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SnapShot: The Hippo Signaling Pathway

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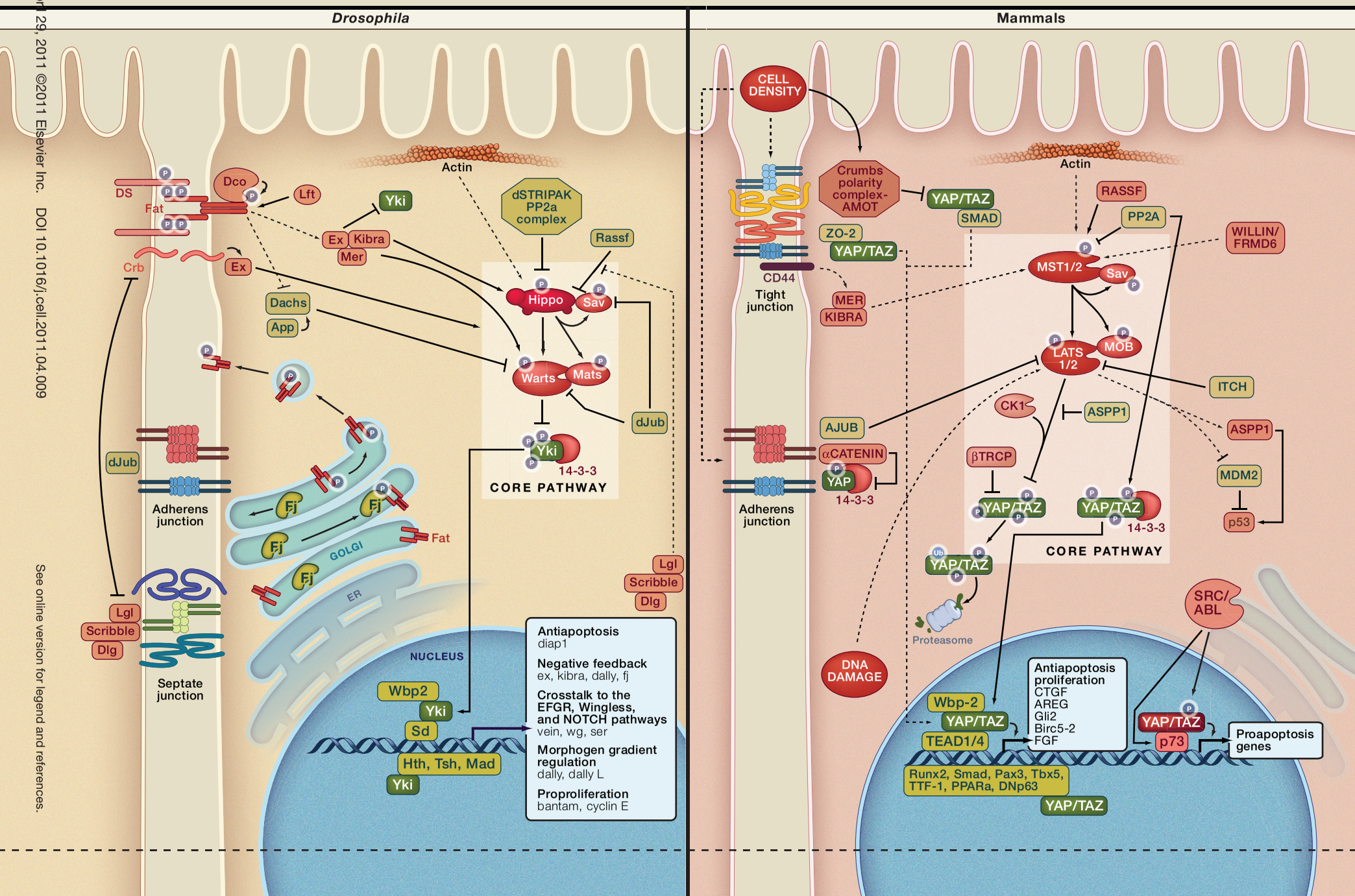

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The Hippo signaling pathway controls tissue growth and appears to be critical in the "organ size" checkpoint. This pathway was first discovered in *Drosophila* and is well conserved in mammals. Mutations in Hippo pathway components have been linked to numerous cancers, including those of the breast, ovary, and liver. In this SnapShot, factors with growth inhibitory/tumor-suppressive functions are colored red, and those that activate growth are colored green.

The Core of the *Drosophila* Hippo Pathway

The core of the Hippo pathway is comprised of a series of phosphorylation events that ultimately lead to the inhibition of the transcriptional coactivator Yorkie (Yki). Initially, the Sterile 20-like kinase Hippo (Hpo) forms a complex with the WW domain scaffolding protein Salvador (Sav) to phosphorylate and activate the DBF family kinase Warts (Wts). This phosphorylation allows Wts, in association with Mob as tumor suppressor (Mats), to phosphorylate and inhibit Yki. Wts phosphorylates Yki on multiple sites, including Ser168, which is the most crucial for creating a 14-3-3 binding site and restricting Yki to the cytoplasm.

