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Neurobiology

How nerve cell axons become mobilised

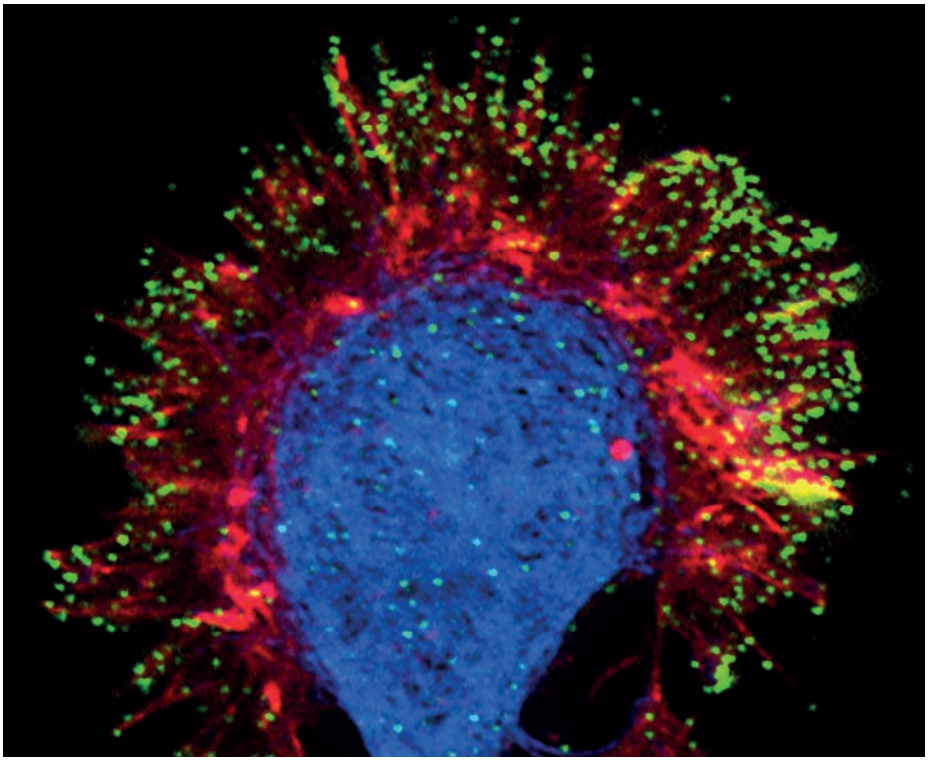
A signalling pathway stimulated in the tips of growing axons triggers the generation of traction forces between an axon and its substrate

Axons are the long, slender projections of the neurons that make up the nervous system and conduct electrical impulses. As they grow towards their targets in the developing nervous system they need to generate traction forces, which allow them to grab onto their substrate and extend themselves to their final location. How do they do it?

A research team led by Naoyuki Inagaki at Japan's Nara Institute of Science and Technology has found that chemo-attractant molecules activate a signalling pathway in growing axons, allowing the generation of mechanical forces that permit axons to propel themselves toward their destination.

Netrin is an extracellular chemo-attractant molecule that induces the growth of developing axons and the researchers thought this could be because netrin induces traction force generation within the tip of the axon. To test this, Inagaki and colleagues grew neurons on elastic gel embedded with fluorescent beads in the presence of netrin and observed the displacement of the beads. This suggested that the axons were grabbing on to the substrate in order to propel themselves forward.

Netrin activates a wide range of intracellular signalling molecules, some of which — such as actin — may be responsible for strengthening the link between structural molecules within the axon and the substrate on which the axon is growing. Pak1 is an enzyme within the axon tip that adds phosphate groups to other proteins to regulate their activity and shootin1 is a linker molecule that couples actin to the substrate.



Naoyuki Inagaki

A fluorescence image of an axonal growth cone (green, shootin1 phosphorylated by Pak1; red, actin filaments; blue, microtubules), showing the sites where the signal-to-force transduction for axon outgrowth occurs.

> **"Our findings provide a key molecular mechanism for understanding the mechanical forces that are involved in human brain development."**

The researchers found that Pak1 can add phosphate groups to shootin1 and that this addition could be increased by adding netrin to the neuronal cultures. When shootin1 contains these phosphate groups that have been added by Pak1, it leads to the strengthening of the link between actin and the substrate.

When Inagaki's team lowered the expression of shootin1 within the neurons, the growing axons seemed to slip on their substrate and netrin was not as effective at inducing axonal outgrowth. If they expressed a modified Pak1 that blocked its ability to add phosphate groups to shootin1, axon growth was also inhibited. However, when they expressed a modified shootin1 that already contained these phosphate groups, traction force generation and axon outgrowth could be restored.

Taken together, the data indicate that the generation of the mechanical forces required for axons to be able to grow requires the induction of a signalling pathway that involves the activation of Pak1 and shootin1. "Our findings provide a key molecular mechanism for understanding the mechanical forces that are involved in human brain development," says Inagaki.

Reference

1. Toriyama, M., Kozawa, S., Sakumura, Y. & Inagaki, N. Conversion of a signal into forces for axon outgrowth through Pak1-mediated shootin1 phosphorylation. *Curr Biol* **23,** 529-34 (2013)

More information about the group's research can be found at the Inagaki Lab webpage:
http://nippon.naist.jp/inagaki_g/english/