REVIEW

# The Hippo pathway: regulators and regulations

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**Control of cell number is crucial in animal development and tissue homeostasis, and its dysregulation may result in tumor formation or organ degeneration. The Hippo pathway in both *Drosophila* and mammals regulates cell number by modulating cell proliferation, cell death, and cell differentiation. Recently, numerous upstream components involved in the Hippo pathway have been identified, such as cell polarity, mechanotransduction, and G-protein-coupled receptor (GPCR) signaling. Actin cytoskeleton or cellular tension appears to be the master mediator that integrates and transmits upstream signals to the core Hippo signaling cascade. Here, we review regulatory mechanisms of the Hippo pathway and discuss potential implications involved in different physiological and pathological conditions.**

Cell proliferation, death, and differentiation are fundamental biological processes. Coordination of these processes is critical for a wide range of physiological and pathological conditions (Pellettieri and Sanchez Alvarado 2007; Galliot and Ghila 2010). During development, an increase in cell number is required to boost organ and body size; meanwhile, proper differentiation of multiple cell types will assure the appropriate function of developed organs. In adulthood, most tissues undergo continuous cell turnover to maintain functionality. Aged or damaged cells are programmed to cell death, whereas adult stem cells may divide and differentiate to replace those dysfunctional cells. Under pathological conditions, such as wound healing and organ regeneration, cell division and differentiation of tissue-specific progenitor cells will be up-regulated to compensate for the lost cells. On the other hand, uncontrolled cell proliferation and decreased cell death lead to hyperplasia or tumorigenesis. Detailed mechanisms underlying cell proliferation, cell death, and cell differentiation have been extensively studied; however, how these processes are coordinated and integrated is poorly understood.

Recently, the Hippo pathway has been shown to promote cell death and differentiation and inhibit cell proliferation; therefore, the Hippo pathway may function as a key node to coordinate these cellular processes (Fig. 1). The Hippo pathway was first defined in *Drosophila* by genetic mosaic screens for tumor suppressor genes. Genetic inactivation of genes, including *Warts* (*Wts*) (Justice et al. 1995; Xu et al. 1995), *Hippo* (*Hpo*) (Harvey et al. 2003; Jia et al. 2003; Pantalacci et al. 2003; Udan et al. 2003; Wu et al. 2003), *Salvador* (*Sav*; also known as *Shar-Pei*) (Kango-Singh et al. 2002; Tapon et al. 2002), and *Mats* (Lai et al. 2005), all resulted in a similar phenotype with robust tissue overgrowth. Yorkie (Yki) is the major downstream effector of the Hippo pathway (Huang et al. 2005), which regulates a transcription program by interacting with the transcription factor Scalloped (Sd) (Fig. 2; Goulev et al. 2008; Wu et al. 2008; Zhang et al. 2008; Zhao et al. 2008). The Hippo pathway is highly conserved in mammals: MST1/2 (Hpo orthologs), Sav1, Lats1/2 (Wts orthologs), and Mob1 (MOBKL1A and MOBKL1B, Mats orthologs) form a kinase cascade that phosphorylates and inhibits YAP/TAZ (Yki orthologs). YAP/TAZ in conjunction with TEAD1–4 (Sd orthologs) mediate major physiological functions of the Hippo pathway (Fig. 2; for reviews, see Pan 2010; Zhao et al. 2010a). The nomenclature of many components of the Hippo pathway in *Drosophila* and mammals is different, and a summary of these components is shown in Table 1.

The core Hippo pathway has been well established in both *Drosophila* and mammals; however, the regulatory mechanisms for this signaling pathway are less understood. Recently, by using both genetic and biochemical approaches, many additional components have been identified to modulate the core Hippo pathway (Table 1). In this review, we briefly describe the components of the Hippo pathway and summarize recent advances with respect to Hippo pathway regulation. In addition, we also discuss the implications of Hippo pathway regulation in different physiological and pathological conditions. The mammalian Hippo pathway is the main focus, although some *Drosophila* works are also covered. For a detailed review on the *Drosophila* Hippo pathway, please refer to Staley and Irvine (2012).

## Core Hippo pathway: a kinase cascade

MST1/2 are STE20 family protein kinases and can phosphorylate Sav1, Lats1/2, and Mob1 (Wu et al. 2003; Chan et al. 2005; Callus et al. 2006; Praskova et al. 2008). The

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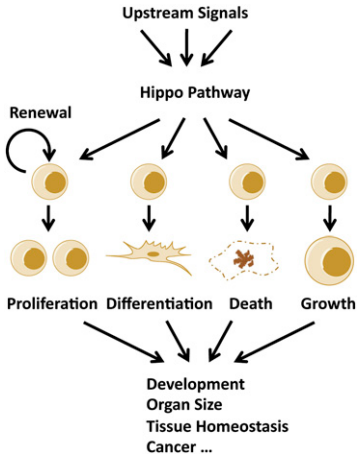


**Figure 1.** Implications of the Hippo pathway in cell biology. The Hippo pathway modulates cell proliferation, differentiation, growth, and death. The coordination of different cellular processes by the Hippo pathway may contribute to diverse physiological and pathological conditions such as development, tissue homeostasis, and tumorigenesis.

kinase activity of MST1/2 is enhanced through interaction with Sav1, which is mediated by SARAH (Sav/Rassf/Hpo) domains present in both MST1/2 and Sav1 (Callus et al. 2006). In addition, the thousand-and-one (TAO) amino acids kinase or TAOK1–3 has been shown to directly phosphorylate and activate Hpo or MST1/2 (Boggiano et al. 2011; Poon et al. 2011). In *Drosophila*, RASSF competes with Sav for Hpo and recruits a PP2A complex (dSTRIPAK) to dephosphorylate and inactivate Hpo (Polesello et al. 2006; Ribeiro et al. 2010). However, multiple RASSF isoforms in mammals showed different roles on the Hippo pathway (Praskova et al. 2004; Ikeda et al. 2009), suggesting a divergent role through evolution.

MST1/2 directly phosphorylate Lats1/2 at the hydrophobic motif (Lats1 T1079 and Lats2 T1041), and this phosphorylation is required for Lats1/2 activation (Chan et al. 2005). Mob1, when phosphorylated by MST1/2, binds to the autoinhibitory motif in Lats1/2, which in turn leads to the phosphorylation of the Lats activation loop (Lats1 S909 and Lats2 S872) and thereby an increase of their kinase activity (Chan et al. 2005; Praskova et al. 2008). Sav1 may function as a bridge to bring MST1/2 and Lats1/2 together (Tapon et al. 2002; Callus et al. 2006) and may enhance or inhibit the activity of Lats1/2 upon phosphorylation by MST1/2 or salt-inducible kinases, respectively (Callus et al. 2006; Wehr et al. 2012). The requirement for MST1/2 to activate Lats1/2 might be cell type-dependent. For instance, MST1/2 knockout in mouse livers does not significantly affect Lats1/2 phosphorylation (Zhou et al. 2009), suggesting that additional kinases may regulate Lats1/2 activity. In addition to protein phosphorylation, the protein levels of Lats1/2 kinases are controlled by Itch E3 ubiquitin ligase-mediated degradation (Ho et al. 2011).

