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# THE BIOLOGY OF YAP/TAZ: HIPPO SIGNALING AND BEYOND

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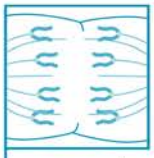

**Piccolo S, Dupont S, Cordenonsi M.** The Biology of YAP/TAZ: Hippo Signaling and Beyond. *Physiol Rev* 94: 1287–1312, 2014; doi:10.1152/physrev.00005.2014.—The transcriptional regulators YAP and TAZ are the focus of intense interest given their remarkable biological properties in development, tissue homeostasis and cancer. YAP and TAZ activity is key for the growth of whole organs, for amplification of tissue-specific progenitor cells during tissue renewal and regeneration, and for cell proliferation. In tumors, YAP/TAZ can reprogram cancer cells into cancer stem cells and incite tumor initiation, progression and metastasis. As such, YAP/TAZ are appealing therapeutic targets in cancer and regenerative medicine. Just like the function of YAP/TAZ offers a molecular entry point into the mysteries of tissue biology, their regulation by upstream cues is equally captivating. YAP/TAZ are well known for being the effectors of the Hippo signaling cascade, and mouse mutants in Hippo pathway components display remarkable phenotypes of organ overgrowth, enhanced stem cell content and reduced cellular differentiation. YAP/TAZ are primary sensors of the cell's physical nature, as defined by cell structure, shape and polarity. YAP/TAZ activation also reflects the cell "social" behavior, including cell adhesion and the mechanical signals that the cell receives from tissue architecture and surrounding extracellular matrix (ECM). At the same time, YAP/TAZ entertain relationships with morphogenetic signals, such as Wnt growth factors, and are also regulated by Rho, GPCRs and mevalonate metabolism. YAP/TAZ thus appear at the centerpiece of a signaling nexus by which cells take control of their behavior according to their own shape, spatial location and growth factor context.



## I. INTRODUCTION

### A. A Young Field With Many Exciting Questions

After 20 years from the discovery of YAP by Marius Sudol (199), research on the biology and regulation of YAP/TAZ has turned into a burgeoning field. At the same time, some of the most fundamental questions relating to the function of YAP/TAZ remain unanswered.

To start, we really do not know how YAP/TAZ realize their most remarkable trademark feature: the growth of organs until they reach their correct size. Then, as detailed in this review, YAP/TAZ are mainly understood as downstream effectors of the Hippo pathway, a kinase cascade that ends up phosphorylating and inhibiting YAP/TAZ. But it is still unclear whether, and to what extent, patterned YAP/TAZ activation in specific tissue niches is really associated with a patterned Hippo signaling. Indeed, Hippo-mediated YAP/TAZ phosphorylation is a fundamental input for YAP/TAZ regulation but clearly not the only one. Mechanical signals represent a second pillar for YAP/TAZ function, conveying individual cells information about their shape and on the structural properties of their microenvironment. These cues may well act as local and organ-level checkpoints that pattern YAP/TAZ activity; yet, the mechanisms behind this biomechanical regulation remain enigmatic.

Another major question in the field is how YAP/TAZ control downstream responses. There are few bona fide YAP/TAZ target genes, but little clues on what mediate the many YAP/TAZ-driven biological effects. Moreover, YAP/TAZ are emerging as central determinants of malignancy in human cancer and thus a better understanding of their upstream regulators and downstream effectors will be essential for the design of innovative cancer treatments.

The horizon of a scientific area can be gauged by questions that are ready to challenge. By this measure, the journey is just at the beginning: we anticipate that great discoveries and many surprises are ahead of us in both the basic and translational aspects of YAP/TAZ biology. We thus hope that this review will inspire the newcomers and promote discussion among specialists.

 

## II. YAP/TAZ REGULATION

### A. An Overview of the *Drosophila* Core Hippo Pathway

The Hippo cascade is one of the fundamental, and first discovered, YAP/TAZ regulators. In *Drosophila*, the key kinase of the Hippo cascade, encoded by the *LATS/warts* gene, was identified in 1995 in a genetic mosaic screen aimed at isolating genes regulating the growth of larval tissues (97, 236). The function of LATS/warts remained rather poorly investigated until 2003, when other core components of the Hippo cascade were identified by similar screens (61, 64, 74, 80, 168). This led to the functional and biochemical characterization of the so-called "Salvador-Warts-Hippo" (SWH) pathway: the Hippo kinase, together with the adaptor protein Salvador, activates the LATS/Warts kinase. *Drosophila* tissues bearing inactivating mutations for these genes invariably display hyperproliferation and reduced apoptosis, causing overgrowth of larval tissues and the emergence of tumors. This led to the notion that the Hippo pathway is a potent tumor suppressor in fly tissues.

The downstream effector of the pathway, Yorkie (the YAP/TAZ ortholog in *Drosophila)*, was isolated in 2005 as Warts-interacting protein, and placed downstream of the Hippo cascade by epistasis experiments (91). *Yorkie* mutants display reduced tissue proliferation, while *Yorkie* overexpression induces tissue overgrowth. Yorkie acts as a transcriptional coactivator, and it is required for the molecular and phenotypic effects caused by mutations of the SWH pathway components.

Mechanistically, the Warts kinase, aided by its cofactor Mats (Mob as tumor suppressor), phosphorylates Yorkie on multiple serine residues and induces its translocation from the nucleus to the cytoplasm, where 14-3-3 proteins contribute to entrap Yorkie and keep it in an inactive state. Thus, when the Hippo cascade is active, Yorkie is inactive. Yorkie, however, does not bear any DNA-binding domain, and thus needs a transcriptional partner to regulate target gene activity, identified in the TEA-domain transcription factor Scalloped (227, 256).

All key components of the SWH pathway are evolutionary conserved. Most of the mammalian homologs of the SWH pathway were known well before they were functionally connected to the Hippo pathway: for example, YAP was isolated as Yes-associated protein in 1995 (199); TAZ is the YAP paralog in vertebrates and was isolated as 14-3-3 binding protein in 2000 (99); TEAD factors, key DNA-binding platforms for YAP/TAZ, were found associated to YAP in 2001 (209). It was however the delineation of the SWH pathway as organ-size regulator in *Drosophila* that pushed forward the notion that factors represented the components of the mammalian Hippo pathway (49).

In this review we have focused our attention mainly into the biology of YAP/TAZ and their regulation in mammalian cells, where Hippo regulations blend with forms of YAP/TAZ control that are independent from Hippo/LATS kinase activity. As such, the reader should be aware of a semantic issue in this field: the term *Hippo signaling* is increasingly used in a very generic fashion to indicate any modality of YAP/TAZ control, if not activity of YAP/TAZ themselves. Instead, we thought to provide a better service by giving different names to distinct regulations, and highlighting their peculiarities.

### B. The Core Hippo Kinase Cassette in Mammals: MST1/2 and LATS1/2

The sterile 20-like kinases MST1/STK4 and MST2/STK3 are orthologs of the *Drosophila* Hippo kinase. As schematized in **FIGURE 2,** MST1/2 bind to their regulatory protein SAV1/WW45 to form an active enzyme that phosphorylates and activates the LATS1/2 kinases (orthologs of Warts) (24). MST1/2 also phosphorylate the MOB1A/B regulatory subunits of LAST1/2 (orthologs of mats) (174). The activated LATS1/2-MOB1A/B complex in turn phosphorylates YAP and TAZ, the two major effectors of the Hippo cascade (49, 114, 266). The mechanisms of YAP/TAZ inhibition by phosphorylation are nuclear exclusion, sequestration in the cytoplasm, and/or proteasomal degradation (114, 123, 264, 266).

LATS1/2 phosphorylate YAP at five serine/threonine residues (defined by the consensus HxRxxS), and TAZ has four of these sites (262) **(FIGURE 1)**. Mutation in these serine residues makes YAP/TAZ insensitive to inhibition by the Hippo pathway. As such, the most potent tools to overexpress YAP or TAZ are, respectively, YAP5SA and TAZ4SA, bearing serine to alanine mutations in all these LATS phosphorylation sites.

Of these sites, the most relevant residues that keep YAP and TAZ inhibited are S127 (S89 in TAZ) and S381 (S311 in TAZ) (262). Phosphorylation of S381 has been linked to regulation of YAP/TAZ protein stability, as it primes for additional phosphorylation by CK1 kinases; this generates a "phosphodegron" recognized by β-transducin repeat-containing protein β-TRCP, a key adaptor for the SCF E3 ubiquitin ligases, leading to YAP/TAZ polyubiquitination (123, 264). Despite the presence of a phosphodegron, endogenous YAP is a relatively stable protein, mainly regulated by nuclear-cytoplasmic shuttling (see below). In contrast, TAZ is a very unstable protein with a half-life of $<2$ h, indicating that protein degradation is the main route for TAZ inhibition.

### C. YAP/TAZ Nucleocytoplasmic Shuttling

YAP and TAZ are found both in the cytoplasm and in the nucleus, where they regulate gene transcription; as such,

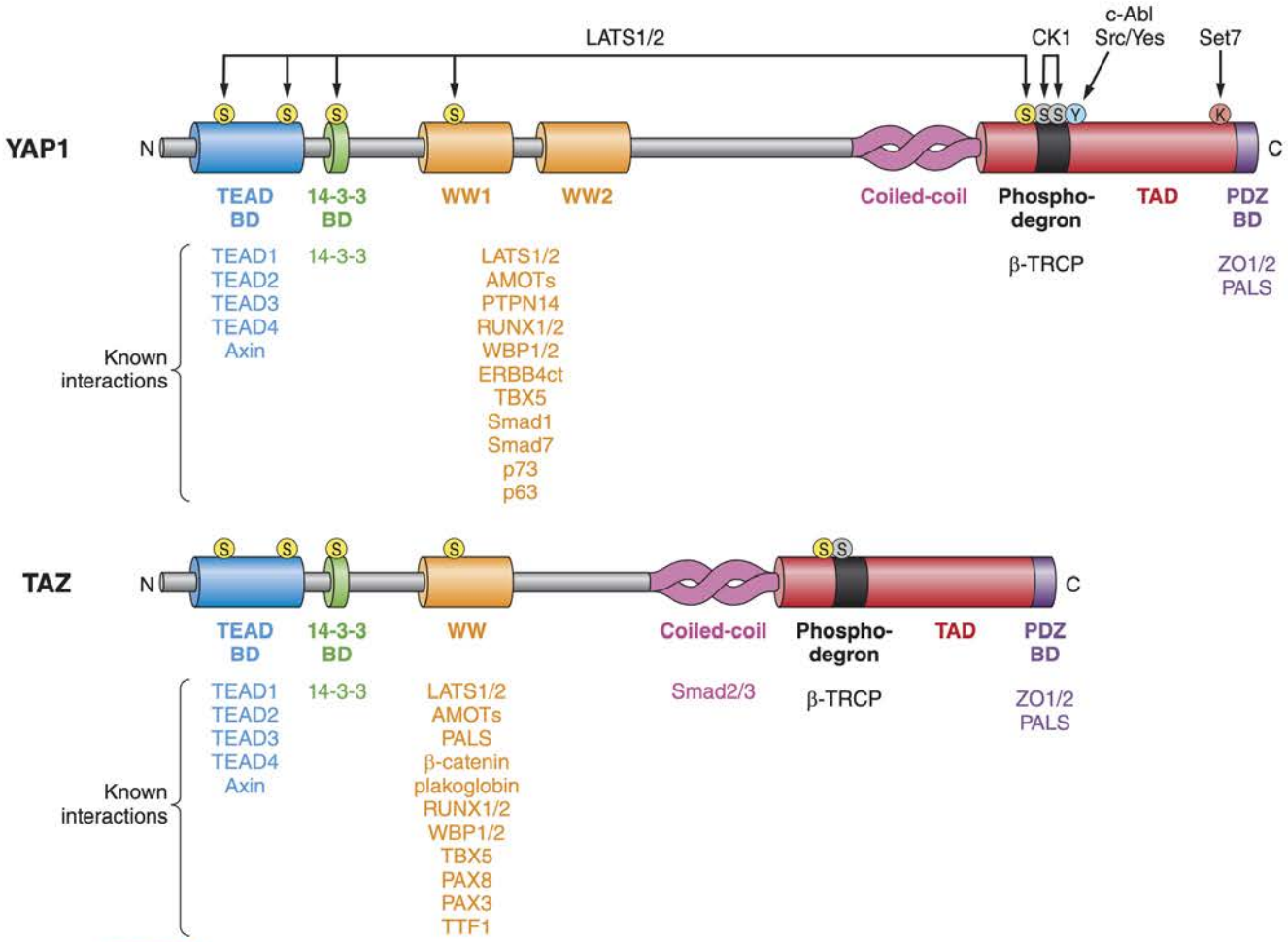


**FIGURE 1.** Schematic representation depicting the multiple domains of YAP and TAZ, the mapped interactions with other proteins, and the residues targeted by posttranslational modifications. The five serines of YAP and the corresponding four serines of TAZ that are targeted by LATS1/2 phosphorylation are shown in yellow, the CK1 phosphorylation sites on both proteins are shown in gray, and the c-Abl phosphorylation site on YAP is shown in cyan. The lysine residue of YAP targeted for methylation by Set7 is also shown. TEAD BD is the TEAD binding domain. 14–3-3 BD is the domain that binds 14–3-3 proteins upon phosphorylation by LATS1/2. TAD is the transcriptional activation domain. PDZ BD is the small COOH-terminal domain able to interact with proteins bearing PDZ domains. See text for details.

