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Cellular Organization and Cytoskeletal Regulation of the Hippo Signaling Network

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Abstract

The Hippo signaling network integrates diverse upstream signals to control cell fate decisions and regulate organ growth. Recent studies have provided new insights into the cellular organization of Hippo signaling, its relationship to cell-cell junctions, and how the cytoskeleton modulates Hippo signaling. Cell-cell junctions serve as platforms for Hippo signaling by localizing scaffolding proteins that interact with core components of the pathway. Interactions of Hippo pathway components with cell-cell junctions and the cytoskeleton also suggest potential mechanisms for the regulation of the pathway by cell contact and cell polarity. As our understanding of the complexity of Hippo signaling increases, a future challenge will be to understand how the diverse inputs into the pathway are integrated, and to define their respective contributions in vivo.

Keywords

Hippo; signaling; cytoskeleton; mechanical force; cell junctions

The Hippo Signaling Network

The Hippo signaling network integrates diverse upstream signals to control cell fate decisions and regulate organ growth. It was first discovered in *Drosophila* through the identification and characterization of genes that, when mutated, cause severe over-growth phenotypes [1]. Hippo signaling is highly conserved amongst animals, and dysregulation of the pathway has been linked to many human cancers [2]. One remarkable feature of Hippo signaling is its role as an integrator of growth control signals. Indeed, Hippo signaling is influenced by, or cross-talks with, multiple pathways that respond to growth factors, that promote growth linked to positional information, or that influence growth in response to nutritional and metabolic status [3-5]. Hippo signaling is also affected by contacts with neighboring cells and the extracellular matrix, and by mechanical forces. In this review, we first briefly describe new insights into core components of the Hippo pathway, and then focus on recent discoveries that have enhanced our understanding of the cellular

organization of the Hippo pathway and its regulation by cell junctions, the actin cytoskeleton, and mechanical force.

Expansion of the Hippo Core

Hippo signaling regulates growth by controlling the localization of a transcriptional co-activator protein that in *Drosophila* is known as Yorkie (Yki) [6-8]. Transcriptional activation by Yki, which is achieved in part by recruiting chromatin and histone modifying complexes [9-11], leads to increased growth. Yki is down-regulated through phosphorylation by the kinase Warts (Wts), which promotes cytoplasmic localization of Yki [6, 7]. As most upstream inputs of Hippo signaling affect Wts, and Wts directly regulates Yki, Wts serves as a central regulatory node within the Hippo pathway. Wts is regulated in several ways, including phosphorylation by the kinase Hippo (Hpo) [12-16], and regulation of Wts abundance [17], Wts localization [18, 19], and Wts interaction with co-factors and inhibitors [20-24]. Activation of Wts is dependent upon two additional core components of the Hippo pathway: Mob-as-tumor suppressor (Mats), which is a Wts co-factor [23], and Salvador (Sav) [25, 26], which promotes Wts activation by acting as a scaffold that links Wts to Hpo [12-16]. The four proteins that regulate Yki, Hpo, Wts, Sav, and Mats, have been generally considered as the “core” of the Hippo network (Fig. 1A).

Mammals have an analogous Hippo network that includes the core components identified in *Drosophila* (though assigned different names). However, mammalian Hippo signaling has greater complexity and includes two Wts homologues, LATS1 and LATS2, two Hippo homologues MST1 and MST2, and two Yki homologues, YAP and TAZ (Fig 1B) [2, 3]. As in *Drosophila,* LATS proteins are the major, though not exclusive, regulators of YAP and TAZ. In mammals, YAP and TAZ localization as well as their stability are both regulated through LATS-dependent and LATS-independent processes [3]. Moreover, several ubiquitin ligases that influence LATS stability have been identified [27-32]. LATS kinases are members of a larger family of protein kinases, the Nuclear Dbf2-related kinases, and two other family members, NDR1 and NDR2, have recently been reported to phosphorylate Yap, and regulate Yap activity [33]. Recent studies have also led to the identification, in both *Drosophila* and in mammals, of multiple MAP4K-type kinases (which, like Hpo/Mst, are within the Ste20 family of protein kinases) that phosphorylate and activate Wts and LATS [34-37]. The identification of these additional “core” components of the pathway explains some instances of MST-independent regulation of LATS, and emphasizes that different inputs into the Hippo pathway could act through regulation of distinct kinases.

