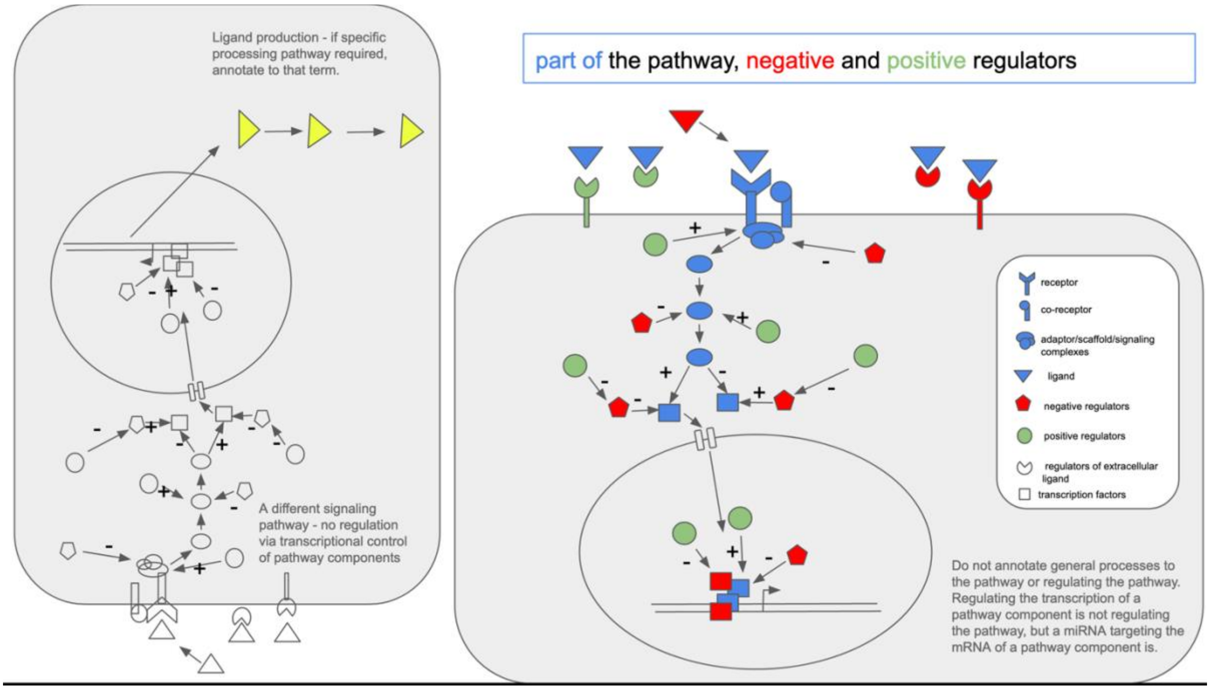
Pathway Curation in FlyBase

General Overview

Pathway components must be curated with particular care as they are used to populate **pathway pages** as follows:
Core: The genes that lie within a pathway, required for executing the defined end-point of the pathway, should be annotated using the GO process term for pathway. Such genes include ligands, receptors and transcription factors that are specific for that pathway. "General process" entities, such as general chromatin modifying proteins, should not be labelled as part of the pathway but annotated with the appropriate process term(s).

Positive regulators: Entities that directly up-regulate the activity of components in the pathway, should be annotated with ‘positive regulation of pathway x’ terms. They should be shown to be acting within the context of the pathway itself.
Negative regulators Entities that directly down-regulate the activity of components in the pathway, should be annotated with ‘negative regulation of pathway x’ terms. They should be shown to be acting within the context of the pathway itself.
Ligand Production: Genes that specifically are involved in the biogenesis or secretion of the ligand (only applicable for certain pathways). This does not include transcription or regulation of ligand mRNA levels by ncRNAs (this is seen as a pathway regulatory event, see below).

General Rules



In the GO, pathways have defined start and end points - usually starting with a ligand binding to a receptor and ending with the binding of a sequence-specific transcription factor to a gene promoter/enhancer region. (Although, there are notable exceptions such as Hippo signaling.)
Genes can be annotated to either being:

1. part of/acting within the pathway by directly annotating to the pathway term (e.g. ‘smoothened signaling pathway’ GO:0007224) - these genes are required for the execution of the pathway, from receptor activation to molecular consequence (but not including transcriptional target genes themselves). Note, this may include protein targets that are negatively regulated by the pathway as part of that e.g. the consequence of the activation of the Hippo pathway is the cytosolic retention of the

transcription factor yki. The yki gene should therefore be directly annotated to 'hippo signaling' GO:0035329.

2. a 'regulator' of the pathway. Regulators of the pathway should target pathway members directly or via another direct regulator, although sometimes it may be difficult to pinpoint the mechanism. As a general rule, the regulator should be in the same cell or extracellular to the cell it is acting on. Regulation of a pathway cannot occur if the components are spatially separated. (Although, location within the same cell, does not mean it is a direct regulator.) Regulation should be specified as 'positive' or 'negative' e.g. 'negative regulation of hippo signaling' and not 'regulation of hippo signaling' in terms of the pathway output. Regulation of mRNA level or translation by a ncRNA (e.g. miRNA) is considered to be a pathway regulatory event. For curation, we consider ncRNAs as if they act at the level of the pathway component's action, rather than at the mRNA. Therefore, if a miRNA is acting to regulate the expression of a ligand, even though this may be spatially separate (e.g. within a different cell), this is still annotated as regulation of the pathway.

   Note: Regulation of the pathway or the membership pathway itself, should not traverse transcription, which should mark a natural breakpoint (i.e. pathway 1 -> transcription -> pathway 2). This is also true of other biological process such as translation. Thus, the curator includes the 'last target' of the pathway e.g. aop and pnt in EGFR signaling, regardless of whether their activity is up or down-regulated. The 'last target' should overlap with the other process or regulation of the next process downstream e.g. 'negative regulation of transcription by RNA polymerase II' GO:0000122

Pathway specificity: When annotating genes to pathways or the regulation of a pathway, the curator should always ask if it is a specific, direct effect? i.e. Is this part of the normal, physiological mode of executing or regulating the pathway?

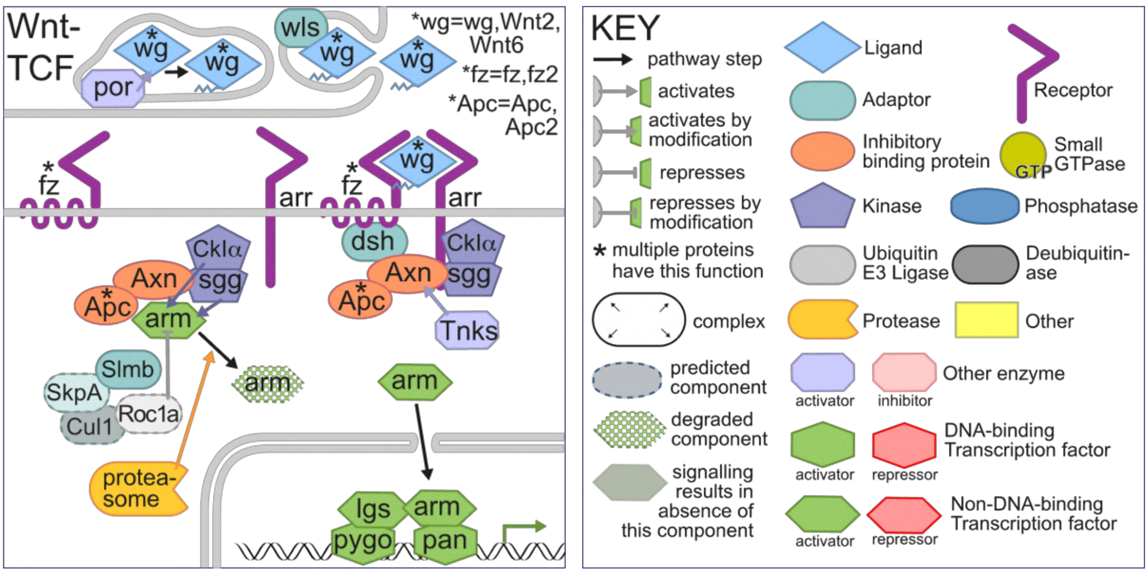
1. **It's ok for a gene product to be annotated to >1 pathway/regulation of a pathway terms:** Although pathways can share regulators and core components, these components can still be considered 'specific' for the pathways in question. Pathway components can be targeted by gene products that also target other pathways e.g. Med is a core component of both activin and BMP signaling. Cbl has been shown to negatively regulate EGFR and Notch signaling pathways. Cbl E3 ligase specifically targets proteins in these pathways and is therefore specific. Note that sometimes a gene product can act within a particular pathway and regulate it or act as a positive and negative regulator. For example, cos, is a considered a core component of the hedgehog signaling pathway, forming part of the signaling complex associated with the activated smo receptor and a negative regulator, promoting the formation of the repressive form of ci in the absence of hh.
2. **Generic or non-specific regulators should not be annotated to a pathway/regulation of a pathway term.** These are gene products that act more "globally", having a similar effect on many different processes and, even though they may be deemed "essential" for a particular pathway by the authors), it is important to view them with a more critical eye. As a general rule, they can be annotated to a more appropriate process term in GO and should NOT be annotated directly to a pathway or pathway regulator term. For example, the activity of chromatin modifiers, such as the NuRD complex or generic transcription regulators e.g. Mediator (MED)

complex, are generally considered non-specific and should be annotated to the correct biological process terms in the 'chromatin organization' GO:0006325 branch, for example.
The curator should try to distinguish between factors that target the pathway and factors that are components of other processes that are downstream or tangential. For example, many receptor-mediated pathways are regulated by endocytic processes - capture the regulatory component e.g. the ubiquitin ligase that directs the component to be endocytosed, but not the downstream endocytic machinery such as ESCRT complex members e.g. Vps28. Some factors, such as the co-repressor gro that act widely, are included in pathway curation as their control of or by the pathway is an essential switch in the execution of that pathway.

