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Review

YAP/TAZ as mechanosensors and mechanotransducers in regulating organ size and tumor growth

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ABSTRACT

Organ size is controlled by the concerted action of biochemical and physical processes. Although mechanical forces are known to regulate cell and tissue behavior, as well as organogenesis, the precise molecular events that integrate mechanical and biochemical signals to control these processes are not fully known. The recently delineated Hippo-tumor suppressor network and its two nuclear effectors, YAP and TAZ, shed light on these mechanisms. YAP and TAZ are proto-oncogene proteins that respond to complex physical milieu represented by the rigidity of the extracellular matrix, cell geometry, cell density, cell polarity and the status of the actin cytoskeleton. Here, we review the current knowledge of how YAP and TAZ function as mechanosensors and mechanotransducers. We also suggest that by deciphering the mechanical and biochemical signals controlling YAP/TAZ function, we will gain insights into new strategies for cancer treatment and organ regeneration.

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1. Introducing YAP/TAZ and Hippo signaling network

1.1. The fast emergence of the Hippo signaling network – a brief retrospective

Over the past 8 years, a new signaling pathway named Hippo tumor suppressor pathway has emerged at an unprecedented speed [1–3]. The orthology and high evolutionary conservation among *Drosophila* fly and mammalian genes, which encode the main components of this pathway, were critical in facilitating the fast pace of the discovery process [2]. The parallel delineation of the Hippo pathway in genetically amenable *Drosophila* flies and mammalian models was, in a way, reminiscent of the successful deciphering of the *Sevenless* pathway, which regulates eye development in the fly and is orthologous to the growth regulating epidermal growth factor pathway [4–6].

The rapid emergence of the Hippo pathway was further driven by a wealth of published biochemical, structural and signaling data that originated from an intense wave of research frequently referred to as the "oncogene revolution" [7]. Here, a number of well-defined complexes were identified from among signaling proteins, and their robust cellular and transcriptional "read-outs" were described in detail. Moreover, modular protein domains and their cognate ligand motifs were characterized, at both functional and structural levels, as regions of protein–protein interaction [8,9]. Often, these structures were solved at a high atomic resolution, which allowed for their use in modeling and simulation studies. Subsequently 'loss of function' mutations were being predicted and these predictions turned out to be useful for the validation of various signals [9]. In more recent years, a plethora of powerful transgenic mouse models became available that allowed for genetic validation of data generated through cell-line models.

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1.2. The essence of the Hippo pathway signaling – canonical and non-canonical

At its core, the mammalian Hippo pathway is composed of two kinases, namely MST and LATS. They control the activity of two closely related transcriptional co-activators YAP and TAZ [2,9]. When MST and LATS kinases respond to various upstream stimuli, the MST-activated LATS kinase phosphorylates YAP and TAZ at a specific Serine residue located in the amino-terminal region of each protein. Following this, 14-3-3 proteins recognize a signature motif around the LATS-phosphorylated Serine, which allows strong protein–protein interactions to be established. The resulting complex then "anchors" or sequesters YAP and TAZ within the cytoplasm [2,10]. If the LATS kinase is inhibited, then YAP and TAZ enter the cell nucleus and, after binding to TEA domain-containing transcription factors known as TEADs, drive a transcription program of overt proliferation [11]. YAP and TAZ themselves do not possess DNA-binding activity.

Today, the Hippo pathway is referred to as the Hippo network because it is subject to regulation by many membrane receptors, including G protein-coupled receptors (GPCRs), LIF receptor [12], E-cadherins [13]. Protein complexes of tight junctions and adherens junctions also play a role, as does the polarity complex 'Crumbs', as well as the LATS kinase [14–16]. A current convention among researchers of the Hippo network is that the regulation of YAP/TAZ *directly* via LATS is called "canonical signaling". This is in contrast to the term "non-canonical signaling", which has been used in a number of recent reports to describe signaling scenarios where YAP and TAZ are regulated independently of the LATS kinase. Although this may represent an oversimplification, the distinction remains useful, especially when discussing the Hippo network in the context of mechanotransduction (Fig. 1).

1.3. YAP and TAZ are of intense interest to biologists and biopharma

YAP and TAZ are the focus of many laboratories because of their remarkable roles in tissue homeostasis, organ development and oncogenic transformation [1–3]. These transcriptional co-activators are potent oncogenes when overexpressed [2,17,18]. Unlike other proto-oncogenes, YAP and TAZ are not usually mutated in cancers. However, as a result of their amplification or overexpression, or a lack of mechanisms that facilitate their cytoplasmic retention, the YAP and TAZ proto-oncogenes are often converted into potent oncogenes [1,17]. Biopsy screening from a number of cancers indicated that preferential nuclear localization of YAP/TAZ, or overall increased expression of YAP/TAZ in both the nucleus and the cytoplasm, is associated with a poor prognosis and reduced survival rate [1,19].

