



An overview of signaling pathways regulating YAP/TAZ activity

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Abstract

YAP and TAZ are ubiquitously expressed homologous proteins originally identified as penultimate effectors of the Hippo signaling pathway, which plays a key role in maintaining mammalian tissue/organ size. Presently, it is known that YAP/TAZ also interact with various non-Hippo signaling pathways, and have diverse roles in multiple biological processes, including cell proliferation, tissue regeneration, cell lineage fate determination, tumorigenesis, and mechanosensing. In this review, we first examine the various microenvironmental cues and signaling pathways that regulate YAP/TAZ activation, through the Hippo and non-Hippo signaling pathways. This is followed by a brief summary of the interactions of YAP/TAZ with TEAD1–4 and a diverse array of other non-TEAD transcription factors. Finally, we offer a critical perspective on how increasing knowledge of the regulatory mechanisms of YAP/TAZ signaling might open the door to novel therapeutic applications in the interrelated fields of biomaterials, tissue engineering, regenerative medicine and synthetic biology.

Keywords Hippo · Mechanisms · Regulation · Signaling pathways · TAZ · YAP

Introduction

YAP and TAZ—homologous transcriptional factors that play key roles in diverse biological processes

YAP (Yes-associated protein, also referred to as YAP1) and its smaller paralog TAZ (transcriptional co-activator with PDZ-binding motif) are transcriptional co-activator proteins that shuttle between the nucleus and cytoplasm, and regulate gene expression through binding with the TEAD (TEA/ATTS domain) family of transcription factors (TEAD1–4)

within the cell nucleus [1, 2]. Due to their close structural similarities, YAP and TAZ share overlapping roles in many key biological functions, but there are important differences. The major structural difference is that the smaller TAZ protein (43 kDa) lacks the SH3-BM proline-rich region and one WW domain present in the larger YAP protein (65 kDa) [1]. Two splice isoforms of the YAP protein have been identified YAP1-1 and YAP1-2, which differ by the presence of an additional WW domain (38 amino acids) on YAP1-2, compared with YAP1-1 [1]. YAP and TAZ are ubiquitously co-expressed in most mammalian tissues, though there are some exceptions. For example, YAP expression is absent in the

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hippocampus and parathyroid gland [3], while the thymus and peripheral blood leukocytes lack TAZ expression [4].

Historically, YAP/TAZ were identified as the penultimate effectors of the Hippo signaling pathway, which plays a crucial role in regulating tissue/organ size [5]. In recent years, many other functions of YAP/TAZ have been found, in development, cell proliferation, tissue regeneration and cell lineage fate determination [6], as well as in mechanosensing and mechanotransduction at cell-ECM/biomaterial interfaces [7]. These functions are all highly relevant to the fields of tissue engineering and regenerative medicine. Furthermore, YAP/TAZ also have roles in other biological processes and disease pathologies, most notably in tissue/organ homeostasis and cancer metastasis [8].

In this review, we will give an overview of the various microenvironmental cues and signaling pathways that regulate YAP/TAZ activity, followed by a brief summary of YAP/TAZ interaction with TEAD and non-TEAD transcription factors. Then, we will present a critical perspective, suggesting how our increasing knowledge of YAP/TAZ signaling might advance therapeutic applications in the fields of biomaterials, tissue engineering, regenerative medicine and synthetic biology.

YAP/TAZ—key mediators of cell interaction with the microenvironment

YAP/TAZ mediate cellular responses to (i) biomechanical cues [9–17], (ii) extracellular ligands, such as growth factors and lipids [18–21], (iii) energy, osmotic and hypoxic stress [22–25], and (iv) inflammation and tissue injury [26–30].

Biomechanical cues refer to the mechanical forces generated upon cell interaction with the extracellular matrix (ECM) or substrata, cell-to-cell contact, and liquid shear forces encountered by cells (best exemplified by the endothelial cells lining blood vessels). The responses to these cues mediated by YAP/TAZ in turn play key roles in orchestrating organ/tissue development and regulating homeostasis, and are therefore of interest in connection with the fabrication of improved biomimetic materials for tissue engineering applications.

Mechanosensing of ECM/substrata stiffness is mediated primarily by focal adhesions (FAs), which influence cell adhesion, spreading, and remodeling of the actin cytoskeleton via RhoA activity [9–11]. The FAs in turn modulate Hippo-dependent and independent signaling pathways that control cell proliferation and differentiation through YAP/TAZ. Generally, with a stiffer substrate, there is increased cytosol to nuclear translocation of YAP/TAZ (Fig. 2), which can be attributed to increased number of FAs per cell, as well as increased tensile force on the stress fibers connecting the FAs that cause the cell to spread over a larger surface area [10, 11]. In the case of bone marrow-derived mesenchymal

stem cells (BMSCs), increased YAP/TAZ activation on a stiff substrate leads to enhancement of osteogenic differentiation [11].

Another biomechanical cue is cell density. With increasing cell density, there is increased cell-to-cell contact, and the cell shape changes from a more flattened to a more rounded geometry, with a consequent reduction in FAs, but an increase in the numbers of tight junctions (TJs) and adherens junctions (AJs) between adjacent cells [12, 13]. This inhibits cytosolic to nuclear translocation of YAP/TAZ through both the Hippo signaling pathway and Hippo-independent mechanisms. Generally, a decrease in YAP/TAZ nuclear-to-cytoplasmic ratio impedes cell proliferation, resulting in the commonly observed phenomenon of contact inhibition of cell proliferation [12, 13].

Cellular response to shear stress is also mediated by YAP/TAZ [14–17]. Studies with a microfluidic perfusion device demonstrated that exposure to shear force enhances YAP translocation to the cell nucleus, which in turn promotes osteogenesis and inhibits adipogenesis in mesenchymal stem cells (MSCs), but initiates dedifferentiation of chondrocytes [14]. In the case of endothelial cells, laminar (unidirectional) shear stress, as is normally encountered in healthy blood vessels, suppresses YAP/TAZ activation, whereas oscillatory (disturbed or turbulent) shear stress, which is associated with choked blood vessels, enhances YAP/TAZ activation [15–17]. These findings have major implications for the pathogenesis of arteriosclerosis [15–17].

The signaling pathways of some cell-surface receptors of extracellular ligands, such as lipids, hormones and growth factors, can also activate YAP/TAZ through interactions with the Hippo signaling pathway (Fig. 1). These ligands include lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), glucagon and epinephrine, which act through G protein-coupled receptors (GPCRs) [18, 19], as well as the receptor tyrosine kinases (RTKs) for nerve growth factor (NGF) and epidermal growth factor (EGF) [20, 21].

Cellular responses to microenvironmental stress factors have also been linked to YAP/TAZ via interaction of various cytosolic factors with the Hippo signaling pathway (Fig. 1). Energy stress induced by inhibitors of glucose or ATP metabolism decreases YAP/TAZ activation through the Hippo signaling pathway via AMPK (AMP-activated protein kinase) [22, 23]. It was reported that osmotic stress promotes YAP translocation to cell nuclei via Nemo-like kinase (NLK)-mediated phosphorylation of the Ser128 residue [24], while hypoxic stress activates YAP through the action of SIAH2 ubiquitin E3 on the Hippo signaling pathway [25].

