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The Hippo Pathway: Biology and Pathophysiology

Shenghong Ma, Zhipeng Meng, Rui Chen, and Kun-Liang Guan

Department of Pharmacology and Moores Cancer Center, University of California, San Diego, La Jolla, California 92093, USA; email: shm049@ucsd.edu, z4meng@ucsd.edu, ruc046@ucsd.edu, kuguan@ucsd.edu

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Abstract

The Hippo pathway was initially discovered in *Drosophila melanogaster* as a key regulator of tissue growth. It is an evolutionarily conserved signaling cascade regulating numerous biological processes, including cell growth and fate decision, organ size control, and regeneration. The core of the Hippo pathway in mammals consists of a kinase cascade, MST1/2 and LATS1/2, as well as downstream effectors, transcriptional coactivators YAP and TAZ. These core components of the Hippo pathway control transcriptional programs involved in cell proliferation, survival, mobility, stemness, and differentiation. The Hippo pathway is tightly regulated by both intrinsic and extrinsic signals, such as mechanical force, cell–cell contact, polarity, energy status, stress, and many diffusible hormonal factors, the majority of which act through G protein–coupled receptors. Here, we review the current understanding of molecular mechanisms by which signals regulate the Hippo pathway with an emphasis on mechanotransduction and the effects of this pathway on basic biology and human diseases.

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INTRODUCTION

Control of organ size in multicellular organisms is an evolutionary milestone and a central question in biology. Despite the extensive interest, our understanding of how organ size is determined during development and maintained in adulthood is still incomplete. The discovery of the Hippo pathway in organ size control has shed light on this mystery (1, 2). Early ingenious genetic studies of the Hippo pathway suggested its role in organ size control and have served to pioneer our understanding of organ size control mechanisms. Decades of research have established a critical role for the Hippo pathway in regulating many fundamental biological processes, from individual cell fate decisions to tissue architecture.

Unlike many conventional signaling pathways that involve dedicated ligand–receptor pairing, the Hippo pathway is influenced by a diverse array of biochemical, physical, and architectural signals, including mechanical cues, cell polarity, cell–cell adhesion, hormones, bioactive chemicals, and cellular stress. The Hippo pathway integrates a broad range of signals to control many key cellular processes. In this review, we discuss our current understanding of the Hippo pathway regulation, including its role in physiological control and dysregulation in human diseases.

THE CORE OF THE HIPPO PATHWAY

The Hippo pathway was first discovered in *Drosophila melanogaster* through genetic mosaic screens designed to identify gene mutations that cause clonal overgrowth phenotypes. This led to the discovery of the Hippo pathway core components, including the NDR family protein kinase Warts (Wts), the WW domain–containing protein Salvador (Sav), the Ste20-like protein kinase Hippo (Hpo), and the adaptor protein Mob as tumor suppressor (Mats) (1, 2). These proteins were grouped into one signaling module—the Hippo pathway—named after *hpo* mutants that

display phenotypes of enormously sized organs and outwardly resemble a hippopotamus. Mutations in these components phenocopy each other in regard to tissue overgrowth of the eye, wing, and limbs. Biochemically, these four tumor suppressors form a kinase cascade, with the Hpo–Sav complex phosphorylating and activating the Wts–Mats complex.

In search for downstream effectors of the Hippo kinase cascade, a transcriptional coactivator named Yorkie (Yki) was identified as an interactor of Wts that controls both cell proliferation and survival (3). Yki functions opposite to Wts, as its overexpression phenocopies loss of the Hippo signaling, whereas inactivation of Yki blocks tissue overgrowth caused by the Hippo pathway inactivation. Yki represents the major functional output of the Hippo pathway and is inhibited by the Hippo kinase cascade.

The Hippo pathway is highly conserved from *Drosophila* to mammals. The mammalian Hpo orthologs MST1/2 belong to the group II germinal center kinases. MST1/2 form heterodimers with SAV1 (Sav ortholog) through their C-terminal SARAH (sav/Rassf/Hpo) domains, and this interaction is required for MST1/2 to phosphorylate SAV1, MOB1 (Mats ortholog), and LATS1/2 kinase (Wts ortholog) (4–6). LATS1/2 directly phosphorylate the Yki orthologs YAP (yes-associated protein) and TAZ (WW domain–containing transcription regulator protein 1) at multiple sites, thereby inhibiting their nuclear localization (3, 7). Mechanistically, phosphorylated YAP/TAZ bind to 14–3–3 and are sequestered in the cytoplasm, resulting in YAP/TAZ inhibition. Further phosphorylation of YAP/TAZ by casein kinase 1 leads to β-TrCP–mediated ubiquitination and proteasomal degradation (7–9) (**Figure 1**).

In parallel to MST1/2, additional kinases, including MAP4K family proteins MAP4K1/2/3/5 [ortholog of *Drosophila* Happyhour (Hppy)], MAP4K4/6/7 [ortholog of *Drosophila* Misshapen (Msn)], and TAO kinases (TAOK1/2/3), can also directly phosphorylate LATS1/2 at their hydrophobic motifs and result in LATS1/2 activation (10–14). Notably, the TAOK kinase can also phosphorylate and activate MST1/2 (15). Although both Hppy and Msn can activate Wts in *Drosophila*, Hpo is likely to be the major Wts regulator because Hpo mutations produce the strongest phenotypes. A comprehensive gene inactivation analysis showed that LATS1/2 and their binding partner MOB1A/B are indispensable for upstream signal–triggered YAP/TAZ phosphorylation and inactivation, whereas deleting MST1/2, MAP4Ks, or TAOK1/2/3 alone only partially blocks YAP/TAZ regulation by upstream signals in mammalian cells. However, combined deletion of MSTs/MAP4Ks/TAOKs severely abolishes YAP/TAZ phosphorylation, suggesting a redundant role for these kinases acting upstream of LATS1/2 in the Hippo pathway (13, 14). The relative contribution of these kinases to LATS activation is likely dependent on both cell types and upstream signals.

YAP and TAZ are transcriptional coregulators that do not contain DNA-binding domains. The primary binding partners of YAP/TAZ are TEAD family transcription factors [ortholog of *Drosophila* Scaloped (Sd)] (16–18). Once activated, the Hippo pathway limits tissue growth and cell proliferation by phosphorylating and inhibiting YAP/TAZ. In contrast, when the Hippo pathway is off, YAP/TAZ are dephosphorylated and translocated into the nucleus, where they bind to TEAD to induce transcriptional programs important for cell proliferation, survival, and migration. In the absence of nuclear YAP/TAZ, TEAD functions as a default repressor by binding to Vg domain–containing protein VGLL4 (transcription cofactor vestigial-like protein 4, ortholog of *Drosophila* Tgi), thus repressing target genes expression (19, 20).

It should be noted that Hippo pathway regulation is not static in either ON or OFF status, but rather it is dynamic. YAP/TAZ is under constant and rapid phosphorylation and dephosphorylation although much less is known about the regulation of the responsible phosphatases. Recent studies in both *Drosophila* and mammalian cells using living cell tracing have shown that YAP/Yki is rapidly trafficking between the cytoplasm and nucleus (21, 22). Alteration in Hippo

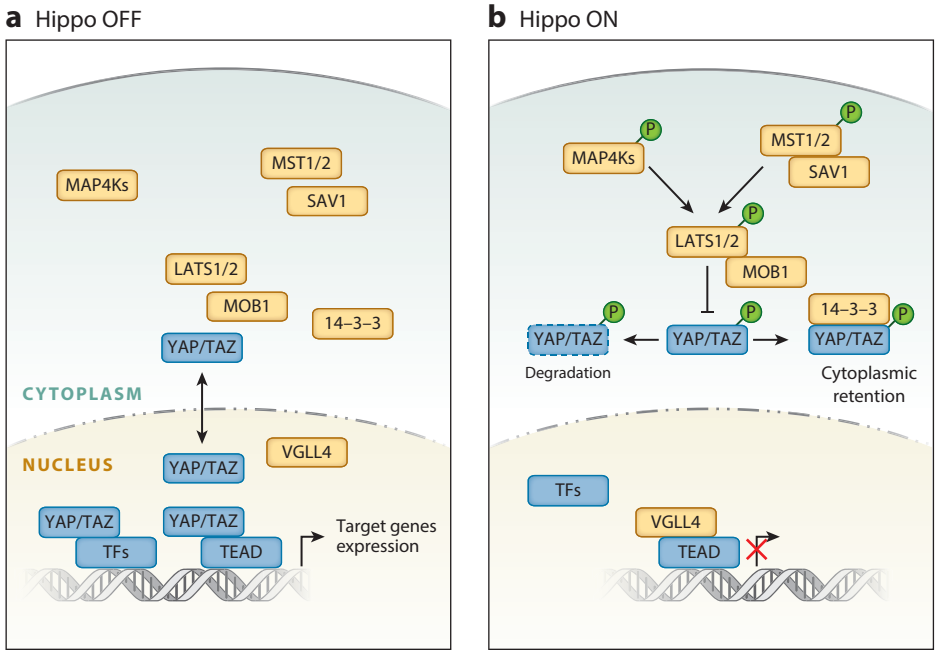

Figure 1

Core components of the Hippo pathway in mammalian cells. The Hippo pathway activity controls the dynamic localization of YAP/TAZ between nucleus and cytoplasm. (*a*) When the Hippo pathway is OFF, YAP/TAZ are dephosphorylated and accumulate in the nucleus, where they bind with TEADs and possibly other transcription factors (TFs) to induce gene transcription. (*b*) When the Hippo pathway is ON, the active LATS kinases phosphorylate YAP/TAZ, resulting in their binding to 14–3–3 and cytoplasmic retention as well as degradation. However, the Hippo pathway does not behave digitally only in ON or OFF status. YAP localization can be partially cytoplasmic and partially nuclear depending on the relative activities of LATS kinase and the opposing phosphatase for YAP. The VGLL4 competes with YAP/TAZ in binding to TEAD and represses the target gene expression.