Recent studies have also identified additional proteins that could act as scaffolds and promote the interaction of core Hippo pathway components. APC, which is best known as a key component of the β-catenin destruction complex, was observed in mammalian cells to have an additional function as a scaffold that promotes association of LATS and SAV [38]. In *Drosophila*, βPix and Git form a scaffold that promotes activation of Hpo [39]. In mammalian cells, βPix was identified as interacting with LATS and YAP/TAZ to stimulate YAP/TAZ phosphorylation [40]. Schip1 was recently identified in *Drosophila* as a protein that activates Hpo by binding to both Expanded and Tao-1 [41], a kinase that induces Hpo [42, 43].

Cellular organization of the Hippo network and regulation at cell junctions

The first decade of Hippo pathway research was characterized by tremendous progress in genetic and biochemical characterization of the pathway. More recently, our understanding of the cell biology of Hippo signaling has advanced significantly – where pathway components localize, where key events happen inside the cell, and how changes in protein localization modulate pathway activity. Many Hippo pathway components localize to cell-cell junctions, such that in addition to their role in maintaining tissue integrity and polarity, these junctions effectively provide a platform for regulation of Hippo signaling. The links between cell junctions and Hippo pathway components can help explain how cell contacts and cell polarity modulate Hippo pathway activity. In both *Drosophila* and mammalian cells, cadherin-mediated cell-cell adhesion occurs at adherens junctions, and these sites of cell attachment are connected to the actin-myosin cytoskeleton through catenins and associated proteins. Apical to the adherens junctions, mammalian epithelial cells have tight junctions, which form a paracellular diffusion barrier. In *Drosophila*, the paracellular diffusion barrier is formed by septate junctions, which are basal to the adherens junctions. Nonetheless, many proteins that are found at tight junctions in mammals, such as Crumbs, are conserved in *Drosophila*, and as in mammalian cells they localize to cell junctions just apical to adherens junctions [44]; in *Drosophila* epithelia this region is referred to as the marginal zone, or sub-apical region. Several of the proteins first identified as upstream activators of Hippo signaling, including Dachsous (Ds), Fat, Expanded (Ex), and Merlin (Mer), localize near the marginal zone [18, 45, 46], suggesting that this could be a site of pathway activation. The concept that activation of core components happens at the membrane was further supported by observations that forced membrane localization of over-expressed Hpo could increase Hpo activity [47], and forced membrane localization of over-expressed Mats or Wts, or their mammalian homologues, could increase Wts/LATS activity [19, 48, 49].

More recently, progress has been made in visualizing the endogenous localization of components in *Drosophila*, and in characterizing mechanisms that contribute to their localization (Fig. 2A). Sav localizes to cell membranes through interaction with the transmembrane immunoglobulin-domain protein echinoid (Ed) [50], which localizes to the membrane overlapping adherens junctions and the marginal zone. Ed participates in homophilic binding to Ed in neighboring cells [51], which serves as a mechanism linking cell-cell contacts to Hippo pathway regulation. Sav, in turn, can recruit Hpo to apical cell-cell junctions, although Hpo is normally found predominantly in the cytoplasm [18, 19, 52]. Crumbs localizes to the marginal zone, and this depends upon homophilic binding between Crumbs proteins in neighboring cells, which serves as another mechanism linking cell contact and polarity to Hippo signaling [53]. Cell contact-dependent regulation of Hippo signaling through Ed and Crb has recently been implicated in maintaining quiescence of neural stem cells in the larval brain [54]. Crumbs, in turn, is required for membrane localization of Ex [53, 55, 56]. By contrast, Wts localizes to adherens junctions, where it is recruited by the *Drosophila* Ajuba LIM family protein, Jub [20]. Jub is an inhibitor of Wts [21, 22], implying that in this context Wts localization to cell-cell junctions is associated with Wts inhibition, rather than activation. Consistent with this role, disruption of adherens junctions in *Drosophila* epithelia can be associated with increased Hippo pathway activity