YAP/TAZ nuclear accumulation is a key determinant of their function. Phosphorylation by LATS also represents a main input for YAP/TAZ subcellular localization, leading to sequestration of YAP/TAZ in the cytoplasm. Phosphorylation of YAP S127 creates a binding consensus for 14-3-3 proteins (14, 49, 266), which would then contribute to keep YAP/TAZ in the cytoplasm. This model is, however, not entirely coherent with some experimental observations. *1*) S127-phosphorylated YAP can be found in the nucleus (217). *2*) Treatment of cells bearing cytoplasmic and phosphorylated YAP with the CRM1 inhibitor leptomycinB induces its nuclear accumulation, indicating that YAP actually keeps on entering the nucleus even when it is phosphorylated (50, 178). *3*) Expression of YAP S127A in tissues of transgenic mice is not restricted to the nucleus (12). It is thus possible that YAP phosphorylation on sites other than S127 may cause YAP sequestration into additional protein complexes. Moreover, it is also possible that LATS-mediated phosphorylation affects YAP/TAZ activity by additional modalities. For example, phosphorylation might hinder part of the transcriptional functions of YAP/TAZ, as some of the putative phosphorylation sites overlap with the TEAD and COOH-terminal transactivation domain, and this may contribute to nuclear exclusion.

It is worth anticipating here that independent pieces of evidence suggest that some classic "LATS" phosphorylation sites, including S127, may be actually targeted by kinases other than LATS1/2: *1*) S127 was originally identified as AKT target; *2*) fractionation of liver extracts revealed that fractions void of LATS1/2 were still able to phosphorylate YAP S127; *3*) in keratinocytes, $\alpha$-catenin works in concert with an unknown kinase to sustain YAP phosphorylation in S127; and *4*) in line, attenuation of the metabolic mevalonate/cholesterol pathway raises YAP S127 phosphoryla-

tion in a LATS1/2-independent manner (see below and Refs. 14, 183, 190, 269).

Irrespectively of the precise mechanisms, a conservative interpretation of the data suggests a scenario in which YAP/TAZ phosphorylation is a bias for their nuclear exclusion, but not necessarily a primary inactivating event. Indeed, any phosphorylation-independent YAP/TAZ inhibitory event may leave YAP/TAZ available for phosphorylation by LATS. The latter may occur either in the cytoplasm or directly in the nuclear compartment (as a nuclear LATS pool can be readily detected in several cell types, Ref. 119). In this perspective, LATS phosphorylation may reinforce the effects of any other YAP/TAZ inhibition, including those that are initially "Hippo independent." LATS-induced phosphorylation can be then reversed by the activity of phosphatases, such as PP1, causing YAP/TAZ reactivation (122, 219).

Apart from serine phosphorylation by LATS and other kinases such as AKT and JNK (14, 42), YAP1 can be also targeted by tyrosine phosphorylation triggered by the Yes/Src and c-Abl kinases (117, 180). In these cases, however, tyrosine phosphorylation is a positive trigger of YAP1 activity; for example, Src-mediated phosphorylation is essential for growth of colon cancer cells. Moreover, YAP1 is a direct target of PTPN14, a protein that contains a tyrosine-phosphatase domain; a PTPN14/YAP complex contributes to retention of YAP1 to the cytoplasm (92, 124, 142, 221). Finally, lysine monomethylation by Set7 also regulates YAP/TAZ subcellular localization **(FIGURE 1)** (166).

Besides posttranslational modifications, YAP/TAZ interaction with other proteins profoundly contributes to their cytoplasmic or nuclear retention. For example, the COOH-terminal PDZ-binding domain plays a role in the regulation of YAP: its deletion induces YAP relocalization to the cytoplasm and inhibits its activity. ZO2 interacts with YAP in a PDZ-dependent manner and was shown to colocalize with YAP into the nucleus, but it is unclear whether ZO2 itself is required for YAP nuclear localization (162, 177). Another example is YAP/TAZ sequestration within the $\beta$-catenin destruction complex, and its regulation by Wnt signaling (see below, Ref. 8).

Altogether, these observations suggest that nuclear localization of YAP/TAZ is the sum of multiple, possibly parallel, regulatory layers. YAP and TAZ lack of a nuclear localization signal (NLS), and the machinery for their nucleocytoplasmic shuttling and nuclear accumulation is unknown.

## D. Upstream Regulations of the Hippo Cascade in Mammals

A central question is how YAP/TAZ are regulated by extracellular cues. Several reports point to cell-cell adhesion and apical-basal polarity as regulators of YAP/TAZ localization and phosphorylation through the Hippo cascade.

### *1. NF2/Merlin is upstream of LATS*

Merlin is an important inhibitor of YAP/TAZ, acting upstream of the Hippo kinases. Merlin is encoded by the NF2 (neurofibromatosis type 2) tumor suppressor locus: germ line mutations of NF2 (1:30,000) lead to schwannomas and other tumors of the nervous system (73). These phenotypes can be recapitulated in NF2 mouse models, that are also predisposed to develop a number of different tumor types (67, 68, 138). In confluent monolayers of mammalian epithelial cells, Merlin/NF2 is preferentially localized in close proximity to adherens and tight junctions (AJs and TJs) **(FIGURE 2)**, and this localization appears essential for Merlin-mediated tumor suppression (107, 151, 195, 241). As such, Merlin also plays a role in the integrity of cell-cell junction (107).

Reconstituting Merlin in NF2 deficient MDA-MB-231 breast cancer cells represents a robust bioassay to inhibit YAP/TAZ activity by activation of the Hippo pathway (4, 50). This inhibition is entirely dependent on LATS1/2 and YAP/TAZ phosphorylation (4).

There are various, perhaps redundant, manners by which NF2/Merlin regulates the Hippo pathway. At cell-cell junctions, Merlin may promote the assembly of the appropriate protein scaffolds that allow LATS activation and YAP phosphorylation. For example, the WW-domain containing protein Kibra may serve as a bridge between LATS and Merlin at AJ (65, 147, 230, 247). Moreover, the Pan laboratory recently reported that, in *Drosophila* and mammalian cells, Merlin directly binds to LATS, recruiting it to the cell membrane where it gets synergistically activated by the Hippo/Sav kinase complex (243). NF2/Merlin also operates in the nucleus: it binds and inhibits the nuclear E3 ubiquitin ligase $CRL4^{DCAF1}$. Loss of NF2/Merlin unleashes this enzyme that ubiquitinates nuclear LATS1/2 fostering YAP/TEAD-dependent transcription (119).

### *2. YAP/TAZ and Hippo regulation downstream of Scribble and epithelial-to-mesenchymal transition*

The normal epithelial architecture, characterized by specialized cell-cell junctions and apicobasal polarity, is a potent suppressor of YAP/TAZ activity, at least in part by activation of the Hippo cascade (134). Mechanistically, this has been linked to membrane localization of Scribble, a key cell polarity determinant. At the cell membrane, Scribble serves as adaptor for the Hippo kinases by assembling a complex containing MST, LATS, and TAZ, required for MST-mediated activation of LAST **(FIGURE 2)** (38). Loss of this epithelial architecture is a hallmark of cancer and represents one of the first events of epithelial-to-mesenchymal

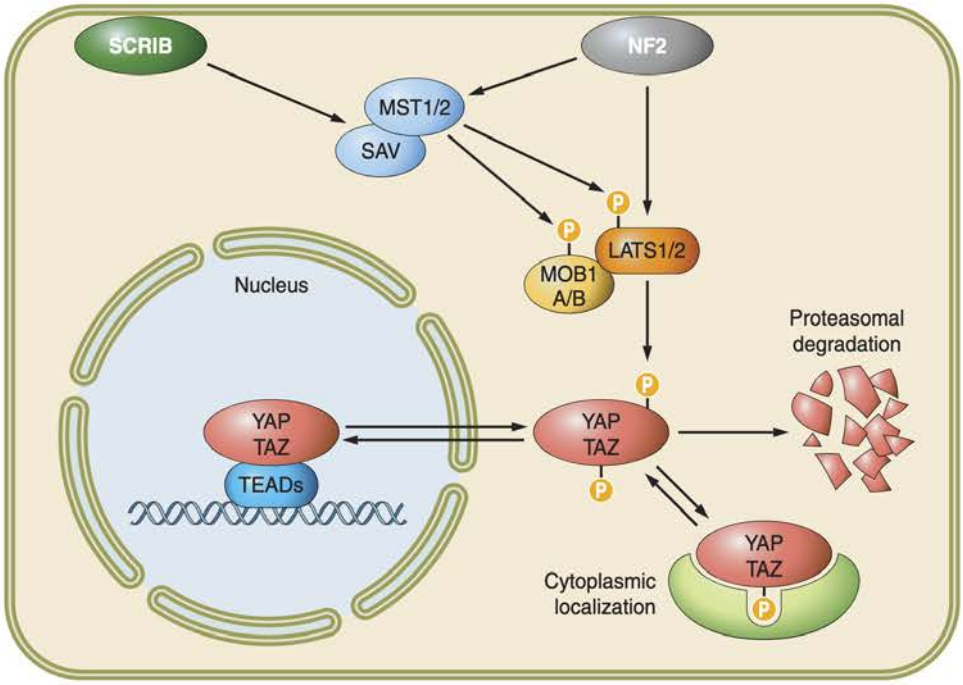


**FIGURE 2.** A model of the Hippo pathway in mammalian cells. See text for details.

transition (EMT) (79). In turn, EMT has been linked to acquisition of stemness and long-living potential (133, 203). In cancer, EMT is critical for tumor progression, to increase the cancer stem cell number, and for tumor dissemination. Interestingly, EMT triggers Scribble delocalization and inactivation of the Hippo cascade (38). This results in TAZ and likely YAP activation that mediate the acquisition of cancer-stem cell like traits typically endowed by EMT in mammary tumor cells (38).

Scribble inactivation or delocalization from the plasma membrane is frequent in cancer, and *Scribble*+/− mouse mutants are tumor prone (53, 134, 172, 251). Consistently, Scribble localization to the plasma membrane is sufficient to oppose YAP/TAZ activity by reactivating the Hippo kinases. For example, this has been shown after overexpression of the LIF-receptor in cells lacking E-cadherin (32). Notably, the functions of Scribble are evolutionary conserved between mammals and arthropods: loss of *Drosophila* Scribble, or of its partner LGL, causes dramatic tissue overgrowths that are genetically *yorkie* dependent (72).

Inputs other than EMT may use Scribble as hub to affect Hippo signaling and YAP/TAZ activity. Very recently, Camargo and colleagues (146) reported that, independently of EMT, the LKB1 tumor suppressor stabilizes the complex between Scribble and the Hippo kinases. This stabilization is mediated by Par1/MARKs, a target of LKB1. Collectively, these results suggest that the Scribble/Hippo axis could represent a main epigenetic event for YAP/TAZ regulation in human tumors.