signaling influences YAP phosphorylation, hence the relative rates of YAP influx into and efflux from the nucleus (**Figure 1**). Such dynamic YAP shuttling can also be achieved by mechanical signal–sensitive nuclear pore complexes (NPCs) mediating nuclear import and export (23). Moreover, the dynamic nature of Hippo regulation is reflected by the fact that each individual signal does not necessary cause a complete ON or OFF switch of Hippo signaling. Furthermore, the pathway has to constantly respond to and integrate a wide range of both positive and negative inputs.

YAP/TAZ REGULATION BY THE HIPPO PATHWAY AND BEYOND

Genetic studies have established YAP/TAZ as the key and major effectors of the Hippo pathway. Deletion of YAP in mice suppressed the overgrowth phenotypes caused by deficiency of MST1/2, NF2 (an important regulator of the Hippo pathway), or other Hippo components (24–26). The TEAD family of transcription factors are the best-characterized binding partners of YAP/TAZ and are comprised of TEAD1–4. Knock-in of a TEAD binding-deficient Yap (S79A) in mice phenocopied the YAP knockout mice (27). The TEAD binding-deficient YAP lost the ability to induce transcription of YAP target genes. Moreover, knockdown of TEAD strongly reduced the

YAP target genes expression (17). Interestingly, a fusion protein of the N-terminal DNA-binding domain of TEAD with VP16, an activation domain from herpes simplex virus, mimicked YAP-activated target gene expression, cell proliferation, and transformation potential (28). Conversely, an alternatively spliced isoform of TEAD, TEAD4-S, which lacks the N-terminal DNA-binding domain but retains the YAP/TAZ interacting domain, acted as a dominant negative, suppressing YAP–TEAD-induced transcriptional activation and tumor formation (29). Deletion of TEAD led to cytoplasmic localization of YAP/TAZ even when they are not phosphorylated. Chromatin immunoprecipitation sequencing experiments showed that genome-wide chromatin binding peaks of YAP/TAZ highly overlap with TEAD binding sites (30–32). However, these YAP/TAZ binding sites represent only a fraction of the TEAD binding sites, raising the question of whether YAP/TAZ choose between different TEAD binding sites as an additional layer of regulation. Collectively, these observations support TEAD as the major nuclear YAP/TAZ binding partner (33).

Considering the role of the Hippo pathway in modulating a wide range of biological functions in multicellular organisms, as shown by several independent gene expression profiling studies performed in *Drosophila*, and the large number of genes affected by YAP/TAZ in each given cell type in mammals, it is surprising that only a small number of genes are commonly regulated by the Hippo pathway among different cell types (17, 30, 34, 35). This suggests that YAP/TAZ transcriptional programs are precisely controlled in a cell context–specific manner. Consistently, other transcription factors, such as AP1, coexist with TEAD binding sites in many YAP/TAZ target genes and collaborate with TEAD in gene regulation (30–32, 36). Furthermore, YAP/TAZ activity is mediated by different chromatin complexes, including the NCOA6 histone methyltransferase complex or the SWI/SNF chromatin remodeling complex (34, 37, 38). These observations may explain why YAP/TAZ targets are highly cell type dependent.

Besides TEAD1–4, YAP/TAZ are capable of forming complexes with other transcription factors. Earlier studies suggested an interaction between YAP/TAZ with SMAD, RUNX1/2, p63/p73, or OCT4 (2). YAP also interacts with PKNOX1, a transcription factor involved in the maintenance of genomic stability, to modulate retinal stem cell genomic stability, as loss of function of YAP leads to DNA damage resulted from DNA replication stress (39). This interaction is evolutionary conserved, as Homothorax (Hth, a potential *Drosophila* ortholog of PKNOX1) binds with Yki to promote cell proliferation and inhibit apoptosis (40). Cinar and colleagues (41) found that YAP-TEAD associate with androgen receptors (ARs) in an androgen-dependent manner. Consistently, disruption of Hippo signaling suppresses AR-dependent gene expression and prostate cancer cell growth. YAP also interacts with the PRDM4 transcription factor to induce integrin expression and cell invasion (42). Though many transcription factors have been implicated as YAP/TAZ binding partners, genetic data strongly support the TEAD family as the major transcription factors for YAP/TAZ to promote cell and organ growth as well as tumorigenesis (27).

The transcriptional activity of YAP/TAZ is negatively modulated by VGLL4, which was named for the presence of the Vg motif similar to the *Drosophila* protein vestigial (Vg), a key regulator of wing development (43, 44). VGLL4 contains two Vg domains in its C terminus, different from VGLL1–3 (orthologs of Vg) that have only one Vg domain in their N termini. Mechanistically, VGLL4 can directly compete with YAP for binding to TEAD, resulting in inhibition of YAP-TEAD–driven target gene expression and suppression of an overgrowth phenotype (19, 20, 45). It has been suggested that TEAD binds to VGLL4 and functions as a default transcriptional repressor, whereas YAP/TAZ binding relieves VGLL4 repression and promotes target gene expression. In addition, several lines of evidence suggest that modulation of VGLL4 is sufficient to affect YAP-TEAD–driven target gene expression. Pu and colleagues (46) showed

that p300-mediated acetylation of VGLL4 disrupts its interaction with TEAD1 in the neonatal heart, leading to increased YAP–TEAD1 interaction and TEAD1 stability. Conversely, disruption of VGLL4 acetylation unleashes the inhibitory effect of VGLL4 toward TEAD and suppresses YAP–TEAD-mediated heart growth. Furthermore, a peptide mimicking VGLL4 potently suppresses YAP-driven gastric cancer (45), supporting an antagonistic effect of VGLL4 on YAP.

In *Drosophila*, the Yki–Sd complex can be disrupted by RB–E2F, a critical regulator of cell-cycle progression (47). However, the functional role of E2F in mediating YAP/TAZ activity is still under debate, as mammalian E2F can cooperate with YAP/TAZ in both promoter and enhancer regions to stimulate target gene expression (30, 48). In addition, HNF4α competes with YAP for binding to TEAD4 in mouse liver, thus inhibiting the transcriptional activity of YAP–TEAD and the expression of their target genes. In line with these findings, overexpression of HNF4α compromises YAP–TEAD-induced hepatocellular carcinoma (HCC) cell proliferation and stem cell expansion (49).

YAP/TAZ also have transcriptional corepressor function by recruiting the NuRD (nucleosome remodeling deacetylase) complex to deacetylate histones and alter nucleosome occupancy of target genes, such as DDIT4 and Trail (50). Thus, YAP/TAZ can be either transcription coactivators or transcription corepressors, depending on the selection of different binding partners. However, the major and best-characterized function of YAP/TAZ is their transcription coactivator activity.

Activation of YAP/TAZ turns on the expression of several Hippo pathway components, including LATS2, AMOTL2, and NF2, which leads to a compensatory reduction of YAP/TAZ activity (51–53). This suggests that YAP/TAZ activity is limited by a Hippo pathway–dependent negative feedback mechanism, which serves to reduce signal noises and ensure tissue homeostasis.

YAP/TAZ activity can be further modulated through Hippo-independent mechanisms. Several proteins, including α-catenin, PTPN14, AMOT, CDK1, and Claudin18, can directly bind and sequester YAP/TAZ in the cytoplasm (2). NF2, a protein acting as an upstream activator of the Hippo pathway, can also physically interact with YAP/TAZ and suppress their nuclear localization in response to cell circumferential actin belt contraction (54). This effect is distinct from the role of NF2 in regulation of the Hippo kinase cascade, as YAP-5SA, a Hippo-resistant YAP mutant with all five LATS phosphorylation sites mutated, can still respond to circumferential actin belt–NF2-mediated cytoplasmic localization.