3. **Do not annotate the components of upstream or downstream processes to a pathway/regulation of a pathway term.**
The phenotypic output/"collateral damage" from the disruption of a general process such as translation or splicing, should not be seen as pathway regulation.
Other examples of upstream processes that should not be annotated to the pathway or regulating the pathway are gene products involved in biogenesis or secretion of signaling components such as the ligand or receptor. There may be specific process terms that can be used (e.g. 'Wnt protein secretion' GO:0061355, 'epidermal growth factor receptor ligand maturation' GO:0038004, 'patched ligand maturation' GO:0007225) when there are specific pathways.
Transcription should be considered the end point of a pathway and should not be traversed in annotation. For example, wnt signaling regulates Notch signaling at a transcriptional level. A component signaling in the Wnt pathway should not be annotated as regulating Notch signaling unless it directly interacts with Notch pathway components.

Pathway specific guidance

1. Wnt-TCF Signaling Pathway



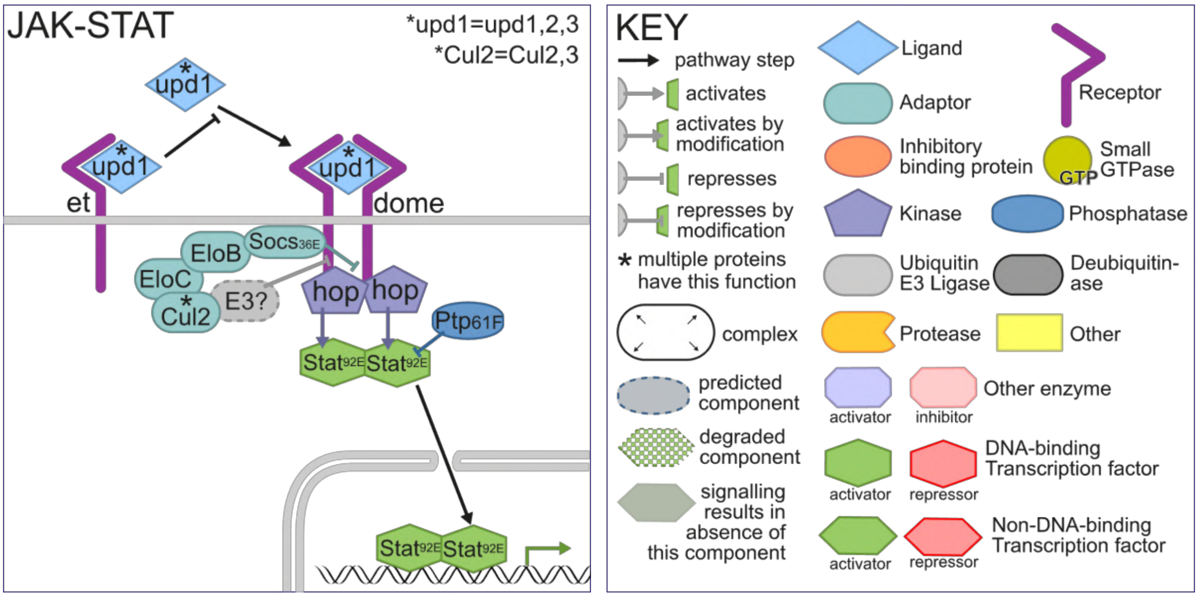
The Wnt-TCF signaling pathway (canonical Wnt signaling) is initiated by the binding of a Wnt ligand to a frizzled family receptor on the cell surface. In the absence of a Wnt ligand, cytoplasmic levels of β-catenin (arm), the transcriptional effector of the pathway, are kept low through its constitutive degradation. Activation of the pathway leads to the inhibition of cytoplasmic β-catenin (arm) degradation and its subsequent accumulation in the nucleus, where it regulates the transcription of target genes (FBrf0218499 and FBrf0223299).
It is the translocation of β-catenin (arm) into the nucleus that is the major diagnostic criteria for assigning a gene product a role in Wnt-TCF signaling.

Pathway Page Terms:
GO:0060070 canonical Wnt signaling pathway
GO:0090090 negative regulation of canonical Wnt signaling pathway
GO:0090263 positive regulation of canonical Wnt signaling pathway
GO:0061355 Wnt protein secretion

Assays used for the Wnt-TCF signaling pathway

I. *In vitro* transcription assay such as TOP-FLASH (FBrf0158721, FBrf0238342)
II. *In vivo* transcription reporters e.g. fz3, neur, 6xTCF binding sites (FBrf0127331)
III. arm translocation into nucleus (FBrf0158859)
IV. Assembly of destruction complex (FBrf0245515)
V. LOF Phenotypic assay (if supported by other evidence):
  a. cuticle/segmentation phenotypes e.g. lawn-of-denticles (FBrf0223299).
  b. Wing/wing disc phenotypes (FBrf0072872) e.g. loss of wing margin bristles and the appearance of notches along the wing margin.

2. JAK-STAT Signaling Pathway



The JAK-STAT signaling pathway is initiated by the binding of an extracellular ligand to a cell surface receptor leading to receptor dimerization and the intracellular activation of a Janus kinase (JAK) family member. JAK phosphorylates cytoplasmic STAT family members which dimerize, translocate into the nucleus and regulate target gene expression. In *Drosophila*, the core pathway is limited to three ligands (the Unpaired family of cytokines), a single receptor (dome), JAK kinase (hop) and STAT (Stat92E) (FBrf0225259).

Pathway Page Terms:
GO:0007259 receptor signaling pathway via JAK-STAT
GO:0046426 negative regulation of receptor signaling pathway via JAK-STAT
GO:0046427 positive regulation of receptor signaling pathway via JAK-STAT

Assays used for the JAK-STAT signaling pathway

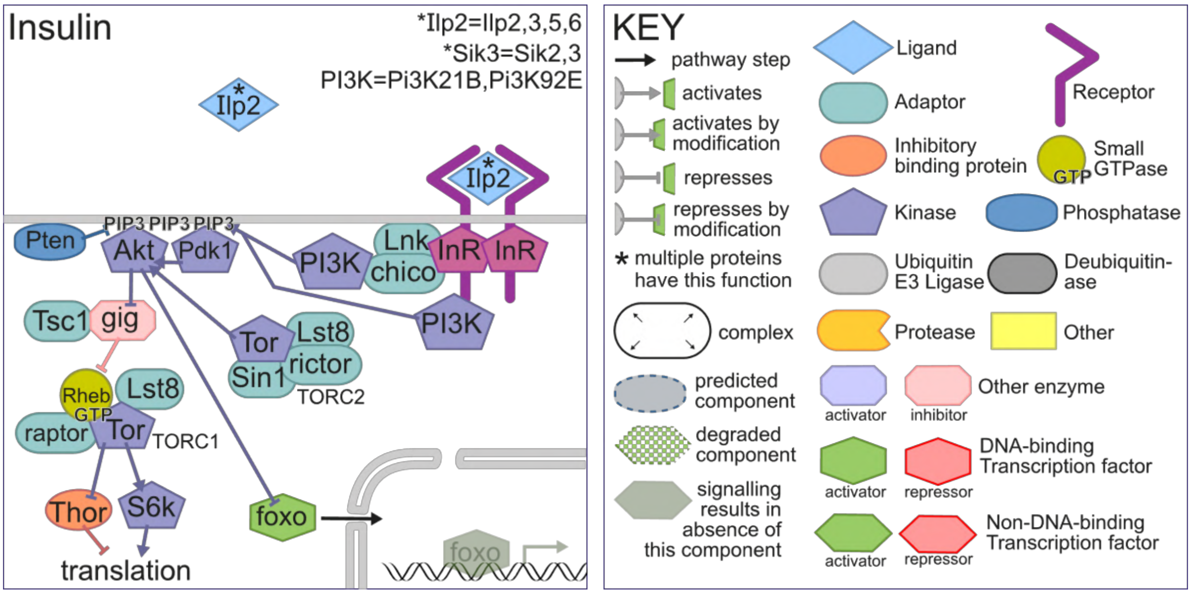
I. *In vitro* pathway reporters e.g. 10xSTAT92E-luciferase, Stat/hop phosphorylation (see FBrf0225259 for extensive list)

II. *In vivo* pathway reporters e.g. 10XSTAT92E-GFP, Anti-Stat92E, Anti-pStat92E (see FBrf0225259 for extensive list)

III. LOF Phenotypic assay (if supported by other evidence):

1. Eye size defects (reduced size)
2. Wing vein defects

3. Insulin-like Receptor Signaling Pathway



The Insulin-like Receptor signaling pathway in *Drosophila* is initiated by the binding of an insulin-like peptides (ILPs) to the Insulin-like receptor (InR). ILPs are important regulators of metabolism, growth, reproduction and lifespan (FBrf0232297, FBrf0230017 and FBrf0229989).

In mammals, activation of the insulin receptor results in the activation of the IP3 kinase pathway and the Erk kinase cascade. Activation of the Erk cascade occurs via SHC-GRB2-SOS-Ras (FBrf0209514). In *D.mel*, although there is some evidence demonstrating the activation of the Erk cascade following insulin-stimulation, the evidence supporting an analogous activation route is patchy and activation of Erk cascade components may be downstream of PI3 kinase (FBrf0180039). It has also been suggested that the activation of these two pathways is separable and that growth and response to nutrients is via the PI3 kinase axis and activation of the Erk axis reduces lifespan (FBrf0228856).

