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Yorkie: the final destination of Hippo signaling

Hyangyee Oh and Kenneth D. Irvine¹

Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway NJ 08854 USA

Abstract

The Hippo signaling pathway is a key regulator of growth during animal development, while loss of normal Hippo pathway activity is associated with a wide range of cancers. Hippo signaling represses growth by inhibiting the activity of a transcriptional co-activator protein, known as Yorkie in *Drosophila* and Yap in vertebrates. In the five years since the first report linking Yorkie to Hippo signaling, intense interest in this pathway has led to rapid increases in our understanding of the action and regulation of Yorkie/Yap, which we review here. These studies have also emphasized the complexity of Yorkie/Yap regulation, including multiple, distinct mechanisms for repressing its transcriptional activity, and multiple DNA-binding partner proteins that can direct Yorkie to distinct downstream target genes.

Yorkie/Yap, the key effector of growth control by Hippo signaling

The control of growth is among the most fundamental aspects of development. A comprehensive understanding of what dictates the size of a particular organ (how does the liver “know” how large it should be), or why a mouse is small and an elephant is large, remains elusive. Nonetheless, insights into how growth is controlled have come from the identification and characterization of intercellular signaling pathways that are required for the normal control of organ growth. Many of these signaling pathways are highly conserved among different phyla. Consequently, studies in simple model systems like *Drosophila* have had a profound influence on our understanding of how organ growth is controlled in humans. Moreover, since in both *Drosophila* and mammals dysregulation of signaling pathways that control growth can result in tumor formation, characterization of these pathways is of fundamental importance to oncology.

Over the past several years, studies of growth control in *Drosophila* have led to the identification of a new signaling pathway that controls growth^{1, 2}. This pathway has been most commonly referred to as “Hippo signaling”, although it is also referred to by a variety of other names, including Fat signaling, Warts signaling, and Salvador-Warts-Hippo (SWH) signaling. The identification of this pathway came about largely through identification and linkage into a common pathway of a series of *Drosophila* tumor suppressor genes. To date, ten tumor suppressors (*warts (wts)*, *hippo (hpo)*, *salvador*, *mob-as-tumor suppressor (mats)*, *expanded (ex)*, *Merlin (Mer)*, *kibra*, *discs overgrown (dco)*, *fat*, and *dachsous*) have been linked to Hippo signaling. Upstream regulation of this pathway is complex, as it encompasses three interconnected branches (Fig. 1). However, these upstream branches all converge and act through a common downstream component, the transcriptional co-activator

¹ Corresponding author, irvine@waksman.rutgers.edu.

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protein Yorkie (Yki). Yki can act as an oncogene, and its potent growth-promoting activity is normally kept in check by the upstream tumor suppressor proteins. Initial studies of Yki identified a simple mechanism for this negative regulation, in which the Warts kinase blocked Yki activity by phosphorylating it³, and other upstream components influenced the levels and activity of Warts^{1, 2}. However, recent studies have begun to uncover increasing complexity in the regulation of Yki and its mammalian homologues, Yap and Taz. This complexity includes distinct roles for multiple phosphorylation sites, phosphorylation-independent regulation, effects on expression and stability, and the identification of additional DNA-binding partner proteins (Fig. 2). In this review, we discuss recent developments in our understanding of Hippo signaling, focusing on those advances that impinge on our understanding of the regulation and function of Yki/Yap.

Regulation of Warts/Lats activity

Crucial phosphorylation-dependant regulation of Yki is mediated by Warts³, a member of Nuclear Dbf2-related (NDR) family of Ser/Thr kinases^{4, 5}. The activity of Warts is controlled through a series of phosphorylation events. Warts is directly phosphorylated by Hippo (Hpo), a member of the Sterile-20 family of Ser/Thr kinases, in a reaction that is facilitated by the Salvador protein⁶. Hpo also phosphorylates the Warts co-factor Mob-as-tumor suppressor (Mats), which promotes Warts-Mats interaction, and thereby Warts activity, and Warts activation also involves autophosphorylation⁷.