Lats1/2 directly interact with and phosphorylate YAP/TAZ (Huang et al. 2005; Dong et al. 2007; Zhao et al. 2007; Lei et al. 2008; Oh and Irvine 2008), in which the interaction may be mediated by PPxY motifs on Lats1/2 and WW domains on YAP/TAZ (Hao et al. 2008; Oka et al. 2008). Lats1/2 are AGC family kinases and recognize the substrate consensus sequence HXRXXS (Zhao et al. 2007). All five HXRXXS sites on YAP are directly phosphorylated by Lats1/2 (Zhao et al. 2010b). The phosphorylated form of YAP is sequestered in the cytoplasm via a 14-3-3 interaction, resulting in inhibition of target gene transcription (Zhao et al. 2007). Also, TAZ and Yki are phosphorylated by Lats1/2 or Wts, respectively, on multiple HXRXXS sites (Kanai et al. 2000; Dong et al. 2007; Lei et al. 2008; Oh and Irvine 2008; Ren et al. 2010b). In contrast, when upstream kinases are inactive, Yki/YAP/TAZ will be hypophosphorylated and translocate into the nucleus to exert their functions on gene expression (Kanai et al. 2000; Dong et al. 2007; Zhao et al. 2007; Lei et al. 2008; Oh and Irvine 2008; Ren et al. 2010b). The phosphorylation status of YAP/TAZ also regulates their protein stability. Phosphorylation of YAP (S381) and TAZ (S311) by Lats1/2 primes subsequent phosphorylation events by casein kinase 1 (CK1δ/ε); this sequential phosphorylation results in recruitment of β-transducin repeat-containing proteins (β-TRCP; a subunit of the SCF ubiquitin E3 ligase) and consequently leads to degradation of YAP/TAZ (CY Liu et al. 2010; Zhao et al. 2010b). Therefore, by affecting YAP/TAZ protein localization and stability, phosphorylation by upstream kinases represents a central regulatory mechanism for YAP/TAZ (Fig. 2).

YAP/TAZ do not contain intrinsic DNA-binding domains but instead bind to the promoters of target genes

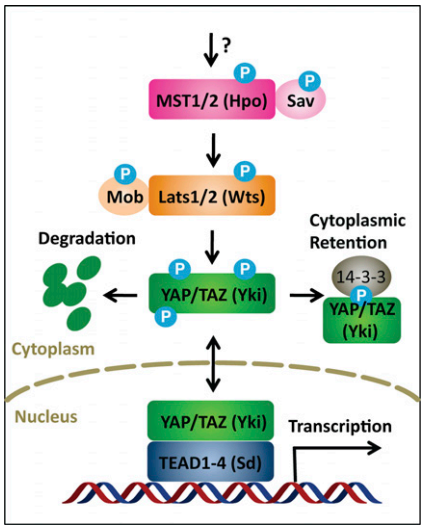


**Figure 2.** The core Hippo pathway. MST1/2 phosphorylates Sav, Lats1/2, and Mob; Lats1/2 phosphorylates YAP/TAZ; and phosphorylated YAP/TAZ interacts with 14-3-3 and results in cytoplasmic retention. Moreover, YAP/TAZ phosphorylation leads to protein degradation. When dephosphorylated, YAP/TAZ enter nuclei and induce gene transcription by interacting with transcription factors TEAD1–4. *Drosophila* orthologs for these core components are shown in brackets.

**Table 1.** *Hippo pathway components in mammals and* Drosophila

| Mammalian | *Drosophila* | Junctional localization | Cytoskeleton interaction |
|---|---|---|---|
| Core components | | | |
| MST1/2 | Hpo (Hippo) | | ✓ |
| Sav1 | Sav (Salvador) | | |
| Lats1/2 | Wts (Warts) | | ✓ |
| Mob1 (a and b) | Mats | | |
| YAP/TAZ | Yki (Yorki) | ✓ | |
| TEAD1–4 | Sd (Scalloped) | | |
| Apical–basal polarity (TJs and AJs) | | | |
| Crb1–3 | Crb (Crumbs) | ✓ | |
| Frmd6 (?) | Ex (Expanded) | ✓ | ✓ |
| NF2 (Mer) | Mer (Merlin) | ✓ | ✓ |
| Kibra | Kibra | ✓ | |
| aPKC | aPKC | ✓ | |
| PAR3 | Baz (Bazooka) | ✓ | |
| PAR6 | Par6 | ✓ | |
| PALS1 | Sdt (Stardust) | ✓ | |
| Scrib | Scrib (Scribble) | | |
| Dlg | Dlg | | |
| Lgl | Lgl (Discs large) | | |
| AMOT (angiomotin) | ? | ✓ | ✓ |
| PTPN14 | Pez | ✓ | ✓ |
| Ajuba/LIMD1/WTIP | Jub | ✓ | ✓ |
| α-Catenin | α-Catenin | ✓ | ✓ |
| β-Catenin | β-Catenin | ✓ | |
| ZO-1 | ZO-1 | ✓ | |
| ZO-2 | ZO-2 | | |
| E-cad (E-cadherin) | E-cad | ✓ | |
| Planar cell polarity | | | |
| Fat1-4 | Fat | ✓ | |
| Dchs1/2 | Ds (Dachsous) | ✓ | ✓? |
| Fjx1 | Fj (four-jointed) | | |
| ? | Dachs | | |
| Zyxin/Lpp/Trip6 | Zyx (zyxin) | | ✓ |
| Lix1, Lix1L | Lft (lowfat) | | |
| CK1δ/ε | Dco (Discs overgrowth) | | |
| ZDHHCs | App (approximated) | | |
| Other components | | | |
| Taok1-3 | Tao | | ✓ |
| RASSF1-6 | RASSF | | ✓ |
| PP2A (STRIPAK) | STRIPAK (PP2A) | | ✓? |
| PP1 | PP1 | | ✓ |
| Itch | Su(DX) | | |
| βTRCP | Slimb | | |
| 14-3-3 | 14-3-3 | | ✓ |

Mammalian Hippo pathway components and their *Drosophila* orthologs are summarized. Check marks indicate localization to tight junctions (TJs), adherens junctions (AJs), or actin cytoskeleton. Question marks indicate unsure information.

by interacting with DNA-binding transcription factors. YAP/TAZ mainly bind to the transcription factors TEAD1–4 to regulate genes involved in cell proliferation and cell death (Vassilev et al. 2001; Goulev et al. 2008; Wu et al. 2008; Zhang et al. 2008; Zhao et al. 2008). Besides TEADs, YAP/TAZ may also interact with other transcription factors, such as Smad1 (Alarcon et al. 2009), Smad2/3 (Varelas et al. 2008), Smad7 (Ferrigno et al. 2002), RUNX1/2 (Yagi et al. 1999), p63/p73 (Strano et al. 2001), and ErbB4 (Komuro et al. 2003; Omerovic et al. 2004); these interactions may mediate transcription of diverse genes involved in proliferation, differentiation, and development.

## Apical–basal polarity: the polarized localization of hippo components

Epithelial cells usually adhere to one another through cell–cell junctions such as adherens junctions (AJs), desmosomes, and tight junctions (TJs). TJs and AJs, with help from different polarity complexes, divide the plasma membrane into an apical domain and a basolateral domain and thereby establish an apical–basal polarity in epithelial cells (Martin-Belmonte and Perez-Moreno 2012). Interestingly, many upstream regulators identified for the Hippo pathway are known components of TJs, AJs, or apical–basal polarity protein complexes (Fig. 3A).

