Want to promote tissue growth? There's an App for that!
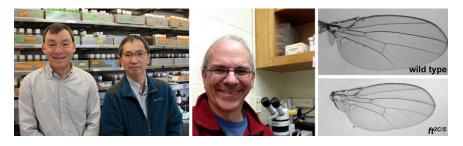
Ben Short

Study describes how a palmitoyltransferase regulates the Hippo pathway in flies.

The Hippo signaling pathway controls the size of developing organs and tissues by regulating the activity of a transcription cofactor (called Yorkie in *Drosophila* and Yap in mammals) that promotes the expression of cell proliferation and anti-apoptotic genes (1). Matakatsu et al. describe how a palmitoyltransferase named Approximated (App) regulates the activity of two key inputs into the Hippo pathway, the protocadherin Fat and the atypical myosin Dachs (2).

Dachs appears to promote epithelial tissue growth by localizing to the apical junction region of epithelial cells, destabilizing a Hippo pathway kinase called Warts so that Yorkie is activated to induce cell proliferation (3). Fat, in contrast, restricts growth by inhibiting Dachs' accumulation at apical junctions (4). In 2008, Hitoshi Matakatsu and Seth Blair discovered that the putative palmitoyltransferase App suppresses Fat signaling and promotes Dachs' localization at apical junctions (5). Matakatsu then continued his work after joining Richard Fehon's group at the University of Chicago as a postdoc. "We wanted to investigate whether App really did function as a palmitoyltransferase and, if so, how palmitoylation regulates this pathway," Fehon says.

One possibility is that App palmitoylates Dachs, thereby promoting its association with the plasma membrane at the apical junction region of cells, but Matakatsu et al. saw no evidence of Dachs palmitoylation. Instead, the researchers found that App palmitoylates two cysteine residues in the intracellular domain of Fat (2). Mutating these cysteines reduced Fat palmitoylation and restricted *Drosophila* wing growth, indicating that Fat is more active and better able to inhibit Dachs when it is not palmitoylated by App.

Fat's intracellular domain can also be phosphorylated by the kinase Discs-overgrown (Dco) (6, 7) and, in the absence of *dco*, Fat is less active, resulting in a greater accumulation of Dachs at apical junctions and tissue overgrowth. Deleting *app*, or mutating the palmitoyltransferase's catalytic domain, suppressed the *dco*-null overgrowth phenotype, producing relatively normal-sized tissues with intermediate levels of junctional Dachs. This suggests that Dco and App antagonistically regulate Fat, with phosphorylation promoting and palmitoylation inhibiting the protocadherin's growth-restricting activity.

Focal Point (Left to right) Richard Fehon, Hitoshi Matakatsu, and Seth Blair describe how the putative palmitoyltransferase App regulates the Hippo signaling pathway to promote tissue growth in *Drosophila*. The researchers find that App directly recruits the atypical myosin Dachs to the apical junction region of epithelial cells, as well as palmitoylating and inactivating the protocadherin Fat, thereby preventing it from displacing junctional Dachs. App therefore promotes Dachs' accumulation at apical cell junctions, resulting in the activation of the growth-promoting transcription cofactor Yorkie. A non-palmitoylatable version of Fat is more active, suppressing Dachs activity and restricting tissue growth to produce flies with smaller wings (bottom) than those of wild-type *Drosophila* (top). PHOTOS COURTESY OF THE AUTHORS.

> **"App's palmitoyltransferase activity is important, but it's not the only thing that the protein is doing."**

Does App only function as a palmitoyltransferase? Matakatsu et al. used CRISPR-Cas9 to introduce mutations in the putative catalytic domain of *app*. Flies carrying these mutations were slightly smaller than normal and showed reduced levels of Dachs at their apical cell junctions. But these phenotypes were not as severe as those seen in mutant flies that completely lack App. "So App's palmitoyltransferase activity is important, but it's not the only thing that the protein is doing to promote tissue growth," Fehon explains.

Matakatsu et al. found that although App doesn't palmitoylate Dachs, it does form a complex with the myosin-like protein and recruits it directly to apical junctions. "So we think that App promotes Dachs accumulation and tissue growth by both recruiting the protein to the apical junction region and by palmitoylating and inactivating Fat," Fehon says.

Matakatsu and colleagues now want to investigate how App is regulated to ensure that tissues grow to the right size during development. To date, tension is the only physiological signal known to influence Hippo pathway activity, but whether mechanical signals regulate App and/or Fat is unknown. In addition, it remains unclear how Fat inhibits Dach's accumulation at apical junctions and how this activity can be inhibited by palmitoylation or promoted by phosphorylation. Answering these questions could be important because a human homologue of Fat, *Fat4*, has a conserved palmitoylation site and is a candidate tumor suppressor, suggesting that finding ways to inhibit palmitoylation and enhance Fat4 activity might be effective in reducing the progression of certain cancers.

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