The insulin PI3 kinase branch pathway is made up of many subprocesses that can also be annotated:
a. The first step is the activation of the PI3 Kinase complex and the production of PIP3 at the membrane (‘phosphatidylinositol 3-kinase signaling’ GO:0014065).
b This activates the 3-phosphoinositide-dependent protein kinase, Pdk1 that phosphorylates and activates Akt (PKB) (‘positive regulation of protein kinase B signaling’ GO:0051897).
c. This is opposed by Pten that converts PIP3 to PIP2 (‘negative regulation of phosphatidylinositol 3-kinase signaling’ GO:0014067).
d. Akt kinase phosphorylates many components in the pathway including foxo, sgg and Tsc1/Tsc2(gig) (GO:0043491 protein kinase B signaling).
e. Akt inhibits the activity of the TSC1-TSC2 complex (‘TSC1-TSC2 complex’ GO:0033596), a Rheb GTPase that stimulates TORC1 signaling (‘positive regulation of TORC1 signaling’ GO:1904263) and therefore the TSC1-TSC2 complex (‘negative regulation of TORC1 signaling’ GO:1904262).
f. The TORC1 complex (‘TORC1 complex’ GO:0031931) is an mTor kinase-containing complex, inhibited by rapamycin, that phosphorylates many downstream targets of the insulin pathway including S6k and Thor (‘TORC1 signaling’ GO:0038202). The TORC1 complex is also activated by amino acids and stress signaling.
g. The TORC2 complex (‘TORC2 complex’ GO:0031932, ‘TORC2 signaling’ GO:0038203) phosphorylates Akt, enhancing Pdk1 phosphorylation of the Akt T-loop and therefore supporting full activation of Akt (‘positive regulation of protein kinase B signaling’ GO:0051897)

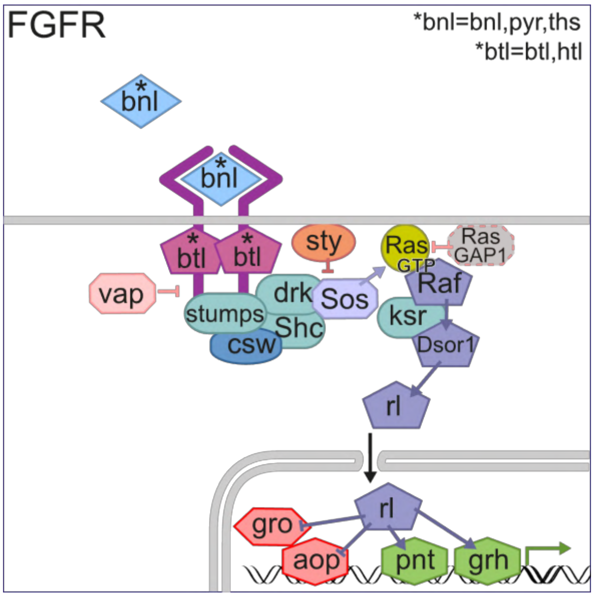
Pathway Page Terms:
GO:0008286 insulin receptor signaling pathway
GO:0046628 positive regulation of insulin receptor signaling pathway
GO:0046627 negative regulation of insulin receptor signaling pathway

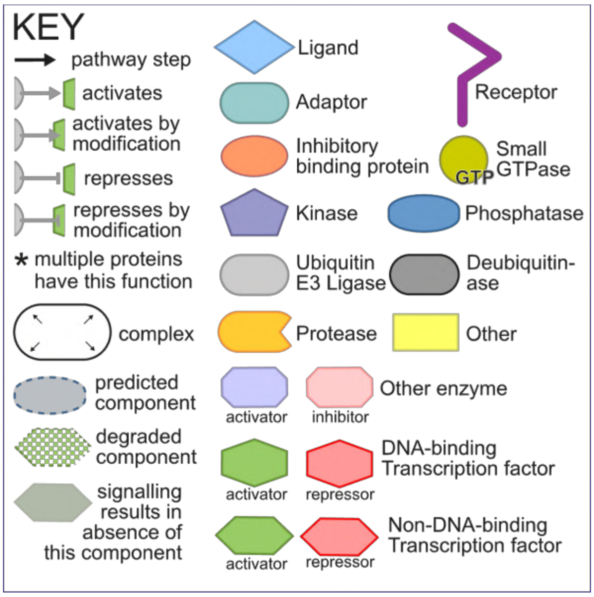
Assays used for the InR signaling pathway

As the insulin receptor pathway has many shared components and intracellular signaling cassettes, we need to make sure the readout lies downstream of InR (either by using insulin-stimulation or mutation of InR. Many readouts are biochemical/cell biology-based rather than a transcriptional readout e.g.

I. Phosphorylation of:
a. Akt (PI3K branch), e.g. ELISA method; FBrf0255740
b. S6k (PI3K branch - it’s downstream of TORC1, so could also be a marker for TOR pathway)
c. foxo (PI3K branch)
d. Cellular activation (phosphorylation) of rl (Erk branch)
II. tGFP (PH domain-GFP fusion protein; FBrf0144797) marker of PI3K activation.
III. Exclusion of foxo from nucleus (PI3K branch)

4. Fibroblast Growth Factor Receptor Signaling Pathway





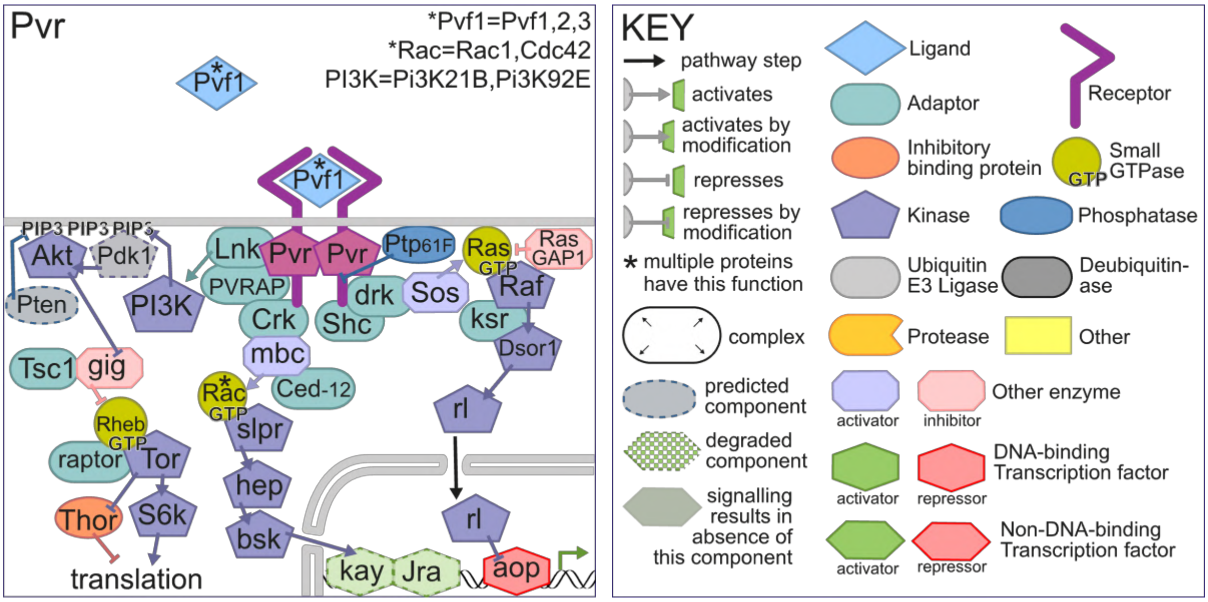
Fibroblast Growth Factor Receptor (FGFR) signaling pathway is initiated by the binding of secreted FGFs - bnl or ths/pyr to receptor tyrosine kinases btl or htl, respectively, to initiate signaling primarily via the canonical Ras/Raf/MAP kinase (ERK) cascade. FGFR signaling is important in several morphogenic events in Drosophila, notably during mesoderm and tracheal development (FBrf0221038).

Pathway Page Terms:
GO:0008543 fibroblast growth factor receptor signaling pathway
GO:0040037 negative regulation of fibroblast growth factor receptor signaling pathway
GO:0045743 positive regulation of fibroblast growth factor receptor signaling pathway

Assays used for the FGFR signaling pathway
Note: there are very few biochemical/*in vitro* or reporter assays for FGFR signaling in *D.mel*. The majority are phenotypic outputs and so should be interpreted with care. Co-annotation and adding extensions are useful here to help differentiate Btl and Htl-mediated pathways.
I. Mesoderm migration/spreading for htl pathway (ths/pyr) (e.g. FBrf0208190)
II. Epithelial migration/branching morphogenesis for btl (bnl) pathway
III. Cellular activation (phosphorylation) of rl (pErk) (e.g. FBrf0208190)

Note on ksr: ksr is a scaffold for the MAPK cascade, binding Dsor and interacting with cnk and Raf to enhance the first step in the cascade. ksr has a kinase domain, and appears to possess the residues required of an active kinase. There are some ideas that ksr may be allosterically activated in the complex and act as a kinase or a kinase in other situations - for now, treat it as a MAPK scaffold.

5. Platelet-Derived Growth Factor-Vascular Endothelial Growth Factor Receptor-Related Signaling Pathway



The Platelet-Derived Growth Factor (PDGF)-Vascular Endothelial Growth Factor Receptor (VEGF)-Related Signaling Pathway is a receptor tyrosine kinase pathway. PDGF/VEGF-receptor related (Pvr) encodes a receptor activated by the binding of PDGF- and VEGF-related factors (Pvf1,Pvf2 or Pvf3). Pvr has been shown to activate the canonical Ras/Raf/MAP kinase (ERK) cascade, the PI3K kinase pathway, TORC1 (FBrf0222697), Rho family small GTPases (FBrf0221764, FBrf0180198) and the JNK cascade (FBrf0180198), in a context-dependent manner (FBrf0222697 and FBrf0221727).