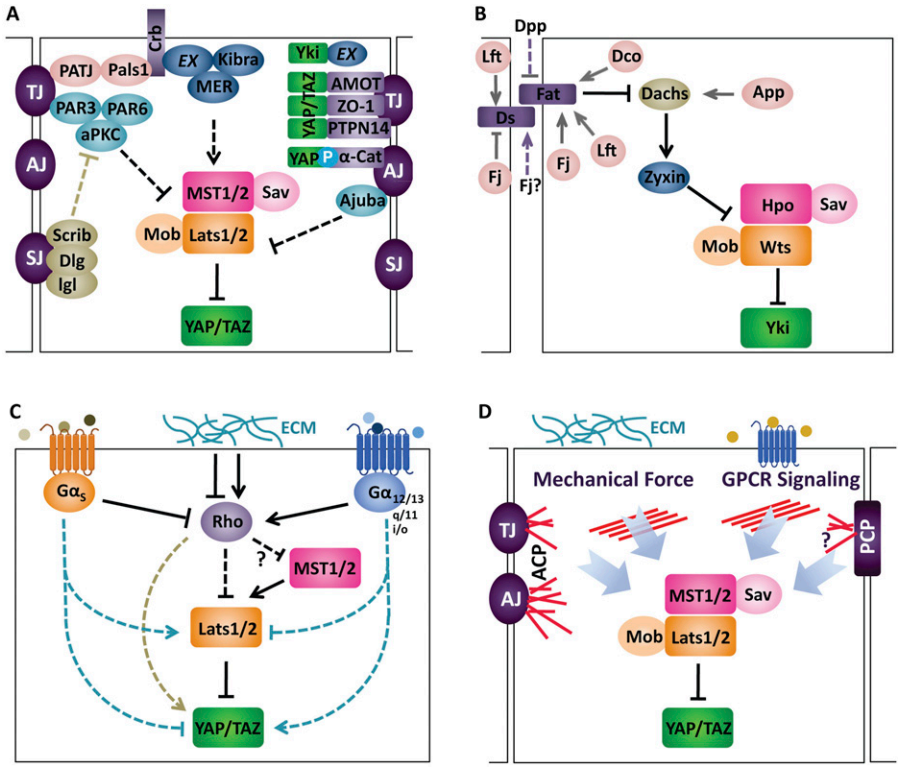


**Figure 3.** Regulatory mechanisms for the Hippo pathway. Regulation of the Hippo pathway by apical–basal polarity (*A*), PCP (*B*), mechanical cues and GPCR signaling (*C*), and actin cytoskeleton (*D*). Arrowed or blunted ends indicate activation or inhibition, respectively. Dashed lines indicate indirect or unknown mechanisms. Red lines in *D* represent actin filaments.

Mer (Merlin; also known as NF2 for neurofibromatosis-2) and Ex (Expanded) are two proteins that belong to the FERM (4.1, Ezrin, Radxin, and Moesin) domain-containing family of proteins. Both Mer and Ex have tumor suppressor functions and work together to regulate cell proliferation and differentiation (McCartney et al. 2000). In *Drosophila*, genetic inactivation of both Mer and Ex revealed a dramatic overgrowth phenotype similar to that of the Hpo mutants (Hamaratoglu et al. 2006). Later, Kibra (a WW and C2 domain-containing protein) was identified to physically interact with Mer and Ex, and these three proteins activate Wts in a cooperative manner (Baumgartner et al. 2010; Genevet et al. 2010; Yu et al. 2010).

Mer, Ex, and Kibra colocalize at the apical domain of polarized epithelial cells (Fig. 3A; Boedigheimer and Laughon 1993; Boedigheimer et al. 1997; Yu et al. 2010). Mer and Ex have been considered as a linker for the apical plasma membrane and actin cytoskeleton (Bretscher et al. 2002). Kibra contains a C2 domain that interacts with phospholipids and may target interacting proteins to the cell surface (Kremerskothen et al. 2003). Sav and Hpo physically associate with Mer and Ex (Yu et al. 2010), and Kibra interacts with Wts (Genevet et al. 2010), suggesting that the Mer/Ex/Kibra complex may recruit the Hippo pathway kinases to the apical plasma membrane for activation. Indeed, it has been shown that Mats is activated at the plasma membrane (Ho et al. 2010), and targeting MST1 to the plasma membrane by adding a myristoylation signal enhances MST1 kinase activity (Khokhlatchev et al. 2002).

*Drosophila* crumbs (Crb) has been identified as a cell surface regulator for the Hippo pathway (Fig. 3A; CL Chen et al. 2010; Grzeschik et al. 2010; Ling et al. 2010; Robinson et al. 2010). In *Drosophila* embryos, Crb is localized at the subapical plasma membrane and plays an important role in organizing apical–basal polarity (Tepass et al. 1990). As a transmembrane protein, Crb has a large extracellular domain and a short intracellular domain. The short intracellular domain contains a FERM-binding motif (FBM) that can interact with Ex, and this interaction modulates Ex localization and stability, which in turn regulates the activity of Hippo pathway kinases and Yki (CL Chen et al. 2010; Ling et al. 2010; Robinson et al. 2010). The connection of Crb with Ex and core components of the Hippo pathway is also reflected by an overgrowth phenotype corresponding to Crb deficiency (Ling et al. 2010). Interestingly, overexpression of Crb leads to Ex mislocalization and inactivation of the Hippo pathway, likely due to a dominant-negative effect of overexpressed Crb (CL Chen et al. 2010; Grzeschik et al. 2010; Robinson et al. 2010).

Similar to the Crb complex, the Par apical complex also regulates the Hippo pathway (Fig. 3A). Overexpression of aPKC, a component of the Par complex, can induce Yki activity and tissue overgrowth (Grzeschik et al. 2010; Sun and Irvine 2011). The activity of the Par complex is

antagonized by the basal Scrib (Scribble) complex (Martin-Belmonte and Perez-Moreno 2012). Indeed, depletion of Scrib or Lgl also resulted in activation of Yki (Grzeschik et al. 2010; Menendez et al. 2010; Sun and Irvine 2011).

The regulation of apical–basal polarity on the Hippo pathway is largely conserved in mammals. NF2 is an extensively studied tumor suppressor, and mutations in the *NF2* gene cause the development of nonmalignant brain tumors, a syndrome called neurofibromatosis type 2. Mice with conditional *NF2* knockout in the liver develop hepatocellular carcinoma, cholangiocarcinoma, and bile duct hamartomas (Benhamouche et al. 2010; Zhang et al. 2010). NF2 patients usually develop cataracts; mice with conditional *NF2* knockout in lens epithelium also develop cataracts (Zhang et al. 2010). Interestingly, the phenotypes of *NF2* knockout in the liver and eye were largely blocked by heterozygous deletion of *Yap* (Zhang et al. 2010). In addition, overexpression of NF2 in mammalian cells results in Lats activation and YAP inhibition (Zhao et al. 2007; Zhang et al. 2010). These results suggest that Mer is also an upstream component of the mammalian Hippo pathway.

Disruption of TJs or AJs in cultured mammalian cells (by depletion of extracellular calcium or knockdown of Crb3 or PALS1) causes induction of YAP/TAZ nuclear localization and target gene expression (Varelas et al. 2010). Moreover, Scrib also positively regulates the Hippo pathway kinases, and down-regulation of Scrib leads to YAP/TAZ activation (Cordenonsi et al. 2011; Chen et al. 2012). In addition, many cell junction proteins, such as LIN7C, PATJ, MPDZ, PTPN14, angiomotin (AMOT), and α-catenin, have also been identified as interacting partners of core Hippo pathway components (Fig. 3A; Varelas et al. 2010; Wang et al. 2011; KL Guan, unpubl.).