Several cancers, including liver cancer, frequently harbor an amplification of the YAP gene as part of the 11q22 amplicon [17]. Organ-specific overexpression of YAP in mice causes excessive organ growth. This is particularly relevant when the protein is overexpressed in the liver, as chronic overexpression in this organ results in hepatocellular carcinoma [2,17,18]. When expressed as transgenes in other mouse organs, YAP and TAZ impair differentiation, while increasing the content of stem and progenitor cells [3].

The main transcriptional target of YAP and TAZ is the TEAD family of transcription factors. These drive the expression of proliferative genes, as well as genes encoding inhibitors of apoptosis [11]. Libraries of small molecules have been screened for compounds that disrupt the YAP/TAZ-TEAD interface and attenuate the oncogenic function of YAP/TAZ [20,21]. Among successful "hits" is Verteporfin, a benzoporphyrin derivative, which is an approved drug, used clinically to treat macular degeneration [20]. Dobutamine is another drug that was shown to expel YAP from the cell nucleus [21]. More recently, PDZ motif-binding compounds were considered as potential inhibitors of YAP/TAZ nuclear translocation, and therefore inhibitors of their oncogenic activity [22]. It is expected that in the near future, new cancer drugs targeting the YAP/TAZ effectors will be identified, and tested preferentially in patients who harbor cancers with amplified or overexpressed YAP/TAZ genes. Innovative cancer drugs will most likely be developed based on the new insights we are gaining now from studying YAP/TAZ as mechanosensors and mechanotransducers.

1.4. Surprising findings illuminate the role of YAP/TAZ in contact inhibition of proliferation

Contact inhibition of proliferation (CIP) is a feature of a cell's normal "social" behavior [23]. CIP is deregulated in cancer and this is one of the major hallmarks of the neoplastic transformation of cells [23]. Interestingly, two laboratories have implicated YAP/TAZ in the CIP process. The team of Kun-Liang Guan has shown that in sparsely populated cells, YAP and TAZ remain in the nucleus where they drive proliferation. In densely populated cells, however, where contact between cells is maintained, YAP and TAZ are inactivated and localized in the cytoplasm [24]. The team of Stefano Piccolo added a new facet to the understanding of the Hippo network by reporting, in two notable publications [25,26], that a stiff matrix substrate will activate YAP and TAZ, and promote their nuclear localization, whereas a soft matrix surface would inactivate them, and promote their cytoplasmic retention. These conditions ultimately promoted or limited growth, respectively. Moreover, the Piccolo lab provided strong evidence that the status of the F-actin cytoskeleton, and Rho GTPase function, underlies the regulation of YAP/TAZ. It was shown that this regulation could also be LATS independent, i.e., non-canonical Hippo signaling [25].

These three reports have strongly reverberated in the signaling field because, intuitively, they provided clues into the mechanism of CIP, the control of organ size and the genesis of cancer. More importantly these reports highlighted that mechanobiology is an important facet of cancer signaling. Intriguingly, YAP/TAZ not only function as effectors of CIP, but they also act as a rheostat to regulate the nuclear localization and function of the phosphatase SHP2 under different cell densities [27].

2. Mechanotransduction and the role of YAP/TAZ at the cell level

Organs and cells are constantly subjected to mechanical stresses. Examples of such stresses are: the force of skeletal muscle contraction, a flow-induced shear stress in circulatory systems, the stretching, strain, compression and pressure arising from different stiffness of extracellular matrices and the geometry of cells. Within cells, the intracellular force/tension generated by the actin-myosin cytoskeleton, as well as the surface tension generated at the membrane, provide intrinsic mechanisms to sense and respond to physical perturbations. Indeed, the classic finding of mesenchymal stem cells developing into specific cell lineages as a function of substrate stiffness, and different tissues and organs adopting distinct physical niches for their differentiation, all highlight that in addition to biochemical signals, the physical environment controls cell fates and organ formation [28–30].