[57]. Wts can also be down-regulated by a signaling pathway initiated by the large cadherin family proteins Ds and Fat, which localize to the marginal zone, and regulate Hippo signaling through the Myosin family protein Dachs [1]. Earlier studies identified an influence of Dachs on Wts protein levels [17], and a more recent study identified an ability of Dachs to inhibit Wts association with Mats [24].

Dynamic localization of Hippo pathway components

The observation that Wts normally localizes with an inhibitor, Jub, raised the question of how and where Wts normally gets activated. Under conditions of pathway activation in *Drosophila* imaginal discs, Wts re-localized from Jub to Ex, where it is activated by Hpo , as revealed by phospho-Wts staining [18] (Fig. 2A). This re-localization requires both Ex, which physically interacts with Wts, and Hpo, which promotes Ex-Wts binding. Ex also physically interacts with Hpo [58], and thus could act as a scaffold to link Hpo to Wts (Fig. 3A). Interestingly, examination of Merlin suggests that it plays a similar role, both in *Drosophila* and mammalian cells [19]. While it was initially thought that Merlin functions as an activator of Hpo, Mer was found instead to promote Wts/LATS activation by bringing Wts/LATS and Hpo/MST together at cell membranes [19]. This scaffolding occurs because, under some conditions, Mer binds to Wts/LATS, and Mer also binds Sav, therefore, linking Mer to Hpo (Fig. 3B). In addition, both APC (in mammals) [38] and Kibra, which in *Drosophila* localizes near Mer and Ex and acts genetically at a similar point in the Hippo pathway, can bind to both Sav and Wts/LATS [58-61]. Together, these observations imply that assembly of an activation complex, in which Hpo and Wts are linked through scaffolding by Ex, Mer, Kibra, or APC, is a key step in Hippo signaling. There has, however, been controversy in mammalian cells over how Merlin influences Hippo signaling, as it has also been reported that Merlin regulates LATS through the ubiquitin ligase, CRL4/DCAF1, and that CRL4/DCAF1 regulates LATS in the nucleus [62, 63]. Merlin might regulate LATS by multiple mechanisms, but immunolocalization studies of LATS proteins are discordant, and further studies are needed to determine whether this reflects differences in experimental conditions, or in the reagents employed.

There are also differences between upstream regulation of the pathway in *Drosophila* and that in mammals. Loss of E-cadherin or α-catenin in mammalian cells has been associated with increased YAP activity [64, 65], rather than decreased Yki activity as in *Drosophila* [57], which suggests that instead of, or at least in addition to, a role in promoting Wts inactivation, adherens junctions in mammalian cells have a role in promoting Wts activation. One possible mechanism for this observation could be physical interactions between α-catenin and Merlin [66], which could localize Merlin to adherens junctions, although Merlin also associates with tight junction proteins [67]. It was also recently reported that α-catenin inhibits a direct activation of YAP by Src [68]. Whether mammalian homologues of Ds and Fat (Dchs1 and Fat4) regulate the Hippo pathway in mammalian cells is also controversial, as there have been conflicting reports about whether they influence Yap activity [69-71]. Moreover, how they might influence Hippo signaling in mammals is unclear, as Dachs, which is essential for Wts regulation by Ds-Fat signaling in *Drosophila* [17], is not conserved in vertebrates [72]. Crumbs is an upstream regulator of Hippo signaling in both *Drosophila* and mammalian cells [53, 55, 56, 73, 74], but may act through distinct

mechanisms: in *Drosophila* it influences Hippo signaling by localizing Ex, which is not fully conserved in mammals, although it has some similarity to mammalian Willin [75]. In mammals, a Crumbs homologue (Crb3) promotes Lats phosphorylation [74], possibly by recruiting tight junction proteins that regulate Lats (Fig. 2B).

