Growth of tissues and organs during animal development involves careful coordination of the rates of cell proliferation and cell death.

Cell proliferation depends on signals to stimulate cell growth and cell division.

In addition, cells compete for intercellular survival signals which are required to prevent them from undergoing apoptosis in response to growth stimuli.

How these cellular processes are coordinated with pattern formation during animal development is a challenging question in developmental biology.
The Hippo Signaling Pathway

• First discovered in Drosophila, the Hippo signaling pathway is a conserved regulator of organ size.
• Central to this pathway is a kinase cascade leading from the tumor suppressor Hippo (Mst1 and Mst2 in mammals) to the oncoprotein Yki (YAP and TAZ in mammals), a transcriptional coactivator of target genes involved in cell proliferation and survival.

• *Nature Cell Biology* 9, 1225 - 1227 (2007)
• The first four components of the Hippo pathway: protein kinase Warts (Wts), the WW domain-containing protein Salvador (Sav), the protein kinase Hippo (Hpo) and the adaptor protein (Mats), were discovered in genetic screens in Drosophila for tumor suppressor genes.

• Loss-of-function mutant clones for any of these four genes lead to a strong tissue overgrowth phenotype characterized by increased proliferation and diminished cell death.
• The prime target of this kinase cascade in growth regulation is transcriptional coactivator Yorkie (Yki), which was isolated as a Hippo pathway component in a yeast two-hybrid screen for Wts-binding proteins.

• Yki functions as an oncogene and its overexpression phenocopies loss of Hippo signaling.

• Genetic analysis placed yki downstream of hpo, sav or wts, and biochemical studies demonstrated that Wts directly phosphorylates and inactivates Yki in a Hpo-regulated manner.
Activation of the Hippo signalling pathway is characterized by phosphorylation of Hippo, Wts and Yki, and results in the inhibition of transcription downstream of Yki.

X= scalloped
Fat activates Hippo signalling by recruiting Expanded to the membrane and by preventing the degradation of Warts through the unconventional myosin Dachsous.
### Box 1 | Mosaic analysis of growth regulators

Temporal induction of mitotic recombination in a heterozygous (*+/−*) cell leads to one homozygous mutant (*−/−*) daughter cell and another homozygous wild-type (*+/+) cell following cell division. Proliferation of the two sister-cell populations is then assessed later in development. Cells that become homozygous for the mutation in a growth-restrictive gene (such as hippo or warts) form larger clones relative to their wild-type sisters.

FRT, FLP recombination target sequence.
Formation de clones mitotiques
Par le système FLP/FRT
Genetic screens for loss and gain of function for growth-regulatory genes using mosaic flies. Mutations of positive or negative growth regulators are expected to produce homozygous mutant clones that are smaller or larger than control clones, respectively.
Saturation screen for growth mutations: The screen of 300,000 mutagenized genomes in genetically mosaic flies (mutations are homozygous in the head and heterozygous in the rest of the fly) led to the identification of 60 genes involved in growth and size regulation.
The Hpo signaling pathway controls organ size in Drosophila. Wild type (A) and flies in which hpo (B) or yki (C) function is specifically inactivated in the head. Increased cell proliferation (D) and decreased cell death (E) in hpo mutant clones in the pupal eye. Wild-type cells (green) (F) shows a wild-type wing imaginal disc (left) and a wing disc that overexpressed the yki gene (right).
hpo mutant eyes that overexpressed Ex showed the same phenotype as hpo mutant eyes (d, e). Therefore, **Ex requires Hpo for its function.** On the other hand, the phenotypes caused by Hpo overexpression were not Suppressed by loss of ex or (f, g).
Together, these results indicate that **Hpo acts downstream of Ex.**

Tests d’épistasie
(A) a wild-type fly
(B) a fly with clones of cells homozygous mutant for hippo

(C) A mouse liver at 2 months of age from a wild-type animal
(D) a liver at 2 months of age from a mouse mutant in which both Mst1 and Mst2 two mammalian Hippo homologs, have been conditionally inactivated
• The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the *Drosophila* homolog of YAP

• Unlike the tumor suppressors of the Hpo pathway, which were isolated in phenotypic screens using genetic mosaics Yki was identified in a yeast two-hybrid screen for Wts binding proteins.

