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Invited Mini Review

Upstream paths for Hippo signaling in *Drosophila* organ development

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Organ growth is fundamental to animal development. One of major mechanisms for growth control is mediated by the conserved Hippo signaling pathway initially identified in *Drosophila*. The core of this pathway in *Drosophila* consists of a cascade of protein kinases Hippo and Warts that negatively regulate transcriptional coactivator Yorkie (Yki). Activation of Yki promotes cell survival and proliferation to induce organ growth. A key issue in Hippo signaling is to understand how core kinase cascade is activated. Activation of Hippo kinase cascade is regulated in the upstream by at least two transmembrane proteins Crumbs and Fat that act in parallel. These membrane proteins interact with additional factors such as FERM-domain proteins Expanded and Merlin to modulate subcellular localization and function of the Hippo kinase cascade. Hippo signaling is also influenced by cytoskeletal networks and cell tension in epithelia of developing organs. These upstream events in the regulation of Hippo signaling are only partially understood. This review focuses on our current understanding of some upstream processes involved in Hippo signaling in developing *Drosophila* organs. [BMB Reports 2018; 51(3): 134-142]

INTRODUCTION

Genetic regulation of cell proliferation and survival is crucial for normal patterning and growth of organs. *Drosophila melanogaster* has been an ideal model animal to dissect the molecular genetic basis of development. Major signaling pathways including Wingless/Wnt, Notch, and Hedgehog signaling discovered in *Drosophila* have provided insights necessary to uncover conserved mechanisms in mammalian development and understand the molecular basis of related human diseases (1).

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In studying organ development, the eye and the wing have been extensively used as model organs. Adult eyes and wings derive from eye and wing imaginal discs, respectively. Imaginal discs are relatively simple epithelial organ primordia. During larval stages, imaginal discs are specified as different organ precursors. They develop by cell proliferation and patterning processes. Genetic defects causing overgrowth or hypotrophy of these organs can be easily detected in larvae or adult flies. Eyes and wings are dispensable for animal's survival, making these organs particularly attractive for genetic analysis of organ development.

Genetic screens using unbiased mutagenesis have been powerful for discovering Hippo pathway mutants. Genetic mosaic techniques have been utilized to search for mutations that affect tissue growth *in vivo*. Mitotic recombination can be induced to generate genetic mosaic tissues in which wild-type cells and mutant sister cells can be visualized. The *lats* tumor suppressor gene was first identified by a mutation that caused tumorous growth in mitotic clones (2). The same gene named *warts* (*wts*) was also found by an independent clonal analysis (3). More recently, a modified genetic strategy has been used to isolate mutations affecting tissue growth. In this approach, mutant clones in the eye are marked by *white* gene mutation. By comparing the size of white mutant clones and red wild-type sister clones, one can identify mutations that affect tissue growth in the eye. From this screen, several mutant alleles for another Hippo pathway gene *salvador* (*sav*) have been isolated (4). In addition, several groups have identified *hippo* mutations that cause massive overgrowth in eye and wing organs (5-8). Identification of Wts, Sav, and Hpo from these studies has laid a foundation for the Hippo signaling pathway that regulates organ growth.

Since the identification of these core factors, there have been expanded investigations in *Drosophila* and mammalian systems to refine the pathway with a number of newly discovered components (9-12) (Table 1). Hippo signaling in mammalian systems and human diseases has been covered by other reviews in this issue. This review begins with a brief overview of the Hippo pathway. It mainly focuses on upstream components and roles of cytoskeleton in Hippo signaling during *Drosophila* organ development.