Recently, TEAD was discovered to shuttle between the nucleus and the cytoplasm in response to cellular stresses, such as hyperosmolarity, high cell density, and cell detachment (33). Unlike YAP/TAZ, the cytoplasmic/nuclear redistribution of TEAD is independent of the Hippo pathway kinase LATS but is dependent on the p38 MAP kinase. Upon hyperosmotic stress, p38 MAP kinase is activated and binds TEAD to drive its cytoplasmic translocation. The nuclear localization of YAP/TAZ depends on nuclear TEAD. When TEAD is cytoplasmic, even the unphosphorylated YAP/TAZ are cytoplasmic. The precise molecular mechanism of TEAD regulation has yet to be fully elucidated.

In addition to LATS1/2, several kinases have been reported to phosphorylate YAP/TAZ and modulate their activities. The phosphorylation of YAP at S128 by Nemo-like kinase (NLK) increases its nuclear localization and transcriptional activity by interfering with YAP and 14–3–3 binding even when the Hippo pathway is activated (55, 56). Moreover, cell-cycle kinase CDK1 inhibits YAP activity independent of the Hippo pathway by targeting sites on YAP upon anti-tubulin drug treatment (57). More recently, the pre-mRNA splicing factor 4 kinase (PRP4K) has been shown to phosphorylate a subset of LATS sites on YAP in the nucleus, thus excluding YAP nuclear localization (58). Although other kinases or binding proteins may influence YAP/TAZ activity, LATS-dependent phosphorylation is clearly the major mechanism most important for YAP/TAZ regulation.

UPSTREAM SIGNALS

Mechanical Signals

Cells in multicellular organisms need to be properly orchestrated in organ development and tissue homeostasis control. The mechanical forces generated from cell–cell contacts, cell–ECM (extracellular matrix) interaction, and the microenvironment have been recognized to regulate gene transcription and thus coordinate cells during growth, proliferation, morphogenesis, migration, and death (59, 60). However, an understanding of how cells sense mechanical forces and process these physical signals into biochemical signals to modulate cell physiology has not been fully achieved at the molecular level. Accumulating evidence from semisynthetic substrates and model systems suggests that YAP/TAZ are two important mechanoregulated transcriptional effectors, relaying physical cues to gene expression and cellular response (**Figure 2**).

Cell–cell contact and cell shape. Contact inhibition is a phenomenon in which cells stop proliferating when they reach confluency in monolayer culture. High cell density is one of the first signals discovered to regulate the Hippo pathway by elevating LATS1/2 kinase activity, leading to YAP/TAZ phosphorylation and cytoplasmic localization (7). Consistently, YAP displays nuclear in outer cells and cytoplasmic in inner cells during early mouse embryonic development (61). However, the mechanism by which cell confluency activates LATS1/2 is only partially understood. One potential mechanism is that the angiomotin (AMOT) complex at the tight junction (TJ) mediates

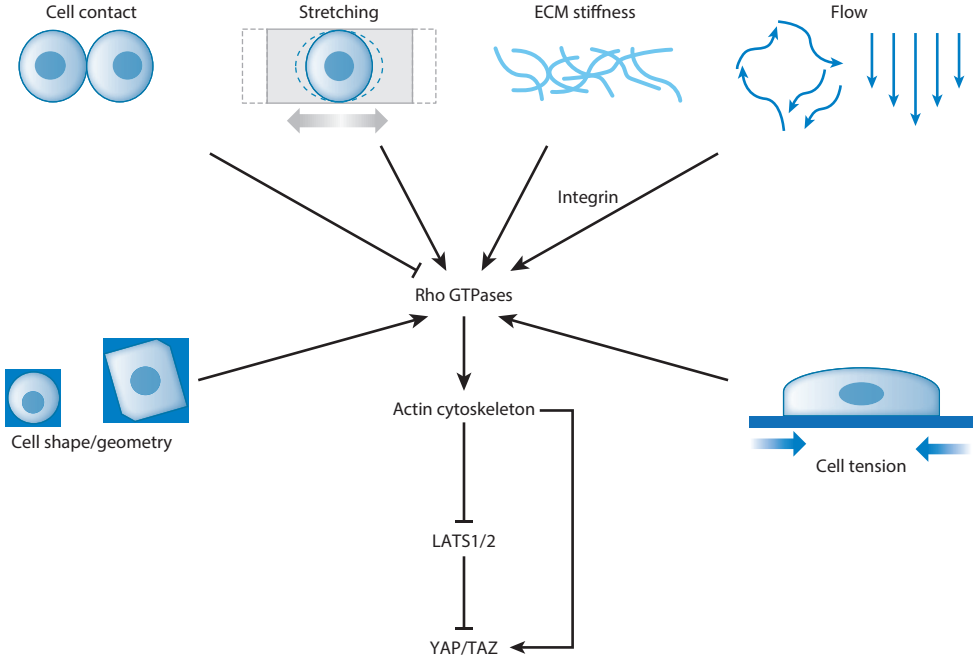

Figure 2

Mechanical cues regulate YAP/TAZ activities through Rho GTPase and the Hippo pathway. Cell–cell contact, cell stretching, cell tension, extracellular matrix (ECM) stiffness, and changes in cell geometry modulate activities of Rho GTPases, which in turn lead to actin cytoskeleton remodeling. The actin cytoskeleton can control nuclear–cytoplasmic shuttling and transcriptional activities of YAP/TAZ through both LATS1/2-dependent and LATS1/2-independent mechanisms.

cell–cell contact signals to regulate the Hippo pathway. AMOT has at least two mechanisms to inhibit YAP. AMOT directly binds YAP regardless of its phosphorylation status, thereby sequestering YAP from the nucleus. Furthermore, AMOT stimulates LATS kinase activity by interacting with and activating NF2. Thus, AMOT can inhibit YAP by stimulating LATS-dependent phosphorylation (62). The second potential mechanism is that *trans*-dimerization of E-cadherin at adherens junctions (AJs) stimulates the Hippo kinase cascade MST1/2-LATS1/2 through α/β-catenin, Kibra, and Merlin (Mer) (63).

It has been shown that relieving mechanical strain in highly confluent cells by stretching the cells and increasing their spreading results in YAP/TAZ nuclear translocation and cell proliferation. The cytoplasmic translocation of YAP/TAZ by high cell confluence involves dynamics of F-actin polymerization and stress fiber formation (64). High cell confluence, as well as soft matrices, results in reduction of adhesive area and altered cell shape. This geometrical change inhibits RhoA activity, reduces stress fiber formation, and thus inactivates YAP/TAZ (64–66). Therefore, cells sense the overall confluence through their own morphological change. However, whether or not cell geometry regulates YAP/TAZ through the Hippo pathway still requires further characterization.

Cell–ECM interaction and matrix stiffness. The integrity and function of tissues rely on not only cell–cell interactions but also adhesion of cells to the ECM, which provides structural support and physical cues to cells for their growth and survival. The stiffness of ECM is one of the primary factors that determines how cells undergo the adhesion process (attachment, spreading) and thereafter how they grow, migrate, and differentiate. Importantly, dysregulation of YAP/TAZ has been associated with pathogenesis in which fibrogenesis contributes to disease progression, such as liver cancer and pancreatic cancer. These diseases exhibit ECM accumulation and remodeling, altering the physical cues and cell–ECM interactions in the tumor microenvironment and thus activating YAP/TAZ in cancer cells and stromal cells. The transcriptional program triggered by YAP/TAZ subsequently further enhances disease progression (2, 67).

Increased adhesive area caused by higher matrix stiffness promotes YAP/TAZ nuclear localization and target gene induction (66). The key player that relays stiffness signals appears to be RhoA GTPase, which controls YAP/TAZ translocation through promoting actin polymerization and stress fiber formation. Though early studies suggested that YAP regulation by stiffness and Rho was independent of the Hippo pathway, recent studies have supported the idea that YAP regulation by matrix stiffening involves the actin cytoskeleton and the Hippo pathway, particularly the LATS1/2-mediated YAP phosphorylation (65). This notion is also supported by a study that matrix stiffness regulates LATS1/2 activities by modulating JNK and its phosphorylation of LIMD1, which directly binds to LATS1/2 and attenuates LATS1/2 kinase activities (68).

