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YAP and TAZ: a nexus for Hippo signaling and beyond

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Abstract

The Hippo pathway is a potent regulator of cellular proliferation, differentiation, and tissue homeostasis. Here we review the regulatory mechanisms of the Hippo pathway and discuss the function of Yes-associated protein (YAP)/transcriptional coactivator with a PDZ-binding domain (TAZ), the prime mediators of the Hippo pathway, in stem cell biology and tissue regeneration. We highlight their activities in both the nucleus and the cytoplasm and discuss their role as a signaling nexus and integrator of several other prominent signaling pathways such as the Wnt, G protein-coupled receptor (GPCR), epidermal growth factor (EGF), BMP/transforming growth factor beta (TGF β), and Notch pathways.

Keywords

extracellular matrix; stem cells; regeneration; primary cilia; Wnt; Notch

The Hippo pathway: signal integrator of cell proliferation and differentiation

Coordinated regulation of stem cell proliferation and differentiation is essential to maintain tissue mass and homeostasis. The Hippo pathway was initially identified through genetic mosaic screens for suppressors of tissue overgrowth in *Drosophila melanogaster* [1,2]. Later studies identified that the general components and functions of the pathway are highly conserved in mammals [3]. Salient properties of the Hippo pathway are in regulating cell proliferation, survival, and differentiation – all of which are particularly important processes in tissue development and regeneration. Deregulation of this pathway is furthermore implicated in cancer development [4–6].

Downstream effectors of the Hippo kinase cascade are the paralogs and transcriptional coactivators YAP and TAZ (also known as WWTR1) [3]. The Hippo kinase cascade is the major regulator of YAP/TAZ by phosphorylating and thereby inhibiting YAP/TAZ nuclear activities. Recent studies have expanded the complexity of YAP/TAZ considerably,

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identifying new upstream regulatory components and revealing its integration with other signaling pathways such as the GPCR [7–13], Wnt [14–17], TGF β [18–20], EGF [21–24], and Notch [25–28]. In addition to expanding the number of inputs that modulate YAP/TAZ, the downstream mechanisms of YAP/TAZ have also grown in complexity. Besides transcriptional regulation, new physiological functions mediated by either nuclear or cytoplasmic localization of YAP/TAZ have been revealed and are providing insights into how YAP/TAZ integrates and modulates the cellular microenvironment.

Drawing on studies in both *Drosophila* and mammals, we highlight recent findings and provide a current overview of the molecular regulation of the Hippo network in mammals, with a particular focus on the key downstream effectors YAP and TAZ. We highlight the role of YAP/TAZ as a nexus and integrator for multiple prominent pathways and signaling organelles that play key roles in the control of cell fate and tissue regeneration.

At the core of the Hippo pathway: phosphorylation-dependent regulation

The Hippo pathway comprises a large network of proteins with the central components of a regulatory kinase module and a transcriptional module (Figure 1). Many components of the Hippo pathway are highly conserved from *Drosophila* to mammals, including mammalian STE20-like protein kinase 1/2 (MST1/2) (Hippo homolog), Salvador family WW domain-containing protein 1 (SAV1) (Salvador homolog), large tumor suppressor 1/2 (LATS1/2) (Warts homolog), MOB kinase activator 1A/B (MOB1A/B) (Mats homolog), MAP4K4 (Misshapen homolog), YAP and TAZ (Yorkie homolog), and TEA domain family members 1–4 (TEAD1–4) (Scalloped homolog). Although the core components of the Hippo pathway are extensively conserved, a divergent role of the upstream regulators through evolution has become apparent (Box 1).

The Hippo pathway responds to various upstream stimuli from the cellular microenvironment including mechanical signals, cellular stress, extracellular stimuli, polarity, and adhesion cues that are integrated through upstream regulators. These upstream regulators promote the activation of the Hippo kinase cascade by activating the mammalian Ser/Thr kinases LATS1/2, which phosphorylate and promote either the cytoplasmic retention of YAP and TAZ via a 14-3-3 interaction or the degradation of YAP/TAZ, ultimately preventing TEAD-mediated transcription [1,3]. Activation of the upstream kinase module thus concludes in inhibition of the transcriptional output module. One of the key questions concerning the Hippo pathway is the molecular mechanism of LATS activation. In a classical view, MST1/2-mediated phosphorylation of LATS1/2 at the hydrophobic motif is necessary for LATS kinase activation [29]. However, the requirement of MST1/2 for LATS1/2 phosphorylation is context- and cell type-dependent. Loss of MST1/2 in mouse embryonic fibroblasts does not significantly affect LATS1/2 phosphorylation and activation [30]. Furthermore, the forced expression of plasma membrane-targeting Warts (the *Drosophila* homolog of LATS1/2) partially rescues the eye disc phenotype of Hippo (the *Drosophila* homolog of MST1/2) mutant flies, suggesting the existence of additional kinases that regulate the activity-dependent phosphorylation of LATS1/2 or Warts [31]. Indeed, an alternative pathway activating Warts was identified [32]. In the *Drosophila* adult midgut, the protein kinase Misshapen activates Warts to negatively regulate the activity of Yorkie and

its target gene *Upd3* (a JAK–STAT pathway ligand) in differentiating enteroblasts, thereby restricting intestinal stem cell division. Notably, depletion of Hippo in enteroblasts had little effect on midgut proliferation. Moreover, an increase in midgut proliferation resulting from *Misshapen* knockdown in enteroblasts was not rescued by Hippo overexpression, suggesting that Hippo and *Misshapen* are not redundant in this cell type [32]. Similarly, the mammalian *Misshapen* homolog MAP4K4 activates LATS2, suggesting *Misshapen*/MAP4K4 is an evolutionarily conserved MST1/2-independent activator of LATS1/2, although it is not entirely clear whether *Misshapen*/MAP4K4 directly phosphorylates and activates LATS [32]. Taken together, these observations suggest a cell type-dependent requirement of Hippo (MST1/2) and *Misshapen* (MAP4K4) for Warts (LATS1/2) activation and Yki (YAP/TAZ) inhibition. We speculate that these direct mediators of LATS activation (and thereby YAP and TAZ inhibition) could be regulated and fine-tuned on cellular stimulation, depending on the cellular ratios and differential regulation of these two LATS-activating kinases. Elucidating this context dependency and the spatiotemporal hierarchy in the dynamics of LATS activation will reveal further insights into the Hippo pathway.

Besides phosphorylation, recent studies have also revealed additional levels of regulation via protein stability of the Hippo kinase/transcription modules [33–37] and by scaffolding the components of the Hippo pathway [38]. Some of these regulatory mechanisms are highlighted in Box 2. This complexity adds an extra layer of divergent regulation of the Hippo pathway.

Upstream components of the Hippo pathway

Spatial regulation of LATS activation

Epithelial cells adhere mutually through cell–cell junctions such as tight, adherens, and basolateral, dividing the plasma membrane into an apical domain and a basolateral domain and thereby establishing an apical–basal polarity. Studies conducted in both *D. melanogaster* and mammals have revealed that many components of cell–cell junction- and cell polarity-related protein complexes regulate the Hippo pathway. Moreover, these regulators of YAP and TAZ are especially important in tissue organogenesis and in preventing cancer progression [39–41].

While YAP and TAZ are found in the cytosol and the tight junctions in dense cells, these transcriptional coactivators translocate to the nucleus and activate TEAD-mediated transcription in sparse cells [42,43]. This regulation is mediated primarily via homophilic cell–cell contacts that activate the kinase module [42,44]. In addition, the basolateral junction proteins, including mammalian Scribble (SCRIB) facilitate the activation of the core kinases MST and LATS, thereby inhibiting the nuclear activities of YAP/TAZ in mammary epithelial cells [45] (Figure 2A). Loss of cell polarity via induction of the epithelial–mesenchymal transition (EMT) causes SCRIB delocalization and TAZ activation, which confers self-renewal and tumor-initiation capacities on breast cancer stem cells [45]. Furthermore, the apical membrane-associated FERM-domain protein neurofibromatosis 2 (NF2) and the WW and C2 domain-containing protein kidney and brain expressed protein (KIBRA) (also known as WWC1) form a complex to activate the core kinase cassette [46–48]. NF2 is a potent tumor suppressor, mutations of which are associated with