Table 1. Members of the Hippo signaling pathway in *Drosophila* and human

Function	Drosophila	Human	Protein family
Transmembrane signaling	Fat (Ft)	Ft1-4	Atypical cadherin
	Dachsous (Ds)	DCHS1,2	Atypical cadherin
	Dachs (D)	?	Atypical myosin
	Crumbs (Crb)	CRUMBS1-3	Transmembrane protein
FERM protein complex	Expanded (Ex)	FRMD1,6	FERM domain protein
	Merlin (Mer)	MERLIN/NF2	FERM domain protein
	Kibra (Kibra)	KIBRA	WW domain protein
	Schip1 (Schip1)	SCHIP1	Ex/Merlin-interacting protein
Kinase cascade	Tao-1 (Tao-1)	TAO1-3	Ste20 family protein kinase
	Hippo (Hpo)	MST1,2	Ste20 family protein kinase
	Salvador (Sav)	SAV1/WW45	Scaffolding protein
	Warts (Wts)	LATS1,2	NDR family protein kinase
Transcription	Yorkie (Yki)	YAP, TAZ	Transcription coactivator
	Scalloped (Sd)	TEAD1-4	Transcription factor
Actin regulators	Cpa	CAPZA1-3	Actin capping protein
	Cpb	CAPZB	Actin capping protein
	Twin star (Tsr)	COFILIN	Actin depolymerization factor
	Ajuba	LIMD1, WTIP, AJUBA	LIM domain protein

HIPPO-WARTS KINASE CASCADE AT THE CENTER

Wts, Sav, and Hpo share function as tumor suppressors for negative growth regulation (5, 8). Genetic analysis of their relationships in developing organs has revealed that they act together to form a core in a pathway (Fig. 1). Hpo and Wts are serine/threonine protein kinases that belong to STE-20 and NDR family kinases, respectively (2, 3, 13). Hpo directly activates Wts by phosphorylation. Wts can also be phosphorylated by an alternative Hpo-like kinase Happyhour (14).

In the search for Wts-interacting proteins, a transcriptional coactivator named Yorkie (Yki) after the Yorkshire Terrier has been identified as a regulator that controls both cell proliferation and survival (15). Yki is required for transcriptional activation of *Cyclin E* (*CycE*) and *Death-associated inhibitor of apoptosis 1* (*Diap1*) that promotes cell cycle and inhibits cell death, respectively (7). Yki also regulates the expression of *bantam* microRNA to increase cell proliferation and survival (16, 17). *Scalloped* (*Sd*) mutations cause characteristic wing notching phenotypes (18). Sd is a TEA domain transcription factor that forms a dimer with Vestigial (Vg) to induce wing development (19, 20). Yki coactivator binds to Sd to activate *CycE* and *Diap1* gene transcription (21-23). Unlike Yki, Sd is not required for all imaginal discs, suggesting that Yki might interact with different transcription factors to induce tissue-specific growth and cell survival. Although Sd is not required for normal eye development, it is critical for compensatory proliferation when cell death is induced in developing eye disc (24).
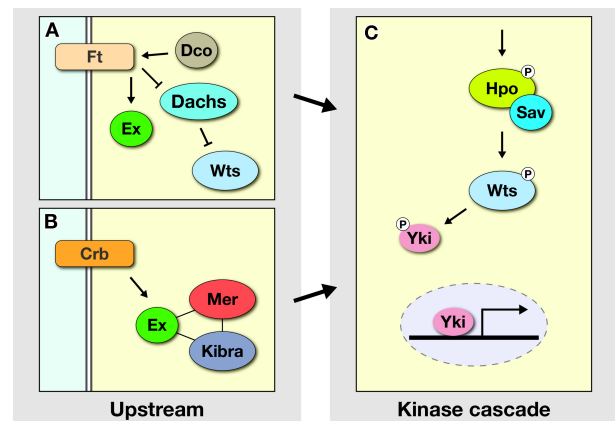
Fig. 1. Upstream pathways for Hippo signaling. Hippo signaling is regulated by two upstream events. (A) Transmembrane protein Ft activates Wts by inhibiting Dachs. Dco promotes Ft activity by phosphorylation. Ft also regulates Ex localization. (B) Crb binds to Ex. Ex, Mer, and Kibra function together to activate the Hippo kinase cascade. (C) The core kinase cascade. Hpo, together with Sav, activates Wts through phosphorylation. Activated Wts phosphorylates Yki to block its nuclear entry.