Two related proteins, Lats1 and Lats2, have been identified as vertebrate homologues of Warts^{8, 9}, whereas Mst1 and Mst2 have been identified as homologues of Hpo^{6, 10-14}. However, a recent study of Yap regulation in mice indicates that additional kinases can influence Yap phosphorylation at its major regulatory site (Ser127). In liver cells mutant for both *Mst1* and *Mst2*, Yap phosphorylation was impaired, but Lats1 and Lats2 phosphorylation were not visibly affected, and instead a distinct kinase appeared to be involved¹⁵. This alternative kinase was not identified, but other members of the NDR kinase family are possible candidates. The same authors also found that even though Mst1 and Mst2 were required for Yap phosphorylation in liver cells, they were not required for Yap or Lats phosphorylation in embryonic fibroblasts, which implies that other kinases provide Mst function in these cells¹⁵. There are additional Ste-20 kinase family members in mice, but whether or not any of them are responsible for Lats activation in fibroblasts remains to be determined. Intriguingly, *hpo* mutant phenotypes are weaker than *wts* mutant phenotypes in some *Drosophila* tissues^{6, 16}, which is consistent with the possibility that additional Hpo-like kinases might also have a role in *Drosophila*. These observations emphasize two themes that are emerging as studies of Hpo signaling intensify – the increasing complexity of the pathway, and the existence of cell-type specific requirements for different components.

Hpo signaling can be regulated by cell contact, and is important for contact-dependent inhibition of proliferation in cultured mammalian cells and regulation of cell fate in the early embryo¹⁷⁻¹⁹. Although the mechanism of contact-dependent signaling remains poorly understood, insights into regulatory steps at the membrane are beginning to emerge. Three interconnected, upstream regulatory branches of Hippo signaling have been identified: Fat-dependent, ex-dependent, and Mer-dependent (Fig. 1)². Fat is a transmembrane receptor protein, and both Fat and its ligand Dachsous are large, atypical cadherins. Dachsous promotes a phosphorylation of the Fat cytoplasmic domain, which is mediated by the CKIε protein Dco^{20, 21}. Fat influences downstream events in Hippo signaling by antagonizing the activity of the myosin Dachs, which accumulates at the membrane in the absence of Fat activity^{16, 22, 23}.

Ex and Mer are members of the FERM-domain containing protein family, which associate with both the membrane and the actin cytoskeleton²⁴. Ex and Mer function in a partially redundant manner to regulate Hippo signaling^{25, 26}. Recently, a new player in the Ex- and Mer-dependent branches has been identified, Kibra²⁷⁻²⁹. Kibra interacts physically and genetically with both Mer and Ex. Kibra binds to Mer and Ex through distinct protein motifs, and the three proteins can form a trimeric complex. Mer and Ex can also bind directly to Sav and Hpo, respectively, consistent with the notion that a regulatory complex among these tumor suppressors forms at the membrane²⁷. Studies of Mats have also emphasized the importance of membrane association for regulation of Wts. A fraction of Mats normally localizes to the membrane, and forced localization of Mats to the membrane induces Wts activation, even in the absence of Hpo³⁰. Similarly, forced membrane localization of human MOB proteins can activate NDR/Lats kinases in cultured mammalian cells^{31, 32}. Thus, although the biochemical mechanisms by which Mer or Ex regulate Wts activation requires further investigation, it seems that activation occurs in a protein complex at the cell membrane.

