



## PERSPECTIVES: DEVELOPMENT

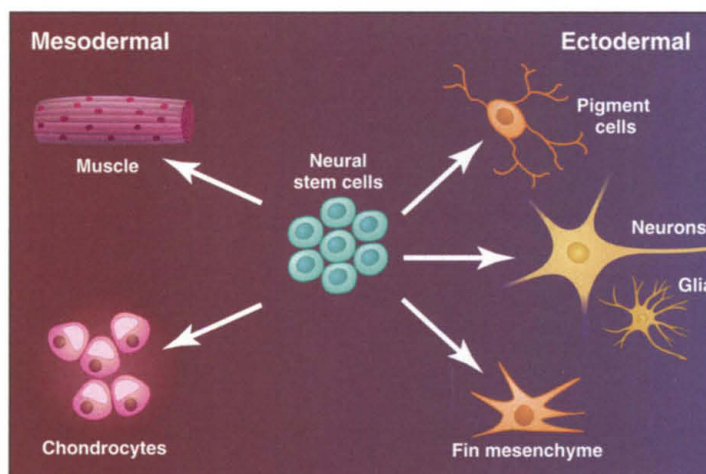
# A Tail of Transdifferentiation

David L. Stocum

Stem cells have the job of replacing tissues that turn over, such as blood and epithelia, or that become damaged by injury or disease, such as muscle and bone. Particularly intriguing is the regenerative capacity of some regions of the mammalian central nervous system—for example, mouse neural stem cells (NSCs) routinely regenerate olfactory bulb neurons (1). However, other regions of the mammalian brain and spinal cord regenerate poorly, even though they harbor NSCs (2). Recent experiments suggest that mouse NSCs (which are of ectodermal origin) as well as other adult mammalian stem cells are developmentally plastic. In vivo, mouse NSCs can be converted (transdifferentiate) into cells of mesodermal or endodermal lineages when injected into mice injured by lethal irradiation (3). But it is still debatable whether transdifferentiation is a real postinjury phenomenon because of questions about the intensity of cell labeling, the accuracy of cell isolation, the identification and purity of test stem cells, the low numbers of transdifferentiated cells detected, and the variable results obtained by different laboratories. Enter Echeverri and Tanaka (4) on page 1993 of this issue, who show in living color that ectodermal NSCs of the regenerating salamander (axolotl) tail transdifferentiate at high frequency into mesodermal muscle and cartilage in vivo.

Amputation of the axolotl tail results in regeneration of all tail structures including the spinal cord, muscle, cartilage, dermis, and skin within several weeks. Regeneration is accomplished by the dedifferentiation of cartilage, muscle, and dermal fibroblasts to embryonic-like mesenchymal cells. These cells form a zone of proliferating progenitor cells (the blastema) over the cut surface of the tail. A separate tube of dividing NSCs, which form neural precursor cells called ependymal or radial glial cells, extends from

the spinal cord into the blastema. The mesenchymal cells in the regenerating tail differentiate into new cartilage, muscle, and dermis, and the ependymal tube regenerates the spinal cord and neural crest derivatives, such as fin mesenchyme and pigment cells (see the figure). The tail of recently hatched salamander larvae is thin and transparent, and so the fate of labeled NSCs can be followed live with a light microscope.



**Cells switch allegiance.** Developmental fate of NSCs in the regenerating axolotl tail. Most of the NSCs in the growing ependymal tube differentiate into ectodermal derivatives: neurons, glia, pigment cells, and fin mesenchyme. However, some ectodermal NSCs transdifferentiate into mesodermal lineages, becoming muscle cells or chondrocytes that form cartilage.

