The Hippo pathway in intestinal regeneration and disease

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Abstract

The Hippo pathway is a signalling cascade conserved from *Drosophila melanogaster* to mammals. The mammalian core kinase components comprise MST1 and MST2, SAV1, LATS1 and LATS2 and MOB1A and MOB1B. The transcriptional co-activators YAP1 and TAZ are the downstream effectors of the Hippo pathway and regulate target gene expression. Hippo signalling has crucial roles in the control of organ size, tissue homeostasis and regeneration, and dysregulation of the Hippo pathway can lead to uncontrolled cell growth and malignant transformation. Mammalian intestine consists of a stem cell compartment as well as differentiated cells, and its ability to regenerate rapidly after injury makes it an excellent model system to study tissue homeostasis, regeneration and tumorigenesis. Several studies have established the important role of the Hippo pathway in these processes. In addition, crosstalk between Hippo and other signalling pathways provides tight, yet versatile, regulation of tissue homeostasis. In this Review, we summarize studies on the role of the Hippo pathway in the intestine on these physiological processes and the underlying mechanisms responsible, and discuss future research directions and potential therapeutic strategies targeting Hippo signalling in intestinal disease.

The mammalian gastrointestinal tract is part of the digestive system responsible for food digestion and absorption. As a result of constant exposure to pathogens and xenobiotics, intestinal epithelial cells (IECs) have a short half-life, and have therefore evolved the ability to rapidly regenerate after damage. Intestinal regeneration is precisely controlled by a myriad of cellular signalling pathways and the crosstalk between them. The Hippo pathway plays a key part in cell-fate decision, tissue homeostasis and control of organ size by regulating cell proliferation, apoptosis and differentiation[1,2], and dysregulation of this pathway has been associated with many human malignancies[3,4]. In the past few years, studies have revealed the importance of the Hippo pathway in gastrointestinal tract physiology[5]. In this Review, we will focus on the role of the Hippo pathway in tissue homeostasis, regeneration and tumorigenesis in the small intestine and the colon. In addition, we will also briefly discuss the functional roles of the Hippo pathway in the liver.

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Hippo pathway overview

The Hippo pathway in Drosophila melanogaster

The Hippo pathway is mostly conserved between *Drosophila melanogaster* and mammals[6,7]; it was initially identified in *Drosophila* during screens for genes that negatively regulate tissue growth. Loss of the serine/threonine-protein kinase warts (Wts) resulted in organ overgrowth in *Drosophila* wing and eye[8,9]. Similar genetic screens later identified that mutation of the Hippo kinase (Hpo) resulted in a similar tissue overgrowth phenotype[10–14]. In addition, the adaptor proteins salvador (Sav) and MOB kinase activator-like 1 (Mats) were found to be partners of Hpo and Wts, respectively, with similar mutation phenotypes[15–17].

Five proteins comprise the core of the Hippo pathway: the Hpo kinase and its binding partner Sav; the Wts kinase and its binding partner Mats; and the transcriptional co-activator yorkie (Yki). Yki mediates the main functional output of the Hippo pathway and binds to the transcription factor Scalloped (Sd), which induces the expression of genes that promote cell proliferation, for example cyclin E (*CycE*) and the *bantam* microRNA (miRNA), or genes that prevent apoptosis, for example death-associated inhibitor of apoptosis (*Diap1*)[18,19]. The Hpo–Sav complex phosphorylates and activates the Wts–Mats complex[10–12,14,17,20], which in turn phosphorylates and inactivates Yki by sequestering Yki in the cytoplasm, thereby negatively regulating Yki–Sd interaction and Sd-mediated gene expression[21]. Three studies published between 2014 and 2015 show that, in addition to Hpo, two kinases known as Happyhour (Hppy) and Misshapen (Msn) also activate the Wts–Mats complex[22–24]. Overexpression of Yki or deletion of Hpo, Sav, Wts or Mats leads to overgrowth phenotypes in *Drosophila* eye, wing, midgut and other tissues as a result of increased cell proliferation and decreased apoptosis[10–12,14,25].

The Hippo pathway in mammals

The Hippo pathway in mammals consists of the core components mammalian STE20-like protein kinase 1 and 2 (MST1 and MST2, homologues of Hpo, also known as serine/threonine-protein kinase 4 and 3, respectively), salvador homologue 1 (SAV1, a homologue of Sav), large tumour suppressor 1 and 2 (LATS1 and LATS2, homologues of Wts), MOB kinase activator 1A and 1B (MOB1A and MOB1B, homologues of Mats), and the two Yki homologues Yes-associated protein 1 (YAP1, also known as transcriptional co-activator YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ, also known as WW domain-containing transcription regulator protein 1, WWTR1)[26]. YAP1 and TAZ have overlapping functions when they are expressed in the same cells[27], but also have divergent functional roles in different organs as YAP1 and TAZ knockout mice show different developmental deficiencies[28]. MST1 and MST2 form homodimers via *C*-terminal Sav/RassF/Hpo (SARAH) domains, resulting in activation loop autophosphorylation[29–32]. MST1 and MST2 can phosphorylate LATS1 and LATS2 at the hydrophobic motif to induce their activation. The scaffold proteins SAV1 and MOB1 are also phosphorylated and activated by MST1 and MST2; activated SAV1 and MOB1 promote LATS1 and LATS2 activity[33–35]. The Hppy homologues MAP4K1, MAP4K2, MAP4K3 and MAP4K5, and the Msn homologues MAP4K4, MAP4K6 and MAP4K7 act in parallel with MST1 and MST2 to

phosphorylate the hydrophobic motifs of LATS1 and LATS2, thereby leading to their activation[22,24]. Activated LATS1 and LATS2 in turn phosphorylate YAP1 and TAZ, leading to the retention of YAP1 and TAZ in the cytoplasm by 14-3-3 family proteins. Cytoplasmic YAP1 and TAZ can undergo further phosphorylation and ubiquitylation-dependent degradation[36,37], preventing their interaction with the mammalian homologues of Sd, transcriptional enhancer factor TEF-1 (also known TEA domain family member 1, TEAD1), TEAD2, TEAD3 and TEAD4. Because the YAP1–TEAD complex activates the transcription of genes involved in cell growth and survival, including *CTGF*, *MYC* and *BIRC5* (also known as survivin), the degradation of YAP1 as a result of phosphorylation by LATS1 or LATS2 reduces expression of these genes[38,39]. Loss-of-function mutation of the Hippo kinase cascade components in mammals, such as inactivation of Merlin (also known as NF2) and deletion of LATS2 in meso-thelioma[40], also leads to overgrowth phenotypes and consequently carcinogenesis in multiple tissues[3,41].

The regulatory signals of the Hippo pathway

The mammalian Hippo pathway responds to a variety of extracellular signals, including cell polarity, contact inhibition, stress, mechanotransduction, cell attachment, hormones and growth factors[7]. More than 20 upstream regulators of the Hippo pathway[42] have been discovered, many of which are involved in intestinal homeostasis and carcinogenesis (FIG. 1).

Polarity and cell-adherence signals—Intestinal epithelial cells, which display apical–basal polarity, are joined together by tight junctions and adherens junctions, forming the intestinal epithelium layer. Intestinal diseases are often associated with disruption of IEC polarity or epithelial junctions[43]. The components of apical–basal polarity complexes attenuate the transcription activities of YAP1 and TAZ. For instance, in addition to stimulating LATS kinase activity, Angiomotin (AMOT) family proteins can directly bind to YAP1 and TAZ, sequestering them at tight junctions and inhibiting their transcriptional activity[44,45]. Studies in *Drosophila* have shown that the FERM (Four-point-one protein, ezrin, radixin and moesin) domain proteins Merlin and Expanded colocalize with Kibra at the apical domain of polarized epithelial cells to promote the activation of Wts by Hpo[46]. Many other key regulators of the Hippo pathway, such as α-catenin, tyrosine-protein phosphatase non-receptor type 14 (also known as PTPN14), and cadherin-1 (also known as E-cadherin), are also components of junction complexes, and attenuate YAP1 and TAZ transcriptional activity by either increasing the kinase activity of LATS or directly sequestering YAP1 and TAZ at cell junctions[26]. A study published in 2014 showed that LKB1, which is a master regulator of the development and maintenance of cell polarity, activates the Hippo pathway in human cell lines through its substrates, the serine/threonine-protein kinase MARK protein family and Scribble[49]. Another LKB1 substrate, 5′-AMP-activated protein kinase (AMPK), directly phosphorylates YAP1, disrupting YAP1–TEAD interaction and inhibiting YAP1 transcriptional activity[50–52]. In *Drosophila*, the planar cell polarity proteins cadherin-related tumour suppressor (also known as Fat) and Dachsous inactivate Yki by promoting the abundance and localization of Expanded at the apical membrane, thereby stimulating Wts activity[53–56].