neurofibromatosis type II [5], and is a potent activator of the core Hippo kinase module [1]. Recent studies have revealed the complexity and multilevel regulation of LATS kinases by NF2 (Figure 2A). NF2 interacts with numerous proteins that are localized at the plasma membrane [31], including α -catenin and Angiomotin (AMOT) at adherens junctions [49], but also localizes in the nucleus [50]. At the plasma membrane of cultured cells, NF2 was shown to recruit LATS kinases and coordinate their activating phosphorylation by the MST-SAV1 kinase complex [31], highlighting the plasma membrane as a critical subcellular compartment for Hippo signal transduction. At adherens junctions in the inner cells of preimplantation embryos, NF2 directly interacts with α -catenin and AMOT to recruit LATS kinases, which in turn activates the kinase module of the Hippo pathway and thereby regulates lineage specification in preimplantation embryos [49]. Interestingly, adherens junctions-localized LATS kinases phosphorylate AMOT to further stabilize the AMOT-LATS interaction [49], establishing a potential positive feedback of Hippo pathway activation. By contrast, however, when LATS kinases are recruited to adherens junctions by the LIM domain-containing protein AJUBA in response to cytoskeletal tension, LATS-mediated phosphorylation of YAP and TAZ is impaired [51,52]. Therefore, it is not only LATS localization but also other unknown factors that affect the activation state of LATS. Moreover, another report showed that NF2 inhibits the E3 ubiquitin ligase CRL4^{DCAF1}-mediated ubiquitylation of LATS1/2 in the nucleus [50]. In NF2-mutant tumors, CRL4^{DCAF1}-mediated ubiquitylation of LATS1 results in its proteasomal degradation, while that of LATS2 leads to loss of its kinase activity potentially as a result of conformational change [50]. Together, these observations suggest that NF2 activates LATS kinases at multiple levels, yet the precise mechanism remains unclear.

Physical regulation of YAP and TAZ

In addition to regulating YAP and TAZ through phosphorylation by LATS, other upstream branches of the Hippo pathway directly inhibit the activity of the transcription module by complexing YAP/TAZ, thus sequestering and preventing their nuclear access (Figure 2B). These branches include AMOT [53–56], protein tyrosine phosphatase 14 (PTPN14) [57,58], and α -catenin [59]. The AMOT-YAP/TAZ interaction is mediated via PPxY motifs of AMOT and WW domains of YAP and TAZ and thus does not require YAP and TAZ phosphorylation by the kinase module [53–55]. This regulatory interaction between PPxY motifs and WW domains is widespread throughout the Hippo pathway [60]. PTPN14 also utilizes its PPxY motifs to directly interact with WW domains of YAP, causing cytoplasmic localization of YAP and decreased YAP activity [57,58]. The adherens junction protein α -catenin also regulates YAP by sequestering YAP-14-3-3 protein complexes in the cytoplasm [59]. However, unlike AMOT, the kinase module that mediates YAP phosphorylation is required for the interaction between α -catenin and YAP, as 14-3-3 binds to phosphorylated YAP only [3]. Notably, α -catenin also provides a scaffold for the LATS activation/inactivation process (Figure 2A).

AMOT is a filamentous actin (F-actin)-binding protein that predominantly localizes at tight junctions and F-actin competes with YAP for binding to AMOT [61]. Interestingly, recent studies revealed that LATS1/2 phosphorylate AMOT within a conserved F-actin-binding domain [49,61–63], which inhibits AMOT binding to F-actin thereby potentiating its

inhibitory effects on YAP and TAZ activity [49,61,62]. LATS kinases can therefore inhibit nuclear YAP and TAZ activity both directly and indirectly. Most studies support an inhibitory role of AMOT on YAP/TAZ [35,49,53–55,61,62], but a recent report indicated that AMOT binds to YAP to support YAP–TEAD mediated transcription in the nucleus and thereby stimulate YAP-mediated hepatic tumorigenesis in NF2-deficient mice [64].

Additional regulatory mechanisms of YAP/TAZ activity, divergent from its main regulation via sequestration, have also become apparent (Figure 2B). WW domain-binding protein 2 (WBP2) interacts with YAP and TAZ in a WW domain- and PPxY motif-dependent manner, enhancing YAP and TAZ transcriptional coactivator properties [65,66]. Suppression of WBP2 expression prevents the overgrowth phenotype of Warts mutant flies and inhibits TAZ-driven transformation of MCF10A mammary epithelial cells [65,66]. Multiple ankyrin repeats single KH domain-containing protein (MASK) has also been shown to potentiate YAP activity through direct binding [67,68]. Recent data suggest that vestigial-like family member 4 (VGLL4) is a natural inhibitor of YAP–TEAD interaction that represses target gene expression [69,70]. The Tondu domains of VGLL4 interact with TEAD transcription factors (TFs) and may compete for common binding sites with YAP and TAZ [69,70]. The physiological/pathological roles of these regulations are primarily characterized in YAP/TAZ-induced tumorigenesis. VGLL4 is downregulated in lung [70] and gastric [71] cancers and high MASK1 expression is associated with poor prognosis for breast cancer patients [67].

Nuclear functions of YAP and TAZ

The most widely studied role of YAP and TAZ is as transcriptional coactivators. YAP and TAZ do not contain any DNA-binding domains and thus bind to and elicit their transcription activation via cognate TFs (Figure 3A). Accumulating evidence shows that the TEAD family of TFs are major mediators of YAP and TAZ nuclear activity and therefore gene output [40,43,70,72,73] (Figure 1). Nonetheless, YAP and TAZ do utilize other TFs, and some contextual transcriptional responses are therefore mediated by additional TFs such as SMADs, p63, RUNX, and PAX [3,74], which diversifies the transcriptional output of the Hippo pathway.

A functional explanation of how YAP and TAZ mechanistically regulate TEAD transcriptional activity has been revealed [75–78]. TAZ (and YAP) interacts with components of the SWI/SNF chromatin-remodeling complex [77,78] and YAP (and TAZ) recruits a methyl transferase complex into proximity with the TF, which leads to increased histone (H3K4) methylation and transcription of target genes [75,76]. These complex formations are functionally mediated via interactions between WW domains of YAP/TAZ and PPxY motifs of components of chromatin-remodeling/methyltransferase complexes [75–78] (Figure 3A). It will be interesting to learn whether the same mechanism holds true for other YAP/TAZ-utilized TFs. In some instances YAP/TAZ also operate as transcriptional corepressors for additional target genes [43,79,80]. Intriguingly this nuclear function also requires TEAD TFs [43,80].

In addition, nuclear YAP has also been found to regulate miRNA processing. Nuclear YAP/TAZ bind and sequester DEAD box helicase 17 (DDX17) (also known as p72) to repress its association with Microprocessor, a complex that regulates miRNA processing (Figure 3B). Therefore, the activation of YAP/TAZ in the nucleus is also a general mechanism of impaired global miRNA biogenesis [81]. Interestingly, nuclear YAP/TAZ conversely stimulate the biogenesis of certain miRNAs, such as miR-16, -21, -23a, -29, -107, and -152, by increasing Dicer via the Let-7 family of miRNAs [82,83]. YAP and TAZ can thus function as both positive and negative mediators of miRNA processing, depending on their targets.

YAP/TAZ as a signaling network

The Hippo pathway does not appear to have a unique extracellular ‘Hippo pathway-specific’ receptor/ligand interaction that exclusively regulates this pathway. Instead, it is integrated with a network of other prominent signaling pathways that impinge and rely on the Hippo pathway for their comprehensive functions. Moreover, YAP/TAZ serve as a cellular manifold and directly mediate transcription of several of these receptors and ligands that when expressed activate YAP/TAZ signaling. As the activity of constitutive positive feedback loops ultimately would lead to futile cycles and to pathological conditions, these must be tightly regulated, but how unrestrained YAP and TAZ activity is sensed and contained at the cellular level is currently not well understood.

Besides their widely recognized role in cancer [4], the physiological importance of YAP/TAZ has also been established in regeneration, development, and stem cell biology. We focus on a few tissues and biological contexts where the Hippo pathway has been most extensively studied and discuss these findings within the framework of other signaling pathways and cellular signaling organelles.