At the molecular level, cells can sense external mechanical signals through various contact points. These contact points include stretch-modulated ion channels, integrin based cell-matrix (focal) adhesions and cell–cell junctions and contacts [31–36]. Upon sensing force, cells react by generating equal but opposite forces by modulating myosin motor activity to balance the tension in the actin cytoskeleton [37–39]. External forces may trigger active changes in the actin cytoskeleton. For example, cell stretching can lead to sustained activation of RhoA and myosin and

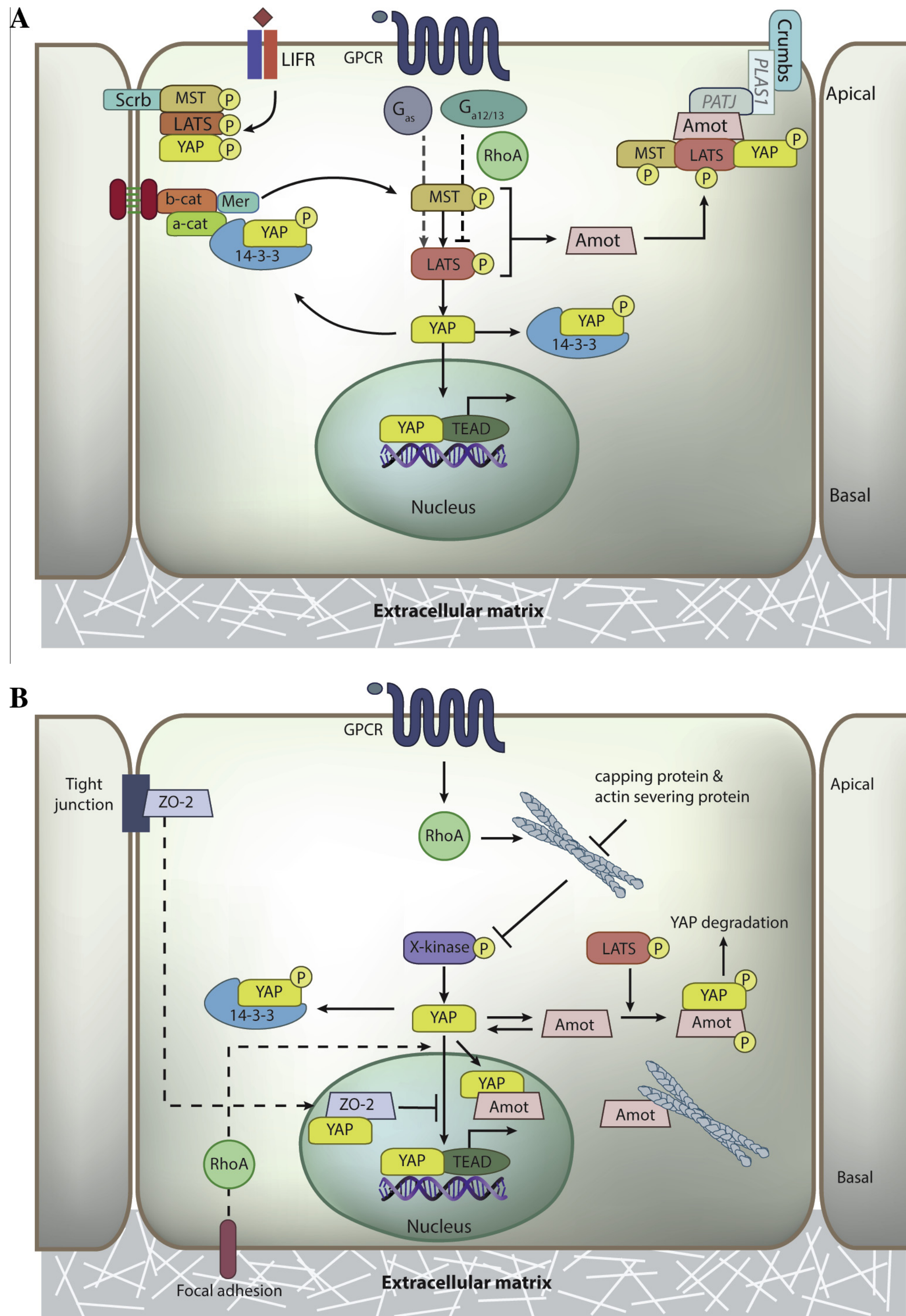

Fig. 1. Schematic representation of the canonical and non-canonical Hippo signaling pathways. (A) In the canonical Hippo signaling pathway, junctional complexes, polarity complexes, G-protein coupled receptors (GPCRs) or leukemia inhibitory factor (LIF) initiate their signals upon cell–cell contact, polarization of epithelial cells or ligand-receptor association. These signals result in the activation of MST kinase (Hippo kinase ortholog), which phosphorylates and activates LATS kinase. In turn, LATS kinase inhibits the activity of YAP/TAZ by Serine phosphorylation. This traps YAP/TAZ in the cytoplasm (by 14-3-3), at the apical cell membrane (by Scribble, Scrb), at adherens junctions (by 14-3-3 and catenins), or at the apical tight junction (by the polarity complex Crumbs and Angiomotins, i.e. AMOT). Note that certain ligands for GPCR inactivate LATS instead. (B) In contrast, non-canonical modes of the Hippo signaling pathway does not involve MST/LATS to act on YAP/TAZ directly. Instead, they involve either an unknown serine kinase (X-KINASE) that has the ability to phosphorylate YAP and TAZ on the same Serine as LATS kinase. Alternatively, YAP/TAZ is inhibited by a non-kinase mechanism, shown by the AMOT protein. AMOT binds F-actin filaments and allows YAP to enter the nucleus. However, upon actin de-polymerization AMOT dissociates from actin and traps YAP/TAZ in the cytoplasm through a strong complex that is mediated by WW domains and PPxY motifs. When phosphorylated by LATS, AMOT can also recruit ubiquitin ligase to the AMOT/YAP complex and target YAP for degradation. Another example of non-canonical regulation of YAP is through the shuttling of a tight junction protein ZO-2 together with YAP to the nucleus where it suppresses YAP activity. On the contrary, the p130 isoform of AMOT promotes nuclear localization of YAP and acts as a transcriptional cofactor of the YAP-TEAD complex. This ternary complex drives the transcription of TEAD target genes for cell proliferation. On the other hand, Rho GTPases could control YAP/TAZ activity through canonical (e.g. GPCR-linked) or non-canonical arms of Hippo signaling (e.g. focal adhesion-linked). For clarity, only YAP was included in this signaling scheme, omitting TAZ. However, in most signaling events, YAP/TAZ could be indicated. Icons representing signaling proteins that were not discussed in the text in detail are shown in italics and in gray color. Please refer to the main text for more details.

subsequently, the induction of stress fiber formation [40–42]. Moreover, it is well known that contractility forces are able to regulate gene expression [43], development [44,45], cell proliferation [46], differentiation [47] and migration [48].

2.1. Substrate rigidity and the extracellular matrix (ECM)

