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Homeostasis of the Drosophila adult retina by actin-capping protein and the Hippo pathway

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The conserved Hippo signaling pathway regulates multiple cellular events, including tissue growth, cell fate decision and neuronal homeostasis. While the core Hippo kinase module appears to mediate all the effects of the pathway, various upstream inputs have been identified depending on tissue context. We have recently shown that, in the Drosophila wing imaginal disc, actin-Capping Protein and Hippo pathway activities inhibit F-actin accumulation. In turn, the reduction in F-actin sustains Hippo pathway activity, preventing Yorkie nuclear translocation and the upregulation of proliferation and survival genes. Here, we investigate the role of Capping Protein in growth-unrelated events controlled by the Hippo pathway. We provide evidence that loss of Capping Protein induces degeneration of the adult Drosophila retina through misregulation of the Hippo pathway. We propose a model by which F-actin dynamics might be involved in all processes that require the activity of the core Hippo kinase module.

Key words: capping protein, Hippo signaling pathway, yorkie, fat, expanded, actin cytoskeleton, Drosophila adult retina, neuronal degeneration

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The conserved Hippo (Hpo) signaling pathway has emerged as a critical regulator of tissue growth both in Drosophila and in mammals. At the center of the pathway are the two Ser/Thr kinases Hpo and Warts (Wts), and their adaptor proteins Salvador (Sav) and dMob as a tumour suppressor (Mats).[1-6] This core kinase module represses tissue growth by sequestering the transcriptional co-activator Yorkie (Yki) in the cytosol through phosphorylation and direct binding.[7-9] Sequestration of Yki prevents formation of complexes between Yki and DNA-binding transcription co-factors and activation of target genes that regulate cell growth, survival and proliferation.[10] In addition to controlling tissue growth, the core kinase module also regulates growth-unrelated events, such as stress-induced apoptosis, cell fate decision and neuronal homeostasis.[11-13] Most of these outputs rely on Yki activity, including the control of tissue growth and the maintenance of neuronal homeostasis of the Drosophila adult retina,[11] while others are Yki-independent.[13]

Multiple upstream inputs have also been shown to regulate the core Hpo kinase module at various levels. Among those, the atypical Cadherin Fat was proposed to transduce signals from the atypical Cadherin Dachsous (Ds) and Four-jointed (Fj), while the two Ezrin/Radixin/Moesin (ERM) family members, Expanded (Ex) and Merlin (Mer) are believed to exert their growth suppressive activity by activating the Hpo kinase.[14,15] These inputs can act in both a coordinated and independent fashion on Hpo pathway activity depending on the tissue context. In the wing imaginal disc, Fat and Ex are major regulators of the pathway.[16,17] In contrast, in the pupal Drosophila eye, Fat and Ex play only minor roles,[18] while in the adult retina, Fat, but not Ex, is absolutely critical to prevent neuronal degeneration.[11]

Despite much progress in understanding the molecular regulation between the core components of this module, key