Liquid shear stress. Cells, such as endothelial cells, are exposed to liquid flow forces, which affect cell proliferation and morphogenesis. It was first reported that YAP expression in chondrocytes and mesenchymal stem cells is correlated with levels of shear stress (69). Two later studies, however, showed that the different patterns of flow could have different effects on the activities of YAP/TAZ. It was found that the atheroprotective laminar (or unidirectional) shear stress inactivates YAP, whereas the proliferative/proinflammatory oscillatory (or disturbed) shear stress sustains YAP activities (70, 71). Integrin, $G_{\alpha 13}$, and Rho function downstream of flow stress to determine the activation status of YAP/TAZ. Rho inhibitors, such as statin, can reverse YAP activation caused by oscillatory flow stress. This finding provides significant therapeutic implications, as targeting YAP/TAZ may be a potential strategy, particularly for atherosclerosis and other related cardiovascular diseases. Interestingly, a later study suggested that even unidirectional flow

stress can activates YAP through the actin cytoskeleton, independently of LATS1/2 kinases in zebrafish and human endothelial cells (72). Future studies are needed to confirm and extend these findings and further characterize YAP/TAZ-inducible genes in response to flow stress.

Besides regulating proliferation and inflammatory response of endothelial cells, flow stress also promotes cancer cell migration and division through dephosphorylating and thus activating YAP and TAZ (73, 74). These responses have important implications in the mechanisms by which cancer cells metastasize through blood and lymph node circulation systems.

The biological output of YAP/TAZ in mechanotransduction. As transcription coactivators, YAP/TAZ mediate the functional output of physical cues by activating gene transcription, particularly genes involved in matrix remodeling and cytoskeleton reorganization. For instance, *CYR61* and *CTGF* (*CCN1* and *CCN2*), two genes that are commonly induced by YAP/TAZ, encode matricellular proteins that serve as ligands for integrins and are functionally important for cell adhesion to matrices (75, 76). YAP/TAZ appear to control a set of matrix proteins or cytoskeleton remodeling genes. Matrix stiffening activates YAP/TAZ in cancer-associated fibroblasts and induces expression of cytoskeletal regulators such as *ANLN* and *DIAPH3* to maintain the activation status of cancer-associated fibroblasts and further increase matrix stiffness (67). In a 3D spheroid model, YAP regulates tissue tension and fibronectin assembly through increasing *ARHGAP18* transcription and subsequently attenuating Rho activity (77). YAP/TAZ also increase expression of miR-130/301 in pulmonary vascular cells to induce collagen deposition and LOX-dependent matrix remodeling (78). In addition to directly *trans*-activating genes involved in inflammation and cell proliferation, under the condition of disturbed flow, YAP/TAZ activation in vascular endothelial cells induces expression of many adhesion molecules (71), thus contributing to atherogenesis (70). Vascular stiffening caused by pulmonary hypertension results in glutaminolysis and anaplerosis by activating YAP-mediated glutaminase transcription (79). YAP/TAZ also crosstalk with β-catenin and MRTF–SRF to regulate cell-cycle and cytoskeleton dynamics (80, 81). Interestingly, a recent study showed that virtually all stiffness-regulating genes are dependent on YAP/TAZ, indicating a major role of YAP/TAZ in mechanotransduction-controlled transcription in HEK293A cells (82). A major theme has emerged that an intricate interplay exists between YAP/TAZ and matrix stiffness, as YAP/TAZ are regulated by and regulate the matrix.

YAP/TAZ play a role in mediating mechanosignaling to stemness as well as cell proliferation and differentiation. In general, YAP/TAZ, when activated by high stiffness or stretching, promote stem cells to differentiate into a rigid cell type (e.g., mesenchymal stem cells are differentiated into osteoblasts at high stiffness, which is dependent on YAP/TAZ activation) (66). However, as YAP/TAZ are also known to promote stem cell self-renewal, certain levels of tension should enhance stem cell expansion rather than differentiation through YAP/TAZ (80). Conversely, epithelial cells use E-cadherin and cell junctions to maintain their quiescence through excluding YAP/TAZ from the nucleus. Mechanical stretch alters cell–cell junctions and leads to re-entry of YAP/TAZ into the nucleus and initiates the cell cycle (80).

Mechanisms of YAP/TAZ regulation by mechanotransduction. The actin cytoskeleton plays an essential role in regulating YAP/TAZ activities by controlling their cytoplasmic–nuclear shuttling. However, the exact mechanism underlying how YAP/TAZ are regulated by various mechanical signals is not entirely clear. Earlier studies used fibronectin-coated 2D hydrogels to investigate the regulation of YAP/TAZ by matrix stiffness (65, 66). The fibronectin–integrin interaction naturally plays an important role and serves as a first responder to receive mechanosignals and relay the signals to the regulatory machinery for YAP/TAZ (83–86). Cell spreading elevated by high stiffness increases the number of focal adhesions (87), which can trigger a myriad of integrin-related

signals. For instance, integrins have been implicated in both positive regulation of YAP/TAZ through the SRC kinase (88) and negative regulation of YAP/TAZ through interacting $G_{\alpha 13}$ (71), in response to different mechanical stimuli. The focal adhesion kinase (FAK) is known to activate YAP/TAZ through RhoA-mediated contractile force (64, 66, 70–72, 89). Another proposed mechanistic link to focal adhesion is that stiff substrates can couple focal adhesions and the nucleus with the actin cytoskeleton, which transmits mechanical force from the stiff matrix to the nucleus, leading to nuclear flattening, nuclear pore stretching, and YAP/TAZ transportation (23). This mechanism is consistent with an earlier study showing that actomyosin-generated cytoskeletal tension regulates YAP/TAZ localization by affecting nuclear shape (90). In parallel to the actin cytoskeleton, the spectrin cytoskeleton is also required to regulate YAP localization in response to cell density and was suggested as a potential mechanosensor (91, 92).

Although many clues have been uncovered, as discussed above, the exact link between the cytoskeleton and YAP/TAZ translocation is still missing. One key question is whether the Hippo pathway mediates YAP/TAZ nuclear–cytoplasmic shuttling downstream of cytoskeleton reorganization triggered by mechanical forces, as F-actin depolymerization stimulates LATS1/2 (15, 65, 89, 93). An early study showed that RNA interference–mediated knockdown of LATS1/2 was not sufficient to block YAP/TAZ regulation by mechanical forces in in vitro cultured cells growing on synthetic substrates (66). However, the LATS1/2 phosphorylation-insensitive mutant of YAP (YAP-5SA) can rescue the expression of the YAP/TAZ target genes in cells at low stiffness. Furthermore, it has also been observed in many in vivo models that the Hippo pathway responded to physical cues and subsequently modulated YAP/TAZ phosphorylation, localization, and transcriptional activities (65, 68, 70, 71, 94). The discrepancy could be due to the form or the nature of the mechanical forces, the magnitude of the forces, and the methods used to generate loss of LATS. A consensus is that RhoA is an important, albeit indirect, player in the inhibition of LATS1/2 (69, 93). Therefore, matrix stiffness and other forms of mechanosignals likely modulate the Hippo pathway through RhoA. Furthermore, the Hippo pathway on its own also contributes to remodeling of the actin cytoskeleton and the ECM (78).

Two recent studies in *Drosophila* and mammalian cells have revealed the key roles of MAP4Ks in relaying mechanical signals to the Hippo pathway and defined a mechanosignaling pathway from plasma membrane to nucleus (82, 95). Li et al. (95) demonstrated that, in *Drosophila* gut epithelium, Msn (homolog of MAP4K4/6/7) is involved in mechanosensing. The phosphorylation and activity of Msn in enteroblasts is suppressed by mechanical stretch, leading to Yki activation and gut cell proliferation. They further identified that Tao is the upstream kinase of Msn and that, upon cell stretch, Msn is dissociated from the plasma membrane, preventing Tao-mediated phosphorylation of Msn and thus resulting in Msn inactivation. Meng et al. (82) discovered that RAP2 is a stiffness-regulated GTPase and plays a key in Hippo regulation. RAP2 activates MAP4K4/6/7 by direct binding and inhibits RhoA through binding to ARHGAP29, leading to LATS activation and YAP/TAZ inhibition in response to low levels of stiffness in mammalian cells. Collectively, these two studies have established a key role of MAP4Ks and the Hippo pathway in YAP/TAZ regulation by matrix stiffness and stretch (96, 97).

Considering the crucial roles of MAP4K4 in focal adhesion dynamics and cell mobility (98, 99), understanding how MAP4K4 regulatory signals are relayed by TAOK and RAP2 should provide important insights into how mechanical tension signals are converted into biochemical signals at focal adhesions. RAP1, a close relative of RAP2 that antagonizes RAP2 in endothelial cells (100), is reported to sense tension and stabilize focal adhesion through promoting actin polymerization and retrograde flow (101). In addition, Rap1 also binds to and inactivates Hpo kinase in *Drosophila* (102). Mechanical cues play a key role in cell morphogenesis. Consistently, Tao, Pdzgef, Msn, and Rap1 are all known to regulate cell shape in *Drosophila* (103–106). Therefore, future studies are

needed to test whether mechanical cues use these molecules to influence cell shape and whether LATS1/2 and YAP/TAZ are involved in this process.