Pro-inflammatory cytokines, such as prostaglandin E2 and tumor necrosis factor alpha (TNF- α), also activate YAP/TAZ, but the underlying mechanisms remain unclear [26, 27]. YAP/TAZ are also activated in response to liver, skin,

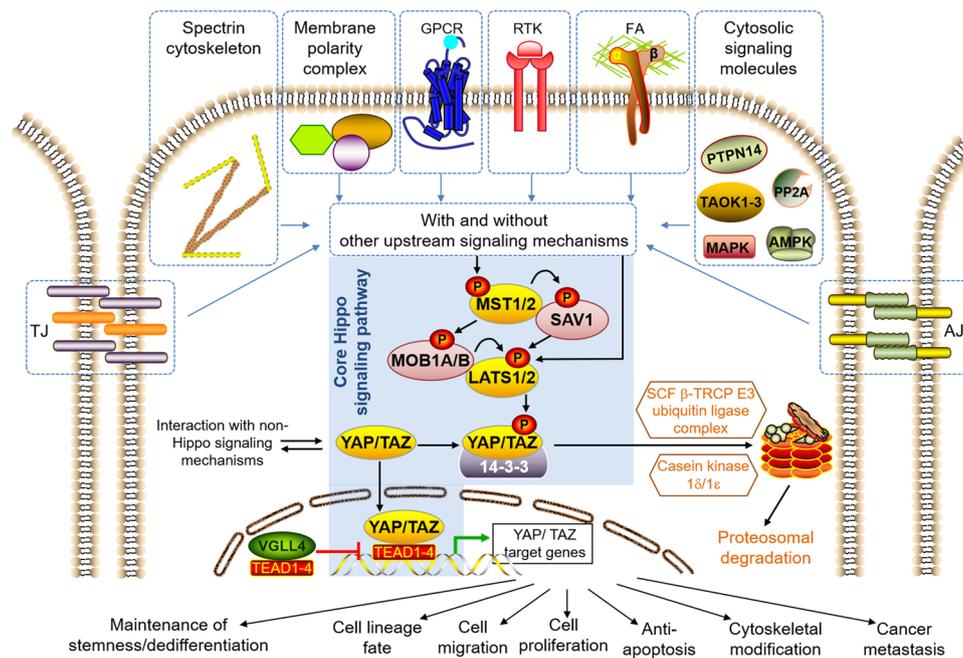


Fig. 1 The core of the canonical Hippo signaling pathway is a kinase cascade that in mammals comprises the MST1/2 complex (MST1 & MST2, also known as STK4 & STK3, respectively), LATS1/2 complex (LATS1 & LATS2, large tumour suppressor kinase 1 & 2, respectively), the adaptor proteins: SAV1 (Salvador 1), MOB1A and MOB1B, the transcriptional co-activators YAP/TAZ, the 14-3-3 protein that binds only to phosphorylated YAP/TAZ, and finally the TEAD transcription factors (TEAD1–TEAD4) that activate the transcription of specific genes upon binding to unphosphorylated YAP/TAZ. The Hippo signaling pathway can be initiated upon phosphorylation of MST1/2 or LATS1/2 by various upstream signaling mechanisms that may involve Focal adhesions (FAs), G protein-coupled receptors (GPCRs), Receptor tyrosine kinases (RTKs), Adherens junctions (AJs), Tight junctions (TJs), Spectrin cytoskeleton, Membrane polarity complexes (i.e. Crumbs, Scribble, aPKC-PAR) and various cytosolic signaling molecules (i.e. PP2A, TAOK1/2/3, MAPK, AMPK, and PTPN14). The activated MST1/2 complex phosphorylates the adaptor proteins SAV1, MOB1A and MOB1B, which in turn assist the MST1/2 complex in recruiting, phosphoryl-

ating and activating the LATS1/2 complex. Nevertheless, it must be noted that the LATS1/2 complex is not necessarily activated only by the MST1/2 complex, but can also be phosphorylated and activated by other upstream signaling mechanisms. Upon activation, the LATS1/2 complex phosphorylates YAP/TAZ, which then binds to the 14-3-3 protein. This in turn prevents the phosphorylated YAP/TAZ from being translocated into the cell nuclei, and it is then targeted for proteasomal degradation through further phosphorylation by casein kinase 1 $\delta/1\epsilon$ and ubiquitination by the SCF β -TRCP E3 ubiquitin ligase. By contrast, when MST1/2 and LATS1/2 are not activated, the unphosphorylated YAP/TAZ remains active and is translocated to the cell nuclei where it binds TEAD transcription factors (TEAD1 to TEAD4) to activate the transcription of specific output genes, which may be involved in cell lineage fate, cell proliferation, cell migration, anti-apoptosis, cancer metastasis, cytoskeletal modification, ‘stemness’ maintenance and dedifferentiation. VGLL4 acts as a competitive inhibitor of YAP/TAZ binding to TEAD, resulting in repression of target gene expression

myocardial and intestinal epithelium injury [28–30], and facilitate healing by enhancing cell proliferation. However, the underlying mechanisms through which YAP/TAZ are activated upon tissue injury are still largely uncharacterized.

Regulatory mechanisms of YAP/TAZ activity

Regulation of YAP/TAZ activity through the canonical Hippo signaling pathway

Core components of the canonical Hippo signaling pathway

The canonical Hippo signaling pathway is highly conserved in different species ranging from fruitflies to mammals [31],

and was so named because deletion mutants in *Drosophila melanogaster* led to loss of control of tissue/organ size, which in turn resulted in a superficial resemblance to the hippopotamus. The core of the canonical Hippo signaling pathway (Fig. 1) is a kinase cascade that in mammals consists of the MST1/2 complex (MST1 and MST2, also known as STK4 and STK3, respectively), LATS1/2 complex (LATS1 and LATS2, large tumor suppressor kinases 1 and 2, respectively), the adaptor proteins SAV1 (Salvador 1), MOB1A and MOB1B, the transcriptional co-activators YAP/TAZ, the 14-3-3 protein that binds only to phosphorylated YAP/TAZ, and finally the TEAD transcription factors (TEAD1–TEAD4) that activate the transcription of specific target genes (Table 1) upon binding to unphosphorylated YAP/TAZ.

