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REVIEW

Mutual regulation between Hippo signaling and actin cytoskeleton

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ABSTRACT

Hippo signaling plays a crucial role in growth control and tumor suppression by regulating cell proliferation, apoptosis, and differentiation. How Hippo signaling is regulated has been under extensive investigation. Over the past three years, an increasing amount of data have supported a model of actin cytoskeleton blocking Hippo signaling activity to allow nuclear accumulation of a downstream effector, Yki/Yap/Taz. On the other hand, Hippo signaling negatively regulates actin cytoskeleton organization. This review provides insight on the mutual regulatory mechanisms between Hippo signaling and actin cytoskeleton for a tight control of cell behaviors during animal development, and points out outstanding questions for further investigations.

KEYWORDS Hippo signaling, actin cytoskeleton, negative feedback, growth control, development

INTRODUCTION

Hippo signaling pathway serves as one of the mechanisms with which cells respond to their microenvironment by controlling proliferation, apoptosis, differentiation, and migration. Hippo pathway is conserved from *Drosophila* to mammals, consisting of a variety of upstream regulators, four core components of a kinase cascade, and a transcriptional co-activator as a key effector. Upstream regulators receive chemical or mechanical signals from the extracellular environment and provide a site on which other Hippo pathway components can assemble. They determine apical-basal polarity, regulate cell adhesion or are located in the apical domain of cells to facilitate the activation of the Hippo pathway core components. The four core components are Hippo (Hpo, Mst1, and Mst2 in vertebrates), Salvador (Sav, Sav1, or WW45 in vertebrates), Warts (Wts, Lats1, and Lats2 in vertebrates), and Mob as tumor suppressor (Mats, MOBKL1a, and MOBKL1b in vertebrates). In receiving a signal from the upstream regulators, Hpo (Mst1/2) phosphorylates Wts (Lats1/2) with the assistance of a scaffolding protein, Sav (Sav1). This phosphorylation activates the kinase activity of Wts (Lats1/2), and along with its adaptor Mats (MOBKL1a/MOBKL1b), Wts (Lats1/2) phosphorylates Yorkie (Yki, Yap/Taz in vertebrates). 14-3-3 proteins interact with the phosphorylated Yki (Yap/Taz) and retain it in the cytoplasm, which suppresses Yki (Yap/Taz)'s function as a transcriptional co-activator. In vertebrates, in addition to the interaction with 14-3-3 proteins, the protein stability of Yap/Taz is controlled by their phosphorus status at a different residue. When the Hippo pathway is off, Yki translocates into the nucleus and binds to its DNA-binding partners, such as Scalloped (Sd, TEAD1-4 in vertebrates), Homothorax, Teashirt, and Mothers against Dpp (Mad), to activate expression of its target genes for regulating cell proliferation and apoptosis (For some recent reviews, see Pan, 2010; Schroeder and Halder, 2012; Staley and Irvine, 2012; Yu and Guan, 2013).

For the past several years, a number of laboratories have focused on what triggers Hippo pathway activation. One of the particularly exciting discoveries is the control of Yki (Yap/Taz) activity by actin cytoskeleton. Filamentous actin (F-actin) is one of the cytoskeletal components and participates in the regulation of numerous cell behaviors, such as morphology, movement, division, endocytosis, and intracellular trafficking. It is a helical polymer of monomeric G-actin subunits, which carry and hydrolyze ATP after joining to F-actin. Formation of F-actin, *de novo* or branching, begins with nucleation where G-actin creates short oligomers in a temporally and spatially regulated

manner. While nucleation is a rate-limiting step due to the instability of oligomers, elongation is fast and spontaneous. F-actin has a polarity with the fast-growing plus (or barbed) end, and the slow-growing minus (or pointed) end. Because actin cytoskeleton conducts a variety of cellular functions, its tight control by regulatory proteins is essential. For instance, Profilin and Thymosin interact with G-actin, promoting and inhibiting F-actin assembly, respectively. WASP/Scar and Arp2/3 provide a hub from which G-actin can nucleate, and Formin recruits Profilin-bound G-actin to facilitate nucleation and elongation of F-actin. Synthesis of F-actin is not the only step in the regulation of its organization; Capping proteins (CP) bind to the plus end of F-actin, blocking its dynamics, and severing proteins, such as Cofilin and Gelsolin, promote depolymerization of F-actin (Pollard and Cooper, 2009).

