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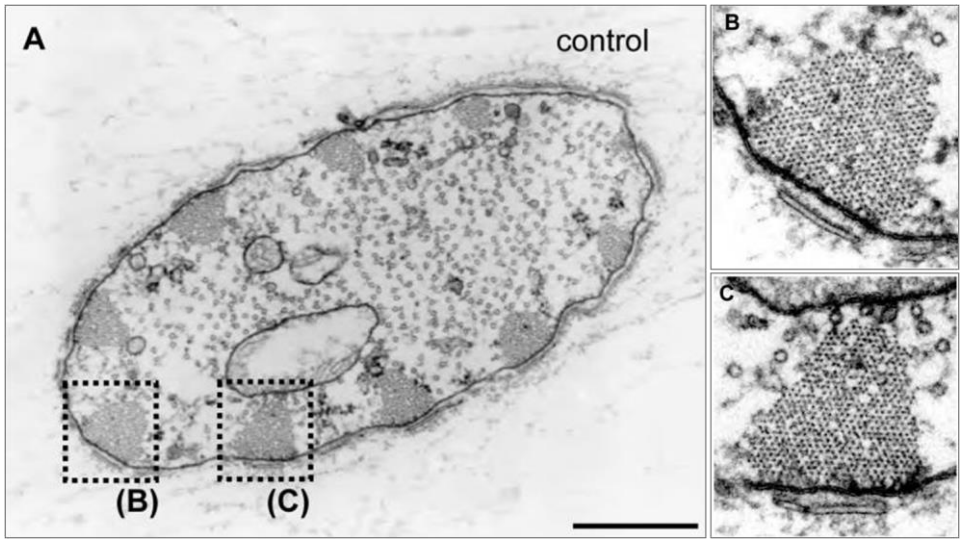
Keeping actin filaments in order in fly bristles

November 18, 2016– The bristles on the external surface of the *Drosophila* fruit fly are mechanosensory organs that form from the robust elongation of single cells during pupal stages of development. On the inside of the bristle, parallel bundles of actin filaments are aligned in an orderly fashion along the long axis of the bristle shaft, nestled close to the cell cortex. A further magnified view of the bristle reveals that the actin filaments making up the actin bundles are aligned in hexagonal paracrystalline lattice, but how this distinct and orderly arrangement, which is essential for maintaining the robust yet supple structure of the bristle, is formed remained relatively unknown.

Now a new study published in *Development* by former CDB scientist Tetsuhisa Otani and colleagues in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Team Leader), working in collaboration with the RIKEN Center for Life Science Technology (CLST)'s Ultrastructural Research Team (Shigenobu Yonemura, Team Leader), reveals that protein kinase IKKε, important for distal tip elongation in *Drosophila* bristles, is also involved in promoting actin bundling by protecting the actin crosslinking protein Fascin from becoming phosphorylated by another protein kinase called, Protein Kinase C (PKC). Fascin, when phosphorylated by PKC is unable to cross-link actin filaments into hexagonal arrangement. IKKε uses a double inhibition mechanism to promote actin bundling by inhibiting PKC activity, which in turn prevents PKC from phosphorylating Fascin and facilitates bundling activity.



Electron-microscopic image of bristle cross section. Actin bundles spaced at regular intervals along the cell cortex (A). Magnified view of actin bundles reveal individual actin filaments are arranged in hexagonal paracrystalline arrangement (B,C).

Otani previously reported that protein kinase IKKε is localized and activated at the distal tip of the elongating bristle, serving as a signaling center regulating the shuttling of molecular cargo required for elongation (see Science News: February 16, 2011; November 24, 2015). They reported that fly mutants for *ikkε* showed kinked or branching bristles as opposed to straight smooth bristles because the signaling center could not be maintained at the elongating tip. These mutants were also seen to display morphological abnormalities along the bristle shaft, and the group turned their attention to uncovering other roles of IKKε in bristle morphogenesis.

The alignment of actin filaments is known to be mediated by two types of actin-binding proteins, Forked which bundles newly formed actin filaments at the bristle tip and Fascin which packages actin filaments into hexagonal arrangement. They found that of the two proteins, which normally distribute along actin filaments, in the *ikkε* mutants, only the localization of Fascin was disrupted, in addition to displaying disorganized actin filament bundles. These results led them to speculate that Fascin functions to bind actin filaments together, and that IKKε regulates Fascin-mediated actin bundling.

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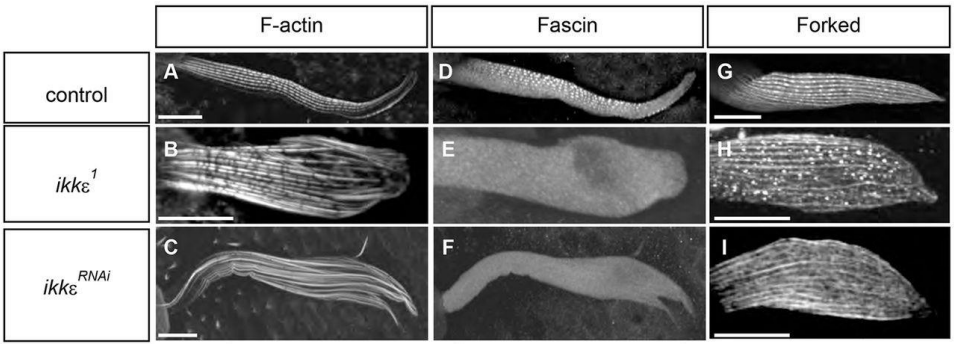

In wildtype flies (top row), Fascin and Forked localize along actin filaments in wildtype flies. In *ikkε* mutant strains (middle and bottom rows), morphology of bristle is abnormal and Fascin localization was disrupted (E, F).

How exactly does IKKε regulate Fascin? Phosphorylation of Fascin by PKC was known to disrupt actin bundling activity in bristles. Thus, the team examined the different mutant fly strains to determine the relationship between IKKε, PKC, and Fascin. They determined that Fascin localizes along actin bundles only in the unphosphorylated state, and that phosphorylation by PKC disrupts Fascin localization along actin bundles. IKKε was also found to play a role in inhibiting phosphorylation of Fascin by PKC. As PKC is known to be activated when it translocates and binds to the cell cortex, the team showed, using cultured cells, that IKKε could prevent PKC activation. These results indicate that IKKε could indirectly maintain Fascin-mediated acting bundling through the inhibition of PKC activity.

“PKC activation is not required for normal bristle formation as *pkc* mutants show no visible abnormalities in bristle morphology. What then is the merit of adopting a double inhibitory mechanism to regulate bristle formation?” says Hayashi. “Having a factor like IKKε keeping PKC activity in check may be the key to maintaining safe and stable cell and body functions, as PKC plays a central role in many intracellular signaling pathways and errors in regulation of PKC activity can lead to cancer or lifestyle diseases, such as diabetes.”