### *3. Role of the apical protein Crumbs and AMOT*

In addition to Scribble, other polarity proteins may impinge on YAP/TAZ function. The apical crumbs complex (CRB) contains the transmembrane protein Crumbs and the associated cytoplasmic proteins Pals and Patj. CRB binds to YAP/TAZ and plays a role in their cytoplasmic localization (208). The underlying mechanistic details of CRB activity are unclear. It is well known that apicobasal polarity factors mutually oppose each other to sustain their asymmetric localization. Thus CRB might be upstream or downstream of Scribble. Moreover, Crumbs may tune the functions of another class of YAP/TAZ binding proteins, the Angiomotin family (AMOTs).

AMOTs have been identified as LATS and YAP/TAZ binding proteins in several mass-spectrometry studies (25, 191, 208, 220, 263). Initial reports supported an inhibitory role for AMOTs as factors promoting either the sequestration of YAP/TAZ (25, 163, 220, 263) or the activation of the Hippo pathway (1, 86). Moreover, AMOTs interact with F-actin (55), and this interaction is regulated by LATS-mediated phosphorylation. This event, directly or indirectly, may modulate YAP inhibition by AMOT (1, 26, 41, 86). An intriguing possibility is that F-actin/AMOT and YAP/AMOT complexes may be alternative to each other such that loss of F-actin may promote AMOT association to YAP (56, 87, 132). Consistently, the phenotypes of *AMOT* and *AMOTL2* deficiency in the early mouse embryo support a role of AMOTs as YAP inhibitors in Hippo pathway activation (86, 115). However, recent results cast doubts on

models envisioning AMOTs only as YAP/TAZ inhibitors. In fact, genetic evidence suggests that AMOTs are required for YAP activation in the context of NF2 deficiencies (240); biochemically, AMOTs may actually serve as YAP positive cofactor, preventing YAP phosphorylation or acting in the nucleus to guide YAP-mediated activation of a specific set of target genes. Thus further work is required to clarify the conflicting and likely context-dependent results on the role of AMOTs in YAP/TAZ regulation (148).

#### *4. Role of E-cadherin and α-catenin*

α-Catenin, a linker between cadherins and the actin cytoskeleton, is a potent inhibitor of YAP activity in keratinocytes, and α-catenin mouse mutants develop skin hyperproliferation and stem cell expansion similar to YAP-overexpressing mice (183, 185). In line, disturbing the E-cadherin/α-catenin complex decreases YAP phosphorylation and promotes YAP nuclear accumulation (102, 208). Distinct mechanisms have been proposed to explain these biologically relevant regulations: Kim et al. (102) proposed that LATS is activated donwstream of E-cadherin-mediated adhesion, while Schlegelmilch et al. (183) proposed that, in keratinocytes, α-catenin sustains the functions of a yet unknown kinase phosphorylating YAP in the same residues targeted by LATS. Clearly more work is required to address this issue in more depth.

The apparent variety in the modalities of Hippo activation by junctional and polarity factors begs the question of whether these represent parallel or rather interdependent events. Disruption of TJs or AJs by EMT or any other mean invariably turns on YAP/TAZ nuclear activities. A tempting, yet untested simplification may thus be the following: disturbing the cell's adhesive properties may inhibit the Hippo cascade indirectly through loss of cytoarchitecture and cell polarization.

### E. Mechanotransduction and the YAP/TAZ Cytoskeletal Pathway

Cells are subjected to different mechanical inputs generated by the elasticity of the extracellular matrix, by the pulling forces of neighboring cells, and by the fact that cells live in tissues that can be stretched or under pressure. In comparison with the classic signaling cascade initiated by a growth factor, which typically displays rapid on-off rates, mechanical signals are omnipresent and pervasive, targeting every cell and every moment of its life. Although these signals have been frequently overlooked, we are now witnessing a renaissance of mechanobiology studies, as it is now clear that cells extract key information from the physical nature of their environment. Cell mechanics and the status of the cytoskeleton are true overarching signals that cells use to make essential decisions, such as proliferation, differentiation, and maintenance of stem cells (48, 95, 131, 225). Strikingly, these mechanical and cytoskeletal inputs represent a central mechanism to control YAP/TAZ activity **(FIGURE 3)**.

A classic binary cellular decision, proliferation versus differentiation, is well known for being profoundly influenced by cell shape (59, 192). However, the underlying mechanisms behind this phenomena remained enigmatic for more than 30 years, until it was showed that when a single cell is allowed to stretch over the ECM, the cytoskeletal adaptation to the spread cell shape (including formation of F-actin stress fibers) causes YAP/TAZ activation and nuclear accumulation, promoting cell proliferation and inhibiting differentiation (50). Oppositely, when cell morphology is manipulated into a round and compact shape (by restricting adhesion to a very small ECM adhesive area), YAP/TAZ get excluded from the nucleus, their transcriptional properties are disabled, and cells stop to proliferate and initiate differentiation **(FIGURE 3)**. Crucially, YAP/TAZ are not just sensors of mechanical cues but also active mediators of their biological effects. For example, endothelial cells die on small adhesive areas (31), but if YAP/TAZ levels are artificially increased in round cells, these start to proliferate; vice versa, attenuation of YAP/TAZ in stretched cells causes them to die (50).

YAP/TAZ also respond to changes in ECM stiffness: a rigid ECM keeps active YAP/TAZ to the nucleus, while more compliant matrices favor YAP/TAZ inactivation.

Mesenchymal stem cells (MSC) can differentiate into distinct cell types depending on the stiffness of the ECM in which they are cultured (54, 137). High stiffness, namely, elevated YAP/TAZ, renders cells competent toward bone differentiation; softer environments progressively disable YAP/TAZ and this allows differentiation into other cell types, such as adipocytes (50) **(FIGURE 3)**. This model was recently linked to the phenotype of mouse knockouts for the secreted metalloprotease MT1-MMP: in the absence of ECM remodeling activity, MSC are entrapped into their ECM and unable to attain a spread geometry. This keeps YAP/TAZ inhibited, finally resulting in osteopenia (200). Fluid shear stress also translates into increased YAP activity (268).

Mechanical regulation of YAP/TAZ occurs in isolated cells and in a variety of cell types, irrespective of their mesenchymal, endothelial, or epithelial origin. This suggests the existence of a universal mechanism for YAP/TAZ control that is independent from the junctional and epithelial polarity complexes connected to the regulation of the Hippo cascade. Indeed, mechanical regulation is not only formally distinct from Hippo-mediated YAP/TAZ phosphorylation, but, in fact, dominates over it. LATS1/2 inactivation is inconsequential, namely, it cannot rescue YAP/TAZ inhibition, in cells with reduced mechanical stress, such as in cells

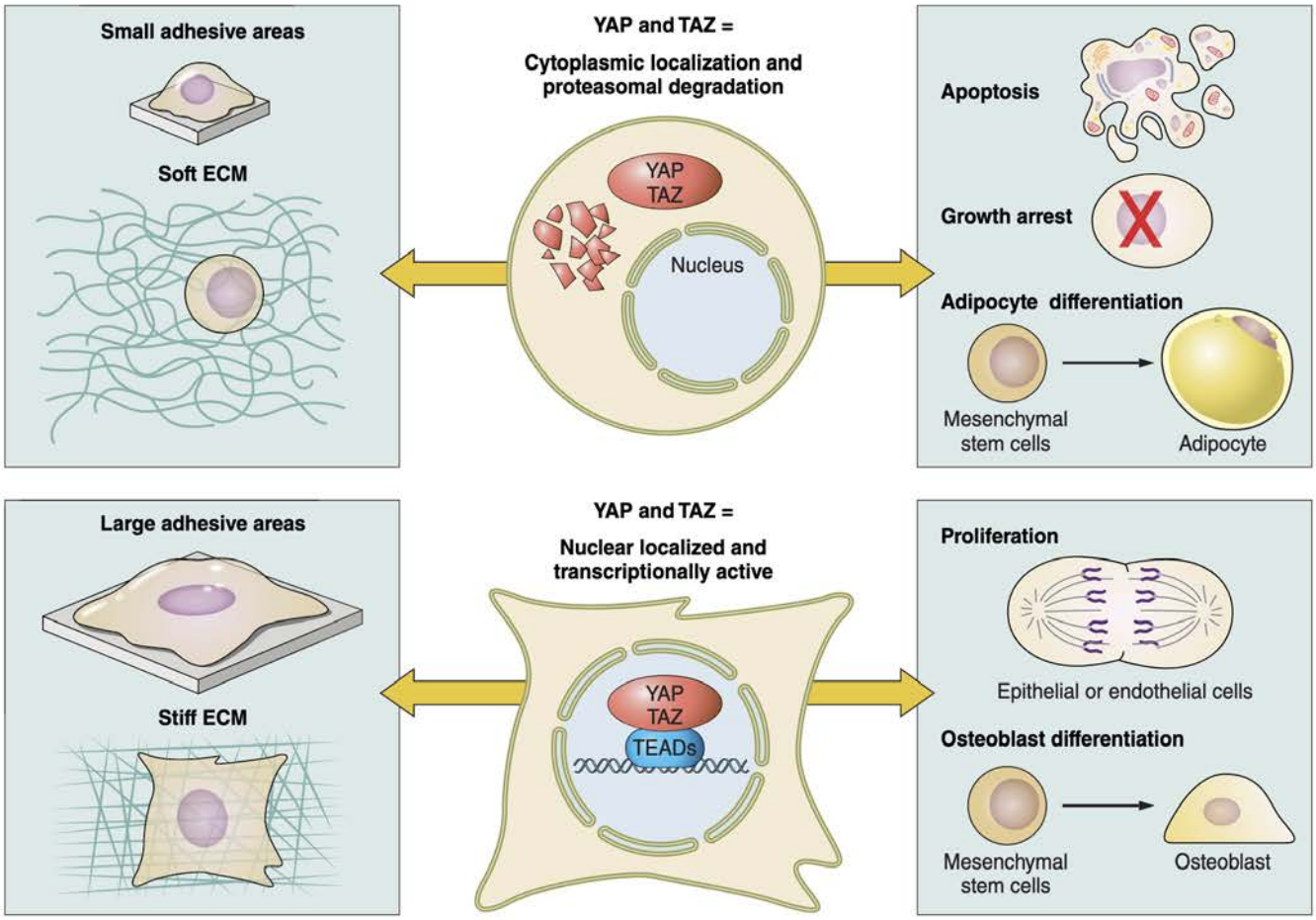


**FIGURE 3.** Representation of two modalities, cell spreading and ECM rigidity, by which mechanical cues affect YAP/TAZ activity and, in so doing, control multiple cell fate decisions. See text for details.

cultured on small adhesive areas or very soft gels, or cells treated with the F-actin inhibitor latrunculin A (4, 50).

Despite the fact that LATS1/2 kinase activity increases upon cytoskeletal disruption, the functional relevance of LATS1/2 as YAP/TAZ inhibitors can be appreciated only in cells experiencing a sufficient mechanical stimulation (217, 265). For example, Aragona et al. (4) showed that LATS1/2 depletion is ineffective at raising YAP/TAZ in cells cultured on soft matrices. However, LATS1/2-mediated inhibition of YAP/TAZ becomes apparent upon regeneration of the F-actin cytoskeleton (4). This can be achieved in cells cultured in soft, small matrices or at high cell density by depletion of F-actin inhibitors, such as Cofilin, CapZ, and Gelsolin, that operate as actin capping and/or severing proteins (4). Similarly, YAP/TAZ activation by Wnt (by itself independent of Hippo) and GPCR signaling (see below) is severely blunted by the cell's mechanical unloading. This suggests that cells "need" an appropriately structured F-actin cytoskeleton to sustain YAP/TAZ transcriptional activities and/or nuclear localization, irrespectively of the YAP/TAZ inducing inputs they experience. Provided a sufficient mechanical stress, a kaleidoscope of YAP/TAZ regulations is then possible: the effects of Hippo, Wnt, and GPCR signaling may be amplified or minimized by the status of the cytoskeleton. In turn, by regulating YAP/TAZ nuclear availability, Hippo, Wnt, and GPCRs may tune YAP/TAZ-mediated mechano-responses. Thus the scale and dynamic of YAP/TAZ activation can be very broad.