AMOT proteins, a family of proteins including AMOT, AMOTL1, and AMOTL2, interact extensively with multiple TJ components and are important for maintaining TJ integrity and epithelial cell polarity (Wells et al. 2006). Recently, an interaction between AMOT and YAP/TAZ has been identified (Chan et al. 2011; Wang et al. 2011; Zhao et al. 2011). The AMOT–YAP/TAZ interaction is not dependent on the YAP/TAZ phosphorylation status and is instead mediated by AMOT PPxY motifs and YAP/TAZ WW domains (Chan et al. 2011; Wang et al. 2011; Zhao et al. 2011). AMOT proteins recruit YAP/TAZ to TJs or the actin cytoskeleton, which consequently results in reduced YAP/TAZ nuclear localization and activity (Zhao et al. 2011). In addition, AMOT proteins also induce YAP/TAZ phosphorylation at Lats target sites (Zhao et al. 2011); this might be due to a scaffolding function of AMOT on Hippo pathway components such as MST2, Lats2, and YAP (Paramasivam et al. 2011). AMOT proteins can therefore inhibit YAP/TAZ activity by both phosphorylation-dependent and phosphorylation-independent mechanisms. Interestingly, AMOT has been shown to interact with NF2 and is required for tumorigenesis caused by NF2 deficiency (Yi et al. 2011). An ortholog of AMOT in *Drosophila* has not been identified, suggesting that regulation of AMOT on the Hippo pathway may be different between *Drosophila* and mammals.

α-Catenin is a component of AJs that functions as a linker for membrane cadherins and the actin cytoskeleton (Drees et al. 2005). An inhibitory role of α-catenin on YAP activity has also been reported, and this inhibition of YAP may contribute to the tumor suppressor function of α-catenin (Fig. 3A; Schlegelmilch et al. 2011; Silvis et al. 2011). In keratinocytes, YAP strongly interacts with α-catenin, and this interaction is mediated by 14-3-3. Unlike AMOT, the phosphorylation of YAP at S127 is required for interaction with α-catenin because 14-3-3 only binds to phosphorylated YAP (Schlegelmilch et al. 2011). The trimeric complex of α-catenin, 14-3-3, and YAP sequesters YAP at AJs and prevents YAP dephosphorylation/activation. In mammary epithelial Eph4 cells, knockdown of α-catenin also induces YAP/TAZ nuclear localization (Varelas et al. 2010), suggesting that the regulation of YAP/TAZ by α-catenin is present in a variety of cell types.

Another AJ component, protein tyrosine phosphatase 14 (PTPN14), has also been shown to be a regulator of the Hippo pathway (JM Huang et al. 2012; Liu et al. 2012; Wang et al. 2012). PTPN14 can directly interact with YAP, and this interaction is mediated by PPxY motifs of PTPN14 and WW domains of YAP (JM Huang et al. 2012; Liu et al. 2012; Wang et al. 2012). PTPN14 also contains an N-terminal FERM domain, and the overall domain organization is similar to that of Ex in *Drosophila*. Moreover, Pez (the *Drosophila* ortholog of PTPN14) has been shown to interact with Kibra and inactivate Yki (Poernbacher et al. 2012). PTPN14–YAP interaction results in cytoplasmic localization of YAP and decreased YAP activity; however, there are contradictory data on the role of the tyrosine phosphatase activity of PTPN14 on YAP (JM Huang et al. 2012; Liu et al. 2012; Wang et al. 2012).

Several other proteins important in establishing or maintaining apical–basal polarity have been shown to modulate the Hippo pathway. In mammalian cells, cell adhesion mediated by homophilic binding of E-cadherin led to YAP inactivation (Kim et al. 2011). Ajuba can interact with Sav and Lats kinases in mammalian cells and *Drosophila* and exhibits an inhibitory effect on YAP/Yki (Das Thakur et al. 2010). LKB1 (liver kinase B1) is able to induce YAP phosphorylation (Nguyen et al. 2012). NPHP4 (nephronophthisis 4) can interact with and inhibit Lats1 (Habbig et al. 2011). ZO-2 (zona occludens-2) can induce YAP nuclear localization (Oka et al. 2010), whereas ZO-1 has been shown to repress TAZ activation (Remue et al. 2010). These results indicate that cell–cell contact, integrity of cell junctions, and apical–basal polarity are important in regulation of the Hippo pathway. Apical–basal polarity can regulate the Hippo pathway by either recruiting the Hippo pathway kinases to the apical domain for activation or sequestering Yki/YAP/TAZ at cell junctions (Fig. 3A), both resulting in inactivation of YKi/YAP/TAZ. However, it is worth noting that the cellular localization of YAP is mainly in the cytoplasm and nucleus (Zhao et al. 2011); the interaction between cell junctional proteins and YAP/TAZ may not result in

a predominant localization of YAP/TAZ at the cellular apical domain.

### Planar cell polarity (PCP): coordinates for the Hippo pathway

Epithelial cells are also polarized along an axis perpendicular to the apical–basal axis, in which clustered cells within an epithelial plate are coordinated, aligned, and orientated to the same direction, and this cell polarity is termed PCP (Simons and Mlodzik 2008). In addition to epithelial cells, PCP is also present in many other cell types, such as mesenchymal cells, and is important in cell migration and cell interchalation (Simons and Mlodzik 2008). Two molecular networks are critical in establishing PCP: One is the Frizzled/Flamingo (Fzi/Fmi) system, and the other is the Fat/Dachsous (Ft/Ds) system (Simons and Mlodzik 2008). The Ft/Ds PCP system has been shown to regulate the Hippo pathway in *Drosophila* (Fig. 3B).

Ft is a tumor suppressor and affects tissue growth (Mahoney et al. 1991). Loss of Ft results in activation of Yki by inactivating either Ex or Wts (Bennett and Harvey 2006; Cho et al. 2006; Silva et al. 2006; Willecke et al. 2006; Feng and Irvine 2007; Tyler and Baker 2007). Ft and Ds are both atypical cadherins, which form intercellular heterodimers (Cho and Irvine 2004; Matakatsu and Blair 2004), and this dimerization is regulated by Fj (four-jointed)-mediated phosphorylation (Ishikawa et al. 2008). Ds and Fj show gradient expression with opposite directions in many tissues, and this expression pattern might be critical for Ft activity (Rogulja et al. 2008; Willecke et al. 2008; Zecca and Struhl 2010). A sharp Ds gradient may inhibit Ft activity, which leads to localization of atypical myosin Dachs to subapical regions (Cho et al. 2006; Feng and Irvine 2007). Polarized Dachs promotes interaction between Zyxin and Wts, which in turn leads to Wts degradation (Fig. 3B; Rauskolb et al. 2011). Several proteins have been reported to modulate the inhibitory effect of Ft on Yki. Dco (discs overgrown), a CK1 homolog, is able to phosphorylate the intracellular domain of Ft and induce Ft activity (Sopko et al. 2009). App (approximated), a palmitoyltransferase, can relieve the Ft inhibition on Dachs and promote its apical localization (Matakatsu and Blair 2008). Lft (lowfat) can bind to Ft and Ds and thereby increases their protein stability (Mao et al. 2009).

The effect of the Ft/Ds PCP system on the Hippo pathway may be modulated by different morphogens. Dpp (decapentaplegic; a BMP homolog) and Wingless (a Wnt homolog) were shown to regulate the expression of Ds and Fj (Rogulja et al. 2008; Zecca and Struhl 2010), suggesting that these morphogens may help in establishing the gradient of Ds and Fj. In addition, Fj is secreted and may function as a morphogen to regulate Ft/Ds phosphorylation (Ishikawa et al. 2008; Tagliabracci et al. 2012).