Recently, several studies unveiled the signal transduction processes that rearrange actin cytoskeleton and regulate the transcriptional activity of Yki (Yap/Taz). In *Drosophila*, modification in actin cytoskeleton caused tissue overgrowth and Yki was epistatic in the regulation (Fernándezet al., 2011; Sansores-Garcia et al., 2011). *In vitro* studies of mammalian cell lines have identified extracellular signals which influence Yap/Taz activity via regulation of actin cytoskeleton (Dupont et al., 2011; Wada et al., 2011; Yu et al., 2012; Zhao et al., 2012; Aragona et al., 2013). G-protein-coupled receptor (GPCR) signaling promotes or inhibits Yap/Taz activity, depending on the types of ligand and G-protein with which the receptor is associated (Yu et al., 2012). Mechanical stress, such as stiffness of extracellular matrix (ECM), cell morphology, and attachment status to ECM and to neighboring cells, also modulates Yap/Taz activity (Dupont et al., 2011; Wada et al., 2011; Zhao et al., 2012; Aragona et al., 2013).

The relationship between actin cytoskeleton and Yki (Yap/Taz) activity is not unidirectional. Several studies indicated that the Hippo pathway regulates actin cytoskeleton in *Drosophila* (Fang and Adler, 2010; Fernández et al., 2011; Lucas et al., 2013). Others demonstrated the interaction of some of the core Hippo pathway components with F-actin regulators and with β-actin itself (Hirota et al., 2000; Yang et al., 2004; Densham et al., 2009; Rauskolb et al., 2011; Visser-Grieve et al., 2011). Although the biological significance of this reverse regulation is not fully understood, it may play an important role in establishing a feedback loop. Furthermore, considering the involvement of actin cytoskeleton in fundamental behaviors of cells, this reverse regulation may influence many cellular activities.

In this review, we first present current evidence regarding the impact of actin cytoskeleton on Yki (Yap/Taz) activity. Then, we look into regulations of actin cytoskeleton by the Hippo pathway. A working model is that a feedback loop exists between actin cytoskeleton and the Hippo pathway which contributes to a tight control of cell behavior and tissue development (Fig. 1). Lastly, by summarizing the reported data, we raise further questions to address the relationship between actin cytoskeleton and the Hippo pathway.

REGULATION OF Yki (Yap/Taz) BY ACTIN CYTOSKELETON

Actin cytoskeleton regulates the transcriptional activity of Yki (Yap/Taz) by directing its subcellular localization (Fig. 1). Interestingly, an increase in the F-actin level can lead to translocation of Yki (Yap/Taz) into the nucleus, promoting the expression of its target genes, while decrease in the F-actin level causes retention of Yki (Yap/Taz) in the cytoplasm (For a recent review, see Yu and Guan, 2013). The transcriptional activity of Yap/Taz affects cell behaviors in various ways, depending on the developmental stage and the cell/tissue type. For instance, in *Drosophila* third instar wing imaginal discs, F-actin accumulation caused by loss-of-function of CP or gain-of-function of a Formin homolog, Diaphanous (Dia), led to cell proliferation and tissue overgrowth (Fernández et al., 2011; Sansores-Garcia et al., 2011). For another, rearrangement of actin cytoskeleton in response to mechanical cues, such as stiffness of ECM and cell morphology, regulates the Yap/Taz activity, directing cell differentiation of human mesenchymal stem cells (MSC) to specific cell lineages (Dupont et al., 2011).

