

Review

The regulation and function of YAP transcription co-activator

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Abstract

The Hippo pathway was initially identified in *Drosophila* by genetic mosaic screens for tumor suppressor genes. Researches indicated that the Hippo pathway is a key regulator of organ size and is conserved during evolution. Furthermore, studies of mouse models and clinical samples demonstrated the importance of Hippo pathway dysregulation in human cancer development. In addition, the Hippo pathway contributes to progenitor cell and stem cell self-renewal and is thus involved in tissue regeneration. In the Hippo pathway, MST1/2 kinases together with the adaptor protein SAV phosphorylate LATS1/2 kinases. Interaction with an adaptor protein MOB is also important for LATS1/2 activation. Activated LATS1/2 in turn phosphorylate and inhibit Yes-associated protein (YAP). YAP is a key downstream effector of the Hippo pathway, and is a transcriptional co-activator that mainly interacts with TEAD family transcription factors to promote gene expression. Alteration of gene expression by YAP leads to cell proliferation, apoptosis evasion, and also stem cell amplification. In this review, we mainly focus on YAP, discussing its regulation and mechanisms of action in the context of organ size control, tissue regeneration and tumorigenesis.

Key words: YAP, Hippo, organ size, proliferation, apoptosis, cancer

Introduction

YAP was originally identified in chicken as a binding protein of non-receptor tyrosine kinase YES1 in 1994. The interaction is mediated by a proline-rich region of YAP and the SH3 domain of YES1 [1]. One year later, the human and mouse homologs of YAP were cloned. Alignment of human, mouse, and chicken Yap uncovered an insertion in mouse Yap that mainly composes a novel protein module, the WW domain. This domain has two highly conserved tryptophans, and is proposed to mediate protein–protein interactions. In addition, another WW domain is conserved in YAP across species. The YAP isoform with one WW domain is called YAP1 and the isoform with two WW domains is called YAP2. These two isoforms are generated by alternative splicing [2] (Fig. 1). Binding assay confirmed that the WW domains of YAP specifically interact with PPXY motifs of WBP-1 and WBP-2 [3]. Details of this interaction were then revealed by the crystal structure of a WW domain–PPXY motif complex [4].

Interestingly, YAP was found to be a transcription co-activator containing a C-terminal transcription activation domain [5]. The most C-terminal four amino acids of YAP interact with PDZ domains in proteins such as zonula occludens 2 (ZO-2) and NHERF2, which may be important in the regulation of YAP subcellular localization [6].

TAZ is a paralog of YAP. They have ~50% sequence identity and very similar topology. It contains a WW domain, a transcription activation domain and a PDZ-binding motif (Fig. 1). TAZ was initially identified as a phosphorylation-dependent 14-3-3 binding protein [7]. Based on their similar structures, YAP and TAZ possess similar functions, for instance, TAZ also acts as a transcription co-activator [7–9]. However, differences are also apparent. This is best shown by distinct phenotypes in *yap* and *taz* knockout mice. *yap* knockout causes early embryonic lethality, shortened body axis, and defects in yolk sac vasculogenesis [10]. In contrast, *taz* knockout mice are viable, but develop renal cysts characteristic of polycystic kidney disease

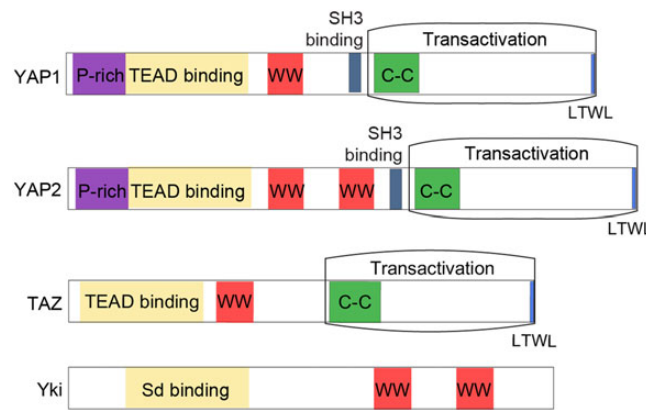


Figure 1. Domain organization of YAP/TAZ/Yki P-rich, proline-rich domain; WW, WW domain; C-C, coiled-coil domain; LTWL, the most C-terminal four amino acids forming the PDZ-binding motif (see text for more details).

(PKD) [11–13]. The regulation and function of TAZ have been reviewed elsewhere [14] and will not be elaborated in this review.

Along an independent line of evidence, mosaic screens in *Drosophila* identified several genes and the mutation of these genes would lead to dramatically enlarged organ size [15–24]. These genes were later grouped into the Hippo pathway. Importantly, there are two kinase complexes, the Hpo/Sav complex and the Wts/Mob complex. Interestingly, Yorkie (Yki), the *Drosophila* homolog of YAP and TAZ, was identified as a downstream target of the Hippo pathway by yeast-two-hybrid screen of Wts-interacting proteins [25]. Yki acts as the major effector of the Hippo pathway in *Drosophila* by transcriptional regulation of target genes such as *diap1*, *cyclin E*, and *bantam* microRNA [26–28]. As a consequence, alteration of Yki activity dramatically affects *Drosophila* organ size [25,29–32]. Importantly, human YAP could functionally rescue the phenotype of *yki* mutation, suggesting evolutionarily conserved functions of the two proteins. Interestingly, the transcription activation domain of YAP is missing in *Drosophila* Yki (Fig. 1), suggesting that the mechanisms of transcription regulation by Yki and YAP are different.