Our understanding of the Ft/Ds PCP system in the mammalian Hippo pathway is limited. There are two Ds orthologs (Hchs1–2) and four Ft orthologs (Fat1–4) in mammals. Among the four Fat genes in vertebrates, Fat4 has the highest homology with *Drosophila* Ft. However, defects in YAP and Lats1 have not been observed in Dchs1 and Fat4 knockout mice with abnormal PCP (Mao et al. 2011). In zebrafish, Fat1 depletion was shown to activate YAP in a Scrib-dependent manner (Skouloudaki et al. 2009). In mammals, an obvious Dachs ortholog is lacking, suggesting that the connection between the Ft/Ds system and the Hippo pathway may not be conserved in mammals. The effect of the Fzi/Fmi PCP system on the Hippo pathway is less well understood. Recently, it has been shown that overexpression of Frizzled 4 can activate YAP and that wnt signaling can activate TAZ in mammalian cells (Azzolin et al. 2012; W Huang et al. 2012; Yu et al. 2012b), suggesting that the Fzi/Fmi system may also regulate the Hippo pathway.

### G-protein-coupled receptor (GPCR) signaling: sensing diffusible signals

A large number of growth factors regulate cell proliferation by activating membrane receptors and intracellular signaling pathways. It is reasonable to speculate that the YAP/TAZ oncoproteins are regulated by growth factors. However, several well-known growth factors, such as insulin and EGF, have no significant effect on YAP phosphorylation (Zhao et al. 2007; Yu et al. 2012b). In mammals, the potential identity of extracellular ligands and their cognate receptors that regulate the Hippo pathway remained elusive until recently. Two independent groups have reported that serum could rapidly activate YAP/TAZ in cultured cells. By extensive biochemical analysis, LPA and S1P were identified as the major components in serum responsible for YAP/TAZ activation (Miller et al. 2012; Yu et al. 2012b). Both reports showed that LPA or S1P bound to their corresponding membrane GPCRs and act through Rho GTPases to activate YAP/TAZ. Consistently, another report showed that thrombin, which activates protease-activated receptors (PARs; a GPCR), also stimulated YAP/TAZ activity via Rho GTPases (Mo et al. 2012). These results suggest that YAP/TAZ can be regulated by diffusible extracellular signals and cell surface receptors (Fig. 3C).

Yu et al. (2012b) further showed that YAP/TAZ is robustly regulated by many GPCRs and their cognate ligands and established a general function of GPCR in YAP/TAZ regulation. GPCRs usually activate downstream signaling through heterotrimeric G proteins. $G\alpha_{12/13}$-, $G\alpha_{q/11}$-, or $G\alpha_{i/o}$-coupled signals induce YAP/TAZ activity, whereas $G\alpha_s$-coupled signals repress YAP/TAZ activity (Fig. 3C). In the latter scenario, glucagon, epinephrine, and a dopamine receptor agonist induce YAP/TAZ phosphorylation. Interestingly, in a screen for YAP inhibitors, dobutamine has been shown to inhibit YAP (Bao et al. 2011). Dobutamine is an agonist for the β1 adrenergic receptor, which likely inhibits YAP by activating $G\alpha_s$. These results suggest that the activity of YAP/TAZ can be either up-regulated or down-regulated by GPCR signaling, depending on which Gα protein is activated. How upstream G-protein signals are transmitted to the Hippo pathway is not fully understood, it is likely that these signals regulate YAP/TAZ by modulating the actin cytoskeleton (also see below).

GPCR represents the largest family of plasma membrane receptors that can be activated or inactivated by a wide range of physiological ligands or pharmaceutical drugs (Lappano and Maggiolini 2011). Therefore, YAP/TAZ activity might be fine-tuned by multiple GPCR signals in a given cellular environment (Fig. 3C). Since most extracellular signals identified in these studies are hormonal factors, it is possible that cells adjacent to blood vessels will be preferentially regulated. Interestingly, YAP activation has been observed at perivascular regions (Fernandez et al. 2009). YAP/TAZ could mediate the physiological functions of LPA or thrombin in inducing gene expression, cell migration, and/or cell proliferation, and YAP/TAZ are activated in breast cancer induced by transgenic expression of LPA receptor in mice (Mo et al. 2012; Yu et al. 2012b). As a downstream branch of GPCR signaling, the Hippo pathway may mediate many biological functions of GPCRs, particularly those related to cell proliferation, cell survival, and tissue growth (Yu et al. 2012a; also see below).

### Extracellular matrix (ECM) and cytoskeleton: sensing mechanical cues

In vivo, cells are experiencing extensive mechanical signals from neighboring cells, the ECM, and surrounding biological fluids. In addition, cell shape and geometry also generate mechanical forces. Cells are able to sense and adapt to these mechanical signals and may undergo proliferation, differentiation, apoptosis, and migration (Vogel and Sheetz 2006). The cellular cytoskeleton can respond to and integrate extracellular mechanical signals. For instance, actin filaments (F-actin) have been considered as important regulators of cell proliferation (Provenzano and Keely 2011), and the dynamics of microtubules are essential for cell division (Sorger et al. 1997). Mechanotransduction is involved in not only normal physiology, but also tumorigenesis and cancer metastasis (Huang and Ingber 2005). Both microtubules and F-actin are critical for cancer development and are targets of cancer therapies (Jordan and Wilson 1998).

Three recent studies have shown that YAP/TAZ is regulated by mechanical cues in mammalian cells (Fig. 3C; Dupont et al. 2011; Wada et al. 2011; Zhao et al. 2012). Dupont et al. (2011) and Wada et al. (2011) showed that YAP/TAZ was regulated by cell geometry, with active YAP/TAZ present in cells that have undergone cell spreading, and inactive YAP/TAZ found in round and compact cells. Dupont et al. (2011) also showed that YAP/TAZ could respond to the stiffness of the ECM, with active YAP (YAP predominantly in the nucleus) in cells seeded on stiff surfaces, and inactive YAP/TAZ in cells seeded on soft surfaces. Zhao et al. (2012) demonstrated that cell detachment and attachment could either repress or induce YAP/TAZ activity, respectively, and inhibition of YAP/TAZ was involved in cell detachment-induced anoikis (a specific type of apoptosis). All three studies revealed that the rearrangement of actin cytoskeleton in response to different mechanical cues is associated with changes of YAP/TAZ activity. Dupont et al. (2011) and Zhao et al. (2012) also revealed that RhoA strongly enhances YAP/TAZ activity. Moreover, Rac and Cdc42 might also regulate YAP/TAZ activity, although less potently when compared with RhoA (Zhao et al. 2012). These results are consistent with a general role of Rho GTPases in regulating dynamics of the actin cytoskeleton and promoting cell proliferation (Jaffe and Hall 2005). In addition to actin cytoskeleton, microtubules have been shown to regulate YAP/TAZ phosphorylation (Zhao et al. 2012), indicating that the Hippo pathway might be regulated by different types of cellular cytoskeleton.