FAT AND CRUMBS AT THE CELL MEMBRANE

Cell-cell communication is essential for controlled growth of epithelial cells during organ development. Growth signaling is mediated by membrane-bound proteins located at cell junctions. Two transmembrane proteins, Fat (Ft) and Crumbs

(Crb), have been identified as important upstream factors that lead to the activation of the Hippo kinase cascade (Fig. 1A, B).

Ft and Dachsous (Ds) are atypical cadherin proteins involved in the regulation of planar cell polarity in developing eye and wing epithelia (25). In Hippo signaling, Ds activates Ft by binding to Ft as a membrane-bound ligand followed by a relay of Ft signal for Hpo activation. Loss of Ft causes overproliferation which results in enlarged organs. Ft activates Hippo signaling in two ways. Firstly, Ft is necessary for the localization of a FERM domain protein Expanded (Ex) at apical junctions to promote Hpo signaling (26-30). Loss of Ft affects the stability of Ex without altering the localization or level of Hpo, suggesting that Ft affects Hpo phosphorylation mainly by regulating the localization of Ex, but not Hpo (26). Secondly, Ft inhibits apical localization of atypical myosin Dachs. Dachs promotes growth by inhibiting Wts function (31). An SH3 domain protein Vamana can directly interact with Dachs. Such interaction mutually facilitates their apical localization (32). Ft activity is promoted by phosphorylation in the intracellular domain through a Casein kinase 1ε Disc-overgrown (Dco) (33). Effects of Dco can be antagonized by palmitoylation of Ft by palmitoyltransferase Approximated (App). Thus, Ft activity and apical Dachs localization are regulated by two opposing posttranslational modifications (34).

Crb is another transmembrane protein that acts upstream of the Hippo pathway in parallel with Ft branch (Fig. 1B, C). Crb is well known for its critical function in the establishment and maintenance of apical basal epithelial cell polarity (35). Evidence suggests that Crb function is regulated by homotypic extracellular binding (36). Crb acts upstream of the Hippo kinase cascade for growth regulation (37-40). Mammalian Crb3, a major isoform expressed in epithelial cells, is also involved in the regulation of Hippo-YAP pathway (41). The intracellular domain of Crb (Crb[intra]) has two functional motifs: the juxtamembrane FERM-binding motif (FBM) and the PDZ binding motif (PBM). Of these motifs, the FBM domain is essential for Crb function in Hippo signaling by binding to the FERM domain of Ex. Mutation in FBM leads to mislocalization of the apical Ex protein to the basal region of cells in imaginal discs. Thus, Crb is necessary for recruiting Ex to the apical plasma membrane.

Overexpression of full-length Crb or Crb[intra] results in overgrowth of imaginal discs, similar to the effect of Crb loss-of-function (LOF). An explanation for this phenomenon has been provided. In the absence of Crb, Hippo signaling is inactivated because Ex cannot be recruited to Crb. Interestingly, high levels of Crb by overexpression promote phosphorylation of Ex which causes proteasome-dependent degradation of Ex by Skp-Cullin-F-Box (SCF) E3 ubiquitin ligase complex (42). Hence, overexpression of Crb can also inhibit Hippo signaling by reducing Ex level. F-box protein Slimb (Slmb) is responsible for Ex degradation induced by high levels of Crb. However, protein kinases necessary for Ex phosphorylation have not been identified.

Collectively, Ft and Crb form major upstream pathways to activate the Hippo kinase cascade. Dachs is recruited to apical membranes by interacting with Ft whereas Ex is localized by both Crb and Ft. Crb-induced degradation of Ex provides a mechanism for controlled growth in response to changes in Crb level.

EX-MER-KIBRA FOR RELAY

In parallel with Ex, Merlin (Mer), another FERM-domain protein, is required for mediating the function of Crb. *Drosophila* Mer is the homolog of mammalian Mer (Schwanommin), a tumor suppressor of neurofibromatosis type 2 (NF2). Mutations in *NF2* are associated with various neural diseases (43). *Drosophila* Mer and *ex* are structurally related. They also show genetic interaction. Mutations in either *Mer* or *ex* show mild defects in wing growth. Double mutations in both genes result in strong synergistic effects on tumorous tissue growth (44-46). Thus, Mer and Ex can act in partially redundant manner for Hpo signaling. Genetic studies have revealed that Mer and Ex act upstream of the Hpo-Wts pathway to negatively regulate Yki activity, consistent with tumor suppressor function of NF2 (47).