YAP/TAZ and Wnt signaling in the intestine

YAP/TAZ, the Hippo Pathway, and Wnt signaling are profoundly integrated [84]. In the absence of a Wnt signal, β -catenin remains phosphorylated, resulting in its degradation and the transcriptional inhibition of Wnt target genes [85]. The cytoplasmic retention of YAP/TAZ via its phosphorylation results in the sequestration of β -catenin in the cytoplasm [86] and in some instances the recruitment of β -TrCP to this complex, leading to the degradation of β -catenin [14,87] (Figure 3C). In addition, cytoplasmic YAP/TAZ interact with disheveled (DVL) to inhibit Wnt target gene transcription [15,16]. The tyrosine phosphatase SHP2 (encoded by the *PTPN11* gene), like YAP and TAZ [42], shuttles between the nucleus and the cytoplasm where its nuclear localization promotes β -catenin-dependent transcription [88,89]. SHP2 is excluded from the nucleus in high-density cells by its interaction with phosphorylated YAP and TAZ [89], suggesting that cytoplasmic YAP/TAZ functionally terminate Wnt signaling through at least three separate mechanisms [15,16,87]. By contrast, nuclear YAP/TAZ promotes nuclear β -catenin, which mediates T cell factor (TCF)-dependent transcription (Figure 3C). Nuclear YAP/TAZ also facilitate nuclear translocation of SHP2, which in turn dephosphorylates CDC73 (also known as parafibromin) to stimulate CDC73- β -catenin binding and ultimately activate Wnt target gene transcription [88–90], providing additional functional links between Wnt and Hippo

differentiate [19,79,107]. In addition, nuclear YAP supports SMAD1 target gene expression downstream of BMP [108]. YAP plays a role in regulating both embryonic and adult lung progenitor cell differentiation by directly specifying the epithelial cell lineage [109,110]. Yap deficiency leads to epithelial progenitors that are unable to properly respond to local TGF β -induced cues [110], resulting in the differentiation of progenitors into epithelial airway cells [109]. Moreover, increased nuclear YAP activity leads to dedifferentiation of secretory cells into basal-like cells, which is directly mediated via a YAP–p63 interaction [109]. However, it remains to be determined whether YAP/TAZ-mediated, TGF β -dependent transcription is regulated exclusively via SMADs [104] or whether it may also require TEADs [18,79] or context-dependent partnering with diverse TFs as has been recently observed. It is a task for the future to discover exactly what is regulated via SMADs, TEADs, and potentially other TFs and how these diverse complexes are regulated.

YAP/TAZ and ligands of the BMP/TGF β family can also create a positive feedback loop that controls the Hippo pathway [20,97,111–113]. TAZ induces BMP4 transcription and promotes cell migration revealing a TAZ/TEAD/BMP signaling axis in mammary epithelial cells [97]. In addition, integrin-linked kinase, an integrin-associated actin and tubulin cytoskeleton-interacting effector that mediates TGF β signaling, was found to be a negative regulator of the Hippo kinase cascade in cancer cells by inhibiting MYPT1, which leads to inhibition of NF2 and nuclear accumulation of YAP/TAZ [21].

GPCR signaling

A prominent role for GPCRs and their cognate ligands as regulators of Hippo signaling has been established [7–10]. GPCRs are coupled to small heterotrimeric G proteins and YAP/TAZ nuclear activities are either activated or inhibited depending on the G proteins coupled to the active receptors. Notable ligands such as the bioactive lipids lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) work through GPCRs [7,9] via G $\alpha_{12/13}$ and are potent inhibitors of the Hippo kinase module and thereby activators of nuclear YAP/TAZ (Figure 4). By contrast, ligands such as epinephrine work via G α_s -coupled GPCRs to inhibit YAP/TAZ through PKA-mediated activation of LATS kinases [114,115]. PKA is also implicated as a widespread activator of LATS on other physiological stimuli [114]. GPCR-driven pathologies can be mediated via dysregulated YAP/TAZ [7,11–13,116] and examples of FDA-approved drugs targeting GPCRs modifying YAP/TAZ activity are known [10,117]. Several notable GPCR ligands play crucial roles in intestinal regeneration [118], development [119], and stem cell pluripotency [120,121] – biological processes where YAP and TAZ are known to be key mediators [6,74,107]. Importantly, S1P–YAP signaling was shown to be important for cardiac precursors in zebrafish [119], but whether these effects by LPA, S1P, and other GPCR ligands in general are mediated via activation of YAP/TAZ transcriptional activity *in vivo* in nonpathological conditions is currently not well understood. It is worthwhile to expand these correlative relationships with loss-of-function experiments in mammalian animal models.

EGF family ligands regulate YAP and TAZ

YAP and TAZ are activated by the EGF family of ligands (Figure 4). Studies in mammalian systems have so far focused on cell culture systems, where YAP/TAZ are facilitators of

EGF-mediated cell migration and transcription [22–24]. The regulation of YAP/TAZ via EGF-like molecules is twofold. EGF inhibits the kinase module via phosphoinositide-dependent kinase-1 and AJUBA proteins, which leads to impairment of SAV1's role in the kinase complex [22,23]. In addition, a cytoplasmic intracellular domain (ICD) of ERBB4 is cleaved and released on neuregulin 1 (NRG1) stimulation that in turn binds to and facilitates nuclear translocation of YAP. This effect is independent of LATS activity [24,122,123] (Figure 4). Moreover, activation of YAP/TAZ results in the transcription of the EGF-like growth factor AREG [21], providing crosstalk between Hippo and EGF signaling [21–23]. The biological role of the EGF-like molecules in the regulation of YAP/TAZ is currently not well understood *in vivo*. As they are prudent modulators of cancer etiology [124] and stem cell biology [103], it will be interesting to delineate precisely what is mediated via the Hippo pathway.

Notch signaling

A direct link between Notch signaling and YAP has become apparent in several systems, such as the developing embryo [28,41,56,125–128], neuronal stem cells [25], mature hepatocytes [26], and hepatocyte-derived stem cells [129]. The ligand Jagged1 (JAG1) [26] and the receptor NOTCH2, both known to be critical determinants of biliary cell fate [130,131], are direct YAP–TEAD target genes [26,27,126,129] (Figure 4). Inducing YAP activity *in vivo* can reprogram hepatocytes via NOTCH activation into progenitor cells [27,129].

Notch signaling is also a well-known regulator of satellite cells [132]. In skeletal muscle, YAP maintains satellite cells undifferentiated via TEAD, partly by repressing the pro-differentiating gene transcription program mediated by MYOD1 and MEF2 [111,133,134]. However, it remains to be elucidated whether YAP mediates this effect in satellite cells directly via NOTCH.

Hippo and NOTCH signaling play an important role during mouse embryogenesis from the morula to the blastocyst stage when the trophoctoderm (TE), which gives rise to the placenta, and the inner cell mass (ICM), which gives rise to the embryo proper, lineages are specified. NOTCH–RBPJ and YAP–TEAD4 combinatorially bind to distinct promoter regions that directly regulate the transcription of the TE lineage determining TF CDX2 [28,41,56,125–128].

Reciprocal interaction with the microenvironment, cellular tension, and the cytoskeleton

Cell shape, cell–cell interactions, mechanotransduction, and matrix adhesiveness and stiffness are all prudent mediators of cellular state, identity, and niche [135–139]. The forces elicited by these interactions are dynamically regulated and are especially important during processes where the cell experiences drastic changes, such as during development, cell differentiation, and regeneration [74,135–138] – all processes where the Hippo pathway has been shown to be an important mediator [3,74,84,137,140]. RHO activity and the actin cytoskeletal organization are robust regulators of YAP/TAZ activity [52,139,141–145]. YAP/TAZ localizes to the nucleus and is transcriptionally active in cells grown on high-rigidity substrate, under static or cyclic high tension, or in cells lacking cell–cell contact

[139–142,146,147]. The regulation of YAP/TAZ by these biological cues has been shown to impact lineage commitment [148], of which directing mesenchymal stem cell (MSC) fate is best described. High tension induced by cell spreading or by culturing on a hard surface promotes the nuclear localization of YAP/TAZ and drives MSCs toward the osteoblast lineage. By contrast, growth on a soft surface retains YAP/TAZ in the cytoplasm and promotes adipogenesis, a phenotype that can be recapitulated by depletion of TAZ [142,147,149]. YAP/TAZ also induce the expression of a range of cellular tension regulators such as zyxin (a component of focal adhesions) and the matricellular protein CYR61, as well as integrins and their ligands [43,113,136,146,150–152]. YAP/TAZ thereby serve as nuclear relays [135] of mechanical signals exerted by extracellular matrix (ECM) rigidity, tension, and cell shape.

These same integrin/ECM signaling proteins can also influence YAP/TAZ regulation [20,43,146,151,152]. For example, in skeletal stem cells the membrane-anchored metalloproteinase MT1-MMP was found to activate the β 1-integrin/RHO GTPase signaling cascade, which triggered the nuclear accumulation of YAP/TAZ and promoted skeletal stem cell lineage commitment [150].