Table 1 Examples of YAP/TAZ effector genes that are activated via TEAD

Function	Genes activated by YAP/TAZ via TEAD	Key references
Stemness/Dedifferentiation	C-Myc	Schutte et al. [114]
	Sox2, Oct4 & Nanog	Lian et al. [115]
Anti-apoptosis/cell survival	CTGF, ANKRD, BCL2 & CYR61	Wang et al. [116]
	MCL-1	Tian et al. [117]
Cell lineage fate decisions/differentiation	Cdx2	Yagi et al. [118]
	MSTN & MyoG	Lv et al. [119]
	GATA3	Ralston et al. [120]
	Myf5	Ribas et al. [121]
Cell migration	CDH2, MACF1, ABL2 & TNS3	Liu et al. [122]
Cytoskeleton/morphology	ARHGAP29	Qiao et al. [123]
Cell proliferation/Cell cycle/Tumorigenesis	MCM3, MCM6 & Cdk1	Ehmer et al. [124]
	CDC6, CDT1, MCM4 & MCM10	Shen and Stanger [125]
	MCM7	Lo Sardo et al. [126]
	RHAMM	Wang et al. [127]
	CTGF	Zhao et al. [128]
	Cyr61	Zhang et al. [129].
	AXL	Xu et al. [130]
	EDN1	Zhang et al. [131]

The Hippo signaling pathway is initiated upon activation (phosphorylation) of MST1/2 by various upstream signaling factors (Fig. 1). The activated MST1/2 complex phosphorylates the adaptor proteins SAV1, MOB1A and MOB1B, which in turn assist the MST1/2 complex in recruiting, phosphorylating and activating the LATS1/LATS2 complex. It should be noted that the LATS1/LATS2 complex is not necessarily activated only by the MST1/2 complex, but can also be phosphorylated and activated by a whole multitude of cytosolic factors that are implicated in other signaling pathways. Hence, there is a rather high degree of overlap with other signaling pathways.

Upon activation, the LATS1/LATS2 complex phosphorylates YAP/TAZ, which then bind to the 14-3-3 protein. This in turn prevents the phosphorylated YAP/TAZ from being translocated to the cell nucleus, and they are then targeted for proteasomal degradation through further phosphorylation by casein kinase 1 δ /1 ϵ and ubiquitination by the SCF β -TRCP E3 ubiquitin ligase complex [32]. In contrast, when MST1/2 and LATS1/2 are not activated, the unphosphorylated YAP/TAZ are translocated to the cell nucleus, where they bind to TEAD transcription factors to activate the transcription of specific target genes (Table 1).

Hence, when the core Hippo signaling pathway is switched 'ON', YAP/TAZ are inactivated through phosphorylation, and their translocation to the cell nucleus is blocked. On the other hand, when Hippo signaling is switched 'OFF', the non-phosphorylated YAP/TAZ can be translocated to the cell nucleus, where they bind to TEAD transcription factors and activate the transcription of specific

target genes (Table 1). Nevertheless, it is important to note that in reality, the Hippo signaling pathway does not behave digitally only in the 'ON' or 'OFF' mode. Depending on the relative activities of MST1/2, LATS1/2, and other cytosolic factors involved in the activation/deactivation of these two complexes (Fig. 1), YAP/TAZ localization may be partially cytoplasmic or partially nuclear. The situation is further complicated by the existence of competitive inhibitors of YAP/TAZ binding to TEAD, such as VGLL4 [33], which represses target gene expression. Hence, depending on the cell type and biological context, variations in the relative activity of the core Hippo signaling cascade and YAP/TAZ may arise from differing balance in the activity/or quantity of the various regulatory kinases, which can function in a complementary manner and which may substitute each other. This in turn would have critical implications for the practical implementation and data interpretation of YAP/TAZ assays.

YAP/TAZ regulation by focal adhesions through the Hippo signaling pathway

Focal adhesions (FAs) are integrin-containing macromolecular assemblies that form a mechanical linkage between the extracellular matrix and cytoskeleton via intracellular actin bundles (stress fibers) [7, 9]. As a consequence of their structural role, FAs are the primary conduits for cellular sensing of ECM/substrata stiffness [7, 9], in addition to being sensors of cell density [34]. At low cell density, cells tend to be more spread out on the substrata surface and have

higher numbers of FAs and associated stress fibers; whereas at high density, cells are more rounded and compact, with fewer FAs and associated stress fibers [34].

Different cell types, such as epithelial and mesenchymal cells, will exhibit different responses to stiff and soft substrates, as well as to high and low cell densities which can be attributed to varying combinations and quantities of TEAD transcription factors present in different cell types that can potentially be activated by YAP/TAZ. Additionally, complex interaction and cross-talk between the core Hippo signaling cascade (Fig. 1) with other signaling pathways in different cell types, will undoubtedly lead to variations in the signaling mechanisms by which ECM/substrata stiffness or cell density is transduced by FAs to modulate YAP/TAZ activity in different cell lineages.

Under conditions of stiff/fibronectin-rich ECM or low cell density, the high tensile force at the interface between FAs and stress fiber is detected by β 1-integrin, leading to sequential phosphorylation of FAK (focal adhesion kinase), SRC (steroid receptor co-activator) and/or P130 Cas via integrin-linked kinase (ILK) [35] (Fig. 2a). These subsequently trigger two distinct parallel signaling pathways that regulate YAP activation: (i) the β 1-integrin-FAK-SRC-PI3K-PDK1 signaling pathway that has been reported for epithelial cell types [36–38], and (ii) the β 1-integrin-FAK-P130-Cas-Rac1-PAK-Merlin pathway that has been reported for mesenchymal cells [39] (Fig. 2a). Activation of either signaling pathway inhibits LATS1/2 activity [36–39], thereby enhancing the nuclear translocation of YAP/TAZ and subsequent transcriptional activation of specific target genes through TEAD transcription factors (Table 1).

On the other hand, with high cell density or soft ECM/synthetic substrata, cells do not spread much, and have fewer FAs per cell with reduced tensile force within the stress fibers connected to FAs. As a result, the two aforementioned signaling pathways are not activated and there is no inhibition of LATS1/2, leading to increased phosphorylation of YAP/TAZ and sequestration within the cytosol. Currently, it is unclear whether in other cell types, these two aforementioned signaling pathways emanating from FAs can act simultaneously in parallel to modulate YAP/TAZ activity.

YAP/TAZ regulation by adherens junctions (AJs) through the Hippo signaling pathway

AJs are macromolecular assemblies that mediate adhesion between cells, and mechanically link the actin cytoskeletons of adjacent cells [40]. They are primarily composed of proteins of the cadherin and catenin families, including α -catenin and β -catenin, which form mechanical linkages

with bundles of actin fibers that connect directly to the cytoskeleton [40]. In addition, proteins that play a role in Hippo signaling bind with AJs. These include Merlin (also known as neurofibromin 2 or NF2), which binds directly to α -catenin [41], and KIBRA (kidney and brain protein) that binds to Merlin [42]. As a result of their structural function in cell-to-cell contact and mechanical linkage of the cytoskeleton, AJs can serve as a means by which cells sense their density and initiate contact inhibition of proliferation [43].