Pathway Page Terms:

GO:0048010 vascular endothelial growth factor receptor signaling pathway

GO:0030948 negative regulation of vascular endothelial growth factor receptor signaling pathway

GO:0030949 positive regulation of vascular endothelial growth factor receptor signaling pathway

note: Use ‘vascular endothelial growth factor **receptor** signaling pathway’ NOT ‘vascular endothelial growth factor signaling pathway’, as we have defined pathway by the receptor rather than ligand!

Assays used for the Pvr signaling pathway

The Pvr pathway is an understudied pathway and the assays for pathway activation are not well-defined. Markers of pathway activation include:

I. Phosphorylation of:

a. Pvr tyrosine

b. Jun kinase (bsk) (for the JNK branch)

c. rl (for Erk branch)

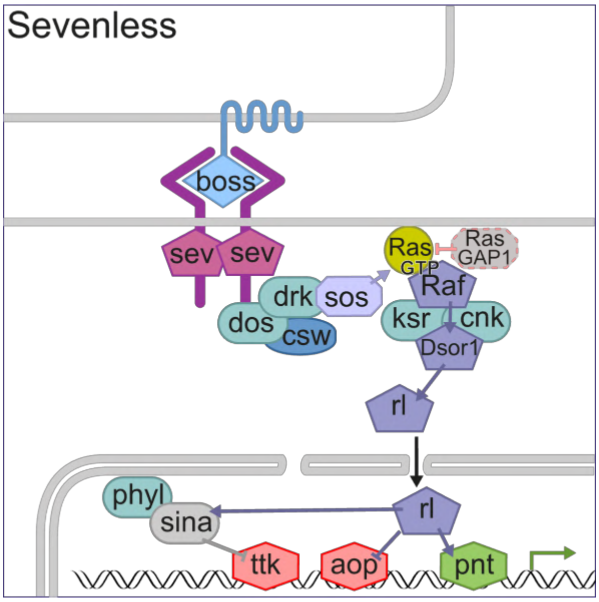
d. Akt (PI3K branch)

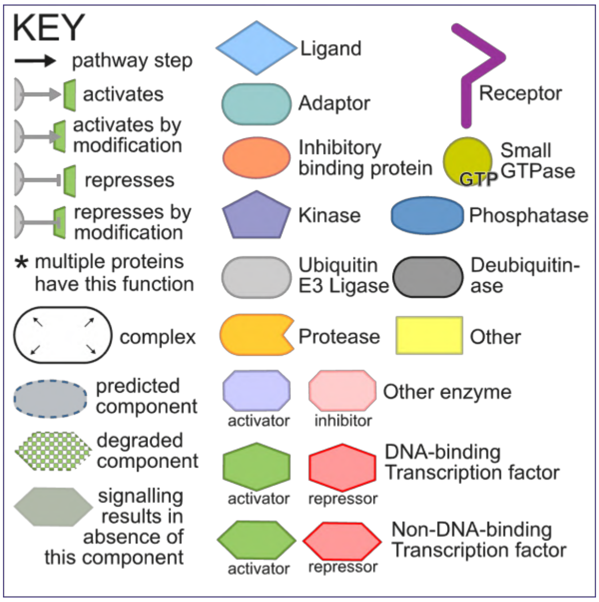
e. S6k (PI3K branch - it's downstream of TORC1, so could also be a marker for TOR pathway)

II. tGFP (PH domain-GFP fusion protein; FBrf0144797) marker of PI3K activation.
III. Rac1, Cdc42 activation assay using a PAK-p21 binding domain (PAK-PBD) pull-down assay. This protein binds specifically to GTP-bound, and not GDP-bound, Rac1 and Cdc42 proteins (FBrf0180198)
IV. Cell size - in cell culture (FBrf0209753)
V. Border cell migration (FBrf0187480)
VI. Hemocyte number (FBrf0180198)
VII. Cell spreading in wounding: In FBrf0252494, a forward genetic screen is used to look for Pvr downstream components in epidermal wound closure and hemocyte spreading

Note on ksr: ksr is a scaffold for the MAPK cascade, binding Dsor and interacting with cnk and Raf to enhance the first step in the cascade. ksr has a kinase domain, and appears to possess the residues required of an active kinase. There are some ideas that ksr may be allosterically activated in the complex and act as a kinase or a kinase in other situations - for now, treat it as a MAPK scaffold.
Note on Cklα: has been curated as a positive regulator of this pathway from FBrf0252494, but may well be part of the pathway. As this pathway has many branches, it is difficult to be sure with this assignment, but as this kinase is a pathway regulator in many instances this was chosen as most likely.

6. Sevenless Signaling Pathway





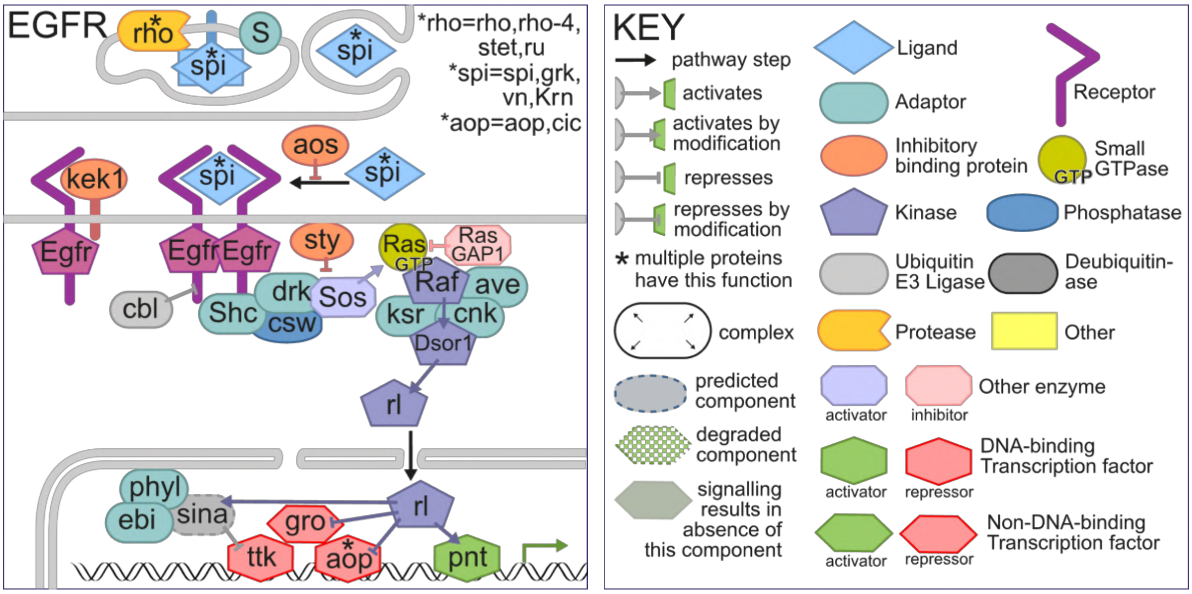
The specification of the R7 photoreceptor cell in each ommatidium of the developing Drosophila eye is dependent on activation of Sevenless receptor tyrosine kinase (sev), which acts via the canonical Ras/Raf/MAP kinase cascade to promote the expression of lz and pros. sev, expressed in presumptive R7 cells, is activated by binding to Bride of Sevenless (boss), a seven-transmembrane protein expressed in R8 cells (FBrf0127283 and FBrf0221727).

Pathway Page Terms:
GO:0045500 sevenless signaling pathway
GO:0045873 negative regulation of sevenless signaling pathway
GO:0045874 positive regulation of sevenless signaling pathway

Assays used for the sevenless signaling pathway
Sevenless signaling results in the specification of R7 photoreceptor cells. In the absence of sev activity, the R7 precursor cells fail to initiate neural development and develop as nonneuronal cone cells. Conversely, expression of a constitutively activated sev under the control of the sev enhancer (sev$^{S11.Tag:MYC}$) or by fusing the cytoplasmic domain of sev to the transmembrane and extracellular domains of a dominant gain-of-function form of the Torso RTK (sev::tor$^{13D.hs.sev}$) in cone cell precursors causes them to become R7 cells resulting in a rough eye phenotype. The number of supernumerary R7 cells is dependent on the expression level of the activated Sevenless protein and can be modulated by altering downstream signaling molecules. Note: rough eye is often used to assay other genetic interactions and constitutively active sevenless has been used to dissect of RTK pathway, so be sure that the phenotype is directly linked to sevenless signaling, if using this for inferring an annotation (e.g. by genetically interacting with sev or boss alleles).
Biochemical assays for activation of sevenless signaling include phosphorylation of erk kinase cascade components: Raf, Dsor and rl.

Note on ksr: ksr is a scaffold for the MAPK cascade, binding Dsor and interacting with cnk and Raf to enhance the first step in the cascade. ksr has a kinase domain, and appears to possess the residues required of an active kinase. There are some ideas that ksr may be allosterically activated in the complex and act as a kinase or a kinase in other situations - for now, treat it as a MAPK scaffold.

7. Epidermal Growth Factor Receptor Signaling Pathway



Epidermal Growth Factor Receptor (EGFR) signaling pathway is used multiple times during development (FBrf0190321). It is activated by the binding of a secreted ligand - the transforming growth factor-α-like ligands:spi, Krn, grk or the neuregulin-like ligand vn, to the receptor tyrosine kinase Egfr. The pathway can be regulated by the maturation and secretion of TGF-α-like ligands. The EGFR signaling pathway acts via the canonical Ras/Raf/MAP kinase (ERK) cascade (FBrf0190321 and FBrf0221727).