YAP Is Inhibited by the Hippo Pathway

As a downstream effector of the Hippo pathway, YAP is best known to be inhibited by direct phosphorylation by LATS1/2. The phosphorylation happens on serine residues in five consensus HXRXXS motifs both *in vitro* and *in vivo* [33–36]. Phosphorylation of YAP on S127 promotes its interaction with 14-3-3 and inhibits its transcriptional activity via cytoplasmic sequestration (Fig. 2). Mutation of S127 into alanine significantly increases YAP nuclear localization. Protein phosphatase-1, a phosphatase that specifically dephosphorylates YAP S127 promotes YAP nuclear accumulation and transcription activity [37]. Interestingly, phosphorylation of YAP on another residue affects its stability. Phosphorylation of YAP S381 triggers subsequent phosphorylation by casein kinase 1 (CK1 δ/ϵ) and activation of a phosphodegron degradation motif. The activated phosphodegron provides a docking site for an E3 ligase SCF $^{\beta\text{-TRCP}}$ and results in ubiquitination and degradation of YAP [38]. Recently, it was reported that TRIB2 functions as an adaptor of $\beta\text{-TRCP}$, inhibiting its E3 ligase activity, thus stabilizes YAP [39].

Neurofibromatosis-2 (NF2), FRMD6 (homolog of *Drosophila* Expanded) and Kibra that co-localize at the apical domain of polarized epithelial cells function upstream of the Hippo pathway, and

may activate the Hippo pathway kinases by recruiting them to the plasma membrane [40–45]. This regulation on the Hippo pathway may be conserved in mammals. It was shown that over-expression of NF2 results in membrane localization and activation of Lats1/2 as well as YAP inactivation [46].

YAP Is Regulated by Mechanical Stress

Mechanical stress, such as that caused by differential extra cellular matrix (ECM) stiffness, cell adhesion, and cell geometry, is a crucial regulator of YAP activity. When cells are cultured on stiff matrix, YAP mainly localizes to cell nuclei and promotes target gene expression. However, when cells are cultured on soft matrix, YAP is cytoplasmic and inhibited. When cells are cultured on soft matrix, cells are round and adhesion with ECM is limited. In a similar scenario, direct manipulation of adhesion surface area results in YAP activation or inactivation. In response to these signals, YAP regulates cell physiology such as adipogenic and osteogenic differentiation [47]. Surprisingly, knock-down of LATS1/2 does not seem to be sufficient to rescue YAP activity [47,48]. In another context, loss of cell adhesion to ECM leads to apoptosis, called anoikis [49]. Anoikis is thought to be a critical defense mechanism against cancer cell metastasis. Similar to that observed in cells on soft matrix, cell detachment leads to YAP inhibition in a phosphorylation and LATS1/2-dependent manner. Consistently, metastatic prostate cancer samples show a clear down-regulation of LATS2 expression. So far, the mechanism of YAP regulation by mechanical stress and the involvement of Hippo pathway kinases LATS1/2 are not completely understood. Nevertheless, F-actin cytoskeleton serves as a sensor to couple mechanical forces to YAP activity (Fig. 2). Studies on *Drosophila* have demonstrated that an increase of F-actin induces Wts-dependent Yki activation and causes tissue overgrowth [50,51]. Depletion of actin-capping protein, expression of F-actin nucleator Diaphanous or inhibition of Capulet, which all induce inappropriate actin-polymerization, lead to activation of Yki. The effect of F-actin in regulation of YAP is likely evolutionarily conserved in mammals because knockout of destrin, an actin-depolymerizing factor, also leads to abnormal actin cytoskeleton and accelerated proliferation of corneal epithelial cells [52]. This was further confirmed by the observation that loss of CapZ or Cofilin promotes YAP nuclear localization and activates YAP transcriptional activity [53]. The Rho family small GTPases, which have great effects on actin cytoskeleton organization, are found to play a role in YAP