Mammals also have a distinct family of proteins, the Motins, which are upstream regulators of Hippo signaling that can localize to tight junctions, and that have some functional similarities to Ex [76]. The Motins include Angiomotin, which exists in distinct p80 and p130 isoforms (created by alternative splicing), Angiomotin-like 1 (AmotL1), and Angiomotin-like 2 (AmotL2). Motins can act as scaffolding proteins that bring together multiple components of the Hippo pathway: Amot-p130, AmotL1 and AmotL2 can bind LATS, YAP, Merlin, and Kibra (Fig. 3C). Their ability to bind YAP could enable them to modulate YAP activity both by promoting its phosphorylation, and by directly sequestering it in the cytoplasm [77-80]; Ex has a similar ability to sequester Yki [81, 82]. Motins have also been implicated in a feed-forward loop that promotes Hippo pathway activation: Motins are substrates of LATS [83-86], and phosphorylation by LATS both stabilizes Motins, and promotes their binding to Merlin. Binding of Motins to Merlin appears to influence Merlin conformation, such that its binding to LATS is enhanced, which presumably promotes further LATS activation [87].

Studies of early cell fate specification in the mouse embryo have identified a role for differential Motin localization in controlling Hippo signaling [88]. At the 32-cell stage, mouse blastomeres are subdivided into inner cells, which form the inner cell mass (ICM), and outer cells, which form the trophectoderm (TE). This subdivision requires Hippo signaling, which is high in the ICM, leading to cytoplasmic YAP, and low in the TE, leading to nuclear YAP [89]. Both cell contacts and cell polarity influence Hippo signaling at this stage. In the ICM, Motins localizes to cell-cell junctions and Hippo signaling is active. However, the outer cells become polarized, causing Motins to localize to the apical domain rather than to cell-cell junctions [86, 90]. Evidently, this loss of Motins from cell junctions prevents formation of the Hippo pathway activation complex needed to promote LATS activation (Fig. 2B). Thus, while there are some differences in the specific proteins involved between *Drosophila* and mammals, a common theme has emerged regarding the existence and importance of platforms for Hippo signal transduction at cell junctions.

Regulation of Hippo signaling by the extracellular matrix

Hippo signaling is also regulated by attachment to the extracellular matrix (ECM). For example, the extent of cell-ECM contacts influences Hippo signaling, and detachment of cells can result in cell death through activation of the Hippo pathway [91-93]. The requirement for cell-substrate attachment has both biochemical and biomechanical components. One biochemical mechanism involves the modulation of Hippo signaling by Integrin-linked Kinase (ILK), which could inhibit Merlin activation by inhibiting the phosphatase MYPT1 [94]. More recently, a link between integrin and Hippo signaling that depends upon Focal adhesion kinase (FAK) was identified [95]. Integrins bound to fibronectin stimulate FAK, thereby activating Src, which activates PI3K. The PI3K downstream kinase PDK1 then disrupts the core kinase cassette, resulting in the inhibition of

Hippo signaling [96]. As activation of FAK by integrins can be modulated by substrate stiffness [97], regulation of Hippo signaling through FAK could also contribute to influences of the mechanical environment on Hippo signaling, . An alternative mechanism by which Src can promote YAP activity, involving direct phosphorylation of tyrosines residues on YAP by Src, has also recently been described [68, 98].

Regulation of Hippo signaling by F-actin levels

Indications of the key influence of the cytoskeleton on Hippo signaling first came from observations that mutations in *Drosophila* that increase F-actin accumulation could be associated with increased Yki activity [99, 100]; this also occurs in mammalian cells [101]. Demonstration of the influence of mechanical force on YAP and TAZ activity then came from observations that cell shape and rigidity of the extracellular matrix could influence YAP/TAZ activity [93], and that this influence requires myosin, which generates tension in the actin cytoskeleton. It was also reported that regulation of YAP/TAZ by cell attachment and cell shape occurs independently of LATS [93], but others have reported LATS-dependent effects [91, 92]. Modulation of the actin cytoskeleton is also correlated with cell density-dependent effects on Yap activity [101, 102].

