Reconstitution of the central and peripheral nervous system during salamander tail regeneration

Levan Mchedlishvili,a,b,1,2 Vladimir Mazurov,a,1 Kathrin S. Grassme,a,3 Kerstin Goehler,a,b Bernhard Robl,a,b Akira Tazaki,a,b Kathleen Roensch,a,b Annett Duemmler,a,b and Elly M. Tanaka,a,b,4

*Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; and †Deutsche Forschungsgemeinschaft Center for Regenerative Therapies Dresden, Cluster of Excellence, University of Technology Dresden, 01307 Dresden, Germany

AUTHOR SUMMARY

The salamander regenerates complex structures, such as an entire limb or tail. The tail consists of muscle, spinal cord, and vertebrae that are organized in repeated segments. Our goals were to determine whether the nervous system and particularly its periodic segmented pattern are reconstructed faithfully during regeneration of the tail and to identify cells that regenerate the central and peripheral nervous systems. This question is important, because tail regeneration in the frog Xenopus and in the lizard does not result in a faithful replica of the original. Here, we show that the segmental organization of the nervous system is regenerated faithfully and that a single stem cell regenerates the central nervous system. These findings may help us understand why such regeneration does not occur in mammals.

To follow the course of nervous system regeneration, we used an antibody to identify cells of the peripheral nervous system as well as other markers of the central spinal cord. Counting repeated cell groups (dorsal root ganglia) in the peripheral nervous system in regenerating tails versus unsevered controls showed that the correct number of ganglia was regenerated. Interestingly, the correct number of ganglia was regenerated before the correct spacing between the ganglia was achieved. To identify the source of the cells in the regenerating nervous system, we successively performed more refined transplantation experiments. We produced a transgenic axolotl that expresses GFP in all cells of its body (1). To determine whether the central spinal cord is the source of the peripheral nervous system during regeneration, we replaced a segment of the spinal cord from a normal animal with a piece of spinal cord from the GFP-expressing animal. When the tail was cut through this green piece, most of the dorsal root ganglia of the regenerated peripheral nervous system consisted of green fluorescent cells, indicating that cells migrated from the spinal cord out to the peripheral tissue to regenerate the peripheral nervous system. We were concerned that contaminating cells might have adhered to the transplanted spinal cord. Therefore, we cultured neural stem cells from the spinal cord of GFP-expressing axolotls. Bulk culturing of these neural stem cells and their implantation showed that these cells are competent to regenerate the central and peripheral nervous system. To refine our experiments further, we expanded single neural stem cells in culture to see if they could reconstitute the nervous system. We observed that the descendents of a single cell could contribute to all parts of the central spinal cord. We did not see any contribution to the dorsal root ganglia from clonally expanded cells. The term “neural stem cell” has been used for such cells previously by other researchers; here we present evidence that the descendents of a single cell indeed can build such substantial parts of the spinal cord.

Identifying the stem cell pools responsible for regenerating the axolotl spinal cord is an important step in understanding how this process occurs in this regenerative vertebrate. Our evidence compels us to conclude that it occurs through the actions of a highly potent stem cell. An interesting question is why this process is blocked in nonregenerating vertebrates such as mammals.

Fig. P1. Neural stems cells expressing a transgene encoding GFP (green) (A) were implanted into the axolotl spinal cord that then was severed distally (B). The implanted cells regenerated both the spinal cord and the peripheral nervous system (C).


The authors declare no conflict of interest.

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1L.M. and V.M. contributed equally to this work.

2Present address: Department of Physiological Genomics, Institute of Physiology, Ludwig Maximilian University of Munich, 80336 Munich, Germany.

3Present address: University of Muenster, Angiogenesis Laboratory, Röntgenstr. 20, 48149 Muenster, Germany.

4To whom correspondence should be addressed. E-mail: elly.tanaka@crt-dresden.de.

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