Regulation of contact inhibition—The Hippo pathway is believed to integrate signals from the spatial organization and the physical state of neighbouring cells, and can regulate contact inhibition of cell growth by phosphorylating and inactivating YAP1, leading to inhibition of YAP1-mediated transcription[36]. Hippo pathway signalling is required for contact inhibition of cell growth mediated by the E-cadherin–catenin complex[57]. In addition, overexpression of constitutively active YAP1 leads to loss of contact inhibition in epithelial cells[36]. Contact inhibition has been shown to control miRNA biogenesis through the Hippo pathway. High YAP1 activity in cancer cells, in part due to loss of contact inhibition, led to a global downregulation of miRNA synthesis and potentially enhanced tumorigenesis[58]. However, another study reported conflicting results[59], showing that YAP1 and TAZ promote processing and biogenesis of miRNA in human cells cultured at low density without contact inhibition. By contrast, cells under high density experiencing contact inhibition showed defective processing of miRNAs owing to loss of YAP1 and TAZ activities[59].

Regulation of Hippo signalling by stress—As a result of its physiological role in digestion, gastrointestinal tract tissue experiences many stresses, including oxidative stress, endoplasmic reticulum stress and hypoxia. These stresses regulate YAP1 and TAZ by various mechanisms[60–62]. In addition, YAP1 and TAZ transcriptional activities are also controlled by mechanical signals through Rho GTPases or JNK1/2 (REFS 63–65), although the involvement of the Hippo pathway in YAP1 and TAZ regulation in this context is still under debate[65,66]. Mechanical forces affect many aspects of intestinal physiology, particularly by promoting the proliferation and migration of gut epithelial cells[67]. However, whether YAP1 and TAZ play any part in the response of IECs to mechanical signals has not been studied.

G protein-coupled receptor regulation—Extracellular signalling molecules such as hormones can regulate the Hippo pathway via G protein-coupled receptors (GPCRs)[68–70]. GPCR overexpression or mutations of guanine nucleotide-binding protein subunit alpha (Gα) proteins have been associated with carcinogenesis in a variety of tissues[71]. Many GPCRs known to promote cell growth and tumorigenesis, such as lysophosphatidic acid receptors (LPARs), sphingosine 1-phosphate receptors (S1PRs) and protease-activated receptors (PARs), are now recognized as cell surface receptors for Hippo signalling[68,70], suggesting that the Hippo pathway mediates GPCR-induced tumorigenesis. Indeed, GPCRs coupled to Gα_{12} or Gα_{13}, or to G$\alpha_{q/11}$, can activate YAP1 and TAZ, leading to tumorigenesis[72–74] On the other hand, Gα_s activation was shown to inhibit YAP1 and TAZ[68,75,76]. Intestinal GPCRs respond to many nutrients and other components in food, and therefore play a crucial part in regulating intestine physiology, particularly in metabolic homeostasis and appetite[77]. It would be interesting to evaluate whether the Hippo pathway is regulated by GPCRs that are highly expressed in the intestine, for instance the GPCRs that respond to fatty acids (which include free fatty acid receptors and GPR120)[77].

Intestinal regeneration and homeostasis

Hippo pathway in Drosophila midgut regeneration

Drosophila has been widely recognized as a powerful and convenient animal model to study cellular mechanisms of human intestinal diseases, owing to its simple structure and well-defined cell populations[78]. The *Drosophila* gut consists of foregut, midgut and hindgut[79], with *Drosophila* midgut and hindgut corresponding to the mammalian small and large intestine, respectively. Studies in the past few years have revealed a crucial role for the Hippo pathway in *Drosophila* midgut regeneration after injury.

Adult *Drosophila* midgut homeostasis is maintained by intestinal stem cells (ISCs). Cell division of ISCs can be either symmetric or asymmetric, depending on the nutrient needs of the animal and the status of the midgut epithelial lining[78]. In asymmetric division, one ISC is divided into an enteroblast and the other remains an ISC. Enteroblasts can further differentiate into enteroendocrine cells and enterocytes, which constitute the epithelium layer of the midgut. Stresses, pathogens and dietary toxins can stimulate enterocytes to secrete ligands that bind the epidermal growth factor receptor (Egfr) or activate the Jak–Stat pathway, stimulating proliferation of neighbouring ISCs in a paracrine manner[78].

Under normal conditions, the Hippo pathway restricts ISC proliferation by inactivating Yki. Staley *et al.*[25] reported that Yki is activated in enterocytes upon tissue damage, leading to increased expression of Unpaired (Upd) target genes. Upd, a secreted hormone for the Jak–Stat pathway, initiates non-autonomous proliferation of ISCs through activation of Jak–Stat signalling[25] (FIG. 2). Yki is activated by stress-activated protein kinase JNK upon intestinal injury; JNK phosphorylates LIM domain-containing protein jub (also known as Jub), and phosphorylated Jub in turn prevents the phosphorylation of Yki by binding to Wts[80]. Although Staley *et al.*[25] did not observe a role for Yki in regulating ISC division, several other studies have demonstrated that inactivation of Hpo in ISCs also leads to ISC overproliferation as a result of Yki-mediated upregulation of downstream genes such as *Cyclin E*, *Diap1* and *bantam* miRNA[81–84]. These studies support the role of the Hippo pathway in regulating the autonomous proliferation of ISCs. Ren *et al.*[83] further showed that in addition to Upd, Yki also promotes the transcription of multiple Egfr ligands to promote non-autonomous proliferation of ISCs (FIG. 2). Besides JNK and Jub, several other proteins play important parts in regulating Yki activities. Pez, the *Drosophila* homologue of PTPN14, negatively regulates Yki-mediated ISC proliferation by binding to Kibra, thereby activating the core Hippo pathway kinase cascade[85]. A later study showed that Pez protein stability is negatively regulated by E3 ubiquitin-protein ligase Su(dx) (also known as Su(dx)), a member of the Nedd4 family of E3 ubiquitin ligases, and overexpression of Su(dx) in enterocytes leads to *Drosophila* midgut epithelium proliferation[86]. Notably, mammalian PTPN14 has been reported to be highly mutated in human colorectal cancers (CRCs)[87]. Exploring the role of PTPN14 in CRCs and whether the Hippo pathway is involved in tumori-genesis would be interesting. In addition, Yki activity in *Drosophila* ISCs is attenuated by Tao, a protein kinase that activates Hpo, to maintain midgut homeostasis[88]. Msn, which seems to activate Wts preferentially in enteroblasts in a Hpo-independent

manner, is involved in controlling ISC proliferation by inhibiting Upd3 expression during intestinal regeneration[23].

Downstream effectors of Yki in midgut regeneration have also been identified, and seem to be crucial in mediating the function of Yki. For instance, Yki is reported to regulate dMyc expression[89]. Inhibition of caspase 3 by Yki target gene *Diap1* is essential for Brahma to participate in ISC proliferation[90]. Future studies are needed to determine whether the mammalian homologues of these Hippo pathway regulators and effectors also have important roles in mammalian intestinal homeostasis.

The Hippo pathway in mammalian intestine

Intestine cellular structure—The functional unit of the mammalian small intestine is the villus, which contains a group of dynamic and self-renewing epithelial cells[91]. Between the villi are invaginations of the epithelium, called the crypts of Lieberkühn. The bottom of the crypt, where ISCs reside, is the main stem cell niche. These stem cells divide to produce highly proliferative transit amplifying cells, which migrate upwards to the tip of the villi and eventually lose their progenitor identity. While moving upwards, transit amplifying cells differentiate into either absorptive enterocytes or secretory cells, which comprise goblet cells, enteroendocrine cells and Paneth cells[91]. Unlike the goblet cells and enteroendocrine cells, which both reside within the villi, Paneth cells migrate towards the base of the crypt and reside beside the ISCs (FIG. 3). Paneth cells are critical for stem cell maintenance as they secrete essential niche signal ligands such as epidermal growth factor (EGF), WNT3a and Notch ligand DLL4 (REF. 92). Although the cellular structure and organization of the colon is mostly similar to that of the small intestine, the colon has a flat surface of epithelia with no villi and no Paneth cells in the crypt[93]. A subpopulation of CD24^{+} cells, located adjacent to colonic stem cells in the colonic crypt, are proposed to provide the stem cell niche by functioning equivalently to Paneth cells[92].