The influences of cell shape, cell attachment, cell density, and matrix rigidity on Hippo signaling are suppressed by inhibiting the key cytoskeletal regulator Rho [91-93, 101]. This is also true for other upstream inputs of Hippo signaling, such as GPCR pathways, which may influence Hippo signaling through cytoskeleton regulation [103]. In *Drosophila*, Zyxin may also regulate Hippo signaling in part through modulation of the cytoskeleton [21, 104]. Initially, how the actin cytoskeleton influences Hippo signaling remained unknown, but a series of recent studies have begun to make progress on identifying molecular mechanisms that link the cytoskeleton to regulation of Hippo signaling.

In mammalian cells, Motins have been identified as a key link between F-actin and Hippo pathway regulation, as knockdown of all three Motins increased Yap activity, even in the presence of cytoskeletal disruption [105]. Motins can physically associate with F-actin, but this association is blocked by phosphorylation of Motins by Lats kinases [83, 84, 105]. Moreover, F-actin competes with YAP for binding to Motins. Thus, when LATS phosphorylates Amot-p130 to inhibit its binding to F-actin, it increases Amot-p130 binding to YAP, and hence inhibition of YAP [105]. Notably, the influence of F-actin on Motin-YAP binding, together with potential sequestration of YAP through direct binding to Motins, also provides a possible explanation for observations of LATS-independent regulation of YAP by the cytoskeleton. Down-regulation of YAP induced by disruption of the actin cytoskeleton also requires Protein kinase A in mammalian cells, which can directly phosphorylate LATS and enhance LATS activity [106].

In cultured *Drosophila* cells, cytoskeletal disruption increased Merlin-Wts binding, suggesting that F-actin accumulation could potentially modulate Hippo signaling by influencing interaction between Merlin and Wts [19]. Regulation of Wts activity by F-actin in *Drosophila* was also partially dependent upon JNK activity [99]. JNK also contributes to influences of cyclic stretch on YAP activity in mammalian cells [107], which occurs over a

time scale that correlates with reorganization of F-actin. JNK has a complex relationship to Hippo signaling, as in *Drosophila*, depending upon the context, it can activate or inhibit Yki [108-110]. A mechanism by which Yki gets activated by JNK involves phosphorylation of Jub, or one of its mammalian homologues, LIMD1; this phosphorylation promotes its ability to bind to, and hence inhibit, LATS [111].

Regulation of Hippo signaling by cytoskeletal tension

In addition to mechanisms that appear to depend upon accumulation of F-actin, mechanisms that could provide a basis for influences of tension within the actin cytoskeleton on Hippo signaling have been identified. As noted above, one such mechanism is the influence of cytoskeletal tension on integrin-dependent signaling. The actin cytoskeleton also forms attachments to the nuclear envelope. Intriguingly, a recent study reported that Nesprin 1 Giant, a protein required for attachment of the actin cytoskeleton to the nuclear membrane, is required for the activation of YAP in response to dynamic stretch in mesenchymal stem cells [112]. How this attachment is able to influence YAP activity remains to be determined.

Epithelial cells are also mechanically coupled to each other at adherens junctions, which are attached to the actin cytoskeleton. In growing *Drosophila* epithelia, these cell-cell junctions are under tension, and this tension promotes Yki activity [20]. Activation of YAP that is promoted by stretching cells, and dependent upon adherens junctions, has also been observed in cultured mammalian cells [113]. A mechanism for how tension at adherens junctions promotes Yki activity has been identified in *Drosophila*, where the localization of Jub to adherens junctions is regulated by myosin activity [20]. This recruitment is mediated through α-catenin, which can act as a mechanotransducer: studies of the association between α -catenin and Vinculin have indicated that α -catenin, which links adherens junctions to the actin cytoskeleton, can undergo a tension-dependent conformational change that exposes, under high tension, a Vinculin binding site [114]. This same conformational change might also influence binding between Jub and α -catenin. The Jub recruited to adherens junctions then recruits Wts to adherens junctions, which, leads to increased Yki activity because Jub is a Wts inhibitor. Conversely, when tension is lowered by reducing myosin activity, Jub and Wts recruitment to junctions is decreased, as is Yki activity [20].

