinal cord (Figs. 2 and 3). It is not known whether this observation reflects differences in innate potential or in the extracellular cues encountered.

In the axolotl, we have observed that GFAP-positive radial glial cells have the ability not only to re-form a functional spinal cord but also to contribute to regenerating tissues outside the spinal cord such as muscle and cartilage. These experiments establish the relevance of neural cell plasticity to regenerating functional tissue in amphibia. The question remains whether mammalian neural stem cells also have the inherent ability to switch lineage. Is the contrast in regeneration ability between amphibians and mammals due to intrinsic differences in neural cell plasticity or to the environment the cells encounter in the injured tissue? In axolotls, tail regeneration occurs through the formation of the blastema—a zone of undifferentiated cells surrounding the regenerating spinal cord. It is likely that mammalian and axolotl neural stem cells are similar, but mammals lack the ability to form an inductive environment like the blastema that induces cells to switch lineage.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5600/1993/DC1

Figs. S1 and S2

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Inhibition of Excess Nodal Signaling During Mouse Gastrulation by the Transcriptional Corepressor DRAP1

Rabah Iratni,[1]*† Yu-Ting Yan,[2]* Canhe Chen,[2] Jixiang Ding,[2] Yi Zhang,[1]‡ Sandy M. Price,[2] Danny Reinberg,[1]§ Michael M. Shen[2]§

The formation and patterning of mesoderm during mammalian gastrulation require the activity of Nodal, a secreted mesoderm-inducing factor of the transforming growth factor–β (TGF-β) family. Here we show that the transcriptional corepressor DRAP1 has a very specific role in regulation of *Nodal* activity during mouse embryogenesis. We find that loss of *Drap1* leads to severe gastrulation defects that are consistent with increased expression of *Nodal* and can be partially suppressed by *Nodal* heterozygosity. Biochemical studies indicate that DRAP1 interacts with and inhibits DNA binding by the winged-helix transcription factor FoxH1 (FAST), a critical component of a positive feedback loop for Nodal activity. We propose that DRAP1 limits the spread of a morphogenetic signal by down-modulating the response to the Nodal autoregulatory loop.

Recent studies indicate that Nodal and related members of the TGF-β family correspond to primary mesoderm-inducing signals in all vertebrates (*1, 2*). Nodal signaling uses an activin/TGF-β–like pathway that is mediated by the signal transducers Smad2 and Smad4 and the winged-helix transcription factor FoxH1 (FAST) (*2*). A primary target of Nodal signaling is the *Nodal* gene itself, resulting in a positive feedback loop that is essential for *Nodal* expression in the visceral endoderm as well as broad expression in the pregastrulation epiblast (*3–5*). Analysis of *FoxH1*-deficient mice indicates that FoxH1 mediates the positive Nodal feedback loop, but not several other Nodal-dependent patterning events (*6, 7*). In addition, members of the *Lefty* subfamily of TGF-β factors can inhibit Nodal signaling and may be induced as part of a negative feedback loop (*1*). Presumably, the activities of these feedback loops are tightly regulated by transcriptional repression, but the molecular mechanisms involved have not been elucidated previously.

Several categories of transcriptional re-

[1]Howard Hughes Medical Institute and Department of Biochemistry, Division of Nucleic Acids Enzymology, [2]Center for Advanced Biotechnology and Medicine and Department of Pediatrics, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, 679 Hoes Lane, Piscataway, NJ 08854, USA.

*These authors contributed equally to this work.

†Present address: Institute Albert Bonniot, Domaine de la Merci, 38706 La Tronche Cedex, France.

‡Present address: Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA.

§To whom correspondence should be addressed. E-mail: reinbedf@umdnj.edu, mshen@cabm.rutgers.edu