Review

EMBO reports

The Hippo signaling pathway in stem cell biology and cancer

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Abstract

The Hippo signaling pathway, consisting of a highly conserved kinase cascade (MST and Lats) and downstream transcription co-activators (YAP and TAZ), plays a key role in tissue homeostasis and organ size control by regulating tissue-specific stem cells. Moreover, this pathway plays a prominent role in tissue repair and regeneration. Dysregulation of the Hippo pathway is associated with cancer development. Recent studies have revealed a complex network of upstream inputs, including cell density, mechanical sensation, and G-protein-coupled receptor (GPCR) signaling, that modulate Hippo pathway activity. This review focuses on the role of the Hippo pathway in stem cell biology and its potential implications in tissue homeostasis and cancer.

Keywords cancer; Hippo pathway; regeneration; stem cell; YAP
DOI 10.15252/embr.201438638 | Received 17 February 2014 | Revised 16 April 2014 | Accepted 16 April 2014 | Published online 13 May 2014
EMBO Reports (2014) 15, 642–656

See the Glossary for abbreviations used in this article.

Introduction: The Hippo signaling pathway

The Hippo pathway is evolutionally conserved and regulates diverse cellular processes, including cell survival, proliferation, differentiation, and organ size. This pathway was initially characterized through clonal genetic screens identifying genes involved in tissue growth control in *Drosophila melanogaster*. In *Drosophila*, the core components of the Hippo pathway include the kinase cascade of Ste20-like kinase Hpo (Hippo) and NDR family kinase Wts (Warts) [1–7]. Hpo complexes with the scaffolding protein Sav (Salvador) to phosphorylate and activate Wts, which then forms a complex with its regulatory protein Mats (Mob as tumor suppressor) [8–10]. When in complex with Mats, Wts directly phosphorylates the transcriptional coactivator Yki (Yorkie), sequestering it in the cytoplasm by promoting its interaction with 14-3-3 [11–15]. Conversely, when the Hippo pathway is inactivated, unphosphorylated Yki translocates into the nucleus where it associates with the TEAD/TEF family transcription factor Sd (Scalloped) to initiate gene expression, promoting cell survival and proliferation [16,17]. Yorkie can also bind to other DNA binding proteins including Mad, Homothorax (Hth), and teashirt to promote gene expression [18,19].

The Hippo pathway is a tumor suppressor pathway because mutations in these regulatory pathway components result in an overgrowth phenotype.

In mammals, the Hippo pathway consists of the serine/threonine kinases MST1/2 (mammalian Ste2-like kinases, Hpo orthologs) and LATS1/2 (large tumor suppressor kinase 1/2, Wts orthologs) [7,20–22]. Activation of the Hippo pathway results in the inactivation of YAP (Yes-associated protein, Yki ortholog) by LATS1/2-mediated direct phosphorylation on YAP Ser127 (in humans). Phosphorylated YAP is sequestered in the cytoplasm via binding to 14-3-3 and is degraded in a ubiquitin-proteasome-dependent manner, which depends on phosphorylation of YAP Ser381 and Ser384 [23]. Conversely, dephosphorylated YAP acts mainly through TEAD family transcription factors to promote cell proliferation and organ growth [24]. TAZ (transcriptional coactivator with PDZ binding motif), a paralog of YAP in mammals, is regulated by the LATS1/2 in a similar manner. YAP/TAZ are the major downstream mediators of the Hippo pathway. Besides the TEAD family transcription factors, YAP/TAZ also interacts with other transcription factors including Smad, Runx1/2, p73, ErbB4, Pax3, and T-box transcription factor 5 (TBX5) to mediate the transcription of a diverse array of genes, although the biological functions of these other transcription factors in mediating Hippo signaling are less clear [25].

Although the core signaling cascade from Hpo (MST1/2) to Yki (YAP) is well understood, the upstream regulators of the Hippo pathway are just beginning to be delineated. Interestingly, accumulating evidence from both *Drosophila* and mammals has shown that apical–basal polarity proteins may regulate the Hippo pathway by controlling YAP/TAZ localization. For instance, earlier studies in *Drosophila* implicated the apical membrane-associated FERM-domain proteins Mer (Merlin) and Ex (Expanded), which are apical tumor suppressors, and the WW and C2 domain-containing protein Kibra (kidney and brain protein) as components upstream of Hpo. The Mer/Ex/Kibra complex recruits Hpo to the plasma membrane to enhance its kinase activity [26–30]. The apical transmembrane protein Crb (Crumbs) also interacts with Ex and modulates its (Ex) localization and stability [31–35]. Similar to Crb, the Scrib (Scribble) complex (Scrib/Dlg/Lgl) and Par3 polarity complex (Par3/Par6/αPKC) have been implicated in the regulation of the Hippo pathway activity [34,36,37]. In addition, a multitude of other cellular junction

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Glossary

BMP	Bone morphogenetic protein
CC	Cholangiocarcinoma
CSNK1	Casein kinase 1
FGF	Fibroblast growth factor
HCC	Hepatocellular carcinoma
Hering canal cells	Origin of liver stem/progenitor cells in adult livers
HIPK2	Homeodomain-interacting protein kinase2
Hippo	Ste20-like kinase Hpo
Id	Inhibitor of DNA binding proteins
LATS1/2	Large tumor suppressor kinase 1/2, Wts orthologs
LIF	Leukemia inhibitory factor
MARK1-4	MAP/microtubule affinity-regulating kinases
MASK	Multiple ankyrin repeats single KH domain-containing protein
Mats	Mob as tumor suppressor
Mob1	MOB kinase activator 1A
MST1/2	Mammalian Ste2-like kinases, Hpo orthologs
PALS1	Membrane-associated palmitoylated protein 5
PATJ	PALS-1-associated tight junction protein
PcG	Polycomb group protein
RASSF	RAS association domain-containing family protein
Sav1	Salvador homolog 1
SIK1-3	Salt-inducible kinases
TAOK1-3	Thousand and one amino acid protein kinases
TAZ	Transcriptional coactivator with PDZ binding motif
TGF-β/activin	Transforming growth factor B
Warts	NDR family kinase Wts
YAP	Yes-associated protein, Yki ortholog

proteins, such as PATJ, PALS1, AMOT (angiomotin), ZO-1 (zona occludens protein 1) [38,39], E-cadherin [40], α/β-catenin [41,42], PTPN14 (protein tyrosine phosphatase non-receptor type 14) [43–45], and Ajuba/Zyxin protein [46,47], have also been identified as regulators or interacting partners of core Hippo pathway components in mammals. Other newly characterized Hippo pathway regulator is the PCP (planar cell polarity) complex, composed of transmembrane cadherins Ft (Fat) and Ds (Dachsous). In *Drosophila*, Ds binds to Ft, which in turn activates the Hippo pathway by inhibiting the interaction between Zyxin and Wts, thus favoring its (Wts) degradation [47]. The vertebrate homolog of Ft is FAT4, but it is still unclear whether FAT4 is involved in regulating the Hippo pathway in vertebrates [48,49]. In any case, further studies are needed to define the exact role of the PCP in regulating the Hippo pathway.

Several studies have reported that YAP/TAZ is regulated by mechanical cues from neighboring cells and the extracellular matrix [50–52]. Moreover, the Hippo pathway is potently and acutely regulated by a wide array of extracellular hormones, including lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P), epinephrine, glucagon, and thrombin [53–55]. These mechanical and hormonal cues appear to be mediated through the actin cytoskeleton. Mechanically, stabilization of F-actin results in YAP/TAZ activation, while the disruption of F-actin leads to YAP/TAZ inactivation [56]. Hormonally, G-protein-coupled receptors (GPCRs) transduce extracellular hormonal cues through RHO GTPases and the actin cytoskeleton to modulate YAP/TAZ. GPCR signaling can either stimulate or inhibit YAP/TAZ activity in a manner dependent on the coupled G-protein. For example, activation of G12/13 or Gq/11 stimulates YAP/TAZ, while Gs inhibits YAP/TAZ. Surprisingly, the LATS1/2 kinase may not be involved in YAP/TAZ regulation by mechanical cues [50], whereas the LATS1/2 kinase is involved in GPCR-medicated hormonal cues to YAP/TAZ and MST1/2 is not required. The precise mechanism by which the actin cytoskeleton relays upstream cues to modulate LATS1/2 kinase activity is still not fully understood and remains a key question in the field.