The Spectrin cytoskeleton also appears to provide a link between tension and Hippo signaling, but the nature of this link remains unclear. Spectrins were found to influence Hippo signaling, both in *Drosophila* and in cultured mammalian cells, in three independent studies [115-117]. Two of these suggested that Spectrins might be regulated by cytoskeletal tension, and help transduce the effects of tension onto Hippo signaling, possibly through upstream regulators of Hippo signaling including Crumbs, Merlin, and Kibra [115, 116]. The other study, by contrast, reported that Spectrins influence myosin phosphorylation, and suggested that Spectrins might influence Hippo signaling by affecting actomyosin contractility [117]. Thus, while Spectrins clearly have an influence on Hippo signaling, defining the mechanism by which they regulate the Hippo network requires further study.

Concluding Remarks

From its simple beginnings as the Salvador-Warts-Hippo pathway, our conception of Hippo signaling has expanded to a complex network including dozens of interacting proteins that cross-talk with numerous other cellular pathways. A key aspect of Hippo signal transduction emphasized by recent studies is the fundamental role of protein scaffolds, like Ex and the Motins, which assemble activation complexes through their ability to bind multiple pathway components. The localization of these scaffolds to cell junctions provides a basis for the sensitivity of Hippo signaling to cell-cell contact and cell polarity. Moreover, modulation of the localization of these scaffolds, or of the ability of other pathway components to bind to them, has emerged as a key mechanism for modulating Hippo signaling.

One of the remarkable features of Hippo signaling is its sensitivity to the cytoskeleton and mechanical forces. Progress has been made recently in identifying molecular mechanisms by which the cytoskeleton can influence Hippo signaling, but among the many outstanding questions that remain to be answered (see Outstanding Questions), a key challenge for the future will be to define the respective contributions of these different mechanisms *in vivo*, and understand how these contributions vary in different contexts to control cell fate decisions and regulate organ growth.

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Outstanding questions

- Are different core components of the Hippo signaling network regulated by different upstream inputs?
- Where do LATS proteins localize in vivo and what does regulation of their localization contribute to Hippo pathway regulation in mammals?
- How are different cytoskeletal-dependent forms of regulation integrated and coordinated?
- What do each of the distinct mechanisms for cytoskeletal regulation of Hippo signaling contribute to modulation of Hippo signaling in vivo during different developmental, physiological or pathological processes?
- What additional cellular sites of Hippo/Mst and Warts/Lats activation remain to be discovered?
- Which of the regulatory mechanisms so far identified only in *Drosophila* or only in mammals are evolutionarily conserved?

Trends box

- Hippo signaling is a complex network that integrates multiple growth control signals through an expanding set of core kinases.
- Recent studies have provided insights into the cellular organization of Hippo signaling, including where pathway components localize, where key events happen inside the cell, and how changes in protein localization modulate pathway activity.
- Scaffolds play an essential role in Hippo signal transduction by assembling kinase activation complexes through their ability to bind multiple pathway components. Localization of these scaffolds to cell junctions provides a basis for the sensitivity of Hippo signaling to cell contact and cell polarity.
- Progress has been made in identifying mechanisms by which the cytoskeleton and mechanical forces can regulate Hippo signaling.