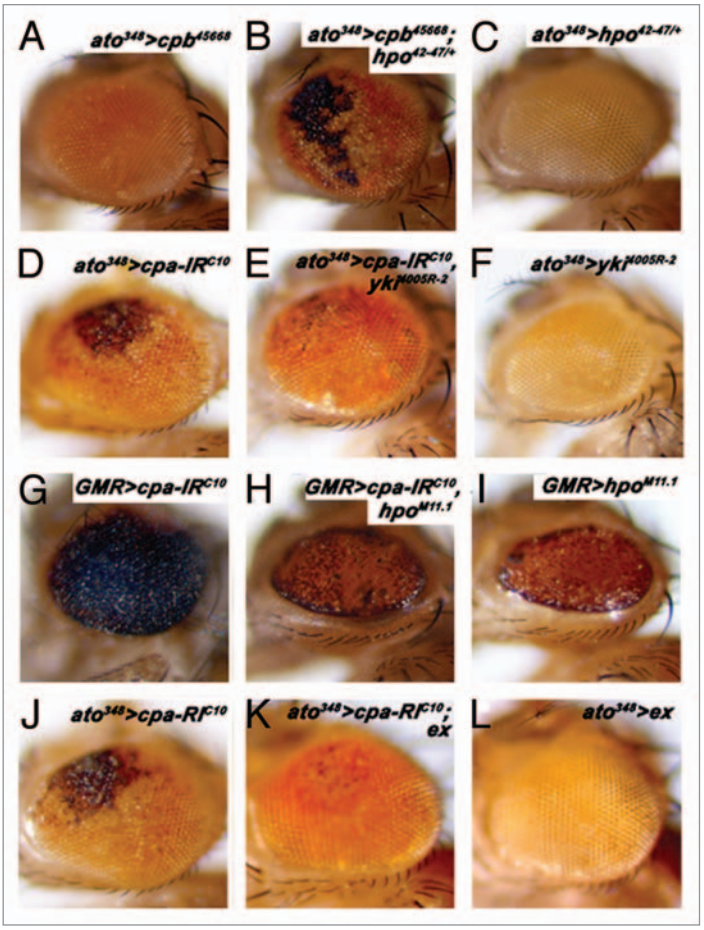

Figure 1. Knocking down Capping Protein triggers retinal degeneration through inhibition of Hippo signaling activity. All panels show adult Drosophila retina. The genotypes of the animals are indicated above the panels. *ato*^{348} or *GMR* refers to *ato*^{348}-Gal4 or *GMR*-Gal4 driving expression of the indicated transgenes (UAS-*cpb*^{45668}, UAS-*cpa-IR*^{C10}, UAS-*yki*$^{3005R-2}$, UAS-*hpo*$^{M11.1}$ or UAS-*ex)* and either wild-type for the *hpo* gene (A) or heterozygote for the *hpo*$^{42-47}$ allele (B and C).

unanswered questions remain, such as how the different inputs are activated and integrated by the core kinase module in various tissues and how this triggers specific developmental outputs.

The Interplay between Hpo Signaling Activity and F-Actin Dynamics

We have recently shown that the actin-Capping protein (CP) heterodimer, composed of an α (Cpa) and β (Cpb) subunits, which regulates actin polymerization, also functions to suppress inappropriate tissue growth by inhibiting Yki activity. Interestingly, Hpo signaling activity, like CP, limits actin filament (F-actin) accumulation at apical sites, independently of Yki. Thus, our findings indicate a novel interplay between Hpo pathway activity and F-actin dynamics, in which regulation of an apical F-actin network by Hpo signaling activity and CP sustains Hpo pathway activity, thereby limiting Yki nuclear import and the activation of proliferation and survival genes.[19] Here, we investigate whether CP is also required for growth-unrelated events controlled by the Hpo signaling pathway. We provide evidence that loss of CP induces neuronal degeneration of the adult Drosophila retina through the control of Hpo pathway activity. Based on our work and the increasing number of reports, implicating F-actin in Hpo signaling, we propose that F-actin might be a central player of the pathway, integrating signals from various inputs and mediating tissue-specific outputs.