YAP/TAZ clearly play a key role in cellular mechanotransduction, particularly in transcription regulation. This is evident by the robust nuclear–cytoplasmic translocation of YAP/TAZ in response to various mechanical signals. However, it is also apparent that both the phosphorylation-dependent and -independent mechanisms are involved in controlling the nuclear–cytoplasmic shuttling of YAP/TAZ, possibly in a manner dependent on the nature of mechanical cues and cell context. Future studies are needed to clarify the precise biochemical processes from mechanosensing to YAP/TAZ regulation and their role in biology.

Cell Polarity and Cell Adhesion

Epithelial cells form cell–cell junctions through TJs, AJs, and desmosomes (107). As noted in early sections, both adherens and TJs relay cell contact signals to the Hippo pathway (**Figure 3**). Desmosomes have also been shown to affect YAP/TAZ activity (108), providing another route

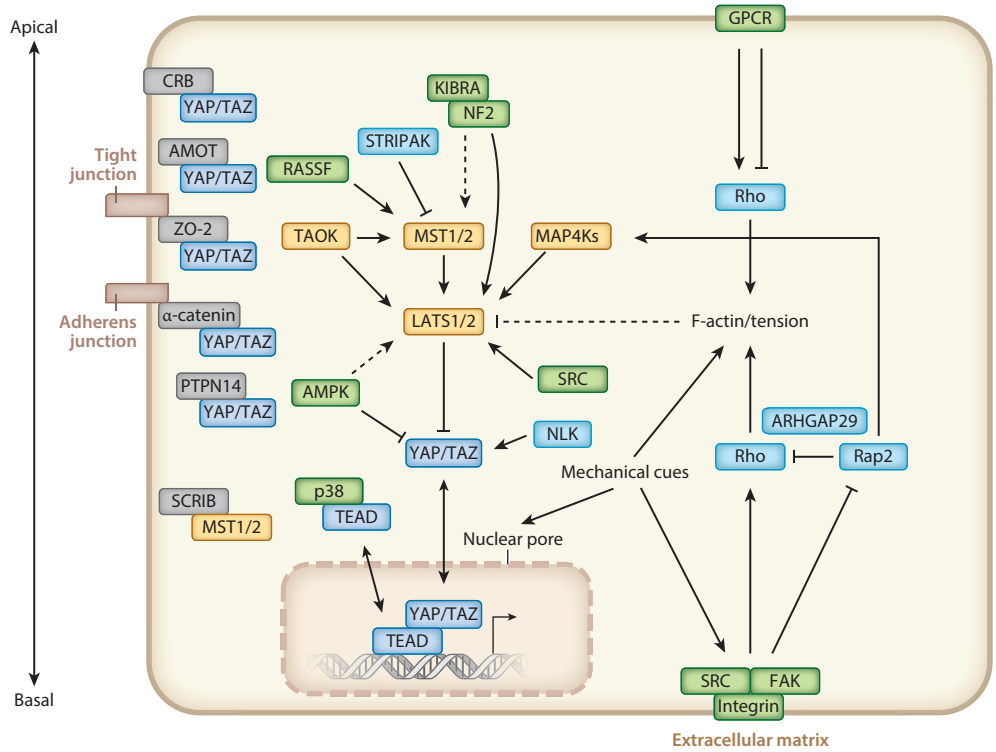

Figure 3

The Hippo pathway integrates multiple signals to regulate the activity of YAP/TAZ. The LATS kinase activity is controlled by cell polarity and cell adhesion through KIBRA/NF2, tight junctions, and adherens junctions. Soluble factors, especially hormones for G protein–coupled receptors (GPCRs), regulate LATS kinase through RhoA and actin dynamics. Mechanical cues also affect YAP/TAZ activity, in both LATS kinase–dependent and –independent manners. The stiffness-regulated GTPase RAP2 directly activates MAP4K4/6/7 as well as inhibits Rho GTPases through RhoGAP ARHGAP29, leading to LATS kinase activation and YAP/TAZ inhibition. In response to stress, the p38 MAP kinase promotes TEAD cytoplasmic localization. Arrows, blunt ends, and dashed lines indicate activation, inhibition, and indirect regulation, respectively.

through which a cell can sense its environment. Deficiency of several desmosome components, including DSP (desmoplakin), JUP (junction protein plakoglobin), and PKP2 (plakophilin 2), activates the Hippo pathway kinase activity, leading to YAP phosphorylation and pathogenesis of arrhythmogenic right ventricular cardiomyopathy (ARVC), a severe heart disease characterized by replacement of the myocardium with fibro-adipocytes and cardiac dysfunction (108). Many cell polarity components, including the Mer–Ex–Kibra complex, the apical transmembrane protein Crumbs (Crb), the Fat–Dachsous complex, and the Par complex, can regulate YAP/TAZ activity in both a Hippo pathway–dependent and –independent manner (1). For instance, the *Drosophila* Merlin (NF2 in mammals) and Expanded (Ex) cooperate with Kibra to activate Hpo in the epithelial apical domain (109, 110). Alternatively, NF2 can directly bind to and recruit LATS1/2 to the plasma membrane for activation by the MST–SAV complex (111). The basolateral component Scribble (SCRIB) interacts with both MST1/2 and LATS1/2, thus promoting LATS1/2 activation (112, 113), whereas dislocation of Scribble from the membrane to the cytoplasm by loss of DLG5, an epithelial polarity component, disrupts the Scribble–MST/LATS interaction and promotes YAP/TAZ activation in breast cancers (114). In addition, the apical transmembrane proteins Crb and TJ protein PTPN14 can inhibit YAP/TAZ activity through a direct interaction (115–118).

It is noteworthy that, although regulation of Hippo–YAP/TAZ signaling by cell polarity and cell adhesion components are similarly observed in both mammals and *Drosophila*, the detailed mechanisms are not entirely conserved (119). For example, mammalian proteins related to the *Drosophila* apical proteins Fat (Ft), Dachs, and Ex, which line upstream of the Hippo pathway, have lost key protein domains important for regulating the Hippo pathway in mammals. However, debate continues as to whether mammalian FAT1/4 (ortholog of Ft) can regulate Hippo–YAP activity, as two studies have shown that FAT1/4 may either assemble a multimeric Hippo signaling complex (signalome), leading to YAP phosphorylation and inactivation, or sequester YAP in cell junctions and cytoplasm in an AMOT-dependent manner (120, 121), although neither of the mechanisms are conserved in *Drosophila*. Furthermore, the junctional adaptor protein AMOT, which binds TJ proteins ZO-1/2 and YAP, is present in vertebrates but is absent in *Drosophila* (119). Therefore, Hippo pathway regulation by cell polarity is evolutionary conserved, whereas the detailed molecular mechanisms appear to diverge.

Soluble Factors

In multicellular organisms, soluble factors, such as hormones and growth factors, mediate organismal and distal signals of the extracellular environment to regulate cellular activities. G protein–coupled receptors (GPCRs) are the largest family of membrane receptors in mammals. Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are the first diffusible hormones discovered to activate YAP/TAZ. LPA and S1P act through their cognate GPCRs to inhibit LATS and activate YAP/TAZ. Further studies showed that GPCR signaling can either positively or negatively control YAP/TAZ activity, depending on the types of coupled heterotrimeric G protein that are induced by the different ligands (93, 122, 123). Specifically, ligand signaling through GPCRs coupled to $G\alpha_{12/13}$, $G\alpha_{i/o}$, or $G\alpha_{q/11}$, such as LPA, thrombin, angiotensin II, and estrogen, activates YAP/TAZ; in contrast, ligand signaling through GPCRs coupled to $G\alpha_{s}$, such as glucagon and epinephrine, suppresses YAP/TAZ activity (93, 124). Mechanistically, dynamic modulations of Rho-GTPases and the F-actin cytoskeleton are required for Hippo pathway regulation by GPCR ligands (**Figure 3**). YAP/TAZ regulation by GPCRs is altered in some human cancers (124, 125). It is likely that many hormones that stimulate GPCRs influence the Hippo pathway and YAP/TAZ activity.

A number of soluble factors that act independently of the GPCRs have also been reported to modulate cell growth, proliferation, and tissue homeostasis through the Hippo pathway; these factors include cytokines, VEGFs (vascular endothelial growth factors), TGF-β (transforming growth factor beta), Wnt, insulin, EGF (epidermal growth factor), and BMPs (bone morphogenic proteins) (2, 126–129). Different regulatory mechanisms have been proposed for Hippo–YAP/TAZ regulation by these soluble factors. Among them, IL-6 (interleukin-6) cytokine coreceptor gp130 associates with Src and Yes tyrosine kinases to induce YAP tyrosine phosphorylation, stabilization, and nuclear translocation. IL-6–induced YAP activation contributes to wound healing and regeneration in the intestine (130). VEGF activates YAP during angiogenesis by PI3K/MAPK activation and LATS inhibition (126, 127).