Beyond the main components of the Hippo pathway described above, many other additional proteins have been reported to modulate the Hippo pathway, including TAOK1-3 (thousand and one amino acid protein kinases) [57,58], MARK1-4 (MAP/microtubule affinity-regulating kinases) [59,60], SIK1-3 (salt-inducible kinases) [61], RASSF (RAS association domain-containing family protein) [61–63], MASK (multiple ankyrin repeats single KH domain-containing protein) [64,65], HIPK2 (homeodomain-interacting protein kinase 2) [66,67], and CSNK1 (casein kinase 1) [23,68] (Fig 1).

Hippo signaling in embryogenesis and embryonic stem cells

The first cell differentiation event in mammalian development occurs during preimplantation, when the outer blastomeres of the embryo form an outer epithelial trophectoderm (TE) that envelops the remaining blastomeres, the inner cell mass (ICM). The TE is necessary for implantation and later contributes to the placenta. Embryonic stem cells (ESCs) are pluripotent cells, derived from the ICM of an early blastocyst, that have the potential to self-renew and differentiate into different cell types and tissues. This pluripotent capacity raises hope for their potential application in regenerative medicine [69].

The association between Hippo signaling and stem cell-like properties has been previously shown. For example, *Yap*$^{-/-}$ embryos arrest during development around E8.5 and display a yolk sac vascular defect [70]. The YAP target transcription factors, TEADs, are the earliest genes expressed at high levels during embryo development, and TEAD4 is required for specification of the TE lineage during preimplantation of the mouse embryo [71–73]. At the blastocyst stage, TEAD4 promotes expression of multiple genes associated with trophoblast specification, including Cdx2 and Gata3, which are selectively expressed only in blastomeres destined to become TE [71,74,75] (Fig 2A). Moreover, it has been shown that this activity of TEAD4 is dependent on YAP localization in the nucleus, which is modulated by cell–cell contact and LATS1/2-mediated phosphorylation. This finding suggests that YAP localization is essential for TEAD4 activity and cell fate specification [76]. Additionally, NF2 (Neurofibromin 2) and AMOT, two upstream components of the Hippo pathway, facilitate YAP phosphorylation via LATS1/2 during cell fate specification of mouse preimplantation development [77,78] (Fig 2B). Although TAZ is also highly enriched in the developing mouse embryo, inactivation of the gene encoding TAZ, *Wwtr1*, results in only minor skeletal defects and the development of renal cysts, and these mice still grow to adulthood [79]. Altogether, these results demonstrate a critical role for YAP and TEADs in the process of cell fate determination in early mouse embryos [67,73].

Recently, the Hippo pathway has also emerged as a crucial regulator of pluripotency *in vitro* [80,81]. Initially, BMP and LIF

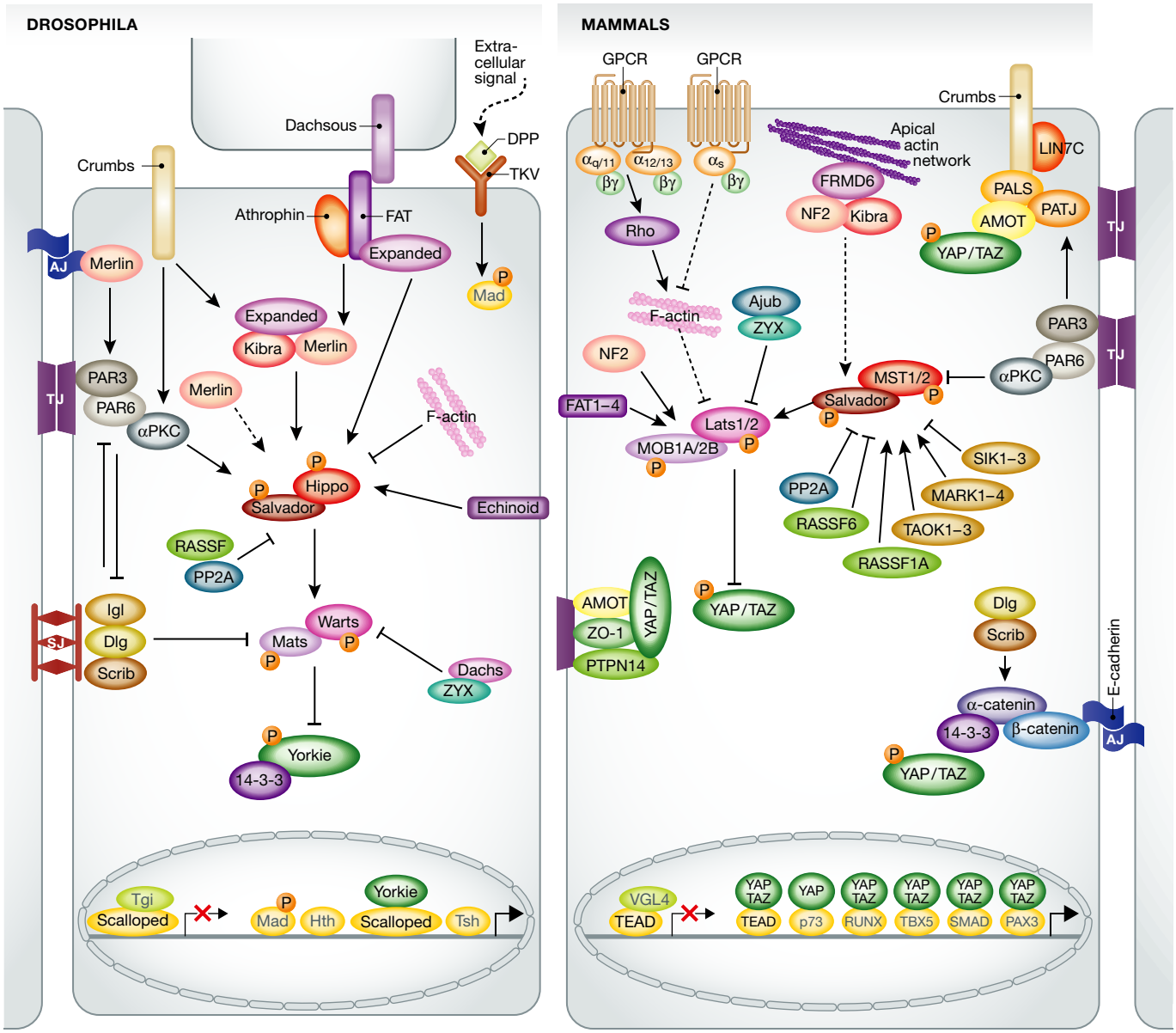

Figure 1. Schematic models of the Hippo pathway in *Drosophila* and mammals.
Cells are shown with respective cellular junctions; adherens junction (AJ), tight junction (TJ), septate junction (SJ). Hippo pathway components in *Drosophila* and mammals are shown in various colors, with arrows and blunt lines indicating activation and inhibition, respectively. The yellow spheres indicate phosphorylation of target proteins by kinase. Continuous lines indicate known interactions, whereas dashed lines indicate unknown mechanisms. See introduction for further details.

signals were shown to maintain mouse ESCs in an undifferentiated, pluripotent state, whereas human ESCs require FGF, BMP, and TGF-β/activin [82–84]. Fine-tuning these multiple signaling pathways is crucial in maintaining the balance between differentiation and self-renewal in ESCs. Supporting the role for YAP and TEADs in maintaining pluripotency, the high expression of YAP and TEAD2 in ESCs, neural stem cells, and hematopoietic stem cells initially placed these genes into a general 'stemness' transcriptional signature based on transcriptional profiling [85]. Tamm *et al* found that YAP and TEAD2 could activate the expression of ESC master transcriptional regulators Oct4 and Nanog in mammalian ESCs. Furthermore, restricting YAP and TEAD2 expression or inhibiting TEAD function resulted in differentiation toward the endoderm lineage [86]. Conversely, YAP protein and mRNA levels are significantly decreased with the loss of pluripotent markers during ESC differentiation [87]. In addition, YAP is sequestered and thereby inactivated in the cytoplasm, and consequently, a large number of genes important for stem cell maintenance and function, including PcG, Nanog, Oct3/4, and Sox2, are repressed.

