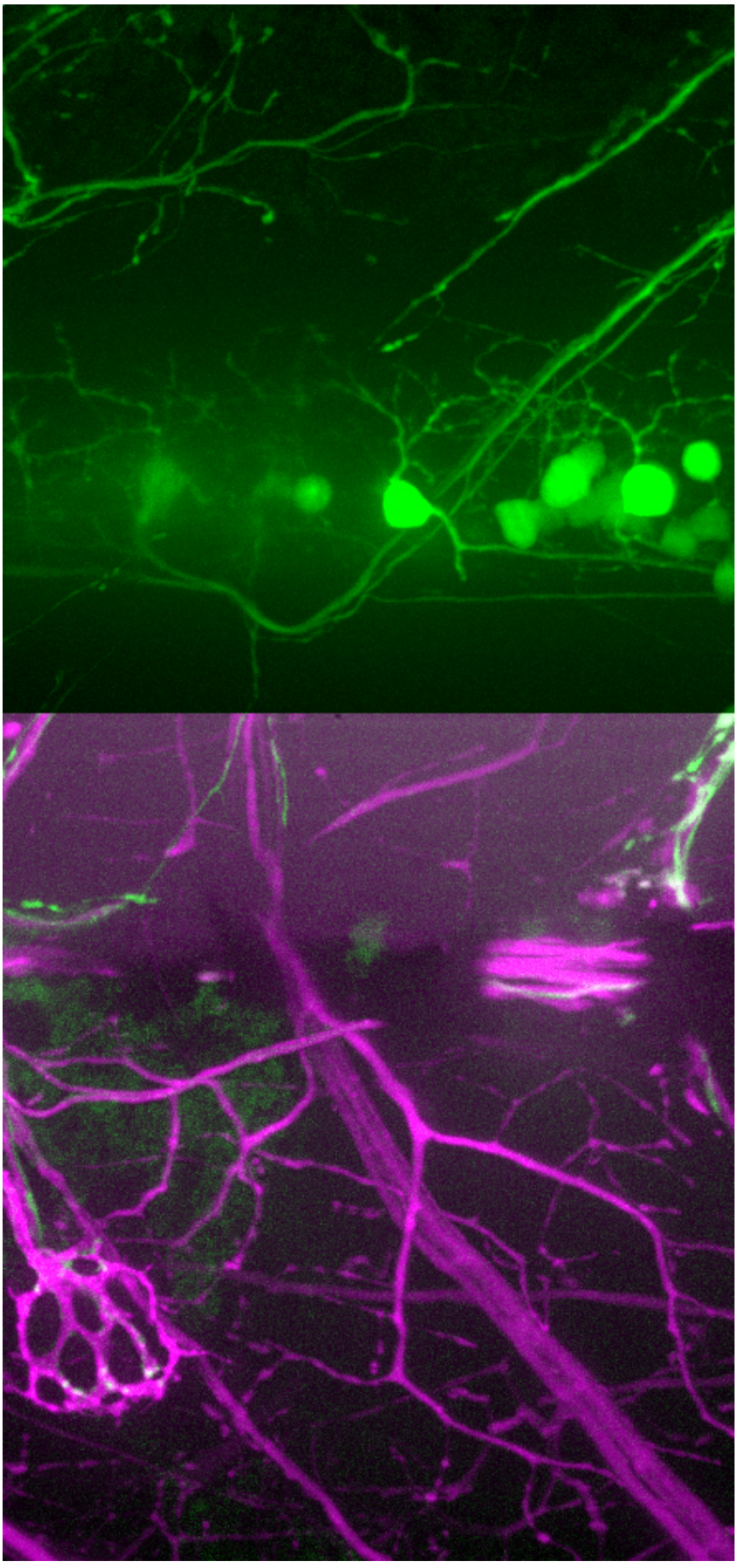
Zebrafish reveal how axons regenerate on a proper path

November 5 2015



Back (green) and abdominal (magenta) peripheral nerve axons in zebrafish. Credit: Michael Granato, PhD, Lab, Perelman School of Medicine, University of Pennsylvania

When peripheral nerves are damaged and their vital synaptic paths are disrupted, they have the ability to regenerate and reestablish lost connections. But what about when a nerve is severed completely, its original route lost? How does a regenerating axon, looking to reconnect with its proper target—with so many possibilities and only one correct path to restore original functioning—know which way to go? Using a transparent zebrafish model, researchers from the Perelman School of Medicine at the University of Pennsylvania, have identified key components of a mechanism that allows the nervous system to heal itself. Their work was published online this week in *Neuron* ahead of the print issue.

"It's been known for over one hundred years that peripheral nerves can regenerate," said senior author Michael Granato, PhD, a professor of Cell and Developmental Biology. However, the mechanics of regeneration, including the question of whether the restoration of axonal branches is random or guided in some way, have remained unresolved issues, partly because of the difficulty of observing the process in live animals. Using zebrafish, which are transparent at larval stages, Granato and his colleagues were able to literally obtain a whole new window into how axons regenerate.

"What really made the difference is the ability to visualize these nerves before and after they were completely cut," he explained. "In no other vertebrate system can you do that, so you can't really be sure what is

going on. For example, in a mouse, you basically have to sacrifice the animal and look at what happened after the injury. You don't know how the situation was before, so you have to extrapolate and make assumptions."

The researchers used fluorescent proteins to label back and abdominal peripheral nerve axons to observe regeneration after nerves were transected by a laser. They found that as regenerating axonal growth cones reach a branch point at which they have to 'choose' to go one way or the other, they will explore both the correct and incorrect paths, but only the proper path will be supported by components of the extracellular matrix (ECM). The ECM is a mix of substances, including collagen, carbohydrates, and fluid, produced by cells and secreted into the environment around them. Cells are embedded in the ECM and it can affect their behavior. In the case of regenerating neuron axons, the ECM keeps axons from 'choosing' incorrect paths and tilts the balance toward the correct growth direction.

The team next investigated the ECM factors that influence this selective regeneration. "The system is heavily influenced by a genetic pathway that starts with the expression of a particular collagen in glial cells," said Granato. "The glial cells that are close to an injury site start expressing the collagen gene 4a5, which has to be modified by a particular enzyme called lh3 to be secreted into the extracellular space."

Collagen 4a5 and the axonal repellent protein Slit1 are strongly upregulated after nerve injury and form a complex. The cells in which the collagen and slit1a are upregulated are along the wrong pathway. They form a barrier because collagen will anchor slit1, present it to the axons, which have the receptor for slit1, and that makes them turn away or stop growing, thereby promoting the regeneration of axons toward their proper paths and towards their original targets. "The specificity really comes from slit1 and its receptor," Granato explained. He also

noted that the same genes are conserved in other vertebrates, including humans.

These experiments are an important step in understanding peripheral nerve regeneration, establishing that it is decidedly not a random process but is controlled by particular genetic pathways. The researchers plan to delve further into the specific mechanisms at work, including the possibility that different nerve cell extensions, such as axons, may control regeneration in separate areas.

"This pathway is highly specific for only the dorsal nerve branch," Granato pointed out. "If we transect the ventral nerves, they are completely unaffected by this genetic pathway. The questions are: Where does this specificity come from? Why are some axons responding to this pathway and others are not? That's basically what's next for us; we want to find out how the specificity is achieved."

While any prospects for clinical applications are still in the future, the work points to some important new research directions. "It tells us there are pathways that we, at some point, will be able to take advantage of to really properly direct axons to regenerate nerves," Granato noted. "Even knowing that in theory one can do that, because there are genes for it, is a significant finding."

Provided by University of Pennsylvania School of Medicine