The base of the intestinal crypt is a specialized ISC niche harbouring two distinct stem cell populations, active stem cells and reserved stem cells. Active stem cells, called crypt base columnar (CBC) cells, express Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5)[94]. LGR5^{+} cells are evenly distributed between Paneth cells and are responsible for normal epithelial homeostasis. The identity of reserved stem cells is less clear. The proteins polycomb complex protein BMI1 (also known as BMI1), homeodomain-only protein (also known as HOPX), telomerase reverse transcriptase (also known as TERT), and Leucine-rich repeats and immunoglobulin-like domains protein 1 (also known as LRIG1) were proposed as markers for +4 reserved stem cells, which reside at the fourth position from the crypt base, but all were later reported to be also highly expressed in CBC cells[95]. Nonetheless, it is widely accepted that reserved stem cells are responsible for repopulating the entire tissue after injury[91,96]. The turnover time of mouse intestinal epithelial cells is ~3–5 days[91]. This high rate of cellular turnover has evolved to maintain optimal intestinal function, and makes the intestine an attractive organ system for the study of cell proliferation, differentiation and regenerative medicine.

Role of the Hippo pathway in normal homeostasis—YAP1 and TAZ have been reported to maintain and promote stem cell properties in various tissues, such as the liver, skin and nervous system[97]. In the intestine, endogenous YAP1 protein expression is restricted to the stem cell niche at the bottom of the crypt, suggesting an association with ISC regulation[98–100]. Several studies have reported that high YAP1 activity can promote ISCs proliferation. Camargo *et al.*[98] and Zhou *et al.*[101] showed that YAP1 overexpression in the intestine leads to expansion of undifferentiated cells and the replacement of differentiated cells at the tips of the villi, similar to the phenotype observed in intestines deficient in MST1 and MST2. In addition, using the intestine-specific gene transfer technique, YAP1 and TAZ were shown to promote proliferation of stem cells and progenitor cells by binding to TEAD transcription factors, and to induce differentiation of progenitor cells into goblet cells by binding to the transcription factor Krueppel-like factor 4 (KLF4)[102]. Under normal homeostasis, protein kinase C zeta type (PKCζ) acts as a tumour suppressor gene, suppressing YAP1 activity through direct phosphorylation in ISCs. Loss of PKCζ in the intestine increases YAP1 nuclear localization, leading to elevated stem cell activity and tumorigenesis[103]. In general, YAP1 and TAZ activity promotes stem cell properties in the intestine. However, Barry *et al.*[104] showed that intestine-specific acute induction of YAP1 expression in mice results in disruption of normal intestinal tissues, such as mislocalized Paneth cells and reduced ISC numbers, resulting in loss of cellular proliferation in crypts. This finding suggests that YAP1 has a growth-suppressive function in healthy intestine. The inconsistency of these results with those of Camargo *et al.*[98] might be due to different experimental settings and mouse models. For example, mice with inducible systemic YAP1 overexpression were used in the study by Camargo *et al.*[98], whereas Barry *et al.*[104] induced YAP1 expression specifically in the IECs. IECs constantly interact with neighbouring stromal cells and immune cells, which might also regulate YAP1 by chemical or mechanical cues. Thus, the opposite proliferation phenotypes observed in the models could result from autonomous and non-autonomous effects. YAP1 cellular localization might also be different after systemic induction of expression compared with localization after intestine-specific induction. In contrast to the activation of Wnt signalling by nuclear YAP1, cytoplasmic YAP1 inhibits Wnt signalling, leading to opposite effects on progenitor cell preservation and proliferation[2]. Moreover, strong YAP1 induction also initiates negative feedback regulation by inducing LATS2 expression, which might exert some growth inhibitory effect[105–107].

The Hippo pathway is important in maintaining normal intestine homeostasis; disrupted Hippo signalling leads to tissue overgrowth and tumorigenesis. Mice with conditional knockout of *Mst1* and *Mst2* specifically in IECs show a disorganized villus structure and expansion of undifferentiated cells. Dysplastic epithelia and adenomas are frequently observed in the colons of these mice[101]. Similarly, mice with *Sav1*-deficient small and large intestines show enlarged crypt structure and increased polyp formation[100]. Surprisingly, various reports show that depletion of YAP1 and/or TAZ protein levels in the intestine has no observable adverse phenotype under normal homeostasis, meaning that YAP1 (REFS 98,100,104) and TAZ[99] might be dispensable under normal conditions. Conversely, the Hippo pathway components MST1, MST2 and SAV1 are indispensable for normal tissue homeostasis, suggesting that the Hippo pathway is constitutively active under normal

conditions to restrict YAP1 and TAZ activity. Disruption of the Hippo pathway leads to hyperactivation of YAP1 and TAZ, which then causes uncontrolled stem cell proliferation and tumori-genesis[100,101]. Currently, only mice with intestine-specific conditional knockout of *Mst1* and *Mst2*, *Sav1*, and *Yap1* and/or *Taz* have been studied, and further investigations could provide new mechanistic insights into how other Hippo pathway components regulate intestinal tissue homeostasis.

Role of the Hippo pathway in regeneration—Although YAP1 is not required during normal intestinal homeostasis, its activity is essential for intestinal regeneration after injury. In mice, an increase in YAP1 protein level is observed 2–5 days after dextran sulfate sodium (DSS)-induced colitis and regeneration[100,108]. *In vivo* and *in vitro* studies show interleukin-6 receptor subunit beta (also known as gp130), a co-receptor for IL-6, activates YAP1 through Src family kinases (SFKs) upon DSS-induced intestinal colitis[108]. Mice with IEC-specific conditional knockout of *Yap1* had a higher mortality rate and more extensive loss of the crypt compartment than wild-type mice after both groups were subjected to DSS-induced intestinal injury[100]. Whole-body irradiation is also widely used to study intestinal regeneration in mice. YAP1 is activated in IECs 2–4 days after irradiation[109], and IEC-specific and ISC-specific *Yap1* knockout mice show reduced crypt proliferation after irradiation-induced injury[109]. These studies suggest that YAP1 positively regulates intestinal tissue regeneration by regulating ISC proliferation. Furthermore, Gregorieff *et al.*[109] found that YAP1 reprogrammes ISCs by transiently inactivating Wnt signalling and activating another regenerative programme, the EGFR pathway. They showed that the EGFR ligand epiregulin is upregulated in the intestines of *Yap1* conditional knockout mice after regeneration of the intestinal epithelia following whole-body exposure to ionizing radiation, which might serve as a compensatory mechanism for *Yap1* loss in some crypts. Indeed, they further showed that exogenous epiregulin can rescue YAP1-deficient organoid formation *ex vivo*[109]. By contrast, Barry *et al.*[104] found that whole-body irradiation of mice with intestine-specific *Yap1* conditional knockout resulted in crypt hyperplasia and overgrowth 7 days later, and suggested that Wnt signalling hyperactivation was responsible for this phenotype. In summary, functional YAP1 is important in the first few days of the regeneration phase. The hyperproliferative phase seen in *Yap1*-IEC-specific conditional knockout mice 7 days after injury could be the result of enhanced Wnt signalling (FIG. 4). The divergent roles of YAP1 in the regenerative response at different time points after injury might be due to the distinct cellular repair mechanisms activated at these time points. For instance, pathways involved in cell cycle and DNA repair are active 2–5 days after injury whereas pathways for tissue development might be activated at subsequent time points, 1 week or later after injury[110]. YAP1 and Wnt signalling work in coordination to maintain proper tissue regeneration after injuries. Mouse genetic studies clearly demonstrate a role of the Hippo pathway in normal intestine homeostasis as well as in the response to tissue injury.

The Hippo pathway and signalling crosstalk

Intestinal epithelial homeostasis is tightly controlled and regulated by interconnected developmental pathways that keep cellular processes such as proliferation and differentiation in balance. Several conserved signalling pathways, such as the Wnt, Notch, Hedgehog and

bone morphogenetic protein (BMP) pathways, have been shown to regulate mammalian intestinal homeostasis and regeneration[111]. Understanding how the Hippo pathway interacts with these signalling pathways can help explain the phenotypes observed in mouse models with disrupted Hippo signalling, and will be beneficial for developing therapeutic strategies (FIG. 5).