• Biochemical and genetic characterization of Yki demonstrates that Yki fulfills all the criteria expected of a Wts effector in growth regulation. Yki is phosphorylated and inactivated by Wts.

• Overexpression of Yki recapitulates loss-of-function wts phenotypes, such as increased diap1 transcription and tissue overgrowth
The bantam MicroRNA Is a Target of the Hippo Tumor-Suppressor Pathway

Current Biology 16, 1895–1904, 2006
Riitta Nolo, Clayton M. Morrison, Chunyao Tao, Xinwei Zhang, and Georg Halder

The Hippo Pathway Regulates the bantam microRNA to Control Cell Proliferation and Apoptosis in Drosophila

Cell 126, 767–774, 2006
Barry J. Thompson and Stephen M. Cohen

* bantam is a bona fide oncogene
• The mi-RNA bantam was first identified as a locus that induced cell growth and proliferation when ectopically expressed in imaginal tissues, and arrested their growth when deleted.

*bantam Encodes a Developmentally Regulated* microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene *hid in Drosophila*

*Cell, Vol. 113, 25–36, 2003*

Julius Brennecke, David R. Hipfner, Alexander Stark, Robert B. Russell, and Stephen M. Cohen
Overexpression of bantam Generates hippo-Like Phenotypes

Excess interommatidial cells indicate that overexpression of bantam mimics loss of hippo in driving cell proliferation and inhibiting apoptosis.
bantam Inhibits Proliferation-Induced Apoptosis
(A’’)-(D’’) show third-instar eye discs stained with α-Drice to mark apoptotic cells. The genotypes of the animals are indicated above the panels. GMR-Gal4 drives overexpression of UAS transgenes in the eye. Expression of bantam suppresses the reduced- and rough-eye phenotype as well as the apoptosis caused by overexpression of Hpo.
Using a computational method for predicting possible target genes of miRNAs, the pro-apoptotic gene hid has been identified as a direct target for regulation by bantam miRNA, suggesting one mechanism by which bantam contributes to controlling cell death.

hid is subject to translational regulation in vivo by the bantam miRNA five bantam binding sites in the 3' UTR of hid mRNA

bantam can block expression of a transgene containing the hid 3’UTR
The **bantam locus of Drosophila** was identified in a gain-of-function screen for genes that affect tissue growth. *Bantam gene encodes a 21 nucleotide microRNA*
bantam Levels Regulate hid Activity in Eye Development

3622 = GMR-bantam

B, E ARN C, F protéine

bantam Regulates Hid Expression

+ hid + ban
Yki overexpression increases bantam levels in vivo
bantam Is Required for Yorkie-Driven Overgrowth in the Retina

Genetic epistasis tests have also placed *bantam* downstream of Yki because the loss of *bantam suppressed* the surplus cells that were generated following Yki over expression.
The insensitivity of Hippo mutants to growth inhibition and apoptosis clearly contributes to the well-defined **hallmarks of cancers**. Perhaps the most exciting future line of investigation will be determining whether the capability of Hippo signalling mutants to outcompete wild-type cells in *D. melanogaster* also has a role in mammalian tumorigenesis.
Yki is required for the overgrowth of hpo or wts mutant cells in vivo, and it was thus postulated that Yki mediates most if not all of the growth effects of Hpo signaling, presumably by driving the expression of transcriptional target genes. When Hpo signaling is reduced, for example by mutations in hpo, Yki is hypophosphorylated and active, driving the expression of target genes that promote cell proliferation and suppress apoptosis, resulting in tissue overgrowth. Normally, the Hpo and Wts kinases limit the growth of tissues through the inactivation of Yki.

increased proliferation, such as that resulting from activation of the microRNA bantam, or inactivation of the tumor suppressors hippo (hpo), salvador (sav), and warts (wts), is accompanied by an inhibition of cell death