

Actin hotspots blaze a trail along axons

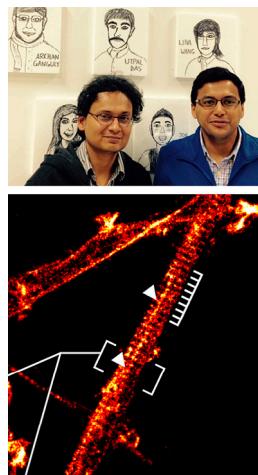
Study identifies a dynamic cytoskeletal network that delivers actin to presynaptic boutons.

Actin's role in guiding axonal growth cones towards their target is relatively well understood, but the cytoskeletal protein's function further back in the axonal shaft remains uncertain. Indeed, even the organization of actin in this part of the neuron is unclear. Ganguly et al. now describe how "hotspots" along the axon produce dynamic "trails" of actin filaments that deliver actin to synapses and help regulate synaptic vesicle recycling (1).

"People in the field have mostly focused on how actin behaves in the growth cones," says Subhajit Roy, from the University of California, San Diego. "When we started this project, very little was known about actin in axons." Recently, however, Xiaowei Zhuang and colleagues at Harvard University used super-resolution microscopy to identify "actin rings" underlying the plasma membrane at regular intervals along the axonal shaft (2). These rings are highly stable structures that give the axonal membrane mechanical support, but seem unlikely to fulfill any of the other functions that actin has in other cell types. Roy and colleagues, led by postdoc Archan Ganguly, therefore looked for more dynamic pools of axonal actin by live imaging hippocampal neurons transfected with fluorescent probes that bind to F-actin (1).

"We saw these 'hotspots' along the axon where actin was polymerizing and spouting out filaments in both directions," Roy explains. "I'd never seen anything like it before." These filaments, which the researchers termed "actin trails," extended rapidly along the axon shaft. Super-resolution microscopy also revealed longitudinally oriented, deep axonal actin filaments that were clearly distinct from the actin rings underlying the axonal membrane.

Ganguly et al. examined the hotspots where the actin trails originated, and found that they often colocalized with endosomes stationed along the axon. Blocking the delivery of endosomal vesicles into axons



FOCAL POINT

Subhajit Roy (left), Archan Ganguly (right), and colleagues identify a pool of dynamic actin filaments, or "actin trails," that extend along axonal shafts and likely deliver actin to presynaptic terminals. These trails emanate in a formin-dependent manner from actin assembly hotspots that seem to be located on, or near, stationary endosomes within the axonal cytoplasm. Blocking actin trail formation coincides with a decrease in presynaptic F-actin and also reduces the efficiency of synaptic vesicle recycling. Super-resolution microscopy of a hippocampal axon stained with phalloidin (bottom) shows several deep axonal actin filaments (white arrowheads) extending through the cytoplasm surrounded by the periodic "actin rings" (comb) that underlie the axonal plasma membrane. The data suggest a two-tier assembly of axonal actin where stable, subplasmalemmal actin rings mechanically support the plasma membrane while dynamic, deep actin polymers sustain other physiological functions.

PHOTO TAKEN IN THE ROY LAB BY DR. D.

strongly reduced the formation of actin trails, suggesting that the filaments might assemble on the endosome surface. Actin trails were also suppressed by an inhibitor of the formin family of actin nucleation and elongation factors. Hotspots of dynamic F-actin still accumulated in the presence of this inhibitor, however, suggesting that small actin filaments are nucleated on endosomes independently of formins but are then extended by one or more of these proteins to form longer actin trails. A precedent for this occurs in mouse oocytes, where actin filaments can assemble on the surface of endosomes in a formin-dependent manner (3, 4).

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"The similarity of our data to the situation in oocytes is striking," Roy says.

Many actin trails appeared to terminate near the presynaptic boutons that form along the long axis of axons. These structures (also known as en passant boutons) are enriched in F-actin, though precisely how actin filaments affect their structure and function is unclear. Photobleaching experiments suggested that the axonal actin trails might help deliver actin to the boutons, and presynaptic actin accumulation was decreased when the researchers blocked

actin trail formation with the formin family inhibitor. Reducing presynaptic F-actin this way also inhibited synaptic vesicle recycling, slowing the rate of both vesicle exocytosis and endocytosis.

Roy cautions that this could indicate a role for formins in assembling actin filaments within the presynapse itself, but the possibility that actin trails nucleated from hotspots within the axon shaft could deliver actin to presynapses or other sites where it is needed is one that excites him. "This two-tier organization of axonal actin is really interesting," Roy says. "You have the rings that support the plasma membrane, and these actin trails that could do everything else: supplying actin to presynaptic terminals and maybe controlling short-range movements along the axon."

Roy now wants to identify the formin or formins responsible for elongating the actin trails. Actin is also conveyed in axons by a mysterious process called slow axonal transport, and Roy plans to continue his long-standing interest (5–6) in this phenomenon.

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