Cellular Stress

The Hippo pathway effectors YAP and TAZ are multifunctional transcription factors involved in a variety of cellular responses. A tight response between cellular stress and the signaling pathway involved in cell survival and proliferation is essential for organ growth and tissue homeostasis. It is expected that many stress signals may impinge on the Hippo pathway. Indeed, regulation of the Hippo pathway by stress signals, including energy stress, osmotic stress, endoplasmic reticulum (ER) stress, and hypoxia, has been characterized in the past few years.

The Hippo pathway can be activated by the inhibition of mevalonate synthesis, which has crucial roles in multiple cellular processes by providing essential bioactive molecules such as cholesterol, bile acid, dolichol, and isoprenyls. Isoprenyl derivatives, such as farnesyl or geranylgeranyl pyrophosphate (GGPP), are substrates for protein modification and membrane anchorage (131, 132). Geranylgeranylation of Rho GTPases is essential for Rho GTPase membrane localization and function. Statin blocks the production of GGPP by inhibiting HMG-CoA reductase, leading to RhoA inhibition. As expected, statin also strongly inhibits YAP/TAZ (131).

Over the past few years, a connection between glucose-mediated cellular metabolic status and the Hippo pathway has been observed. Glucose deprivation or inhibition of glycolysis induces a robust inhibition of YAP/TAZ activity in, at least partially, an AMP-activated protein kinase (AMPK)–dependent manner (133–136). However, AMPK appears to use multiple mechanisms to inhibit YAP/TAZ, as it can phosphorylate AMOTL1, which increases LATS1 activity, to inhibit YAP. AMOTL1 knockdown blocks YAP cytoplasmic localization induced by phenformin treatment, which activates AMPK (133). AMPK also directly phosphorylates YAP at several sites, including the S94 residue, which is crucial for YAP–TEAD complex formation (135, 136). Therefore, YAP can be inhibited directly by AMPK. Furthermore, LATS kinase can still be activated by energy starvation in AMPK knockout cells, indicating an AMPK-independent mechanism of Hippo pathway regulation in response to energy stress.

Hexosamine biosynthesis pathway, one of the downstream pathways of glucose metabolism, regulates YAP/TAZ activity in response to glucose levels (137). When glucose is sufficient, YAP is O-GlcNAcylated by O-GlcNAc transferase (OGT) at serine 109 or threonine 241. YAP O-GlcNAcylation activates its transcriptional activity through disrupting the LATS-mediated phosphorylation. Interestingly, *OGT* is also a YAP target gene (138). Besides YAP, AMOT is another Hippo signaling component that could be O-GlcNAcylated in high-glucose conditions, which stimulates YAP nuclear accumulation, interaction with TEAD, and transcriptional activity (139).

Hypoxia inhibits the Hippo pathway, leading to YAP/TAZ activation (140). Mechanistically, hypoxia stimulates an E3 ubiquitin ligase SIAH2, which binds to and destabilizes LATS2. Loss

of SIAH2 suppresses tumorigenesis in a LATS2-dependent manner. Furthermore, YAP forms a complex with HIF1α and is important for HIF1α stability and function.

Osmotic pressure has also been shown to regulate YAP in a rather complex manner. Osmotic stress acts via the NLK kinase to induce acute YAP-S128 phosphorylation, which interferes with YAP's ability to bind to 14–3–3, resulting in YAP nuclear accumulation and induction of downstream target gene expression (55, 56). Interestingly, osmotic stress also induces activation of p38, which can directly bind to TEAD and induces TEAD cytoplasmic translocation and suppression of YAP–TEAD-dependent transcription in a Hippo-independent manner (33). However, the p38-mediated TEAD cytoplasmic translocation occurs at a late time point. Furthermore, hyperosmotic stress also strongly activates LATS kinase, although the molecular mechanism of LATS activation by osmotic stress is unknown. So, the hyperosmotic effect on YAP appears to be biphasic, an acute activation mediated by NLK followed by a sustained inhibition mediated by p38 and LATS. Further research is needed to clarify the exact role of YAP/TAZ regulation in osmotic stress response. The unfolded protein response (UPR) is a cellular stress response induced by accumulation of misfolded/unfolded proteins in the ER. The UPR also has a biphasic effect on YAP/TAZ activity (141). In the initial stage, UPR can activate YAP by a PERK (PKR-like ER kinase)–eIF2α (eukaryotic initiation factor 2α) axis, whereas prolonged ER stress inhibits YAP and promotes apoptosis.

Several members of Hippo signaling, including LATS1/2 and YAP, are phosphorylated in a cell cycle–dependent manner (15). Cytokinesis failure resulting from failed cell division and tetraploidy activates LATS2, which in turn inhibits YAP/TAZ transcriptional activity (142). Conversely, activation of Yki regulates the cell-cycle phosphatase Cdc25/string, which is able to bypass cytokinesis failure induced by cell proliferation defect and apoptosis in *Drosophila* (143). In fact, the Hippo signaling–YAP axis is able to regulate cell polyploidy, as both inactivating Hippo components and activating YAP are able to trigger polyploidy phenotype in mouse liver (144).

BIOCHEMICAL MECHANISMS OF HIPPO KINASE REGULATION

Regulation of MST Kinase Activities by Autophosphorylation, Tao Kinase, and STRIPAK

As discussed above, a wide range of extracellular signals regulate YAP/TAZ activities through the Hippo pathway. However, the molecular mechanisms by which the Hippo core kinase cascade senses and responds to the signals, in many cases, are not exactly the same. An early study suggested that phosphorylation in the MST activation loop can be achieved by autophosphorylation (145). Homodimerization of MST1/2 enhances the activation loop phosphorylation (146). Although phosphorylation of the activation loop is certainly important for MST activity, a big puzzle in the Hippo field is that the level of MST phosphorylation appears to be very low and not dramatically changed during the Hippo pathway activation, whereas the corresponding phosphorylation in LATS and YAP/TAZ is strongly altered under various conditions. This discrepancy raises an important and perplexing question on how the Hippo pathway activation is initiated. In *Drosophila*, Hpo dimerization and autophosphorylation can be enhanced by the Rho-type guanine nucleotide exchange factor Pix (PAK-interacting exchange factor) and GPCR kinase–interacting protein (Git) (147). In addition, MAP4Ks also undergo autophosphorylation upon activation. However, whether they form homodimers to promote the phosphorylation is not known (148).

In *Drosophila*, Tao kinase phosphorylates Hpo at its activation loop and thus could initiate the Hippo kinase cascade (149, 150). The Hpo upstream components, Merlin and Ex, require

Tao to induce Hpo phosphorylation (149). Tao has recently been reported to phosphorylate MAP4K4/6/7 homolog Msn at its activation loop in the *Drosophila* gut (95), which is consistent with the high homology in the kinase domains of Msn and Hpo. In mammalian cells, TAO kinases can bypass MST1/2 and directly activate LATS1/2 by phosphorylating its hydrophobic motif (14). Considering that TAO kinases are activated by a lot of stress signals (151), it is possible that TAO kinases may mediate YAP/TAZ inhibition by relaying those signals to the Hippo core kinase cascade. However, the loss of function of Tao kinases generates a much weaker phenotype than Hpo deletion (149, 150), indicating that there are other layers of Hpo kinase regulation.

MST1/2 activation loop phosphorylation is tightly controlled by phosphatases. A proteomics study has discovered that MST1/2 dynamically interact with the PP2 complex STRIPAK (striatin-interacting phosphatase and kinase) (152), which dephosphorylates MST. RASSF1A is a tumor suppressor and can directly interact with MST. It has been proposed that RASSF1A prevents MST dephosphorylation (153). SAV1, the regulatory subunit of MST, also promotes MST1/2 activation by antagonizing STRIPAK (154, 155). The SLMAP subunit in STRIPAK binds to the phosphorylated MST1/2, thereby recruiting MST1/2 to STRIPAK for dephosphorylation and inactivation. This biochemical mechanism is conserved from *Drosophila* to mammals. In addition, the MAP4K4/6/7 family has been known to strongly interact with STRIPAK, and kinase activities from this family are thus inhibited by STRIPAK (156), supporting a prominent role of STRIPAK in Hippo pathway regulation.

Phosphorylation and Activation of LATS1/2 by MST and Others

The hydrophobic motif phosphorylation and the activation loop phosphorylation have been commonly used as readouts for LATS kinase activity. The hydrophobic motif of LATS is phosphorylated by MST1/2 or MAP4Ks. This phosphorylation by upstream kinases increases the autophosphorylation of the activation loop and therefore LATS activation.