Wts/Lats-mediated regulation of Yki/Yap localization

Investigations of the regulation of Yki/Yap by Wts/Lats identified Ser168 of Yki (Ser127 of Yap) as a key site^{18, 33-35}. Phosphorylation of this Ser enables interaction with 14-3-3 proteins, which promote cytoplasmic retention and nuclear export, and phosphorylation of Yki/Yap decreases its nuclear localization. The expectation that 14-3-3 proteins should thus be functionally involved in Yki regulation has recently been confirmed by knock-down experiments in *Drosophila*³⁶. These authors also showed that both wild-type and phosphorylation site mutant isoforms of Yki are subject to CRM1-mediated nuclear export, which implies that normal pathway regulation may involve additional, as yet undefined, nuclear export processes.

Although Ser168/127 of Yki/Yap is a crucial Wts/Lats phosphorylation site, there are two additional Wts sites within Yki (Ser111 and Ser250)^{36, 37}, and four additional sites within Yap (Ser61, Ser109, Ser164 and Ser381)^{18, 38}. Mutations of Yki Ser111 and Ser250 give mild overgrowth phenotypes on their own, and reduce Ser168 phosphorylation, which presumably contributes to their influence on Yki activity, and implies that Yki phosphorylation is cooperative. However, since combinations of mutations in the different sites result in additive phenotypes, each site also makes independent contributions to Yki regulation^{36, 37}. In the case of Yap, mutation of all five sites results in stronger activation than just mutation of Ser127^{18, 39}, but most of this can be accounted for by the site at Ser381^{39, 40}.

Unlike Ser168/127, the other Wts/Lats phosphorylation sites lack consensus 14-3-3 binding motifs, and biochemical and genetic experiments argue against a role for 14-3-3 proteins in regulating Yki/Yap through these sites^{18, 33, 34, 36, 37}. They do influence Yki/Yap localization, which might be mediated through interactions with additional binding partners. Alternatively, they might act through distinct mechanisms, such as the effect of Ser381 of Yap on protein stability⁴⁰.

Phosphorylation-independent regulation of Yki localization by upstream tumor suppressors

Protein-protein interactions mediated by binding between WW-domains and Pro-rich motifs are found in a range of biological processes (see Box 1), but appear to be especially common in Hpo signaling. Recent studies have implicated direct binding of Yki to Ex, Wts or Hpo, mediated by WW domains of Yki and PPxY motifs of Ex, Wts or Hpo, in a

phosphorylation-independent mechanism of Yki repression^{41, 42}. Over-expressed Ex or Wts can repress Yki mediated transcriptional activation, *in vivo* and S2 cell experiments, even when all of the Wts phosphorylation sites on Yki are mutant^{36, 37, 41}. Moreover, this repression requires PPxY motifs of Ex and Wts^{41, 42}. A fraction of Yki localizes to the sub-apical membrane in an Ex-dependent fashion^{41, 42}, and this apical localization of Yki requires its WW domains⁴³.

Assessing the respective contributions of phosphorylation-dependent versus phosphorylation-independent repression *in vivo* is complicated by the fact that both modes are mediated by the same upstream tumor suppressors, and by the dual positive and negative roles of the WW domains in Yki regulation (discussed below). Although phosphorylation-independent repression has not yet been demonstrated in vertebrates, its discovery in *Drosophila* suggests a dual repression model, with the same upstream tumor suppressors repressing Yki activity by two distinct mechanisms, and emphasizes the importance of the relative levels of Yki/Yap to its upstream tumor suppressors. As discussed below, these two themes also apply to the regulation of Yap in vertebrates.

Additional mechanisms of Yki/Yap Regulation

In addition to Wts-mediated phosphorylation of Yki and Yap, there is also evidence for additional forms of phosphorylation-mediated regulation. Two Ser residues near Ser168 of Yki (Ser169 and Ser172) are highly conserved in Yap, and mutating these to Ala in Yki elevated phosphorylation of Ser168 *in vivo*, whereas phosphomimetic mutations (Ser to Asp) inhibited Ser168 phosphorylation³⁷. Although the functional significance of phosphorylation at these sites has not been directly demonstrated, a phosphoproteomic analysis of *Drosophila* embryos established that at least one, and possibly both, of these sites can be phosphorylated *in vivo*⁴⁴. Interestingly, this study also identified several additional amino acids of Yki that can be phosphorylated, the significance of which remains to be determined.