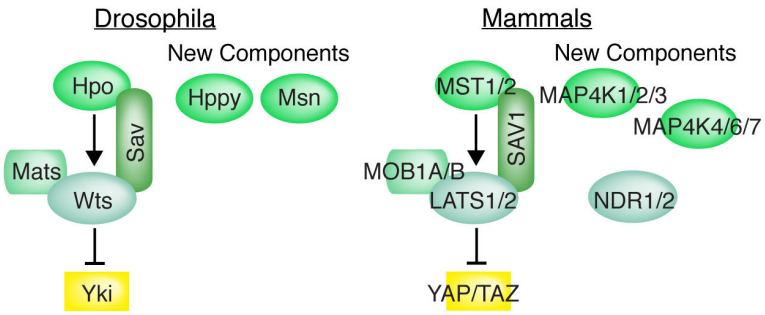

Figure 1. Core proteins of the Hippo network

In Drosophila (left), the kinase Hpo phosphorylates and activates the kinase Wts; the kinase Wts phosphorylates and inhibits the transcriptional co-activator Yki. This requires the Wts co-factor Mats, and is facilitated by the scaffolding protein Sav. The Hppy and Msn kinases can also phosphorylate and activate Wts. In mammals (right), the kinases MST1 or MST2 phosphorylate and activate the kinases LATS1 and LATS2; the kinase LATS1 and LATS2 phosphorylate and inhibit the transcriptional co-activators YAP and TAZ. This requires the LATS co-factors MOB1A or MOB1B, and is facilitated by the scaffolding protein SAV1. MAP4K kinases can also phosphorylate and activate LATS kinases, and NDR kinases can also phosphorylate and inhibit YAP.

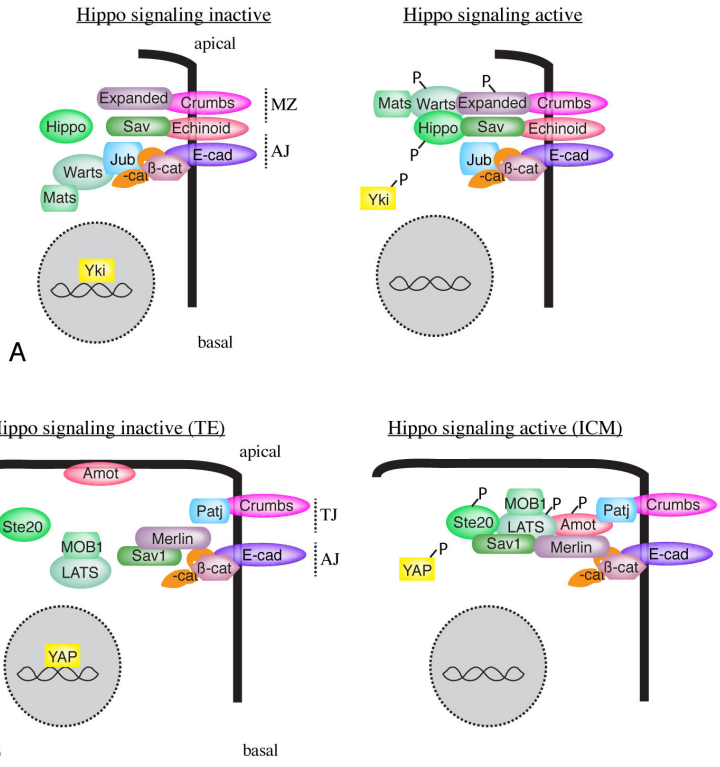

Figure 2. Localization and re-localization of core Hippo pathway components

A) Localization of core components in *Drosophila* epithelia. Under conditions of low Hippo pathway activity (left), Wts is associated with its inhibitor, Jub, at adherens junctions (AJ), and Hpo is predominantly cytoplasmic, while Yki is nuclear. Under conditions of high Hippo pathway activity, Wts localizes with Ex at the marginal zone (MZ), and Hpo is recruited to Sav, causing Yki to be cytoplasmic. B) Localization of core components in 32 cell mouse blastocysts. In outer TE cells, Hippo signaling is low, YAP is nuclear, and Amot is localized to the apical membrane. This is presumed to prevent the formation of a LATS activation complex, although the localization of LATS proteins has not been determined in this tissue. In inner ICM cells, Hippo signaling is high, YAP is cytoplasmic, and Motins (Amot) are localized to cell-cell junctions, where, together with Merlin/NF2, they promote phosphorylation and activation of LATS [118].