The subcellular localization and activity of YAP/TAZ are tightly regulated by cell substrate rigidity and topography [15,16,25], actin cytoskeleton remodeling [26,49,50], and by specific regimens of cell stretching [26]. These observations directly highlight that YAP and TAZ may serve as novel mechanotransducers and mechnaosensors (Table 1). As reported by the Piccolo laboratory, substrate rigidity tightly controls the subcellular localization of YAP/TAZ. When cells are grown on a soft matrix (circa 0.7 kPa), YAP/TAZ localize to the cytoplasm, whereas when cells are grown on a hard matrix (circa 40 kPa), YAP/TAZ localize to the nucleus and drive the transcription of proliferative genes [25]. In addition to regulating the subcellular localization of YAP/TAZ, substrate rigidity may also control their expression in some cells. Differential substrate stiffness was shown to modulate the expression of YAP/TAZ in human trabecular meshwork cells [51]. Interestingly, the expression of YAP/TAZ was upregulated in these cells when grown on 75 kPa hydrogel as compared to 5 kPa hydrogel.

Importantly, the notion that the subcellular localization of YAP/TAZ is controlled by substrate stiffness, can also apply to 3D cultures. Mammary epithelial cells seeded onto a 3D soft matrix tend to form growth-arrested acini with YAP/TAZ being localized in the cytosol. In contrast, when these cells grow on a stiff matrix, larger spheroids with tubules and organoid-like structures are observed with YAP localized to the nucleus [26]. Overall, the dynamic shuttling, transcriptional activity of YAP/TAZ and in some cases the expression of YAP/TAZ, are tightly linked to matrix stiffness. Although our understanding of the underlying mechanism is still nebulous, new findings have begun to provide clues into how the F-actin cytoskeleton acts as a critical intermediary between mechanical cues from the ECM and the control of YAP/TAZ activity.

Cells are not simply regulated by the ECM in a "passive" manner. They remodel the ECM by secreting collagen to promote matrix stiffening. Such changes in ECM stiffness provide further mechanical signals for cells to adapt to their environment. Deregulation of such processes leads to pathological abnormalities. For instance, many cancers, such as breast cancer, are associated with the development of tumor masses that are more rigid than their surrounding tissue. This has been attributed to an altered composition of the ECM. By softening the tumor microenvironment one can actually attenuate the growth and progression of the tumor [52]. Intriguingly, remodeling of the ECM is in part dependent on the activity of YAP. The activation of YAP in cancer-associated fibroblasts (CAFs) promotes matrix stiffening through an extensive deposition of collagen [50]. Such matrix stiffening in turn creates tension within CAFs, leading to the activation of Src kinase and the nuclear translocation of YAP. Consequently, YAP (and possibly TAZ) promotes the expression of cytoskeletal regulators, such as ANLN and DIAPH3, and stabilizes actomyosin proteins. This leads to further matrix stiffening, thereby establishing a self-enhancing loop during tumorigenesis. This may explain why organ specific overexpression of YAP in transgenic mice initially causes hypertrophy, but gradually leads to cancer. In this scenario, prolonged expression of the YAP transgene, and the ensuing changes in the ECM, may promote a metastatic phenotype.

