

# Log of Research Documents

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1. Tumaneng, et al.: YAP mediates crosstalk between the Hippo and PI3K-TOR pathways by suppressing PTEN via miR-29.
  - YAP is main downstream target of mammalian Hippo pathway, promotes organ growth
  - YAP activates mTOR (major regulator of cell growth)
  - YAP is phosphorylated and inhibited by LATS
  - Cell density is known to regulate YAP phosphorylation and activity
  - Yorkie (*Drosophila*) induces expression of bantam microRNA that serves as a critical mediator of Yorkie's biological functions
  - Establishes a functional link between Hippo and TOR in mammals
2. Kockel, et al.: Dynamic Switch of Negative Feedback Regulation in *Drosophila* Akt-TOR Signaling
  - Three basic concepts of downregulating signaling pathways: control via specific inhibitory ligands/receptors, negative cross-regulation by distinct signaling pathways, auto-regulation by negative feedback mechanisms
  - TORC1 and TORC2 both participate in Akt-TOR signaling but act at different levels in the pathway and integrate distinct stimuli: TORC2 responds to growth factors and might determine substrate specificity of Akt, TORC1 mediates signaling by amino acids and cellular energy stress.
  - dAkt-TOR pathway in *Drosophila* regulates cell proliferation, developmental timing and sizing of cells, organs, and whole fly
  - *Drosophila* has only one dAkt gene (mammals have 3)
  - Phosphorylation of dAkt is regulated by negative feedback from Tsc1/Tsc2-TOR-S6K but independent of FoxO
  - Negative feedback regulating dAkt activity is independent of S6K under normal TORC1 activity or dependent on S6K when TORC1 activity is high. Thus, S6K is a sensor of TORC1 that provides additional suppression of the signal when TORC1 is highly active.
3. Zhao, et al.: The Hippo pathway in organ size control, tissue regeneration, and stem cell self-renewal

- Hippo pathway limits organ size by phosphorylating and inhibiting Yki, a key regulator of proliferation and apoptosis
- Hippo pathway is regulated by cell polarity, cell adhesion and cell junction proteins
- Core components of Hippo pathway: warts (wts), hippo (hpo), salvador (sav), all tumor-suppressor genes
- Wts directly phosphorylates and inhibits Yki
- Merlin (Mer) and Expanded (Ex) were found to activate the Hippo pathway
- Fat protocadherin, a cell surface molecule, is an upstream regulator of Hippo pathway. Its activity is regulated by binding to Dachshous (Dachs) and is modulated by Dco, Fj, and Lft. Dpp and Wg affect expression of Fj and Ds.
- Yki induces cycE and E2F1 (regulation of cell proliferation), EGFR ligands and Jak-Stat ligands
- An imbalance of Hippo pathway activity in neighboring cells may induce cell competition through differential expression of dMyc in Drosophila
- Mechanism by which upstream regulators of the Hippo pathway are integrated to initiate or terminate signaling is not yet fully understood

#### 4. Sun, Irvine: Cellular Organization and Cytoskeletal Regulation of the Hippo Signaling Network

- Cell-cell junctions serve as platforms for Hippo signaling by localizing scaffolding proteins that interact with core components of the pathway
- Hippo was first discovered in Drosophila through the identification and characterization of genes that when mutated cause severe overgrowth phenotypes
- Hippo signaling is influenced by or crosstalks with multiple pathways that respond to growth factors, that promote growth linked to positional information, or that influence growth in response to nutritional and metabolic status.
- Hippo signaling is also affected by contacts with neighboring cells and the ECM, and by mechanical forces.
- Yki is downregulated by phosphorylation by the kinase Wts which promotes cytoplasmic localization of Yki (doesn't allow it into the nucleus)
- Core of the Hippo network: four proteins that regulate Yki, Hpo, Wts, Sav, and Mats
- Dachs influences Wts protein levels and inhibits Wts association with Mats
- Mer promotes Wts activation by bringing Wts and Hpo together at cell membranes.
- cell-cell junctions are under tension in Drosophila epithelia, promoting Yki activity
- Outstanding questions: Are different core components of the Hippo signaling network regulated by different upstream inputs?; How are different cytoskeleton dependent forms of regulation integrated and coordinated?; What additional cellular sites of Hippo and Warts activation remain to be discovered?

5. Vidal, Cagan: *Drosophila* models for cancer research

- The excess proliferation observed in cancer can be attributed to both a deregulation of the mechanisms underlying cell growth and a loss of inducers of apoptosis
- Yorkie is normally inactivated by Warts and mediates the transcription of cyclin E and DIAP1 (*Drosophila* inhibitor of apoptosis 1)
- Border cells migrate as an epithelial patch without undergoing an EMT.
- In a homotypic environment where all cells in the tissue are scrib negative, cells display neoplastic growth and produce tumors
- In discrete clonal patches where scrib negative cells are mixed with wild type ones, the scrib negative cells undergo apoptotic death

6. Hemalatha, Prabhakara, Mayor: Endocytosis of Wingless via a dynamin-independent pathway is necessary for signaling in *Drosophila* wing discs