Without inhibition from the Hippo pathway, Yki translocates into the nucleus, where it associates with several transcription factors to induce the expression of genes promoting proliferation (*bantam* micro-RNA, *cyclin E*) and inhibiting apoptosis (*diap1*). The primary transcription factor interacting with Yki is Scalloped (Sd). Other known Yki interactors include the transcription factors Homothorax (Hth), Teashirt (Tsh), and Mad, as well as the WW domain binding protein 2 (Wbp-2). Activated Yki increases the expression of several upstream components of the Hippo pathway (e.g., *kibra crb* and *fj*), constituting a negative feedback loop. In addition, Yki controls the expression of genes involved in the Notch (*ser*), EGFR (*vein*), and Wingless (*wg*) signaling pathways, providing one level of crosstalk between these pathways.

Regulation of the *Drosophila* Hippo Pathway

Although we have a relatively complete picture of the core Hippo signaling cassette, our view of what lies upstream of the pathway is incomplete. To date, the inputs feeding into the core pathway fall into the two main categories: cell-cell interactions and cell polarity. The atypical cadherins, Fat and Dachsous (Ds), are thought to act as receptor and ligand, respectively. Fat activates the Hippo pathway by at least two independent mechanisms. In one instance, Fat inhibits the atypical myosin Dachs, which binds Wts to induce its degradation. Dachs localization and activity are regulated by the palmitoyltransferase Approximated (App). Fat also recruits the FERM domain-containing protein Expanded (Ex) to the apical surface. Ex, along with its partners Merlin (Mer) and Kibra, promotes Hippo and Wts activation. Ex can also directly bind and inhibit Yki, restricting it to the cytoplasm. Regulators of Fat and Ds activity include: the Golgi-localized kinase Four-jointed (Fj), which regulates the Fat-Ds interaction by phosphorylation of both extracellular domains; the cytoplasmic kinase Discs overgrown (Dco), which promotes Fat activation by phosphorylating its intracellular domain upon Ds binding; and the cytoplasmic protein Lowfat (Lft), which binds to both Fat and Ds intracellular domains and regulates their stability.

Polarity cues also impinge on Hippo signaling. The transmembrane apical determinant Crumbs (Crb) interacts with Ex and, like Fat, is important for apical localization of Ex. Another polarity protein, the basolateral determinant Lgl, can feed into the Hippo pathway, modulating Hpo activation. In addition, recent data indicate that the actin cytoskeleton, via actin capping proteins, can promote the activity of the Hippo pathway.

The Hippo pathway is regulated by other proliferation-linked signaling molecules. For example, the *Drosophila* Ras association domain family protein, dRASSF, inhibits Hpo by competing with Sav for a binding site. Additionally, dRASSF induces the recruitment of a PP2A-containing complex named STRIPAK, responsible for Hpo inhibition by dephosphorylation. In addition, the *Drosophila* LIM protein Ajuba (dJub) interacts with both Sav and Wts, inhibiting Yki phosphorylation.

The Core of the Mammalian Hippo Pathway

Similar to the invertebrate cascade, a series of phosphorylation events via MST and LATS ultimately leads to the phosphorylation of two Yki homologs: YAP and TAZ. In addition to cytoplasmic retention by 14-3-3, YAP/TAZ phosphorylation by the kinases LATS and CK1 leads to βTRCP-dependent proteasomal degradation of YAP/TAZ.

In the nucleus, both YAP and TAZ can bind to the Sd homologs TEAD1/4 and activate the transcription of genes required to promote cell growth and inhibit apoptosis (*CTGF*, *AREG*, *BIRC5-2*, *FGF*, and *GLI-2*). Upon phosphorylation by the SRC/ABL kinases, YAP and TAZ acquire the capacity to bind p73 and activate the transcription of proapoptotic genes. Numerous transcription factors are described as YAP/TAZ binding partners, but their functional significance is still unclear.

Activation of the Mammalian Hippo Pathway

In vertebrates, cell density information feeds into the Hpo pathway, which is transmitted, in part, through the Crumbs polarity complex. The Crumbs complex contains Angiomotin (AMOT), a protein that binds YAP/TAZ and SMAD to inhibit their nuclear activity. The adherens junction protein α-CATENIN also inhibits YAP by restricting its localization in a complex with 14-3-3. This complex also protects YAP from dephosphorylation by the PP2A phosphatase. Another junction protein, the tight junction protein ZO-2, binds to YAP/TAZ, facilitating their nuclear translocation. In addition to cell density sensing, DNA damage is another input that activates the vertebrate Hippo signaling via LATS activation.

Another level of Hpo regulation occurs through MER, KIBRA, WILLIN\FRMD6 (Ex homolog), RASSF, PP2A, AJUB, and the actin cytoskeleton. Their roles in mammals appear similar to their *Drosophila* orthologs, with the exception of RASSF, which is described as an MST activator.

Another set of Hippo pathway regulators, CD44, ITCH, and ASPP, has been identified only in mammals. CD44 is a transmembrane protein that promotes Mer activity. ITCH is an E3-ubiquitin ligase that regulates growth by inducing LATS degradation. LATS also promotes the activation of the tumor suppressor protein p53 via ASPP and MDM-2. The apoptosis-stimulating protein of p53-1, ASPP-1, can also act as an oncogene by inhibiting the binding between LATS and YAP/TAZ.

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