Table 1
Mechanical factors that influence YAP/TAZ activity.

Treatment	Impact on YAP/TAZ	References
Stretching	YAP/TAZ enters the nucleus in contact-inhibited cells upon stretching	[26]
Geometry	YAP/TAZ re-localizes to cytoplasm on small surface area	[25,26,56]
Cell density	YAP/TAZ localizes to cytoplasm in contact-inhibited cells	[24,26,62]
Substrate rigidity	YAP/TAZ re-localizes to cytoplasm on soft substrate matrix. Expression of YAP/TAZ is upregulated on hard substrate matrix	[25,51]

2.2. Strain forces and YAP/TAZ

The influence of strain force on cells was recently outlined in an elegant study by LeGoff et al. which analyzed "endogenous" cell stretching. In this study, the authors unveiled the global pattern of mechanical stresses that polarize cell divisions and cell shape in growing *Drosophila* wing discs [53]. By supplementing an innovative quantitative approach with computer modeling, they showed that cells in the periphery of the tissue are mechanically stretched while the inner cells are compressed. Remarkably, a perturbation of the Hippo signaling pathway was shown to result in an overgrowth of the tissue, specifically from induced tissue strain and cell position-dependent changes in proliferation [53]. It was proposed that this may be important in the regulation of cellular behavior during organogenesis. In connection to this, phosphorylated active ERK1/2 (nuclear effector for proliferation) was observed at the periphery but not in the inner layer of zebrafish liver [54]. This suggests that mechanical strain and stress can regulate the function of nuclear effectors.

CIP restricts the proliferation of cells by activating Hippo to ensure that YAP/TAZ is inactive and localized in the cytosol [24], however, it remains unclear if mechanical forces play a role in CIP. While investigating this very point, the Piccolo laboratory demonstrated that mechanical stretching can indeed induce the entry of YAP/TAZ into the nucleus to stimulate proliferation of contact-inhibited mammary epithelial cells [26]. This implied that an external strain force can overcome YAP/TAZ inhibition in growth arrested, contact-inhibited cells. This seemingly simple observation, where mechanical stretching was able to override CIP via YAP/TAZ, is of paramount importance. If the precise mechanism behind this regulation is deciphered, it will provide a rationale for the development of new tools to facilitate organ regeneration and manage cancer. A simple hypothesis that may explain the effect of stretching the cell monolayer is that YAP/TAZ could be activated after the integrity of cell–cell junctional complexes is modulated. Being directly connected to these junctional complexes, the F-actin cytoskeleton would be critically involved in this process.

2.3. Cell geometry and YAP/TAZ

The wide diversity in cell geometries reflects the assortment of cellular morphologies that arise during tissue and organ morphogenesis, remodeling and planar polarization. It is known that cell proliferation can be regulated by changes in cell geometry and YAP/TAZ sense these changes [39,55]. For example, single cells plated on a small adhesive micro-patterned surface (300 μm^2) will assume a more bulky geometry with YAP/TAZ localized in the cytoplasm, while single cells plated on a large adhesive micro-patterned surface (10,000 μm^2) will have 'flattened', epithelial cell-like geometry with active YAP/TAZ localized in the nucleus [25,26]. This observation was replicated in a study confirming that YAP was mostly cytoplasmic in cells spread on a small surface area but distinctly localized in the nucleus in cells spread on a large surface area [56]. How YAP/TAZ sense changes in the cell geometry is not known. It is possible that adhesion sites, and their associated F-actin cytoskeleton, are affected differently in rounded cells

compared to spread cells. It is within these differences that clues may exist that begin to explain how YAP/TAZ sense cell geometry. It is important to note here that cell geometry also imposes a physical constraint on the structure of the nucleus, as well as on the organization of chromatin. It can also affect the activity of some chromatin remodeling enzymes [57,58]. Whether or not YAP/TAZ activity could indeed be modulated by nuclear and adhesion mechanics remains to be established.