The Hippo pathway and signaling organelles

Cellular organelles that serve as signaling hubs are important integrators of YAP/TAZ activity. The Hippo pathway both controls and is regulated by the primary cilia [94,153–155], a microtubule-based solitary sensory signaling organelle comprising a special part of the plasma membrane in most eukaryotic quiescent cells [156]. The primary cilia respond to mechanical signals [157] and dynamically localize and regulate a range of receptors mediated by YAP/TAZ, such as GPCRs, sonic hedgehog (shh), the TGF β family, receptor tyrosine kinases, and Wnt receptors [7,10,14,17,22,23,158]. This regulation of receptors is predominantly mediated through the cilia transition zone, the cellular gatekeeper for receptors destined for ciliary localization [156]. Members of the nephrocystin (NPHP) family, established primary cilia-associated proteins [159], induce TAZ (and YAP) activity. NPHP4 binds to LATS and negatively regulates LATS activity, whereas NPHP3 and NPHP9 bind directly to TAZ and potentially compete with 14-3-3 binding to relieve cytoplasmic retention [160,161]. Therefore, members of the NPHP family increase transcriptional activity of YAP and TAZ [153]. Furthermore, TAZ binds the primary cilia-associated protein GLIS family zinc finger 3 (Glis3), facilitating Glis3-mediated transcription [154]. In addition, SHH, the prototypical primary cilia signaling ligand [162], activates YAP transcription, abundance, and activity [158]. SHH binds to the inhibitory transmembrane protein Patched and thereby relieves the GPCR-like molecule Smoothed from its inhibition [163], an effect that could be mediated via GPCR signaling. The upstream Hippo kinase module likewise integrates with the primary cilia, as MST and SAV1 (Figure 1) inhibit the disassembly of primary cilia by MST-mediated phosphorylation of AURKA, an important regulator of the primary cilia disassembly complex, and by association with the NPHP transition-zone complex, thereby inducing an increase in steady-state primary ciliary abundance [153]. A range of diseases caused by dysfunctional primary cilia are termed ciliopathies [156,164] and, intriguingly, TAZ knockout animals develop polycystic kidneys [94] and express lower levels of multiple genes important for primary ciliary morphogenesis;

thus, these mice have malfunctioning cilia and exhibit a phenotype reminiscent of known human ciliopathies [94,164]. By contrast, TAZ depletion in tissue culture cells such as RPE1 cells induce ciliogenesis [155]. It is worth noting that the primary cilia are turned over at each cell cycle and that YAP/TAZ causes cell cycle progression; however, since the primary cilia are present in quiescent cells only, it needs to be fully determined which of these effects are causative or correlative.

The endolysosomal system is also a mediator of the Hippo pathway [165–167]. Through a genetic screen for conditional growth suppressor mutants, Myopic [the *Drosophila* homolog of the endosomal regulator His-domain protein tyrosine phosphatase (HD-PT) also known as PTPN23] was identified as a regulator of Yorkie endosomal localization [168]. A series of Hippo interactome studies also link endosomal compartments to the Hippo pathway [165–167]. Yorkie (the fly YAP/TAZ homolog, see Box 1) is recruited onto endolysosomes via Leash, a member of the arrestin family, which leads to degradation and inhibition of Yorkie nuclear activity [165]. Overexpression of Warts induces Yorkie-containing vesicular structures, suggesting that phosphorylation of Yorkie facilitates its vesicular localization [165]. Although it remains unclear whether similar regulation of YAP and TAZ occurs in mammalian systems, a role for the autophagosome/lysosome system in YAP protein stability in mice has been observed [169]. YAP accumulates in the kidney of tuberous sclerosis complex 1 (TSC1)-deficient mice due to impaired autophagy and thereby promotes abnormal cell proliferation and polycystosis, reminiscent of human perivascular epithelioid cell tumors from TSC patients. How lysosomal regulation of YAP can be reconciled with previous reports [170] that indicate proteasomal degradation of YAP is currently unclear. However, these findings suggest an intriguing new mode of regulation, as this organelle structure has been shown to not only downregulate surface receptors but also to dynamically respond to intra- and extracellular stimuli [171,172] and serve as an integrator for diverse cellular signaling events [124,172].

With the realization that multiple signaling pathways converge and integrate the Hippo pathway, it will be important to further delineate the interactions and dynamic spatiotemporal reciprocal regulation of the Hippo pathway to and from cellular signaling hubs such as the primary cilia and the endolysosomal system to fully understand the contextual signaling mediated by YAP and TAZ and the complete Hippo pathway.

Concluding remarks

The Hippo pathway extends beyond a simple kinase cascade targeting the transcription module and several proteins have been discovered to either completely or partially bypass the core kinase module to regulate YAP and TAZ. Studies in both *Drosophila* and mammals have broadened the Hippo pathway network, which now extends to components of cell–cell junction protein complexes, additional kinases, cytoskeletal regulators, cellular organelles, and, most recently, cellular stress responses (Box 2). Numerous pathways converge on multiple levels with YAP/TAZ and it is being revealed that YAP/TAZ serve as potent manifolds by partnering with seemingly distant signaling molecules for contextual cellular decision making.

Some of the initial *in vivo* Hippo pathway studies in flies [173] and mammals [27,174] highlighted the importance of this pathway in organ growth control as well as its therapeutic potential in regeneration and in tumorigenesis. These studies laid the foundation for the immense interest in and research into this pathway. Many important questions remain to be answered (Box 3). Recent findings showing that increasing YAP activity in various organs can reprogram fully differentiated cells into an undifferentiated state *in vivo* [77,109,129] clearly indicate a powerful therapeutic capacity in tissue regeneration and repair. Inhibiting the upstream Hippo kinase module should be a prime target in regenerative medicine [100] and research in cellular plasticity should be explored in the future. A clear challenge for this therapeutic strategy will be to fine-tune the activation state of YAP/TAZ, recollecting the interdependence between cancer and regeneration [4,6,100].

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Box 1. Evolutionary divergence of the Hippo pathway

The general structure of the Hippo pathway was drafted by studies conducted in *Drosophila melanogaster* (reviewed in [1]) and a wealth of studies in both flies and mammals since then has considerably expanded the realization of the Hippo pathway network as an evolutionarily conserved growth- and cell fate-controlling integrator. The core architecture of the Hippo pathway is highly conserved in evolution and homologous components are found in most major animal phyla, predating the origin of the Metazoa [175,176].

Multiple upstream branches of the Hippo pathway also regulate the core components. In *D. melanogaster*, Tao [thousand and one amino acid protein kinases (TAOKs) in mammals] phosphorylates Hippo (the *Drosophila* homolog of MST1/2) to activate its kinase activity [177,178], Par-1 [MAP/microtubule affinity-regulating kinases (MARKs) in mammals] phosphorylates Hippo to restrict its activity [179], Sik [salt-inducible kinases (SIKs) in mammals] phosphorylates Salvador (the *Drosophila* homolog of SAV1) to inhibit its association with the core kinase module [180], and RAS association domain-containing family protein (RASSF) competes with Salvador for Hippo and recruits a *Drosophila* PP2A phosphatase complex (dSTRIPAK) to dephosphorylate and inactivate Hippo [181,182]. However, except for TAOK [175,176], the functions of these upstream regulators do not appear to be conserved in mammals: MARK acts downstream of liver kinase B1 (LKB1) to activate the core kinase module through SCRIB [183]; the phosphorylation site in Salvador by Sik is not conserved in human SAV1 [180]; and multiple RASSF isoforms in mammals have different roles in the mammalian Hippo pathway [184,185]. Also, while YAP and TAZ are targeted for degradation in mammals, the phosphodegron responsible for this degradation is not conserved in Yorkie [170]. A plausible explanation for this divergence is an evolutionary shift at the base of the arthropod lineage [186]. In this transition, fly Fat gained novel domains that are required for restricting Dachshous to regulate the levels of Warts (the *Drosophila* homolog of LATS1/2) [186]. Unlike fly Fat, mouse Fat4 does not appear to regulate the Hippo pathway in murine liver [186]. Nevertheless, an additional mechanism linking mouse Fat4 to Yap is possible because a genetic interaction between Fat4 and Yap in mouse embryonic neuroepithelium has been reported [187]. In mammals, CRBs (crumbs family members) polarity complex forms a complex with AMOT [104], which binds and sequesters YAP and TAZ through its PPxY motif [53–55]. AMOT is likely to have been present in the last common ancestor of all bilaterian animals but was lost during arthropod evolution [186]. By contrast, the fly Crumbs-binding protein Expanded [188–190] gained a novel C-terminal domain that contains a PPxY motif and is thus able to interact with Yorkie [186,191,192]. The Crumbs complex thereby exchanges AMOT for Expanded at the base of the arthropod lineage. Expanded also regulates the Hippo pathway by forming a complex with Merlin (NF2 in mammals) and Kibra in flies [48,193]. Therefore, although groundbreaking discoveries continue to be pioneered in the fly [32,144], fundamental differences exist in the regulatory mechanisms of core Hippo components between flies and mammals.