Zhao et al. (265) showed that cell detachment, a well-known inducer of cell death by anoikis, induces cytoskeletal reorganization and induction of LATS activity; however, while YAP overexpression fully rescued detachment-induced anoikis, depletion of endogenous LAST1/2 did not, indicating that a combination of LATS-dependent and -independent events concur to this phenomenon (4).

YAP has been investigated also in the context of the mechanical activation of cancer-associated fibroblasts (CAFs) (19). Here YAP is activated by aberrant ECM stiffening to promote a number of CAF protumorigenic properties. Interestingly, YAP self-sustains its own activity in CAFs, by promoting the expression of myosin regulatory light chain (MYL9) and actin contractility, that in turn incites "inside-out" ECM stiffening. Also in this case, mechanotransduction occurs independently of MST1/2 and of LATS-medi-

ated phosphorylation. In contrast, this regulation requires Rock, myosin, and Src activity.

Two studies in *Drosophila* support the notion that F-actin controls Yki activity and that this contributes to organ growth: depletion of capping protein A (ortholog to one of the subunits of CapZ), of Capulet (another inhibitor of actin polymerization) or overexpression of an activated form of the formin Diaphanous (Dia) all lead to increased F-actin bundles and wing imaginal disc tissue overgrowth (57, 181). Of note, overexpression and inactivation of LATS/Warts respectively counteracts and enhances these F-actin-associated phenotypes. The results are consistent with a scenario in which Yorkie activity is regulated in parallel, rather than epistatically, by F-actin and Hippo signaling. Interestingly, *Drosophila* mutants for Hippo, Warts, and Sav also display increased F-actin levels, suggesting that Hippo signaling and F-actin regulation are deeply untangled in fly tissues.

Finally, the existence of independent layers of YAP/TAZ regulation, mutually overseeing each other, is suggested by the pattern of YAP/TAZ activation in mammalian tissues: YAP/TAZ are enriched and active in the nucleus of stem/progenitor cells (20, 22, 110, 183, 253), yet there is no evidence that this localization is due to a patterned Hippo pathway. Moreover, whole-tissue depletion of the Hippo pathway does not cause widespread YAP/TAZ activation; in contrast, YAP/TAZ remains localized in the nuclei of stem/progenitor cells. This suggests that other contextual signals dominate over Hippo signaling, spatially templating the competence to activate YAP/TAZ to specific niches. It is tempting to propose that the physicality and architecture of these niches, including cell deformation and ECM composition, may provide a mechanical competence for stemness.

Clearly, the mechanism by which F-actin control YAP/TAZ activity remains one of the most important questions yet to be answered in the field.

### F. Regulation by Cell Density and Epithelial Architecture

Guan and colleagues (266) pioneered the concept that YAP and TAZ inactivation underlies the contact inhibition of proliferation (CIP), a classic paradigm of epithelial biology. During this process, cultured cells stop dividing when they become confluent, occupying the entire space allotted to them. CIP recapitulates the growth-arrested state of most epithelia, and loss of this "crowd" control is a hallmark of cancer. YAP and TAZ are nuclear and active in cells growing at low density but become cytoplasmic in confluent cultures (266). YAP and TAZ phosphorylation in S127 increases during CIP, and overexpression of a nonphosphorylatable form of YAP delays CIP, allowing cells to reach saturation densities higher than those of control cells. Collectively, the data support the notion that the Hippo pathway is activated in the course of CIP. However, at odds with this conclusion, other data indicate how the regulation of YAP/TAZ by contact inhibition is more complex; for example, double knockouts of MST1/2 in mouse embryonic fibroblasts (MEFs) or keratinocytes revealed that the Hippo pathway is dispensable for CIP in these contexts (183, 269).

Aragona et al. (4) recently provided a solution to this conundrum, by proposing a two-step model for CIP. When cells start making contact with each other, the E-cadherin/catenin system triggers LATS activation and YAP/TAZ phosphorylation (4). This contributes for only ~30% of growth inhibition and YAP/TAZ relocalization. E-cadherin, $\alpha$-catenin, and LATS1/2 depletions rescue such inhibition. In other words, "all around" cell-cell contact partially cripples YAP/TAZ activity through Hippo signaling, but leaves cells with sufficient nuclear YAP/TAZ to continue proliferation. For overt growth arrest and quantitative YAP/TAZ nuclear exclusion, a second, mechanical inhibitory step must occur: as proliferation continues, cell crowding in the monolayer progressively boxes individual cells into smaller areas. Reducing cell spreading to a tiny adhesive area impairs the YAP/TAZ mechanical pathway in a way not dissimilar from YAP/TAZ inhibition in isolated single cells plated on small ECM islands or soft ECM (see above). As expected from its mechanical nature, this second step does not involve E-cadherin, $\alpha$-catenin, and LATS1/2; instead, YAP/TAZ inhibition entails the remodeling of the F-actin cytoskeleton mediated by the F-actin capping and severing proteins Cofilin, CapZ, and Gelsolin(4). These proteins thus represent a form of "tissue-level" checkpoint of mammalian epithelial sheets.

Aragona et al. (4) also provided the first proof of principle that cells "read" the architectural topology of a tissue as patterns of local mechanical stresses, translating into "peaks and valleys" of YAP/TAZ activity. Similarly, information coming from changes in the three-dimensional rigidity of the ECM scaffold also reaches individual cells through YAP/TAZ-mediated mechanotransduction. In so doing, tissue conformation may use YAP/TAZ to literally template the patterns of cell proliferation and growth arrest within an epithelial monolayer. Although still speculative at this stage, mechanical forces transmitted through the interconnected mesh of ECM and cytoskeletal cables may serve as messengers of a global control whereby tissue architecture represents a new form of epigenetic memory, a template that perpetuates spatial cell organization through cellular generations.

### G. Regulation by Rho-GTPases

YAP/TAZ activity can also be regulated by Rho-GTPases: Dupont et al. (50) originally showed that treatment with the C3 toxin, an ADP-ribosyltransferase from *Clostridium bot-*

*ulinum* that potently oppose the function of Rho-GTPases, is also a potent inhibitor of YAP/TAZ activity.

These results suggested that a number of inputs upstream of Rho could also serve as upstream YAP/TAZ regulators. A first demonstration in this direction has been the work of Yu et al. (246), on GPCR, the largest family of membrane receptors in eukaryotes. A number of G protein-coupled receptors (GPCRs) were shown to signal through YAP/TAZ. Indeed, GPCRs couple to different combinations of heteromeric G proteins consisting of Gα, Gβ, and Gγ subunits. Although $G\alpha_s$-coupled signals (i.e., glucagon, epinephrine) repress YAP/TAZ, signaling through $G_{12/13}$ (stimulated by serum LPA or thrombin) or $G_{q/11}$ turns on YAP/TAZ transcriptional responses and reduces YAP-S127 phosphorylation (246). Depletion of YAP/TAZ inhibits cell migration and proliferation triggered by GPCR ligands (17, 145). One context in which the GPCR/YAP axis is particularly relevant is proliferation of uveal melanoma cells, carrying activating mutations in $G_{q/11}$ (56, 245).

Mechanistically, regulation of Rho-GTPases is part of the transduction cascades activated by GPCR, by impacting on YAP/TAZ phosphorylation. This correlates with the fact that LATS1/2 show reduced kinase activity upon Rho inhibition (246). Yet, the functional involvement of LATS1/2 for the biological effects of GPCR/Rho signaling, and YAP phosphorylation itself, is contentious (56, 245).

Two recent studies confirmed the positive role of Rho in YAP/TAZ activation and dephosphorylation, but excluded LATS1/2 as target of Rho activity. Sorrentino et al. (190) and Wang et al. (222) found that the metabolic pathway initiated by the mevalonate/HMG-CoA reductase supports YAP/TAZ activity in a variety of mammalian cell lines, inducing cell proliferation and migration, and sustains Yki-induced tissue overgrowth in *Drosophila* embryos. This is because the geranylgeranyl pyrophosphate produced by the mevalonate cascade is required for proper membrane tethering and thus activity of Rho-GTPases. Statins, the popular cholesterol reducing drugs that inhibit this pathway, downregulate YAP/TAZ activity in cell cultures and in in vivo assays, in a manner that could be rescued by forcing RhoA membrane recruitment. While YAP/TAZ phosphorylation increased upon statin treatments or Rho inhibition (as in the study of Yu et al., Ref. 246), Rho-regulated YAP phosphorylation in S127 turned out surprisingly to be LATS independent. This suggests that the activity of Rho-GTPases is critical for YAP/TAZ function by inhibiting a yet unknown kinase, distinct from LATS1/2.

An open issue related to the function of Rho GTPases in the YAP/TAZ world is the relationship between Rho GTPases and the actin cytoskeleton. F-actin depolymerization with latrunculin A inhibits GPCR-mediated induction of YAP/TAZ activity, apparently suggesting that the actin cytoskeleton may be downstream of GPCR/Rho signaling (246). However, the interpretation of these results is hampered by the fact that integrity and organization of the F-actin cytoskeleton is essential for any YAP/TAZ activity, blocking the effects of ECM stiffness but also of Wnt stimulation or Hippo inactivation (4). The conclusion from Sorrentino et al. (190) instead suggests that the Rho and cytoskeletal pathway can be formally distinguished, as Rho regulates YAP through phosphorylation, while the cytoskeleton is phosphorylation-independent (4). A potential confounding issue in this respect is the well-established function of RhoGTPases in F-actin remodeling, contractility, and stress fibers formation, which are known mediators of the cytoskeletal pathway. It is possible that the F-actin subpool required for YAP/TAZ activity in the cytoskeletal pathway may not overlap with the subpool of F-actin regulated by Rho. This scenario may be consistent also with the finding that Rac1 and CDC42 have been implicated in YAP regulation (56, 265), despite the fact these proteins regulate distinct types of F-actin structures and are generally considered to play mutually antagonistic functions. Intriguingly, mice bearing kidney-specific inactivation of CDC42 display defective nephrogenesis, mirroring the effects of YAP knockout in the same tissue (176) (see below). YAP is inactivated in CDC42-mutant cells in a Hippo/MST-independent manner.

## H. Wnt Signaling Through Nuclear YAP/TAZ and Hippo/Wnt Cytoplasmic Crosstalk

### 1. YAP/TAZ orchestrate the Wnt response

YAP/TAZ are not only messengers of the cell's structural features, but also of Wnts, a leading family of growth factors involved in cell proliferation, stem cell expansion, regeneration, and tumorigenesis (36, 154). Recent work highlighted a deep integration of YAP/TAZ in the Wnt pathway that mechanistically explains the extensive overlaps between Wnt and YAP/TAZ biology **(FIGURE 4)**. The core of the Wnt pathway is the regulation of its nuclear transducer β-catenin by a cytoplasmic destruction complex, consisting of a central scaffold protein, Axin, that interacts with other factors, such as adenomatous polyposis coli (APC), CK1, and glycogen synthase kinase-3 (GSK3). In absence of Wnt signaling, the destruction complex targets β-catenin to degradation through β-catenin phosphorylation by GSK3 and its ubiquitination by β-TrCP. The arrival of a Wnt ligand causes functional inactivation of the destruction complex, resulting into β-catenin accumulation and formation of nuclear complexes with the β-catenin DNA-binding partners TCF/Lef (36).