Pathway Page Terms:
GO:0038004 epidermal growth factor receptor ligand maturation
GO:0007173 epidermal growth factor receptor signaling pathway
GO:0042059 negative regulation of epidermal growth factor receptor signaling pathway
GO:0045742 positive regulation of epidermal growth factor receptor signaling pathway

Assays used for the EGFR signaling pathway

I. Activation of rl (pErk) (e.g. FBrf0223725, FBrf0098244, FBrf0210285)

II. Phenotypes associated with EGFR analysis:

a. Wing vein phenotype: loss of EGFR function impedes vein differentiation, and the increase in EGFR activity causes the formation of extra veins (FBrf0221826).

b. Formation of dorsal appendage formation (FBrf0162227)

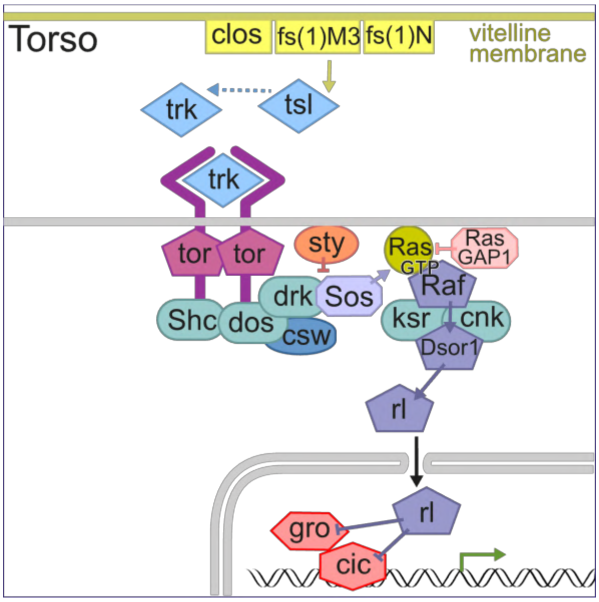
c. Eye development: EGFR signaling is essential for the correct patterning and specification of all cell types in the *Drosophila* eye. Various assays - R8 specification, rough eye from over-expression of pathway components.

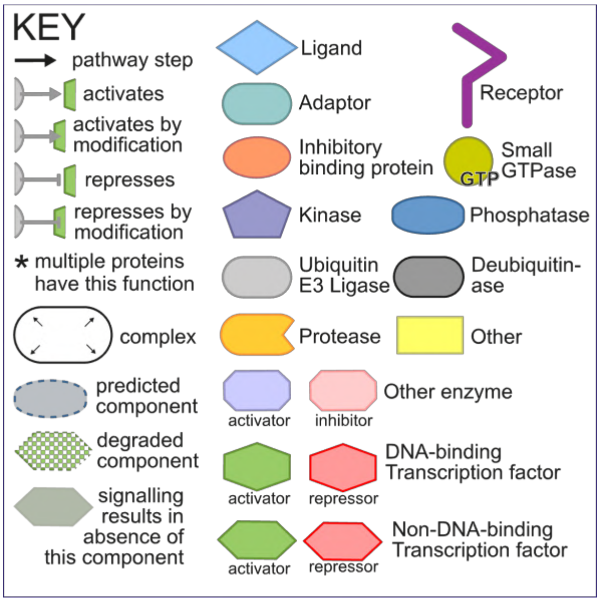
Note that EGFR signaling is involved with a myriad of developmental processes in *Drosophila*, often overlapping or sequential with other RTK pathways. Thus, it is important to be sure that the phenotype of any RTK pathway component mutants is in the EGFR pathway and not another RTK.

III. Expression of aos (FBrf0085111, FBrf0221826) pnt and rho) (FBrf0221826).

Note on ksr: ksr is a scaffold for the MAPK cascade, binding Dsor and interacting with cnk and Raf to enhance the first step in the cascade. ksr has a kinase domain, and appears to possess the residues required of an active kinase. There are some ideas that ksr may be allosterically activated in the complex and act as a kinase or a kinase in other situations - for now, treat it as a MAPK scaffold.

8. Torso Signaling Pathway





The formation of Drosophila embryonic termini is controlled by the localized activation of Torso (tor) receptor tyrosine kinase. The Torso signaling pathway acts via the canonical Ras/Raf/MAP kinase cascade (FBrf0157176.)

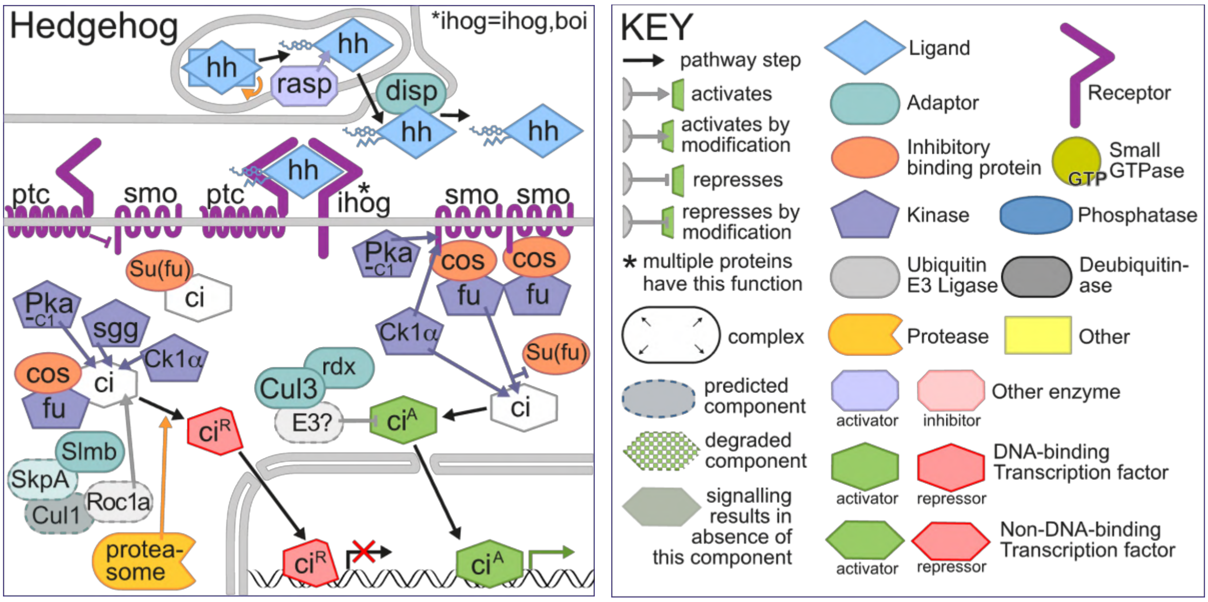
Pathway Page Terms:
GO:0008293 torso signaling pathway
GO:0120177 negative regulation of torso signaling pathway
GO:0120176 positive regulation of torso signaling pathway

Assays used for the Torso signaling pathway
For conventional torso signaling (ie excludes that mediated by Ptth), the key feature is that it is restricted to the embryonic termini.:

I. Activation of rl (pErk) (FBrf0157176)
II. cic excluded from nucleus (FBrf0157176)
III. Expression of tll and hkb (FBrf0157176)
IV. Mutant phenotype: lack of embryonic terminal structures (FBrf0135732)

Note on ksr: ksr is a scaffold for the MAPK cascade, binding Dsor and interacting with cnk and Raf to enhance the first step in the cascade. ksr has a kinase domain, and appears to possess the residues required of an active kinase. There are some ideas that ksr may be allosterically activated in the complex and act as a kinase or a kinase in other situations - for now, treat it as a MAPK scaffold.

9. Hedgehog Signaling Pathway



The hedgehog signaling pathway is initiated by hedgehog (hh) ligand binding to the extracellular domain of patched receptor (ptc), leading to the derepression of smoothened (smo) activity. Activation of the atypical GPCR smo results in the accumulation of the transcriptional activator form of cubitus interruptus (ci) (Ci(A) /Ci155) and the derepression/activation of hh target genes.
In the absence of hh ligand, ptc inhibits smo activity, probably by preventing its cell surface localization. Suppressor of Fused (Su(fu)) binds to ci and retains it in the cytoplasm. ci is proteolytically processed, facilitated by a cytoplasmic signal transducer complex consisting of cos, fu and sequential phosphorylation by Pka-C1, sgg, CkIα to produce a transcriptional repressor form of ci, (Ci(R) /Ci75), for hh target genes (FBrf0220683 and FBrf0231236).

Many gene products that are either part of the process, can also regulate it and some, both positively and negatively regulate the pathway, depending on the presence or absence of hh.

hh is a morphogen. At different levels of hh, different genes are activated. During embryonic and limb development in *Drosophila*, hh is produced by posterior compartment (P) cells and diffuses to reach target cells in anterior (A) compartment. In the A compartment hh acts as a morphogen by activating responsive genes differentially depending on its levels.