Mass-spec analysis of Yap expressed in cultured cells identified five phosphorylation sites (Thr63, Ser138, Ser289, Ser351, and Ser384) in addition to the five Lats sites⁴⁰. Notably, Ser384 is phosphorylated by Casein kinase I in a reaction that is dependent upon a priming phosphorylation of Ser381 by Lats⁴⁰. Phosphorylation of Ser384 then recruits an E3 ubiquitin ligase complex to promote Yap degradation. This study thus identified an additional mechanism by which Wts-mediated phosphorylation negatively regulates Yap. The existence of this mechanism *in vivo* is supported by the observation that Yap levels are elevated in the livers of *Mst* mutant mice^{15, 45}.

The frequency with which the Yap locus is amplified and/or Yap levels are elevated in human cancers emphasizes the importance of Yap levels to tumorigenesis^{34, 46-50}. Although transcriptional regulation of Yap has not received much attention so far, a recent study identified Yap as a key mediator of Shh-associated medulloblastoma⁴⁶. In addition to increased nuclear localization, the authors found that Yap expression is transcriptionally upregulated in Shh-driven medulloblastoma, which should stimulate a greater focus on transcriptional regulation of Yki/Yap in other contexts.

Mammals have a single Yap locus, but through alternative splicing it can generate three different polypeptides, Yap1 and Yap2, and Yap2L⁵¹. These isoforms are structurally related, and can be regulated by Hpo signaling^{18, 34, 38, 52, 53}, but Yap2 isoforms differ in the inclusion of a C-terminal PDZ binding domain, which is also found on the Yap-related gene Taz⁵⁴. The PDZ domains are important for Yap2 and Taz activity and regulate their nuclear localization^{54, 55}, which thus identifies another mode of Yap/Taz regulation.

Drosophila Yki does not have a C-terminal PDZ domain, so this form of regulation may be specific to vertebrates.

Role of the WW domains in regulating Yki/Yap activity

Yki was identified as an interactor and substrate of Wts³. Although it was initially assumed that this binding would be required for Yki/Yap phosphorylation by Wts/Lats, subsequent studies have revealed that this is not the case^{37, 39}, raising the possibility that it may serve other functions. Indeed, as noted above, the PPxY motifs of Ex and Wts are required for phosphorylation-independent repression^{41, 42}. However, if the WW domains of Yki were only required for Yki repression, then mutation of these domains should result in elevation of Yki activity, but instead the opposite occurs—mutation of the WW domains abolishes the activity of Yki^{34, 37, 43}. Since the WW domains are not required for nuclear localization of Yki, yet they are required for its transcriptional activity, it appears that the WW domains may recruit an as yet unidentified PPxY-motif containing transcriptional co-factor to Yki. Indeed, even when Yki was bound to the promoter of a synthetic reporter gene, using a Gal4-DNA binding domain-Yki fusion protein, the WW domains were required³⁷. WW domain-PPxY interactions play multiple roles in Hippo signaling (see Box 1), and the dual role of the WW domains, facilitating repression of Yki in the cytoplasm, but participating in its activity in the nucleus, raises the possibility that competitive binding between PPxY-containing proteins for Yki might contribute to Yki regulation.

In the case of Yap, the requirement for the WW domains appears to be more complex. They are required for transcriptional activation by Yap with some DNA binding partner proteins, and on some target genes, but not others^{39, 43, 53}. For example in they are required for transformation of NIH3T3 cells, but in breast epithelial cells, Yap without WW-domains was even more potent at promoting cellular transformation than wild-type Yap⁴³, which implies that in this context, the major function of the WW domains is to contribute to Yap repression. Such cell-type specific responses presumably reflect the differential expression and regulation of Yap partner proteins in different cells.