Additional evidence for the role of YAP in pluripotency is seen in induced pluripotent stem cells (iPSCs). The seminal findings by Yamanaka's group demonstrated that mouse somatic cells can be reprogrammed into iPSCs by inducing the activity of four

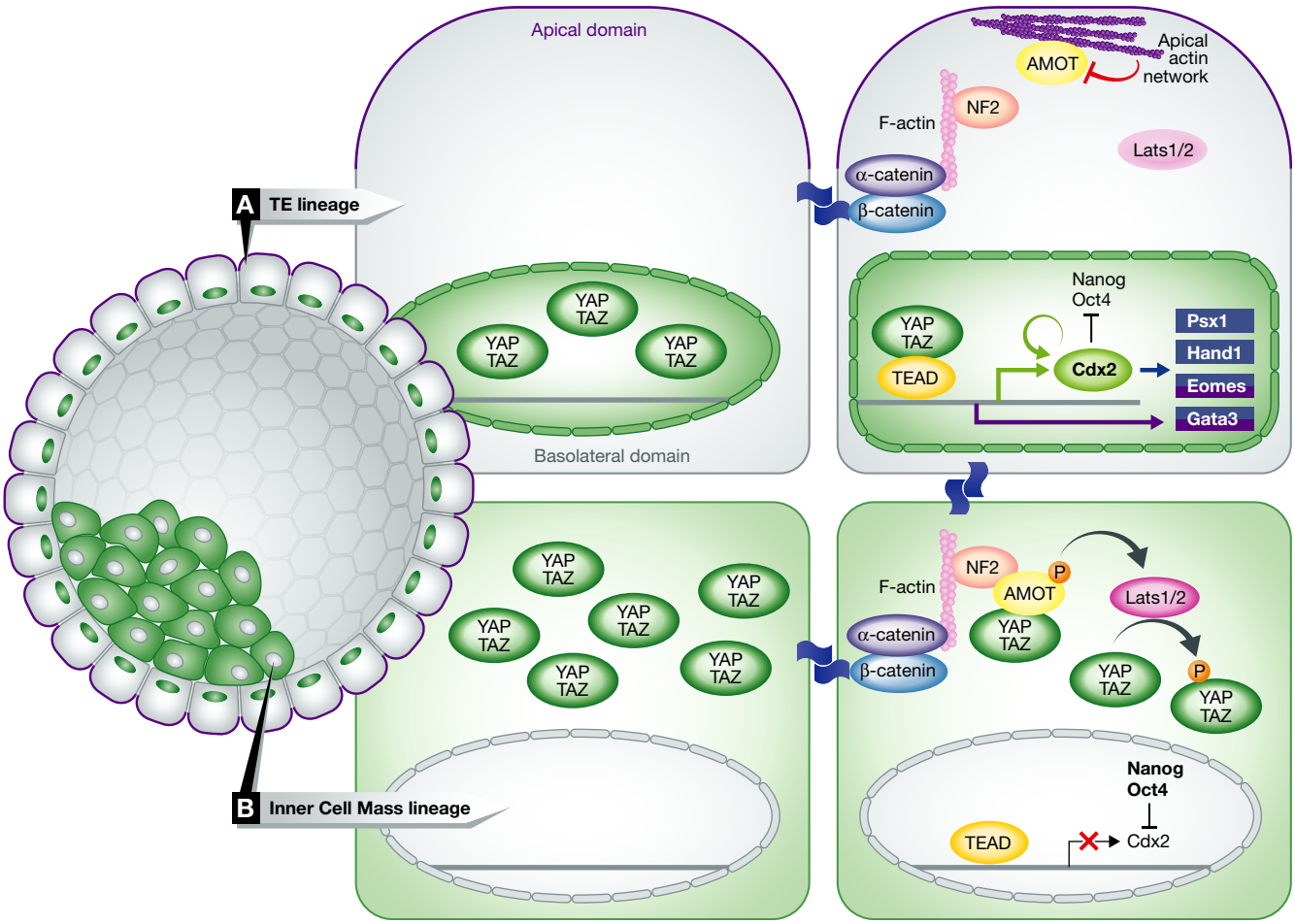

Figure 2. A model of TE and ICM specification regulated by Hippo-YAP pathway in preimplantation embryo.
During preimplantation, the outer blastomeres of the embryo form an outer epithelial trophectoderm (TE) that envelopes the remaining blastomeres, the inner cell mass (ICM). The Hippo pathway plays important roles in this cell fate specification. The outer cells have an outside exposed surface and are composed of plasma membranes with apical and basolateral domains, whereas inner cells are completely surrounded by outer cells. (A) In the outer cells, the nuclear localization of YAP and TEAD4 regulates specification of the TE lineage through activation of the TE-specific genes such as Gata3, Cdx2, and Eomes. (B) In the inner cells, cell–cell adhesions influence Hippo signaling. Activated Hippo pathway impairs YAP nuclear localization in the ICM lineage, thereby limiting TEAD4 transcription and abrogating expression of other TE-specific genes. Activation of Oct4 and Nanog maintains pluripotency and generates the ICM in mouse embryos. The yellow spheres indicate phosphorylation of target proteins by kinase.

transcription factors: Sox, Oct3/4, c-Myc, and KLF4 [88]. YAP is activated during the reprogramming of human embryonic fibroblasts into iPSCs, and the addition of YAP to Sox2, Oct4, and KLF4 increases iPSC's reprogramming efficiency in mouse embryonic fibroblasts, further confirming a positive role of YAP in stemness [87].

Moreover, it has been reported that the Hippo pathway can interact with other pathways to promote and maintain pluripotency. For example, TAZ associates with Smad2/3 to maintain the nuclear accumulation of Smad complexes, thereby promoting expression of pluripotency markers (Oct4, Nanog) in response to TGF-β stimulation [80]. Another piece of evidence linking the Hippo and TGF-β/BMP pathways is the finding that YAP binds Smad1 to regulate the induction of Id family members for mESC maintenance upon stimulation with BMP [81]. Finally, TAZ has been identified as a coactivator of Pax3-dependent transcription, which influences the expression of various genes during embryogenesis [89].

Thus, in ESCs, YAP/TAZ promotes stemness directly, as well as indirectly by mediating TGF-β/BMP or LIF signaling, through regulating the expression of genes responsible for maintaining pluripotency both *in vivo* and *in vitro* [86,87]. These studies implicate the Hippo pathway with those involved in maintaining ESC pluripotency and controlling cell fate specification in development. In conclusion, YAP, TAZ, and TEAD proteins seem to be key regulators for maintaining the pluripotent properties of both ESCs and iPSCs in mammals. The transcription coactivator activity of YAP/TAZ is similarly required for promoting stem cells as well as normal cell proliferation. In addition, TEAD is likely to be involved in both the stemness and proliferation function of YAP/TAZ. However, depending on cell context, YAP/TAZ must induce expression of different genes between stem cells and differentiated cells. It is also possible that besides TEAD, different transcription factors may be used by YAP/TAZ in stem cells compared to differentiated cells to induce downstream target gene expression, thereby promoting and maintaining stem cells.