Kibra is another protein that acts upstream of Hpo-Wts core kinases. Human KIBRA was initially found as a WW domain protein enriched in the kidney and the brain (48). *Drosophila kibra* was identified in a genetic screen for cell polarity factors in the oocyte. Kibra physically interacts with Mer and Ex to form a protein complex localized to the apical cell membrane (49) (Fig. 1). Kibra can enhance the level of Wts phosphorylation induced by Mer and Ex *in vitro*, consistent with synergistic tissue overgrowth by mutations in *kibra* and *ex* (or *Mer*). Furthermore, Kibra, Mer, and Ex can directly bind to the Hpo-Sav complex, thus activating Wts kinase activity (50, 51). Kibra also interacts with Wts. Interestingly, when Yki is not phosphorylated by Wts, Ex can directly sequester Yki (52). Because depletion of Ex or Kibra does not affect the interaction between Wts and Yki, Ex and Kibra seem to be mainly required to activate the Hippo kinase cascade rather than promote Wts-Yki interaction (51). Depletion of Kibra also reduces the strength of Ex-Mer interaction, indicating a role of Kibra in stabilizing the Ex-Mer complex (51).

Although Ex, Merlin, and Kibra function together to activate the Hippo pathway, each of these factors may have tissue-specific contribution *in vivo*. Effects of *ex* mutations are more severe in imaginal discs than those of *Mer* mutations. On the contrary, Mer is essential for regulation of cell polarity and growth suppression in ovarian follicle cells (53). Ex and Mer also display differential roles in regulating cell cycle and apoptosis in different tissues and organs (47). Thus, Ex, Mer, and Kibra proteins might form distinct protein complexes with tissue-specific factors for context-dependent regulation of Hippo signaling.

It is interesting to note that Kibra and Mer are not only

detected at the junctional domain of wing disc epithelial cells, but also detected in the apical medial region between junctions (54, 55) (Fig. 2). Depletion of Kibra and Mer leads to strong reduction of Sav at the medial domain, but not at junctions. Kibra and Mer also recruit Hpo to the medial region for Sav-dependent activation of Hpo signaling. In contrast to Mer, Ex is only localized to the junctional membrane, but not to the medial region. Junctional localization of Kibra requires neither Mer nor Ex. Instead, Crb plays a key role in sequestering Kibra to apical junctions, consistent with medial accumulation of Kibra in *crb* mutant clones. This leads to a proposal that Hippo signaling is organized at two distinct sites of the apical membranes: junctional and medial regions. Thus, Crb has dual functions to suppress Hpo signaling by degrading Ex and sequestering Kibra away from the medial domain. It remains to be determined whether the apical medial localization of Kibra is a general phenomenon in the epithelia of other organs.

SCHIP1 AND TAO-1 BRIDGE EX TO THE HPO CASCADE

Ex, Mer, and Kibra form a protein complex with Hpo to activate the kinase cascade. However, they are not directly linked to Hpo kinase, suggesting that additional factors are needed to connect Ex-Mer-Kibra to Hpo. Two independent studies have identified Tao-1 kinase as a direct regulator of Hpo (56, 57). Human TAO1 can suppress the activity of YAP in human cells, suggesting that human TAO kinases might function as tumor suppressors (57).

