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Previews

Harness the Power: New Insights into the Inhibition of YAP/ Yorkie

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YAP/Yorkie (Yki) is a transcriptional coactivator that controls organ size; dysregulation causes tumorigenesis by stimulating cell proliferation and inhibiting apoptosis. The Hippo pathway restrains YAP/Yki activity, but it remains unclear how cellular regulation at the plasma membrane influences the Hippo-YAP/Yki pathway. Two papers in this issue on *Developmental Cell*, by Badouel et al. and Nishioka et al., address this question.

Yes-Associated protein (YAP) is a potent transcription coactivator that normally promotes cell proliferation and inhibits apoptosis. YAP is also a candidate oncogene and its expression and nuclear localization are elevated in multiple human cancers (Dong et al., 2007; Zhao et al., 2007). Increased/ectopic expression of YAP causes anchorage-independent and serum-independent growth as well as epithelial-mesenchymal transition (EMT) (Overholtzer et al., 2006; Zhao et al., 2007). In mice, spontaneous amplification of YAP cooperates with *myc* to promote liver tumor growth (Zender et al., 2006), and transgenic overexpression of YAP dramatically increases liver size and eventually causes liver tumors (Camargo et al., 2007; Dong et al., 2007). Therefore, ample evidence supports a role of YAP in organ size control and tumorigenesis.

Obviously, as such a strong growth promoter, YAP activity has to be tightly regulated in order to avoid pathological processes like hypertrophy and cancer. How is YAP activity restricted? *Drosophila* genetic studies and mammalian biochemical analyses have revealed that the core of the Hippo (Hpo) pathway is a kinase cascade starting from the Ste20 family kinase Hpo in association with a scaffold protein Salvador (Sav) (reviewed by Edgar, 2006) (Figure 1). The Hpo-Sav kinase phosphorylates and activates the NDR family kinase Warts (Wts), which interacts with another adaptor protein Mats. Yki, the *Drosophila* YAP homolog, was identified as a Wts-interacting protein inhibited by Wts and functioning downstream of the Hpo pathway. All these proteins, including MST1/2 (Hpo homolog), WW45 (Sav homolog), Lats1/2 (Wts homolog), MOB1 (Mats homolog), and YAP (Yki homolog) are highly conserved in mammals (Figure 1). At the end of the cascade, phosphorylation of YAP by Lats results in 14-3-3 binding to YAP and retaining it in the cytoplasm, thus separating YAP from its transcription factor partners such as Tead (Figure 1) (Dong et al., 2007; Zhao et al., 2007).

Signals upstream of the Hpo pathway core components, on the other hand, are much less clear. Expanded (Ex) and Merlin (Mer), two ezrin/radixin/moesin (ERM) family cytoskeleton-related proteins, were placed upstream of the Hpo pathway mainly by *Drosophila* genetic study, though biochemical evidence is elusive (Hamaratoglu et al., 2006). In this issue of *Developmental Cell*, McNeill and her colleagues reported an unexpected finding that Ex directly interacts with and inhibits Yki (Badouel et al., 2009). This interaction was identified by affinity purification of Ex-interacting proteins; the Yki-Ex interaction is nearly stoichiometric. Further characterization showed that binding is mediated by the WW domains of Yki and the PPxY motifs of Ex. This interaction is suggested to keep Yki in the cytoplasm. The notion of Ex directly inhibiting Yki is further supported by the observation that Ex overexpression can partially rescue the overgrowth induced by the loss of Wts. However, Ex overexpression did not induce apoptosis in a *wts* mutant background, suggesting that blocking Yki functions in cell survival are more strictly Hpo/Wts dependent, as proposed by previous genetic studies. Further studies are needed to determine which mechanism (Ex directly binds to Yki versus Ex signals through Hpo to Yki) is primarily responsible for the inhibitory role of Ex on Yki. As modeled in the report, the WW domain of YAP/Yki should have inhibitory function. However, WW domains of YAP/Yki have been shown to be required for YAP/Yki function in promoting cell proliferation and oncogenic transformation in vitro and sustaining tissue overgrowth in vivo. To reconcile these observations, one may propose that the WW domains have dual roles in mediating the binding of YAP/Yki inhibitory components in the cytoplasm and positive target transcription factors in the nucleus. Notably, there is no obvious Ex ortholog in mammals. Mammalian FRMD1 and FRMD6 contain sequences homologous to the N-terminal FERM domain of Ex, but lack the entire C-terminal domain, which harbors the PPxY motifs required for interaction with Yki, raising the question of whether such a direct regulation of Yki by Ex is conserved for mammalian YAP.