Hippo and Wnt signalling—Wnt signalling is essential for intestinal tissue homeostasis and stem cell maintenance[112]. Canonical Wnt signalling depends on β-catenin as its key effector. β-catenin binds to the TCF and LEF families of transcription factors to activate the transcription of target genes. Nuclear localization of β-catenin, which represents active Wnt signalling, has been observed in mice in the ISCs at the base of the crypt[113]. Also in mice, inhibition of canonical Wnt signalling results in loss of transit amplifying cells and disrupted crypt structure, leading to defects in intestinal homeostasis and function[114]. Interplay between the Hippo pathway and Wnt signalling has been intensively studied in the context of stem cell maintenance and damage repair. In the mammalian intestine, YAP1 maintains the proliferation and stemness of crypt stem cells by activating Wnt signalling pathways[98,101]. YAP1 overexpression induces nuclear β-catenin accumulation and expression of β-catenin target genes[98]. In addition, the Wnt target genes and stem cell markers *Lgr5* and *Ascl2* are upregulated in the intestines of mice with intestine-specific conditional double knockout of *Mst1* and *Mst2* (REF. 101). *In vitro* studies show that downregulation of YAP1 in the SW480 human colon cancer cell line results in a decrease in β-catenin-dependent transcription[101]. These studies indicate that YAP1 activity potentiates Wnt signalling. On the other hand, several studies have shown that cytoplasmic YAP1 and TAZ inhibit canonical Wnt signalling, and several mechanisms have been proposed to explain this regulation. Firstly, cytoplasmic YAP1 and TAZ bind to segment polarity protein dishevelled homologue DVL-1 (known as DVL), a positive regulator of Wnt, and suppress its activity[104,115]. Secondly, β-catenin nuclear translocation is supressed by direct binding with YAP1 and TAZ[116]; and thirdly, YAP1 and TAZ incorporate into the β-catenin destruction complex to recruit F-box/WD repeat-containing protein 1A (also known as β-transducin repeat-containing protein, β-TrCP), which ubiquitylates β-catenin and leads to its degradation[99]. YAP1 overexpression in mice causes an increase in cytoplasmic YAP1 levels and downregulates intestinal β-catenin target gene expression[104]. Reciprocally, several studies have shown that YAP1 and TAZ can be activated by Wnt signalling, and are required for some Wnt responses in the intestine[99,117,118]. Notably, the non-canonical Wnt signalling pathway, which is β-catenin-independent, has also been shown to have a critical role in intestinal regeneration[119]. YAP1 and TAZ can be activated by noncanonical Wnt signalling, and many proteins induced by active YAP1 and TAZ can antagonize canonical Wnt signalling *in vitro*. This study provides a mechanism by which noncanonical Wnt signalling might antagonize canonical Wnt signalling through YAP1 (REF. 120). As a result, testing whether the effect of noncanonical Wnt signalling in intestinal regeneration is mediated by YAP1 and TAZ would be interesting. Crosstalk between Wnt and the Hippo pathway is highly complex and future investigation is needed to fully understand their interplay.

Hippo and Notch signalling—In addition to Wnt, the Notch pathway is also required to maintain ISCs. Mammals possess four Notch receptors (Notch1, Notch2, Notch3 and

Notch4), each of which consists of a large extracellular ligand binding domain, a transmembrane domain and an intracellular domain. Upon ligand activation, the Notch intracellular domain (NICD) is cleaved from the receptors and translocates into the nucleus[121]. Nuclear NICD associates with recombining binding protein suppressor of hairless (RBPJ, also known as CSL (CBF1/RBPjκ/Su(H)/Lag-1)), to induce the expression of target genes such as the *HES* transcription factor family, *CDKN1A* (also known as *p21*) and *MYC*. Pharmacological inhibition of Notch signalling in mice results in decreased expression of the stem cell markers olfactomedin 4 (*Olfm4*) and *Lgr5* and reduced numbers of crypt base columnar stem cells[122]. Besides preserving stemness, Notch signalling is able to determine cell fate during differentiation. High Notch activity promotes the differentiation of transit amplifying cells along the absorptive lineage[123], whereas inhibition of Notch signalling leads to loss of stem cell properties and induces secretory lineage differentiation[124]. Notch signalling is also regulated by the Hippo pathway. Mice with intestine-specific conditional double knockout of *Mst1* and *Mst2* have intestines with increased NICD nuclear localization and upregulated *Hes1* gene expression[101]. The expansion of undifferentiated intestinal progenitor cells resulting from YAP1 overexpression in transgenic mice is attenuated when γ-secretase inhibitors that block Notch signalling are administered[98]. In summary, these data suggest that YAP1 can positively regulate Notch signalling in the intestine, and that Notch is a mediator of YAP1-induced ISC expansion. The molecular mechanism by which Hippo regulates Notch in the intestine has not yet been elucidated. Nevertheless, several members of the Notch pathway, such as *Notch1* and *Notch2*, *Sox9* and *Hes1*, are transcriptionally upregulated in hepatocytes with YAP1 overexpression, and *Notch2* was identified as a direct target gene of the YAP1–TEAD complex[125]. This notion might provide insights into the interplay between Hippo and Notch pathways in the intestine.

Hippo and BMP signalling—Although not in the context of intestinal cells, the existence of crosstalk between Hippo and BMP signalling or between Hippo and Hedgehog signalling has also been established. BMPs regulate cellular differentiation, apoptosis and cell growth[126]. Inhibition of BMP signalling by over-expressing its antagonist Noggin in the mouse intestine results in hyperproliferation of ISCs, an increased number of crypts, and eventually intestinal polyposis[127,128]. These data suggest that BMP signalling negatively regulates stem cell proliferation in the crypt. BMP signalling is initiated by BMP ligands binding to type I and type II serine/threonine kinase receptors. Activated receptors then phosphorylate the *C*-termini of SMADs (SMAD1, SMAD5, and SMAD8), which then bind to their cooperative partner SMAD4, and translocate into the nucleus[126]. This transcriptional complex of SMAD1, SMAD5, SMAD7 and SMAD4 is fully activated by phosphorylation at the linker region by cyclin-dependent kinase (CDK)8 and/or CDK9. In mouse embryonic stem cells, the phosphorylated SMAD1–SMAD4 complex recruits YAP1 to the enhancer region of BMP-responsive genes, resulting in BMP-induced gene expression[129].

Hippo and Hedgehog signalling—Hedgehog signalling has also been implicated in intestinal homeostasis. Indian hedgehog and sonic hedgehog, two key Hedgehog signalling proteins, are expressed in mouse intestinal epithelium[130]. Ablation of Indian hedgehog protein in the mouse small intestine causes overexpansion of the stem cell niche and reduces

stem cell differentiation along the absorptive lineage[131]. Although the crosstalk between Hippo and Hedgehog signalling has not been characterized in intestinal tissue, there is evidence of crosstalk between the two pathways in other tissues. For example, in medulloblastoma, cerebellar granule neuron precursors (CGNPs) are implicated as the origin of the tumours, and their proliferation depends on Sonic hedgehog signalling[132,133]. Activation of Sonic hedgehog in CGNPs upregulates YAP1 protein levels, and direct binding between YAP1 and insulin receptor substrate 1 (IRS1), a Sonic hedgehog effector, results in nuclear localization of YAP1. In addition, YAP1 depletion abolishes Sonic-hedgehog-induced CGNP proliferation. GLI2, a downstream effector of Sonic hedgehog signalling, is identified as a target gene of the YAP1–TEAD complex, suggesting a mechanism by which YAP1 drives tumorigenesis in medulloblastoma[134]. However, Indian hedgehog is the predominant Hedgehog protein expressed in the gastrointestinal tract. Understanding the crosstalk between Hippo and Hedgehog signalling in the intestine, which might be mediated by Indian hedgehog, remains an interesting question and needs further exploration. In summary, the crosstalk between Hippo and other signalling pathways is complex and might be context-dependent. The Hippo pathway has been shown to communicate with the BMP pathway and the Hedgehog pathways in other biological contexts, and one can speculate that these interactions would also be present in the intestine and contribute to tissue homeostasis.

Hippo pathway in intestinal diseases

The Hippo pathway in colon cancer

Uncontrolled tissue regeneration can lead to malignant transformation[135]. Dysregulation of the Hippo pathway has been observed in many cancer types[41], including CRC. Genetically engineered mouse models serve as useful tools to study how the Hippo pathway influences tumorigenesis. Because global knockout of many genes encoding Hippo pathway components, such as *Nf2*, *Sav1*, *Mst1*, *Mst2* and *Lats2,* results in embryonic lethality in mice, intestinal-epithelium-specific conditional knockout mice have been developed. Mice with conditional knockout of both *Mst1* and *Mst2* in the intestinal epithelium develop dysplastic small and large intestine and spontaneous adenomas in the colon[101]. The increased tumour risk might be the result of elevated YAP1 protein expression in these mice, resulting in strong activation of Wnt and Notch signalling. Intestinal-epithelium-specific S*av1*-knockout mice develop colonic polyps by 13 months of age, whereas wild-type littermates do not develop polyps[100]. This study further showed that DSS-induced intestinal injury and repair greatly exacerbated the tumorigenicity of the S*av1*-deficient crypts. This effect was YAP1-dependent, as mice with intestinal epithelium-specific knockout of both *Sav1* and *Yap1* had no colonic polyp formation. In addition to canonical Hippo pathway components, the NDR protein kinases have been identified as tumour suppressors upstream of YAP1. Mice with intestinal epithelium-specific knockout of both *Ndr1* and *Ndr2* show increased YAP1 expression level and are more sensitive to colon carcinogenesis induced by DSS or azoxymethane[136]. These data support the notion that dysregulation of Hippo pathway leads to tumorigenesis and that active YAP1 is oncogenic in CRCs.