Tao-1 is a serine/threonine protein kinase that promotes Hpo activation by phosphorylation. Genetic evidence indicates that Tao-1 acts downstream of the Ex-Mer-Kibra complex. However, given that Ex-Mer-Kibra does not directly interact with Tao-1 (56), there might be additional protein(s) that can bind to the Ex-Mer-Kibra complex. *Drosophila* Schip1 (hereafter Schip1), a homolog of human Schwanommin (Merlin) interacting protein 1 (hSchip1) (55), turns out to be a factor that connects Ex-Mer and Tao-1 (58). Loss of Schip1 increases Yki activity and induces organ overgrowth (58). Schip1 is recruited apically to Ex by direct binding. Furthermore, Schip1 directly promotes Tao-1 activity by physical interaction, leading to increased phosphorylation of Hpo. Therefore, Schip1 and Tao-1 play critical roles in connecting Ex to Hpo, hence facilitating the activity of the Hpo kinase cascade (Fig. 2).

Tao-1 was initially found as a protein that could control cell shape by destabilizing the microtubule plus-end (59). A recent study has revealed new functions of Tao-1 in germline cell development (60). Interestingly, alternative promoters of *tao-1* produce two different proteins (Tao-L and Tao-S) that act antagonistically. The long form Tao-L, but not the short form Tao-S, contains the protein kinase domain in the N terminal region. Tao-L and Tao-S can have distinct effects on cell shape by inducing lamellapodia and filopodia, respectively. Thus, Tao-1 can affect both microtubule and actin cytoskeletons by Tao-L and Tao-S.

The function of Tao-1 in organ growth depends on its kinase activity (56). Therefore, it is likely that Tao-L plays a major role
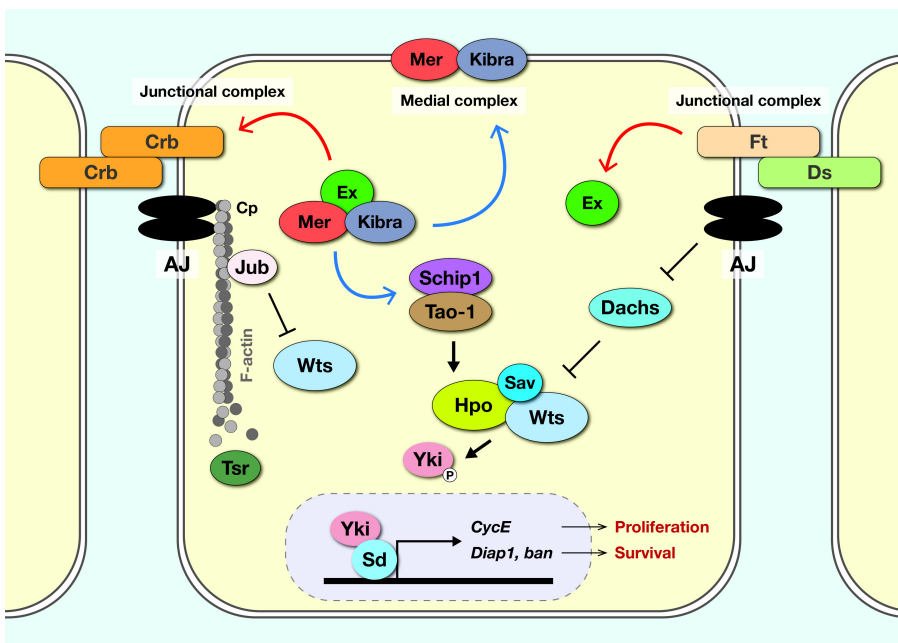
Fig. 2. Overview of the Hippo signaling pathway in *Drosophila*. Key steps of Hippo signaling. (1) Membrane proteins Ft and Crb regulate junctional localization of Dachs and Ex-Mer-Kibra, respectively. (2) Ex-Mer-Kibra function together to activate the Hippo kinase cascade. Mer and Kibra can form a complex at cell junction (Junctional complex) and at apical medial region (Medial complex). Ex binds to Schip1 which then promotes Tao-1 kinase activity for Hpo phosphorylation. (3) Hpo-Wts core kinase cascade. Activated Wts phosphorylates Yki to block its nuclear entry. F-actin formation and Yki activation are negatively regulated by capping proteins (Cp) and Tsr/cofilin. (4) When Wts is inactive, Yki induces target gene expression to promote cell proliferation and cell survival.