Despite an increasing amount of data supporting regulation of Yki (Yap/Taz) by actin cytoskeleton, it is not entirely clear as to how the signal is passed down and what molecules play in between. Especially, involvement of the Hippo pathway, a signaling pathway having Yki (Yap/Taz) as its effector, is not completely known. In *Drosophila* wing discs, the Hippo pathway might be a mediator between F-actin and Yki. Cells overexpressing Wts rescued the overgrowth phenotype caused by overexpression of Dia and by F-actin accumulation (Sansores-Garcia et al., 2011). In addition, a very recent study showed that Merlin (Mer, NF2 in vertebrates), an upstream regulator of the Hippo pathway, may be required in the regulation of Yki phosphorylation by actin cytoskeleton (Yin et al., 2013). The group demonstrated that Mer (NF2) brings Wts (Lats1/2) to the plasma membrane and this interaction and subcellular localization of Wts activate the Hippo pathway. While NF2 was capable of interacting with Lats1/2 in its wild-type form in a human cell line, only constitutively active Mer with a short region deleted at its C-terminus was able to associate with Wts in *Drosophila*. What turns out to be compelling is that disruption of actin cytoskeleton by Latrunculin B (LatB) or inhibition of Rho GTPase by C3 facilitated the wild-type Mer to interact with Wts. Cells depleted of Mer did not phosphorylate Yki upon the treatment of LatB or C3, indicating the requirement of Mer in the regulation of Yki by actin cytoskeleton (Yin et al., 2013). As the conformation of Mer (NF2) determines its functions, it is speculated that actin cytoskeleton influences Mer (NF2)'s functions by regulating its conformation.

Similar conclusions on the involvement of the Hippo pathway were deduced in mammalian cell lines. Treatment with Cytochalasin D (CytoD) or LatB disrupted F-actin, retaining Yap in the cytoplasm (Wada et al., 2011; Zhao et al., 2012). However, this retention was blocked in cells overexpressing the kinase-dead form of Lats2 (Lats2KD) which is dominant negative