Studies of samples from patients with colon cancer and human colon-cancer-derived cell lines also support the oncogenic property of YAP1 and TAZ in CRCs. A positive correlation

between YAP1 protein expression level and poor prognosis has been observed in patients with CRC[101,137,138], and TAZ has also been found to be a prognostic indicator for CRC outcome[139,140]. In human colon cancer cell lines, YAP1 knockdown by small-hairpin RNA resulted in a dramatic decrease in cell proliferation, metastasis and invasion, whereas over-expression of YAP1 resulted in an increased proliferation rate[101,139]. Knockdown of TAZ resulted in decreased cell proliferation, both *in vitro* and in xenograft mouse models[141]. These studies support YAP1 and TAZ as oncogenes in CRCs.

Several underlying mechanisms of Hippo-pathway-driven tumour transformation have been proposed. The Wnt–β-catenin pathway has a crucial role in initiating CRC. Loss of function mutations in the gene encoding adenomatous polyposis coli (*APC*) are observed in most colorectal tumours[142]. *APC* mutation increases the risk of CRC through the constitutive activation of the β-catenin–TCF4 complex in IECs[143]. Proteins encoded by β-catenin–TCF4 complex target genes, including matrilysin (also known as MMP-7), Myc, cyclin D1 and PPARd, have been shown to contribute to CRC tumour progression[144–148]. YAP1 and TAZ are activated in APC-deficient cells both *in vitro* and *in vivo*[99,117,149], and mouse models with intestine-specific *Apc*-knockout do not show intestinal hyperplasia if TAZ or YAP1 are also knocked out in the intestines[99,149]. Different mechanisms have been proposed for how APC regulates the activity of YAP1 and TAZ. Azzolin *et al.*[99] reported that YAP1 and TAZ are sequestered in the cytoplasm by the β-catenin destruction complex when Wnt signalling is not active. When Wnt is activated by APC depletion, YAP1, TAZ and β-catenin separate from the destruction complex and become active. Cai *et al.*[149] reported that the activity of YAP1 and TAZ can also be regulated by APC via a mechanism independent of the β-catenin destruction complex. They showed that APC serves as a scaffolding protein to facilitate LATS activation. Loss of APC inactivates the Hippo pathway, leading to YAP1 and TAZ activation[149]. Other studies have shown that the β-catenin–TCF4 complex directly binds to the *YAP1* enhancer region to promote *YAP1* gene expression, thereby identifying *YAP1* as a TCF4 target gene[118]. In addition, β-catenin can complex with YAP1 and TBX5 to induce anti-apoptotic gene expression; this complex is essential for survival of β-catenin-driven colon cancers[150]. Together, these results suggest that YAP1 and TAZ can be mediators downstream of APC, and that their activities contribute to colonic tumorigenesis. The dispensable nature of YAP1 and TAZ under normal conditions make them an attractive therapeutic target for treating CRC.

In addition to Wnt signalling, JNK signalling has also been implicated in inducing tumorigenesis in the intestine[151,152]. Several studies indicate that JNK is an upstream regulator of the Hippo pathway. In the *Drosophila* midgut, bleomycin-induced injury activates JNK, leading to Yki nuclear translocation. Activated Yki upregulates Upd expression and Jak–Stat signalling, which induces cellular proliferation[25,153]. In mammals, JNK induces YAP1 activity when cells are exposed to mechanical strain[66] and DNA damage[154]. Mechanistically, JNK activates YAP1 through inhibition of LATS by increasing binding between LATS proteins and Ajuba (the mammalian homologue of Jub)[80]. Although no studies have established a connection between JNK signalling and the Hippo pathway in the context of the mammalian intestine, this mechanism could be worthy of further exploration in colorectal tumorigenesis.

The Hippo pathway in other intestinal diseases

Peutz–Jeghers syndrome (PJS) is characterized by the development of benign polyps in the gastrointestinal tract. In PJS, the tumour suppressor *LKB1* is frequently mutated, leading to intestinal polyp formation[155]. These polyps can cause bleeding, bowel obstructions and pain. Patients with PJS are at an increased risk of developing various cancers, especially gastrointestinal tumours[156,157]. The polyps in these patients were observed to exhibit increased YAP1 nuclear localization, prompting the investigation of the connection between LKB1 and the Hippo pathway. As a result, LKB1 has been identified as an upstream regulator of Hippo signalling. *In vitro* studies show that loss of *LKB1* leads to YAP1 nuclear localization in intestinal epithelial cell lines, and depleting YAP1 expression reduces cancer phenotypes, including decreasing cell proliferation rate, colony formation and tumour size[49,158]. In addition, LKB1 is a major positive upstream regulator of AMPK activity. Activated AMPK inhibits YAP1 through several mechanisms, including: activation of LATS proteins; direct phosphorylation of YAP1 at serine 94, disrupting YAP1 interaction with TEAD proteins; and stabilizing Angiomotin-like protein 1 (AMOTL1), which inhibits YAP1 (REFS 50,52). Loss of LKB1 might contribute to tumorigenesis by lowering AMPK activity, resulting in YAP1 hyperactivation.

IBD, including Crohn's disease and ulcerative colitis, is a chronic idiopathic inflammatory disorder that affects the gastrointestinal tract. Appropriate intestinal epithelial regeneration is required to improve the outcomes of treatments for IBD. YAP1 regulation has been identified as a responsive mechanism for mucosal regeneration in IBD using a DSS-induced colitis mouse model[100,108]. IL-6 is a key modulator of the intestinal inflammatory response that can promote intestinal epithelial cell proliferation and regeneration[159]. As mentioned earlier, gp130 activates YAP1 upon DSS treatment. *Villin-gp130^{Act}* mice, which express constitutively active gp130 exclusively in IECs, have increased YAP1 nuclear localization, and IEC-specific *Yap1* depletion in *gp130^{Act}* mice reverses their DSS resistance[108]. This study establishes a mechanism by which YAP1 is activated upon intestinal injury and provides insights into potential therapeutic strategies for IBD.

The Hippo pathway in the liver

The liver is the central organ controlling metabolism of carbohydrates, lipids and amino acids, as well as detoxification of both exogenous and endogenous compounds[160]. The liver is capable of rapidly renewing its parenchymal cells (hepatocytes) and non-parenchymal cells (cholangiocytes, Kupffer cells, stellate cells and sinusoidal endothelial cells) after injury to resume its physiological functions[161].

Regulation of liver size by Hippo signalling

Liver regeneration and homeostasis are considered a classic model for mammalian organ size control[162]. The discovery that Hippo signalling is essential in the regulation of tissue homeostasis in *Drosophila* was followed immediately by speculation over whether the Hippo pathway similarly regulates mammalian organ size[163]. In mammalian models, overexpression of YAP1 in the liver leads to tissue overgrowth, and sustained over-expression of YAP1 eventually results in hepatocellular carcinogenesis[39,98]. In human

hepatocellular carcinoma specimens, high YAP1 protein levels and increased YAP1 nuclear localization have been observed[36,39,164]. Because liver cancer results from uncontrolled proliferation of hepatocytes, increased YAP1 expression in human liver cancer and mouse models supports the notion that YAP1 can serve as a major driver of hepatocyte proliferation. Mouse models bearing deletion of Hippo pathway proteins, including MST1, MST2, SAV1, Merlin, LATS1 and LATS2, have been generated and characterized for the roles of these components in liver size control and carcinogenesis[106,165–169] (FIG. 6). Hepatomegaly and spontaneous hepatocellular carcinogenesis were consistently observed in liver-specific *Mst1* and *Mst2* knockout mice[165–168]. Liver-specific *Sav1*-knockout mice exhibit more complex tumorigenesis, and display both hepatocellular carcinoma and cholangiocarcinoma[165,168]. SAV1-deficient mice have been speculated to be more prone to the malignant transformation of liver progenitor cells than wild-type mice, whereas hepatocytes are unaffected by loss of SAV1 (REF. 165). Similarly, liver-specific *Nf2*-knockout mice develop hepatocellular carcinoma and bile duct hamartoma at 1 year of age[169]. A study published in 2013 showed that combined liver-specific deletion of *Nf2* and *Sav1* causes an exaggerated liver overgrowth phenotype in mice at a very early age (8 days old), whereas deletion of either *Nf2* or *Sav1* alone did not result in any abnormality in liver at that age[170]. This result indicates the synergistic actions of Merlin and SAV1 attenuate YAP1 and TAZ activity during liver development. Consistently, mice with knockout of other Hippo pathway components, such as *Mob1a* and *Mob1b,* also induce spontaneous hepatocellular carcinogenesis[171] (FIG. 6).