Capping Protein and Neuronal Degeneration through the Hippo Signaling Pathway

Independently of its role in growth control, the Hpo signaling pathway prevents neuronal degeneration of the adult Drosophila retina.[11] CP has also been shown to prevent retinal degeneration.[20,21] Therefore, CP might have a general requirement in controlling the different outputs of Hpo signaling activity. To test whether loss of CP triggers neuronal degeneration through inhibition of Hpo pathway activity, we carried out genetic interactions between *cpa* or *cpb* and components of the *hpo* signaling pathway. To perform this study, we expressed independent double-stranded RNAs to knock down Cpa (UAS-*cpa-IR*) or Cpb (UAS-*cpb-IR*) using the *ato*^{348} or *GMR*-Gal4 drivers, which drive expression in committed G_1-arrested cells prior to photoreceptor differentiation in the differentiating retina respectively. While expressing *cpb-IR*^{45668} under *ato*^{348}-Gal4 control did not induce morphological defects (**Fig. 1A**), driving *cpa-IR*^{C10} with *ato*^{348}-Gal4 (**Fig. 1D and J**) or *GMR*-Gal4 (**Fig. 1G**) or *cpb-IR*^{45668} with *GMR*-Gal4 (data not shown) triggered the appearance of black omatidial clusters, which reveals retinal degeneration. Reducing one copy of *hpo* strongly enhanced neuronal degeneration of Cpa (**Fig. 1B**) or Cpa-depleted photoreceptor cells using either *ato*^{348} or *GMR*-Gal4 (data not shown). While heterozygote mutant animals for *hpo*, carrying either *ato*^{348}-(**Fig. 1C**) or *GMR*-Gal4 (data not shown) showed no visible defects of the adult retina. This indicates that *CP* and *hpo* genetically interact to maintain neuronal homeostasis.

The neuroprotective function of Hpo signaling required Yki activity.[11] Expressing an RNAi construct against Yki with *ato*^{348}-Gal4 had no visible effect on eye morphogenesis (**Fig. 1F**) but suppressed the neurodegenerative phenotype due to Cpa depletion (**Fig. 1E**). We observed a similar suppression with GMR-Gal4 (data not shown). This indicates that Yki activity is part of the signaling cascade, which trigger neuronal degeneration of Cpa-depleted photoreceptors cells.

We then investigated the epistatic relationship between *CP* and components of

the Hpo pathway. Adult eye overexpressing *hpo* (**Fig. 1I**) or expressing both *hpo* and *cpa-IR* (**Fig. 1H**) under *GMR*-Gal4 control displayed identical morphological defects. This indicates that Hpo activity overcomes the effect of CP loss. Moreover, *ex* overexpression, which had no visible effects in the adult eye (**Fig. 1L**) suppressed the neuronal degeneration of Cpa-depleted tissues (**Fig. 1K**). Taken together, we conclude that retinal degeneration as a result of CP loss is mediated, at least in part, by inhibiting Hpo pathway activity.

The Actin Cytoskeleton: A Central Role in Hippo Signaling Activity

All outputs of the Hpo signaling pathway seem to be dependent on the activity of the core kinase module, whereas the strength and identity of the upstream inputs depend on tissue context.[14,15] The core kinase module is therefore central to the pathway, integrating multiple signals that are translated into tissue-specific responses. We propose that F-actin dynamics may also be a central player of the Hpo signaling pathway (**Fig. 2**). CP restricts the accessibility of the filament barbed end, inhibiting addition or loss of actin monomers.[22] In addition to CP, other actin regulators have also been shown to regulate Hpo signaling activity. Thus, overexpression of a constitutive active form of the actin-nucleator Diaphanous inhibits the pathway activity upstream of Wts but in parallel to Hpo.[23] The MST1/2 Hpo orthologs also co-localize with F-actin structures and are activated upon F-actin depolymerization;[24] and, Ajuba, which negatively regulates Hpo signaling activity downstream of Hpo and upstream of Wts, belongs to an actin-associated family of LIM-domain-containing proteins.[25] In addition, CP appears to be required not only to control Hpo-dependent tissue growth but also Hpo-dependent growth-unrelated events, such as neuronal homeostasis. Thus, CP and consequently F-actin, may mediate all functions of Hpo signaling. Consistent with this, in addition to facilitate Yki phosphorylation by the Hpo kinase cassette in the wing disc tissue,[19] CP may also regulate non-phophorylated Yki since Wts-dependent phosphorylation of Yki does not appear to be involved in neuronal homeostasis.[11] Moreover, the role of F-actin may be independent of tissue-specific inputs since F-actin seems to be involved in all processes that require the activity of the core kinase module, whereas Ex, which has a critical role in regulating Hpo-dependent tissue growth,[16,17] is not involved in neuronal homeostasis.[11]