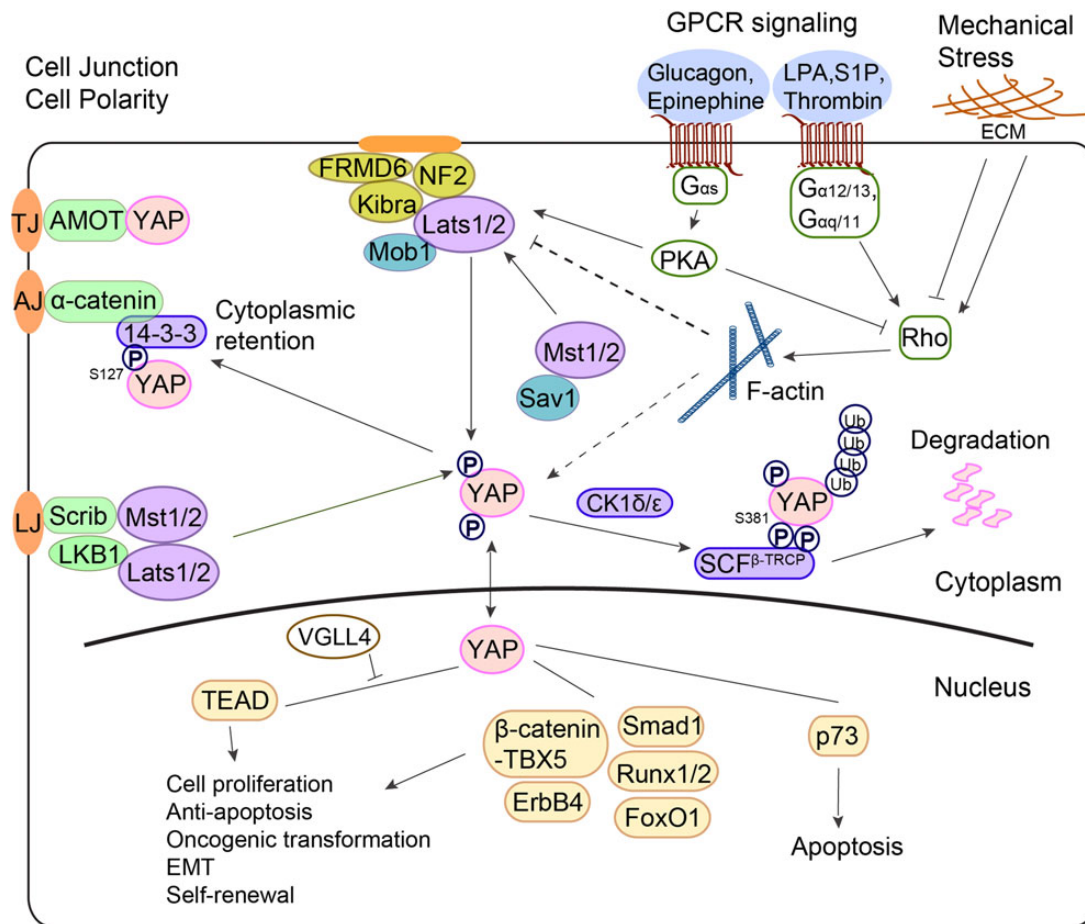


Figure 2. Regulation and function of YAP TJ, tight junction; AJ, adherens junction; LJ, lateral junction; ECM, extra cellular matrix. Arrowed or blunted ends indicate activation or inhibition, respectively. Dashed lines indicate unknown mechanisms.

activation. For instance, botulinum toxin C3, a specific inhibitor of RhoA, significantly inhibits YAP nuclear translocation and activity [47–49]. In *Drosophila*, Ajuba-mediated inhibition of Wts might be involved in response of the Hippo pathway to tension on actin cytoskeleton. Whether such a mechanism is conserved in mammalian cells is unknown [54]. Regulation of YAP and the Hippo pathway by mechanical stress has also been reviewed in detail elsewhere [55–57].

YAP Is Regulated by G-protein-coupled receptor Signaling

G-protein-coupled receptors (GPCRs) are cell surface signaling proteins transducing diffusible extracellular signals into intracellular effector pathways through the activation of cytoplasmic heterotrimeric G proteins. GPCRs have broad physiological functions and are important drug targets. Recent researches identified GPCRs as novel upstream regulators of the Hippo pathway potentially modulating YAP activity [58]. It was found that lysophosphatidic acid and sphingosine 1-phosphate in serum strongly inhibit YAP phosphorylation and increase YAP nuclear localization and transcriptional activity. Further investigation revealed that these ligands bind to their cognate GPCRs and then activate $G_{\alpha 12/13}$ and $G_{\alpha q/11}$, which in turn activate Rho and inhibit LATS1/2 (Fig. 2). Consistently, activation of another GPCR, protease-activated receptor, by thrombin also stimulates $G_{\alpha 12/13}$ and

Rho, which similarly leads to YAP activation [59]. Other processes that activate Rho could also result in YAP activation. For example, the mevalonate pathway, a metabolic pathway that activates Rho by the production of geranylgeranyl pyrophosphate, also leads to YAP activation. This finding links cellular metabolism to the Hippo pathway [60].

However, not all GPCRs cause YAP activation. Activation of $G_{\alpha s}$ -coupled GPCRs, such as that induced by glucagon, leads to activation of protein kinase A (PKA) and then activation of LATS1/2, which finally represses YAP. PKA may directly phosphorylate LATS1/2 and thereby enhance its activity [61]. In addition, PKA activation in *Drosophila* inhibits the expression of Yki target genes involved in cell proliferation and apoptosis [62].

YAP Is Regulated by Protein–protein Interactions

Besides phosphorylation-dependent inhibition of YAP by the canonical Hippo pathway, YAP is also regulated by protein–protein interactions, mainly through its WW domains. Angiomotin (AMOT) proteins, which are involved in maintaining tight junction (TJ) integrity and epithelial cell polarity, directly bind to YAP through WW–PPXY interactions and cause YAP junctional localization and cytoplasmic retention [63–67]. In addition, AMOT also inhibits YAP nuclear accumulation and activation through promotion of

YAP phosphorylation [65–67]. Alpha-catenin, an integral component of adherens junction (AJ), is also identified as an interacting protein and inhibitor of YAP by affinity purification and siRNA screening. The interaction may be mediated by 14-3-3, and require YAP phosphorylation. In addition, α -catenin–YAP–14-3-3 complex suppresses dephosphorylation of YAP by PP2A [68,69]. Another AJ protein, PTPN14, also has direct physical interaction with YAP through WW–PPXY binding, thus directly or indirectly causes YAP cytoplasmic localization and inactivation [70–72]. In addition, another PDZ domain-containing TJ protein ZO-2 that shuttles between cytoplasm and nucleus, interacts with the PDZ-binding motif of YAP, and promotes YAP nuclear localization [6]. Physical interaction of YAP with cell junctional proteins suggests the role of cell–cell junction in modulating YAP activity. Indeed, YAP phosphorylation and subcellular localization are known to be regulated by cell density and cell contact. In addition, YAP activation leads to evasion of cell contact inhibition of cell proliferation. Moreover, depletion of extracellular calcium or knockdown of Crb3 or PALS1, which causes the disruption of TJs and AJs, promotes YAP nuclear localization and activation [73].