The role of Hippo signalling in liver development is further supported by evidence that the level of nuclear YAP1 correlates with cyclin D1 expression and hepatocyte proliferation during postnatal development in mice[172]. Mice with liver-specific *Yap1* deficiency have smaller livers and a reduced hepatocyte proliferation rate compared with wild-type mice[169]. In addition, liver-specific *Yap1*-knockout mice show impaired bile duct formation, suggesting a critical role for YAP1 in bile duct development[169]. A study published in 2015 found that the Hippo pathway might be a major downstream effector of the transcription factors SOX4 and SOX9, which regulate liver and bile duct development[173]. Another study published in 2014 showed that the main function of the Hippo pathway in the liver is to maintain the differentiation status of hepatocytes[125]. The activation of YAP1 dedifferentiates adult mouse hepatocytes into progenitor or ductal-like cells. In summary, YAP1 induces cell proliferation and suppresses differentiation during liver development, and reduced Hippo pathway activity causes uncontrolled organ growth and therefore tumorigenesis.

Hippo signalling is also involved in liver repair after injury in mouse models. A few studies have implicated that the Hippo signalling regulates liver regeneration after partial hepatectomy in rats and mice[174–176]. In addition, studies in the past few years have demonstrated the essential role of YAP1 in liver repair after injury. Deletion of *Yap1* in mice potentiates cholestatic liver injury caused by bile-duct ligation[144]. Similarly, mice with liver-specific knockout of *Yap1* exhibit deficient liver repair after carbon-tetrachloride-induced liver injury[177]. Notably, this study used a mosaic knockout mouse model to show that YAP1 activation alone in a fraction of hepatocytes is not sufficient to drive growth of the liver, whereas previous reports showed an organ hyperplasia phenotype as a result of YAP1 over-expression in the entire liver[39,98]. A possible explanation for this finding is that relative

YAP1 activation in the two models is different. In conjunction with high YAP1 activity, inflammatory signals, which are always present in the context of liver injury, are essential to induce proliferation of hepatocytes[177]. Upon liver injury, YAP1 is also activated in cells besides hepatocytes. In the livers of carbon-tetrachloride-treated mice and tissue samples from human fibrotic livers, activated YAP1 causes activation of hepatic stellate cells. Prolonged activation of these cells leads to liver fibrogenesis[178].

Hippo signalling and human liver tumorigenesis

In the healthy adult liver, YAP1 is expressed and active in the bile duct cells, whereas it is expressed at low levels and is inactive in hepatocytes[125]. However, numerous immunohistochemistry and immunoblot analyses show raised YAP1 protein levels and increased nuclear localization in human liver cancer cells compared with adjacent normal tissues[36,39,164,179–182]. YAP1 activity, defined either as protein levels or the degree of nuclear localization, can serve as a prognostic marker for overall survival and disease-free survival of patients with hepatocellular carcinoma[164,183].

YAP1 activation in liver cancer could result from many factors. The *YAP1* gene was observed to be frequently amplified in human hepatocellular carcinoma tumours[184]. YAP1 can also be activated by carcinogenic compounds such as 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene[185], which mimics the xenobiotic Phenobarbital to activate the nuclear receptor constitutive androstane receptor (CAR). The tumour-promoting role of CAR has already been implicated in liver cancer[186,187]. However, a direct link between YAP1 and CAR has not been clearly established. HBV is a major cause of liver cancer, and the viral HBx protein can increase hepatocyte protein levels of YAP1 and TAZ *in vitro*, which might contribute to tumorigenesis induced by HBV infection[188,189]. In addition, bile acids, which play an important regulatory part in liver injury and injuryrelated carcinogenesis[190,191], also activate YAP1 to promote spontaneous liver tumorigenesis in a mouse model of hepatocellular carcinoma[192].

Mechanistically, the tumour-promoting property of YAP1 in liver cancer mainly relies on TEAD-mediated gene transcription. The YAP–TEAD complex can upregulate the expression of genes directly involved in promoting cell proliferation and tissue overgrowth, for instance the Notch ligand Jagged1 and the amino acid transporters SLC38A1 and SLC7A5 (REFS 193,194). In addition, YAP–TEAD also increases expression of the microRNA miR-130a, which in turn activates YAP1 by repressing the translation of the YAP1 antagonist VGLL4, resulting in a positive feedback loop that reinforces TEAD-mediated transcription[195]. Using gene-set enrichment analysis of liver tissue from a mouse model of liver cancer, a study published in 2015 reported that the YAP–TEAD complex colocalizes with other transcription factors, such as E2F1, HNF4α and β-catenin, to coordinate the expression of genes that modulate hepatocyte quiescence and differentiation[196]. Disruption of YAP–TEAD interaction using verteporfin abolishes tumour growth *in vitro* and in mouse models[74,197]; targeting YAP–TEAD interaction could therefore be a potential therapeutic strategy for hepatocellular carcinoma.

Conclusions

The Hippo pathway has an important role in intestinal homeostasis and regeneration in both *Drosophila* and mammals, and its dysregulation often leads to tumorigenesis. In general, YAP1 and TAZ activity is associated with ISC maintenance and progenitor cell proliferation. Although YAP1 and TAZ might be dispensable under normal intestinal homeostasis, their activation is crucial during tissue regeneration, particularly in response to injury. Negative regulation of YAP1 and TAZ activity by upstream components of the Hippo pathway, such as MST1, MST2 and SAV1, is required to maintain normal intestine homeostasis and prevent tissue overgrowth. The Hippo pathway is integrated with other key pathways that are important for intestinal homeostasis, such as the Wnt signalling pathway. YAP1 acts either as a downstream effector and/or an inhibitor of Wnt signalling to contribute to ISC maintenance and differentiation of transit amplifying cells. The complex communications between the Hippo pathway and other pathways, especially the Wnt pathway, are still being explored and remain an important question in the gastrointestinal field. Notably, *in vitro* studies show the Hippo pathway can be activated by tight junctions and adherence junctions. Intestinal diseases are often associated with disruption of the intestine lining and junction structures. One can speculate that the intactness of IEC junction structure is a major signal acting via the Hippo pathway to modulate intestine homeostasis. Thus, it would be interesting to investigate the regulation of the Hippo pathway by junctions in the intestine, and how the junction structures contribute to intestinal tissue regeneration in the context of Hippo signalling pathway.

Acknowledgments

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Key points

- The Hippo pathway plays an important part in intestinal homeostasis and regeneration in both *Drosophila melanogaster* and mammals, and its dysregulation often leads to uncontrolled tissue growth

- In general, YAP1 and TAZ activity promotes intestinal stem cell properties, and overexpression of YAP1 induces stem cell expansion

- YAP1 and TAZ are crucial for intestinal tissue regeneration after injury; YAP1 depletion leads to disturbed tissue formation during regeneration

- Crosstalk between the Hippo pathway and other signalling pathways, such as Wnt and Notch, regulates intestinal tissue homeostasis and regeneration, although detailed mechanisms have not yet been elucidated

- Dysregulation of the Hippo pathway leads to tumorigenesis; *YAP1* and *TAZ* are oncogenic in colorectal cancers, as illustrated by data from mouse models and patient specimens