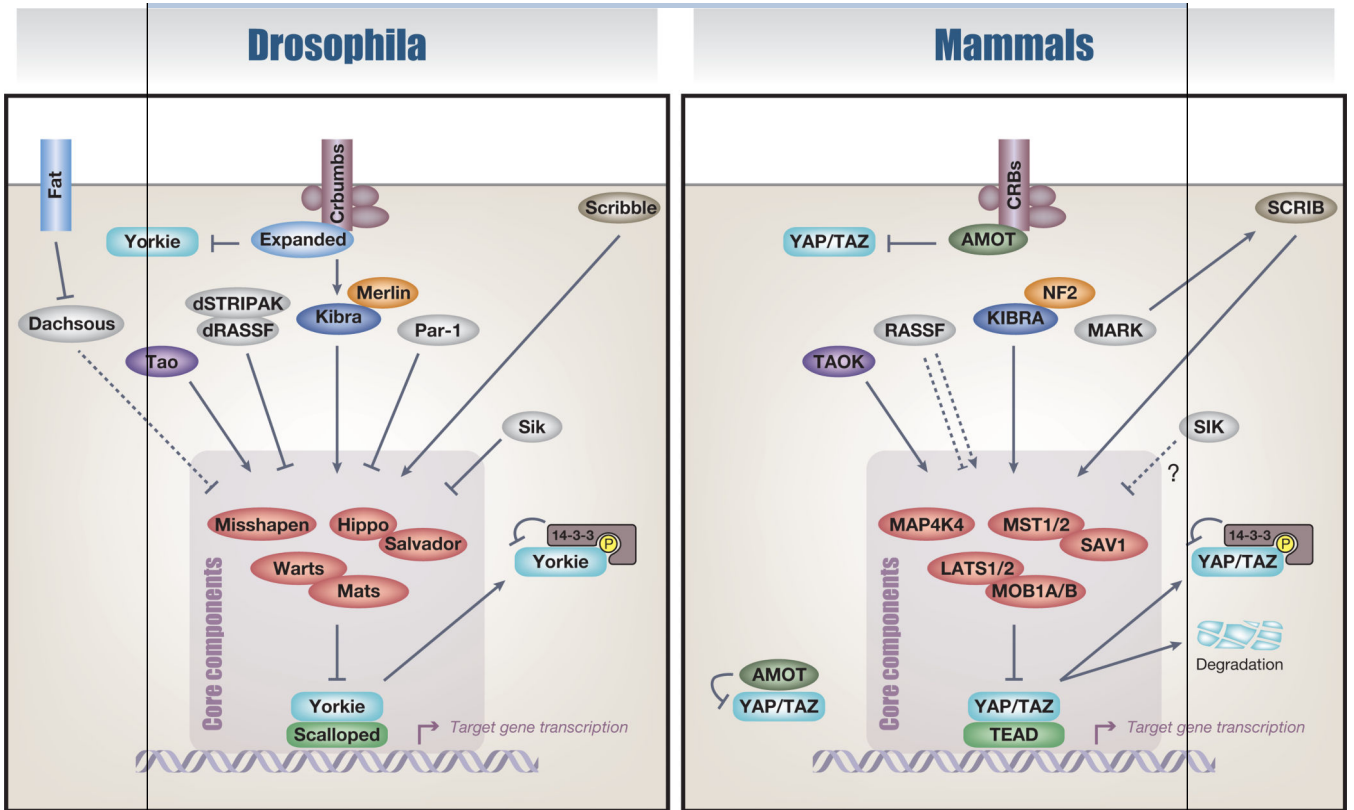
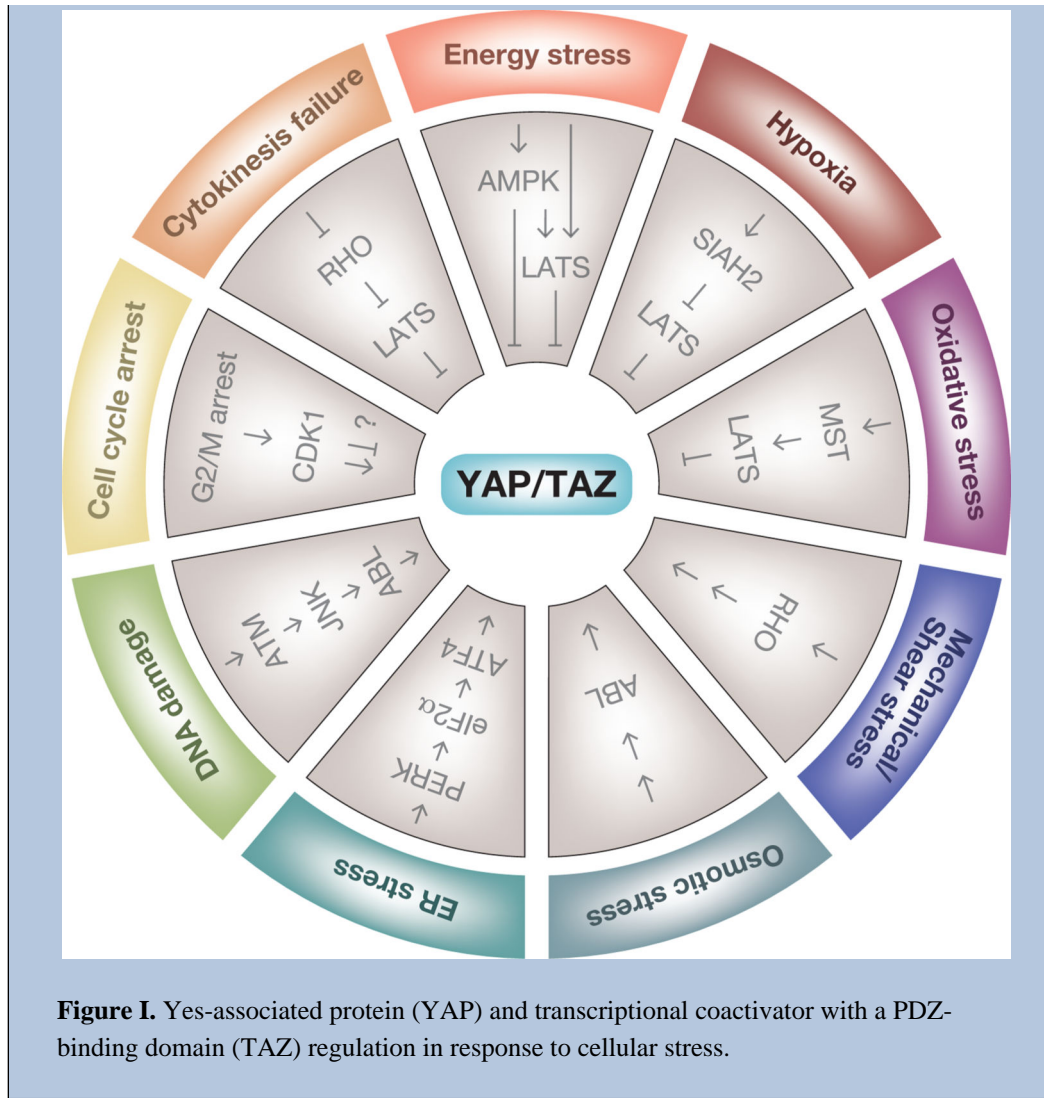


Figure I. Evolutionary divergence of the Hippo pathway.