Azzolin et al. (8) discovered that YAP and TAZ are components of the β-catenin destruction complex. The significance of this is twofold: *1*) YAP/TAZ are sequestered in the cytoplasm in the destruction complex, and 2) cytoplasmic

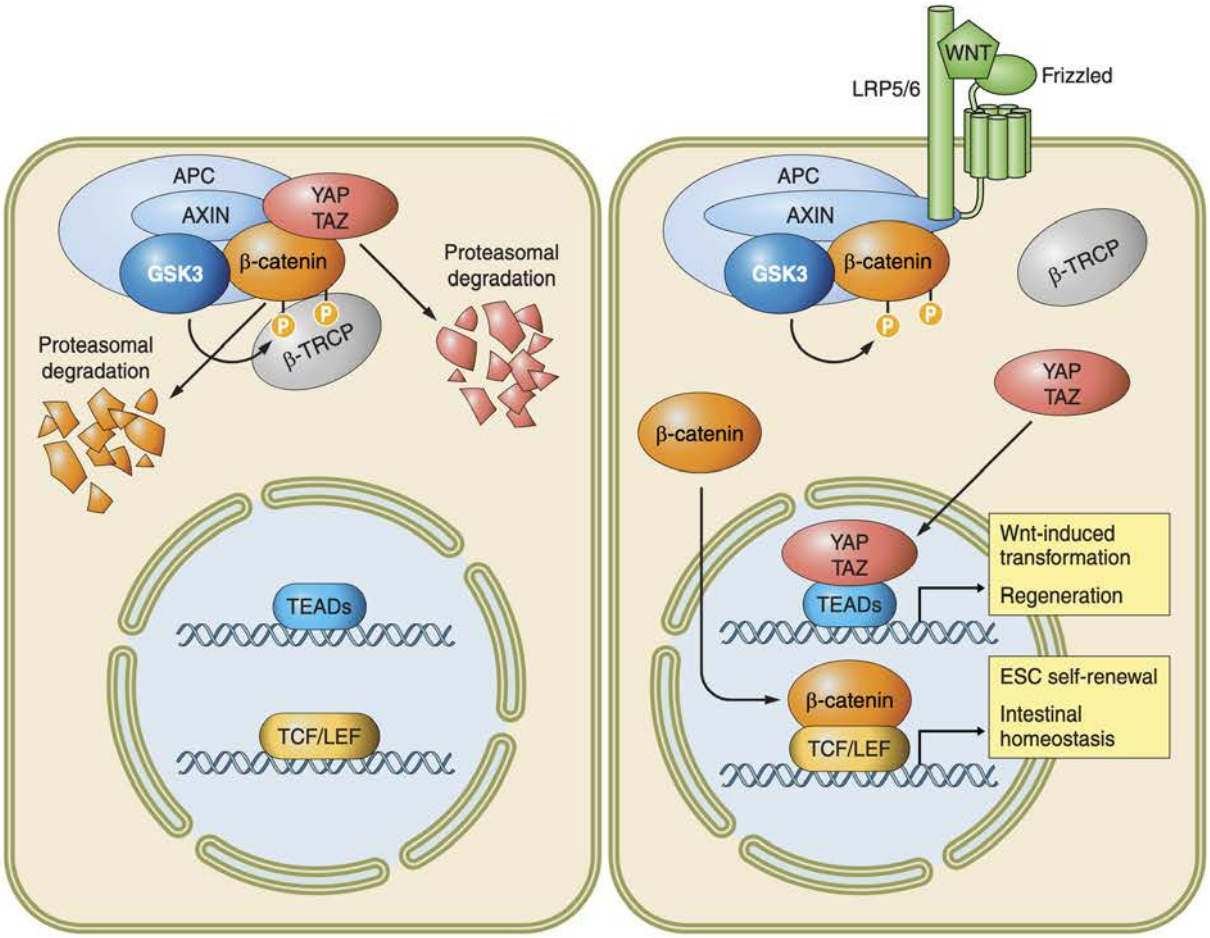


**FIGURE 4.** Role of YAP and TAZ in Wnt signaling. See text for details.

YAP/TAZ associate to Axin and are required for recruitment of β-TrCP to the complex. As such, in "Wnt OFF" cells, YAP/TAZ are critical for β-catenin degradation, and depletion of YAP/TAZ leads to the activation of β-catenin/TCF transcriptional responses **(FIGURE 4)**. This model has been biologically validated in mouse ES cells where YAP/TAZ knockdown favors β-catenin-dependent self-renewal. Similarly, TAZ knockout mice develop polycystic kidneys whose cells accumulate β-catenin in the nucleus (206). Consistently, overexpression of a cytoplasmic and transcriptionally defective version of YAP in the mouse intestine can rapidly turn off β-catenin/TCF targets and cause crypt degeneration (12). The arrival of a Wnt ligand triggers the association between the Wnt receptor LRP6 and Axin with concomitant release of YAP/TAZ from the destruction complex (8). The consequence of such release is again twofold: *1*) without YAP/TAZ, the destruction complex is now "invisible" to β-TrCP, favoring β-catenin accumulation; and 2) YAP/TAZ can now accumulate in the nucleus leading to the activation of Wnt-induced, YAP/TAZ-dependent transcriptional responses **(FIGURE 4)**.

As such, YAP/TAZ can serve either as nuclear, transcriptional mediators of Wnt signaling or as antagonists of Wnt/β-catenin signaling in the cytoplasm. Such duality is reinforced by additional regulatory mechanisms: on the one hand, cytoplasmic YAP/TAZ can inhibit β-catenin nuclear entry, and oppose phosphorylation of the Wnt transducer Dvl (94, 206). On the other hand, the destruction complex assembles a phospho-β-catenin/TAZ/β-TrCP association that leads to TAZ (but not YAP) degradation (9). In other words, the presence of YAP/TAZ and phospho-β-catenin in the destruction complex allows β-TrCP recruitment leading to TAZ and β-catenin inhibition. By disassembling that complex, Wnt does not only promote nuclear accumulation of YAP/TAZ but also TAZ stabilization.

### *2. Hippo/Wnt crosstalk*

While TAZ phosphorylation is not required for its degradation by phospho-β-catenin, it remains unclear whether the Hippo kinases are required for a stable incorporation and sequestration of YAP/TAZ in the destruction complex. Interestingly, LATS2 has also been linked to inhibition of oncogenic Wnt/β-catenin signaling: independently of its kinase function, nuclear LATS2 interacts with β-catenin interfering with the interaction between β-catenin and its essential cofactor BCL9 (120).

Collectively, the data indicate a deep integration of YAP/TAZ into the Wnt cascade as well as a role of YAP/TAZ as Wnt transcriptional mediators in parallel to, or independently from, β-catenin itself. The data also configure interesting scenarios for future studies by which cells compute various inputs into coherent responses: a Hippo ON state would blunt total levels of nuclear YAP/TAZ, short-cutting Wnt/TAZ/YAP signaling. At the same time, by promoting cytoplasmic retention of YAP/TAZ, a Hippo ON state would also coherently bar Wnt/β-catenin signaling. Conversely, the Hippo OFF state would license both Wnt/β-catenin and Wnt/YAP/TAZ signaling. This crosstalk may ensure that some cellular responses are induced only in the presence of coherent inputs: for example, activation of stem cell amplification only when Hippo is OFF and WNT is ON. That said, a Hippo OFF scenario, with ensuing YAP/TAZ release from the destruction complex and nuclear localization, may be actually sufficient to activate nuclear β-catenin signaling: in support of this hypothesis, genetic ablation of Hippo pathway components is sufficient to localize β-catenin in the nucleus of cardiomyocytes and to foster their proliferation, leading to development of oversized hearts, in a manner that is YAP/TAZ as well as β-catenin dependent (84).

### *3. Role of YAP/TAZ in Wnt biology*

What are the biological consequences of YAP/TAZ in the Wnt cascade? The final outcome of a Wnt treatment will ultimately depend on the relative relevance of the gene expression programs activated by TCF/β-catenin and YAP/TAZ/TEAD signaling branches for a given Wnt-regulated process (8). There may be contexts or conditions in which the Wnt response is mainly sustained by just one of these branches; in other contexts, both branches might be essential. For example, in mesenchymal stem cells, Wnt-induced bone differentiation requires nuclear YAP/TAZ (9), while the role of β-catenin remains unclear (11). Instead, one example in which YAP/TAZ transcriptional responses are irrelevant for the Wnt response is intestinal homeostasis. Inactivation of the receptors LGR4/5 for the Wnt agonist R-Spondin leads to crypt degeneration in vivo and in vitro (43), highlighting the requirement of the Wnt cascade for intestinal homeostasis and regeneration (36). YAP and TAZ are localized in the nucleus of intestinal progenitor cells located at the bottom of the crypt. However, intestinal-specific double knockout of YAP/TAZ in adult mice is ostensibly inconsequential for normal intestinal physiology. That said, YAP/TAZ are absolutely required for crypt growth induced by aberrant Wnt activation in vivo (upon loss of the APC tumor suppressor) or during crypt regeneration in organoid cultures (8). The latter result correlates with the requirement of YAP for intestinal regeneration after inflammatory insults. The key role of YAP and TAZ as downstream effectors of Wnt-induced cell proliferation is also supported by studies in colorectal cancer cells: YAP/TAZ-target genes represent a large portion of Wnt targets, and essential for the proliferation of APC-deficient colorectal cancer cell lines (9, 180). It is tempting to speculate that β-catenin and the YAP/TAZ transcriptional programs may mediate distinct outcomes of Wnt signaling, with β-catenin/TCF controlling normal homeostasis and YAP/TAZ playing a central role in conditions characterized by intense intestinal proliferation, such as during APC deficiency, tumorigenesis, and regeneration.

The here described interlace between YAP/TAZ and β-catenin regulation may also help to explain some intriguing, but seemingly confusing observations recently reported by the field. Barry et al. (12) showed that absence of YAP in the intestinal epithelium synergized with R-Spondin in triggering robust crypt cell proliferation and regeneration after irradiation (12), results hardly compatible with the requirement of YAP for intestinal regeneration reported by Cai et al. (18) and with the combined role of YAP/TAZ for crypt regeneration reported by Azzolin et al. (8). However, the model shown in **FIGURE 4** can in principle reconcile these conflicting findings. Partially lowering YAP/TAZ dosage, as described by Barry et al. (12) with a single YAP knockout, may on the one hand unleash TCF/β-catenin-dependent transcription, while on the other hand still preserving the nuclear function of TAZ. In other words, the opposing functions of YAP/TAZ as mediators and inhibitors of Wnt signaling may actually coexist, adding sophisticated regulatory opportunities.

A further sophistication is that some Wnt target genes are under joined control of both YAP/TAZ and β-catenin, in a sort of "double-assurance" mechanism. For example, in cardiomyocytes, both YAP/TEAD and β-catenin/TCF bind, perhaps cooperatively, to cognate promoter elements in the *Sox2* and *Snai2* genes (84); and in colorectal cancer cells, YAP and β-catenin might share a common DNA-binding platform, the transcription factor TBX5, to transcribe the *Birc5* and *Bcl2l2* pro-survival genes (180).

Thus we just started to explore the intimate and complex relationships between YAP/TAZ and β-catenin in the context of Wnt signaling. A better understanding of the biological contexts in which these relationships are relevant may have far reaching implications, particularly in the fields of stem cells and cancer.

## I. Nuclear YAP/TAZ Complexes

The notion that YAP and TAZ act in the nucleus as transcriptional coactivators was originally suggested in mammalian cells, when they were isolated as binding proteins of the RUNX or TEAD transcription factors and shown to enhance the activation of the corresponding luciferase reporters upon overexpression. Fusion of YAP/TAZ with the heterologous GAL4 DNA binding domain provided formal evidence of their transcriptional activation potential and

indicated a requirement for the COOH-terminal portion of YAP and TAZ for transcription (99, 209).

TEADs have been repeatedly isolated as main YAP and TAZ interacting transcription factors in mass-spectrometry experiments (29, 209, 252), in line with results in *Drosophila*, in which the TEAD homolog Scalloped was identified as key (although not exclusive) partner for Yorkie transcriptional activity in imaginal discs (69, 227, 256). A series of studies suggests that TEAD factors mediate a number of YAP/TAZ functions in mammalian cells. First, phenotypes obtained by overexpressing YAP and TAZ are abolished by point mutations in key amino acidic residues of the $NH_2$-terminal TEAD binding domain (29, 165, 204, 252, 267) (see **FIGURE 1**). This includes clonal growth of epithelial stem cells and YAP/TAZ-dependent growth in soft agar. Moreover, a constitutive-repressive form of TEAD fused to the engrailed repressor domain blocked YAP-induced liver overgrowth (125). Second, loss of TEADs cause superficially similar phenotypes to those caused by loss of YAP, or by activation of the Hippo cascade (156, 157, 165, 182, 261). Third, in chromatin-immunoprecipitation experiments, YAP- and TEAD-bound genes display an extensive overlap (261); finally, TEAD1 was found mutated in a cohort of patients affected by the Sveinsson's chorioretinal atrophy genetic disease, where all patients bear a point mutation (Y421H) that abolish YAP/TEAD interaction, thus potentially explaining lack of tissue growth/regeneration (60, 103).