3. Mechanosensing and intracellular signals

3.1. Actin integrity and Acto-myosin contractility

Myosin motor proteins cross-link and slide along actin filaments to generate contractile forces and tension in cells. Recent studies have demonstrated that such tensile forces are important in regulating the activity of YAP/TAZ. For example, the role of YAP/TAZ in sensing differences in substrate stiffness is dependent on the tension of the actin cytoskeleton [25]. This was shown by treating cells grown on a rigid substrate with Blebbistatin, an inhibitor of non-muscle myosin II. Here, not only was the intracellular tension released, but YAP/TAZ re-localized from the nucleus to the cytosol. Besides the tensile strength, the actual integrity of the actin cytoskeleton can also regulate the activity of YAP/TAZ. For example, treatment of cells with Latrunculin A, an inhibitor of actin polymerization, results in cytosolic localization of YAP/TAZ [25], increased phosphorylation of YAP at Serine 127 and decreased expression of CTGF; a target gene of YAP/TAZ [51]. In contrast, F-actin regulates the activity of YAP in a LATS-dependent, i.e., canonical manner [49,56]. Extensive polymerization of actin will inactivate Hippo (MST ortholog in Drosophila) and subsequently allow Yki (YAP ortholog) to go to the nucleus to drive proliferation. Interestingly. Aragona et al. demonstrated that the presence of actin modulating proteins such as Cofilin, CapZ and Gelsolin regulate YAP/TAZ activity and maintain growth arrest in contact inhibited cells [26]. For example, when CapZ was depleted, contact inhibition of cell proliferation was derepressed and cells started to proliferate.

Alternative pathways also exist to regulate YAP/TAZ activity. For example, when cells are grown on stiff substrate, RhoA, which is an activator of F-actin cytoskeleton and actomyosin contractility, regulates YAP/TAZ activity independently of LATS activity [25]. Different signals from G-protein coupled receptors (GPCRs) can also modulate LATS activity to varying degrees. For example, lysophosphatidic acid stimulates $G\alpha_{12/13}$-coupled receptor to induce YAP/TAZ activity by inhibiting LATS, whereas $G\alpha_s$-coupled signals activate LATS, thus inhibiting YAP/TAZ [14] (Fig. 1A). The $G\alpha_{12/13}$-induced YAP/TAZ activation can be blocked by the F-actin disrupting agent, Latrunculin A [14], suggesting that GPCRs and RhoA act upstream of LATS to regulate YAP/TAZ. Another study by Zhao et al., demonstrated that cytoskeletal reorganization caused by cell detachment can activate *anoikis* through the Hippo pathway to induce cell death [59].

RhoA and YAP/TAZ were also implicated in the mechanism by which statins affect cell signaling [60]. In this recent study, statins, which are very popular cholesterol-lowering drugs that target HMG-CoA-reductase, were shown to inhibit nuclear translocation of YAP/TAZ. This inhibition was further potentiated by the inhibition of RhoA [60]. The RhoA-mediated increase in YAP/TAZ phosphorylation was LATS independent, suggesting an unknown kinase is involved in mediating non-canonical signaling by Hippo. It should be noted that the doses of statins used in the reported cell culture studies significantly exceeded the recommended therapeutic doses for individuals undergoing cholesterol-lowering therapies. Thus, the overt effects of statins on YAP and TAZ observed may be minimal and not affect the stem and progenitor cells that control organ homeostasis.

There has also been genetic validation of the crosstalk between 'contractility' and the function of YAP. Helen McNeill and colleagues [61] have shown, for example, that kidney-specific conditional knockout of the *YAP* in mice results in a defective organ with abnormal glomeruli, ducts without lumen, and therefore empty bladders. Interestingly, the kidney-specific knockout of another gene, *Cdc42*, which encodes a small GTPase of the Rho family, phenocopied the *YAP* knockout. This result strongly suggests that CDC42 and YAP function in the same pathway and support the signaling interface between actin polymerization and YAP, at least in the kidney. Taken together, these biochemical and genetic data strongly support an intricate link by which the actin cytoskeleton network regulates YAP/TAZ signaling (Fig. 2).

3.2. Signaling by scaffold proteins at cell–cell contacts, and the role of AMOT

Cell–cell contacts are crucial in the maintenance of the apical-basal polarity of epithelial cells, and to mediate CIP. Generally, cell adhesions experience high compression and tensile forces. Several signaling components of the Hippo network, including Merlin, Kibra, as well as YAP/TAZ, have been reported to localize at the apical membrane. There they may serve as sensors of mechanical tension through the status of polarity and the integrity of junctional complexes. In addition, various junctional proteins such as α- and β-catenins, Crumbs polarity complex [62], Angiomotins (AMOTs) [62–67] and Zonula Occludens proteins can physically interact with YAP/TAZ and regulate its activity [67,68] (Fig. 1A and B). Many of these proteins, including Scribble also serve as scaffolds that direct the precise placement of various components of the Hippo network in order to achieve a versatile crosstalk in signaling [69]. For example, α-catenin is a component of adherens junctions that mediates cell–cell contact and couples the adhesion complex to the actin cytoskeleton. Several studies have shown that in the epidermis, α-catenin negatively regulates YAP by localizing the phosphorylated YAP-14-3-3 complex to adherens junctions [70,71]. Furthermore, in densely-packed cells, Crumbs, which is a trans-membrane polarity complex, is known to couple cell density sensing to the activation of the Hippo pathway and the inactivation of YAP [62]. This process is mediated by another regulator of YAP, AMOTs, which forms a canonical Hippo core complex with MST and LATS (Fig. 1A).