- Wingless forms a spatial gradient across the boundary and activates distinct concentration-dependent transcriptional programs ensuing coordinated tissue growth
- Study provides evidence for a mechanism wherein cells leverage multiple endocytic pathways to coordinate signaling during patterning
- Honestly not sure what the importance of this paper is in the scheme of looking at the *Drosophila* Hippo pathway

7. Shimobayashi, Hall: Making new contacts: the mTOR network in metabolism and signalling crosstalk

- TOR pathway integrates stimuli from growth factors, nutrients, and cellular energy status to activate the metabolic pathways that ultimately drive cell growth
- Gives an overview of downstream effectors and upstream regulators of mTOR signaling, but (obviously) focused on mammalian

8. Harvey, Zhang, Thomas: The Hippo pathway and human cancer

- Nice figure detailing the components of the Hippo pathway in *Drosophila*
- Else, mainly focuses on mammalian Hippo pathway

9. Lin, Othmer: A model for autonomous and non-autonomous effects of the Hippo pathway in *Drosophila*

- Hippo pathway controls cell proliferation and apoptosis in *Drosophila* and mammalian cells
- Growth control in wing disc involves both local signals within the disc and system-wide signals such as insulin that coordinate growth across the organism
- pathways are tightly linked so strengths of interactions determine outcome – Boolean model insufficient, need quantitative model

- Hippo pathway is a highly-conserved kinase cascade made of Hippo (Hpo), Warts (Wts), and adaptor proteins Salvador (Sav) and Mob as tumor suppressor (Mats)
- Key effector is Yorkie (Yki) and Wts is its master regulator
- Yki controlled by Wts through phosphorylation—once Yki is phosphorylated, it cannot enter the nucleus, so it cannot control cell proliferation or expression of genes upstream of Hippo module
- Hippo has two upstream modules: one based on Crumbs, Expanded, Merlin, and Kibra which affect Hippo kinase, and one based on Fat (Ft) and Dachsous (Ds) that regulate Wts. Input to Hippo assumed to be constant, so more important is second upstream module
- Ft and Ds are cadherins (calcium-dependent adhesion, bind cells together) which have intracellular, transmembrane, and extracellular domains. The ICDs mediate signaling within a cell while the ECD on adjacent cell membranes can associate to strengthen signaling and mediate cell-cell interaction
- Binding between Ft and Ds is regulated by Fj (Fj phosphorylates ECD of Ft, increasing its affinity to Ds, and phosphorylates ECD of Ds, decreasing its affinity to Ft)
- Signaling from ICD of Ft suppresses growth via Dachs (Dh): overexpression of Dh increases wing size, while Dh loss of function mutant decreases wing size
- Amount of Dh localized on membrane controls cell growth
- Effect of Ft on growth is not a strictly decreasing function of Ft level: overexpression of Ft above wild type levels decreases wing size and complete knockout of Ft increases wing size, but partial knockout of Ft decreases instead of increases wing size
- Effect of Ds is non-monotonic: loss of Ds enlarges wing discs but overexpression can either reduce or enhance growth
- When Fj and Ds are co-overexpressed, the reduction in wing size is greater than for either separately
- **Central components for model: Ft, Ds, Dh, Riq, Wts, Yki**
- Phosphorylation: phosphate group (provided by ATP) added to a protein by kinases. This alters the activity of a protein after the protein has already been formed.
- Dh degraded more rapidly when bound to Ds so in the absence of Ds, Wts inhibition by Dh is increased since there is more of it around, leading to higher Yki bc less Wts to phosphorylate it (?)
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#### 10. NIH proposal

- Primary morphogens in the wing disc are wingless (Wg, a segment polarity gene), and decapentaplegic (Dpp, a bone morphogenic protein)
- **Goal: develop a multi-scale, 3D computational model of signaling in the wing disc**