Abnormality of junctional proteins not in TJ or AJ may also affect YAP activity. For example, down-regulation of Scribble, a basal lateral junctional protein, attenuates the formation of MST1/2, LATS1/2, and YAP complex, and finally leads to YAP nuclear translocation and activation [74,75]. Liver kinase B1 (LKB1) is a tumor suppressor mutated in many human cancers especially in the Peutz-Jeghers syndrome. The role of LKB1 in cell junction and polarity has long been recognized. Recent observations suggest that LKB1 acts as a negative regulator of YAP by regulating scribble localization in a microtubule affinity-regulating kinases (MARKs) and MST/LATS-dependent manner [76]. LKB1 has also been reported to regulate YAP through an MST/LATS-independent manner [77]. In addition, MARKs have also been reported to be YAP activators in both *Drosophila* and mammalian cells [78].

YAP Is Regulated by Other Post-transcriptional Modifications

Several other modifications have been reported to play regulatory roles on YAP in various contexts, although their roles in organ size control are not yet clear. For example, YAP is known to be tyrosine phosphorylated by several kinases. C-Abl directly binds and then phosphorylates YAP on Y357, which stabilizes YAP and promotes YAP interaction with p73, resulting in selective proapoptotic target gene expression and cisplatin-induced apoptosis [79]. YES1 is the first kinase known to interact with YAP, so comes the name Yes-associated protein [1,80]. In colon cancer cells, phosphorylation of YAP by YES1 is observed to translocate YAP– β -catenin–TBX5 complex to promoters of anti-apoptotic genes such as *BCL2L1* and *BIRC5* and thus inhibits apoptosis [81]. SRC, in the same tyrosine kinase family with YES1, is also discovered to phosphorylate YAP, which is required for later interaction with Runx2 in the nucleus. Formation of YAP–Runx2 complex attenuates the transcriptional activity of Runx2 on osteocalcin gene promoter [82]. However, YAP is mostly known as a transcription co-activator. The mechanism of this transcriptional repression is unknown. It has also been suggested that phosphorylation of YAP by SRC suppresses YAP interaction with 14-3-3 and promotes its interaction with TEAD family transcription factors in cancer-associated fibroblasts in an S127 phosphorylation-independent manner [83]. However, the exact biochemical mechanism is unknown. Some other serine/threonine phosphorylation events also contribute to

YAP regulation. CDK1 may directly phosphorylate YAP on T119, S289, and S367 during G2–M cell cycle phase, which induces mitotic defects and promotes neoplastic transformation [84]. CDK1 is later discovered to directly phosphorylate YAP and then induce cancer cell apoptosis. Such a mechanism is found to be defective in anti-tubulin drug-resistant cancer cells [85]. Therefore, YAP phosphorylation by CDK1 may be a critical mechanism in cell death induced by this kind of chemotherapy.

Furthermore, YAP is also reported to be methylated. Methylation of YAP lysine 494 by SET-7 leads to cytoplasmic retention of YAP, but the upstream signal for SET-7-induced methylation and the mechanism of cytoplasmic retention are still unknown [86]. Besides phosphorylation and methylation, it was recently discovered that under DNA-damaging stimuli, YAP translocates to cell nuclei and is acetylated or deacetylated by CBP/p300 acetyltransferase and SIRT1 deacetylase, respectively. Acetylation may be essential for YAP transcription co-activator activity [87].

Regulation of YAP Expression Level

In general, YAP mRNA is ubiquitously expressed in a wide range of tissues, except peripheral blood leukocytes [88]. However, there are very few reports on the regulation of YAP mRNA transcription. A recent study reported that an Ets family transcription factor GABP directly binds to YAP promoter and induces YAP transcription under inhibition by oxidative stress. Furthermore, a correlation between the protein levels of YAP and GABP is observed in human liver cancers [89]. Other reports unveiled an AP-1 binding site and a c-Jun recognition element in the promoter of YAP and knockdown of c-Jun results in down-regulation of YAP expression. Consistently, YAP is responsible for c-Jun-mediated apoptosis [90]. Further investigation of YAP transcriptional regulation under various physiological or pathological conditions may provide invaluable insights into regulation of organ size and cancer development.

YAP mRNA is also targeted by microRNAs. miR-375, a microRNA down-regulated in hepatocellular carcinoma (HCC), has been observed to repress the expression of YAP [91]. Furthermore, a negative correlation between YAP expression and miR-375 has been observed in lung cancer cell lines and in a chemically induced HCC mouse model [92,93]. Consistently, expression of miR-375 suppresses YAP mRNA and protein levels and inhibits YAP-induced proliferation and invasion of HCC cells. It remains possible that more microRNAs regulating YAP expressing would emerge in the future.

TEADs Are Major Transcription Factor Partners of YAP

The C-terminal transcription activation domain provides YAP with a strong transcriptional activity. However, YAP could not bind to DNA directly. It is brought to certain gene promoters by partner transcription factors (Table 1). Interestingly, TEAD family transcription factors were identified as potent DNA-binding partner of YAP by both affinity purification and transcription factor library screening [29–32,94]. Point mutation of YAP (S94A) that eliminates interaction with TEADs strongly abolishes YAP-induced gene expression as well as YAP-induced cellular transformation [32]. Furthermore, tissue-specific knock-in of this mutation in mouse skin or heart generates a phenotype similar to *yap* knockout. This notion is supported by genetic studies in *Drosophila in vivo* showing the function of Scalloped (Sd), the *Drosophila* homolog of TEADs, in Yki-induced tissue growth