Another protein that predominately functions at cell–cell adhesion sites, yet was also shown to interact with YAP/TAZ, is Zona Occludens 2 (ZO-2). It was suggested that this protein facilitates the shuttling of YAP/TAZ between junctional complexes and the cytoplasm as well as between the cytoplasm and the cell nucleus [67,68,72]. A complex reportedly resulted from interactions between the first PDZ domain of ZO-2 and a conserved carboxy-terminal motif on YAP/TAZ [68,72]. When ZO-2 was overexpressed and forced into the cell nucleus, it attenuated proliferative activity of YAP in MDCK cells (Fig. 1B). The discovery of a YAP/TAZ/ZO-2 complex was interesting as it provided a lead for the investigation into the role of tight junction proteins in the direct regulation of the transcriptional activity of YAP/TAZ. We hope to investigate this signaling further using mouse models that harbor mutations of *YAP/TAZ* and *ZO* genes.

The scaffold protein Scribble also plays an important role in the Hippo signaling network. This protein can assemble MST and LATS kinases together with TAZ, independently of the known Hippo scaffolding protein Salvador. Scribble assembles the complex at the apical cell membrane, thereby promoting the activation of LATS by MST kinase, which subsequently inactivates TAZ [73]. Like

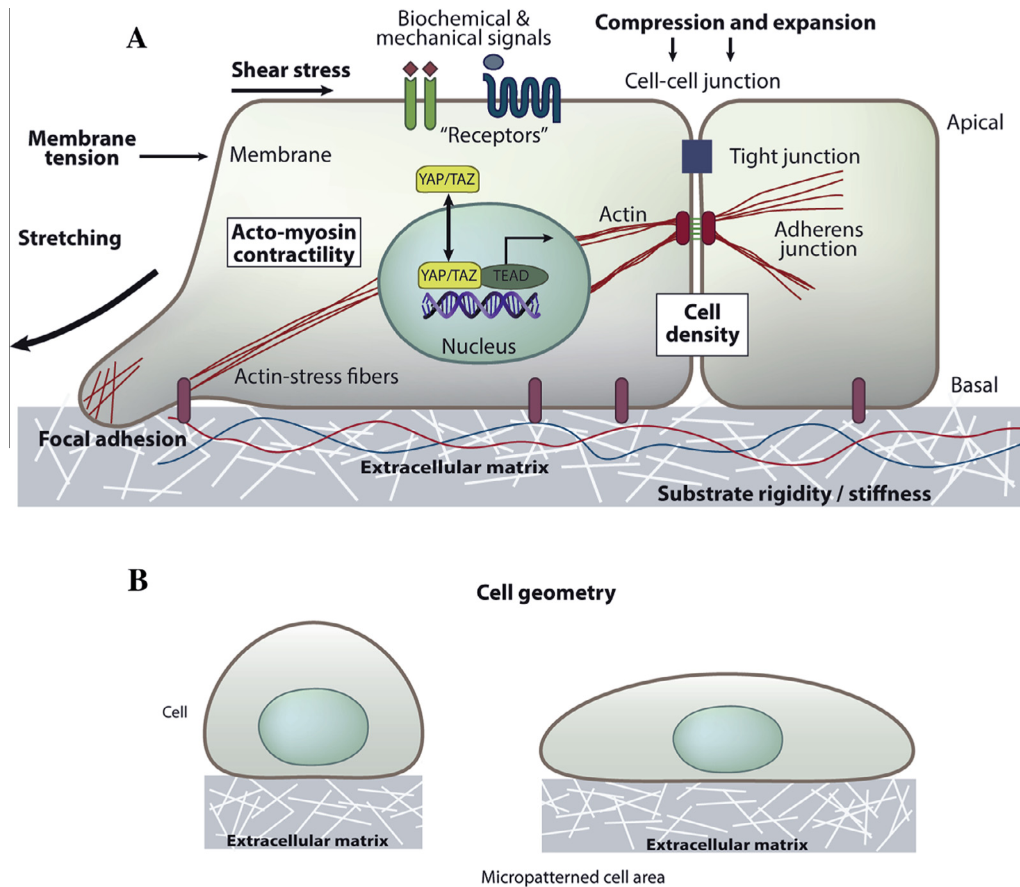

Fig. 2. YAP/TAZ effectors as mechanosensors and mechanotransducers. (A) It is hypothesized that the presence of F-actin and stress fibers is critical for the activation of YAP and TAZ. When translocated to the nucleus, they associate with TEAD transcription factors to drive transcription of proliferative genes. Rho GTPases and actin modifiers such as CAP-Z, Cofilin and Gelsolin can affect F-actin network stability and directly or indirectly regulate YAP/TAZ translocation to the nucleus (please see Fig. 1 and text for details). Although increasing amount of data support the impact of physical perturbation on YAP/TAZ function (as summarized in Table 1), our main challenge now is to achieve a detailed understanding of the concerted roles of F-actin, actin regulators and Hippo-YAP signaling in response to various physical cues. These include stretching and strain, compression and expansion, membrane tension and shear stress as well as different topography, cell density, cell–cell contacts, extracellular matrix/substrate stiffness and cell geometry which can be experimentally controlled by micro-patterned area (B).

Scribble, AMOT is also able to negatively regulate TAZ/YAP, but does so in a canonical as well as non-canonical fashion [63–65].

AMOT is a notable signaling partner of YAP/TAZ, and is of particular interest because of its ability to bind both actin and adhesion complexes (Fig. 1B) [74–76]. In its cytoplasmic form, AMOT can promote phosphorylation of YAP and induce its cytoplasmic retention by forming a stable complex with YAP/TAZ. This ultimately reduces the transcription of YAP/TAZ target genes such as CTGF and Cyr61 [63,64,66]. Importantly for our discussion, like YAP/TAZ, AMOT is also phosphorylated by LATS kinase. This leads to the dissociation of AMOT from actin and to the inhibition of cell proliferation [76–78] as YAP activity is suppressed via the recruitment of 14-3-3 and ubiquitin ligase AIP4 [79]. Such a ternary complex can stabilize AMOT and promote the ubiquitination and degradation of YAP, thus leading to the suppression of cell growth. As such, AMOT serves as an anchor of YAP/TAZ, to ensure these effectors are retained in the cytoplasm through WW domain-PPxY link-mediated complexes [9]. This function is redundant with 14-3-3 proteins.