Echeverri and Tanaka inserted the cDNA for green fluorescent protein (GFP) into individual spinal cord ependymal cells after tail amputation. They chose the glial fibrillary acidic protein (GFAP) promoter to control GFP expression, because GFAP is expressed in the ependymal cells of the mature axolotl spinal cord. This allowed them to track spinal cord ependymal cells throughout the process of regeneration. Control sections stained with antibody to GFAP showed that only ependymal cells expressed GFP. The labeled cells were seen to divide and their progeny differentiated into neurons and glia in the regenerating spinal cord, as well as neural crest derivatives, such as fin mesenchyme and melanocytes. But some NSCs migrated out of the ependymal tube and transdifferentiated into muscle (identified with antibody to muscle-specific myosin heavy chain) and morphologically distinct chondrocytes, at frequencies of 24% and

12%, respectively. In salamanders, transdifferentiation occurs during regeneration of limbs, neural retina, and lens, but only within mesodermal or ectodermal lineages (5). This is the first unequivocal demonstration that an ectodermally derived stem cell, the NSC, is converted into mesodermal cell types during the regeneration of amphibian tissues. As yet unanswered is the question of whether tail blastema cells derived from dedifferentiating muscle, cartilage, and dermis can transdifferentiate into neurons, glia, and neural crest derivatives.

What is the mechanism of NSC transdifferentiation in the regenerating axolotl tail? Muscle cells are formed by fusion of myoblasts, so one mechanism might be the fusion of NSCs with blastema cells that are

destined to become muscle, resulting in the activation of muscle-specific genes in the NSC nuclei (6). Alternatively, the NSCs, in response to signals released by the injured region, might transdifferentiate into myoblasts that then fuse to become muscle myotubes. Purified GFP-labeled mouse NSCs cocultured with a muscle cell line participate in muscle formation, perhaps through both of these mechanisms (7). The significance of transdifferentiation to axolotl tail regeneration is unknown, but it may ensure that the proper spatial pattern of tissues is regenerated in lieu of strictly controlling the position of NSCs within a blastema derived from multiple cell sources.

The big question is whether the developmental potential of mammalian NSCs is restricted compared with that of salamander NSCs. The answer seems to be no. The greater capacity of axolotl NSCs to generate new neurons and undergo transdifferentiation after tail amputation is most likely due to an injury environment that is more favorable to regeneration. Consistent with this idea, providing a more permissive environment boosts the capacity of mammalian NSCs to produce new neurons. NSCs in the periventricular region of the adult rat hippocampus regenerate only small numbers of pyramidal neurons after ischemic injury in vivo, but treatment with epidermal growth factor and fibroblast growth factor-2 boosts the number of regenerated neurons to nearly 50% of normal (8). These neurons are functionally integrated into the normal hippocampal circuitry as determined by microscopy, the electrophysiological properties of synapses, and the rat's performance on behavioral tasks. Furthermore, clonally derived mouse

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NSCs injected into early chick or mouse embryos are able to make a wide range of nonneuronal cell types, showing that they have the innate capacity for transdifferentiation when exposed to appropriate developmental signals (9).

Differences in the injury environments of salamanders and mammals suggest a strategy for understanding the biology of regeneration that will contribute to progress in regenerative medicine. The strategy is to compare and contrast the patterns of gene activity in regeneration-competent versus regeneration-deficient tissues to define which molecular signals and injury products determine whether regeneration rather than scar formation takes place. Several types of

comparative models are useful: regenerating versus nonregenerating species, for example, salamanders versus frogs; regeneration-competent versus regeneration-deficient stages of the life cycle, such as frog tadpoles versus froglets; or mutant versus normal tissues, for example, the ear and heart tissue of wild-type versus MRL mutant mice (10). The data obtained can then be used to design molecular "cocktails" of genes or proteins that mimic an injury environment permissive for regeneration by the body's own tissues. The feasibility of this approach is indicated by the fact that mammalian muscle cells can be induced to dedifferentiate by newt limb regeneration blastema extracts (11). Although stem cell transplantation is

likely to be the first therapeutic wave of regenerative medicine, the ability to induce regeneration of new tissues from our own cells will not be far behind.

#### References

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#### PERSPECTIVES: ECOLOGY

## Looking Beneath the Surface

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It is widely accepted that the release of greenhouse gases into the atmosphere will have profound impacts on Earth's climate, including global warming, altered precipitation patterns, and increased storm intensities (1). The likely ecological impact of global change is typically assessed in experiments conducted in various ecosystems subjected to one or at most two such environmental changes. The paucity of multiple-factor, multiple-year global change studies limits our understanding of how ecosystem processes will respond to global climate change.