- The Hippo pathway also has an important role in liver damage repair and tumorigenesis, and dysregulation of the Hippo pathway leads to uncontrolled growth in the liver
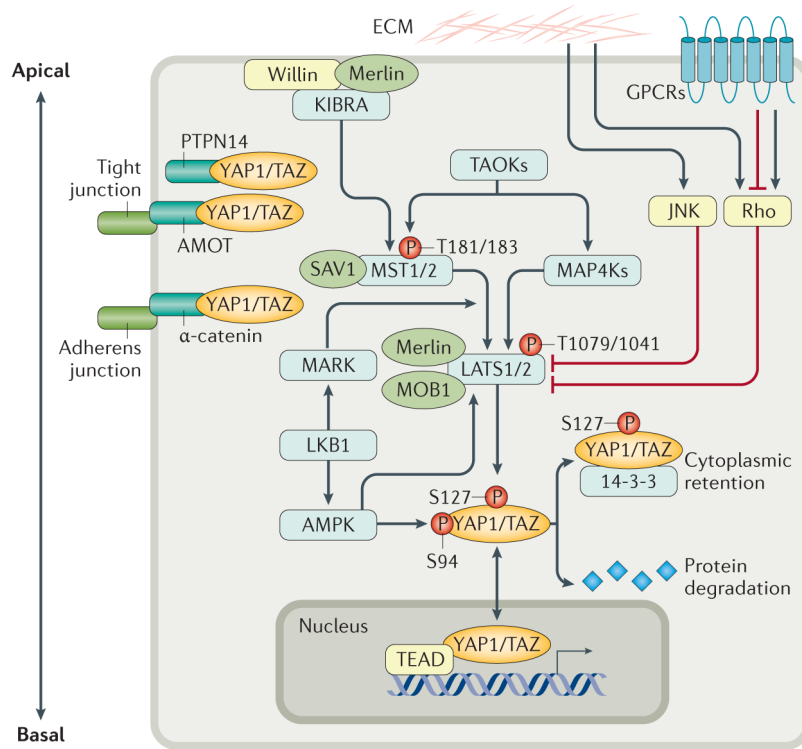
Figure 1. The Hippo pathway integrates signals to regulate the activity of YAP1 and TAZ
The core of the Hippo pathway is the kinase cascade of MST1 and MST2 and LATS1 and LATS2. MST proteins, activated by upstream signals, phosphorylate and activate LATS proteins directly, and also activate the scaffold proteins MOB1 and SAV1. MAP4Ks also phosphorylate and activate LATS proteins. Activated LATS proteins phosphorylate YAP1 and TAZ at multiple sites, triggering 14-3-3-mediated cytoplasmic retention and protein degradation. YAP1 and TAZ function as transcriptional co-activators of TEAD proteins to induce expression of genes involved in cell proliferation and apoptosis. Merlin, Willin and KIBRA form a complex to recruit MSTs and LATS to the apical plasma membrane, activating LATS proteins. AMOT and PTPN14 sequesters YAP1 and TAZ at tight junctions, and α-catenin sequesters YAP1 and TAZ at adherens junctions, to prevent their nuclear translocation. LKB1, which has important roles in both cell polarity and cellular stress response, regulates the activity of YAP1 and TAZ through activation of its substrates MARK and AMPK, both of which activate LATS. Activated AMPK also phosphorylates YAP1 and disrupts the YAP1 TEAD complex. The Hippo pathway is further regulated by ECM stiffness and mechanotransduction through Rho GTPases or JNK. GPCRs mediate many extracellular signals to either activate or inhibit LATS via Rho GTPase. AMOT, Angiomotin; AMPK, AMP-activated protein kinase; ECM, extracellular matrix; GPCRs, G protein-coupled receptors; JNK, c-Jun *N*-terminal kinase; LATS, large tumour suppressor; LKB1, liver kinase B1; MAP4K, mitogen-activated protein kinase kinase kinase kinase; MARK, microtubule affinity-regulating kinase; MOB1, Mob1 homologue; MST, mammalian ste20-like kinase; P, phosphorylation of stated amino acid; PTPN14, protein tyrosine phosphatase non-receptor type 14; SAV1, salvador family WW domain-containing protein 1; TAZ,