DNA binding co-factors and targets of Yki/Yap

Yki/Yap function as transcriptional co-activators, and lack their own DNA binding domain. Before Yap was linked to the Hpo pathway, several different DNA binding partners for it were identified^{56, 57}. However, the biological significance of many of these interactions was unclear, and it was not known which might function as partners for Yki/Yap's role in Hpo signaling. This began to be addressed with the discovery that Sd could function as partner for Yki in Hpo signaling in *Drosophila*, and its mammalian homologues the Tead/Tef proteins could function as partners for Yap in Hpo signaling in vertebrates^{19, 35, 58-61}. The significance of Sd/Tead as Yki/Yap co-factors was further solidified by the demonstration that they bound directly to regulatory sequences of a known Yki target gene, Diap1 (*thread*)^{35, 58}, and a known Yap target gene, *Connective Tissue Growth Factor* (CTGF)⁶⁰.

The identification of Sd as a partner for Yki in *Drosophila* was unexpected, because most *Drosophila* tissues that require *yki* for normal growth don't require *sd*^{62, 63}. Recently, a new DNA-binding partner for Yki, has been identified, Homothorax (Hth)⁶⁴. Together with two other DNA-binding proteins (Extradenticle and Teashirt), Hth regulates the proliferation of uncommitted progenitor cells in the eye imaginal disc. Genetic experiments revealed that Yki and Hth require each other to promote growth in the eye, and also interact physically. Hth does not detectably influence expression of Diap1, but instead the authors identified another Yki target, the micro RNA gene *bantam*^{65, 66}, as a direct target of the Yki:Hth complex⁶⁴. The identification of both Sd and Hth as DNA binding partners of Yki thus

reveals a surprising complexity in Hpo signaling. On the one hand, since they are expressed in different patterns, it suggests that one resolution to the limited requirements for *sd* may be that Yki interacts with multiple, distinct partner proteins in different tissues. However, this raises the question of how Yki executes a common developmental function, promoting tissue growth, in combination with distinct DNA binding proteins that appear to regulate distinct sets of genes.

Another class of Yki/Yap partners that have been further characterized recently are the Smad proteins, which function as transcription factors for TGF β signaling pathways⁶⁷. Smad7 was identified as a Yap-binding protein several years ago⁶⁸. More recent studies have identified Smad1 as a Yap partner⁶⁹, Mad as Yki partner⁶⁹, and the Smad2/3-4 complex as a Taz partner⁷⁰. These recent studies focussed on the ability of Yap or Taz to potentiate transcriptional activation by Smads to regulate Smad target genes. The significance of these interactions to growth control by Hippo signaling remains to be determined, but their detection identifies the potential for cross-talk between TGF β and Hippo signaling pathways.

The interaction between Yki/Yap/Taz and Smads is partially dependent upon WW-domains in Yap-related proteins and PPxY motifs in SMADs⁶⁸⁻⁷⁰. In addition to the Smads, several other transcription factors have been reported to interact with Yap through WW-domain-PPxY interactions^{56, 57}. These partners appear to utilize Yap to execute diverse functions. For example, while activation of Yki/Yap in Hpo signaling inhibits apoptosis, Yap can have a pro-apoptotic role in conjunction with p73 under some conditions^{53, 71}, and this pro-apoptotic activity of Yap can also be regulated by Hpo signaling⁵³.

The Yki/Yap partners that have been linked to its role in Hpo pathway-regulated growth control, namely Sd/Tea and Hth, lack PPxY motifs. Sd/Tea interacts with an N terminal region of Yki/Yap that is well separated from its WW domains (see Box 1 Figure)^{35, 58, 59, 72}. Thus, different regions of Yki/Yap associate with different DNA binding partners. One question that needs further study is how Yki or Yap interact with distinct partners to mediate transcriptional activation. For example, since Yki/Yap appear to employ their WW domains to recruit factors required for transcription when they are bound to Sd/Tea, when complexed with DNA binding partners that contain PPxY motifs, do they still employ one of their WW domains to recruit other transcription factors, or do they employ a distinct mechanism of transcriptional activation? The observation that at least in some contexts the WW domains of Yap are not required for transcriptional activation suggests that Yki/Yap may be able to act as a versatile scaffold to assemble distinct transcriptional regulatory complexes.