Table 1. Target transcription factors and target genes of YAP

Transcription factors	Target genes	Functions	Reference
TEADs	<i>CTGF</i>	Cell proliferation, metastasis	[32,94]
	<i>Cyr61</i>	Cell proliferation	[95,96]
	<i>Axl</i>	Cell proliferation, metastasis	[97]
	<i>CDK6</i>	Senescence	[98]
	<i>Oct4/Nanog</i>	Stemness	[80,99]
	<i>Cdx2</i>	Trophoblast differentiation	[100,101]
	<i>Gata3</i>	Trophoblast differentiation	[102]
	<i>BCL2/BIRC5</i>	Anti-apoptosis	[36]
	<i>c-Myc</i>	Cell proliferation, metastasis	[36,103]
	<i>EDN1/2</i>	Cell proliferation, metastasis	[104]
	<i>Pax3</i>	Embryogenesis	[8,105,106]
	<i>RHAMM</i>	Cell proliferation, metastasis	[107]
	<i>SOX9</i>	Cancer stem cell maintain	[108]
	<i>ZEB2</i>	Barrier for lung cancer cell fate conversion	[109]
Smad1		Stemness	[73,110]
Smad2/3-TEADs	<i>Snail/Twist1/Slug/NEGR1/UCA1/CTGF</i>	Cell proliferation and EMT	[111,112]
p73/p63	<i>PML</i>	Cell apoptosis	[113,114,115]
KLF5	<i>FGF-BP/ITGB2</i>	Cell proliferation	[116]
Runx1/2		Osteoblast differentiation in MSCs	[5,47]
ErbB4		Cell proliferation	[88,117]
TBX5	<i>BCL2L1/BIRC5</i>	Anti-apoptosis and cardiac development	[81,118]
β-catenin	<i>Sox2/Snai2</i>	Cardiomyocyte proliferation	[119]
FoxO1		Antioxidant	[120]
Unknown	<i>AREG</i>	Cell proliferation, cell migration	[121]

[29–31]. In addition, the structure of YAP–TEAD complex has been solved, showing critical interfaces mediating the interaction which may be targeted by small molecules for therapeutic purposes [122–124]. Interestingly, the structure reveals a direct hydrogen bond between YAP S94 and TEAD1 Y406. Y406 is mutated in a human genetic disease Sveinsson’s chorioretinal atrophy [124]. Thus, defective YAP–target gene expression and tissue growth might be the underlying cause of this disease.

Recent research identified Tgi as a novel cofactor of Sd that mediates default repression of gene expression [125,126]. Yki competes with Tgi for Sd binding thus relieves the default repression and promotes tissue growth. When the Hippo pathway is activated, Yki is strongly inhibited and Tgi becomes the dominant occupant of Sd that strongly suppresses Sd activity. Importantly, the function of Tgi is conserved in its mammalian homolog VGLL4. VGLL4 acts as a tumor suppressor in both lung and gastric cancers by repressing YAP–TEAD activity. In addition, a peptide mimicking VGLL4 function acts as a YAP antagonist and shows prominent anti-cancer activity in gastric cancer models [127,128].

In *Drosophila*, Yki/Sd activate transcription of target genes such as *diap1* and *dMyc*, which are important for Yki-mediated tissue overgrowth [129,130]. However, in mammals, the target genes of YAP–TEAD are quite different. YAP–TEAD induces expression of connective tissue growth factor (*CTGF*), which is important for YAP-mediated proliferation and anchorage-independent growth *in vitro* [32]. YAP–TEAD also induces expression of *CDK6*, which may mediate YAP-dependent cellular senescence [98]. Additionally, Yki/Sd cause direct transcriptional up-regulation of genes that regulate mitochondrial fusion, including *opa1-like (opa1)* and *mitochondria assembly regulatory factor (Marf)*, and causes extensively fused mitochondria formation and Yki-induced tissue overgrowth. The role of Yki in regulation of mitochondria is proposed to be conserved in humans [131]. Thus, in addition to the direct role of YAP in cell

proliferation and apoptosis, regulation of cell energy metabolism might be another function of YAP to coordinate fast tissue growth.

Other Transcription Factor Partners and Target Genes of YAP

YAP also interacts with PPXY motif-containing transcription factors through the WW domains. These transcription factors include Smad1, RUNX1/2, ErbB4, and p73 [5,88,110,113,117]. Interaction of Smad1 with YAP depends on Smad1 phosphorylation and is required for BMP-mediated suppression of neural differentiation of mouse embryonic stem cells [110]. p73 is a p53 family pro-apoptotic transcription effector that may interact with YAP [114,132]. The promyelocytic leukemia (PML) tumor suppressor gene is a direct target of p73/YAP. Conversely, PML is believed to stabilize and activate p73/YAP, which induces p73/YAP-mediated apoptosis. Although over-expression of YAP stimulates p73-mediated apoptosis in certain context, apoptosis is suppressed in conditional *yap* transgenic liver or heart [36,133,134]. YAP was also reported to interact with KLF5 transcription factor through WW–PPXY binding and stabilize it by competing with the E3 ligase WWP1, which then led to up-regulation of KLF5-related gene expression [116]. In addition, some other transcription regulators interact with YAP in WW domain-independent manner. For example, YAP can interact with β-catenin and up-regulate Wnt signaling by inducing its canonical target genes such as *Sox2* and *Snai2* [119]. YAP has also been reported to interact with FoxO1 through TEAD-binding region and the C-terminal transactivation domain, and promote antioxidant gene expression [120].