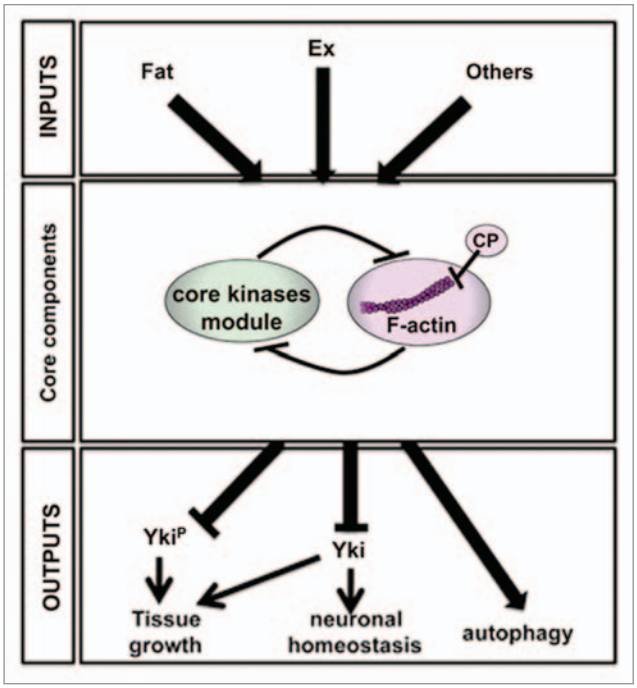

Figure 2. Model for the role of F-actin in Hippo signaling activity. The core kinase module of the Hpo pathway regulates multiple outputs, including tissue growth, neuronal homeostasis and autophagy. Hpo-dependent tissue growth is mediated through inhibition of Yki by a phosphorylation-dependent and independent mechanisms. The neuroprotective effect of Hpo signaling also required Yki inhibition, independent of its phosphorylation by Wts. In contrast, Yki is not involved in Hpo-dependent autophagy. The core kinase complex is regulated by multiple inputs, including Fat and Ex. The interplay between Hpo signaling activity and F-actin dynamics may be a general requirement in Hpo signaling.

In conclusion, the interplay between Hpo signaling activity and F-actin dynamics may be a general requirement in Hpo signaling. Consistent with this, in the wing disc tissue, the core kinase module inhibits F-actin accumulation, prevents excess F-actin of CP-depleted cells[19] and we observed similar epistatic relationships between CP, *ex* or *hpo* in the adult retina. Nevertheless, further studies will be required to validate the central role of F-actin in the Hpo pathway.

Materials and Methods

Fly stocks used were UAS-*cpa-IR*^{C10},[19] UAS-*cpa-IR*$^{10540R-2}$; UAS-*yki-IR*$^{4005R-2}$ (National Institute of Genetics, NIG); UAS-*cpa-IR*^{7009} and UAS-*cpb-IR*^{45668} (Vienna Drosophila Research Center, VDRC); UAS-*hpo*$^{M11.1}$;[2] UAS-*ex*;[4] *GMR*-Gal4;[26] FRT42D, *hpo*$^{42-48}$, a gift from D. Pan.[1] Crosses with the *ato*^{348}-Gal4 or GMR-Gal4 drivers were maintained at 25°C or 18°C respectively. Each cross was performed in parallel with the appropriate controls. Adult flies were collected and photographed 24 to 48 h after hatching. To generate *ato*^{348}-Gal4 transgenic lines that express Gal4 within, and anterior to, the morphogenetic furrow in the eye disc, the minimal *3'-ato* eye enhancer[27] was cloned in the pChs-Gal4 vector. Transgenic flies were generated by standard methods.

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