The regulation of the Hippo pathway by GPCR signaling and mechanical cues strongly suggests that the actin cytoskeleton may function as a mediator and integrator of various upstream signals to the Hippo pathway. Indeed, two studies in *Drosophila* have revealed a link between the actin cytoskeleton and the Hippo pathway (Fernandez et al. 2011; Sansores-Garcia et al. 2011). Depletion of actin-capping protein, which inhibits actin polymerization, resulted in Yki activation and tissue outgrowth. Similar phenotypes were observed when Capulet (an actin-binding protein that inhibits polymerization) was inactivated or a constitutively active Diaphanous (Dia; induces actin polymerization) was overexpressed. All of these manipulations led to an increase in F-actin, suggesting that F-actin can induce Yki activity. Overexpression of Wts, but not Ex or Hpo, significantly reversed the phenotype of constitutively active Dia, indicating that the effect of F-actin on Yki is mediated by Wts. In mice, knockout of destrin, which is an actin-depolymerizing factor, resulted in an abnormal actin cytoskeleton and accelerated proliferation of corneal epithelial cells (Ikeda et al. 2003), suggesting that the role of F-actin in regulating YAP/TAZ activity might be conserved in mammals.

Dupont et al. (2011) also showed that YAP/TAZ could sense cellular tension. Actomyosin (composed of myosin and actin) is present in bundles in nonmuscle cells, where it can generate contraction and tension following RhoA activation (Clark et al. 2007). When cells were treated with the nonmuscle myosin inhibitor blebbistatin, Rho kinase inhibitor Y27632, or myosin light chain kinase inhibitor ML-7, nuclear YAP/TAZ localization was reduced (Dupont et al. 2011; Wada et al. 2011). Y27632 had also been shown to block the effect of S1P on YAP/TAZ (Miller et al. 2012). However, in other reports, these drugs were unable to block the effect of LPA-induced, S1P-induced, thrombin-induced, and cell attachment-induced YAP activity (Mo et al. 2012; Yu et al. 2012b; Zhao et al. 2012). These discrepancies might be due to different experimental settings, such as cell lines and conditions of treatment. However, it is also possible that additional signaling pathways evoked by these mechanical or diffusible signals are involved in the regulation of YAP/TAZ activity (Fig. 3C). Therefore, further investigation is required to clarify whether cellular tension is responsible for YAP/TAZ regulation.

It is still unclear how the actin cytoskeleton transmits upstream signals to the Hippo pathway. Both MST1/2 and Lats1 have been shown to bind or colocalize with F-actin (Densham et al. 2009; Visser-Grieve et al. 2011), suggesting

a model in which F-actin may directly regulate the activity of Hippo pathway kinases. In mammalian cells, the kinase activity of Lats1 and the phosphorylation status at the hydrophobic motif and activation loop of Lats1/2 are clearly sensitive to GPCR signaling and cell detachment; however, the phosphorylation and in vitro kinase activity of MST1/2 are not significantly regulated by these upstream signals (Mo et al. 2012; Yu et al. 2012b; Zhao et al. 2012), indicating that MST1/2 are not direct targets of these upstream signals. On the other hand, Lats kinases were required for YAP/TAZ regulation by GPCR signaling, cell attachment, and cell geometry (Wada et al. 2011; Mo et al. 2012; Yu et al. 2012b; Zhao et al. 2012). Meanwhile, actin cytoskeleton may also regulate YAP/TAZ phosphorylation via a Lats-independent mechanism (Dupont et al. 2011; Miller et al. 2012). In addition, the role of F-actin in Hippo pathway regulation might be indirect. F-actin may function as a platform and facilitate signal transmission between upstream regulators and core Hippo pathway components. Protein kinases or phosphatases downstream from G proteins and Rho GTPases may regulate Hippo pathway components in an actin cytoskeleton-dependent manner (Fig. 3C). Clearly, the mechanism linking F-actin to the Hippo pathway is one of the most important questions yet to be answered in the field.

As mentioned above, the Hippo pathway is regulated by apical–basal polarity in epithelial cells, PCP, mechanical cues, and GPCR signaling. One common feature of these regulatory mechanisms is the involvement of actin cytoskeleton (Fig. 3D). The integration of multiple upstream signals to the Hippo pathway by F-actin may explain the YAP/TAZ activity regulation under diverse conditions. Actin dynamics is tightly regulated by Rho GTPases when cells are experiencing mechanical forces or stimulation by extracellular ligands (Sah et al. 2000; Vogel and Sheetz 2006). It is well known that some GPCR ligands, such as LPA, S1P, and thrombin, also induce contractive actin bundles in cells (Miller et al. 2012; Mo et al. 2012; Yu et al. 2012b). On the other hand, some ligands for $G\alpha_s$-coupled receptors, such as vasopressin, dopamine, and parathyroid hormone, have been shown to counteract the formation of actin bundles (Ding et al. 1991; Egan et al. 1991b; Roma et al. 1998; Nguyen et al. 1999; Zhang et al. 2006). Mechanical cues such as cell spreading, cell geometry, and matrix stiffness all result in rearrangement of the actin cytoskeleton (Dupont et al. 2011; Wada et al. 2011; Zhao et al. 2012). In cultured osteoblasts, the cross-linked actin and myosin are reduced when cells are plated at high density (Egan et al. 1991a), indicating that cellular F-actin level is cell density-dependent, which may provide a mechanism for cell density-dependent YAP phosphorylation (Zhao et al. 2007).

In epithelial cells, the actin cytoskeleton is more complex due to polarization and cell–cell junctions. There are apical or basal actin networks, and also connected actin cables that span multiple pairs of cells (Baum and Georgiou 2011). Some actin bundles are linked to apical junctions via adaptor proteins such as catenins, NF2, and AMOT, and these physical connections will create continuity between actin cytoskeletons of neighboring cells (Gjorevski et al. 2012); in addition, these adaptor proteins may regulate activity of cdc42 and PAK1-Rac, thereby modulating the apical actin dynamics (Kissil et al. 2003; Perez-Moreno and Fuchs 2006; Wells et al. 2006). Therefore, it is possible that the phenotypes of NF2, AMOT, α-catenin, and β-catenin deficiency on Yki/YAP/TAZ activity and cell proliferation are mediated by local actin rearrangements. In keratinocytes and mammary epithelial cells, calcium depletion led to disruption of cell junctions and YAP/TAZ nuclear enrichment (Varelas et al. 2010; Schlegelmilch et al. 2011). Calcium-chelating agents may regulate intracellular actin dynamics, as disruption of cell junctions by calcium depletion resulted in an increase of actomyosin contraction in bovine corneal endothelial cells (Ramachandran and Srinivas 2010). However, it is also possible that the effect of actin rearrangements on the Hippo pathway is mediated by other cellular processes; for instance, the actin cytoskeleton is required for the establishment of cell polarity (Li and Gundersen 2008).

Actin cytoskeleton may also mediate the effect of PCP on the Hippo pathway. Dachs in the Fat/Ds system is a myosin-like protein, which may directly or indirectly affect actin dynamics (Rauskolb et al. 2011). In addition, RhoA is a well-known downstream player of the non-canonical wnt pathway (Habas et al. 2001); thus Fzi/Fmi may regulate YAP/TAZ activity via RhoA and actin organization. Collectively, actin dynamics emerges as a central mediator for YAP/TAZ regulation by a wide range of stimuli (Fig. 3D).