Generally, the higher the cell density, the higher will be the number of AJs between adjacent cells. It is thought that Merlin (NF2) associated with AJs can bind and sequester LATS1/2 at the plasma membrane, which in turn facilitates LATS1/2 phosphorylation and activation by MST1/2 and MAP4Ks [44, 45]. Subsequent phosphorylation of YAP/TAZ by the activated LATS1/2 prevents their translocation to the cell nucleus, thereby leading to inactivation of target genes, some of which are involved in cell proliferation. This accounts for contact inhibition of cell proliferation at high cell densities. It has also been reported that homophilic dimerization of E-cadherin, which takes place during formation of AJs between adjacent epithelial cells, leads to phosphorylation of LATS1/2 and subsequent inhibition of YAP/TAZ translocation into the cell nucleus, thereby resulting in contact inhibition of proliferation [12]. The underlying mechanisms are unclear, but appear to involve NHERF (Na^+/H^+ exchange regulatory factor), Merlin, Kibra and LATS1/2 [12].

Dutta et al. [46] reported yet another signaling mechanism for contact inhibition of cell proliferation mediated by AJs. At high cell densities, increasing mechanical tension of actin stress fibers at AJs facilitates the recruitment of TRIP6 to AJs by vinculin, which subsequently binds to and phosphorylate LATS1/2, thereby inhibiting YAP/TAZ translocation into the cell nucleus [46].

Hence, increasing numbers of AJs and heightened mechanical tension within their associated actin stress fibers exert an opposite effect in downregulating YAP/TAZ activity, as compared to signaling mechanisms associated with FAs that promote YAP/TAZ activation. This in turn can be attributed to the opposite but complementary roles of AJs and FAs in sensing cell density and effecting contact inhibition of cell proliferation. AJs are localized at the interface between adjacent cells, whereas FAs by contrast, are localized at the interface between cells and ECM/substrata. Consequently, with higher cell density, there is an increasing number of AJs, but decreasing number of FAs per cell, which therefore explains their antagonistic effects on regulating YAP/TAZ activity that effects contact inhibition of proliferation at high cell densities.

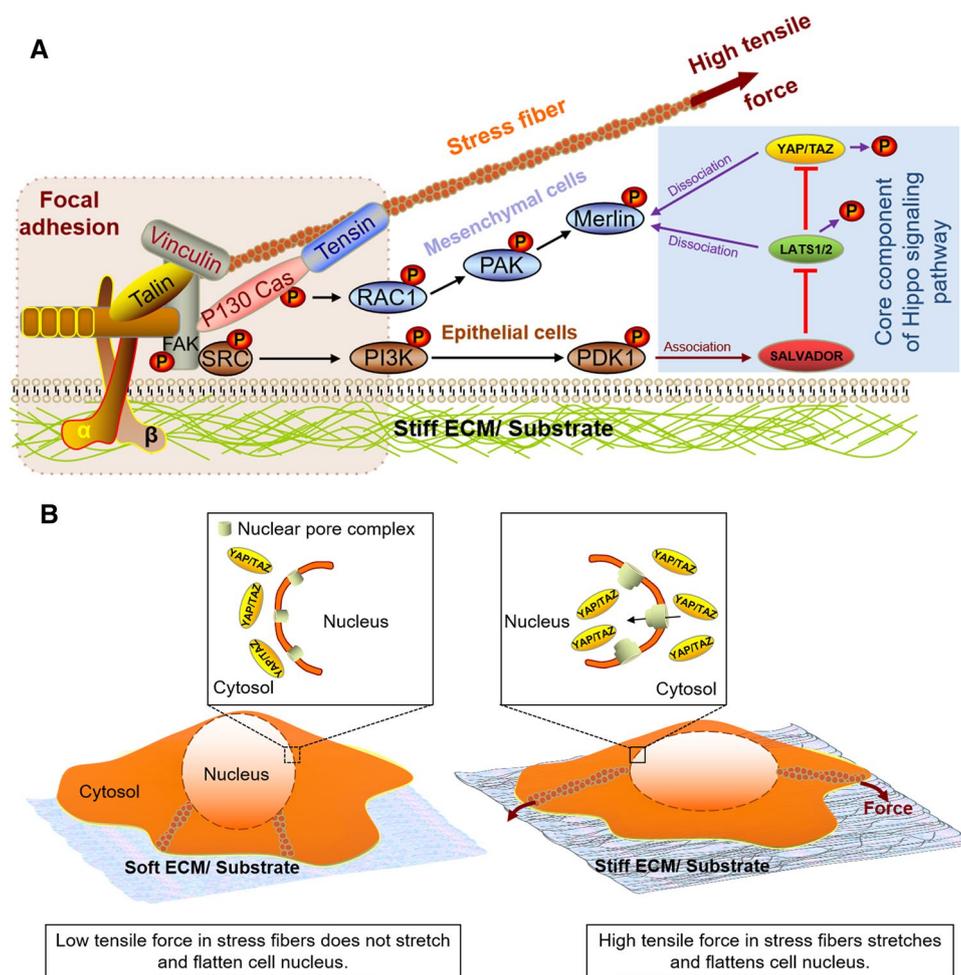


Fig. 2 The underlying mechanisms by which ECM/substrata stiffness or cell density is transduced by FAs to effect YAP/TAZ activation or inactivation involve both the Hippo-signaling pathway (a), as well as a Hippo-independent mechanism (b). In the Hippo-dependent mechanism (a), high tensile force at the interface between FAs and stress fibers with stiff substrata is detected by β 1-integrin, which leads to sequential phosphorylation of FAK (focal adhesion kinase), SRC (steroid receptor coactivator) and/or P130 Cas. In epithelial cells, phosphorylated SRC activates the PI3K–PDK1 signaling pathway. Upon activation, PDK1 associates with the core Hippo pathway-kinase complex through the scaffold protein Salvador, leading to inhibition of LATS1/2 phosphorylation of YAP, thereby enhancing its nuclear translocation [38]. On the other hand, in mesenchymal cells, the phosphorylated P130 Cas activates the Rac1–PAK–Merlin pathway. In the unphosphorylated state, Merlin normally interacts with both YAP and LATS1/2 via its C-terminal moiety and FERM domain, respectively, facilitating phosphorylation of both proteins. PAK1-mediated Merlin phosphorylation on Ser-518 reduces Merlin's interactions with both LATS1/2 and YAP1, resulting in YAP dephospho-

phorylation and nuclear translocation [39]. In the Hippo-independent mechanism (b), increased contractile force generated by the actinomyosin stress fibers when cells encounter a stiff substrate, flattens the cell nuclei. This in turn increases the curvature of the lateral part of the nuclear membrane, which enlarges the diameter of the nuclear pore on the cytoplasmic side of the nuclear membrane, while at the same time reducing the pore diameter at the opposite side. Such asymmetric deformation of the nuclear pores in turn favors nuclear import, rather than export, of YAP/TAZ. Additionally, it is also thought that increased curvature of the lateral portion of the nuclear membrane that occurs through cell nuclei flattening via contractile force of the stress fibers, exposes the inner surface of the nuclear pore to the cytosol, which in turn favors import rather than export of YAP/TAZ. This is thought to be mediated by the disorganized meshwork of flexible FG-nups comprised phenylalanine–glycine (FG) repeats on the inner lumen of nuclear pores, which facilitates protein unfolding and subsequent passage through the nuclear pore via repulsive interaction between the FG repeats and proteins