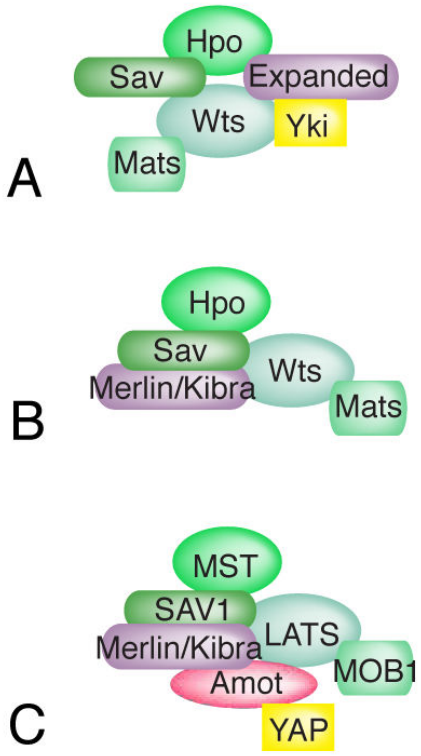

Figure 3. Hippo activation complexes

Hippo activation appears to require the participation of multiple scaffolds that assemble multi-protein complexes. A) In *Drosophila*, both Sav and Ex can interact with both Hpo and Wts. B) Ex is partially redundant with Merlin and Kibra (depending upon the tissue), which can interact with Wts and Sav. C) In mammals, Merlin (NF2) and Motins play key roles in Hippo pathway activation. Merlin and Kibra can each interact with SAV1 and LATS, and Motins can interact with LATS, Merlin, and Kibra.

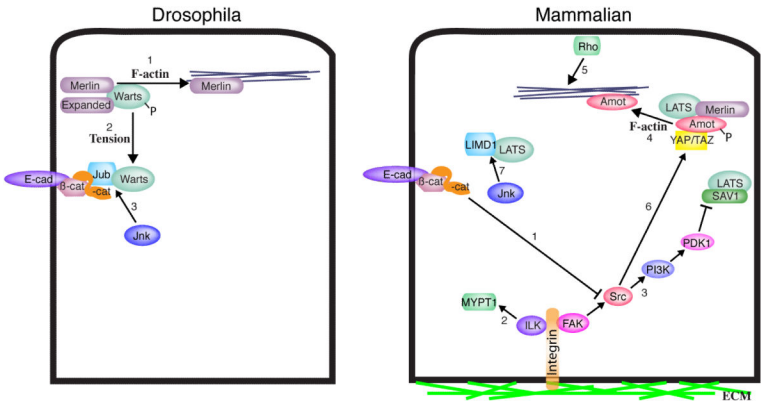

Figure 4. Regulation of Hippo signaling by F-actin and cytoskeletal tension

Hippo signaling is regulated by levels of F-actin, tension within the actin cytoskeleton, and cell attachments. Several processes that could contribute to these effects have been identified, some of which are illustrated here. In *Drosophila*, these include 1) inhibition of Merlin-Wts interactions by F-actin, 2) cytoskeletal tension dependent recruitment of Wts into a complex with Jub at adherens junctions, and 3) promotion of Jub-Wts binding by Jnk phosphorylation. In mammals, these include 1) α-catenin-mediated inhibition of Src activation by integrins, 2) activation of MYPT1 by ILK, 3) inhibition of Sav-Lats association through a FAK-Src-PI3K-PDK1 pathway, 4) association of Amot with F-actin, which prevents Amot from associating with YAP/TAZ, 5) increases in F-actin promoted by Rho, 6) activation of YAP through phosphorylation of YAP by Src, and 7) promotion of LATS-LIMD1 binding by Jnk phosphorylation. In most cases potential conservation of these processes between *Drosophila* and mammals has not yet been investigated.