Box 2. YAP and TAZ regulation in response to cellular stress

Cells are constantly subjected to external and internal stresses that endanger their integrity. YAP and TAZ are regulated in response to a range of these cellular stresses. Cytokinesis failure and extra centrosomes alter small G protein signaling and thereby activate LATS2 kinase. LATS2 in turn inhibits YAP and TAZ and stabilizes p53, thus arresting cells at G1 phase to prevent tumorigenesis [143]. Treatment of cancer cells with antitubulin drugs exerts a mitotic block, which activates cyclin-dependent kinase 1 (CDK1) [194]. CDK1 directly phosphorylates and inhibits YAP function to induce apoptosis. However, the role of CDK1-mediated YAP phosphorylation is controversial as mitotic phosphorylation of YAP by CDK1 promotes mitotic defects and potentiates oncogenic functions of YAP [195]. DNA damage activates the tyrosine kinase ABL through ATM–JNK signaling, which in turn phosphorylates YAP to stimulate proapoptotic functions of YAP in complex with p73 [196]. Endoplasmic reticulum (ER) stress has also recently been implicated in YAP regulation. The ER stress transducer PKR-like ER kinase (PERK) phosphorylates eukaryotic initiation factor 2 α (eIF2 α) during the adaptive stage of the unfolded protein response (UPR), which suppresses general protein synthesis and specifically induces the translation of ATF4, which regulates YAP transcription [197]. ER stress thus induces YAP expression through the PERK–eIF2 α –ATF4 axis to prevent cell death during the adaptive stage of the UPR, while prolonged ER stress activates Hippo signaling to inhibit YAP and promote apoptosis [197].

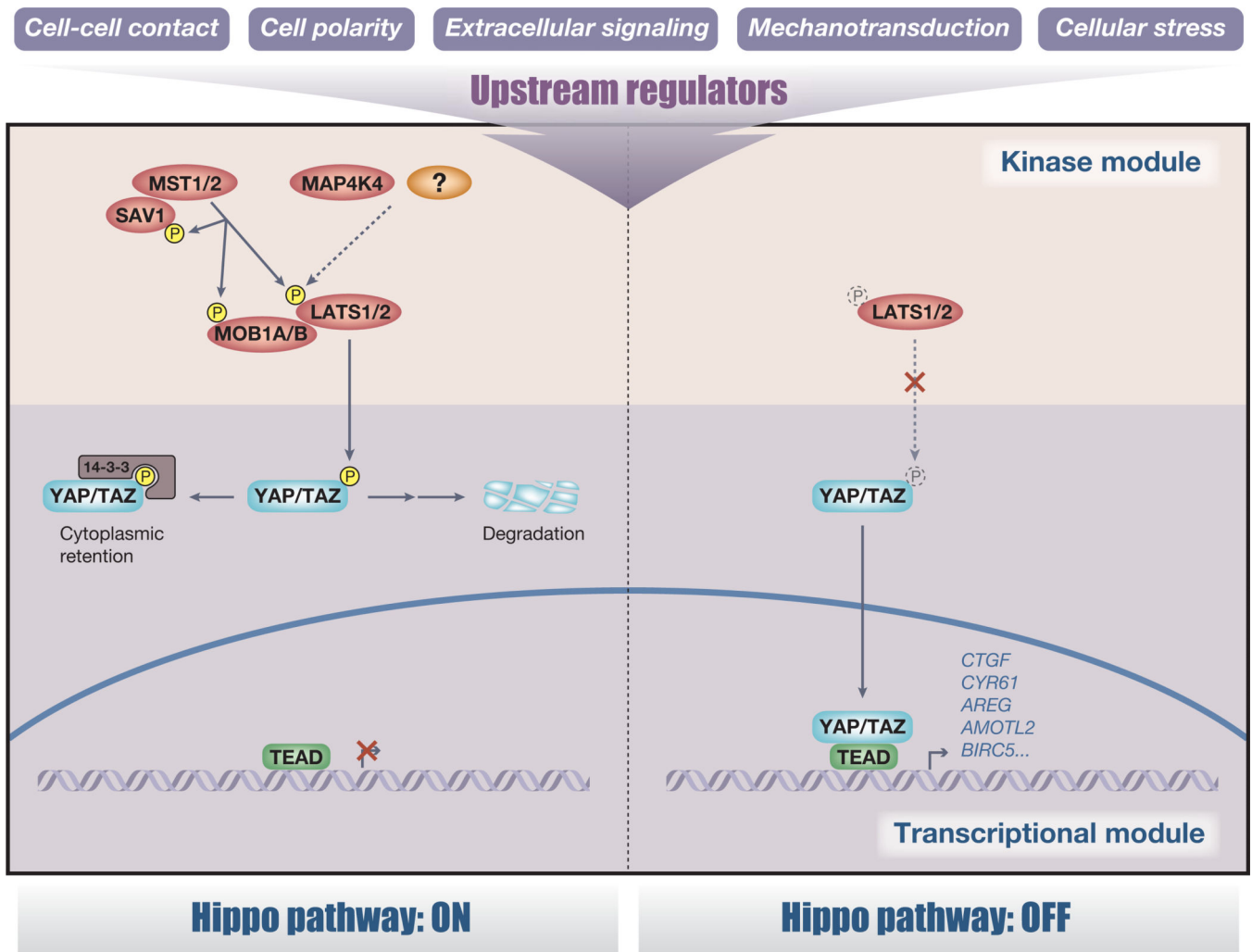
YAP and TAZ likewise respond to external stresses evoked by these cellular microenvironments. Hyperosmotic stress induces tyrosine phosphorylation of TAZ by the ABL kinase, which facilitates the interaction between TAZ and nuclear factor of activated T cells 5 (NFAT5) to inhibit NFAT5 function in osmoregulatory transcription [198]. YAP/TAZ are activated by interstitial flow-driven shear stress and promote osteogenic differentiation of mesenchymal stem cells [147,199]. Rho GTPase appears to be involved in this regulation, although the precise mechanism remains unknown [148]. Oxidative stress evoked by ischemia and reperfusion in the mouse heart activates the Hippo pathway to antagonize a functional YAP–FOXO1 complex, leading to enhanced oxidative stress-induced cell death [200]. By contrast, hypoxia deactivates the Hippo pathway by destabilizing LATS2 through SIAH2 ubiquitin ligase-induced degradation [33]. Energy stress, such as inhibition of glucose metabolism and ATP production, induces AMP-activated protein kinase (AMPK)-mediated phosphorylation of Angiomotin-like 1 (AMOTL1) to stabilize and increase AMOTL1, which in turn stimulates LATS [35]. Furthermore, LATS also senses and is activated by glucose starvation in an AMPK-independent manner [201]. In addition, AMPK inhibits YAP by directly phosphorylating it at least two distinct sites [201,202]; therefore, energy stress inhibits YAP nuclear activity by several mechanisms [35,201,202].



Box 3. Outstanding questions

- How is LATS activated in different contexts?
- How does the temporal and spatial organization and regulation of the Hippo pathway occur?
- Does the Hippo pathway regulate additional effectors besides YAP and TAZ?
- How do YAP and TAZ differ and how are their nuclear/cytoplasmic functions regulated?
- What underlies the context-dependent physiological roles and activities of YAP and TAZ?
- How can the Hippo pathway be therapeutically utilized in regeneration and cancer?

- YAP and TAZ are major downstream effectors of the Hippo pathway.
- YAP and TAZ function as signaling manifolds in the nucleus and the cytoplasm.
- The Hippo pathway interacts on multiple levels with many other signaling pathways and organelles.
- The Hippo network is a potent regulator of tissue regeneration and stem cell biology.

**Figure 1.**

The core of the Hippo pathway. The core components of the Hippo pathway comprise a regulatory serine–threonine kinase module and a transcriptional module. In mammals, this kinase module comprises two groups of kinases: mammalian STE20-like protein kinase 1 (MST1) (also known as STK4) and MST2 (also known as STK3) and large tumor suppressor 1 (LATS1) and LATS2 in combination with their activating adaptor proteins Salvador family WW domain-containing protein 1 (SAV1) and MOB kinase activator 1A/B (MOB1A/B), respectively. MAP4K4 has recently been identified as an alternative activator of LATS independent of MST. The transcriptional module comprises the transcriptional coactivators Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ) and several transcription factors, of which the most prominently studied are TEA domain family members 1–4 (TEAD1–4). In a classical view, when the upstream kinase module is activated, MST1/2 phosphorylates SAV1, MOB1A/B, and LATS1/2 to activate the LATS1/2 kinases, which in turn directly phosphorylate YAP and TAZ on multiple serine residues leading to cytoplasmic retention of YAP/TAZ via a 14-3-3 interaction. Furthermore, the phosphorylation of YAP/TAZ by LATS1/2 primes YAP/TAZ for proteasomal degradation. The requirement for MST1/2 to phosphorylate LATS1/2 is

cell-type dependent, suggesting the existence of additional kinases (drawn as a question mark) that regulate the activating phosphorylation of LATS1/2. By contrast, when the kinase module is inactivated, hypophosphorylated YAP/TAZ translocate into the nucleus and induce target gene expression.