Also in this issue of *Developmental Cell*, an article by the Sasaki lab (Nishioka et al., 2009) demonstrated that the Hpo pathway, presumably by sensing cell-cell contact, regulates YAP-Tead4 activity to specify trophectoderm (TE) lineage during mouse blastocyst development. The Hpo pathway was reported to inhibit YAP in response to increased cell density in cell culture, mediating the cell contact inhibition phenomenon (Zhao et al., 2007). This

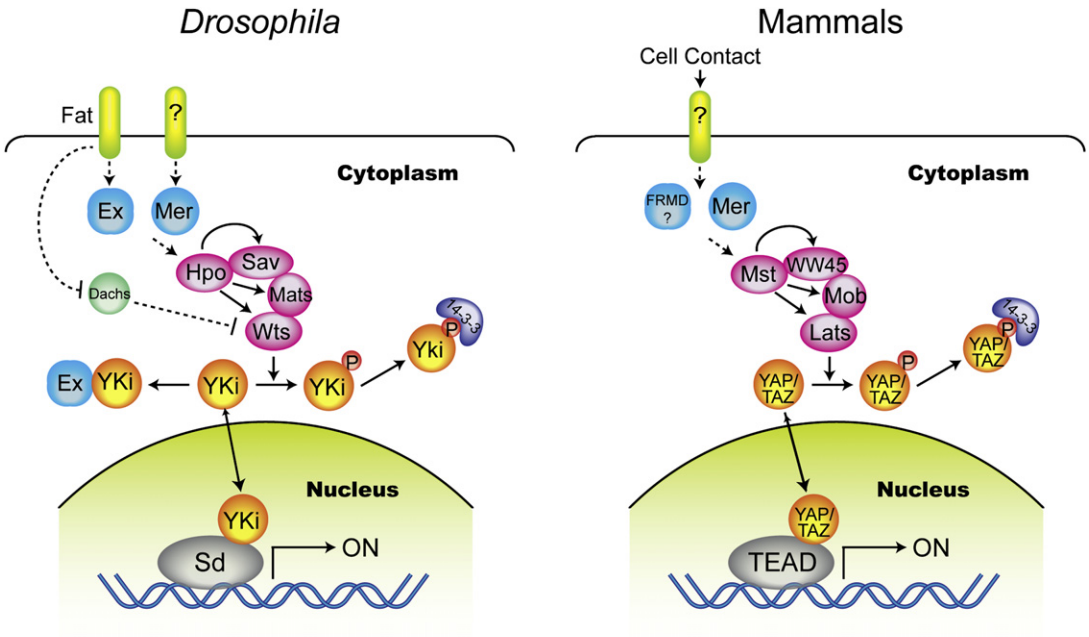

Figure 1. The Hippo Pathway in *Drosophila* and Mammals
Corresponding components in *Drosophila* and mammals are shown in the same color. Dashed arrows indicate unknown biochemical mechanism and question marks denote unknown or uncertain components.

report provides in vivo evidence to support the hypothesis that YAP is regulated by cell contact. During blastocyst formation, YAP localizes in the nucleus of the outer cells and in the cytoplasm of the inner cells in a Lats-dependent manner, which is reminiscent of YAP phosphorylation by Lats and 14-3-3-mediated cytoplasmic retention in cultured cells in vitro. Nishioka et al. further demonstrated that the cytoplasmic localization of YAP in inner cells is due to cell-cell contact because disruption of adherens junctions by an E-cadherin-neutralizing antibody brought YAP back to nucleus. Moreover, fusion of multiple blastomeres reprograms YAP localization, as all the cells inside of the chimera, of which some were originally on the surface before fusion, now show a cytoplasmic YAP localization. This study further supports that cell-cell contact generates a "positional cue" to modulate YAP localization via the Hpo pathway in vivo. However, the key molecule sensing the positional cue is still elusive. Is it Fat and its ligand or does something else provide the cue? This is surely a key question in the Hpo pathway field and this report provides a good in vivo model to address this question.

In the same report, the authors demonstrated that the active YAP in the outer cells of a blastocyst activates Tead4 to sustain expression of Cdx2 and other TE markers. Thus, YAP-Tead4 specifies the TE lineage as opposed to the inner cell mass (ICM) from which embryonic stem cells derive. These data indicate that the Hpo pathway can be used not only to control cell growth and survival, but also to confer spatial pattern on embryonic tissues. Indeed, this mechanism provides the key stimulus that distinguishes extraembryonic TE from the embryonic ICM—arguably the most important developmental decision in mammals. Interestingly, YAP-Tead has also been shown to expand progenitor cell populations (Cao et al., 2008). YAP overexpression expands multipotent undifferentiated progenitor cells in mouse intestine (Camargo et al., 2007), and TAZ, a YAP paralog, has also been shown to modulate mesenchymal stem cell differentiation and maintain stem cell self-renewal. Therefore it will also be very interesting to further explore whether the Hpo pathway functions in the specification, self-renewal, and differentiation of stem cell lineages during development.

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