On page 1987 of this issue, Shaw *et al.* (2) take an important step toward a more integrated approach to understanding multiple global changes. The study raises questions about our ability to design and interpret studies for understanding long-term ecosystem responses to global change.

The authors report that simulated global changes—warming, increased precipitation, and increased nitrogen deposition—all increased net primary productivity (NPP) of a California annual grassland, but that elevated CO<sub>2</sub> reduced these global change enhancements in the third year of this field study. These findings are in sharp contrast to many earlier studies and to other findings presented in their report (2), which indicate that rising atmospheric CO<sub>2</sub> will either enhance production in grasslands or have minimal or no effects on production (3, 4).

Photosynthesis of almost all grass species is stimulated in the short term by increasing atmospheric CO<sub>2</sub> concentra-

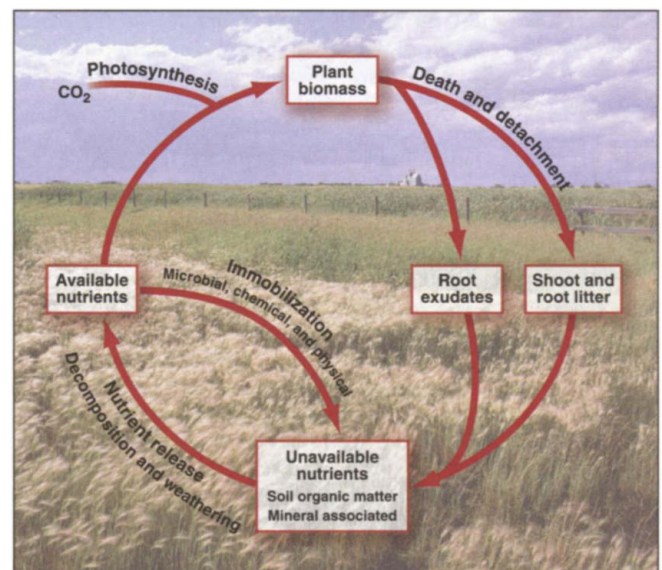
tions (3), suggesting that the potential exists for productivity responses of most grasses to increased CO<sub>2</sub>. However, this potential is often unrealized or declines over time because of plant metabolic adjustments that optimize resource use (4), or because the soil cannot keep pace with the greater demand for soil nutrients by faster growing CO<sub>2</sub>-enriched plants.

Longer term adjustments to CO<sub>2</sub> involve changes in soil nutrient cycling, which may further modulate plant responses. Under CO<sub>2</sub> enrichment, greater amounts of carbon may enter the soil organic pools, either as litter or root exudates, and may fuel microbial growth and demand for soil nutrients. This, in turn, can immobilize soil nutrients, making them less available to plants, and can reduce or eliminate a plant's ability to respond to CO<sub>2</sub>.

But can it lead to lower productivity? The answer is, apparently, yes. Shaw *et al.* (2) are not the first to report CO<sub>2</sub>-induced reductions in plant productivity. Although rare, inhibition of plant growth by elevated CO<sub>2</sub> has also been observed by others (5–7). Further, periods of

reduced production under elevated CO<sub>2</sub> have been predicted in plant simulation models that incorporate soil feedback mechanisms (8).

Microbial immobilization of nitrogen is a common feature in many CO<sub>2</sub> enrichment studies (9) and is probably involved in some of the negative growth responses to CO<sub>2</sub>. Elevated CO<sub>2</sub> inhibited NPP in the California grassland only under favorable growth conditions. Such conditions of high plant growth often deplete the soil of one or



**Complex feedbacks.** Litter from decaying plants and root exudates enters a large, diverse pool of nutrients that are unavailable to plants until they have been decomposed by microbes. Weathering also releases small amounts of nutrients over long time scales. Some of the available nutrients become immobilized by microbial growth; others may be rendered chemically or physically unavailable. The balance between nutrient release and immobilization determines the level of nutrients available to the plant, and hence the ultimate plant response. Increases in atmospheric CO<sub>2</sub> may initially stimulate photosynthesis and plant production, but soil nutrient feedbacks may constrain or eliminate that response. The long-term consequences of global change on these interactive processes are poorly understood and are likely to vary among ecosystems.