## Regulation of the Hippo pathway: implications in physiological and pathological conditions

Recently, the Hippo pathway has been shown to regulate the functions of stem cells. YAP and TAZ are required for the maintenance of mouse and human embryonic stem cell pluripotency, respectively (Varelas et al. 2008; Alarcon et al. 2009; Lian et al. 2010; Qin et al. 2012). In transgenic or knockout mice, elevated YAP/TAZ activity leads to an expansion of tissue-specific stem cells and the blockage of cell differentiation (Camargo et al. 2007; Cao et al. 2008; Lee et al. 2008, 2010; Zhou et al. 2009, 2011; Benhamouche et al. 2010; Lu et al. 2010; Song et al. 2010; Schlegelmilch et al. 2011; Zhang et al. 2011). YAP/TAZ are important for mesenchymal stem cell (MSC) differentiation; knockdown of YAP/TAZ in MSCs decreases osteogenesis and increases adipogenesis (Hong et al. 2005; Dupont et al. 2011). In addition, YAP/TAZ have been shown to be important in myogenesis (Jeong et al. 2010; Watt et al. 2010; Judson et al. 2012). These results suggest that the Hippo pathway plays important roles in cell differentiation (Fig. 4A). Both mechanical forces and GPCR ligands contribute to the cell microenvironment. Stiff ECM and LPA have been shown to promote osteogenesis and inhibit adipogenesis (McBeath et al. 2004; YB Liu et al. 2010). On the other hand, soft ECM and cAMP signaling (e.g., IBMX treatment) could induce adipogenesis and repress osteogenesis (McBeath et al. 2004; Yang et al. 2008). Cell density and serum concentration are also critical during cell differentiation; for instance, myogenesis requires

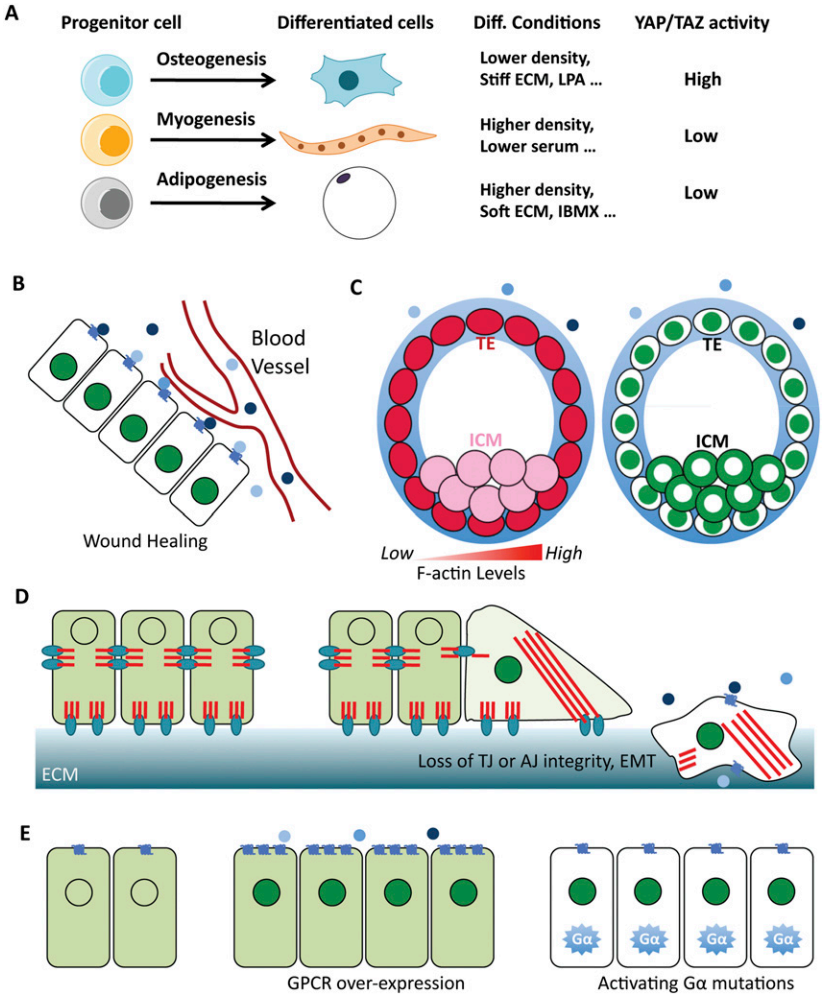


**Figure 4.** YAP/TAZ activity under different physiological and pathological conditions. (*A*) YAP/TAZ activity in cell differentiation. YAP/TAZ activity is regulated by cell density, ECM, and GPCR signaling. (*B*) Cells close to a wound may experience different mechanical forces and a higher concentration of GPCR ligands from blood vessels. This may induce YAP/TAZ activity and promote wound healing or tissue regeneration. (*C*) In a blastocyst, cells in the trophectoderm (TE) and inner cell mass (ICM) have distinct YAP localization and F-actin/tension distribution. (*D*) Disruption of cellular junctions may induce mechanical stress and result in changes in cell morphology, which can enrich YAP/TAZ in nuclei under these conditions. Activated YAP/TAZ may promote epithelial–mesenchymal transition (EMT) and cell migration. Cells that have escaped from epithelium may also encounter more GPCR ligands. Red lines represent actin filaments. (*E*) Aberrant GPCR signaling may activate YAP/TAZ. Elevated GPCR expression or activating Gα mutations may induce YAP/TAZ nuclei localization and activation and result in hyperproliferation that may contribute to cancer development. YAP/TAZ localization is represented by green in *B*–*E*.

higher cell density and lower serum concentration—conditions that favor YAP/TAZ inactivation. Altogether, a variety of microenvironmental signals may determine cell differentiation by regulating YAP/TAZ activity.

The Hippo pathway is also involved in tissue regeneration. In the intestines of mice, YAP protein levels were dramatically increased following dextran sulfate sodium (DSS)-induced injury, and the damaged intestinal epithelium underwent regeneration; however, inactivation of YAP severely impaired regeneration (Cai et al. 2010). Furthermore, Yki is required for tissue regeneration in *Drosophila* midgut and wing discs (Karpowicz et al. 2010; Ren et al. 2010a; Shaw et al. 2010; Staley and Irvine 2010; Grusche et al. 2011; Sun and Irvine 2011). Interestingly, TEAD1 Y406H (tyrosine-to-histidine) mutation abolishes YAP–TEAD interaction (Zhao et al. 2008; L Chen et al. 2010; Li et al. 2010), and this mutation is associated with Sveinsson chorioretinal atrophy (a human degeneration disease with absence of retinal pigment epithelium and additional retinal structures) (Jonasson et al. 2007), suggesting the importance of YAP/TEAD transcriptional activity in tissue growth and homeostasis. Moreover, tissue damage may result in changes of mechanical or biochemical environments, and a local increase of YAP/TAZ activity may facilitate wound healing processes (Fig. 4B). For instance, thrombin is increased during blood clotting, a process directly associated with wounds, and may promote wound healing by activating YAP/TAZ. Moreover, LPA has been shown to facilitate wound closure in a mouse model (Balazs et al. 2001). Therefore, Hippo pathway regulation is important for wound healing and regeneration.