Liver: Liver progenitor cells and tumorigenesis

The liver is the most important metabolic organ and has a high regenerative capacity and is able to regenerate after more than 70% hepatectomy. Hepatocytes are the predominant cell type in the adult liver and are mitotically quiescent. The regenerative capacity of the liver depends on hepatocyte proliferation, although the liver also contains oval cells (OCs) which are capable of generating a transit precursor compartment. Liver regeneration has been known for many years, although the underlying mechanisms and how the liver senses when it has reached its original size are still poorly understood [90].

Previous studies in *Drosophila* have implicated the Hippo pathway as a central mechanism that restricts tissue overgrowth during development and it derailed under pathological conditions contributes to tumorigenesis [91]. The Hippo pathway impinges on the transcriptional coactivator Yki to regulate the transcription of target genes involved in cell growth, proliferation, and survival. Conservation of mammalian homologs for all the known components of the *Drosophila* Hippo pathway has facilitated investigation of the physiological roles of Hippo signaling in mammals. While it was already suggested based on the *Drosophila* data that the Hippo pathway is involved in mammalian tumorigenesis, Dong *et al* [12] provided functional evidence that the mammalian Hippo pathway is a potent regulator of organ size and that its dysregulation leads to tumorigenesis in the liver. Induction of YAP overexpression using a conditional YAP transgenic mouse resulted in massive hepatomegaly via an increase in the number, but not the size, of the liver cells. Interestingly, the YAP-induced enlarged livers reverted back to their original size without any gross abnormalities when the expression of transgenic YAP was repressed. These data clearly establish a predominant role of YAP in organ size control in mice [92,93]. However, when YAP overexpression was maintained for an extended period of time, the transgenic mice develop liver tumors similar to hepatocellular carcinoma, suggesting a role of hyper-YAP activation in cancer development.

More recently, other components of the Hippo pathway have been shown to repress proliferation and restrict liver growth. Deletion of both *MST1 and MST2* results in embryonic lethality [94–96]. However, a single copy of *MST1* or *MST2* (mice with genotype of either *MST1*−/−, *MST2*+/− or *MST1*+/−, *MST2*−/−) is sufficient to support normal embryonic development. During later stages of development, loss of *MST1* or *MST2* promotes proliferation of liver stem cells/progenitor cells such as oval cells. Proliferation of liver progenitor cells gives rise to both hepatocytes and cholangiocytes (biliary epithelial cells, BECs), which are the prominent epithelial cells of the bile duct. This eventually leads to the development of liver tumors due to the loss of heterozygosity of the remaining *MST1* or *MST2*. These mice display characteristics of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) with expansion and transformation of a mixed population of tumor-associated liver progenitors [94]. Interestingly, YAP protein levels were increased, while YAP phosphorylation and LATS1/2 phosphorylation were significantly reduced, relative to wild-type, in the absence of *MST1/2*, indicating that YAP is a downstream effector of *MST1/2* in the liver. In contrast, TAZ protein levels and phosphorylation status are decreased in the *MST1/2*-knockout liver and tumors, suggesting that TAZ, a potential oncogene, is unlikely to play a major role in overproliferation and tumorigenesis in this model [95].

Intriguingly, liver-specific deletion of *Sav1* enhanced proliferation and expansion of hepatic progenitor cells (OCs) and these mice eventually developed liver tumors with a mixed HCC and CC phenotype, distinct from HCC which originates from the aberrant proliferation of hepatocytes only. However, the levels of phosphorylated YAP and phosphorylated LATS1/2 were not affected in the *Sav1* KO livers, suggesting that Sav is likely to play an essential role in OC expansion and tumorigenesis in this model but, surprisingly, acts independently of LATS1/2 and YAP [96,97].

Studies of *NF2* conditional knockout mice also support a role for YAP in liver tumorigenesis [93,98,99]. Inactivation of NF2 results in hepatocyte and BEC proliferation, widespread hepatocellular carcinoma, and bile duct hamartomas comprising cytokeratin-positive biliary epithelial cells. Zhang *et al* [98] reported that NF2 and YAP act antagonistically to each other in the Hippo pathway to regulate liver development and physiology. Deletion of only one copy of *YAP* was sufficient to reverse the expansion of liver progenitor cells and tumorigenesis driven by the loss of *NF2*. Consistent with this finding, the *NF2*-deficient liver showed reduced phosphorylation of YAP and LATS1/2 and increased YAP nuclear localization, providing functional evidence that the main tumor suppressive mechanism of NF2 is mediated through inactivating YAP. On the other hand, EGFR signaling has also been implicated in *NF2* deletion-induced tumorigenesis. Pharmacologic inhibitors of EGFR blocked OC expansion and tumorigenesis triggered by *NF2* deletion [99]. Benhamouche *et al* also showed that liver-specific deletion of *NF2* leads to an early and dramatic expansion of progenitor cells without any detectable alteration in YAP localization and phosphorylation, arguing against a role for YAP in NF2 KO-induced tumorigenesis. Future studies are necessary to clarify the discrepancy of these two reports regarding NF2 deletion-induced YAP activation [98,99]. However, the general consensus is that NF2 acts upstream of YAP and that other downstream effectors of NF2 may also contribute to tumorigenesis.

Collectively, these data suggest that Hippo pathway components may play an important role in maintaining hepatocyte quiescence and regulating organ size in mammals, yet their dysregulation can lead to stem cell expansion, overgrowth, and tumorigenesis through multiple mechanisms. There are differences in the phenotypes observed in the conditional knockout mouse models of various Hippo pathway components (Fig 3). Thus, further studies are needed to fully elucidate the roles of these Hippo pathway components and their mechanisms of action in regulating of liver progenitor cells.

Skin: Epidermal progenitor cells

The skin (epidermal tissue) in the human body undergoes constant replenishing, completely replacing itself every 2 weeks throughout an individual's life [100]. The epidermal stem cells are located within the basal layer and have a high proliferative capacity to continuously produce new epidermis while still maintaining structural integrity. During development, the basal epidermal cells generate proliferative progenitor cells, which can only divide for a limited number of cycles; these cells then leave the basal layer, migrate