Pathway Page Terms:
GO:0007224 smoothened signaling pathway
GO:0045879 negative regulation of smoothened signaling pathway
GO:0045880 positive regulation of smoothened signaling pathway
GO:0007225 patched ligand maturation

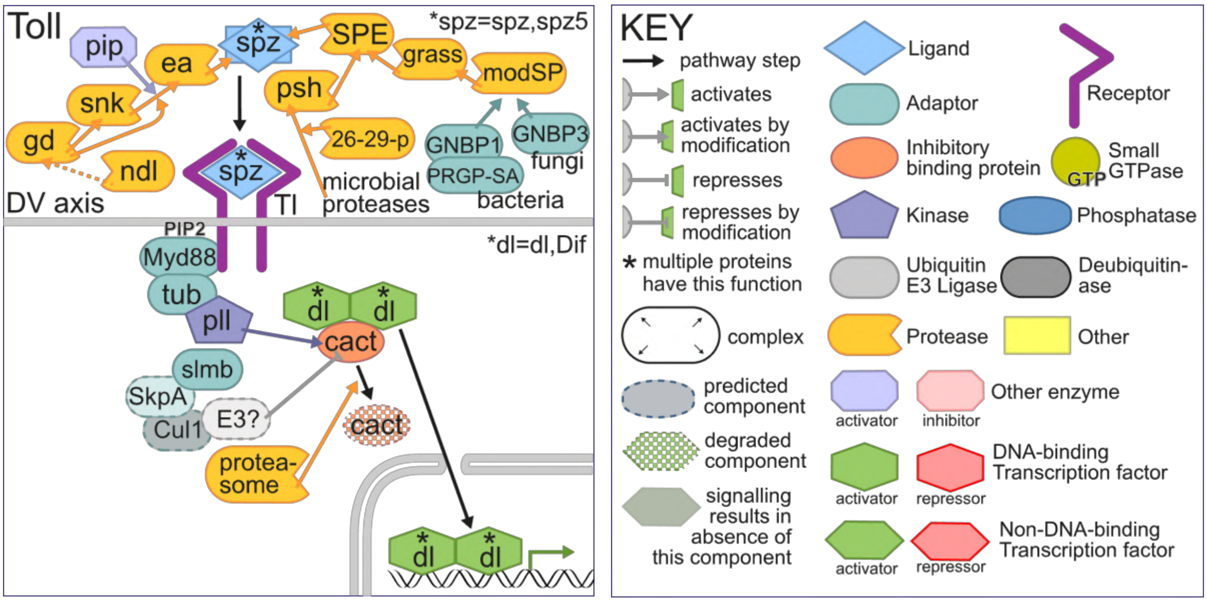
Assays used for the hedgehog signaling pathway

I. ci nuclear accumulation of the full-length version.
II. Cleavage of ci to the repressor form, Ci(R) (for negative regulation)

III. Phosphorylation of downstream components e.g. cos phosphorylation at Ser-57 (FBrf0211312), smo phosphorylation.
IV. Reporter genes/expression of genes - hh is a morphogen. At different levels of hh, different genes are activated (note that the definition of low-intermediate-high levels of expression seems to vary between authors).
a. Intermediate levels: dpp and ara
b. High levels: ptc and kn (often referred to as col)
V. Width wing disc, the width of the Ci(A)//kn domain is often used as a readout of activity.
VI. ci cleavage state: e.g. antibodies which recognize the full-length but not the truncated form of ci (FBrf0211312, FBrf0123234).
VII. *ptc-luc* reporter assay in cell culture (FBrf0245753)

Useful notes:
There are two common reagents used when looking at PkA signaling in the hh pathway:
UAS-mC* or C* (Mmus\PrkacamC.UAS) - a constitutively active MOUSE Pka catalytic subunit.
UAS-R* or R* (Dmel\Pka-R1BDK.UAS, FBal0086779) - the D.mel Pka-R1 subunit, dominant negative for PKA signaling.

10. Toll Signaling Pathway



In Drosophila, the canonical Toll signaling pathway is initiated by the binding of a spatzle (spz) ligand to Toll (Tl) or a Toll-like receptor leading to the nuclear localization of the NF-κB (dl or Dif) transcription factor. Activation of the pathway is controlled by the generation of a cleaved, active, Toll-binding form of spatzle ligand. Proteolytic activation of spatzle ligand lies downstream of several zymogen activation cascades that are initiated by different cues. The canonical Toll pathway is best characterised in the establishment of embryonic dorsal-ventral pattern and innate immunity. In dorsal-ventral patterning, localized activation of spz results in ventral nuclear accumulation of dl. During gram-positive bacterial, viral and fungal immune challenge, a zymogen cascade is activated by extracellular pattern recognition receptors or virulence factor-mediated cleavage of the zymogen persephone (psh) (FBrf0091014, FBrf0223077).

Pathway Page Terms:
GO:0008063 Toll signaling pathway
GO:0045751 negative regulation of Toll signaling pathway
GO:0045752 positive regulation of Toll signaling pathway
GO:0160032 Toll receptor ligand protein activation cascade
GO:0160035 negative regulation of Toll receptor ligand protein activation cascade
GO:0160034 positive regulation of Toll receptor ligand protein activation cascade*

*no D.mel genes annotated to this term as yet and so corresponding pathway group not made

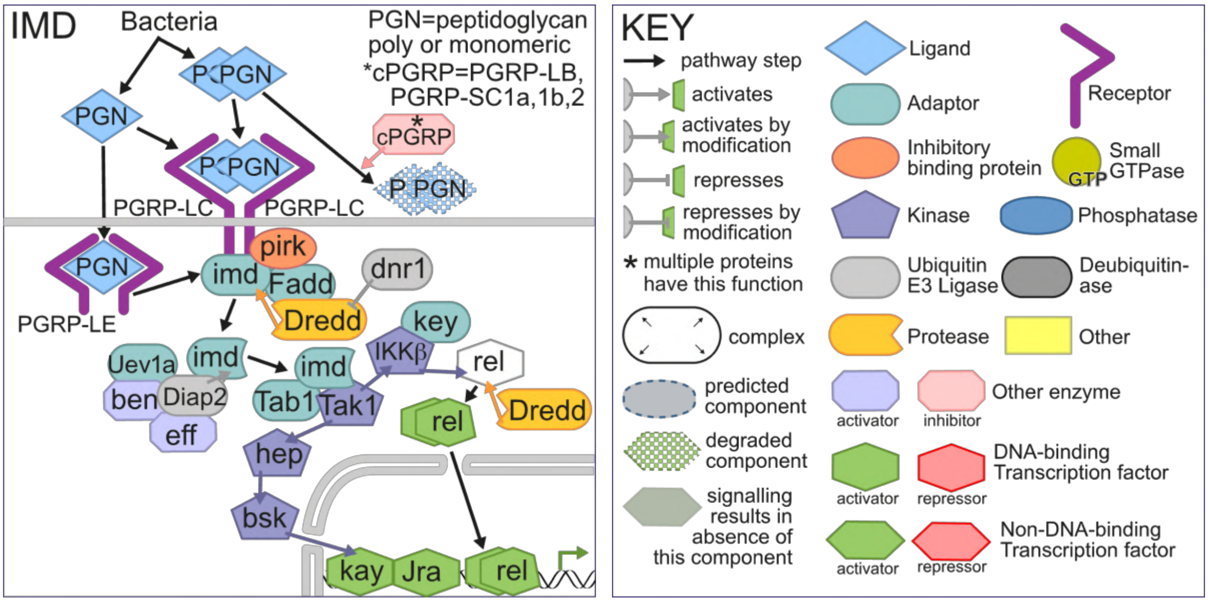
Assays used for the Toll signaling pathway

I. Production of antimicrobial peptides - Drs (also induced by Imd, but to a much less extent), BomS1,
II. NFκB luciferase reporter (cell culture, also a reporter for Imd signaling FBrf0234632)
III. Susceptibility to fungal and gram-positive bacterial infections (FBrf0190205)
IV. Disrupt the formation of pattern elements along the dorsal–ventral (DV) axis (FBrf0225950) of the embryo, for example, loss-of-function mutants displaying dorsalization of the embryo as seen with the maternal effects of the Dorsal group genes.
V. Nuclear localization of dl, FBrf0217797.
VI. Cleavage/activation of components of the zymogen cascade (FBrf0135928).

Note on accommodating organism-level differences in Tl/Tlr signaling: Toll signaling was first described and dissected in *Drosophila* (FBrf0252634) leading to the discovery of vertebrate Toll-like receptors (TLRs) (FBrf0100706). There is extensive evolutionary conservation between fly Toll and human TLR signaling pathways, between receptors and the intracellular signal transduction pathways and primary effector transcription factors of the pathway: NF-kB family members. However, the fly Toll and vertebrate TLR pathways differ in some major ways. In flies, the Toll was first described in its role in dorsoventral patterning of the embryo. Later it was shown to have a non-developmental role in innate immunity. Although these are very different contexts: one requiring precise spatial control and one requiring a disseminate response, the activation in both contexts is controlled by the proteolytic activation of Toll-ligand, spz. Active spz production is controlled by zymogen activation cascades that are initiated by different cues – in dv patterning, localised activation of spz results in ventral nuclear accumulation of the NF-kB transcription factor dorsal (dl), whereas during immune challenge, zymogen cascades are activated by extracellular pattern recognition receptors or virulence factor-mediated cleavage of the zymogen persephone (psh). Vertebrate TLRs are pattern recognition receptors (PRRs) of the innate immune system which directly bind pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs). They may signal via a MyD88-NF-kB axis orthologous to the fly Toll intracellular signal transduction, or alternative signaling axes that leads to the activation of IRF3 and MAPKs. Additionally, as the TLR pathway is not tied to an extracellular zymogen cascade, some act as intracellular PRRs ([reviewed here](#)).

In the GO such differences can be tricky to accommodate. The GO term definitions can be made sufficiently broad to accommodate cross-species differences to facilitate comparative work, and where this is not possible, more granularity may be generated by creating new child or sibling terms. However, when this involves defining different start and end points of a process or its biological role, such a resolution not be possible. This is true of the TLR vs Toll pathways and in the GO they are separated into toll-like receptor signaling pathway (GO:0002224), classified as a ‘pattern recognition receptor signaling pathway’ (GO:0002221) and the Toll signaling pathway (GO:0008063), classified as a ‘cell surface receptor signaling pathway’, both of which group under signal transduction (GO:0007165). Although this solution works to accommodate the differences in these pathways, it fails to accommodate the zymogen cascade components that leads to the activation of spaztle, thus in the past curation these important components have been missed or incorrectly associated with the Toll signaling pathway (GO:0008063). We therefore created new terms to capture this process: Toll receptor ligand protein activation cascade (GO:0160032), regulation of Toll receptor ligand protein activation cascade (GO:0160033) and the positive (GO:0160034) and negative (GO:0160035) regulation terms. In the FlyBase Pathway groups, GO:0160032 maps to the ‘Extracellular Spatzle Activating Pathway Core Components’ page and GO:0160035 to the ‘Negative Regulators of Spatzle Activating Pathway’ page (there are currently no gene products annotated to GO:0160034. This mirrors the approach taken to pathways with ‘Ligand Production’ pages (EGFR ligand, Hedgehog and Wnt Production), where separate GO terms can define these processes which require very specific components.

