Memory of Fate and Position, Colorized

Stephen L. Johnson[1,*]
[1]Department of Genetics, Washington University Medical School, St. Louis, MO 63110, USA
*Correspondence: sjohnson@genetics.wustl.edu
DOI 10.1016/j.devcel.2009.07.005

Many of our ideas about cellular memory of fate and position come from regeneration studies in salamanders. A popular notion is that cells of the blastema transdifferentiate to different fates during limb regeneration. In a recent issue of *Nature*, Tanaka and colleagues challenge this notion. Using transplant experiments with GFP-expressing axolotl, they show vividly which cells of the blastema remember their fate and position of origin.

One of the enduring questions in developmental biology is whether and how cells know what and where they are. Thus, it is thought that the process of development endows cells with notions of their fate and also with notions of their position. Mechanisms of homeostasis, wound repair or regeneration in the adult might then use these fate and positional identities to maintain the mature form. Evidence that cells remember their fate and position comes most clearly from regeneration studies, including studies of the regenerating salamander limb. The report by Kragl et al. (2009) in a recent issue of *Nature* uses GFP or RFP transgenic axolotl in transplantation and regeneration studies to extend our confidence that cells know their fate and retain it through the blastema. The authors then build on these experiments to show that cartilage cells also retain and act on memory of their original positions but that neural crest-derived Schwann cells do not.

When salamander limbs are amputated, they first heal the wound by generating a wound epidermis (WE). Mesenchymal cells underneath the WE divide and form a mass of dedifferentiated cells referred to as the blastema. A variety of labeling experiments show that the blastema is derived from most or all of the mesenchymal tissues at the amputation plane and that after contributing to the apparently homogeneous blastema, the cells differentiate to the fate of their precursors in the stump (reviewed in Mescher, 1996). Thus, cells of the muscle lineage give rise to muscle, Schwann cells give rise to Schwann cells, and connective tissue gives rise to connective tissue during regeneration (Hay and Fishman, 1961; Gardiner et al., 1986). Other experimental approaches suggest that blastema cells can transdifferentiate, given the right opportunity (for instance, Thornton, 1938). Although the previously mentioned labeling experiments indicated that the vast majority of cells from muscle, connective tissue or Schwann cell lineages are lineage restricted during regeneration, some contribution from transdifferentiation to the regenerate might have been overlooked due to imprecision in the labeling technique. For instance, a commonly used labeling technique in axolotl has been to transplant triploid tissue into a diploid host, or vice versa. Since triploid cells often have three nucleoli and diploid cells always have two nucleoli, triploid cells can often, but not always, be distinguished in these regenerates. Such imprecision in the labeling does not invalidate the conclusions of lineage restriction drawn by earlier investigators but may contribute to the persistent belief in popular science that salamander cells transdifferentiate when passed through the regeneration blastema.

Kragl et al.'s use of genetically marked strains expressing fluorescent markers for donor tissue allows a much finer resolution and higher confidence to be brought to questions of lineage restriction. In addition to using GFP-expressing donor cells, the investigators preformed their transplants during embryonic development to generate chimeric juvenile limbs with discretely labeled tissues for their regeneration work. Thus, transplantation of GFP-labeled neural fold (including neural crest) into an unlabeled host embryo results in juvenile salamanders with label in the neural tube and neural crest derivatives. Since the only neural crest derivatives in these limbs are Schwann cells (these experiments were performed in the *white* mutant background, that ablates melanocytes, the other neural crest derivative in the limb), the finding that only Schwann cells are labeled in these regenerates reveals with high confidence that Schwann cells do not typically transdifferentiate to other fates during regeneration. Similar transplants of labeled lateral plate mesoderm to generate labeled dermis and connective tissue, or presomitic mesoderm to label muscle, followed by amputation and regeneration, reveal that dermis and connective tissue are restricted to dermis and connective tissue, and muscle derives only from the muscle lineage. These experiments now clearly demonstrate fate restriction in salamander limb regeneration with fluorescent glory. We can now retire the popularly held notion of extensive transdifferentiation in the regeneration blastema.

These first experiments from Kragl et al. (2009), which demonstrate that the blastema is a mix of different lineages that remember their fate, now compel the question of whether each of these lineages also remembers its positional identity. When blastema from distal, wrist level amputation sites are grafted onto blastema of more proximal amputation sites, the donor (wrist blastema) cells contribute mainly to the wrist or more distal structures, rather than to the more proximal structures in the upper arm (Stocum, 1975; Echeverri and Tanaka, 2005). These and similar experiments have led to the model that the blastema remembers its proximodistal (PD) position. With outgrowth of the regenerate, cells of the blastema first form structures appropriate to the PD position from which they originated and then acquire successively more distal identities with

further outgrowth (a property referred to as distalization).