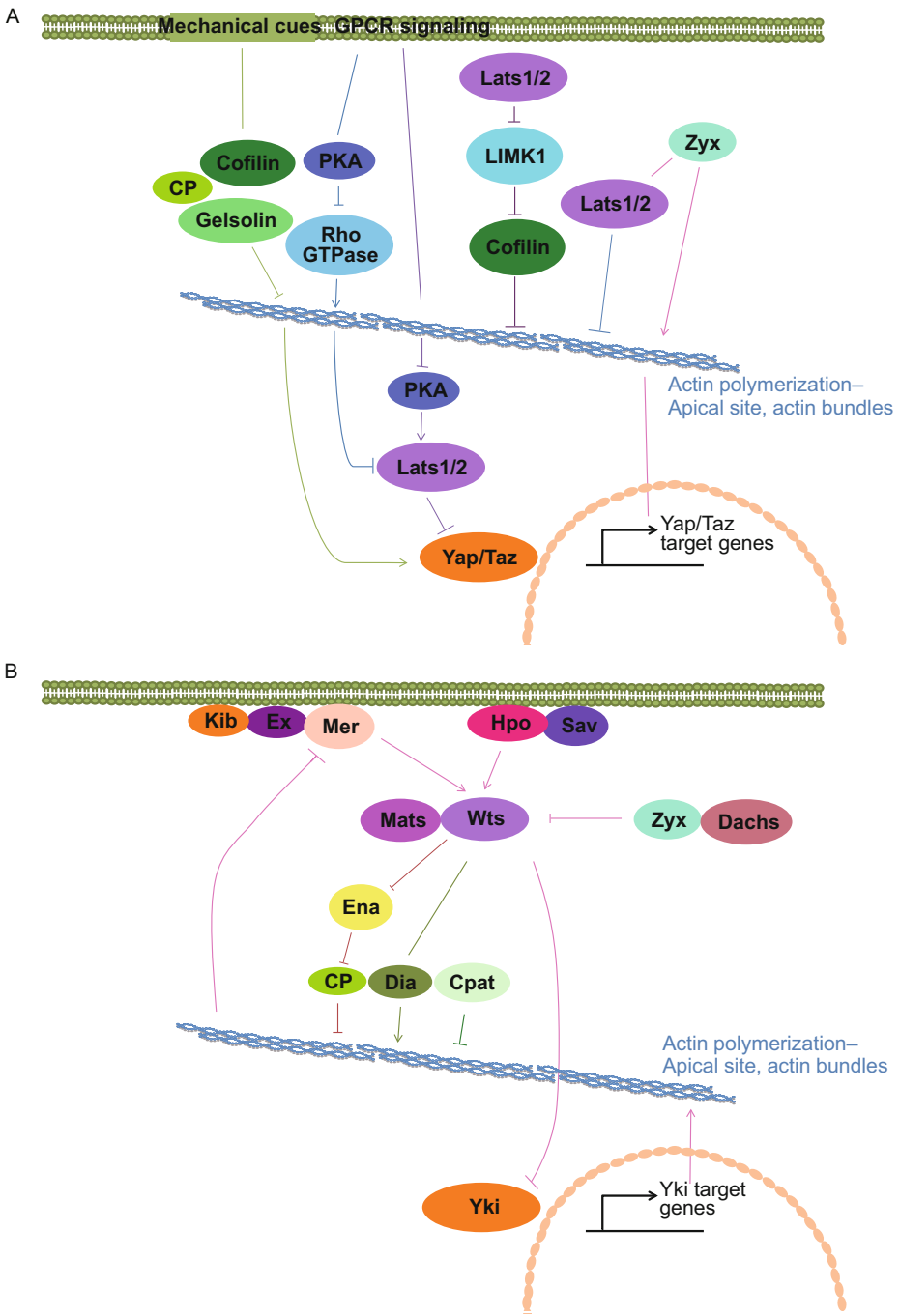

Figure 1. Mutual regulation between actin cytoskeleton and the Hippo pathway in mammalian cells and in *Drosophila*. (A) In mammalian cells, mechanical cues control actin cytoskeleton and Yap/Taz activity independently from the Hippo pathway. Negative regulators of F-actin, such as CapZ, Cofilin, and Gelsolin, are required in this regulation. GPCR signaling also influences actin cytoskeleton and the activity of Yap/Taz, but in a Lats1/2-dependent manner. Involvement of Rho GTPase and PKA is reported in this regulation. In the reverse regulation, interaction between LIMK1 and Lats1/2 was reported to regulate the F-actin level at the contractile ring and the periphery of the cells for cytokinesis. Interaction between Zyx and Lats1/2 has also been observed during mitosis. Lats1/2 proteins can directly bind to β-actin, suppressing F-actin polymerization. (B) In *Drosophila*, manipulating F-actin level by its regulators, such as Capping proteins (CP), Diaphanous (Dia), and Capulet (Capt), affects Yki activity via the Hippo pathway. An upstream Hippo pathway component, Merlin (Mer), acquires an ability of interacting with Wts upon disruption of F-actin. In the regulation of actin cytoskeleton by the Hippo pathway, Wts phosphorylates Enabled (Ena), which blocks the inhibitory effect on Capping proteins (CP). A positive F-actin regulator, Zyxin (Zyx) presumably undergoes conformational change when interacting with an upstream Hippo pathway component, Dachs. This conformational change facilitates binding between Zyx and Wts, which negatively regulates the Hippo pathway. Lines with arrowed or blunted end indicate activation or inhibition, respectively. Dashed lines indicate either indirect or unknown mechanism.

for endogenous Lats1/2. Furthermore, in mouse embryonic fibroblast NIH3T3 cells, when one of the Lats1/2 phosphorylation residues of Yap, Serine 112, was mutated to Alanine (Yap-S112A, S127 in human YAP), Yap-S112A remained in the nucleus and no longer translocated to the cytoplasm following the treatment (Wada et al., 2011). In the upstream of Yki (Yap/Taz) and Wts (Lats1/2), Mst2 can also react to the change of actin cytoskeleton. In NIH3T3 cells, immunohistochemistry identified the co-localization between actin cytoskeleton and Mst2. Moreover, inactivation of Rho GTPase by C3 or disruption of F-actin by LatB or CytoD induced the kinase activity of Mst2 (Densham et al., 2009). Understanding if this regulation of Mst2 by actin cytoskeleton activates Hippo pathway needs more examinations.