11. Imd Signaling Pathway



The [immune deficiency (Imd) pathway](#) primarily mediates the humoral immune response to Gram-negative bacteria. Activation of the Imd pathway by diaminopimelic acid-type (DAP) peptidoglycan (PGN) initiates a signaling cascade that ultimately results in the release of the

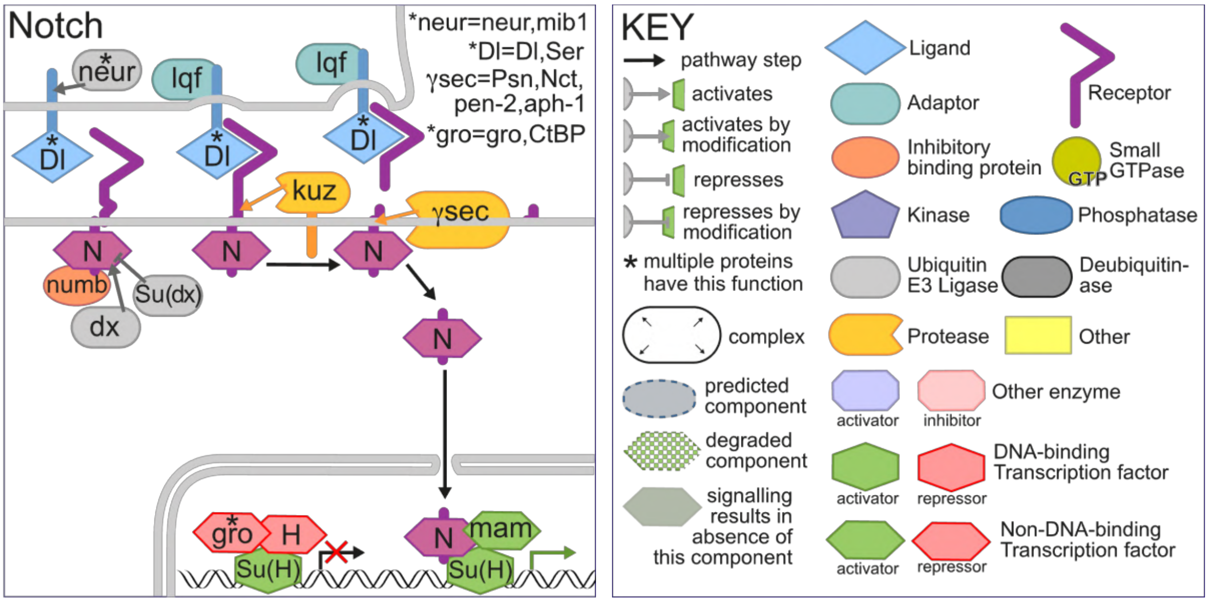
NFκB-like factor Rel from auto-inhibition and its translocation into the nucleus to activate the transcription of antimicrobial peptides (FBrf0224587, FBrf0238555.)
There are two DAP-PGN receptors in *D.mel*, a transmembrane receptor, PGRP-LC, and intracellular receptor PGRP-LE, that binds monomeric PGN (aka tracheal cytotoxin, TCT) that has been transported into the cell.
Activation of the pathway results in the cleavage of imd and the downstream activation of the IKK complex and activation of Rel.
Unlike mammalian NF-κB proteins, Rel possesses an N-terminal Rel homology domain (RHD), characteristic of NFκB transcription factors, and a C-terminal IκB-like domain. In unstimulated cells, Rel is auto-inhibited - sequestered in the cytosol. Activation of the Imd pathway leads to the cleavage of Rel, releasing the C-terminal IκB domain and allowing translocation of the active, RHD-containing N-terminal portion into the nucleus to regulate transcription of target genes (FBrf0233452).
The immune deficiency (Imd) pathway can also activate the JNK cascade (FBrf0151904, FBrf0204462) .

Pathway Page Terms:
GO:0061057 peptidoglycan recognition protein signaling pathway
GO:0061060 negative regulation of peptidoglycan recognition protein signaling pathway
GO:0061059 positive regulation of peptidoglycan recognition protein signaling pathway

Assays used for the Imd signaling pathway

I. Production of antimicrobial peptides - DptA, DptB, AttA-D (FBrf0234632).
II. NFκB luciferase reporter (cell culture, also a reporter for Toll-mediated signaling, FBrf0234632).
III. AttA-Luc reporter gene in cell culture (FBrf0227121)
IV. Cleavage and/or nuclear localization of Rel (FBrf0190362).
V. Survival rates/bacterial levels after infection with gram negative bacterial infection are also used to report on the integrity of the pathway, but should not be used as an assay in isolation (FBrf0234032).
VI. JNK pathway activation e.g. transcription of puc and Sulf1 (FBrf0204914).

12. Notch Signaling Pathway



The Notch receptor signaling pathway is activated by the binding of the transmembrane receptor Notch (N) to transmembrane ligands, Delta or Ser, presented on adjacent cells. This results in the proteolytic cleavage of N, releasing the intracellular domain (NICD). NICD translocates into the nucleus, interacting with Su(H) and mam to form a transcription complex, which up-regulates transcription of Notch-responsive genes. Notch cell-cell signaling is important in many cell fate decisions during development and in tissue homeostasis (FBrf0225731, FBrf0192604).
Notch signaling occurs between neighbouring cells and pathway components are required for signaling from the sending cell and response in the receiving cell. The reasoning behind annotating components in the sending cell (as regulators; besides the membrane-bound ligands which are annotated to the pathway term), is that some of these stimulate the cleavage of Notch in the receiving cell, possibly by generating tension forces.
GO:0007219 'Notch signaling pathway' should be reserved for **ligand-dependent** notch signaling between cells. The existence of ligand-independent/non-canonical signaling is not so well evidenced and, for some experimental systems, may be a non-physiologically relevant artefact e.g. manipulation of Vha subunits can result in the acidification of endosomal compartments, resulting in cleavage of Notch ligand and generation of NCID.

Pathway Page Terms:
GO:0007219 Notch signaling pathway
GO:0045746 negative regulation of Notch signaling pathway
GO:0045747 positive regulation of Notch signaling pathway

Assays used for the Notch signaling pathway (Reviewed in FBrf0225258)

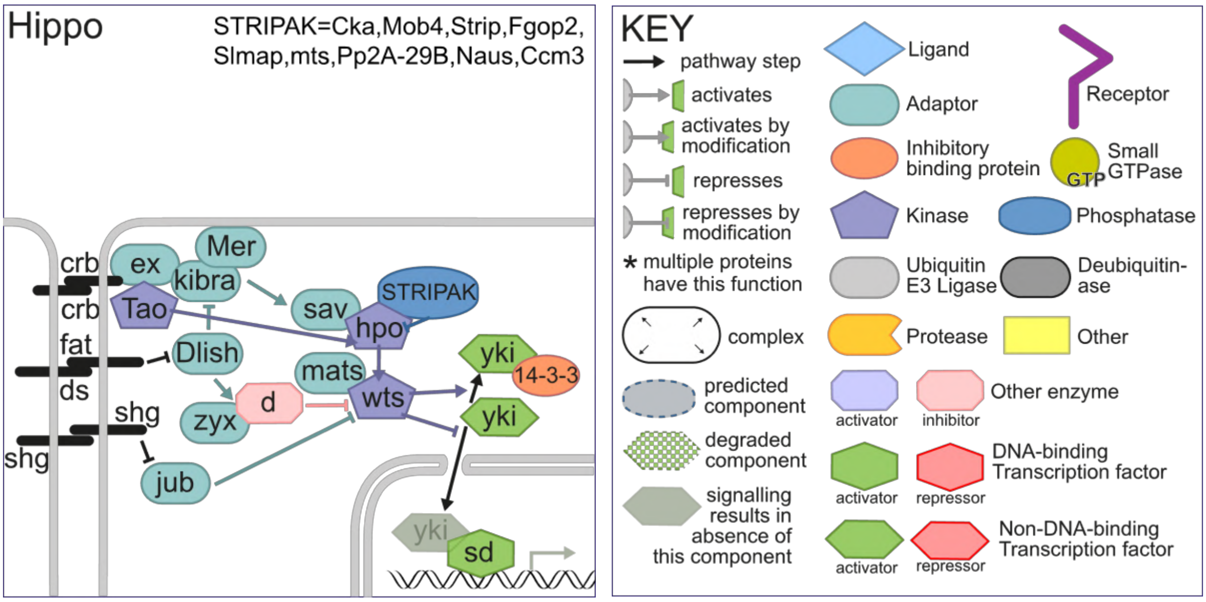
I. Cleavage of Notch.

II. Reporters with multimerised Su(H) binding motifs (FBrf0102729) such as the NRE element which comprises 2 paired Su(H) binding-sites (4 Su(H) sites total) and with grh binding-sites FBrf0134524, FBrf0217660).

III. HES genes present in the *Enhancer of split [E(spl)] locus*: *E(spl)mγ* (FBrf0102729), E(spl)m7-HLH (FBrf0195377), E(spl)mβ-HLH (FBrf0127044), E(spl)mδ-HLH (FBrf0106363), E(spl)m8-HLH (FBrf0073637). Expression of ct and wg at the wing disc D-V boundary. (In imaginal wing discs, Notch signaling is in a very thin strip at the D-V boundary. This is because the N activation is suppressed by cis-interactions when not adjacent to cells presenting ligand in trans).

IV. Phenotypes: wing margin notching, thickened veins, ectopic sensory bristles, misorientation of ommatidia (FBrf0237921).