YAP and Yki also up-regulate some other genes, whose responsible transcription factors have not been firmly established. For example, Yki activates *cycE* and *E2F1*, which are involved in cell-autonomous regulation of cell proliferation [18,29]. Yki also activates the EGFR

ligands *Vein* (*Vn*), *Keren* (*Krn*), and *Spitz* (*Spi*), and the jak-Stat pathway ligands *Unpaired* (*Upd*), *Upd2*, and *Upd3*, which are involved in non-cell-autonomous regulation of cell proliferation [121,135–138]. Target genes mediating non-cell-autonomous functions of the Hippo signaling in mammalian cells were also reported, such as *amphiregulin* (*AREG*) and *fibroblast growth factor 1* [34,121]. YAP and Yki may also induce expression of Hippo pathway genes as a feedback mechanism. For example, in *Drosophila*, Yki stimulates the expressions of *Ex*, *Kibra*, *Crb*, and *Fj* [40,139–141]. It should be kept in mind that some of these YAP/Yki target genes might be indirectly induced.

Mechanisms of YAP in Transcription Activation

Although the strong transcription activity of YAP C-terminal domain has been observed for a long time, the biochemical nature of this activity remains elusive. Recently, it was found that the Brahma (BRM) complex, a catalytic ATPase component of the SWI/SNF chromatin remodeling complex, physically interacts with Yki and is required for Yki/Sd-dependent intestinal stem cell (ISC) proliferation [142]. In addition, genome-wide association analysis showed that Yki recruits the BRM complex to chromatin [143]. In mammalian cells, TAZ was found to directly recruit BRM to target gene *CTGF* promoter and regulate its expression in MCF10A cells. This suggests a potentially conserved mechanism of YAP/Yki in inducing transcription. Besides BRM, Irvine's lab also identified that Yki, in association with GAGA factor (GAF) and the Mediator complex in cell nucleus, contributes to Yki-induced transcriptional activation of target genes [143]. TAZ has also been reported to interact with the mediator complex in the nucleus [144].

Back in 2005, it was proposed that TAZ can associate with histone acetyltransferases p300 and PCAF, and dramatically activates TBX5-dependent promoters, but whether YAP has the same activity has not been confirmed [118]. Moreover, it was recently reported that Yki regulates transcription through WW domain-mediated interaction with NcoA6 (a subunit of the Trr methyltransferase complex) and then modification of local chromatin [145,146]. NcoA6 is required for Yki/Sd-induced tissue overgrowth. However, the important difference between YAP and Yki is that the C-terminal transcriptional activation domain of YAP is missing in Yki. Thus, the function of the Yki WW-domain in mediating transcription activation might be weakened in YAP due to evolution of the new transcription activation domain. It would therefore be unsurprising if later investigation reveals different mechanisms in transcription regulation by YAP and Yki.

YAP in Organ Size Control

Organ size control is a long-standing mystery in biology. Previous research provided limited information that both systemic factors and organ-autonomous mechanisms are involved in size control of specific organ. However, detailed molecular mechanisms are missing. One of the most amazing functions of the Hippo pathway is its remarkable effect on organ size [36,147]. In fact, this pathway is largely established by genetic screen for mutations that affect organ size. In *Drosophila*, mutations of Hippo pathway genes *warts* (*wts*), *hippo* (*hpo*), *salvador* (*sav*), and *mats* or compound inactivations of Merlin (Mer) and Expanded (Ex) were found to dramatically promote tissue overgrowth and organ size enlargement [15–23]. Similar effect has been observed in *yki* transgenic lines [31,32]. Careful examination indicated that the enlargement of organ size is due to both inhibition of apoptosis and increase of cell proliferation, which together cause

increased cell number. Importantly, roles of the Hippo pathway in organ size control are evolutionarily conserved in mammals. Transgenic expression of *yap* in mouse liver causes increase of liver size up to one fourth of the mouse body weight [36,133]. Sustained expression of *yap* finally leads to tumor development. However, if *yap* expression is turned off before tumorigenesis, liver size goes back to normal due to apoptosis. Thus tuning of Yap activity results in reversible regulation of liver size. Systemic ablation of the Hippo pathway kinases *Mst1/2* indicates that the sizes of different organs are differentially sensitive to the Hippo pathway [35,119,148,149]. For example, the sizes of the liver, stomach, heart, and spleen are affected by *Mst1/2* knockout, but the sizes of limb, kidney, and lung are not obviously affected. This organ specificity might be due to expression pattern of another kinase sharing redundant functions with *Mst1/2* upstream of the Hippo pathway, or due to functional variation of the Hippo pathway in organ size determination in different developmental stages. Of note, several studies have provided strong evidence of the Hippo pathway and YAP in cardiomyocytes in regulating heart size [119,134,150,151].

Enlargement of organ size obviously needs ectopic protein synthesis. Interestingly, besides promoting cell cycle, YAP coordinately enhances protein synthesis through the PI3K–TOR pathway. It was demonstrated that YAP activates the expression of miR-29, which then inhibits PTEN translation and finally activates the mammalian target of rapamycin (mTOR) [152]. Thus, the Hippo pathway and the mTOR pathway may coordinate to regulate organ size. Although the Hippo pathway clearly plays important roles in organ size control, the origin of organ size information is still elusive. In fact, in *Drosophila*, mutation of genes involved in cell polarity and adhesion results in the most dramatic change of organ size. Future investigation of the mechanisms of Hippo pathway regulation by mechanical stress and GPCRs and their ligands *in vivo* may provide insights into the mystery of organ size control.