YAP/TAZ regulation by tight junctions (TJs) through the Hippo signaling pathway

Tight junctions (TJs), also known as occluding junctions, are the closely associated areas of two adjacent cells where the membranes are joined together via strands of transmembrane

proteins, such as claudins and occludins, to form an impermeable barrier. TJs are expressed exclusively by epithelial cells and function as a selective barrier to regulate the permeability of epithelial cell sheets to diffusing molecules. During differentiation and maturation of the epithelial cell, increasing numbers of TJs are formed between adjacent

cells, in tandem with increasing cell density within the epithelial sheet [47]. It has been shown that increasing formation of TJs during maturation of the epithelial cell reduces cell proliferation via downregulation of YAP/TAZ activity [13]. Paramasivam et al. [13] identified a tight-junction protein of the angiomin family, AMOTL2, which serves as an activator of LATS2 in the Hippo signaling cascade, leading to phosphorylation and sequestration of YAP within the cytosol. Subsequently, it was found that AMOTL2 can also bind to MST2, LATS2, and YAP, facilitating the sequestration and activation of Hippo pathway components at TJs. This in turn might lead to inhibition of YAP translocation into the cell nucleus, thereby leading to inhibition of cell proliferation in response to increasing numbers of TJs within the maturing epithelial cell sheet.

YAP/TAZ regulation by the spectrin cytoskeleton through the Hippo signaling pathway

Spectrins are filamentous proteins that are organized into a polygonal meshwork underneath mammalian cell membranes [48]. They play key roles in the formation of cell membrane microdomains, axonal growth, and synapse development [48]. Knockdown of spectrin in mammalian cells abrogates contact inhibition of proliferation through maintenance of YAP translocation into the cell nucleus [49, 50], via deactivation of LATS1/2 [50]. Currently, the underlying mechanisms are still unclear, but is thought to involve the role of spectrin as an actin cross-linking protein, which enables it to modulate YAP/TAZ activity by effecting tension within actin stress fibers [49, 50].

YAP/TAZ regulation by surface receptors of soluble ligands (GPCRs and RTKs) through the Hippo signaling pathway

Yu et al. [19] reported that YAP/TAZ are regulated by soluble ligands of G protein-coupled receptors (GPCRs), which are the targets of about 40% of pharmaceutical drugs on the market [51]. GPCRs are a family of cell-surface receptors characterized by seven transmembrane helical domains. Upon binding to their cognate ligands, intracellular signals are transduced through their heterotrimeric G-proteins (G_α , G_β & G_γ subunits) [51]. GPCRs can be classified into 4 groups according to their heterotrimeric G-proteins: $G_{12/13}$, $G_{q/11}$, $G_{i/o}$ and G_s . These groups are associated with different intracellular signaling pathways [51]. GPCRs that activate $G_{12/13}$, $G_{q/11}$, and $G_{i/o}$ inhibit LATS1/2 phosphorylation, thus leading to increased nuclear translocation of YAP/TAZ [19]. In contrast, GPCRs that activate G_s signaling enhance the phosphorylation of LATS1/2, leading to increased YAP/TAZ sequestration within the cytosol. The underlying mechanisms involved remain unclear, but Yu et al. [19] provided some evidence for the involvement of Rho GTPases

and actin cytoskeleton organization in mediating LATS1/2 phosphorylation by GPCRs.

Besides GPCRs, the receptor tyrosine kinase (RTK) family has also been implicated in YAP/TAZ regulation through the Hippo signaling pathway. Yang et al. [20] demonstrated that inhibition of the nerve growth factor (NGF) receptor tyrosine kinase (NTRK1) decreases YAP-driven transcription, proliferation and migration of cancer cells, through increased phosphorylation of LATS1/2. In contrast, Fan et al. [38] showed that activation of the epidermal growth factor receptor (EGFR), which also belongs to the RTK family, instead leads to increased translocation of YAP to the cell nucleus. This is achieved by activation of the PI3K-PDK1 (phosphoinositide 3-kinase-phosphoinositide-dependent kinase-1) signaling pathway by EGFR upon ligand binding, and this in turn inhibits MST1/2 phosphorylation of LATS1/2 [38, 52].

YAP/TAZ regulation by the aPKC-PAR, Crumbs and Scribble polarity complexes through the Hippo signaling pathway

Cell polarity is a distinctive feature of various cell lineages and is crucial for various developmental and physiological functions [53–58]. Membrane protein complexes that play key roles in cell polarity include aPKC-PAR [53–55], Crumbs [56, 57] and Scribble [58].

A number of studies have established that the aPKC-PAR (atypical protein kinase C-partitioning defective) polarity complex regulates YAP/TAZ activity via the Hippo signaling pathway. Archibald et al. [59] studied the mechanism by which elevated aPKC expression leads to malignant transformation in epithelial cells, and proposed a model in which aPKC binding to MST1/2 blocks its ability to phosphorylate LATS1/2. This in turn increases nuclear translocation of YAP, which leads to activation of proliferation-related genes. Zhou et al. [60] found that elevated expression of Par3, a key component of the PAR complex, is involved in prostate cancer metastasis. They proposed that Par3 inhibits phosphorylation of LATS1/2 by sequestering KIBRA, leading to enhanced nuclear translocation of YAP [60].

The Crumbs polarity complex also regulates YAP/TAZ activity via the Hippo signaling pathway. Szymaniak et al. [57] showed that Crb3, a Crumbs isoform, promotes the interaction between LATS1/2 and YAP at apical cell junctions, which in turn promotes YAP phosphorylation and retention within the cytoplasm. Narimatsu et al. [61] also showed that the Crumbs complex promotes phosphorylation and inactivation of YAP/TAZ, which in turn inhibits TGF β -induced Smad nuclear accumulation and activity.

Liu et al. [62] reported that the disks large homolog 5 (DLG5) protein facilitates the interaction of Scribble with MST1/2 and LATS1/2 in the core Hippo signaling cascade, which in turn promotes phosphorylation and

inactivation of YAP, resulting in inhibition of breast cancer cell proliferation.