Another key question is how the association of Yki/Yap amongst distinct DNA-binding partners within the same cell is modulated. Related to this is the identification of downstream target genes regulated by Yki/Yap. A comprehensive discussion of target genes is beyond the scope of this review, but we note that even in *Drosophila*, which seems to have fewer DNA-binding partners for Yki/Yap than vertebrates, not all downstream targets are related to growth control, as exemplified by the recent discovery that Yki activity influences the apical membrane of epithelial cells^{73, 74}. Moreover, even in the context of its growth control functions, we can consider distinct types of downstream target genes, as both autonomous and non-autonomous effects are important. Autonomous growth promoting targets of Yki/Yap include genes that promote cellular growth (*Myc*, *bantam*), genes involved in cell cycle progression (*CyclinB*, *CyclinD*, *CyclinE*, *E2F1*), and inhibitors of apoptosis (*Diap1*, *clap1*)^{6, 34, 59, 65, 66, 75-79}. Non-autonomous growth promoting targets include genes that encode ligands for other signaling pathways. In *Drosophila*, the Wnt pathway ligand Wg is regulated by Hippo signaling in the proximal wing, where it is a major

contributor to growth regulation^{16, 23}. The Notch pathway ligand *Serrate*, and the EGF-R pathway ligand *vein*, can also be regulated by Yki in some contexts^{16, 22, 80}. Additionally, the glypicans *Dally* and *Dally-like*, which influence the movement of secreted signals through tissues, can be regulated by Yki⁸¹. Studies in mammalian cells identified *Connective Tissue Growth Factor (CTGF)* as a Yap target^{52, 60}, and the importance of non-autonomous growth promoting targets in vertebrates has been emphasized recently by the discovery that the ability of Yap to transform breast epithelial cells is dependent upon its ability to upregulate expression of the EGFR ligand *amphiregulin*⁸⁰. Thus, the biological effects of Yki/Yap on growth control *in vivo* appear to reflect a combination of autonomous and non-autonomous effects, which may vary in different cell types.

Conclusions and future directions

Hpo signaling is an evolutionarily conserved signaling pathway that controls organ size from flies to humans. The novelty of the pathway, and its fundamental importance to growth control and oncogenesis, has attracted many researchers to the field, and the pace of discovery has been rapid. These discoveries have identified sophisticated, multi-layered mechanisms for controlling Yki/Yap activity. Nonetheless, many steps in Hippo signaling remain poorly understood, and it is likely that core components are still missing. For example, despite the fundamental importance of kinases and protein phosphorylation at multiple steps of Hippo signaling, phosphatases that act on pathway components have not yet been identified.

As emphasized by the existence of multiple DNA binding partners for Yki and Yap, a particularly important challenge for the future will be to elucidate the logic of the transcriptional regulatory network relevant to Hpo signaling. What is the full complement of Yki/Yap partner proteins for transcriptional activation? How is the availability or activity of the partner proteins controlled, and what modulates the association of Yki among different possible partners within the same cell? How are different partner proteins employed to effect common or distinct transcriptional responses to Hpo signaling in different cell types? The answers to these questions should provide new insights into growth control during development, and could, in the future, help to guide diagnoses and therapies and for cancers or other diseases involving dysregulation of Hpo signaling.

