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http://dx.doi.org/10.1016/j.cub.2014.12.044

Growth Control: Re-examining Zyxin's Role in the Hippo Pathway

The Hippo pathway is a conserved regulator of organ growth that computes information from the cellular microenvironment. A new study examines the role of the Hippo pathway protein Zyxin and finds that it antagonises Expanded to modulate F-actin and organ size.

Kieran F. Harvey

Cellular signalling pathways commonly transmit information from the extracellular environment to the nucleus to modulate transcription and elicit a biological response. The Hippo pathway is one of the most recently identified and intensely studied signalling pathways. Unlike most pathways, which transmit information downstream of diffusible extracellular ligands that bind to transmembrane receptor proteins, the Hippo pathway appears predominantly to convey information about the 'cellular neighbourhood' of tissues [1–3]. Hippo signalling has been shown to be influenced by G-protein-coupled receptors [4], which are regulated by diffusible ligands, but is also controlled by transmembrane proteins that form ligand–receptor pairs between neighbouring cells (e.g. the Fat and Dachsous cadherins, Crumbs and Echinoid) [5]. The Hippo pathway also responds to key cell biological properties, such as cell polarity and cell adhesion [1–3]. In addition, this pathway is sensitive to mechanical properties of cells and tissues, and it has been touted as a key integrator of tissue mechanics, in the context of both organ size control and tumorigenesis [6]. In this role the Hippo pathway is thought to be controlled by the tensile state of the actin cytoskeleton [2,6].

Two of the best-studied upstream regulators of the Hippo pathway are Fat and Expanded. Fat engages in bidirectional signalling with its ligand Dachsous and signals via several proteins, including the atypical myosin Dachs and the casein kinase Discs overgrown [7,8]. Expanded forms complexes with multiple Hippo pathway proteins, including the upstream regulators Merlin and Kibra and the core pathway members Warts, Hippo, Salvador and Yorkie [9–11]. Expanded can repress Yorkie by direct binding and also by activating the kinase Warts, which phosphorylates and inhibits Yorkie [9–11]. Despite rapid advances in the past decade or so, many aspects of Hippo signalling are still shrouded in uncertainty.

In a study published in this issue of *Current Biology*, Gaspar *et al.* [12] address the role of Zyxin, a protein that has been independently linked to both Hippo signalling and mechanotransduction, and suggest that Zyxin might present a nexus between the two. Zyxin possesses a triple LIM domain that has been shown to mediate its association with focal adhesions and actin fibres. It also binds to the F-actin polymerisation factors Enabled and VASP in both *Drosophila melanogaster* and mammalian cultured cells [13,14]. Zyxin is recruited to F-actin that has been compromised by mechanical force and is proposed to induce actin fibre repair at least in part by recruiting Enabled/VASP [15]. Zyxin also controls tissue growth via the Hippo pathway [16]. Based largely on RNA interference studies, Zyxin had been proposed to function downstream of the Fat branch of the Hippo pathway, but independently of Expanded [16]. In particular, biochemical experiments showed that Zyxin bound to both Dachs and the key kinase Warts and that Zyxin functions with Dachs to limit Warts levels via an as yet unknown mechanism [16].

The genomic localisation of *D. melanogaster zyxin* (on the relatively small and less genetically tractable fourth chromosome) has hindered the ability to study this gene using traditional loss-of-function alleles. Gaspar *et al.* [12] have now overcome this challenge by using genome editing to generate null *zyxin* mutant flies, which allowed them to reappraise its role in Hippo signalling and tissue growth. Based on several phenotypes of *zyxin* null tissue, and the fact that *zyxin* overexpression strongly rescued phenotypes caused by *expanded* overexpression, they conclude that Zyxin has a major role in antagonising Expanded. Key data supporting their claims include the demonstration that *zyxin* loss strongly suppressed imaginal disc overgrowth and defects in eye differentiation associated with *expanded* loss but not *fat* loss, while *zyxin* overexpression more robustly counteracted growth retardation induced by *expanded* than that induced by *fat.* Perhaps most compelling is the finding that *zyxin* loss brought *expanded*, but not *fat*, mutant flies back to life [12].