YAP/TAZ regulation by other cytosolic signaling molecules (PP2A, TAOK1/2/3, MAPK, AMPK, PTPN14) through the Hippo signaling pathway

YAP/TAZ are also regulated by other cytosolic signaling molecules that enable overlap and cross-talk between the Hippo signaling pathway and other signaling pathways. Protein phosphatase 2A (PP2A) is a ubiquitously expressed serine threonine phosphatase that has diverse roles in tumor suppression, apoptosis, cell proliferation and signal transduction. It acts by dephosphorylating various cytosolic signaling molecules, such as Akt, p53, c-Myc and β -catenin. Bae et al. [63] showed that PP2A regulates YAP/TAZ through dephosphorylation of MST1/2, and its activity may be inhibited by SAV1, which is a part of the core Hippo signaling cascade. Thousand and one kinases 1/2/3 (TAOK1/2/3), which have diverse functions in various signaling pathways relating to inflammation, microtubule dynamics, and stress response, also have a role in modulating YAP/TAZ activity. Boggiano et al. [64] showed that TAOK1/2/3 regulate YAP/TAZ activity by facilitating phosphorylation of MST1/2 within the Hippo signaling cascade. MAP kinase kinase kinases (MAP4Ks) are a family of serine threonine kinases that regulate a diverse array of biological processes including cell survival, proliferation, motility and differentiation, and also regulate YAP through the Hippo signaling pathway [65]. Meng et al. [65] showed that several members of the MAP4K family act in parallel to MST1/2 to activate LATS1/2 within the Hippo pathway, leading to increased YAP phosphorylation and sequestration in the cytosol. Hence in this respect, MAP4Ks functionally overlap in part with MST1/2. Adenosine monophosphate kinase (AMPK) is a key regulator of cellular metabolism and a sensor of energy/nutrient stress. Mo et al. [23] showed that in the presence of nutrient starvation or energy stress, AMPK activates LATS1/2, leading to YAP phosphorylation and inhibition of nuclear translocation. They further showed that AMPK can also phosphorylate YAP directly at the Ser 94 residue, which is essential for its binding to TEAD, thus inhibiting target gene activation by blocking YAP-TEAD interaction. A notable example is the inhibition of gene expression of glucose-transporter 3 (GLUT3), which is involved in glucose metabolism [66]. In addition to AJs and TJs, non-receptor tyrosine phosphatase 14 (PTPN14) also mediates contact inhibition of cell proliferation by regulating YAP/TAZ activity through the core Hippo signaling pathway [67, 68]. At high cell densities, PTPN14 is abundantly expressed and forms a complex with KIBRA, which activates the core Hippo signaling cascade to phosphorylate YAP/TAZ via

MST1/2 and LATS1/2, and this in turn represses TEAD-specific genes involved in cell proliferation [67, 68].

Regulation of YAP/TAZ activity through Hippo-independent mechanisms

Hippo-independent regulation of YAP/TAZ through stress fibers connected to focal adhesions (FAs)

Besides FA-mediated mechanotransduction through the canonical Hippo signaling pathway (Fig. 1), there is also a Hippo-independent pathway mediated through contractile force generated by actinomyosin stress fibers, which directly connect FAs to the apical surface of the cell nuclei via the LINC (linker of the nucleoskeleton and cytoskeleton) complex of the nuclear envelope [69]. Elosegui-Artola et al. [69] proposed that increased contractile force generated by the actinomyosin stress fibers when cells encounter a stiff substrate flattens the cell nuclei (Fig. 2b). This in turn increases the curvature of the lateral part of the nuclear membrane, which induces asymmetric morphological changes to the openings of nuclear pores on both the cytoplasmic and nuclear side of the membrane. This in turn favors nuclear import, rather than export, of YAP/TAZ (Fig. 2b). It is hypothesized that morphological changes to the nuclear pore exposes the disorganized meshwork of flexible FG-nups comprising phenylalanine–glycine (FG) repeats [70] on the inner lumen of the nuclear pores to the cytosol, facilitating protein unfolding and subsequent passage of YAP/TAZ through the nuclear pore via repulsive interaction between the FG repeats and the protein.

Hippo-independent regulation of YAP/TAZ through adherens junctions (AJs) and tight junctions (TJs)

As previously discussed, AJs mechanically link the cytoskeleton of adjacent cells, and serve as a means by which cells sense and react to their density via the Hippo-signaling pathway through the AJ-associated protein Merlin (also known as neurofibromin 2 or NF2). Although Merlin is closely associated with the Hippo signaling pathway, it is not considered to be a central component of the core signaling Hippo signaling cascade (Fig. 1). Besides the mechanism described in 2.1.3, it was also reported that Merlin can act independently of the core Hippo signaling cascade to repress YAP/TAZ activation [71]. At high cell densities, increased tensile force exerted on the actomyosin-based stress fibers tethered at AJs induces dissociation of Merlin from AJs. Subsequently, the dissociated Merlin is translocated to the cell nucleus, where it directly binds and forms a complex with YAP. This Merlin-YAP complex is translocated from the cell nuclei to the cytosol via the nuclear export signals of Merlin, leading to repression of

TEAD-specific genes involved in cell proliferation and thus, contact inhibition of proliferation.

As previously discussed, TJs regulate YAP activity through the Hippo signaling pathway via AMOTL2. On the other hand, Domínguez-Calderó [72] found that TJs can also regulate YAP activity through a Hippo-independent mechanism via the tight-junction protein zona occludens 2 (ZO-2), which binds to and sequesters YAP within the cytosol. Upon ZO-2 gene silencing, hypertrophy of renal cells was observed concurrently with increased YAP accumulation within the cell nuclei [72].

Hippo-independent regulation of YAP/TAZ through the Wnt- β -catenin pathway

The regulation of β -catenin through a cytoplasmic destruction complex forms the crux of the Wnt signaling cascade [73]. This β -catenin destruction complex is a multi-protein complex that includes Axin, adenomatous polyposis coli (APC), glycogen synthase kinase-3 (GSK3), and casein kinase 1 (CK1) [73]. When Wnt signaling is inactive, this destruction complex captures not only cytosolic β -catenin [73], but also cytosolic YAP [74], leading ultimately to degradation by the β -TrCP ubiquitin ligase. The activation of Wnt signaling inactivates the destruction complex and allows escape of both β -catenin [73] and YAP [74] from degradation, which in turn enables their translocation and accumulation in the cell nucleus.

Hippo-independent regulation of YAP/TAZ through methylation and phosphorylation

SET7 (also known as SETD7) is a SET-domain-containing lysine methyltransferase that methylates and alters the function of a variety of proteins. Oudhoff et al. [75] demonstrated that SET7 methylates the lysine 494 residue of YAP, which inhibits its translocation to the cell nucleus and results in its sequestration within the cytosol. Knockout of SET7 in mice resulted in a larger progenitor compartment in the intestine, concomitantly with increased expression of YAP target genes.

As previously discussed, PTPN14 forms a complex with KIBRA protein to regulate YAP/TAZ activity via the core Hippo signaling pathway. Liu et al. [76] showed that PTPN14 can also directly phosphorylate and inactivate YAP, through interaction between the WW domain of YAP and the PPxY domain of PTPN14. The presence of a YAP-PTPN14 complex was validated by co-immunoprecipitation [76].