BOX 1

WW domain – PPxY interactions in Hpo signaling

WW domains are 35-40 amino acid protein-protein interaction domains, which are named for their pair of conserved Trp residues⁸², and which generally interact with Pro-rich sequence motifs. WW domain-Pro motif interactions are found among proteins that function in a wide range of biological processes, including cytoskeletal regulation, signal transduction, protein trafficking, protein degradation, and transcriptional regulation^{83, 84}. They appear to be especially common in Hpo signaling. WW domains have been classified into different subgroups based on either on differences in sequence composition of the WW domain, or in the sequence of the Pro-rich motif to which they bind^{83, 84}. Most of the WW domains found in Hpo pathway components are of the group I type, which bind to PPxY sequence motifs. Three core components of Hpo signaling (Yki, Kibra, and Sav) contain WW domains, whereas three other components (Wts, Ex, and Hpo) contain PPxY motifs (see Figure). Moreover, several DNA binding transcription factors have been identified that contain PPxY motifs and interact with WW domains of Yap or Taz^{56, 57}. The existence of binding between many of the possible WW-PPxY pairs within the pathway has been confirmed, at least in cultured cells. Recently it has been reported that certain atypical WW domains, found in Sav and Kibra,

can dimerize, which can mediate homodimerization of Sav or heterdimerization between Kibra and Sav^{27, 85}. The prevalence of this protein interaction module among pathway components suggests that sequential or competitive interactions amongst proteins with these motifs might be important. Indeed, competitive binding between Ex and Wts for Yki was demonstrated by titration experiments in S2 cells⁴². Moreover, interaction between Mer and Kibra does not involve WW-PPxY interactions, but was enhanced when the WW-domains of Kibra were deleted²⁹, which suggests that interactions between Mer and Kibra are impeded by other WW-domain interacting proteins. Figure I depicts linear schematics of *Drosophila* components of the Hpo pathway with WW domains and PPxY motifs, and also indicates the location of other key motifs in these proteins (WW* = atypical WW domain that can dimerize, C2 = C2 domain, Sarah = Sarah domain, NH = Sd binding domain, P = Wts phosphorylation sites).

Figure I.
Linear schematics of *Drosophila* components of the Hpo pathway

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Figure 1. The Hippo signaling pathway

Schematic depictions of the regulatory interactions among genes linked to Hippo signaling. Pointed arrows indicate a positive regulatory connection, blocks indicate an inhibitory regulatory connection. Genes depicted in red can function as tumor suppressors (loss of function mutations can cause overgrowth), genes depicted in green can function as oncogenes (over expression and/or activation can cause overgrowth), genes depicted in black have not been shown to function as tumor suppressors or oncogenes. At the bottom, genes depicted in gray encode DNA binding proteins that can partner with Yki/Yap to influence transcription of downstream target genes. Hippo signaling influences growth by influencing the transcription of target genes, but transcriptional targets that are not directly related to growth control have also been identified. A) Depicts genes and regulatory links identified in *Drosophila*. B) Depicts some of the genes identified in mammals. The Fat regulatory branch is shaded by a grey box to indicate that it is not yet known whether these genes actually influence Hippo signaling in mammals.

Figure 2. Multiple modes of Yki/Yap regulation

This schematic depicts some of the different ways in which Yki/Yap is known to be regulated within the cell, gray oval represents the nucleus. 1) Yki/Yap can be transcriptionally regulated; increased transcription has been implicated in medulloblastoma. 2) Phosphorylation of Yki/Yap by Warts/Lats (green arrows) creates a 14-3-3 binding site, which promotes cytoplasmic localization of Yki/Yap. Wts activity is regulated by Hpo-dependent phosphorylation (blue arrows). 3) Phosphorylation of Yap by Lats makes it a substrate for CKI phosphorylation, which recruits SCF, leading to Yap ubiquitination and degradation (small yellow boxes). 4) Direct binding of Yki to Wts and Ex can retain Yki in the cytoplasm. 5) Yki/Yap can be exported from the nucleus in a CRM1-dependent fashion. 6) Nuclear Yki/Yap can associate with distinct DNA-binding transcription factors to regulate distinct downstream target genes.