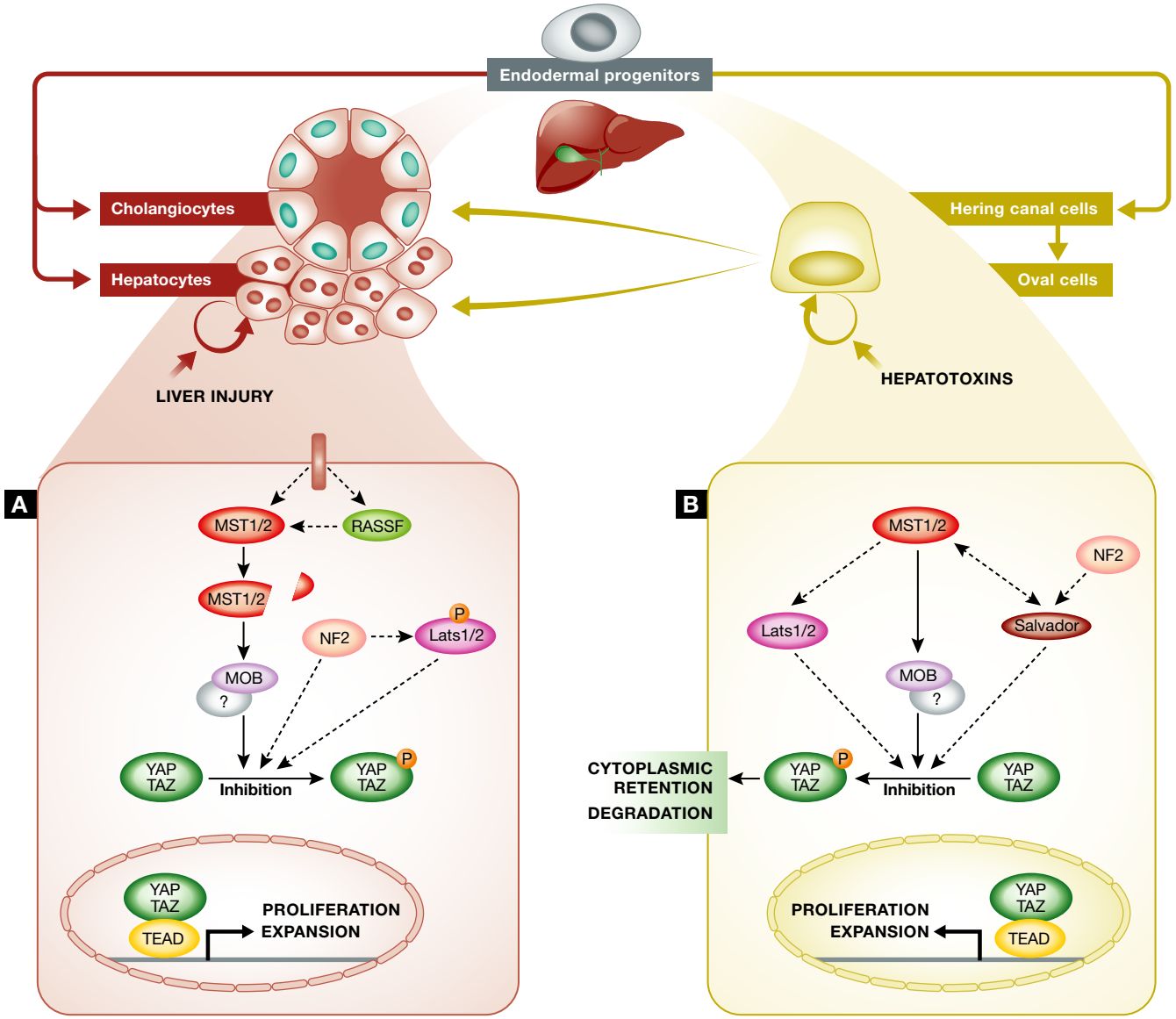

Figure 3. Schematic illustration of Hippo pathway in liver.
Endodermal progenitors generate hepatocytes and cholangiocytes that surround the bile duct system in adult liver. The Hering canal cells give rise to bipotential oval cells, which are capable of generating both hepatocytes and cholangiocytes. Hepatocyte regeneration is responsible for liver growth after partial hepatectomy. The exposure of the adult liver to hepatotoxins induces the proliferation of oval cell, but hepatocytes are slow to respond or do not respond at all to toxic injury. (A) In the hepatocytes, MST1/2 is activated by proteolytic cleavage that resulted in the loss of the Sav1 interacting SARAH domain. Cleaved MST1/2 is required to phosphorylate Mob1, but SAV1 is not required for Hippo pathway activity. This facilitates YAP phosphorylation, resulting in cytoplasmic retention by 14-3-3 binding and degradation by ubiquitin-proteasome-dependent manner. Lats1/2 activity is unaffected by MST1/2 inactivation in hepatocytes. But loss of NF2 decreases Lats1/2 and YAP phosphorylation, suggesting that the existence of unknown kinase other than MST1/2. Additionally, RASSF family proteins seem to have a role in MST1/2 regulation. Lats1/2 might indirectly inhibit YAP by activating unidentified kinase distinct from MST1/2. (B) In the oval cells, MST1/2-regulated phosphorylation on YAP Ser127 is unaffected by Sav1 inactivation. However, Sav1 regulates YAP protein level and localization via as yet defined mechanisms. There are no clear links between MST1/2 and Lats1/2 activation in oval cells. The mechanism underlying the inactivation of YAP inhibits oval cell proliferation. However, the role of oval cells in liver regeneration remains controversial.

toward the skin's surface as they terminally differentiate, eventually leading to constant skin remodeling. When the skin is injured, wound healing greatly accelerates this regenerating process by which these inner progenitor cells migrate outwards. Epidermal growth must be carefully balanced, because inadequate proliferation results in the thinning of skin and loss of protection, whereas excessive growth leads to hyperproliferative disorders.

Recent findings have implicated the importance of the Hippo pathway in epidermal development and homeostasis. It has been shown that inactivation of *Sav1 (WW45)* alleles leads to early embryonic lethality, and histological examination displayed a thickening of the epidermal skin layer in the embryos [101]. *WW45*-null primary keratinocytes show hyperproliferation, progenitor expansion, decreased apoptosis, and inhibition of terminal differentiation.

These observations suggest that the Hippo pathway restricts the pool of these progenitor cells.

Through molecular and genetic studies, two groups have independently shown that YAP overexpression results in expansion of the epidermal stem cells and progenitor cells in the epidermis [42,102]. Mice carrying the YAP transgene reveal epidermal thickening, hyperkeratosis, and squamous cell-like carcinoma in skin grafts. Conversely, deletion of YAP in the epidermis or disruption of the YAP–TEAD interaction during epidermal development resulted in epidermal hypoplasia and loss of keratinocyte proliferation. This phenotype was attributed to the gradual loss of the epidermal stem/progenitor cells and the progenitor cells' limited capacity for self-renewal.

Surprisingly, deletion of *MST1/2* did not lead to epidermal hyperplasia, indicating that YAP is regulated through an alternative mechanism that is not dependent on canonical Hippo pathway components MST1/2 in the skin [41]. Consistent with an MST1/2-independent regulation of YAP, recent studies have shown that MST1/2 is not required for YAP activation by G-protein-coupled receptor (GPCR) signaling. Cell adhesion and α-catenin have also been implicated in YAP regulation. Interestingly, skin-specific deletion of *α-catenin*, a component of adherens junctions and an important tumor suppressor in epithelia, resulted in keratinocyte hyperproliferation and squamous cell carcinoma that resemble the phenotypes observed in YAP transgenic mice [41]. α-Catenin is considered a critical sensor for cell density and provides the cell with neighborhood information through the formation of density-dependent cell–cell junctions (adherens junctions). Similar to α-catenin, the Hippo signaling pathway has been implicated in cell contact inhibition of proliferation as well as tissue growth control [103]. Notably, α-catenin can directly interact with YAP and suppress YAP function, possibly by sequestering YAP at the plasma membrane and preventing it from entering the nucleus [41]. These findings provide a mechanistic explanation for how α-catenin modulates YAP activity by translating context-dependent information to regulate stem cell proliferation and tissue expansion. It should be noted that there is strong evidence supporting that angiomotin mediates cell–cell contact and tight junction signals to inhibit YAP function by both increasing YAP phosphorylation and physical binding [78,104].