- Essential to incorporate detailed structure in a horizontal slice of the disc, and transport and signaling in the vertical direction
  - The morphogens Hh, Dpp, and Wg control the downstream network (they are each at the top of a signaling pathway)
  - Local inputs to Hippo pathway are from links to adjacent cells via Ft and Ds, and the key effector is Yki, a cotranscription factor whose nuclear localization is controlled by the kinase Wts (when Yki phosphorylated by Wts, cannot enter nucleus)
  - WAMND—what are my neighbors doing model
  - Binding of Ds to Ft activates Wts by relieving the inhibition of Wts by Dachs. Activated Wts phosphorylated Yki to prevent entry into nucleus, controlling cell growth
  - Incorporated in Hippo pathway: Ft, Ds, Fj, Dachs, Wts, Riq, Ex, and Yki
  - Test whether feedback loops created by Yki control of Fj and Ds expression leads to the observed spatial profiles of Ft and Ds (how do these loops affect Yki activity within a cell)
11. Zhang, Lai: Mob as tumor suppressor is regulated by bandtam microRNA through a feedback loop for tissue growth control
- Yki upregulates transcription of Expanded and Merlin in Drosophila
  - bantam microRNA is a downstream target of Hippo pathway; promotes tissue growth by stimulating cell proliferation and inhibiting cell apoptosis.
  - Show: ban indirectly regulates mats expression in developing tissues
  - ban is sufficient to increase level of Mats expression, but does not regulate mats at the transcript level
  - mats is the only core component of the Hpo pathway to be a potential target of ban miRNA.
  - ban miRNA functions to increase the level of mats expression through a feedback loop, but indirectly—not sure what the factor between ban and mats is
  - Yki also mediates transcriptional regulation of several upstream genes such as ex and mer
12. Djiane, et al.: Notch Inhibits Yorkie Activity in Drosophila Wing Discs
- Notch is required throughout the wing disc for growth but the effects of its overactivation are most noticeable at the edge of the wing disc
  - Notch activation releases intracellular part of Mcd receptor which enters nucleus, binds to transcription factor SuH, and turns on transcription of target genes
  - Epithelial cells require Notch and depend on Sav/Wts/Hpo pathway to regulate growth
  - DNA binding transcription factors of Yki: Scalloped/TEAD, Homothorax, p53
  - By modifying the ratio between Sd, Vg, and Yki, Notch signaling prevents Yki from activating its targets in the wing pouch

- Notch could either act upstream of Yki by activating the SWH pathway or downstream of Yki by inhibiting Yki's transcriptional activity...results suggest Notch acts either at the level or downstream of Yki
- Vg mediates the repressive effects of Notch on Expanded expression
- Vg inhibits expression of two Yki targets (ex-lacZ, IAP2B2C-lacZ) in the wing pouch
- Notch activity can inhibit Yki under circumstances where Yki acts together with Sd by promoting expression of Vg, a cofactor for Sd, counteracting the effects of Yki
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### 13. Konarska, Yochum: Intersection of Hippo/YAP and Wnt/ $\beta$ -catenin signaling pathways

- Mutation in Wingless gene causes loss of wings in Drosophila (hence the name...)
- Wnt is the human homolog of Wg
- Wnt genes mediate short range paracrine signaling
- Loss of function mutations in Warts resulted in strong tissue overgrowth
- Loss of function mutations in each of Warts, Hippo, Salvador, and Mats proteins gave a similar phenotype so hypothesized that they functioned as part of a signaling pathway
- "Yki acts as a transcriptional coactivator protein and it interacts with the transcription factor Scalloped to drive the expression of genes that promote cell growth and inhibit apoptosis"
- Fat senses cell-cell contacts by binding Dachshaus
- Border cells in the fly imaginal disc express Wg and Vestigial (Vg), which is a transcription factor that specifies wing cells
- Border cells provide a feed forward signal that involves Wg which recruits non-wing cells from surrounding tissue and instructs them to become wing cells
- Scalloped/Yki directly activate Vg which results in the conversion of nonwing cells to wing cells
- E-cadherin expression is lost during the progression and metastasis of tumors (because tumors want to be able to grow even if they're not in contact with other cells or a substrate)

### 14. Alexandre, Baena-Lopez, Vincent: Patterning and growth control by membrane-tethered Wingless

- Spread of Wingless is dispensable for patterning and growth even though it contributes to increasing cell proliferation
- Wg initially expressed throughout wing and then later only at a narrow stripe at the boundary between front and back of body
- Near this boundary, High activity activates senseless expression → wing margin fates. Further away, low signal stimulates vestigial, Distal-less and frizzled, which are more sensitive to signalling.

- Reporters of Wg signaling: senseless, vestigial, Distal-less, frizzled 3
- Wild type Wg can spread and signal over a few cell diameters
- Wg knockout flies are morphologically normal but have a delay in entering fly puberty and are less fit (fail to eclose)
- Wg is first expressed throughout wing precursor and eventually is restricted to a narrow stripe at the dorsoventral boundary, where it will spread to form a gradient

15. Attisano, Wrana: Signal integration in TGF- $\beta$ , Wnt, and Hippo pathways

- Smads are direct receptor substrates that upon phosphorylation accumulate in the nucleus to regulate transcription through interactions with DNA-binding partners
- Smad pathway is key for directing TGF- $\beta$  transcriptional responses
- Hippo pathway is activated through cell-cell contact or upon polarization of epithelial cells
- Smads homolog in Drosophila? Mad, Medea, Dad, Smox

16. Hemalatha, Prabhakara, Mayor: Endocytosis of Wingless via a dynamin-independent pathway is necessary for signaling in Drosophila wing discs

- DFrizzled2 is the receptor of Wingless
- Wg is endocytosed from the apical surface devoid of DFz2 via a dynamin-independent CLIC/GEEC pathway, regulated by Arf1, Garz, and class 1 PI3K
- Wg containing CLIC/GEEC endosomes fuse with DFz2-containing vesicles resulting in low pH-dependent transfer of Wg to DFz2 within the merged and acidified endosome to initiate Wg signaling
- Employment of two distinct endocytic pathways exemplifies a mechanism wherein cells in tissues leverage multiple endocytic pathways to spatially regulate signaling
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