How muscle develops: A dance of cellular skeletons

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Revealing another part of the story of muscle development, Johns Hopkins researchers have shown how the cytoskeleton from one muscle cell builds finger-like projections that invade into another muscle cell's territory, eventually forcing the cells to combine.

Such muscle cell fusion, the researchers say, is not only important for understanding normal muscle growth, but also muscle regeneration after injury or disease. The work, they believe, could further development of therapies for muscular dystrophy or age-related muscle wasting.

Their report on muscle cell cytoskeletons, published in *Developmental Cell* May 17, adds detail to a previous study last year showing that actin — a main building block of the cell's cytoskeleton — is required to form those finger-like projections and stimulate muscle cell merges. The new discovery outlines the intricate dance required among cytoskeleton-regulating proteins to precisely construct protrusions that promote muscle cell merging. Specifically, the Johns Hopkins team uncovered the activity of a regulatory protein known as "Blown Fuse," aptly named because muscle cells lacking this protein fail to fuse.

"Blown Fuse was found to play a role in muscle cell fusion 14 years ago," says Elizabeth Chen, Ph.D. assistant professor of molecular biology and genetics, "and now we know how Blown Fuse regulates the dynamics of the cytoskeleton to facilitate the invasion of one muscle cell by another."

In a test tube, the researchers showed that the protein, Blown Fuse, disrupts the complex formed by the protein duo WASP and WIP, which are known regulators of the actin cytoskeleton. "Blown Fuse does so by a competitive binding mechanism — it binds to the same site in WIP as WASP does," says Rui Duan, a postdoctoral fellow in Chen's lab and a co-first author of the study.

The researchers knew that the WASP-WIP protein duo binds to the growing ends of actin filaments, protecting these ends from being capped by proteins that prevent further actin growth. Apart from its protective role, WASP also has to come off the end of the actin filaments from time to time to start new actin branches. The intricate balance between actin filament growth, capping and branching, determines the dynamics of the cytoskeleton. Armed with this knowledge, the researchers tested whether Blown Fuse competes with this process to change how WASP simultaneously protects and builds the cytoskeleton.

The test began with researchers putting fluorescent actin in fruit fly muscle cells that incorporated themselves into the growing actin branches in the finger-like protrusions. Then, the researchers used a laser beam to bleach the fluorescent actin in the region of the finger-like protrusions and waited to see whether and how long it would take for new, unbleached actin to spread from other parts of the cell and be taken up by the growing branches in the "fingers." In normal muscle cells, it took about two minutes for the fluorescence to return. In muscle cells that lacked Blown Fuse, the fluorescence never fully recovered and the cytoskeleton failed to project finger-like protrusions, probably because the WASP-WIP complex does not come off the ends of the actin filaments to start new actin branches.

"These results suggest that the growing ends of the actin cytoskeleton are occupied by the WASP-WIP protein duo and that without Blown Fuse to

dissociate with the WASP-WIP complex and push WASP off the ends, new actin branches cannot be started," says Chen. "And these shorter and stiffer new branches are critical for generating the finger-like membrane protrusions."

Through a microscope, the Hopkins team compared the finger-like projections from normal cells with cells lacking Blown Fuse. Normal muscle cells form pointy finger-like protrusions that push into the other muscle cell, but cells without Blown Fuse have fewer and floppier protrusions that don't push their way in to other muscle cells.

"Modulating the stability of the WASP-WIP complex may represent a general mechanism in regulating cytoskeleton dynamics and generating membrane protrusions," says Chen.

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