Nervous system: neural progenitor cells

YAP and TEAD2 are highly expressed in neural stem cells (NSCs), which are multipotent progenitors present in the nervous system. NSCs are capable of self-renewing and produce multiple neural lineages which ultimately compose the central nervous system (CNS) [85,105]. In the vertebrate's developing neural tube, YAP is expressed by ventricular zone progenitor cells and co-localizes with Sox2, a neural progenitor marker [106,107]. Overexpression of either YAP or a transcriptionally active form of TEAD in the neural tube leads to reduced neural differentiation and a marked increase in neural progenitor cell numbers due to accelerated cell cycle progression and recurring cell cycle exit. These effects are associated with the induction of cyclin D1 and the down-regulation of NeuroM. Conversely, loss of YAP triggers cell death and promotes premature neuronal differentiation in the chick neural tube [106]. Both YAP gain-of-function and loss-of-function studies in *Xenopus* demonstrate that YAP is required for expansion of $Sox2^+$ neural plate progenitors and $Pax3^+$ neural crest progenitors at the neural plate border and for maintaining these progenitor cells in an undifferentiated state. The effects of YAP on $Pax3^+$ neural crest progenitors are through the direct regulation of Pax3 transcription. YAP acts through TEAD to stimulate Pax3 expression. Previous studies have also suggested that mouse TEAD is responsible for activating the Pax3 promoter and neural crest expression in the mouse as well [108]. It is well documented that the expansion of mouse neural progenitors is mediated by the activation of the Notch pathway; however, in the frog embryo, YAP's ability to repress neural differentiation is likely independent of Notch signaling [107]. It has been shown that YAP is amplified or up-regulated in human Shh-dependent medulloblastoma, a brain tumor in children. Similarly, it was observed that YAP and its target transcription factor TEAD1 are highly expressed in mouse Shh-dependent medulloblastomas [109]. In addition, YAP is a target of Shh signaling in the developing cerebellum. YAP expression and nuclear localization are induced in proliferating cerebellar granule neural precursors, which are thought to be the cells of origin for certain medulloblastomas. Additionally, it has been suggested that mutation of Patched1 (PTCH1), which encodes an inhibitor of hedgehog pathway, leads to the activation of YAP in a non-cell-autonomous manner and alters hedgehog pathway in medulloblastoma cells and tissue samples [110]. These studies show a critical role for YAP and TEAD in neuronal progenitor cells and medulloblastoma development.

Large-scale RNAi screens reveal that FatJ cadherin, the closest homolog of the *Drosophila* dFat, is spatially restricted to the intermediate regions of the neural tube and acts though YAP to regulate the number of neural progenitor cell pools within the dp4-vp1 domain [111]. Loss of NF2 also caused an overexpansion of the neocortical progenitor pool by increasing YAP/TAZ protein levels, enhancing nuclear localization of both these proteins, and up-regulating their target genes in the mammalian dorsal telencephalon [112]. In addition, Hippo signaling had previously been implicated in Ft/Ds signaling through its regulation of cell proliferation and differentiation in *Drosophila*, although there was no direct evidence to implicate Ft/Ds signaling in regulating the vertebrate Hippo pathway [113]. Cappello *et al* recently suggested a connection of FAT4/DCHS1 and YAP in mammals. They reported that knockdown of FAT4 or DCHS1 promotes neural progenitor cell proliferation and malpositioning of cells in the developing cerebral cortex [114]. These mouse data demonstrate that reduced levels of FAT4 and DCHS1 increase the activity of unphosphorylated YAP and a YAP-responsive transcriptional reporter. Together, these findings reveal a novel function of NF2 and FAT4 signaling in inhibiting neural progenitor expansion during brain development and establish YAP/TAZ as key effectors.

To date, the proposed model is that YAP promotes NSC proliferation by serving as an effector of the Shh pathway in the brain. A full understanding of the role of the Hippo pathway in NSC requires future studies to examine crosstalk between Hippo and other signaling pathways such as the MAPK, Ephrin, Wnt, and Notch pathways that are also thought to control brain development.

Cardiac progenitor cells and muscle progenitor cells

The fetal heart grows through the proliferation of cardiomyocytes, and following birth, postnatal cardiomyocytes undergo hypertrophy to reach an optimal size. Although it was traditionally believed that the adult human heart lacks adequate myocardium regenerative potential for repair, recent studies have identified endogenous stem cells with the regenerative capacity to repair lost or damaged heart tissue during the late cardiac development of the adult heart [115].

Unlike other tissues such as the liver, the role of Hippo signaling in the heart is less well understood. It has recently been shown that a cardiac-specific deletion of *Sav1* or overexpression of a constitutively active YAP mutant in embryos results in embryos within cardiomegaly due to increased cardiomyocyte proliferation. Ablation of either the *MST1/2* or *LATS1/2* kinases, the upstream inhibitory kinases of YAP, causes perinatal lethality resulting from an overgrown heart due to elevated cardiomyocyte proliferation, similar to the *Sav* cKO heart [116,117]. Genetic interaction studies have shown that nuclear YAP interacts with β-catenin in cardiomyocytes, directly activating β-catenin target genes to promote Wnt signaling, which has already been implicated in cardiac repair and cell reprogramming. Loss of β-*catenin* in the *Sav* cKO hearts suppressed the overgrowth phenotype caused by Hippo pathway inactivation, suggesting that the Hippo pathway restrains cardiomyocyte proliferation and heart size by inhibiting Wnt signaling [118]. Another recent study showed that YAP activates the IGF pathway during heart development, resulting in the inactivation of GSK3b, which in turn inhibits β-catenin degradation [119]. More recently, Xin *et al* have reported that expression of constitutively active YAP promotes proliferation of adult cardiomyocytes and enhances adult heart regeneration in response to injury. YAP-expressing cardiomyocytes behave similar to embryonic cells with regard to their regenerative potential [120].

Conversely, loss of *YAP* leads to embryonic lethality through myocardial hypoplasia, due to reduced cardiomyocyte proliferation in the embryonic heart [119,121]. Thus, YAP connects Hippo signaling and other growth-promoting pathways, such as IGF and Wnt signaling, to regulate embryonic and neonatal cardiomyocyte proliferation. This is mediated at least in part by its interaction with β-catenin, directly promoting a stemness gene expression program [117–119,121].

A role for the Hippo pathway in skeletal muscle is beginning to be delineated. YAP overexpression in C2C12 myoblasts and primary mouse muscle stem cells blocks the progression of myoblasts through the myogenic program and preserves the progenitor-like and proliferative properties [122,123]. High YAP expression and activity expands the pool of activated satellite cells, the resident stem cells in skeletal muscle, and prevents the differentiation of this cell population. Interestingly, overexpression of TAZ increases myogenic gene expression in a MyoD-dependent manner, thereby promoting myogenic differentiation [124]. Despite the high level of sequence identity between YAP and TAZ, their opposite effects on muscle progenitor fate is a nice illustration of the complexity and context specificity associated with Hippo pathway activation or inhibition and the resulting transcriptional response. Obviously, further studies need to be carried out *in vivo* to conclusively determine the role of Hippo signaling, particularly the opposing functions of YAP and TAZ, in cardiac and skeletal muscle biology.

Intestine: Intestinal stem cells

Intestinal stem cells (ISCs) are responsible for the constant renewal and repair of the intestinal epithelia to maintain tissue homeostasis [125,126]. Recent studies have highlighted the role of the Hippo pathway and its effectors YAP and Yki in intestinal regeneration following tissue injury in both mice and *Drosophila*, respectively. In general, the loss of Hippo signaling and/or the elevated YAP activity is associated with stem cell expansion in various organs [125,127]. However, in the intestine, there are contradictory reports regarding the role of YAP in ISC expansion and intestinal regeneration across different species and experimental settings.

The function of the Hippo pathway and YAP in ISCs has mostly been studied in the context of intestinal regeneration following tissue injury in transgenic animal models (Fig 4). In the DSS-induced colonic regeneration model by Cai *et al*, YAP protein levels are elevated following tissue injury. In addition, the specific deletion of *YAP* in the intestinal epithelium prevented DSS-induced intestinal regeneration, suggesting that YAP is required for these processes [128]. Correlating with the function of the Hippo pathway to suppress YAP activity, loss of Hippo signaling in *Sav1*-deficient crypts displayed accelerated regeneration upon DSS-induced injury in a YAP-dependent manner [128]. Similarly, Zhou *et al* [129] showed that deletion of the core Hippo kinase *MST1/2* in the intestinal epithelium resulted in a marked expansion of the ISC compartments due to YAP hyperactivation. Ubiquitous overexpression of YAP-S127A, which lacks the phosphorylation site required for inactivation by the Hippo pathway, also resulted in the loss of differentiation markers and expansion of an undifferentiated cell population in the mouse intestine [92].