in activating Hpo for growth suppression. However, it is unknown whether both Tao-L and Tao-S are expressed in imaginal discs to affect microtubule and actin cytoskeletons or whether they act in an antagonistic manner. Tao-1 kinase is required for the restriction of larval brain size by regulating neuroblast proliferation through Hippo signaling (61). Reduction of Mer causes enlargement of the adult head that can be suppressed by Schip1 overexpresssion (58, 62). It needs to be determined whether effects of Mer and Schip1 on head size depends on Tao-1 function. Like Tao-1, Mer is also associated with microtubules. Recent studies have shown that Hippo components are required for microtubule polarity, axial patterning, and asymmetric cell division in developing tissues (63-65). Hence, functional relationship between microtubules and Hippo signaling is an intriguing topic to be explored in the future.

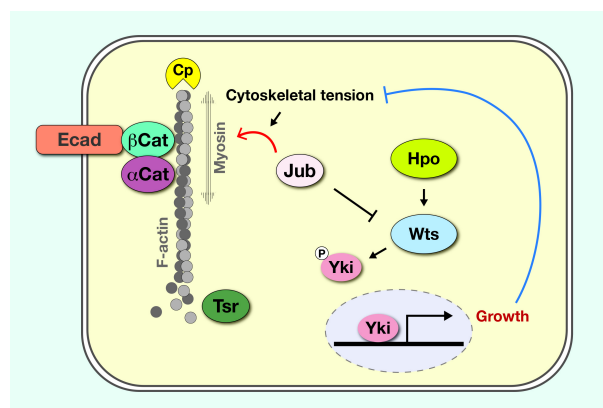## ACTIN CYTOSKELETON IN HIPPO SIGNALING

Crb is essential for epithelial integrity by regulating adherens junctions (AJs). The FERM domain of Ex can bind to the FBM region of the intracellular domain of Crb (39, 40). This raises the possibility that actin cytoskeleton associated with the FERM domain might be involved in the regulation of Hippo signaling.

Multiple proteins involved in actin dynamics have been identified as regulators of the Hippo signaling pathway in *Drosophila*. Loss of actin capping protein alpha (Cpa) and beta (Cpb) or overexpression of an active form of a formin family protein Diaphanous induces F-actin formation and overgrowth in imaginal discs (66). F-actin-induced overgrowth depends on Yki activity. Importantly, tissues overgrown by excess F-actin show relatively normal cell polarity, suggesting that F-actin accumulation has direct effects on tissue growth by modulating the Hpo pathway rather than through disrupting epithelial cell polarity. The role of F-actin in Hippo signaling seems to be evolutionarily conserved based on the findings that increased F-actin assembly or mDia overexpression causes activation of mammalian Yap activity (66).

Independent studies have found similar roles of actin dynamics. *cpb* mutant clones in wing discs show overgrowth without causing any defects in cell polarity (67). Interestingly, clone boundaries are smooth and round, suggesting that mutant clone cells and adjacent wild-type cells have different tension and affinity properties. This seems to be consistent with previous observation showing that junctional tension is modulated by loss of tumor suppressors in mutant clones (68). Capulet (Capt) is a cyclase-associated protein that inhibits actin polymerization by sequestering actin monomers. Knockdown of Capt results in F-actin accumulation and increases Yki target gene expression. *Drosophila* cofilin encoded by *twin star* (*tsr*) severs actin filaments by depolymerizing actin from the pointed end of F-actin. Knockdown of Tsr by RNAi potently increases the Yki transcriptional activity in cell culture assay

(66). Clones of weak *tsr* mutant alleles in imaginal discs show increased F-actin accumulation as in *cpb* mutant clones without inducing overgrowth or activation of Yki target genes. This suggests that accumulation of F-actin is insufficient to induce Yki activation (67). However, clones generated by stronger *tsr* alleles display F-actin accumulation and upregulation of *ex-lacZ* reporter, suggesting Yki activation (69). Cells mutated in *tsr* or *cpa* show basal extrusion from the epithelia, suggesting that both genes are required for the maintenance of epithelial integrity. Despite similarities between effects of *cpa* and *tsr* mutations, they have apparent differences. In contrast to increase in Arm level of *cpb* LOF mutant clones, reduced Tsr causes strong reduction of Arm and extensive cell death. Hence, Tsr not only restricts cell proliferation, but also promotes cell survival, consistent with the observation that *tsr* RNAi induces overproliferation when cell death is blocked by expressing baculovirus cell death inhibitor gene *p35* (69). In contrast, although Cpa is required for cell survival, dying cells are not rescued by p35 overexpression, indicating that dying cells in *cpa* mutants are independent of programmed cell death (70).