YAP in Stem Cell and Progenitor Cell

YAP could promote cell proliferation and inhibit apoptosis thus regulating cell number in certain organ. However, organ size could also be regulated by the number of tissue-specific progenitor cells, which are capable of differentiating into all cell types in a specific tissue [153]. The role of YAP in progenitor cells has been examined. In skin, the expression and nuclear localization of YAP is much higher in the basal epidermis progenitor cells, and YAP translocates to the cytoplasm in differentiating cells [68,95]. In addition, over-expression of YAP results in marked expansion of basal progenitor cells and inhibition of terminal differentiation through α -catenin-dependent or independent ways [69,95]. Consistently, knockout of *yap* inhibits progenitor proliferation and leads to the failure of skin expansion. In neural stem cells, YAP also functions as a stimulator of cell proliferation and an inhibitor of differentiation possibly downstream of Notch signaling or Sonic hedgehog pathway [105,154,155]. The role of YAP in progenitor cells has also been shown in the context of intestine, lung, and liver [109,133,156–160]. Of particular interest is the recent finding that activation of YAP could convert hepatocytes to liver progenitor cells called oval cells, which provides insights into the cells of origin of HCC [161].

Following the observation of YAP function in progenitor cells, it is also found to play a role in embryonic stem cells (ESCs). YAP together with TEADs induces ESC self-renewal. When mouse ESCs are induced to differentiate, YAP is inactivated with decreased protein level and increased phosphorylation. And over-expression of YAP inhibits mouse

ESCs from differentiation even under differentiating conditions such as leukemia inhibitory factor withdrawal [80,99]. In addition, chromatin immunoprecipitation (ChIP) experiments indicated that YAP–TEAD binds to promoters of many stemness-promoting genes such as *Oct4*, and YAP knockdown leads to loss of pluripotency. Consistently, it was found that the expression of *Lats2* is significantly repressed during reprogramming from fibroblasts to iPSCs, and knockdown of *Lats2* increases the efficiency of human iPSC generation about three-fold without accelerating cell proliferation [162]. Co-expression of YAP was also shown to improve the efficiency of human iPSC generation by *Oct4*, *Sox2*, and *Klf4* [99]. However, in a separate study, YAP did not show significant effect when all four factors *Oct4*, *Sox2*, *Klf4*, and *Myc* were used [163]. The role of YAP in ESCs *in vivo* is more complex. It was shown that during embryogenesis YAP and TEADs play an essential role in the process of trophoblast (TE) specification [164,165]. However, in inner cell mass composed of ESCs, YAP is inhibited to prevent TE-specific gene expression. Thus how would the roles of YAP in ESCs observed *in vitro* and *in vivo* be reconciled still awaits further study.

YAP in Regeneration

The functions of YAP in proliferation and stem/progenitor cell expansion suggest a role of YAP in tissue regeneration. Indeed, it was found that YAP protein level is elevated during intestinal regeneration and *yap* knockout largely impairs damage-induced regeneration program, although it does not seem to affect general intestinal development [158]. However, it was also demonstrated that in irradiation-induced intestine regeneration model, loss of YAP leads to over-expansion of ISC and development of microadenomas, which correlates with hyperactive Wnt signaling [159]. This finding would support YAP as a suppressor on intestine regeneration through Wnt signaling inhibition. One possible explanation of the phenotype is that loss of YAP promotes Paneth cell differentiation and thus increases Wnt secretion, therefore elevates Wnt signaling.

Heart regeneration is another context where the function of YAP in tissue repair has been examined. Although it is now recognized that mammalian hearts have renewal and regeneration capacity, the rate is normally too low and would be easily overwhelmed by injury such as that in myocardium infarction. In mouse, postnatal hearts have regeneration ability, which is quickly lost after day P7. Interestingly, knockout of *yap* impairs regeneration of hearts infarcted on P2 and over-expression of *yap* improves regeneration of hearts infarcted on P28 [166]. Knockout of Hippo pathway components such as *save* also leads to increased regeneration capacity [167].

However, it was observed that injury of one area of the heart induces cell cycle re-entry of cardiomyocytes throughout the whole organ in Hippo-deficient mouse hearts. Similar phenomenon has also been observed in regenerating hearts of zebrafish [167,168]. Therefore, these findings suggest that some diffusible signals may exist to propagate damage signals to instruct cardiomyocyte proliferation distant from the site of injury. Whether Hippo–YAP is directly responsive to myocardium injury or simply regulates cardiomyocyte proliferation needs to be further examined.

YAP as an Oncogene

YAP controls cell number and organ size through regulation of cell proliferation, apoptosis, and progenitor cell amplification. However, when dysregulated, these processes could all contribute to human