Genome-wide analyses of YAP and TAZ transcriptional targets have been carried out by several groups both in vitro and in vivo, leading to the identification of important target genes that are now widely used to monitor YAP/TAZ activity (such as *CTGF*, *CYR61*, *ANKRD1*, *BIRC5*, *AXL*, *InhA*, *Col8a1* and others), and to the definition of useful YAP/TAZ target gene signatures (19, 38, 50, 249). These studies relied on YAP/TAZ overexpression potentially highlighting only a specific fraction of YAP/TAZ targets. In the future, it will be important to couple YAP/TAZ loss-of-function experiments with ChIP-seq data to determine the set of direct YAP and TAZ target genes. Moreover, these types of studies should also be important to clarify to what extent the transcriptional activity of YAP and TAZ overlaps that of TEADs and/or other factors.

Finally, it must be considered that both YAP/TAZ and TEADs bind to other nuclear proteins. For example, TEADs factors interact with the Vestigial family of transcriptional coactivators (75, 210). A role as TEAD corepressor has been recently proposed for Vestigial-like 4 (Vgll4), opposing hepatomegaly and liver tumor development in YAP transgenics and NF2-null mice (105). This correlates with findings in *Drosophila*, whereby TEAD/Scalopped functions as a repressor, with Yorkie relieving such repression rather than acting as a pure coactivator. This offers a simple explanation for the observation that *yki*, but not *Scalloped*, is essential for growth of most fly tissues: in the presence of Yorkie, Scalloped is inactive, thus resulting genetically dispensable. Whether a similar "default repression" mechanism operates with TEAD and YAP/TAZ in mammals remains to be investigated.

YAP and TAZ were repeatedly isolated as binding proteins for Smads, key transducer of the TGF-$\beta$ and BMP signaling pathways (2, 207, 208). Cytoplasmic YAP/TAZ participate in Smad2/3 cytoplasmic retention, even overruling the effects of high levels of TGF-$\beta$ ligands (207, 208). Moreover, YAP1 binds Smad7, enhancing the inhibitory activity of Smad7 against the TGF-$\beta$ receptors (58). Interestingly, the pattern of Smad activity and YAP phosphorylation appears inversely correlated in early mouse embryos (208). The interaction with Smad2/3 entails the TAZ coiled-coil domain (207). The WW domains are essential for YAP-induced cell proliferation (261) and mediate binding of YAP/TAZ to PPxY motif-containing transcription factors such as RUNX, p73, and the cytoplasmic domain of ERBB4 or with the transcriptional cofactor WBP2 (28, 40, 104, 140, 164, 197, 214).

The interaction of YAP with p73 is particularly interesting as it mediates a proapoptotic function of YAP in the context of DNA damage. Indeed, c-Abl directly phosphorylates YAP upon genotoxic insults leading to YAP association with the p53-family member p73 (117, 196). In turn, YAP sustains p73 stability (118) and selectively coactivates p73 proapoptotic target genes. This chain of events is a main tumor suppressor pathway in hematological cancers: YAP expression is reduced in these tumors blocking p73 activation by c-Abl and chemotherapy (39).

Other YAP and TAZ binding transcription factors are as follows: PPAR-$\gamma$, with which TAZ works as corepressor during MSC adipogenic differentiation (89); PAX8 in the context of thyroid development (45); TTF1/Nkx-2.1 in lung alveolar type II epithelial cells (143, 170); and Tbx5 in colorectal cancer cells (180).

A major area of future studies relates to the role of YAP/TAZ on chromatin. Some advancements have been recently reported, such as the role of the chromatin-remodeling complex Brg1 for TAZ-mediated activation of vimentin and CD44 transcription in MCF10A cells (186). In *Drosophila*, protein-protein and chromatin-immunoprecipitation data also suggest that Yorkie pairs with chromatin remodeling complexes and with the Mediator complex to regulate transcription (160). That said, the epigenetic control of YAP and TAZ function remains largely unexplored.

The function of YAP/TAZ in the nucleus may go beyond association with transcription factors and direct control of promoter transcription. Two papers recently proposed that

nuclear YAP is a general regulator of the microRNA processing machinery (30, 149), although with opposite conclusions. On the one hand, YAP binds and sequesters the p72/DDX17, a key cofactor of Drosha. This occurs in a manner that does not require the association of YAP to TEAD, and possibly explains the global miRNA downregulation by low cell density in keratinocytes (149). On the other hand, YAP and TAZ control the levels of Dicer by acting posttranslationally and are thus required for efficient pre-miRNA processing in cells cultured at low density (30). Irrespectively, the functional implications of these findings for YAP/TAZ biology remain to be explored, also taking into consideration the body of literature on the widespread requirement of YAP/TEAD binding for a number of phenotypes.

### J. Pro-apoptotic Functions of MST and LATS

MST1/2 have long been recognized as pro-apoptotic kinases in response to a number of cellular stresses, including exposure to DNA damage, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and presence of activated oncogenes (135, 188). In this context, MST1/2 are cleaved by caspases, that clip a COOH-terminal autoinhibitory domain leading to enhanced kinase activity and autophosphorylation (70, 71, 173). Self-activated MST1/2 translocate in nucleus, ultimately leading to cell death. In this pathway, the downstream targets of MST1/2 include the stress-induced JNK and p38 kinases, the histones H2B and H2AX, and FOXO transcription factors (35, 113, 202, 229, 248). Adding a new twist to this story, Maejima et al. (128) recently provided evidence that MST1 phosphorylates Beclin, inhibiting autophagy and biasing cells to apoptosis. MST1-phosphorylated Beclin dissociates from its authophagy partners Atg14L and Vps34, causing blockade of the normal autophagy flux and accumulation of protein aggregates and damaged mitochondria. Moreover, MST1-phosphorylated Beclin detaches Bax from Bcl2, leading to Bax-induced apoptosis.

MST1/2 are positively regulated by heterodimerization with members of the RASSF protein family, frequently inactivated in human tumors through epigenetic silencing (179). Association to RASSF releases MST1/2 from an inhibitory complex with Raf1 and increase MST1/2 autoactivation (101, 136, 159, 161, 169). MST1/2 are also required for centrosomal integrity, regulating both centrosome duplication and disjunction, ensuring accurate chromosome alignment and segregation during mitosis. Interestingly, part of these functions of MST1/2 are mediated by the LATS-related kinases Ndr1/2 (211).

Lats2 is also primarily localized at centrosomes, where it is thought to contribute to the organization of the mitotic spindle (93, 212). Indeed, Lats2(−/−) embryos and MEFs display centrosome amplification and genomic instability (141, 237). Coherently, a Lats2-Mdm2-p53 pathway has been shown to represent a checkpoint by which p53 blocks the propagation of tetraploid cells. Activation of oncogenic stress (for example, RasV12 overexpression) or damage to the mitotic apparatus cause Lats2 to depart from the centrosome and accumulate in the nucleus, where it binds and inhibit Mdm2, stabilizing p53 (5–7). Collectively, LATS1/2 concur in the processes that govern maintenance of mitotic fidelity and genomic stability.

The tumor suppressive functions of MST1/2 and LATS1/2 mentioned in this paragraph appear largely distinct and parallel to those impinging on YAP/TAZ regulation; yet, an involvement of YAP/TAZ has not been formally excluded so far. Indeed, the paradoxical proapoptotic and anti-apoptotic functions of YAP remind us that potential unorthodox roles of YAP/TAZ may only need to be discovered, particularly in cells experiencing specific forms of stress. Also unclear is to what extent these putative "YAP/TAZ-unrelated" functions contribute to Hippo-regulated organ growth and stem cell biology.

## III. YAP/TAZ FUNCTIONS IN ORGANS AND TISSUES

### A. Germ Line Knockouts

In *Drosophila*, Yorkie is required for proliferation of several embryonic tissues, and loss of Hippo pathway components or F-actin inhibitors induces tissue overgrowth (57, 77, 81, 97, 129, 181, 201). The first hint that this function was conserved in mammals came from observations in YAP transgenic mice generated independently by two groups (20, 49). These mice showed widespread cell proliferation and tissue overgrowth. Subsequent mouse genetic studies then focused on the role of Hippo pathway components. Germ line mutants of NF2, MST1/2, and LATS2 were not particularly informative as death occurred at relatively early embryonic stages: in NF2−/− and MST1/2−/− caused by defective development of the extraembryonic tissues, and in LATS2−/− by multiple defects (139, 141, 269).

A major breakthrough in the Hippo field has been the use of conditional knockout alleles and inducible transgenic mice leading to the current appreciation of the various functions of Hippo signaling in organ growth, cell proliferation, stem cell amplification, and cell fate decision

### B. Liver

The simple overexpression of YAP in the liver of transgenic animals is sufficient to induce a fourfold increase in liver mass caused by proliferation of mature hepatocytes (20, 49); this also leads to the acquisition of biliary duct/liver progenitor cell traits by the hepatocytes (242). This over-

growth phenotype is dependent on TEAD-mediated gene responses, as it is rescued by treatment of YAP transgenics with Verteporfin, a small-molecule inhibitor of YAP/TEAD interaction, or coexpression of a dominant-negative version of TEAD2 (125). Of note, YAP-mediated activation of the Notch pathway is important for the observed phenotypes (242).

Liver overgrowth was also observed upon tissue-specific genetic inactivation of *MST1/2, Salvador/Sav/WW45*, or *NF2/merlin* by using the embryonic liver "deleter" albumin-Cre (15, 112, 127, 188, 257, 269). In these cases, enhanced proliferation was interpreted mainly as an expansion of oval cells, a population of liver cells that serves as progenitors of both hepatocytes and bile-duct epithelial cells after liver damage, although recent lineage-tracing data strongly suggest that additional oval cells in Hippo mutant mice were in fact derived by dedifferentiation of the hepatocytes (242). In all cases, with age, hepatomegaly is followed by development of tumors of mixed characteristics, resembling both hepatocellular carcinomas and cholangiocarcinomas, in keeping with aberrant mobilization of a progenitor compartment such as oval cells. Moreover, oval cells were expanded after hepatotoxin injury in *Sav* mutants (127). *Nf2*-null livers also develop bile duct hamartomas with very early onset (as soon as 1 mo), well before signs of liver overgrowth (125, 257). Interestingly, *MST1/2* knockouts, but not *NF2* and *Sav* mutants, display liver damage and inflammation, likely contributing to accelerated tumor growth. Thus Hippo pathway components are suppressors of liver growth and liver tumor development at least in part by regulating the number of liver progenitor cells.

Importantly, the phenotypes due to *NF2* inactivation were rescued by the combined deletion of one *YAP* allele, establishing NF2 as a bone fide upstream regulator of YAP in mammals (257). The same epistatic relationship between YAP and NF2 was also validated in the eye lens epithelium, where *NF2* inactivation induces the formation of cataracts, and *Yap* co-deletion rescues it (257). The requirement for *YAP* or *TAZ* in *MST1/2*- or *WW45*-deficient livers is likely but remains formally unproven.

Biallelic, liver-specific inactivation of *YAP* causes the expected decrease in hepatocyte proliferation and increase in apoptosis, but it also induced defective bile duct morphogenesis due to dysfunction of the cholangiocytes; as a result, mice display a mild liver enlargement, steatosis, and progressive fibrosis (257). Upon bile duct ligation (a model of cholestatic injury), *YAP* liver knockout mice display decreased duct cell proliferation and enhanced parenchymal damage, suggesting a positive role for YAP in liver regeneration (10). The phenotypes associated with combined deletion of *YAP* and *TAZ* for liver development, homeostasis, and regeneration are unknown.

Similarly to the YAP requirements, *NF2−/−* liver phenotypes can be also rescued by codeletion of *AMOT*, and *AMOT* is required for the response to hepatotoxin injury (240). These results are consistent with a positive role of AMOT for YAP/TAZ function in vivo.

Metabolites may serve as ideal sensors of organ total mass, and recent evidence suggests that bile acid flux may indeed serve as endogenous regulator of liver mass. Mice with a severe defect in bile acid homeostasis have enlarged livers, progenitor cell proliferation, and YAP activation and develop spontaneous liver tumorigenesis, recapitulating the defects of Hippo mutants (3).