Early studies in *Drosophila* showed that the changes in Wts phosphorylation are due to the relocalization of Hpo and Wts and altered accessibility between them. The Mer–Ex–Kibra complex recruits Hpo (by Mer–Ex) and Wts (by Kibra) to the apical plasma membrane, where Hpo can access Wts and phosphorylate it (109, 110). In mammalian cells, targeting MST1/2 to the plasma membrane greatly promoted LATS1/2 kinase activities (5, 157). Another study suggested that, although cytoplasmic MST1/2 and LATS1/2 are inactive, NF2 and Sav, respectively, recruit LATS1/2 and MST1/2 to the plasma membrane to initiate the MST–LATS kinase cascade (111). In *Drosophila*, however, inactive Wts is localized at AJs and needs to be relocated to Crb–Ex apical junctions to be phosphorylated by Hpo (158). Notably, Msn appears to be inactive at the plasma membrane (95), indicating that Wts activations by Msn and Hpo are different. Although not all observations regarding the mechanism of Hippo pathway activation are in line with one another, the spatial regulation of LATS1/2 activation by MST1/2 is a generally accepted model.

LATS1/2 activities are also affected by posttranscriptional modifications such as ubiquitination, which has been reviewed previously (15). Notably, in addition to phosphorylation by MST1/2 and MAP4Ks, tyrosine phosphorylation by SRC leads to LATS1/2 inactivation (159). Therefore, phosphorylation can be either activating or inhibitory for LATS.

Rho and Actin

Many signals, such as matrix stiffness, contact inhibition, cell attachment, and GPCR signals, are highly dependent on RhoA to modulate the Hippo pathway (7, 66, 89, 93). RhoA inhibitor statin can strongly induce LATS phosphorylation, although statin may also be able to bypass LATS1/2

to inhibit YAP/TAZ (71). How RhoA inhibits the Hippo kinase cascade is largely unresolved, although it is known that ROCK (Rho-associated protein kinase) is partially involved. It appears that F-actin is essential and largely mediates the effect of Rho on Hippo regulation. Consistently, disruption of F-actin by latrunculin or cytochalasin D strongly induces LATS1/2 phosphorylation (89, 93). However, the molecular mechanism by which the actin cytoskeleton controls the Hippo pathway is essentially unknown. One key open question is whether and how the actin cytoskeleton affects the activity of MST1/2, MAP4Ks, or TAO kinases. Moreover, it is also unclear whether all RhoA effects are mediated by the actin cytoskeleton. Notably, disruption of microtubules by nocodazole decreases YAP/TAZ phosphorylation, an effect opposite to F-actin disruption (89). Considering that MST1/2 and LATS1/2 are spatially regulated through membrane targeting, one may speculate that cytoskeleton-based intracellular trafficking could have a critical role in regulating the Hippo kinase cascade.

THE HIPPO PATHWAY AND DISEASE

The Hippo Pathway in Cancer

Hippo pathway dysregulation is common in many human tumors, in which YAP/TAZ have been shown to be essential for multiple hallmarks of cancer. Recently, a systematic profiling of 9,125 tumor samples revealed a widespread dysregulation of Hippo pathway components in multiple human cancer types, including glioma, colorectal cancer, and endometrial cancer (160). In this section, we discuss the functional role of the Hippo pathway in different aspects of cancer cell hallmarks (**Figure 4**).

Uncontrolled cell proliferation is a fundamental aspect of neoplasia. The role of Hippo–YAP/TAZ dysfunction in promoting cancer cell proliferation is well documented in the literature (2). In *Drosophila* imaginal discs, Yki active cells progress through the cell cycle more rapidly and proliferate precociously. YAP overexpression or hyperactivation strongly induces cell proliferation in multiple cells types in vitro. Similarly, YAP activation in mice also causes excessive proliferation in multiple tissues (161).

In addition to promoting tumor cell proliferation, the Hippo pathway circumvents the negative regulators of cell proliferation, thus evading growth suppressors. Contact inhibition is an

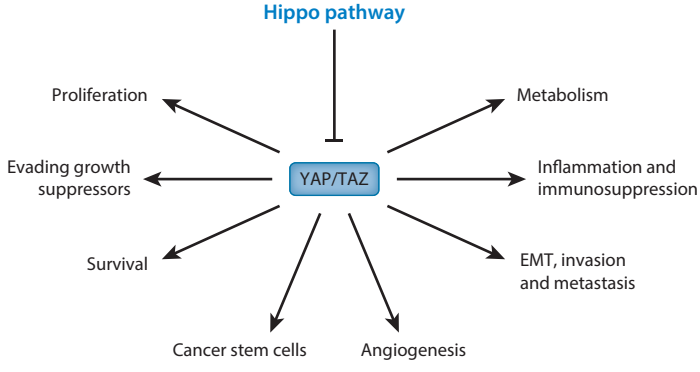

Figure 4

YAP/TAZ and cancer hallmarks. The Hippo pathway effectors YAP/TAZ directly or indirectly control multiple cancer hallmarks, including proliferation, survival, evading growth suppressors, reprogramming energy metabolism, angiogenesis, invasion and metastasis, and cancer stem cells, as well as inflammation and immunosuppression. Abbreviation: EMT, epithelial-to-mesenchymal transition.

important regulator of tissue growth and homeostasis, which is formed by dense populations of normal cells, thus suppressing further cell proliferation. Such contact inhibition, however, is thought to be abolished in a variety of cancer cells. The Hippo pathway has been found to have an important role in contact inhibition. Activating YAP/TAZ is sufficient to sustain cell proliferation even after the cell has reached confluence (7). It remains to be seen how frequently Hippo pathway dysregulation contributes to loss of contact inhibition of malignant cancer cells as well as how the Hippo pathway interferes with other contact-induced proliferative barriers.

One of the reasons that Hippo pathway dysregulation promotes tumorigenesis is that its inactivation leads to insensitivity to apoptosis. In *Drosophila*, the Hippo pathway controls apoptosis partly by regulating the expression of DIAP1 (inhibitor of apoptosis) (162). The mechanisms of YAP in suppressing mammalian cell apoptosis are more complicated, but *Survivin* and growth factors are among the YAP target genes that possibly contribute to cell survival (30, 163).

Tumor cells with dysregulated Hippo signaling not only escape the intrinsic cell death barriers but also display resistance to chemotherapeutic drugs or molecular targeted therapies, further promoting cancer relapse (161). In response to therapy, cancer cells may also reduce their dependence on one particular oncogenic driver and switch to another oncogenic driver, which confers resistance to the original therapy. It has been observed that YAP amplification rescued Kras-mutant pancreatic cancer cells when Kras was inhibited (48). YAP acts as a survival input to promote drug resistance to RAF- and MEK-targeted cancer therapy. Inhibiting YAP with RAF or MEK is synthetic lethal in BRAF- and RAS-mutant cancers (164). Notably, the role of Hippo–YAP/TAZ in sustaining cell survival may be cancer type–specific, as evidenced in studies in hematological cancers that showed that inactivation of MST1 or expression of YAP leads to growth inhibition and apoptosis (165).

Invasion and metastasis are multistep processes that are essential for tumors to progress to high malignancy. Anoikis (cell death induced by the loss of cell–matrix interactions) has been suggested as a barrier to metastasis, as tumor cells have to survive in circulation for distant metastasis to occur. YAP/TAZ inactivation by cytoskeleton reorganization and induced LATS1/2 activation contributes to detachment-induced anoikis, whereas activating YAP can inhibit anoikis (89). Cancer cells use EMT (epithelial-to-mesenchymal transition) to acquire the abilities to increase migration and invasion. Several EMT-inducing signals, including LKB1 (liver kinase B1) deficiency or TGF-β, can suppress Hippo signaling partially through Scribble, which leads to the YAP/TAZ activation (166). Importantly, YAP/TAZ are active inducers of EMT. High activity of YAP or TAZ induces expression of ZEB1/2, a key transcription regulator in EMT, to stimulate EMT (167).

Tumorigenesis is dependent on the reprogramming of cellular metabolism to acquire the necessary nutrients for building materials for growth and maintaining viability. The Hippo–YAP/TAZ axis is regulated by and also regulates cancer-associated metabolic changes. YAP activation enhances the expression of glycolytic regulatory enzymes HK2 (hexokinase 2) and PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3), thus promoting glycolysis in breast cancer cells (168). Conversely, active glycolysis promotes the direct binding of glycolysis enzyme PFK1 (phosphofructokinase 1) with TEAD and promotes their functional cooperation with YAP/TAZ (169). Mitochondrial dysfunction coupled with Ras activation induces tumor formation in a cell nonautonomous manner, which is mediated by the inactivation of the Hippo pathway (170). The Hippo pathway can also affect nucleotide biosynthesis and lipid metabolism through the regulation of de novo purine/pyrimidine biosynthesis, gluconeogenesis, amino acid uptake, and cholesterol and lipid biosynthesis (171, 172). All these findings support a function of the Hippo pathway in the regulation of cancer metabolism and suggest that YAP/TAZ oncogenic activity must be tightly coordinated with cell metabolism to efficiently control cellular homeostasis and tumorigenesis.