Kragl et al. (2009) use their fluorescently labeled axolotl to ask whether two lineages, cartilage, and Schwann cells, remember their PD level of origin after transplant into unlabeled hosts. When they grafted GFP-labeled cartilage from proximal levels of the upper arm onto unlabeled upper arms and then amputate through the graft, they find that donor-derived labeled cells contribute to the entire length of the regenerate distal of the amputation plane. That the upper-arm cartilage cells can contribute to the distal lower-arm regenerate illustrates the property of distilization discussed above. In contrast, when distal cartilage of the finger tips are grafted into the upper arm then amputated through the graft, cells from the distal cartilage are generally excluded from the proximal regenerate and are found instead in the cartilages of the hand. These experiments reveal that the cartilage lineage retains and acts on positional memory when passed through the blastema.

Other lineages need not retain their positional memories during regeneration. To explore this, Kragl et al. (2009) transplanted GFP-labeled Schwann cells from hands into upper arms and then amputated through the graft. Rather than homing to the hand regenerate, labeled Schwann cells were found throughout the PD axis of the regenerated limb. These results indicate that unlike cartilage, the Schwann cell does not retain or act on memory of its position but rather can acquire new positions. The findings now raise the question of which other lineages in the limb retain and act on PD positional memory when passed through the regeneration blastema.

REFERENCES

Echeverri, K., and Tanaka, E.M. (2005). Dev. Biol. *279*, 391–401.

Gardiner, D.M., Muneoka, K., and Bryant, S.K. (1986). Dev. Biol. *118*, 488–493.

Hay, E.D., and Fishman, D.A. (1961). Dev. Biol. *3*, 26–59.

Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H.H., and Tanaka, E.M. (2009). Nature, in press. Published online July 2, 2009. 10.1038/nature08152.

Mescher, A.L. (1996). Int. J. Dev. Biol. *40*, 785–795.

Stocum, D.L. (1975). Dev. Biol. *45*, 112–135.

Thornton, C.S. (1938). J. Morphol. *62*, 219–235.

TGF-β: A New Role for an Old AktTOR

Pankuri Goraksha-Hicks[1,2] and Jeffrey C. Rathmell[1,2,3,*]
[1]Department of Pharmacology and Cancer Biology
[2]Sarah W. Stedman Center for Nutrition and Metabolism
[3]Department of Immunology
Duke University, Durham, NC 27710, USA
*Correspondence: jeff.rathmell@duke.edu
DOI 10.1016/j.devcel.2009.07.004

Nutrient overabundance is known to promote cellular hypertrophy, a significant pathological event in diseases like diabetes and cancer, although mechanisms have remained unclear. In this issue of *Developmental Cell*, Wu and Derynck provide a new model that links metabolism and cell growth by demonstrating that hyperglycemia can increase TGF-β-dependent activation of the mTOR pathway to promote cellular hyperplasia.

Control of cell size is a central feature of tissue homeostasis. If cells atrophy, cell metabolism decreases, and cell size and growth potential are reduced (Rathmell et al., 2000). Conversely, cellular hypertrophy is associated with increased metabolism, which can lead to inflammation, reduced cellular or tissue function, and pathologies like diabetes, obesity, and cancer (Conlon and Raff, 1999). Cell hypertrophy represents an increase in cell size through elevated protein synthesis without DNA duplication and can be induced by growth factors, hormones, extracellular matrix protein accumulation, and hyperglycemia. The phosphatidylinositol-3 kinase (PI3K)/mTOR pathway has been genetically and biochemically shown to regulate cell size (Plas and Thompson, 2005). Activation of this pathway promotes cell growth, while inhibition prevents hypertrophy and can often lead to cellular atrophy.

While mechanisms that control the PI3K/mTOR pathway in growth-factor-stimulated hypertrophy have been well defined, the mechanisms by which hyperglycemia (elevated glucose levels) promotes hypertrophy are unclear (Wolf and Ziyadeh, 1999). A manuscript by Wu and Derynck (2009) in this issue of *Developmental Cell* now highlights a vital contribution by transforming growth factor beta (TGF-β) to this process, identifying a role for TGF-β in glucose-induced hypertrophy through activation of matrix metalloproteinases (MMPs) and the Akt/mTOR pathway. This novel connection between glucose overabundance and cellular pathology may provide new directions in understanding both control of cell growth and the spectrum of pathologies that characterize the