Proteases help nerve cells to navigate

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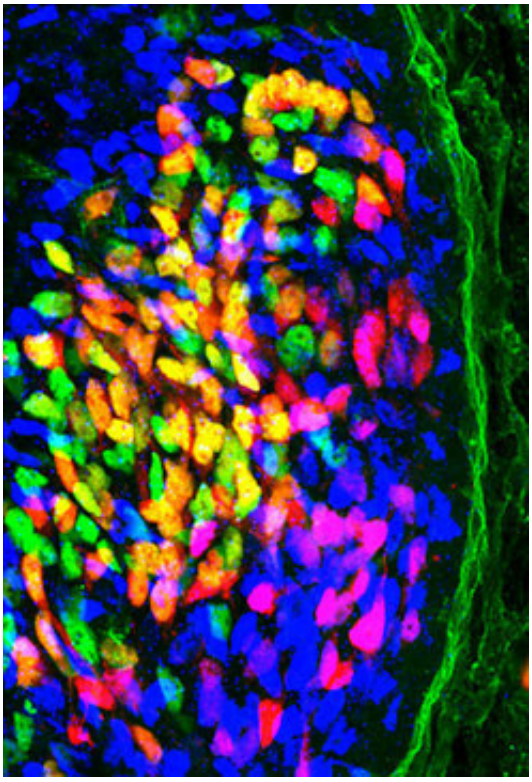

During development, nerve cells (shown in blue, green, violet and yellow) extend their axons to target leg muscles. If the EphA4 receptors of the growing nerve cells no longer encounter freely accessible ephrins, the axons of many nerve cells (violet) are no longer able to find their partner cells. Credit: MPI of Neurobiology / Gatto

Our ability to move relies on the correct formation of connections between different nerve cells and between nerve and muscle cells. Growing axons of nerve cells are guided to their targets by signposts

expressed on the surface of other cells. Very prominent are "do not enter" signs that push axons away. Cell culture studies suggest that protein-cutting enzymes (proteases) remove these signs as soon as they are recognized by the growing axons. In this way, the "bond of recognition" between the axon and the sign is quickly broken, and the axons are more easily guided in a new direction. Scientists from the Max Planck Institute of Neurobiology in Martinsried and the Institut de Recherches Cliniques de Montréal have now shown that proteases indeed control the navigation of growing axons. However, contrary to the current belief, they do so by regulating the number of existing signs. Without proteases, the signposts would be masked and the axons would grow in the wrong direction. These findings clarified how cells form connections during development and may also improve our understanding of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS).

The human brain consists of about 100 billion nerve cells. During embryonic development each of these cells connects with other cells by means of a long extension, known as axon. Some axons need to navigate long distances through the body to find their correct targets, for example from the spinal cord down to the foot. Only if all these connections are correctly established we can perform basic and fine-tuned movements, such as walking or playing the piano.

It is therefore essential that each nerve cell finds its correct target. But how does an axon navigate and find the appropriate partner cells among billions of other possible targets? "We have now identified a few dozen guidance molecules and their receptors that help axons orient themselves," says Rüdiger Klein, Director at the Max Planck Institute of Neurobiology. "However, these few receptor-guidance molecule pairs need to control a very large number of navigational decisions. Therefore, there must be some mechanisms to amplify and modulate the effects of these protein pairs".

Cutting for speed?

Over the last decade, Rüdiger Klein and his team have been studying how nerve cells find their way during development. They are focusing on "do not enter" signs, e.g. ephrin guidance molecules and their Eph receptors. Ephrins and Eph receptors, being present on almost all cell surfaces: on axons as well as on cells in the surrounding tissues, help the growing axons to explore their surroundings and locate their partner cells.

As an axon travels through the body, it docks again and again to other cells via the ephrin/Eph system. This triggers cellular processes, in one or both cells, that eventually cause the connection to be severed and the cells to repel each other, preventing the axon to grow in the wrong direction. It has been hypothesised that this cellular repulsion is accelerated by proteases. Proteases are enzymes that cut Eph receptors and/or ephrins, thus by severing the Eph/ephrin bond between two opposing cells they might expedite the repulsion process. "In this way, proteases could contribute to changes in the guidance process – but this has not yet been experimentally proven." says Rüdiger Klein.

Not faster, but better

To address this question, the neurobiologists studied how proteases affect the rate of cellular repulsion controlled by EphA4 receptors and ephrins. "Although the experiments in cell culture initially appeared to confirm the theory, we discovered something quite different in living organisms," states Rüdiger Klein. Contrary to expectations, cellular repulsion proceeded with undiminished accuracy in animals whose axons expressed EphA4 receptors resistant to protease severing. On the other hand, in animals whose axons and muscles expressed EphA4 receptors resistant to protease cutting many axons grew in the wrong direction.

Because no cutting occurred, more and more functional EphA4 receptors accumulated on cell surfaces of the leg tissues. This accumulation caused EphA4 receptors to bind to the ephrins on the same cell surface, a phenomena termed as "masking". Consequently, the ephrins could no longer act as "do not enter" signs for the growing axons. Thus, axons, being no longer repelled, are misguided in a "no entry zone" and are unable to find their correct targets.

These results show that the cleavage of Eph receptors by proteases does not, as expected, accelerate the repulsion reaction. Instead, it regulates the number of functioning receptors and indirectly the number of available ephrins on cells, where they serve as navigational aids. If the balance is disrupted, growing axons are misdirected.

This is an important finding, as EphA4 receptors perform essential functions during the development of neural networks in the brain and in the spinal cord. They are also involved in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). In the absence of EphA4 receptors, ALS manifests itself later and develops more slowly in a number of animal models. "It's possible that the number of EphA4 receptors is kept low by the regulatory activity of proteases," Rüdiger Klein reflects. "This could provide a way to exert a positive influence on the course of ALS."

More information: Graziana Gatto, Daniel Morales, Artur Kania, Rüdiger Klein, EphA4 Receptor Shedding Regulates Spinal Motor Axon Guidance, *Current Biology*, 20 October 2014 (DOI: dx.doi.org/10.1016/j.cub.2014

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