On the other hand, Piccolo group argues that actin cytoskeleton modulates the Yap/Taz activity independently from Lats1/2 in human cell lines (Dupont, et al., 2011; Aragona et al., 2013). Upon Latrunculin A (LatA) treatment, an inhibitor of F-actin polymerization, the phosphorylation level of Yap at S127 where Lats1/2 phosphorylation did not change. Reduction in the protein stability of Taz upon LatA treatment also turned out to be Lats1/2-independent, as the deletion of Lats1/2 failed to bring back the stability. Moreover, in another set of experiment, the endogenous Yap/Taz was deleted and a mouse Taz construct with four Lats1/2-target residues mutated (4SA-mTAZ) was overexpressed. However, LatA treatment was still able to suppress the 4SA-mTAZ activity, indicating 4SA-mTAZ remained to be responsive to the change of actin cytoskeleton (Dupont et al., 2011).

Precisely, F-actin accumulation *per se* is not sufficient to upregulate the activity of Yki (Yap/Taz). In the clonal analysis of *Drosophila* wing discs, clones mutant for a Cofilin homolog, *twinstar* (*tsr*), accumulated F-actin in the entire cortical region. These mutant clones showed no difference in the Yki target gene expression compared to wild-type cells. By contrast, cells depleted of a cyclase-associated protein, Capulet (Capt), accumulated F-actin near the apical surface. In these cells, there was an increase in the Yki target gene expression (Fernández et al., 2011). These data implicate that it is the apical surface where actin cytoskeleton needs to be modified for the regulation of Yki. Likewise, in human HeLa cells, the ratio of G-actin to F-actin does not affect Yap/Taz activity. When R62D mutant Actin or V159N mutant Actin was overexpressed to increase the amount of G-actin or F-actin, respectively, there was no difference in the expression level of Yap/Taz target genes. Rather, the specific structures of F-actin regulated by Rho GTPase are required for modulating Yap/Taz activity. Treatment of human cell lines with the Rho GTPase inhibitor, C3, caused the translocation of Yap/Taz into the cytoplasm, decreasing the expression levels of their target genes (Dupont et al., 2011). Among the F-actin structures regulated by Rho GTPase, formation of F-actin bundles (stress fibers), not actin meshwork, is associated with Yap/Taz activity. Chemically inhibiting a positive regulator of F-actin bundles, Formins, reduced the expression of their target genes, while inhibiting Arp2/3 which promotes more branched structure, did not affect their expression levels (Aragona et al., 2013). Formation of focal adhesions, a bridge between ECM and actin cytoskeleton, does not influence Yap/Taz activity, either. In MCF10A cell line, seeding cells onto the FA-forming substrate (Fibronectin) or the FA-non-forming substrate (poly-lysine) both led to Yap nuclear localization (Zhao et al., 2012). The group further examined if actin tension manipulates Yap/Taz activity and showed releasing the actin forces by the suppression of ROCK or myosin II ATPase did not induce Yap phosphorylation. However, alteration in actin tension affected Yap/Taz localization in other human cell lines, calling for more data to clarify its involvement in regulating Yap/Taz activity (Dupont et al., 2011).