Hippo-independent regulation of YAP/TAZ through the Notch signaling pathway

The Notch signaling pathway is a major signaling pathway with diverse biological functions, which regulates

cell-to-cell communications in a juxtacrine manner, via interaction between Notch receptors (Notch 1 to 4 isoforms) and their corresponding ligands (JAG1, JAG2, DLL1, DLL3, and DLL4) expressed on adjacent cells [77]. Notch receptor-ligand binding triggers proteolytic cleavage and subsequent release of the Notch intracellular domain (NICD), which translocates into the cell nucleus, where it binds and activates the transcription factor RBPJ (recombining binding protein suppressor of hairless), as well as the nuclear effector Mastermind-like (MAML), thereby initiating transcription of Notch target genes [77].

Although, it is well-known that there is much cross-talk between YAP/TAZ and the Notch signaling pathway [78], and numerous examples whereby these can work synergistically together to regulate the expression of various genes and hence biological processes [78], there have only been a few studies to date, which have reported upstream regulation of YAP/TAZ activity via the Notch signaling pathway [79–81]. In the study of Li et al. [79] on murine neural stem cells, it was shown by gain- and loss-of-function experiments that the Notch signaling pathway exert positive upstream control of YAP activity, which in turn regulated the proliferation of these cells. Further investigations revealed that the RBPJ transcription factor of the canonical Notch signaling pathway directly control transcription of the YAP1 protein by binding to its promoter sequence [79]. Although, Li et al. [79] observed that RBPJ could also bind to the promoter sequence of TEAD2, this by itself was insufficient to initiate transcription of TEAD2. Very similar results were reported by the study of Slemmons et al. [80] on human rhabdomyosarcoma cells, which also utilized gain- and loss-of-function experiments to demonstrate positive regulation of YAP activity by the Notch signaling pathway. Again, it was demonstrated that RBPJ can bind directly to the YAP promoter and activate its transcription. Additionally, Slemmons et al. [80] showed that the Notch signaling pathway can also facilitate YAP nuclear translocation, but the underlying mechanism of how this occurs is currently unknown, and it is unclear whether this involves the core Hippo signaling cascade. Contradictory results were, however, reported by the study of Lu et al. [81] on murine hepatic stem cells. Although, RBPJ was again shown to bind directly to the YAP promoter, it repressed rather than activated transcription of the YAP protein [81]. This in turn led to Notch signaling promoting the differentiation of hepatic stem cells, while at the same time inhibiting their proliferation [81] by reducing YAP activity. Hence, it is likely that specific upstream regulation of YAP/TAZ activity by Notch signaling may vary according to the particular cell type, being dependent on complex interactions with other signaling processes present in different cell lineages.

Hippo-independent regulation of YAP/TAZ through the TGF- β signaling pathway

Transforming growth factor beta (TGF- β) with five known isoforms (TGF- β 1 to 5), together with other members of the TGF- β superfamily, i.e. bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activin and inhibin, play key roles in numerous diverse biological processes, such as embryonic development, cell proliferation, differentiation and migration [82]. Nevertheless, the focus here will be solely on the TGF- β signaling pathway [83].

As in the case of the Notch signaling pathway, the TGF- β signaling pathway is also known to have extensive cross-talk and interactions with YAP/TAZ, in the regulation of numerous genes and biological processes [84, 85]; but again, there are few studies that have reported upstream modulation of YAP/TAZ activity by the TGF- β signaling pathway. It must be noted that to date, studies have reported that TGF- β signaling can only exert upstream regulation of TAZ, but not YAP [86, 87], even though it is known that YAP can promote TGF- β signaling by binding to and facilitating the nuclear translocation of SMAD2/3 [88]. For example, in the study of Miranda et al. [86] on rodent mesenchymal and epithelial cell lines, it was shown that TGF- β signaling can induce only robust expression of TAZ, but not YAP. Further investigations revealed that the underlying mechanism was independent of SMAD2/3, and involved sequential activation of p38 MAPK and its major downstream target MK2 by TGF- β signaling, which in turn activates myocardin-related transcription factor (MTRF) that drives the TAZ promoter in a CC(A/T-rich)6GG (CArG) box-dependent manner and induced TAZ protein expression [86]. Additionally, Miranda et al. [86] also identified a positive feedback loop mechanism that amplified TGF- β -induced TAZ expression. This involved potentiation of Nox4 expression by MTRF, with NOX4 in turn enhancing MTRF activation via phosphorylation, thereby amplifying TGF- β -induced TAZ expression [86]. The study of Wang et al. [87] on murine hepatic stellate cells uncovered another mechanism of upstream regulation of TAZ activity by the TGF- β signaling pathway, at the post-translational level. Utilizing co-immunoprecipitation, immunofluorescence, and nuclear fractionation assays, Wang et al. [87] showed that TGF- β 1 signaling promoted binding of SMAD2/3 and TAZ to the co-activator p300, leading to formation of a p300/SMAD2/3/TAZ heterocomplex that more readily translocate into the cell nucleus. Hence in this case, the p300 co-activator induced by TGF- β 1 signaling, acted as a shuttle for the nuclear transport SMAD2/3 and TAZ, as both proteins lacked nuclear localization signals (NLS). Further investigations by Wang et al. [87] revealed that both the protein scaffolding function and acetyltransferase activity of p300 were essential for its nuclear transport function, and that p300 played no role in YAP nuclear translocation.

Interaction of YAP/TAZ with TEAD transcription factors

As already noted, YAP/TAZ controls the expression of target genes primarily by acting as a co-activator of TEAD transcription factors. There are four isoforms of TEAD in mammals (TEAD1–4), all of which contain a highly conserved TEA DNA-binding domain (DBD) and a YAP-binding domain (YBD), separated by a proline-rich region (PRR) [89]. There is evidence that early mammalian development may be associated with distinct spatio-temporal expression patterns of TEAD1–4 [90]. The various target genes regulated by YAP/TAZ through TEAD1–4 have been linked to a diverse array of biological processes. These can be broadly classified into the following six categories [91]: (i) cell proliferation, cell cycle and tumorigenesis, (ii) cell migration, (iii) stemness/dedifferentiation, (iv) cell lineage fate determination and differentiation, (v) cytoskeleton and cell morphology, and (vi) anti-apoptosis and cell survival (Table 1). The YAP/TAZ regulation of TEAD1–4-activated target genes appears to be precisely controlled in a tissue- and cell type-specific manner, possibly through expression of specific TEAD isoforms in different tissue/cell types [90]. Epigenetic mechanisms may also be involved, since there is evidence that YAP/TAZ activity can be mediated by various chromatin complexes, including the NCOA6 histone methyltransferase complex and SWI/SNF chromatin remodeling complex [92, 93]. Currently, the precise mechanisms by which YAP/TAZ mediates tissue/cell-type specific activation of target genes via TEAD1–4 remain unclear.