As detailed in the previous sections, most of what we know about YAP/TAZ regulation is centered on their posttranslational regulation. However, an issue largely overlooked is the contribution of *YAP/TAZ* transcription and mRNA levels to YAP/TAZ biology. For example, transcription of the *YAP* gene is controlled in the liver by the GABP transcription factors, and transgenic GABP overexpression causes increased *YAP* expression and liver overgrowth (226).

### C. Heart

Conditional deletion of YAP in embryonic cardiomyocytes affects their proliferation leading to severe heart hypoplasia, and a similar phenotype has been reported in TEAD1 knockouts (34, 182, 216, 232). Consistently, overall heart size is increased by YAP overexpression, in a TEAD-dependent manner. *Salvador/WW45*, *Mst1/2*, and *Lats2* inactivation in developing mouse hearts also caused severe heart enlargement (84, 188).

Deletion of *YAP* postnatally (i.e., after cardiomyocyte maturation) was associated with progressive dilated cardiomyopathy, with thinning of the ventricular walls eventually causing lethal heart failure (232). In this case, an exquisite dosage-dependent function for *YAP* and *TAZ* was demonstrated: while *TAZ* inactivation has no effect, the inactivation of *TAZ* together with *YAP* accelerated the onset of the cardiac disease, with complete *YAP/TAZ*-null hearts being unable to sustain postnatal life. Very interestingly, overexpression of activated YAP in the adult heart enhanced, while deletion of *YAP* impaired, the regenerative response to myocardial infarction (44, 232). Similarly, deletion of *Salvador/WW45* and *LATS1/2* in adult cardiomyocytes enabled their proliferation in postnatal life, facilitating heart regeneration upon infarction or partial surgical resection (83). These findings hold important consequences for the development of new therapeutic approaches that, by inhibiting Hippo kinases, could facilitate heart regeneration in patients suffering cardiac damage.

### D. Intestinal Epithelium

YAP overexpression in transgenic mice by means of an inducible and ubiquitous promoter potently expands intestinal cell proliferation at the expense of differentiation, without affecting whole organ size (20). Intriguingly, nuclear YAP is endogenously restricted to intestinal progenitor cells at the bottom of intestinal crypts, and this cell population expands up to the tip of the villus after YAP overexpression.

Conditional knockout of *Salvador/WW45* and *MST1/2* in the intestine also induces crypt hyperplasia accompanied by decreased differentiation, in a manner that genetically depends on *YAP* (18, 111, 270). Moreover, conditional knockout of Sav displayed a potent and YAP-dependent regenerative response in a colitis model [i.e., treatment with dextran sulfate sodium (DSS)]. Thus, similarly to that described in heart and liver, the Hippo-YAP axis is an important regulator of intestinal tissue proliferative homeostasis.

As more extensively discussed in section II*H*, epithelial-specific inactivation of both *YAP* and *TAZ* has no effects on normal tissue homeostasis (8, 12, 18). The requirement of *YAP* and *TAZ*, however, can be revealed during intestinal regeneration. After DSS treatment, lack of *YAP* impairs epithelial proliferation and crypt repopulation leading to rapid death (18), whereas combined deletion of *YAP/TAZ* blocked crypt growth in an ex vivo culture set-up, eventually leading to the demise of the mutant crypts (8). Moreover, combined deletion of *YAP* and *TAZ* rescues intestinal hyperplasia caused by acute activation of the Wnt pathway after *APC* depletion (8).

### E. Epidermis

YAP/TAZ play important roles in skin homeostasis: overexpression of activated YAP in the basal layer of the epidermis causes thickening and increased proliferation of keratinocytes, with defective stratification and reduced terminal differentiation. Gain of YAP can specifically expand the epidermal stem cell compartment, as shown by clonogenic assays (183, 185, 253). Oppositely, when *YAP* is deleted from the basal layer of the embryonic epidermis, mice display reduced stratification caused by reduced keratinocyte proliferation and reduced stem cells' self-renewal leading to skin loss or thinning in E18.5 pups (183, 185). Interestingly, knock-in of a mutated YAP isoform unable to interact with TEADs was unable to rescue a YAP null allele, suggesting that TEADs are required for these phenotypes (183). The roles of YAP or YAP/TAZ in the postnatal epidermis or hair follicle dynamics are still unknown.

Among the multiple defects of *Salvador/WW45* knockouts, one of the best characterized is the hyperplasia of the epidermal tissue, accompanied by hyperproliferation of basal keratinocytes, appearance of suprabasal proliferating cells, and delayed or imperfect terminal differentiation (111). These phenotypes recapitulate those of YAP overexpression phenotypes and correlate in vitro with deficient MST1/2 activity and inhibition of YAP. A similar phenotype was observed upon deletion of *MOB1A/B* (155). Surprisingly however, inactivation of *MST1/2* in the skin does not result in any phenotype, and in HaCaT keratinocytes, knockdown of LATS1/2 has no effect on YAP activity or phosphorylation (183, 185). This suggests that alternative kinases might be responsible for YAP inactivation in this tissue.

Also mice lacking $\alpha$-catenin, a core component of Ajs, display phenotypes consistent with YAP activation. Keratinocytes deleted of $\alpha$-catenin overproliferate in a YAP-dependent manner and display enhanced YAP nuclear accumulation and activity (183, 185).

### F. Nervous System

Evidence for the involvement of YAP in brain development comes from inactivation of *NF2* in the dorsal telencephalon, causing severe malformations due to expansion of neural progenitor cells (NPC) in the cortical hem, hippocampus, and neocortex. Transgenic overexpression of YAP induces a hippocampal phenotype similar to *NF2* inactivation, while combined loss of *NF2* and *YAP* rescues this phenotype (110). These data are consistent with results obtained in the developing neural tube of chick and *Xenopus* embryos, whereby overexpression of YAP caused overproliferation and decreased differentiation of neural progenitor cells, while dominant-negative YAP and TEAD proteins led to premature differentiation and apoptosis (22, 63). Similarly to that observed in other organs, *YAP* inactivation had no detectable phenotypic consequences (110).

Other recent reports in the mouse and chicken indicate a role for the giant protocadherin *Fat4* in neural epithelial progenitors, possibly through YAP/TAZ (23). Similar to *NF2* inactivation or YAP overexpression, *FAT4* deficiencies cause an increase in progenitor cell proliferation, and a corresponding decrease in neuron differentiation. In line with this, downregulation of YAP activity by use of overexpressed dominant-negative molecules or of YAP-targeting microRNAs counteracted the phenotypes due to *Fat4* deficiency.

### G. Pancreas

Pancreatic deletion of *MST1/2* did not induce organ overgrowth but instead a reduction of organ size. This was accompanied by altered tissue architecture, acinar cell atrophy, expansion of ductlike cells, and inflammation, reminiscent of acute pancreatitis (62, 66). In accordance with increased YAP activity, ductal cells of MST1/2 knockouts were shown to maintain a proliferative status throughout

adult life, but their overgrowth was likely balanced by tissue autodigestion due to defective formation of ductal structures (absence of terminal intercalated ducts). Overexpression of active YAP caused the expansion of ductal cells at the expenses of acinar structures, while deletion of one allele of *YAP* in the context of *MST1/2* deficiency was sufficient to rescue these phenotypes. MST1/2 deficiency had no effect on the endocrine pancreas.

Notably, pancreatic tissue remains histologically normal after homozygous deletion of *YAP* (259) from adult tissues, indicating that YAP is not involved in the homeostasis of the normal pancreatic epithelium, or that TAZ could compensate for YAP deficiencies. In contrast, deletion of both *YAP* alleles blocks the development of pancreatic ductal adenocarcinomas after conditional mutation of KRAS and p53, although it has no effect on KRAS-induced acinar-to-ductal metaplasia (259).

### H. Kidney

During organogenesis, inactivation of *YAP* or *TAZ* in kidney precursor cells (metanephric mesenchyme) produces very different phenotypes: *YAP* is required for efficient nephron morphogenesis, while *TAZ* inactivation causes polycystic kidney disease (90, 130, 176). This clearly indicates that, at least in this tissue, YAP and TAZ have distinct and specific functions. A link between YAP and kidney development was also observed in zebrafish (187) and has been mechanistically associated with CDC42 GTPase activity in mouse models(176).

### I. Early Embryonic Development

YAP/TAZ double null mutants die before implantation (156). *YAP*−/− embryos die shortly after gastrulation, at stage E8.5 (150). Embryos display a shortened and highly disorganized body axis, abnormal neural morphogenesis, defects in the formation of the yolk sac vasculature, and defects in chorioallantoic fusion. These embryos bear superficial similarities to the phenotype observed in compound *TEAD1/2* knockout embryos, where the axial mesoderm is formed but not maintained (182). *TAZ* knockouts display high rates of embryonic lethality, but a fraction of TAZ mutants develop to term and die of polycystic kidney disease and pulmonary emphysema (90, 130).

In mammals, the first cell fate decision occurs in the embryo at the blastocyst stage (250). The outer cells of the blastocyst, in contact with the zona pellucida and the external fluids become the trophoectoderm (TE); TE cells acquire strong apicobasal polarity, establish tight junctions, and express the TE master gene Cdx2. Cells of the inner cell mass are nonpolar and stabilize expression of pluripotency transcription factors such as *Oct4* and *Sox2*.

Genetic data in mice indicate that *cdx2* expression and TE development require the *TEAD4* gene (157, 238). Studying the role of *YAP/TAZ* in the ICM versus trophoblast decision has not been possible, as double knockouts do not survive past the morula stage. That said, immunofluorescent stainings at the morula stage indicate that YAP and TAZ are enriched in the nucleus of TE cells, while ICM cells display cytoplasmic YAP/TAZ. Overexpression of activated YAP and TEAD, or LATS1/2 inactivation, are sufficient to induce Cdx2 expression in ICM; conversely, LATS2 overexpression suppresses Cdx2 expression in TE (156). These findings suggest a role for YAP/TAZ activity as inhibitor of embryonic pluripotency; in contrast, YAP/TAZ have been reported to play pro-stemness roles in mouse ES cells (121).

The mechanisms that pattern YAP/TAZ at these early stages of development are incompletely understood but likely involve a combination of factors: a role for NF2 and angiomotins in preventing YAP activation within inner cells (37, 86, 115), and possibly mechanical activation of YAP/TAZ in external cells during compaction (224).

### J. YAP/TAZ and Stem Cells

YAP/TAZ have been extensively portrayed as "stemness factors" (reviewed in Ref. 175). However, we would like to note that this idea is based on the combination of two distinct and so far poorly connected observations: the embryonic organ overgrowth induced by YAP/TAZ activation in Hippo mutants or YAP transgenics and the localization of YAP/TAZ proteins in cells at the stem cell niche of distinct adult organs. On the one hand, the cellular targets of YAP/TAZ activity during fetal growth remain largely unexplored; more generally, it is unclear whether a connection exists between adult, tissue-specific stem cells and their embryonic counterparts. On the other hand, the evidence linking YAP/TAZ function to normal adult stem cells is surprisingly scant: conditional genetic inactivation of YAP in adult mammary gland, pancreas, liver, and even dual knockout of YAP and TAZ in the intestine was largely inconsequential (8, 33, 257, 259). Intriguingly however, YAP and TAZ are strikingly essential in conditions in which stem cells need to be amplified for tissue regeneration or after oncogenic transformation (8, 33, 257, 259). Although these analyses are in their infancy (mainly due to the lack of double YAP/TAZ knockout studies), the data indicate that YAP/TAZ regulate stem cells only when they are stimulated by specific intrinsic or extrinsic cues.

## IV. ROLE OF YAP/TAZ IN CANCER

### A. Control of Proliferation, Survival, and Cancer Stem Cells

A number of studies suggest that human tumors use the biological properties of YAP/TAZ to foster their own proliferation, progression, migration, and metastasis (with the exception of hematological tumors, see section III*I*). As detailed in **TABLE 1**, immunohistochemical characterization of human tumor samples suggests that these proteins are activated at very high frequency during progression toward malignancy. YAP has been detected in late-stage ovarian, colon, gastric, liver, esophageal, non-small-cell lung cancers and lobular type of invasive breast cancers (see **TABLE 1** and references therein). TAZ has been detected in the nucleus of most of, if not all, malignant breast, lung, and colon cancers. Aberrant YAP and/or TAZ nuclear localization, or high expression of YAP/TAZ target genes (i.e., gene signatures), are associated with poor outcome (i.e., recurrence and short disease-free survival) in large datasets of breast and colon cancer patients **(TABLE 1)**.