It is difficult to fully reconcile the seemingly different results presented in this study and that from Rauskolb *et al.* [16], but it should be recognised that, whilst RNA interference has revolutionised the study of gene function in many organisms, including *D. melanogaster*, it has many

limitations. Two possible issues that could cloud *zyxin* RNA interference phenotypes are off-target effects and hypomorphism. It is formally possible that Zyxin functions downstream of both Fat and Expanded, however. Genetic epistasis studies can be misleading, especially when used to study interconnected upstream regulators of a signalling network that ultimately converge on the same downstream proteins. Therefore, further biochemical and cell biological analyses are necessary to fully define Zyxin's role in the Hippo pathway.

How does Zyxin function downstream of Expanded? Three main possibilities exist, based on the known roles of Expanded. Firstly, Zyxin could regulate the activity of the Hippo pathway core kinase cassette. This is possible but was not favoured as a major function of Zyxin by Gaspar *et al.* [12], as *zyxin* loss only partially suppressed overgrowth resulting from *hippo* loss, whilst Warts-dependent phosphorylation of Yorkie was unaltered in fly tissues overexpressing *zyxin* [12]. Given that Expanded binds to Hippo, Warts and Yorkie and regulates their phosphorylation [9–11], and that Zyxin has been reported to bind to Warts and influence Warts levels [16], this could be examined further. For example, it would be ideal to be able to monitor even subtle changes in Warts activity/levels in organs that overexpress or lack *zyxin*.

Secondly, Zyxin could interfere with Expanded's ability to bind directly to Yorkie and repress it. This possibility was ruled out, however, by the finding that *zyxin* loss strongly suppressed phenotypes associated with tissues that were nullizygous for *expanded* [12].

Thirdly, Zyxin could regulate Yorkie activity by modulating the actin cytoskeleton. Evidence for this possibility was presented by Gaspar *et al.* [12], who found that both Zyxin and Expanded influence each other's ability to modulate F-actin [12]. Given known links between actin and Hippo, they proposed that Expanded and Zyxin regulate Yorkie-mediated organ growth by antagonising each other's influence on F-actin. This assertion was supported by functional links between Zyxin and Enabled, a regulator of F-actin polymerisation. The Enabled-binding domain of Zyxin was required for it to suppress Expanded-induced growth retardation and to cooperate with Enabled to induce tissue overgrowth. Gaspar *et al.* [12] also showed that actin capping proteins, which block F-actin growth and are known to limit Yorkie-driven tissue growth [17,18], antagonised Zyxin's ability to stimulate tissue growth.

Thus, Gaspar *et al.* [12] favour a scenario whereby Zyxin modulates Yorkie independent of Warts, primarily by promoting F-actin assembly, a function that is counteracted by Expanded [12]. A related mechanism has been proposed for the regulation of Yorkie's mammalian orthologues YAP and TAZ. In human cultured cells, F-actin assembly promoted YAP/TAZ activity independent of the Warts orthologues LATS1 and LATS2 [19]. Furthermore, in uveal melanoma cells, hyperactive G proteins (G_q and G_{11}) were found to stimulate YAP activity via F-actin polymerisation, but independent of LATS1 and LATS2 [20]. Therefore, actin-dependent regulation of Yorkie/YAP/TAZ, independent of Warts/LATS1/LATS2, appears to be a conserved regulatory mechanism important for both normal and neoplastic tissue growth. Important future questions on the role of Zyxin in Hippo-pathway-dependent tissue growth include: when and where does Zyxin function in growing tissues? Is Zyxin regulated by mechanical properties of cells in growing and/or regenerating organs? And how does actin regulate Yorkie/YAP/TAZ independent of Warts/LATS1/LATS2?

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http://dx.doi.org/10.1016/j.cub.2015.01.019