Mechanisms underlying tissue overgrowth triggered by misregulation of F-actin have not been clearly identified. Reduction of Cpa does not affect subcellular localization of Ex, Mer, or Hpo, but increases nuclear localization of Yki. Phenotypes of *ex* mutants can be rescued by Hpo overexpression, but not by active Dia, suggesting that F-actin acts in parallel with Ex and Hippo to regulate the localization of Yki (66). Loss of Ex, but not Mer, reduces the level of F-actin in the wing disc (67), supporting that Ex regulates actin cytoskeleton *in vivo* by binding to F-actin. On the other hand, clonal loss of *hpo*, *sav*,

Fig. 3. Role of actin cytoskeleton in Hippo signaling. Cell adhesion is regulated by Ecad, β-catenin (βCat), and α-Catenin (αCat) at the adherens junction. α(Cat) interacts with actomyosin networks. Under cell tension, Jub is apically localized with Wts to inactivate Wts, increasing Yki activity for growth. Fast growth reduces cytoskeletal tension by feedback to decrease junctional localization of Jub and Wts, thus decreasing Yki activity.

or *wts* induces F-actin accumulation, implying the presence of negative feedback regulation between F-actin and the Hpo kinase cascade activity (67). How Hpo pathway activity regulates F-actin and *vice versa* are intriguing issues to be studied further.

Cells in developing tissues are influenced by extracellular environments including neighboring cells. Cells respond to environmental changes by sensing mechanical forces and transmitting surface tension signals into cells. Mechanical conditions of a tissue can affect the progression of diseases. Loss of tensional homeostasis can enhance cancer risk (71). Recent studies in *Drosophila* and mammals have provided strong evidence that cell tension affects organ growth by modulating Hippo signaling (72-74). In this process, actin cytoskeleton and its regulators play key roles in mediating cell tension signals. In the wing disc, a LIM domain protein Ajuba (Jub) plays a role in relation to cytoskeletal tension and tissue growth (75-79). α-Catenin, a key factor for mechanosensing and adherens junction development (80, 81), is required for localizing Jub to junctions (Fig. 3). Jub is increased at compartment boundaries that are sites of increased tension between two different groups of cells. Reducing tension by depleting Rho-associated kinase (ROCK) encoded by *rho kinase* gene decreases the level of apical Jub. Conversely, enhancing tension by overexpression of ROCK or an active form of the regulatory light chain of Myosin II encoded by *spaghetti squash* (*sqh*) increases apical Jub (77). Apical Jub recruits Wts to AJs, thereby inhibiting Wts function in a tension-dependent manner.

Zyxin (Zyx) is another LIM-domain protein associated with actin. Zyx is recruited to actin compromised by mechanical stress. It repairs actin fibers by recruiting actin polymerase Enabled (Ena) (82). This interaction is mediated by the L/FPPPP motif of Zyx (83). RNAi-based studies have suggested that Zyx functions downstream of Ft independent of Ex (84). However, analysis using *Zyx* null mutants has provided evidence that Zyx antagonizes Ex (83). Although *Zyx* null mutants are viable and fertile, they display smaller body sizes than normal ones. *Zyx* LOF phenotypes are suppressed by *ex* LOF mutation, but not by *ft* LOF mutation. Phenotypes of Ex overexpression can also be suppressed by Zyx overexpression, supporting that Zyx antagonizes Ex (85). These studies have led to a model that Zyxin can promote tissue growth by binding to Ena, thus facilitating F-actin polymerization. On the other hand, Ex and Cpa/Cpb capping proteins inhibit tissue growth by preventing actin polymerization. Thus, the antagonistic relationship between Zyx and Ex on Yki activation appears to be mediated through their opposing effects on F-actin polymerization (83). In contrast to the role of Jub in Wts inhibition, function of Zyx in promoting Yki activation is independent of Wts. This seems to be consistent with mammalians studies showing that YAP regulation by mechanical stretching in mammalian cells is independent of LATS (72, 86, 87).