Recently, more mediators linking the actin cytoskeleton and the activity of Yap/Taz have been identified in human mammary epithelial cells (MEC). They are negative regulators of F-actin: Cofilin, Gelsolin, and CapZ (or Capping proteins) (Fig. 1). When cells were grown on the soft ECM substrates, the F-actin level decreased, which suppressed the expression level of Yap/Taz target genes. However, knockdown of Cofilin, Gelsolin, or CapZ brought back the expression level of Yap/Taz target genes by increasing the F-actin level. Phosphorylation of Yap may be biologically irrelevant in the suppression of its activity in this context, as knockdown of CapZ did not decrease the phosphorylation level of Yap. Intriguingly, although knockdown of Lats1/2 *per se* did not increase the expression level of Yap/Taz target genes in the cells with the low F-actin level, knockdown of both CapZ and Lats1/2 synergistically increased the expression level of these genes. This indicates not only that Lats1/2 and actin cytoskeleton can regulate Yap/Taz activity independently, but that the proper F-actin organization is the prerequisite for the functions of Lats1/2. Another factor which may transduces a signal from actin cytoskeleton to Yap/Taz activity is cyclic AMP (cAMP)-dependent protein kinase (PKA). In the downstream of actin cytoskeleton, PKA phosphorylates and activates Lats1/2, inducing phosphorylation of Yap. Interestingly, PKA-activated Lats1/2 phosphorylates Serine 381 of Yap, a residue for controlling the protein stability of Yap, more dramatically than Serine 127, a residue for directing localization of Yap. In mouse embryonic fibroblasts (MEFs), endogenous Lats1/2 was removed and either wild-type Lats2 or a Lats2 construct with four PKA putative phosphorylation sites-mutated (Lats2-4SA) was overexpressed. Upon the disruption of F-actin by LatB, Lats2-4SA failed to phosphorylate Yap at either S127 or S381, pointing out the necessity of phosphorylation of Lats2 by PKA for the regulation of Yap (Kim et al., 2013). Yet, there is a contradictory study as to where cAMP/PKA signaling can be placed in the pathway. PKA has been reported to negatively regulate actin cytoskeleton via suppression of Rho GTPase pathway and this model places cAMP/PKA upstream of actin cytoskeleton. Biochemical and genetic experiments supported this model; in human cell lines, overexpression of wild-type or constitutively active RhoA blocked the phosphorylation of Yap induced by forksolin treatment, a PKA activator. In addition, suppression of Rho GTPase by overexpressing its

inhibitor (RhoGDI) retained the phosphorylation of Yap even in the presence of a PKA inhibitor, KT5720 (Yu et al., 2013). The discrepancy could be partly due to different cell types that were used in these studies.

REGULATION OF ACTIN CYTOSKELETON BY THE HIPPO PATHWAY

Several studies noted that the Hippo pathway reduces the F-actin level, which may play important biological roles (Fang and Adler, 2010; Fernández et al., 2011; Visser-Grieve et al. 2011; Lucas et al., 2013). In *Drosophila* wing discs, clones mutant for the Hippo pathway components, such as *ex*, *hpo*, *sav*, *mats*, and *wts*, accumulated F-actin at the apical surface. This is one of the earliest evidence of Hippo pathway providing its negative feedback in the fly epithelial cells. However, whether this regulation involves Yki is still questionable. In *Drosophila* larval wing discs, the F-actin level did not change in the mutant clones with gain- or loss-of-function of Yki, while in *Drosophila* pupal wings, Gal4 flip-out clones overexpressing wild-type Yki increased the F-actin level (Fang and Adler, 2010; Fernández et al., 2011). In addition to the feedback, Hippo pathway can bring about distinct biological consequences by regulating actin cytoskeleton. In *Drosophila* border cells in adult ovaries, the Hippo pathway plays an essential role in organizing actin cytoskeleton and controlling proper border cell migration. Border cells, derived from follicle cells, normally migrate through nurse cells from the anterior pole to the oocyte at the posterior pole in each egg chamber. The proper migration depends on the polarization of F-actin. However, border cells mutant for Hippo pathway components, such as *ex*, *kibra*, *hpo,* or *wts*, migrated in a "tumbling motion" and failed to reach the oocyte at the appropriate stage of oogenesis. This was presumably due to the accumulation of F-actin and loss of its polarity by the mutation (Lucas et al., 2013). In human mammary epithelial MCF10A cell line, involvement of Yap/Taz in epithelial-mesenchymal transition (EMT) has been reported. Cells overexpressing the constitutively active forms of Yap/Taz with Lats1/2 phosphorylation-sites mutated (Yap-5SA or Taz-4SA) promoted EMT. The features of EMT which cells exhibited included the disorganization of adherens junctions and conversion from cortical actin to stress fibers (Lei et al., 2008; Zhao et al., 2008).