YAP/TAZ expression in human tumors pairs with the protumorigenic phenotypes of mouse knockouts of Hippo pathway components (155, 193, 269); together, these data support the notion that aberrant YAP/TAZ are instrumental for tumor growth as they are of normal organ growth. Indeed, YAP/TAZ function may underlie some of the key hallmarks of cancer (79), such as uncontrolled proliferation, escape of cell death, and induction of cancer stem cells.

#### *1. Proliferation*

In *Drosophila*, clones of imaginal disk cells bearing hyperactive Yorkie grow faster than wild-type cells; conversely, clones bearing inactive *yorkie* alleles are outcompeted by faster growing wild-type cells (91). This function is conserved in mammalian cells: overexpression of nonphosphorylatable forms of YAP or TAZ increases the proliferation of several human or mouse cell lines in vitro (114, 165, 167, 267). As discussed previously, this is nicely paralleled in vivo, as YAP overexpression or loss of Hippo control increases organ size at least in part by sustaining their cell proliferation (49, 112, 188, 257). Conversely, depletion of YAP/TAZ by RNA interference blocks growth of various human cancer cell lines (9, 27, 144, 244, 270).

How YAP/TAZ control cell proliferation remains a mystery. The interaction with TEAD cofactors has been shown to be essential for promotion of cell growth in some contexts (165, 252, 267). It is thus conceivable that proliferation may rely on some YAP/TAZ direct target genes: several candidates have been in fact suggested, ranging from signaling molecules such as AREG or AXL, to cell cycle regulators like FOXM1 and CyclinD1 (144, 234, 254). However, the functional involvement and general relevance of these genes for YAP/TAZ-induced proliferation are uncertain. Of note, aberrant YAP/TAZ may not need to accelerate the cell cycle; enhanced proliferation may also result from the bypass of tissue-level checkpoints. For example, this may relate to the capacity of overexpressed YAP/TAZ to overcome contact inhibition and leaves cells competent to respond to oncogene or external mitogens (4).

#### *2. Cell survival*

YAP and TAZ can repress programmed cancer cell death induced by loss of cell-substrate contacts (anoikis) (265). Moreover, overexpression of YAP blocks TNF-$\alpha$ and FAS-induced cell death in mouse liver (49). Conversely, TAZ knockdown in breast cancer cells strongly reduces their ability to survive treatments with chemotherapeutic drugs, such as taxol (106). The molecular mediators of these effects are unknown.

Two recent papers studied the mechanism of tumor recurrence after K-Ras inhibition and intriguingly linked the rescue of cell survival of otherwise K-Ras-addicted tumor cells to YAP1 activation (100, 184). This bears important clinical implications, as it suggests that YAP/TAZ activation may be a common late event in human tumors, favoring escape from targeted-chemotherapy.

#### *3. Endowing cancer stem cell traits*

The growth of solid tumors has been also proposed to rely on cells with properties of stem cells, called cancer stem cells (CSCs) (213). These properties include sustained proliferative potential, capacity to resist stress and chemotherapy, and loss of differentiation markers. Importantly, elevated TAZ activity has been shown to be required and sufficient to endow these CSC characteristics. First, TAZ is required for self-renewal and tumor-initiation capacities of breast cancer cells, as measured by the capacity of cells to grow as self-regenerating mammospheres and to form tumors once cancer cells are injected as limiting dilutions in immunocompromised mice (13, 38). Second, gain-of-TAZ endows CSC properties to otherwise non-CSC populations, enabling them to generate high-grade, undifferentiated tumors that resist chemotherapy (13, 38). Third, YAP/TAZ have been recently reported to be instrumental for breast and lung cancer metastasis in animal models, and associated with metastatic diseases in human patients (13, 38, 85, 108, 109).

The provocative concept emerging from these studies is that, just like organ growth may be ascribed to YAP/TAZ-dependent maintenance of the stem cell pools and inhibition of differentiation, tumor progression and malignancy may be associated with YAP/TAZ-mediated increase in the cancer stem cell representation driving tumor growth. The two

**Table 1.** *Role of YAP/TAZ in tumors*

| Tumor Type | YAP/TAZ Expression | YAP/TAZ Functions | Genetic Alterations on *YAP/TAZ* and *TEAD* | Genetic Alterations on the Hippo Pathway Components | Other Genetic Alterations | Reference Nos. |
|---|---|---|---|---|---|---|
| Breast cancer (BC) | High TAZ expression and nuclear activity in high-grade and triple-negative BCs; nuclear YAP is associated with invasive lobular BC; elevated YAP/TAZ activity is prognostic of metastatic proclivity | TAZ is a determinant of CSC state; YAP and TAZ support metastatic dissemination of human BC cells; genetic ablation of YAP reduces mammary tumor formation in PyMT mice | *WWTR1* (encoding TAZ) is frequently amplified in some triple-negative BCs | None reported | No relevant alterations reported | 33, 38, 108, 186, 215, 194 |
| Colorectal cancer (CRC) | YAP/TAZ expression and activity are elevated in malignant cancers and are prognostic for poor outcome | Knockdown of YAP reduces tumor growth and impedes metastatic dissemination of human CRC cells; TAZ is required for human CRC cell growth; genetic deletion of YAP and TAZ abolishes intestinal tumor formation in APC knockout mouse models | None reported | None reported | Constitutive activation of the Wnt pathway in 97% of CRCs, mostly because of mutations inactivating APC | 8, 9, 21, 180, 194, 218, 249 |
| Gastric carcinoma (GC) | Nuclear YAP correlates with bad prognosis; low VGLL4 expression correlates with bad prognosis | YAP is required for growth and invasive abilities of human GC cells; YAP overexpression promotes in vivo tumor growth of human GC; VGLL4 overexpression inhibits in vivo tumor growth of human GC | None reported | None reported | Activating mutations on RHOA are found in 28% of diffuse-type GC | 96, 98, 189, 255 |
| Glioblastoma (GB) | High TAZ expression correlates with the mesenchymal subtype of GBs | TAZ overexpression confers malignant traits to glioma cells; TAZ knockdown impairs tumor growth of human GB cells of the mesenchymal subtype | None reported | None reported | No relevant alterations reported | 16 |
| Hepatocellular carcinoma (HCC) | High YAP expression is prognostic for poor outcome | YAP is required for human HCC cells to grow as tumors in vivo; YAP overexpression in mouse liver leads to the development of HCC | *YAP1* (encoding YAP) is rarely amplified | None reported | The components of the β-catenin destruction complex are mutated in ~20% of HCC cases | 49, 78, 205, 233–235 |
| Melanoma | YAP and TAZ are found in both benign lesion as well as in malignant melanomas | Knockdown of YAP or TAZ impairs invasive and metastatic abilities of human skin melanoma cells | *YAP1* is amplified in 4% of malignant skin melanomas | None reported | Genes encoding $G_{q/11}$ are mutated in 83 and 6% of uveal melanomas and skin melanomas, respectively; activating mutations of *RAC1* have been identified as driver mutations in 9% of skin melanomas | 56, 88, 153, 245 |
| Mesothelioma | No reports | Knockdown of YAP impairs human mesothelioma cell growth | YAP1 is amplified in a minority of cases | NF2 is inactivated by mutation or genetic deletion in 45% of mesothelioma cases; LATS2 is inactivated in a minority of cases | No relevant alterations reported | 152, 244 |
| Nonsmall cell lung cancer (NSCLC) | YAP and TAZ expression are prognostic of bad outcome | YAP is required for tumor formation by human NSCLC cells; YAP and TAZ are required for metastatic dissemination of malignant mouse lung cancer cells; YAP overexpression confers metastatic abilities to mutant-KRAS-induced mouse lung adenocarcinoma cells | YAP1 is amplified in ~25% of NSCLCs; WWTR1 amplification is found on a fraction of NSCLCs | None reported | *STK11* (encoding LKB1) is mutated in ~20% of lung adenocarcinomas; APC is mutated in 7% of lung adenocarcinomas | 47, 109, 126, 146, 158, 194, 198, 231, 258 |
| Osteosarcoma | YAP expression correlates with tumor stage | Yap knockdown inhibits tumor formation by human osteorsacoma cells | None reported | None reported | No relevant alterations reported | 239, 258 |
| Ovarian cancer (OC) | YAP and TEAD4 expression are prognostic for bad outcome | YAP overexpression promotes tumor growth and metastasis of human OC cells | *YAP1* is rarely amplified | None reported | No relevant alterations reported | 76, 194, 228, 260 |
| Pancreatic ductal adenocarcinoma (PDAC) | Yap expression does not change from normal ducts to PDAC | YAP is required for human PDAC cell growth; YAP is required for progression to PDAC in the mutant-KRAS/p53 mouse model; YAP expression overcome KRAS dependency in human and mouse PDAC cells | None reported | None reported | No relevant alterations reported | 46, 100, 259 |

processes may actually represent a continuum, at least in epithelia, as tumors invariably develop after YAP/TAZ-induced organ overgrowth. In other words, tumors and metastases are, by all means, aberrant secondary organs, that still need to build themselves by corrupting the universal YAP/TAZ-centered mechanisms that control organ size and tissue replenishment. Perhaps not by chance, tumors have long been recognized as "caricatures" of normal organs, or as "wounds that never heal" (51).

It will be now very important to extend these results to other tumor types and to find validations in mouse tumor models. In this line, TAZ has been recently shown to play essential roles in the growth, self-renewal, and differentiation of the mesenchymal subtype of glioblastomas (16).

### B. What Is Inducing YAP/TAZ in Cancer?

The widespread and pervasive upregulation of YAP/TAZ in human cancer suggests that YAP/TAZ may represent a common end point of various pathways involved in cell transformation. Much research is required to clarify this aspect, but, surprisingly, YAP/TAZ activation cannot be explained by mutations in Hippo pathway components in human cancer. Indeed, with the exception of inherited disorders associated with *NF2*, somatic mutations in the core Hippo members are extremely rare in human tumors (82).

This suggests that either we still have to discover essential YAP/TAZ pathway components that are disrupted in cancers, and/or that the Hippo pathway may be targeted nongenetically during cell transformation. In this respect, disturbed cell polarity or EMT may represent common modalities to blunt the Hippo cascade. In turn, loss of cell architecture is part of a more general loss of normal tissue organization that typifies cancer. This includes altered tissue mechanics, increased ECM rigidity due to collagen deposition and inflammation, and structural changes in the stem cell niche (51, 52). As such, epigenetic Hippo inactivation blends with aberrant pro-YAP/TAZ mechanical signals to disrupt the potent tumor suppressive properties of normal tissues. Classic work using transformed mammary cells showed that once these cells are grown as spheres within a soft ECM, tumor cells displayed a remarkable normalization of their behavior (116, 171, 223). Oppositely, when nontransformed cells where grown in three dimensions in an abnormally rigid ECM, they started to change shape, lose polarity, and display malignant characteristics (116). Mechanical YAP/TAZ modulation is at the root of these behaviors (4), indicating that loss of tissue architecture and/or altered ECM composition convey a wrong set of instructions to individual cells, wreaking havoc spatial control of proliferation.

## V. CONCLUSIONS

In reviewing the current status of the Hippo field, we could highlight just some of the many exciting questions generated by research in this area. Some of the most critical ones relate to possible therapeutic applications: can we combat cancer by tackling YAP and TAZ? Can we improve organ regeneration by tuning them at will? It is hard to predict whether the answer to these questions will be affirmative, but there is room for optimism: only few years ago, all we knew about YAP/TAZ regulation was the presence of two inhibitory Hippo kinases. The discoveries of other upstream inputs, such as the cytoskeleton, Rho, Wnt, and GPCR greatly expanded the complexity of YAP/TAZ regulation and, as such, the repertoire of possible routes for therapeutic intervention.

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## DISCLOSURES

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