Spectrin is part of the cytoskeleton that supports the integrity of cell structures. *Drosophila* encodes one α subunit (α-Spec) and two β subunits (β$_{Heavy}$-Spec or β-Spec) that generate two heterodimers: αβ and αβ$_H$. α-Spec is localized along the apical basal membrane whereas β- and β$_H$-Spec are localized at basolateral and apical membrane, respectively (88). Cell tension by stretching of wing disc reduces Crb and apical Spec, resulting in increased Yki activity and organ size. In contrast, compression of wing tissue by overexpression of Crb reduces Yki activity (89). Apical β$_H$-Spec encoded by *karst* gene binds to FERM domain proteins such as Ex and Mer to inhibit Yki activity. Thus, spectrin is required as mechnosensor and upstream regulator of Yki in several tissues, including the eye, wing, and oocyte. The link between spectrin and Hippo signaling varies depending on tissues. Basolateral β-Spec is essential for Yki repression in ovary and intestinal epithelium. However, apical α-Spec is dispensable (90). In contrast, loss of α-Spec increases organ size and expression levels of Yki target genes in eye and wing discs (91). Interestingly, loss of α-Spec causes increased phosphorylation of myosin II light chain (MLC), suggesting that α-Spec affects Yki signaling by modulating actomyosin activity in parallel with the Crb-Ex pathway. Spectrin is also essential for YAP regulation in response to cell density in human cells (89).

Epithelial cells in growing organs are subjected to mechanical tension. In normal wing disc development, cell proliferation occurs relatively evenly without distortion of the epithelia, although secreted growth signal such as Decapentaplegic (Dpp)/TGFβ is locally expressed along the anterior-posterior boundary. Mechanical feedback hypothesis has been proposed to explain such homeostatic regulation of growth in developing organs. In this hypothesis, higher proliferation signaling in fast growing cells are counterbalanced by higher compression and reduced mechanical tension as more cells are produced. A recent study has provided evidence that supports this hypothesis *in vivo* (92). In normal development, fast growth induced by local mitogenic signals results in a mechanical feedback which activates Hippo signaling by reducing the level of junctional Jub and Wts (Fig. 3). However, when mechanical feedback is blocked by overexpressing Yki target gene *ban* or depleting Wts, clones of fast-growing cells will lead to increased cell proliferation, consistent with the proposed model.

CONCLUDING REMARKS

Hippo signaling has emerged as a major conserved mechanism for controlling organ growth. This review is mainly focused on upstream paths that lead to the regulation of Hpo kinase cascade. Hpo signaling is activated by two upstream paths involving Ft and Crb. Crb function is in part relayed to Hpo through a complex of Ex, Mer, and Kibra and Schip1-Tao1 activity. Functions of Ex, Mer, and Kibra are redundant or independent in different tissues. Thus, regulation of Hippo signaling in cellular and developmental contexts is an

important issue.

Hippo signaling is also modulated by cytoskeletons. It remains to be studied how actin and spectrin cytoskeleton regulates apical-basal localization and levels of Hippo upstream components. Homeostatic control of growth signaling is critical for organ development. Molecular mechanisms for sensing extracellular environments and transducing cytoskeletal tension to Hippo signaling merit further studies. Roles of microtubules associated with Hippo components are also open questions. Lastly, cross-talks between the Hippo pathway and other signaling networks are important for organ development. *Drosophila* is an excellent system for genetic dissection of such complex interactions *in vivo*. Studies in flies and mammals are complementary in providing novel insights into growth regulation.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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