Cancer stem cells (CSCs) are a specialized subset of tumor cells that harbor unique properties, such as unlimited self-renewal and an ability to create a heterogeneous tumor population. The CSCs play an essential role in tumor initiation, metastasis, recurrence, and drug resistance and are thus emerging as an important target for anticancer drugs. The Hippo–YAP/TAZ pathway has been reported to be a crucial regulator of embryonic stem cells, various tissue-specific progenitors, and CSCs. Transient expression of YAP/TAZ is sufficient to reprogram differentiated cells into somatic stem/progenitor cells (173). Similarly, YAP/TAZ activation promotes the CSC properties in a wide range of cancers. Both YAP and TAZ have been reported to be important regulators of breast CSCs in self-renewal and tumorigenic potential (174, 175). YAP/TAZ directly bind to the promoter regions and induce expression of mammary stem cell signature genes, which promotes the formation of high-grade tumors through the enrichment of CSCs. In addition, YAP/TAZ can bind to and cooperate with a variety of cofactors in regulating CSCs properties, including SOX2, SOX9, and OCT4 (176, 177). These findings indicate that the Hippo–YAP/TAZ pathway in CSCs could be a potential therapeutic target in cancer treatment.

Recent studies have shown that the Hippo pathway can modulate tumor immune response. Hyperactivated YAP in prostate tumors promotes the recruitment of MDSCs (myeloid-derived suppressor cells), which play a tumor-promoting role by maintaining a state of immunological tolerance (178). Consistently, Lats1/2 knock out, Mst1/2 knock out, and expression of active YAP in mouse liver recruit type II macrophages through the induction of cytokines, leading to the establishment of an immunosuppressive microenvironment (179). Also, the immune checkpoint molecule, PD-L1, has been identified as a target of Hippo signaling. YAP/TAZ–induced PD-L1 upregulation in human cancers is sufficient to inhibit the antitumor immune response of T cells (180). The functional role of LATS1/2 in cancer immunity can be either positive or negative, as loss of LATS1/2 can inhibit tumor growth in syngeneic mouse models through the recruitment of CD45-positive leukocytes and enhance antitumor immunity. Mechanistically, LATS1/2 knockout cancer cells induce a type-I interferon response nonautonomously in a manner dependent on the Toll-like receptor–MYD88–TRIF pathway (181). Thus, the role of the Hippo pathway in tumor immunity is clearly more complex than previously recognized. A comprehensive understanding of how the Hippo pathway influences the interplay between cancer cells and immune system in vivo is a critical goal going forward.

The Role of MST in Immunity

Early studies into the immunological properties of the Hippo pathway revealed a role of MST1/2 as controllers of lymphocyte adhesion, migration, and $CD4^+$ antigen recognition (182–184). A recent study showed that MST1/2 are also crucial for $CD8\alpha^+$ dendritic cell–mediated antigen presentation to $CD8^+$ T cells, which requires a balance of metabolic activity and cytokine signaling depending on MST1/2 activity (185). However, YAP/TAZ are generally absent or lowly expressed in immune cells. Furthermore, LATS1/2 may not be involved in the immune regulatory functions of MST1/2. Given the essential function of LATS and YAP/TAZ for the canonical Hippo pathway in regulation of organ size and tissue homeostasis, one may propose the role of MST in immune modulation as noncanonical Hippo signaling independent of LATS and YAP/TAZ.

Recently, a link between Hippo–Yki signaling and host immunity response has been reported in *Drosophila*. The Hippo and Toll pathways are functionally intertwined in mediating antimicrobial response in *Drosophila*. Activation of Yki–Sd directly regulates the transcription of Cactus, the *Drosophila* IκB ortholog, thus inhibiting the production of antimicrobial peptides and vulnerability to infection by Gram-positive bacteria. Furthermore, activation of the Toll pathway suppresses Yki–Sd by Toll–Myd88–Pelle-mediated Hippo pathway activation (186).

YAP has been shown to play an inhibitory role in innate immunity because it serves as a negative barrier in the innate antiviral response in mammals. Two mechanisms have been proposed, in which YAP binds to either TBK1 or IFR3 (187, 188). In the first mechanism, YAP binds and inhibits TBK1, which is essential for NF-κB (nuclear factor κB) activation in the innate immune response. In the second mechanism, YAP inhibits nuclear translocation and activation of IFR3, which is an important transcription factor in antiviral response. Mechanistically, virus-activated kinase IKKε (inhibitor of NF-κB kinase ε) phosphorylates YAP at Ser403, which triggers degradation of YAP in lysosomes and consequently relieves YAP-mediated inhibition of the cellular antiviral response (187). TAZ, but not YAP, has been reported to promote helper T cell differentiation but inhibit T_{reg} cell differentiation, thus modulating the balance of immune response (189). Again, the functions of YAP and TAZ in immune response are independent of TEAD and therefore should be considered as noncanonical Hippo signaling.

The Hippo Pathway in Cardiovascular Diseases

The Hippo–YAP/TAZ pathway has emerged as an important regulator of homeostasis in the heart and cardiovascular system, including cardiomyocyte proliferation, heart size control, and regeneration. Heart tissues, especially in adult hearts, lose regeneration potential. Deletion of *Sav1* in the mouse heart causes elevated cardiomyocyte proliferation in the developing as well as postnatal stages (190, 191). Conversely, YAP inactivation by dystrophin glycoprotein complex–mediated sequestration inhibits cardiomyocyte proliferation (192). The function of Hippo–YAP/TAZ in cardiomyocyte proliferation and regeneration is due in part to promoting the expression of several YAP/TAZ target genes, including *IGF*, Wnt target genes, and *PARK2* (190, 191, 193).

The Hippo pathway is an important factor in cardiovascular pathophysiology. Mechanical cues generated by blood flow can either activate or inhibit YAP activity in endothelial cells. Disturbed blood flow stimulates YAP/TAZ and promotes atherosclerosis, whereas unidirectional shear stress inhibits YAP/TAZ, thereby being atheroprotective (70, 71). YAP/TAZ promote atherogenic response of endothelial cells in vivo by stimulating proinflammatory gene expression and monocyte infiltration. Treatment with simvastatin, the main anti-atherosclerotic drug, retards plaque formation, which is at least partially through inhibition of YAP/TAZ activity (71).

Targeting the Hippo pathway in the heart could have valuable clinical benefit. Inhibition of the Hippo pathway prolongs the time window in which regeneration after resection of the apex is still possible (192, 194). Also, inhibition of dystroglycan 1 by agrin or impairment of the dystrophin glycoprotein complex leads to increased YAP activity and extends the regenerative window. In both studies, neomyogenesis was even reported in adult hearts after induction of myocardial infarction. Moreover, genetic inactivation of *Sav1* improved heart function recovery after ischemic heart failure following myocardial infarction (193). Collectively, these observations suggest an exciting potential of targeting the Hippo pathway for tissue regeneration.

CONCLUDING REMARKS

The Hippo pathway plays a critical role in sensing intrinsic and extrinsic signals and regulating multiple aspects of growth at both cellular and organ levels. Notably, various mechanical cues impinge upon this pathway. Given the diverse modes of upstream signals feeding into the Hippo pathway and the large number of genes regulated by YAP/TAZ, many important questions remain unanswered. Although many inputs to Hippo–YAP/TAZ have now been identified, it is still a puzzle as to how these regulators affect the activity of the core Hippo components, particularly the regulation of MST1/2 and MAP4Ks. F-actin remodeling and cellular tension seem to

play a key role in Hippo pathway regulation and act as an integration point relaying different upstream signals to modulate YAP/TAZ activity. However, the molecular linker(s) between the cytoskeleton and Hippo pathway activation is still missing. Perhaps old-fashioned biochemistry studies will provide insights into the regulatory mechanisms of MST1/2 and MAP4Ks and how their kinase activity links to upstream signals. The phosphorylation and dephosphorylation of LATS and YAP/TAZ are very dynamic. Therefore, phosphatases are likely to play an active role in Hippo pathway regulation. This area has been largely neglected and certainly deserves serious future attention. Moreover, YAP/TAZ may be regulated by mechanisms independent of the Hippo pathway kinase cascade, including direct protein–protein binding-mediated sequestering and LATS-independent posttranslational modifications. An integrated understanding of the relative importance of these signals and the cellular contexts is urgently needed. Another major point of Hippo–YAP/TAZ research going forward, however, will be to address whether these molecular insights can improve therapeutic targeting of Hippo–YAP/TAZ in the clinic.

DISCLOSURE STATEMENT

K.L.G is a cofounder and has equity interest in Vivace Therapeutics, Inc. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict of interest policies.

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