Importantly, AMOT is present in cells at relatively high concentrations and its binding to YAP/TAZ is of high affinity. Immunoprecipitation of YAP or TAZ, even in "harsh buffers", which contain SDS detergent and high concentrations of salt, are known to pull down AMOT or one of the members of the AMOT family of proteins, in a stoichiometric complex [67]. When AMOT is engaged in a complex with F-actin, YAP/TAZ may be free to enter the cell nucleus and be active. We suggest that AMOT, and members of the family of AMOT proteins, play an important role in conveying the status of the F-actin cytoskeleton to YAP/TAZ, through competitive binding between F-actin and YAP/TAZ. Thus, increased polymerization of the F-actin cytoskeleton could lower AMOT levels, releasing YAP/TAZ to travel to the nucleus and activate cell proliferation (Fig. 1B). Surprisingly, besides its role in the cytoplasm, one isoform of AMOT, AMOT-130 can promote the nuclear localization of YAP and act as a transcriptional cofactor of YAP-TEAD to drive the transcription of YAP target genes [80]. It remains unclear if such a complex can be regulated upon mechanical stress or upon contact inhibition.

4. Concluding remarks

Despite the fast progress in the field, we still do not know how YAP and TAZ work in concert with the complex mechanical milieu to regulate organ size. From the large sets of data in systems biology, we appreciate that in addition to the Hippo signaling network multiple other pathways play important roles in the organ homeostasis; these including the mTOR pathway, the WNT pathway and several other major signaling pathways [81]. Although YAP and TAZ can play dominant roles in organ size control they can also function as potent oncogenes in certain organs. We do not

understand why cancer develops in those organs after transgenic overexpression of YAP/TAZ genes, since both mechanical and biochemical factors could be important. However, modern tools of mechanobiology together with fine "omic"- and systems biology-based approaches, should enable dissection of the functions of YAP and TAZ in the background of other signaling pathways involved in organ size control. Once we decipher the mechanical and biochemical signals that control the function of YAP and TAZ, we will then gain further insights to new strategies for modulating cell growth in cancer treatment, tissue regrowth or organ regeneration.

This review is meant to stimulate discussion in the exciting research area that literally "exploded" in the span of the past three years at the interface of cancer signaling and mechanobiology. We hope that the precise mechano-molecular signaling that underlies organ size control will be soon deciphered. We believe that paradigm-shifting discoveries are imminent in this area of research because of the need for interdisciplinary observations of both mechanical and biochemical changes. Deregulation of organ size control results in many pathologies, including cancer. Therefore, an understanding of these controls at the molecular and organismal level holds the promise of better public health.

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