Some nuclear proteins can regulate YAP/TAZ activity by modulating their binding interaction with TEAD transcription factors within the cell nucleus. Two prominent examples are p38 MAPK (mitogen-activated protein kinase) and VGLL4. In the presence of cellular stresses, such as hyperosmolarity, high cell density, and cell detachment, increased shuttling of TEAD from the cell nucleus to the cytosol takes place [94]. Subsequently, it was found that these cellular stresses activate p38 MAPK, which in turn binds to TEAD and drives the translocation of TEAD from the nucleus to the cytosol, thus repressing the expression of YAP/TAZ target genes [94]. This translocation of TEAD does appear to involve p38 MAPK-mediated phosphorylation of TEAD. However, p38 MAPK has no binding interaction with YAP/TAZ [94]. VGLL4 blocks the YAP-TEAD interaction by competing directly with YAP for binding to TEAD [33, 95]. In addition, Lin et al. [96] found that p300-mediated acetylation of VGLL4 disrupts its interaction with TEAD1 in the neonatal heart. This results in increased expression of target genes activated by YAP-TEAD1 interaction, which promotes the growth of heart tissue. On the other hand, disruption of VGLL4

acetylation led to suppression of YAP–TEAD binding interaction by VGLL4, slowing the growth of heart tissue [96].

Interaction of YAP/TAZ with non-TEAD transcription factors

Although TEAD1–4 are the major transcription factors that interact with YAP/TAZ to regulate the expression of specific target genes in the Hippo signaling pathway, it is also known that YAP/TAZ can also bind to and modulate the activity of various other non-TEAD transcription factors. These regulate a diverse array of biological processes, with YAP/TAZ demonstrating different effects on different cell types. Nevertheless, there are some commonalities among different cell types, particularly in the case of major key signaling pathways. For example, it is well-known that YAP/TAZ binding to the transcription factor Smad2/3 mediate cross-talk between the Hippo and TGF- β signaling pathways in diverse cell types ranging from epithelial, mesenchymal, keratinocyte and colon cancer cell lines [88, 97].

Very often, YAP and TAZ interaction with the same transcription factor, modulate different signaling pathways in different cell types, thus eliciting different biological effects, for example, in the case of RUNX2. Brusgard et al. [98] demonstrated that the binding interaction of RUNX2 with TAZ initiated ectodomain shedding of an oncogenic soluble E-cadherin fragment (sE-Cad), which worked synergistically with human epidermal growth factor receptor-2 (HER2/ErbB2) to stimulate the growth of breast cancer cells; whereas, Lin et al. [99] reported that RUNX2 binding interaction with YAP can inhibit osteogenic differentiation of mesenchymal stem cells, and that this inhibition can be overcome through competitive binding of YAP with AP2a.

Many of the non-TEAD transcription factors that have binding interactions with YAP/TAZ are implicated in oncogenesis and cancer metastasis. For example, Tomlinson et al. [100] and Roperch et al. [101] established that apoptosis can be regulated by the binding of YAP/TAZ with the p53-related proteins p63 and p73. Bora-Singhal et al. [102] showed that YAP can bind to and enhance OCT4 activity, which in turn upregulated SOX2 expression to promote self-renewal and vascular mimicry of stem-like cells from non-small cell lung cancer. Kuser-Abali et al. [103] identified YAP as a physiological binding partner and positive regulator of androgen receptors in prostate cancer. Liu et al. [104] showed that the binding of YAP with PRDM4 induced leukocyte-specific integrin β 2 (ITGB2) expression in cancer cells, which in turn promoted cell invasion through the endothelium in a similar manner to leukocytes.

Conclusion

Recent years have seen an exponential growth of scientific knowledge on the intricate molecular mechanisms regulating YAP/TAZ and their associated signaling pathways. Despite commonalities in the various upstream and downstream signaling pathways related to YAP/TAZ, it should be noted that the specific biological effects elicited by YAP/TAZ ultimately depend on the specific cell type and lineage in question, simply because different cell types and lineages have variable amounts and proportions of diverse signaling and effector molecules that interact with YAP/TAZ. For example, variable proportions of the TEAD 1 to 4 isoforms in different cell types lead to activation of different subsets of effector genes by YAP/TAZ. Moreover, different cell types and lineages may have differing sensitivity to YAP/TAZ, so that what may be considered ‘low’ YAP/TAZ activity in one particular cell type or lineage, may in fact work as ‘high’ or ‘moderate’ activity in another cell type or lineage.

New insights into the regulatory mechanisms of YAP/TAZ can potentially lead to novel therapeutic applications. First, they may offer clues to solving one of the most difficult and intractable challenges in tissue engineering and regenerative medicine, i.e., to quickly obtain sufficient numbers of tissue-specific adult stem cells or progenitors for transplantation therapy. Due to the key roles of YAP/TAZ in activating the proliferation and self-renewal of various adult stem cell lineages during the regeneration process, and in maintaining the ‘stemness’ of these cells [105, 106], it may be useful to employ high-throughput drug screening technology to screen various natural-product-derived or artificially synthesized small molecules for the capacity to activate proliferation or enhance ‘stemness’ of specific adult stem cell types via upregulation of YAP/TAZ activity (e.g., by utilizing fluorescent reporter genes). Such newly identified small molecule drugs might not only find application to facilitate the expansion of transplantable adult stem/progenitor cells *in vitro*, but also might be delivered into the human body directly or via controlled release from biomedical implants to aid tissue regeneration via activation and mobilization of the body’s endogenous pool of adult stem cells.

Second, new knowledge on YAP/TAZ should help to improve the biomimetic properties and therapeutic efficacy of implant materials. There are already much data on how the biomechanical properties of substrata, such as stiffness [9–11] and topography (i.e. micro- and nanopatterning) [107–111], can modulate YAP/TAZ activity. Much research has been focused on the role of YAP/TAZ in the mechanosensing of substrata biomechanical properties via both Hippo-dependent and Hippo-independent

mechanisms. Increasing knowledge of how YAP/TAZ activity correlates with the healing and regeneration processes at specific tissue/organ sites, may enable us to fine tune the biomechanical properties of implant materials. For example by screening the YAP/TAZ activity of relevant cell lineages cultured on newly-developed biomaterials in vitro.

Third, the rapidly progressing field of synthetic biology may open up intriguing new possibilities for applying our increasing knowledge of YAP/TAZ in hitherto unimagined ways. Synthetic biology is the science of creating and engineering artificial biological systems (often for therapeutic applications) through reassembling biological items with known functions (i.e. genes, proteins) in a systematic and rational manner [112, 113]. Because YAP/TAZ are key mediators of cellular interaction with the microenvironment, including (i) biomechanical cues [9–17], (ii) extracellular ligands, such as growth factors and lipids [18–21], (iii) energy, osmotic and hypoxic stress [22–25], and (iv) inflammation and tissue injury [26–30], it is possible that YAP/TAZ and related effector and signaling molecules could be integrated into complex synthetic gene networks that would activate the transcription of specific therapeutic transgenes in response to changes in YAP/TAZ activity [112, 113]. Alternatively, synthetic gene circuits might be designed to activate YAP/TAZ in response to specific stimuli, such as molecular markers of tissue injury or metabolic stress. We hope that the present review will inspire new advances in these and other areas.

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