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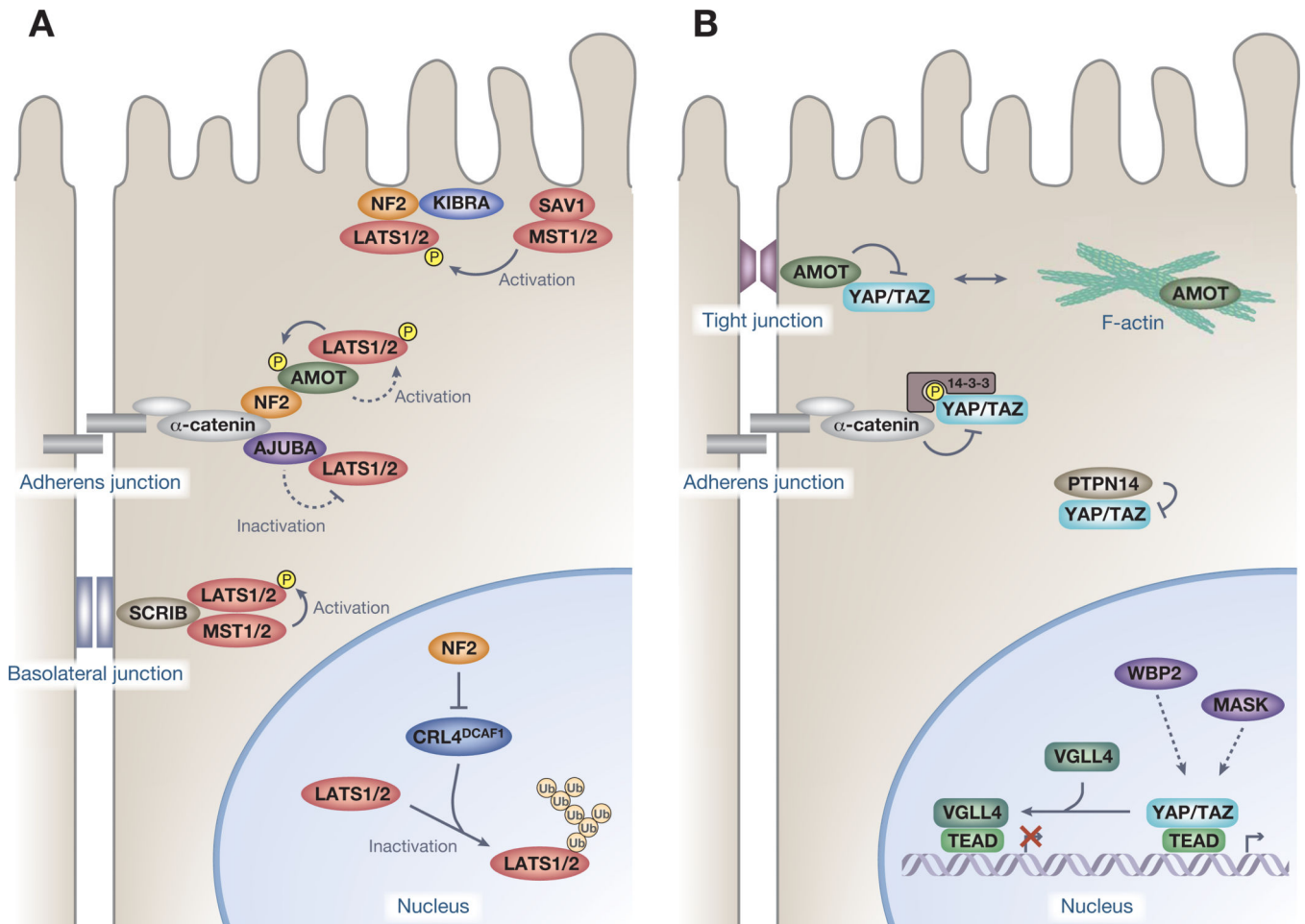


Figure 2.

Regulators of Yes-associated protein (YAP)/transcriptional coactivator with a PDZ-binding domain (TAZ) activities. **(A)** Spatial organization and regulation of the kinase module. Neurofibromatosis 2 (NF2) (also known as Merlin) activates large tumor suppressor (LATS) kinases at multiple levels. NF2 recruits LATS at the plasma membrane to coordinate the activating phosphorylation by the mammalian STE20-like protein kinase (MST)–Salvador family WW domain-containing protein 1 (SAV1) kinase complex. NF2 directly interacts with α-catenin and Angiomotin (AMOT) to recruit LATS kinases at the adherens junction, which in turn activate LATS. NF2 inhibits the E3 ubiquitin ligase CRL4^{DCAF1}-mediated ubiquitylation of LATS1/2 in the nucleus to inhibit proteasomal degradation of LATS1 and ubiquitylation-induced conformational change of LATS2. Scribble (SCRIB) recruits the core kinases MST and LATS to facilitate LATS activation at the basolateral junction. LATS recruitment by AJUBA at adherens junctions inhibits LATS kinase activity. **(B)** Physical regulation of the transcription module. AMOT, protein tyrosine phosphatase 14 (PTPN14), and α-catenin inhibit the activity of the transcription module by complexing YAP and TAZ, thus sequestering and preventing their nuclear access. AMOT interacts with and thereby causes cytoplasmic sequestration of YAP and TAZ by recruiting them to tight junctions. PTPN14 also causes cytoplasmic localization of YAP and decreased nuclear YAP activity. The adherens junctions protein α-catenin also regulates YAP by sequestering YAP–14-3-3

protein complexes in the cytoplasm. WW domain-binding protein 2 (WBP2) and multiple ankyrin repeats single KH domain-containing protein (MASK) interact with nuclear YAP and TAZ, which enhances YAP and TAZ transcriptional coactivator properties, but the precise mechanism remains unknown. Vestigial-like family member 4 (VGLL4) represses target gene expression by competing with YAP-TEA domain family (TEAD) interactions.

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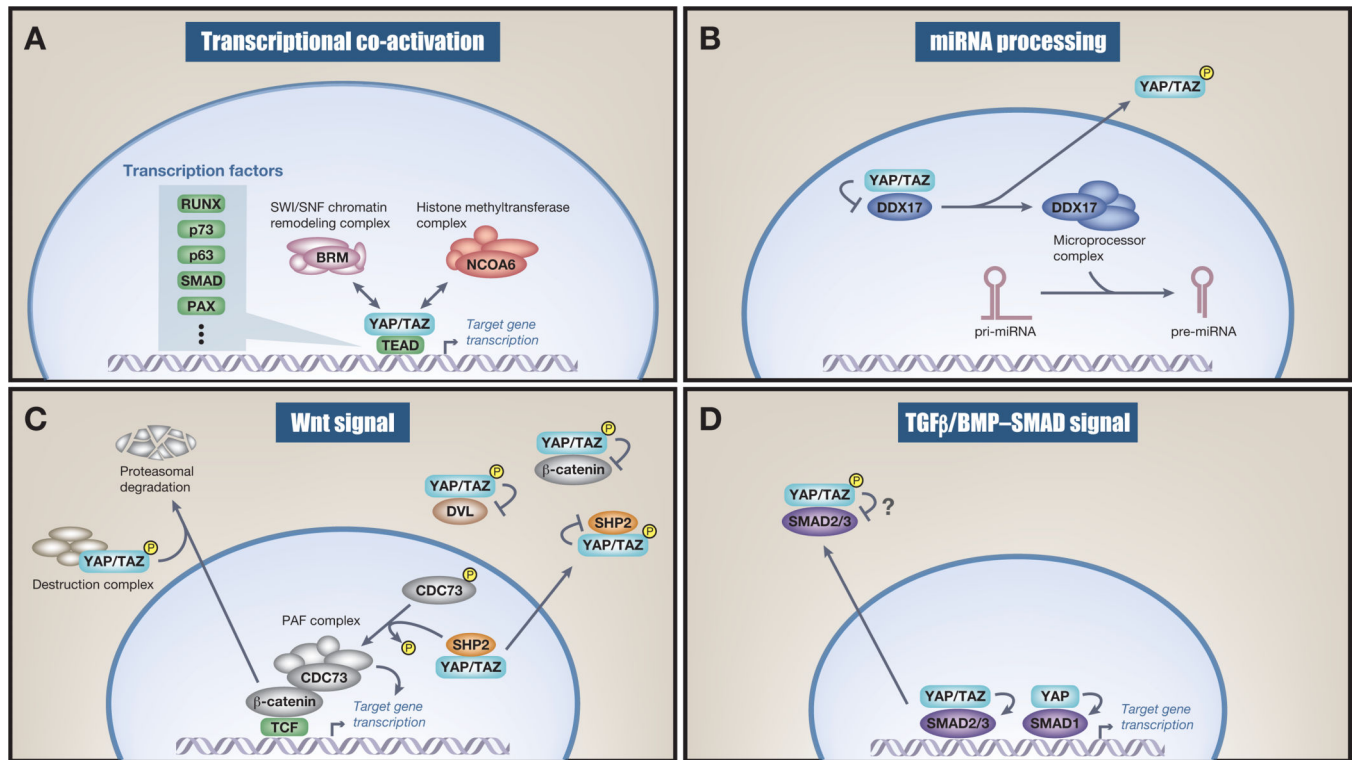


Figure 3.