On the other hand, Barry *et al* [130] reported that specific expression of YAP in the intestinal epithelium suppresses intestinal renewal and reduces the ISC population by restricting Wnt/β-catenin signaling. Intestinal regeneration after irradiation is characterized by hyperactivation of Wnt/β-catenin signaling. Consistently, deletion of *YAP* resulted in Wnt hypersensitivity and led to ISC expansion and crypt hyperplasia after injury by irradiation. These results are at odds with the role of YAP in the DSS-induced colonic regeneration model.

Another inconsistency is in the crosstalk between YAP and Wnt/β-catenin signaling and their role in intestinal regeneration. The *Sav1*-deficient mouse colons developed polyps after DSS-induced regeneration, which showed nuclear accumulation of YAP, but not β-catenin [128]. This is consistent with the observation by Barry *et al* [130] that YAP-S127A expression restricts Wnt/β-catenin signaling during intestinal regeneration. In contrast, Zhou *et al* [129] reported that in the *MST1/2*-deficient intestinal epithelium, nuclear accumulation of YAP correlates with β-catenin activation. Uncontrolled tissue regeneration after injury can become oncogenic, like in colon cancer. In this context, Barry *et al* underscored that YAP is silenced in a subset of highly aggressive human colorectal carcinomas, whereas Zhou and co-workers showed a striking prevalence of YAP overexpression in 95% of colonic cancer specimens [129,130]. The complex nature of YAP in the context of ISC expansion, intestinal regeneration, and its relation to Wnt/β-catenin signaling certainly requires further investigation. Nevertheless, these studies point to a role of YAP in ISC, either positively by

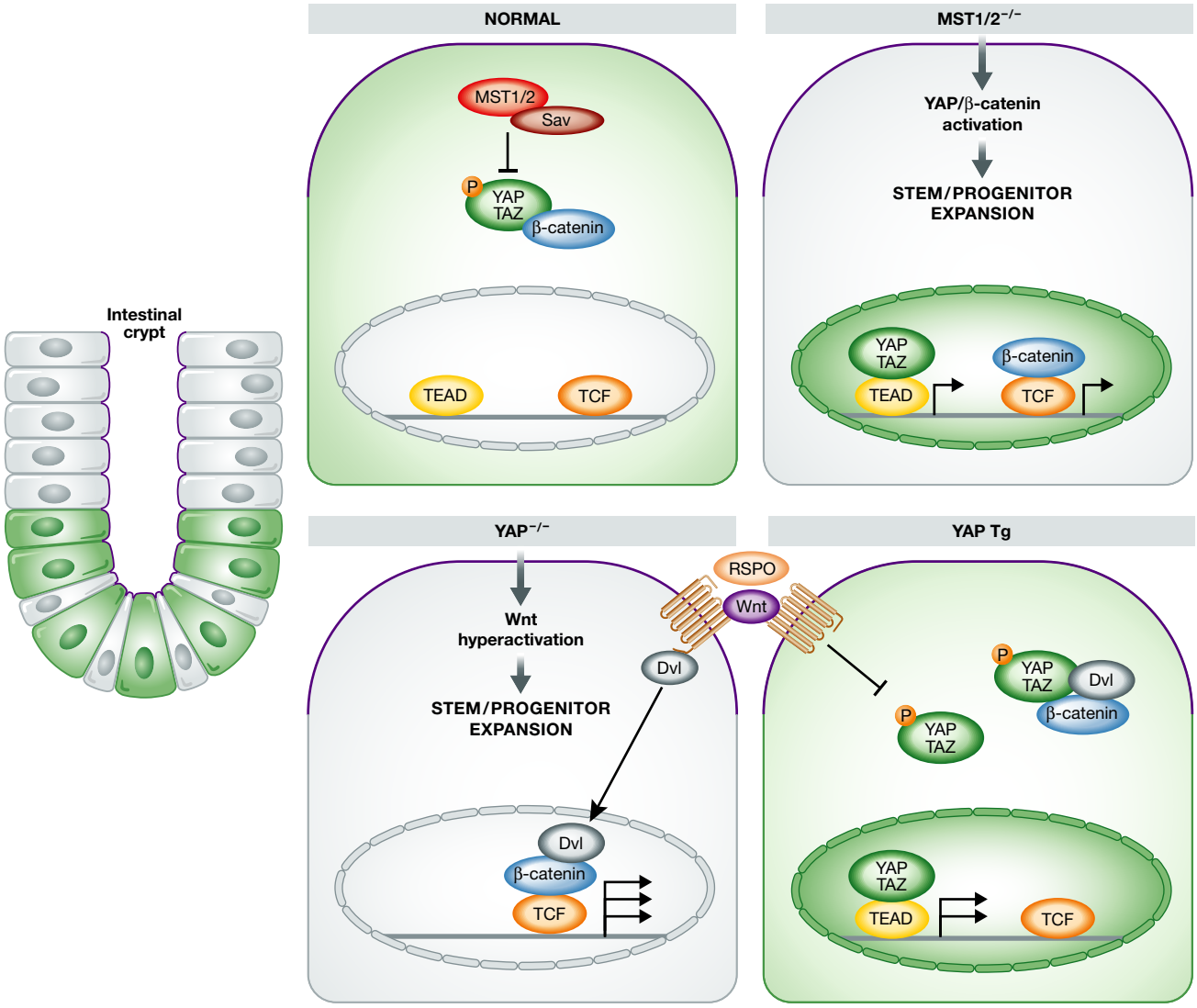

Figure 4. The context-dependent role of YAP in intestinal stem cell expansion.
In the intestinal stem cells (ISC), the Hippo pathway inhibits YAP activity by phosphorylation and cytosolic retention of YAP. The cytosolic YAP directly binds to β-catenin and subsequently inhibits the canonical Wnt signaling. In Mst1/2$^{-/-}$ intestinal epithelia, loss of Hippo pathway regulation promotes dephosphorylation and nuclear translocation of YAP/β-catenin and induces their target gene expression. Activation of YAP/β-catenin results in the expansion of ISC. However, a controversial role of YAP has been demonstrated in the context of Wnt-induced intestinal regeneration. In YAP$^{-/-}$ intestinal epithelia, hyperactivation of Wnt/β-catenin signaling results in ISC expansion, whereas YAP overexpression represses Wnt/β-catenin signaling, which leads to the loss of ISC and epithelial self-renewal. In this context, YAP functions to inhibit the nuclear translocation of disheveled (Dvl).

directly promoting ISC or negatively by indirectly inhibiting Wnt signaling.

In the *Drosophila* midgut, the Hippo pathway and Yki facilitate intestinal regeneration after tissue injury [131–133]. Perturbation of Hippo signaling or overexpression of a constitutively active Yki mutant (Yki-S168A) induced the expression of the Upd (Outstretched), which is a cytokine that stimulates expansion of ISC through the JAK/STAT pathway. However, further investigation is required to address whether Upd acts in an autocrine fashion via Hippo-Yki signaling in the ISC [131] or whether it triggers a non-autonomous increase in ISC expansion via Hippo-Yki signaling in the enterocytes [132].

Hippo signaling and cancer stem cells

As discussed above, the Hippo pathway plays a key role in regulating organ size and tumorigenesis by inhibiting cell proliferation, promoting apoptosis, and regulating stem/progenitor cell expansion [134,135]. Phosphorylated YAP/TAZ localizes to the cytosol, decreasing tumor growth, whereas unphosphorylated YAP/TAZ is localized mainly in the nucleus and promotes cell and tumor growth. Indeed, there is considerable evidence that abnormal Hippo signaling is associated with tumor progression. As expected, elevated expression and activity of YAP/TAZ correlates with various human cancers [103,136–138].