Although in what way the Hippo pathway negatively regulates actin cytoskeleton remains to be investigated, current data provide some hints (Fig. 1B). First, a simple biochemical assay differentiated two possible mechanisms of regulating F-actin by the Hippo pathway: promoting depolymerization or inhibiting polymerization. *Drosophila* pupal wings were treated with LatA which inhibits F-actin polymerization, leaving its depolymerization unaffected. Following the treatment, *wts* mutant clones and wild-type cells had the same rate of loss of F-actin signal, indicating that *wts* mutation promoted F-actin polymerization (Fang and Adler, 2010). Second, genetic data using *Drosophila* wing discs suggest that the Hippo pathway is capable of suppressing the F-actin level through multiple mechanisms. In case of the F-actin accumulation at the apical surface caused by the loss-of-function of Capping protein alpha (Cpa), overexpression of the Hippo pathway components, such as Ex and Hpo, was still able to partially rescue the phenotype (Fernández et al., 2011). Interestingly, overexpression of the Hippo pathway components, such as Ex, Hpo, and Wts, failed to rescue the F-actin accumulation when constitutively active Dia (Dia^{CA}) was overexpressed (Sansores-Garcia et al., 2011). Third, in *Drosophila* border cells, the Hippo pathway lies upstream of Enabled (Ena), an inhibitor of Capping proteins. Border cells mutant for *ena* or overexpressing Capping protein beta (Cpb) rescued the delayed migration phenotype caused by the mutations of the Hippo pathway core components. *In vitro* analysis further revealed that Wts phosphorylates Ena and this phosphorylation suppresses Ena's inhibitory activity on Capping proteins (Lucaset al., 2013). Fourth, Lats1 directly interacts with LIMK1, a positive regulator of F-actin, in HeLa cells. Upon association with Lats1, LIMK1 can no longer phosphorylate and inactivate Cofilin, a severing factor of F-actin. The interaction may be independent of the kinase activity of Lats1 since it failed to phosphorylate LIMK1 and a kinase-dead Lats1 remained to be able to suppress the inhibitory activity of LIMK1 on Cofilin. This negative regulation of Lats1 on LIMK1 controls F-actin level specifically at the contractile ring and the periphery of cells for the proper progression of cytokinesis (Yang et al., 2004). If this mechanism has anything to do with the Hippo pathway remains to be examined. Fifth, Zyxin (Zyx), another positive F-actin regulator, directly interacts with Lats1 in mammalian cell lines. This interaction takes place at mitotic apparatus during mitosis, when Cdc2 phosphorylates Zyx, triggering the interaction with Lats1 (Hirota et al., 2000). Intriguingly, this interaction also occurs in *Drosophila* wing discs with a different biological consequence, or negative regulation of the Hippo pathway. Dachs, an upstream Hippo component, binds to Zyx and may induce the conformational change of Zyx. This enables the interaction between Zyx and Wts at the sub-apical region and partially regulates the stability of Wts. Cells depleted of Zyx rescued the reduction of Wts protein caused by the loss of an atypical cadherin, Fat. Removal of Zyx in wing discs did not decrease the F-actin level; however, considering Zyx binds to focal adhesions and promotes F-actin polymerization in sensing the increase of mechanical tension within the cell, it is possible that interaction between Zyx and Wts occurs in response to change in actin cytoskeleton (Rauskolb et al., 2011).

Although the biochemical and genetic approaches have identified indirect regulations of actin cytoskeleton by the Hippo pathway, the possibility of direct regulation cannot be excluded (Fig. 1A). Lats1 interacts with β-actin, which is one of the two isoforms constituting non-muscle actins. *In vitro* actin polymerization assay showed that the addition of GST-Lats1 into pyrene-actin decreased the polymerization rate compared to the rate in the GST control. Furthermore, the addition of N-terminus of GST-Lats1 inhibited the polymerization more strongly than full-length Lats1, while C-terminus of GST-Lats1, where kinase activation sites are found, had almost no inhibitory effect on

actin polymerization. It implicates that the negative regulation by Lats1 may be independent from its kinase activity (Visser-Grieve et al., 2011).