Function of nuclear and cytoplasmic Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ). **(A)** Transcriptional coactivation. Nuclear YAP and TAZ bind to transcription factors (TFs) and recruit the NCOA6 methyltransferase complex as well as the BRM-containing SWI/SNF chromatin-remodeling complex, which mediates TF target-specific gene transcription. **(B)** miRNA processing. Nuclear YAP inhibits DDX17, a regulatory component of the microprocessor complex. This nuclear inhibition decreases global miRNA on YAP stimulation. Of note, some specific miRNAs are upregulated on YAP nuclear activation. **(C)** Wnt signaling. Cytoplasmic YAP and TAZ sequester β -catenin and in some instances recruit β -TrCP to the destruction complex. This leads to nuclear β -catenin downregulation and therefore dampens Wnt signaling. Cytoplasmic YAP and TAZ also negatively regulate Wnt signaling by sequestering β -catenin and disheveled (DVL). YAP and TAZ dictate nuclear and cytoplasmic localization of SHP2 and therefore its function. This feeds into the Wnt signaling pathway because nuclear SHP2 dephosphorylates parafibromin (CDC73), which leads to increased β -catenin transcriptional activity. **(D)** Transforming growth factor beta (TGF β)/BMP-SMAD signal. Cytoplasmic YAP and TAZ sequester SMAD2/3 in the cytoplasm and therefore inhibit SMAD2/3-mediated signaling, although these mechanisms seem not to be universal and somewhat controversial. Nuclear YAP activates SMAD1-mediated transcription.

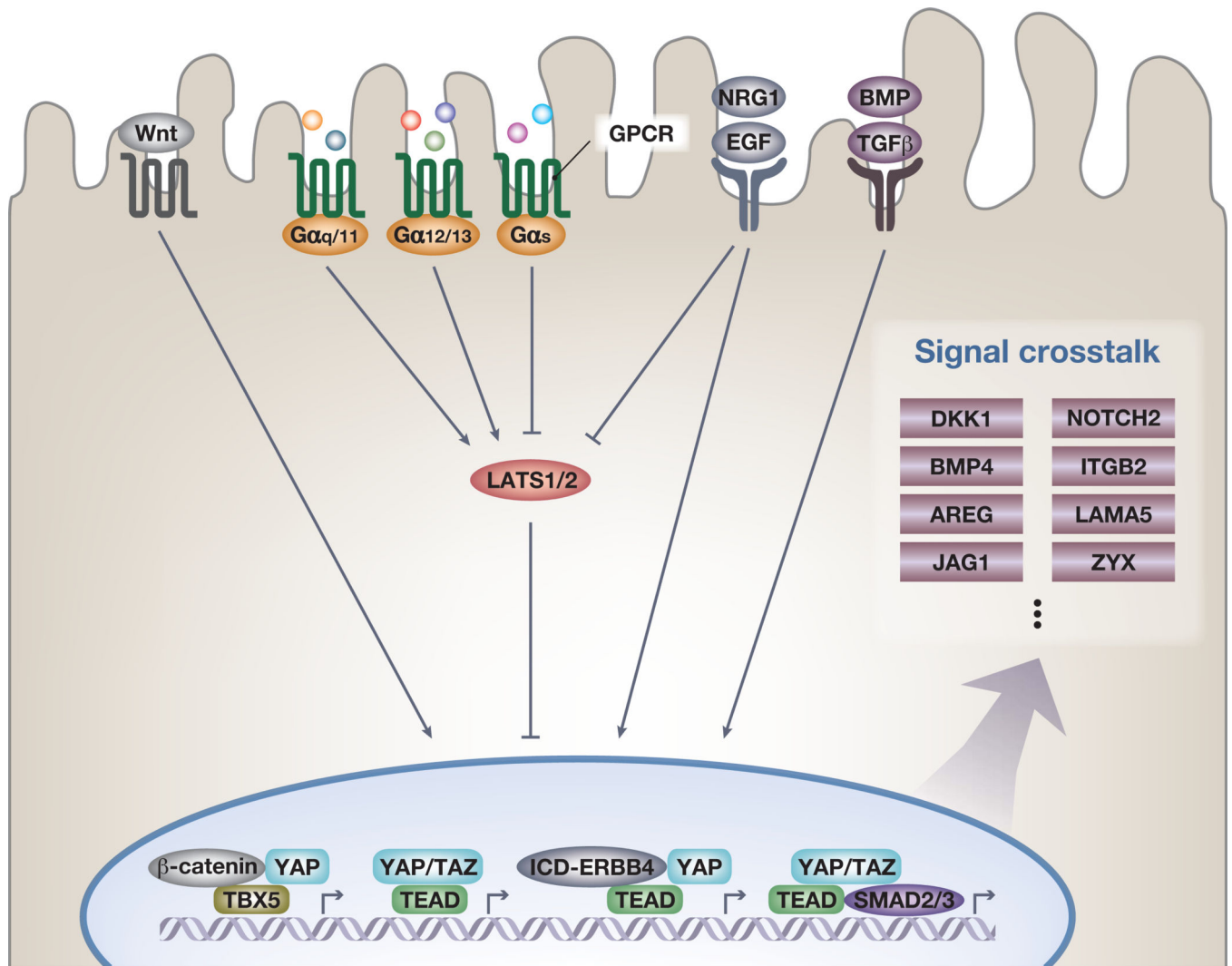


Figure 4.

Signal crosstalk with Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ). Wnt signaling activates YAP and TAZ by inhibiting the formation of the β -catenin destruction complex that in some instances functions as a cytoplasmic sink and causes degradation of YAP and TAZ. Nuclear YAP can form a complex with β -catenin and the transcription factor TBX5 to promote antiapoptotic gene expression, including BCL2L1 and BIRC5. G protein-coupled receptor (GPCR) signaling is relayed via small G proteins. Activation of $G_{\alpha_{12/13}}$ and $G_{\alpha_{q/11}}$ induces YAP and TAZ nuclear translocation and activity, whereas active G_{α_s} represses YAP and TAZ nuclear activity, which causes either activation or inhibition of large tumor suppressor (LATS) kinase activity. The family of epidermal growth factor (EGF) ligands activates YAP and TAZ in both a LATS-dependent and -independent manner. EGF receptor (EGFR) stimulation inhibits the kinase module by dissociating phosphoinositide-dependent kinase-1 (PDK1) from the Hippo pathway components [LATS, mammalian STE20-like protein kinase 1 (MST), and Salvador family WW domain-containing protein 1 (SAV1)], preventing activation of LATS by MST. EGFR–Ras–MAPK signaling also enhances the AJUBA

family protein Wilms' tumor protein 1-interacting protein (WTIP) binding to LATS, which inhibits LATS activity toward YAP. In addition, a cytoplasmic intracellular domain of the ERBB4 receptor (ICD-ERBB4) is cleaved and released on neuregulin 1 (NRG1) stimulation, which in turn binds to YAP and promotes target gene expression. On transforming growth factor beta (TGF β) signal activation, nuclear YAP and TAZ form a complex with SMAD2, SMAD3, and TEA domain family (TEAD) transcription factors, coordinating a protumorigenic transcriptional program. YAP and TAZ also directly promote transcription of several receptors and ligands to mediate endocrine and paracrine signal crosstalk and potential feedback loops. Notable examples are the Wnt signal inhibitor DKK1, ligands of the BMP/TGF β family BMP4, the EGF-like growth factor AREG, NOTCH2, the Notch ligand Jagged1 (JAG1), integrin/extracellular matrix (ECM) signaling components such as β 2-integrin (ITGB2) and the α 5 subunit of laminin 511 (LAMA5), and the focal adhesion component zyxin (ZYG).