YAP plays important roles in early embryonic development. Systematic knockout of YAP in mice is lethal, and the embryo stops developing at embryonic day 8.5 (E8.5) with defects in the yolk sac, vasculogenesis, chorioallantonic fusion, and body axis elongation (Morin-Kensicki et al. 2006). In a normal blastocyst, YAP shows nuclear localization in the trophectoderm (TE) and cytoplasmic localization in the inner cell mass (ICM), and this distinct distribution of YAP is important for lineage specification in the preimplantation mouse embryo (Fig. 4C; Nishioka et al. 2009). However, the underlying mechanism for this patterned YAP localization is not clear. In blastocysts, the F-actin and myosin staining is strong in TE but not detectable in the ICM, suggesting that contractive actin bundles are abundant in the TE (Slager et al. 1992). The distribution of F-actin is in nice correlation with YAP

localization in blastocysts (Fig. 4C; Nishioka et al. 2009). It is possible that the actin cytoskeleton is the determinant of YAP localization in this context. The distribution of F-actin in blastocysts might be regulated by multiple means: (1) Cells in the ICM are not polarized, whereas cells in the TE are polarized and may have more F-actin and tension. (2) Like in cells growing in high density, cells in the ICM contact one another in all directions and may have less F-actin and tension. (3) The outside of the TE is exposed, and GPCRs that are expressed on the surface might be influenced by maternal hormones, whereas the access of these signals to ICM cells is prevented by the epithelium (TE). It would be interesting to further investigate the relationship between F-actin and YAP localization and the upstream cues during early embryogenesis.

The Hippo pathway is widely recognized as a signaling pathway that regulates organ size. In *Drosophila*, loss-of-function mutants of Hpo, Sav, Wts, and Mob all lead to significant tissue outgrowth, as indicated by enlarged eyes, wings, or other appendages (Justice et al. 1995; Xu et al. 1995; Kango-Singh et al. 2002; Tapon et al. 2002; Harvey et al. 2003; Jia et al. 2003; Pantalacci et al. 2003; Udan et al. 2003; Wu et al. 2003; Lai et al. 2005). Overexpression of Yki also revealed similar tissue outgrowth phenotypes (Huang et al. 2005). The function of the Hippo pathway in organ size control is conserved in mammals. For instance, tissue-specific overexpression of YAP in the mouse liver or heart resulted in a dramatic increase of liver or heart size (Camargo et al. 2007; Dong et al. 2007; von Gise et al. 2012). Consistently, knockout of MST1/2 or Sav in the liver or heart also induced organ size (Zhou et al. 2009; Lee et al. 2010; Lu et al. 2010; Song et al. 2010; Heallen et al. 2011). Recently, a connection between the Hippo pathway and the PI3K–TOR pathway has been demonstrated (Strassburger et al. 2012; Tumaneng et al. 2012b; Ye et al. 2012). This is significant because TOR signaling is important in cell growth; thus, the Hippo pathway may control organ size in part by modulating cell growth (Fig. 1; Tumaneng et al. 2012a). It is possible that multiple upstream signals work together to modulate the Hippo pathway to determine the final organ size. The mechanical forces may correlate with organ size and convey the organ size information to the Hippo pathway. Similarly, tissue-specific GPCRs may also play a role. For example, acetylcholine (a ligand for $G\alpha_{q/11}$-coupled receptor) signaling was required for salivary organogenesis (Knox et al. 2010). Knockout of luteinizing hormone receptor resulted in dramatically smaller testis (Zhang et al. 2001), indicating a possible role of GPCR signaling in tissue growth and organ size control.

Hippo pathway members have been identified in the search for tumor suppressor genes in *Drosophila*. Kinases in the Hippo pathway are generally tumor suppressors, whereas Yki/YAP/TAZ have oncoprotein-like functions. Elevated YAP or TAZ expression and nuclear localization are frequently observed in human cancers (Zhao et al. 2007; Chan et al. 2008; Steinhardt et al. 2008; Fernandez et al. 2009; Xu et al. 2009). YAP transgenic mice display hyperplasia and tumors (Camargo et al. 2007; Dong et al. 2007; Zhang et al. 2011; von Gise et al. 2012). Similarly, inactivation of upstream core components of the Hippo pathway leads to tumor development in mice (St John et al. 1999; Lee et al. 2008, 2010; Zhou et al. 2009, 2011; Lu et al. 2010; Song et al. 2010; Nishio et al. 2012). Moreover, genetic inactivation of NF2, a well-known tumor suppressor that acts upstream of the Hippo pathway (Rouleau et al. 1993; Ruttledge et al. 1994), resulted in tissue overgrowth in *Drosophila* and cancers in mice (Hamaratoglu et al. 2006; Benhamouche et al. 2010; Zhang et al. 2010), and the phenotype was blocked by down-regulation of YAP (Zhang et al. 2010). YAP and TAZ induced an epithelial–mesenchymal transition (EMT), a phenomenon crucial for the initiation of cancer metastasis (Overholtzer et al. 2006; Lei et al. 2008; Thiery et al. 2009). The role of YAP in promoting cancer metastasis has also recently been demonstrated in mice (Chen et al. 2012; Lamar et al. 2012). In addition, TAZ has been shown to sustain self-renewal and induce tumor initiation of breast cancer stem cells (Cordenonsi et al. 2011). These data suggest an important function of the Hippo pathway in cancer development.

The connection between the Hippo pathway, mechanical forces, and GPCR signaling also provides new insights in cancer development. Following disruption of cell–cell junctions and loss of cell polarity, cells may form extensive focal adhesions with the ECM and thereby generate cellular tension, and these cells may furthermore encounter stimulation by GPCR ligands (Fig. 4D). Activation of YAP/TAZ under these conditions may facilitate cell proliferation and cell migration or induce EMT. GPCRs are crucial players in cancer development, and dysregulated GPCR signaling has been identified in many types of human cancers (Dorsam and Gutkind 2007). GPCR signaling can contribute to cancer in a variety of ways (Fig. 4E). Increased production of some GPCR ligands may promote cancer development; for instance, overexpression of autotaxin (an enzyme critical for LPA synthesis) in mouse mammary glands has been shown to induce breast cancer (Liu et al. 2009). Up-regulated expression of GPCRs may activate intracellular signaling automatically, as indicated by high PAR1 expression in high-grade breast cancer patients (Hernandez et al. 2009). Activating mutations of GPCRs have been identified in different type of cancers, such as the metabotropic glutamate receptor mutations in melanoma and thyroid-stimulating hormone receptor in thyroid carcinomas (Paschke and Ludgate 1997; Prickett et al. 2011). Activating mutations of Gα are also present in different types of cancers: $G\alpha_q$/11-activating mutations have been identified in >80% of uveal melanomas (Van Raamsdonk et al. 2009, 2010); $G\alpha_i2\alpha$ and $G\alpha_s$ mutations have been identified in ovarian and endocrine tumors (Lania et al. 2001). In addition, aberrant activity of Rho GTPases and GPCR regulatory molecules, such as GPCR-related kinases, may also contribute to cancer by regulating YAP/TAZ activity (Sahai and Marshall 2002; Metaye et al. 2005). Altogether, activation of YAP/TAZ by dysregulated GPCR signaling may play an important role in the development of human cancers.

## Concluding remarks

The Hippo pathway plays critical roles in normal physiology and pathogenesis, and pharmacological interventions of this pathway have diverse clinical implications; therefore, it is very important to understand how this pathway is regulated. The dynamics of the actin cytoskeleton appears to act as a focal point of different signals pathways to modulate YAP/TAZ activity in either a Lats-dependent or Lats-independent mechanism (Fig. 3D). However, several critical questions regarding the regulation of the Hippo pathway by these upstream regulators still wait to be answered. How are these regulators, especially F-actin or cellular tension, sensed by the core components of the Hippo pathway? To what extent does the Hippo pathway contribute to the physiological and pathological functions of these upstream regulators? Are one or more of these regulators the determining factors for organ size control? Answers to these challenging questions will advance our understanding of the regulation and function of the Hippo pathway.

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# The Hippo pathway: regulators and regulations

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