transcriptional co-activator with PDZ-binding motif; TEAD, TEA domain family member; TAOK, thousand and one amino acid protein kinase; YAP1, Yes-associated protein 1.
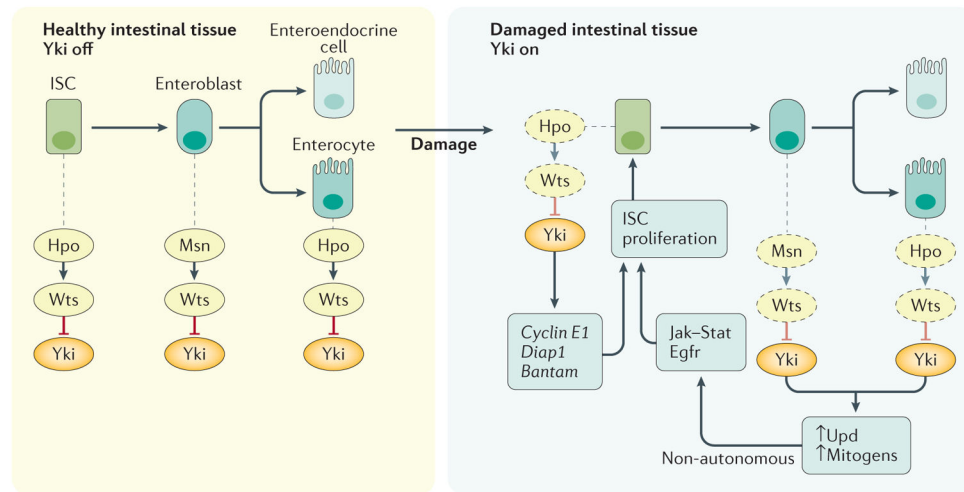
Figure 2. The Hippo pathway controls *Drosophila* midgut homeostasis by restricting Yki activity
In healthy ISCs and enterocytes, the Hpo Wts kinase cascade (homologous to the MST LATS kinase cascade in mammals) suppresses Yki activity. Upon intestinal damage Wts is inactivated, in part by JNK signalling, resulting in Yki activation. Yki activation in enterocytes leads to expression and secretion of Upd protein, a Jak Stat ligand, and Egfr ligands, which promote non-autonomous ISC proliferation in a paracrine manner. Yki activation in ISCs results in expression of *cyclin E1*, *bantam* and *diap1*, promoting autonomous ISC proliferation and survival. In enteroblasts, Msn activates Wts in a Hpo-independent manner, and therefore negatively regulates Yki. Egfr, epidermal growth factor receptor; Hpo, Hippo; ISC, intestinal stem cell; LATS, large tumour suppressor; Msn, misshapen; MST, mammalian ste20-like kinase; Upd, unpaired; Wts, warts; Yki, transcriptional co-activator yorkie.
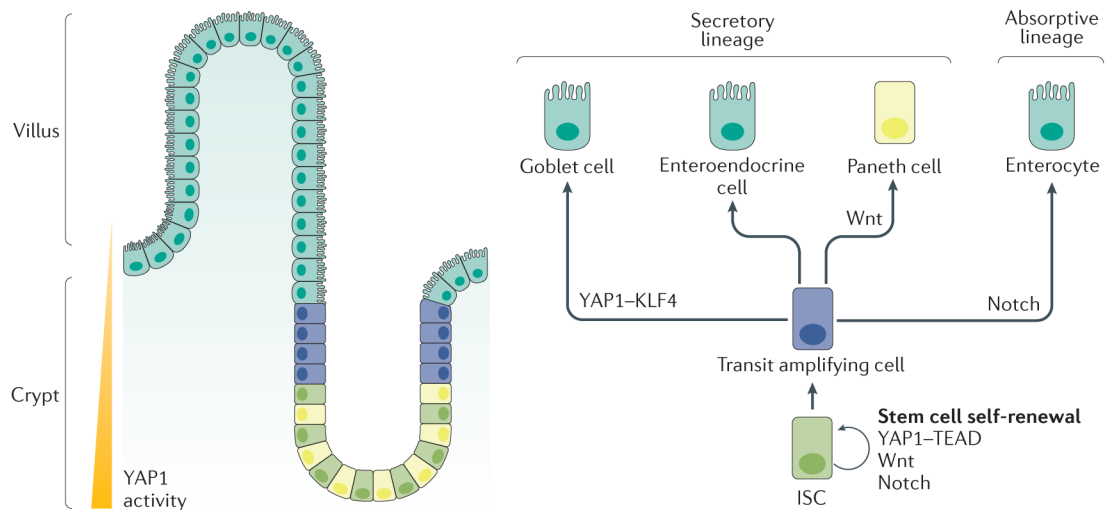
Figure 3. Mammalian intestine cellular structure and cell fate decision
The mammalian intestinal mucosa is to form villi and crypts. YAP1 activity is highest at the bottom of the crypt, where ISCs and Paneth cells reside. The villus consists of differentiated cells. ISCs in the crypt base differentiate into transit amplifying cells, which further differentiate along a secretory lineage (into goblet cells, enteroendocrine cells and Paneth cells) or an absorptive lineage (into enterocytes). After differentiation, Paneth cells move down to the stem cell niche and reside beside ISCs at the bottom of the crypt. YAP1, Wnt signalling and Notch signalling play important parts in stem cell self-renewal and progenitor cell differentiation. YAP1 interacts with KLF4 to induce progenitor cell differentiation into goblet cells, whereas Wnt signalling is involved in Paneth cell differentiation, and high Notch signalling induces differentiation along the absorptive lineage. ISC, intestinal stem cell; KLF4, krueppel-like factor 4; TEAD, TEA domain family member; YAP1, Yes-associated protein 1.
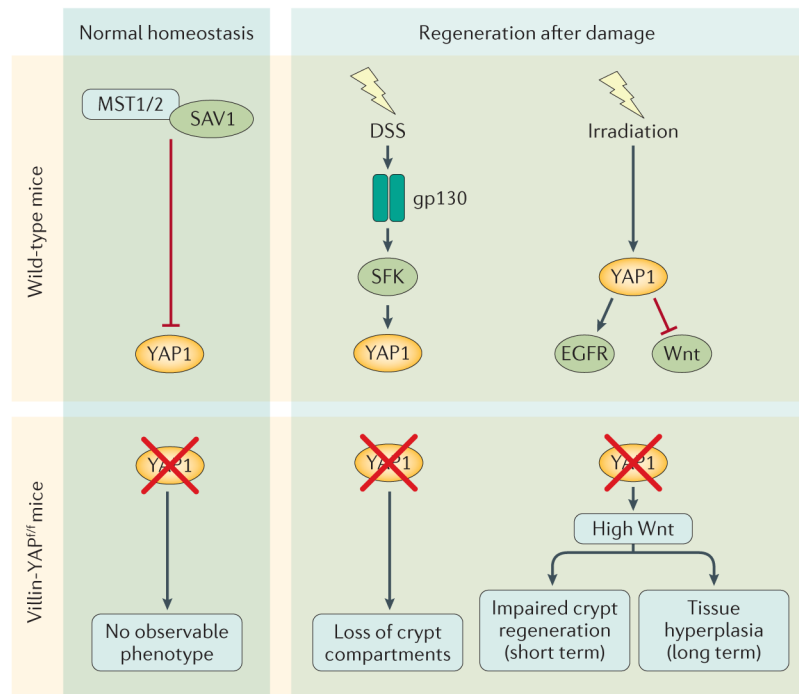
Figure 4. The Hippo pathway in intestinal homeostasis and injury-induced regeneration
In normal homeostasis, the Hippo pathway is constitutively active to keep YAP1 activity at low levels. Mice lacking YAP1 in intestinal epithelial cells (Villin-YAP$^{f/f}$ mice) have no observable phenotype. During tissue regeneration induced by damage caused by DSS, YAP1 is activated by gp130 signalling to stimulate cell proliferation. Mice with *Yap1* deficiency in intestinal epithelial cells have higher mortality and greater loss of crypt compartments than wild-type mice. Whole-body irradiation of mice causes YAP1 activation, which transiently inhibits Wnt and stimulates EGFR signalling to promote regeneration. Loss of YAP1 in intestinal epithelial cells leads to Wnt hyperactivation, impairing crypt regeneration shortly after injury but inducing tissue hyperplasia in the long term. DSS, dextran sulfate sodium; EGFR, epidermal growth factor receptor; gp130, interleukin-6 receptor subunit beta; SAV1, salvador family WW domain-containing protein 1; SFK, Src family kinases; YAP1, Yes-associated protein 1.
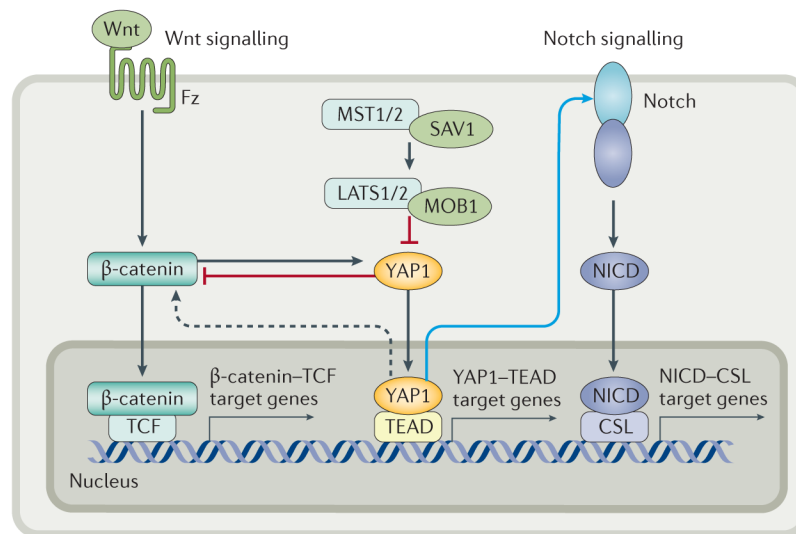
Figure 5. Crosstalk between the Hippo pathway and Wnt and Notch signalling in the intestine
When the Hippo pathway is activated, cytoplasmic YAP1 inhibits Wnt signalling by sequestering β-catenin in the cytoplasm. When the Hippo pathway is inactive, nuclear YAP1 upregulates Wnt signalling through an unknown mechanism. In addition, Wnt activation leads to β-catenin-induced activation and upregulation of YAP1. Activated YAP1 transcriptionally upregulates expression of Notch receptors, leading to increased activation of Notch signalling and translocation of the NICD to the nucleus. Notch is considered to be a mediator of the Hippo pathway. Black arrows indicate direct activation, blue arrows indicate transcriptional regulation, dotted lines indicate processes supported by experimental evidence but without a known mechanism. CSL, (CBF1/RBPjκ/Su(H)/Lag-1) transcription factor; Fz, Frizzled; LATS, large tumour suppressor; MOB1, Mob1 homologue; MST, mammalian ste20-like kinase; NICD, Notch intracellular domain; SAV1, salvador family WW domain-containing protein 1; TCF, T-cell factor; TEAD, TEA domain family member; YAP1, Yes-associated protein 1.
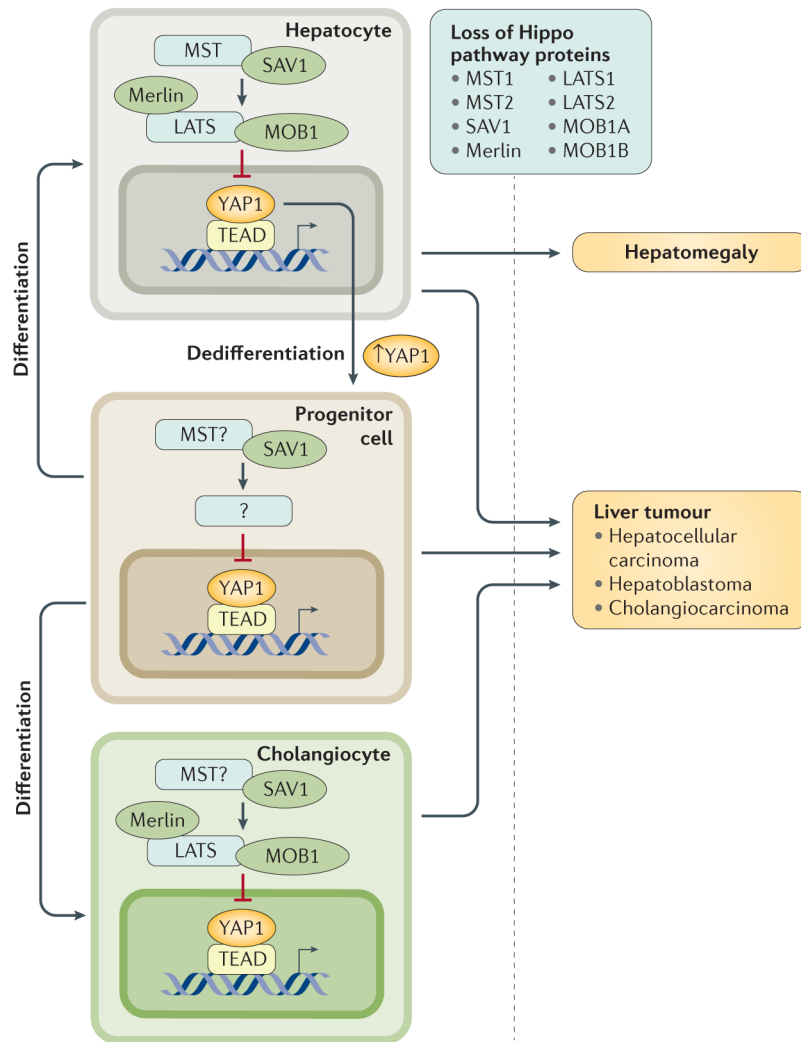
Figure 6. Hippo pathway has roles in organ size control and carcinogenesis in different types of liver cells

Disruption of Hippo signalling by deleting core kinase components (MST1, MST2, SAV1, MOB1A, MOB1B, LATS1, LATS2 and Merlin) leads to uncontrolled proliferation of hepatocytes and bile duct epithelial cells (cholangiocytes). Transgenic mice with deficiency in Hippo pathway show spontaneous liver tumours derived from hepatocytes and/or cholangiocytes, as well as hepatomegaly caused by expansion of hepatocytes. Notably, *Sav1*-knockout mice exhibit expansion of hepatic progenitor cells, which is not observed in mice with deficiency of other Hippo pathway components. On the other hand, induced ectopic expression of YAP1 converts hepatocytes into progenitor or ductal-like cells. These observations indicate a unique role of SAV1 in regulating YAP1 activities in hepatic progenitor roles. LATS, large tumour suppressor; MOB1, Mob1 homologue protein; MST, mammalian ste20-like kinase; SAV1, salvador family WW domain-containing protein 1; YAP1, Yes-associated protein 1.