Moreover, TAZ has been shown to be a key regulator of cancer stem cells (CSCs) in breast cancer [139]. In addition, YAP and TEAD are highly expressed in CSCs of medulloblastomas [109]. Increasing evidence has suggested that tumor growth is dependent on CSCs, which represent a small subset of cells within a tumor but have the ability to self-renew, differentiate into other tumor cell types, and initiate tumor formation. CSCs are also thought to be resistant to chemotherapeutic agents and are responsible for cancer recurrence and metastasis. High-grade tumors are characterized by a higher population of CSCs within the tumor. Microarray analysis of 993 primary human breast tumors has identified a list of genes highly expressed in G3 (tumors that poorly differentiated tumors) compared to G1 (benign tumors) [139]. Interestingly, elevated YAP/TAZ activity is observed in G3 tumors, which are also characterized by the expression of embryonic and normal mammary stem cell genes. Using a model for tumor progression, Cordenonsi *et al* demonstrated a role for TAZ in breast cancer cells [139]. Upon injection in mice, MII cells, which are Ras-transformed MCF10A-T1k, generate low-grade tumors. On the other hand, MIV cells, which are malignant MCF10A-CA1a cells derived from the *in vivo* spontaneous evolution of MII cells, readily formed tumors resembling G3 tumors. TAZ was highly expressed in the MIV cells, but not the MII cells, whereas YAP levels were comparable across both cell lines. Overexpression of active TAZ increases MCF10A proliferation and the formation of invasive carcinomas. These observations support an important role of TAZ in breast cancer stem cells.

Other studies have shown that nuclear TAZ is highly expressed in high-grade glioblastomas. Ectopic expression of TAZ leads to increased invasion, self-renewal, and tumor initiating capacity to generate properties similar to mesenchymal-like stem cells [140]. Conversely, knockdown of TAZ expression in mesenchymal-like stem cells decreases their mesenchymal properties and limits their capacity to self-renewal and initiate in glioma. Collectively, it is clear that TAZ enhances the self-renewal capacity and tumorigenic potential contributing to both the initiation and progression of breast cancer and glioma. Therefore, TAZ could be a potential molecular target for treating aggressive tumors that have uncontrolled TAZ activation.

Dysregulation of the Hippo pathway has been identified in a broad range of human cancers, including liver, lung, colorectal, ovarian, and prostate [12,103,137]. Studies have shown that YAP activity is increased as a result of increased expression and nuclear localization in human tumor samples. This is consistent with inactivation of the Hippo pathway which is known to inhibit YAP and TAZ activity mainly by promoting these transcriptional coactivators' cytoplasmic localization and ubiquitin-mediated degradation. In addition, YAP gene amplification (somatic mutation) has been reported in various human and murine tumor models [136,141]. Collectively, these data suggest that unrestrained YAP activity can counteract classical tumor suppressor checkpoints.

Compared with other well-known oncogenic signaling pathway, only few cancers are known to be associated with a direct mutation of a Hippo pathway component. Of note, Lats2 is mutated in approximately 40% of mesothelioma cases [142]. Interestingly, Mst1/2 and Lats1 are tumor suppressors in mice, and although mutation in these genes have not been identified in human cancer, silencing of these genes have been reported to data, suggesting that these genes may be inactivated by non-mutational mechanisms [94–96,129,143–146]. NF2 is a potent upstream regulator of the Hippo pathway, and an inactivating mutation in NF2 is associated with several human cancers including acoustic neuromas, meningiomas of the brain, and schwannomas of the dorsal roots of the spinal cord [146,147]. A high frequency of NF2 mutations has also been reported in mesothelioma [148,149]. Recently, a *TAZ* and calmodulin-binding transcription activator 1 (*CAMTA1*) fusion gene has been reported in epithelioid hemangioendothelioma, a rare form of sarcoma [150,151]. The role and mechanism of this fusion protein in cancer progression is still unclear, but may relate to the transcriptional regulatory functions ascribed to both TAZ and CAMTA1.

Many studies have reported a high frequency of mutations in various GPCRs (*GPR98*, *GRM3*, *AGTRL1*, *LPHN3,* and *BAI3*) and G-proteins (*GNAS*, *GNAQ,* and *GNAO1*) across a wide range of cancers, particularly in melanoma [152–154]. Notably, activating mutations in *GNAQ and GNA11* have been observed in approximately 50% of uveal melanomas. And in these uveal melanomas with activating mutations in GNAq or GNA11, we found that YAP is constitutively activated and its activation is pathologically critically important.

Collectively, extensive studies have established a critical role for the Hippo pathway in human tumorigenesis. Inhibiting YAP/TAZ may be a new therapeutic area for treating cancers with a dysregulated Hippo pathway.

Conclusions

Although most of the Hippo pathway components were initially identified in *Drosophila*, much research has recently been done in mammalian cells and animal models, revealing this pathway's important contribution to tissue homeostasis, organ size control, cancer development, and stem cell biology. As the key downstream effectors of the Hippo pathway, YAP/TAZ is involved in embryonic stem cells as well as tissue-specific stem cell self-renewal, and tissue regeneration and homeostasis of the liver, intestine, pancreas, heart, skin, and central nervous system. Moreover, compelling evidence supports a role for YAP/TAZ in cancer stem cells. Therefore, components of the Hippo pathway may be good therapeutic targets in diseases such as degeneration and cancer.

Since the discovery of the Wrts kinase in *Drosophila* in 1995, for the first decade research in the Hippo pathway was largely limited to *Drosophila*. However, rapid progress, especially in the last several years, has been made regarding the identification of upstream components, signals, and mechanisms of regulation in both *Drosophila* and mammalian systems. Cell polarity, adhesion, mechanotransduction, as well as diffusible signals acting through GPCRs, have all been identified as regulators of Hippo pathway activity. However, many key questions remain to be addressed. The function of YAP/TAZ has been investigated in only a few cell types. Further studies to uncover the physiological roles of YAP/TAZ in a broad range of tissue-specific stem cells and various types of cancer stem cells will likely expand our knowledge of the Hippo pathway in regulating tissue homeostasis during development and adulthood as well as cancer initiation and metastasis. Organ size regulation is a fundamental question in biology, though the signals critical for sensing organ size control, with each organ presumably having its own specific signals, are unknown. Research into the molecular signals controlling organ size will be of paramount importance not

Sidebar A: In need of answers

(i) Does the stemness function of Hippo-YAP pathway differ from its more classic role in cell growth regulation? Are different downstream target transcription utilized in both processes?

(ii) What are the molecular bases for the differential functions of YAP and TAZ in stem cells? Is this simply due to differential expression of these two proteins?

(iii) How is YAP/TAZ regulated by stemness signals? Is there hippo kinase cascade MST-Lats independent regulation of YAP/TAZ?

(iv) YAP/TAZ clearly plays different roles in different stem cell types, either being inhibitory or activating. What determines the specificity and complexity of the Hippo-YAP in tissue-specific stem cells?

only for the Hippo pathway but also for the field of developmental biology. Because the Hippo pathway is regulated by a wide range of signals, both physical and chemical, how the Hippo pathway integrates all of these inputs from multiple signaling pathways to generate a concerted cellular response remains a question of high interest. Understanding the molecular mechanisms by which the Hippo pathway controls development, regeneration, tissue homeostasis, and injury/repair will require the input of researchers across multiple disciplines, including genetic, genomic, developmental, systems biology, cell biology, biochemistry, and cancer biology. Given the increasing research interest in this pathway, continued rapid progress is eagerly anticipated.

Acknowledgments

We apologize for the many important contributions to the Hippo pathway field that we could not cite owing to space constraints. The authors would like to thank Steven W Plouffe and Dr. Carsten G. Hansen for critical reading of this manuscript. This work is supported by grants from the National institutes of Health to K.L.G. (CA132809 and EYO226116).

Conflict of interest

The authors declare that they have no conflict of interest.

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