CONCLUDING REMARKS

There appears no simple model representing the molecular interactions among actin cytoskeleton, the Hippo pathway and Yki (Yap/Taz). When we look at the regulation of Yki (Yap/Taz) activity by actin cytoskeleton, F-actin accumulation in response to GPCR signaling activates Yap/Taz by inhibiting Lats1/2 kinase activity. On the other hand, mechanotransduction regulates Yap/Taz independently from Lats1/2. Moreover, the level of F-actin *per se* does not guarantee a response; instead, an increase in the F-actin bundles at the apical surface of the cells activates Yki (Yap/Taz). These observations take us to further inquiries about this regulation. How the actin cytoskeleton controls Wts (Lats1/2) or Yki (Yap/Taz) remains to be addressed. In particular, how the disruption of actin cytoskeleton initiates the interaction between Mer and Wts in *Drosophila* is a place of interest (Yin et al., 2013). As Zyx affects Wts stability as well as actin polymerization, how F-actin might be involved in mediating the effect of Zyx on Wts needs to be clarified. Additional outstanding questions include how Hpo and Mats proteins are recruited to the plasma membrane for the activation of the Hippo pathway (Deng et al., 2013). As is the case of Mer which interacts and recruits Wts to the plasma membrane upon disruption of actin cytoskeleton, it would be intriguing to test a possibility of actin cytoskeleton as a negative factor for the recruitment of Hpo and Mats to the plasma membrane. Also, we need more systematic ways to understand how multiple inputs that modulate actin cytoskeleton cooperate or compete for the response in Yki (Yap/Taz). Identification of additional factors and comparative analysis on Yki (Yap/Taz) target gene expression should help address this issue.

Integrating the reported mechanisms of regulation of actin cytoskeleton by the Hippo pathway or Wts (Lats1/2) alone results in a significantly more complex model. *Drosophila* studies identified some F-actin regulators which function either upstream or downstream of the Hippo pathway and affect such biological consequences as migration and growth control. In studies using mammalian systems, several F-actin regulators were revealed to associate with Lats1/2 for mitotic control. The collective model brings new questions to light. For example, in *Drosophila*, whether and how Yki participates in the regulation of actin cytoskeleton needs further investigations. For another, in addition to Ena and Capping proteins, if any other F-actin regulator functions downstream of Wts should be analyzed.

An increasing number of studies have uncovered the upstream signals associated with Yki (Yap/Taz) activity, such as the stiffness of ECM substrate, cell morphology, cell attachment/detachment, the cell density in a culture, GPCR signaling, to name a few (Dupont et al., 2011; Wada et al., 2011; Yu et al., 2012; Zhao et al., 2012; Aragona et al., 2013). At the same time, these seemingly unrelated upstream signals were found to share a common denominator: the actin cytoskeleton. Actin cytoskeleton integrates multiple signals from inside and outside of the cells by rearranging its organization. This enables cells to have precise control of their behaviors, such as transcriptional regulation by Yki (Yap/Taz). What attracts our attention even more is the mutual monitoring of the activities between the Hippo pathway and actin cytoskeleton which takes a form of a feedback loop. Accumulation of F-actin inhibits Hippo pathway and activates Yki (Yap/Taz), and activation of the Hippo pathway decreases the F-actin level. This negative relationship ensures that the important cell behaviors, such as proliferation, apoptosis, and differentiation, are regulated in a strict manner. In addition to a negative feedback, the regulation of actin cytoskeleton allows Hippo pathway to be involved in the control of many important developmental processes. The Hippo pathway turned out to be essential in directing the polarity of actin cytoskeleton and the migratory pattern of the border cell cluster in adult ovaries (Lucas et al., 2013). Additional molecular mechanisms by which Hippo signaling regulates actin cytoskeleton are expected to be elucidated in coming years. Uncovering the interplay between the Hippo pathway and actin cytoskeleton has opened up a new branch of study in this field. Understanding its detailed mechanisms will define specific roles that the Hippo pathway plays in development and diseases.

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ABBREVIATIONS

CP, Capping proteins; Cpa, Capping protein alpha; Cpb, Capping protein beta; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; F-actin, filamentous actin; GPCR, G-protein-coupled receptor; MEC, mammary epithelial cells; MSC, mesenchymal stem cells

COMPLIANCE WITH ETHICS GUIDELINES

Yurika Matsui and Zhi-Chun Lai declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

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