cancer development. Thus, the potential roles of YAP in human cancers have been extensively explored. First, evidence from cultured cells supports that YAP has transforming ability and oncogenic roles. Over-expression of YAP drives cells to reach the higher saturation density and to evade cell contact inhibition [33]. YAP also empowers cells for anchorage-independent growth, another well-known indicator of oncogenic transformation. In MCF10A mammary epithelial cells cultured on matrigel, expression of YAP promotes formation of larger and filled acini, suggesting defects in apoptosis and also in proliferation control [169]. In addition, YAP induces epithelial–mesenchymal transition (EMT), which may contribute to cancer metastasis [75,170]. The mechanism for YAP to induce EMT could involve multiple downstream targets and is complicated [111]. However, both YAP and TAZ were shown to induce expression of ZEB1/2, strong inducers of EMT [109,171]. A genome wide RNAi screen also identified YAP as an essential gene in β -catenin-driven cancer cell lines [81]. Oncogenic roles of YAP are well supported by mouse models [36,133,134,154]. *Yap* cooperates with *Myc* to stimulate tumor growth in nude mice. More interestingly, enlarged livers in *yap* transgenic mice finally develop tumors. Consistently, conditional knockout of *mst1/2* also quickly leads to tumorigenesis in the liver [148]. In fact, upstream components of the Hippo pathway such as NF2 are well-known human tumor suppressors [172–176]. Oncogenic functions of YAP have also been demonstrated in other organs such as skin, lung, and stomach. More importantly, YAP and the Hippo pathway are deregulated in human cancers in various ways. It was observed in human HCC samples that YAP is in the 11q22 amplicon [169,177]. Similar amplicons have also been found in human intracranial ependymomas, oral squamous cell carcinomas, medulloblastomas, and mouse mammary tumors [33,36,169,177]. A recent analysis of large cohorts of esophagus cancer samples also revealed that YAP was genomically amplified in ~6% of cases [178]. YAP activity is also up-regulated through other mechanisms. Elevated expression and nuclear localization of YAP have been observed in multiple types of human cancers such as liver, colon, ovarian, lung, and prostate cancers [33,36,169,177,179,180]. Microarray analysis on 993 primary human breast cancers from clinical data has identified high expression of YAP in poorly differentiated cancers, which supports YAP as an oncoprotein [75]. Following the discovery of GPCR signaling mediated by $G_{\alpha q/11}$ as potent inhibitor of the Hippo pathway, it was noticed that activating mutation of $G_{\alpha q/11}$, which happens in 80% of uveal melanoma, correlates with YAP dephosphorylation and activation [181,182]. Furthermore, inhibition of YAP blocks tumor growth of these uveal melanoma cells. Thus, YAP might be a good therapeutic target of this specific cancer in the eye. YAP could also be important for relapse of certain cancers. RAS is one of the most common oncogenes in human cancers, such as pancreatic ductal carcinoma. It was known that inhibition of Ras in these tumors would cause regression of the tumor. However, relapse normally happens. Surprisingly, it was recently found that YAP is amplified and over-expressed in some of these relapsed tumors, which bypasses Ras-addiction [183,184]. Moreover, recent findings also indicated that YAP functions in inhibiting global miRNA biogenesis in a cell-density-dependent manner, which may also contribute to tumorigenesis [185,186]. Taken together, evidence from tissue culture, animal models, and clinical samples supports a prominent role of YAP in human cancer.

YAP-targeted Cancer Therapy

YAP as an oncogene significantly induces cancer development mainly through TEAD-dependent gene expression. Thus, YAP–TEAD

interaction is thought to be the most direct drug target to suppress YAP-induced tumor growth. Luckily, the crystal structure of YAP–TEAD complex has already been solved, which lays a foundation for screening and designing of specific compounds to disrupt or weaken the YAP–TEAD complex [122,123]. A screening of >3300 drugs from Johns Hopkins Drug Library discovered 71 drugs that may down-regulate YAP–TEAD interaction. Among them, verteporfin (VP), hematoporphyrin, and protoporphyrin IX (PPIX) stood out which all belong to the Porphyrin family. Further coIP assay confirmed VP and PPIX as functional compounds that directly destroy the YAP–TEAD complex stability [187]. Profoundly, VP abrogates liver overgrowth induced by YAP over-expression *in vivo*, verifying the therapeutic potential of targeting YAP–TEAD interaction. More recently, it was demonstrated that VP inhibits esophageal cancer development *in vivo* by down-regulating SOX9, another YAP–TEAD target gene [108]. Simvastatin, which is originally prescribed to reduce cholesterol in patients with cardiovascular disease, is recently found to inhibit YAP–TEAD activity and leads to suppression of RHAMM transcription in breast cancer cells [107]. In addition, Super-TDU, a short peptide mimicking VGLL4 and YAP binding to TEADs, also showed promising therapeutic effects in inhibiting YAP activity in gastric cancer models [127]. Thus, further development of strategies with higher efficiency and lower toxicity in targeting YAP–TEAD is still an important future direction.

Concluding Remarks

YAP is a well-known primary downstream effector of the Hippo pathway crucial for organ size control, tumorigenesis, stem cell amplification, and regeneration. Intensive investigations in the past decade have provided insights into the regulation of this protein, mainly by post-translational modifications and protein–protein interactions, and its mechanisms of action largely based on its transcriptional activity. Examination of genetically engineered *Drosophila* and mouse strains also provided strong evidence for the function of this protein in organ size control and regeneration. More importantly, recent findings of the key roles of YAP in tumorigenesis of several human cancers, especially uveal melanoma, and in cancer relapse have generated new excitements in the field. Based on the current knowledge on YAP, we believe that several key questions would be important directions for future research. The first would be how YAP is coordinately regulated by physical signals such as cell adhesion, matrix stiffness and chemical signals such as various GPCR ligands. In the context of organ size control, it is known that mutation of cell adhesion and polarity proteins results in the most dramatic alterations of organ size. On the other hand, during regeneration after injury, proliferation spread to sites distant from the injury, and could even be transferred to other individuals by blood circulation, suggesting the involvement of soluble molecules. Whether and how YAP is involved in both scenarios are thus interesting questions. Although YAP is known to be deregulated in cancers, the prevalence and mechanisms are unknown in most types of human cancers. With accumulating cancer genome and transcriptome projects getting done, we expect invaluable information about the deregulation of the Hippo pathway and YAP to be extracted. In addition, many efforts have been made to target YAP for cancer therapy. Some potentially useful strategies have already emerged. We anticipate more translational researches on YAP inhibition or activation being reported for cancer therapy or regenerative medicine, respectively. Hopefully, with all these efforts, the evolutionarily conserved Hippo pathway may be controlled for the purpose of human health.

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