13. Hippo Signaling Pathway



The Hippo signaling pathway is an intracellular kinase cascade in which hpo kinase in complex with sav, phosphorylates wts kinase which, in turn, phosphorylates yki transcriptional co-activator leading to its cytosolic retention. Activation of the Hippo pathway results in the down-regulation of cell proliferation and up-regulation of apoptosis, limiting tissue size (FBrf0224870).

Pathway Page Terms:
GO:0035329 hippo signaling
GO:0035331 negative regulation of hippo signaling
GO:0035332 positive regulation of hippo signaling

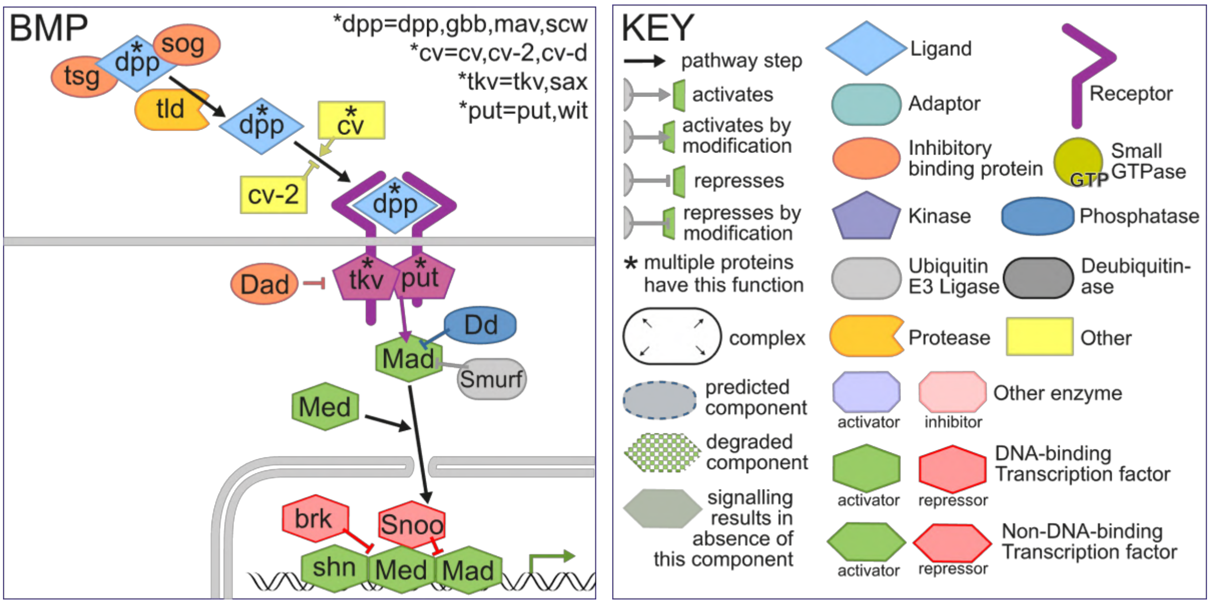
Assays used for the Hippo signaling pathway

Frequently, authors refer to hippo pathway activation and target genes when they are actually referring to the activation of yki and the expression of yki targets i.e. negative regulation of the pathway. Only genes that lie upstream of or directly influence yki cytosolic retention have been annotated as being within or regulating the Hippo Signaling Pathway. Nuclear factors that regulate yki-mediated transcription or DNA-binding transcription factors that act with yki such as sd, tsh and hth (FBrf0209052) should be annotated for their role in transcription not the pathway.

Much of the hippo signaling pathway depends on subcellular localization/clustering of components. Mutants that mis-direct components can produce regulatory effects that do not reflect a genuine LOF cellular phenotype. For example, cell polarity defects can affect the pathway due to the mis-localization of membrane components. Do not annotate these as regulating the pathway as this does not represent a biological phenomenon. Equally, when some membrane proteins have their membrane or extracellular domains removed, they act in a very different manner - dominant negative or having non-physiological effects, so try to avoid annotating incorrectly.

I. yki exclusion from the nucleus and phosphorylation (FBrf0204358).
II. wts phosphorylation on T1077 (FBrf0210017).
III. Down regulation of transcriptional of Diap1, ex, CycE (FBrf0194966) and mir-ban
IV. With other supporting evidence: tissue-overgrowth when core components or positive regulators removed (FBrf0230705).

14. BMP Signaling Pathway



The Bone Morphogenetic Protein (BMP) signaling pathway is one of two branches of Transforming Growth Factor-β family signaling in Drosophila. The binding of a BMP family dimer to a heterodimeric serine/threonine kinase receptor complex (composed of type I and type II subunits), results in the phosphorylation and activation of the type I receptor by the type II subunit. In the BMP branch, the downstream target of the type I receptor is Mad, a member of the Smad family. Mad forms a complex with the co-Smad, Med. This complex translocates into the nucleus and regulates the transcription of target genes in concert with other nuclear cofactors (FBrf0236482.)
BMPs signaling is used multiple times during development. For example, in the follicle cells to influence eggshell patterning and axis formation, in embryonic development; particularly as a morphogen in patterning and cell fate specification. In the wing disc, it controls growth and patterning and acts in cell movements e.g. tracheal cell migration and branching, dorsal closure It is also involved in regulating growth and morphogenesis of the NMJ (FBrf0236482).

BMP and activin signaling pathway are the only two branches of Transforming Growth Factor-β superfamily signaling in *Drosophila*. The GO term 'transforming growth factor beta receptor signaling pathway' (GO:0007179) should not be used as a generic term - it is not a parent term for these pathways in GO and represents a class of ligands that do not exist in flies.

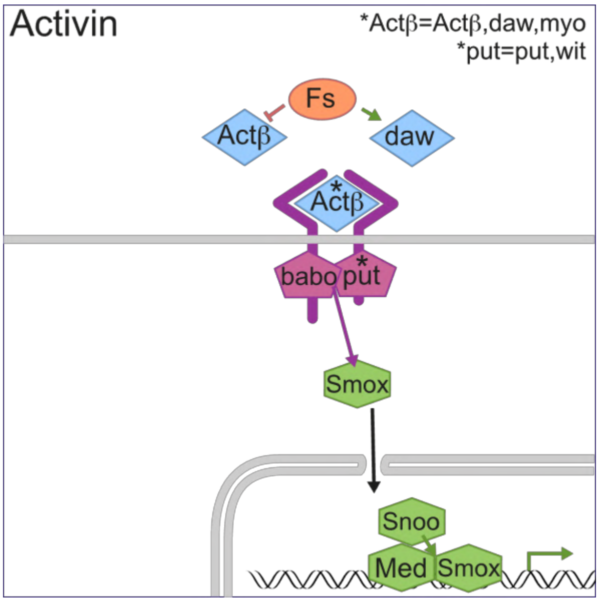
Pathway Page Terms:
GO:0030509 BMP signaling pathway
GO:0030514 negative regulation of BMP signaling pathway
GO:0030513 positive regulation of BMP signaling pathway

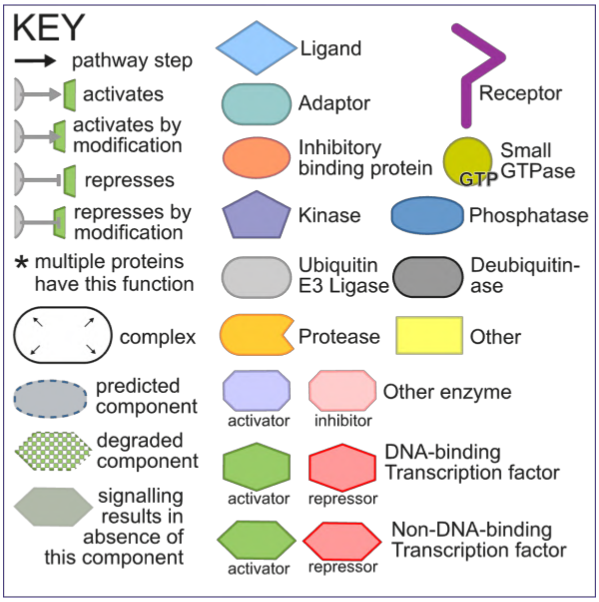
Assays used for the BMP signaling pathway

There are common components used in activin and BMP signaling: e.g. co-SMAD, Med and the type II receptors (put/wit). These pathways can be differentiated by the downstream SMAD (Mad for BMP signaling and Smox for activin signaling) and the type I receptors (sax/tkv for BMP signaling and babo for activin signaling). The receptor complexes bind different sets of ligands. The various combinations of these specific pathway components can be used to distinguish between BMP and activin signaling when combined with an assay which reports on any TGF-β-type signaling pathway.

I. The phosphorylation of Mad (FBrf0240051).
II. Increased transcription of target genes bi (FBrf0098897, FBrf0240051, FBrf0087626), Dad (FBrf0098897), lab (FBrf0051544), salm (FBrf0220378).
III. Decreased transcription of target genes brk (FBrf0107889, FBrf0158763)
IV. Phenotypes: Wing development LOF - diminished wing size and lack of crossveins (FBrf0187398).

15. Activin Signaling Pathway





The activin signaling pathway is one of two branches of Transforming Growth Factor-β family signaling in Drosophila. The binding of an activin family dimer to a heterodimeric serine/threonine kinase receptor complex (composed of type I and type II subunits), results in the phosphorylation and activation of the type I receptor by the type II subunit. In the activin branch, the downstream target of the type I receptor is Smox, a member of the Smad family. Smox forms a complex with the co-Smad, Med. This complex translocates into the nucleus and regulates the transcription of target genes in concert with other nuclear cofactors (FBrf0236482.)

Activin signaling has a less prominent role in development than BMP. It has roles in guidance, remodelling and proliferation on the nervous system and regulates the production of some hormones (FBrf0236482).

BMP and activin signaling pathway are the only two branches of Transforming Growth Factor-β superfamily signaling in *Drosophila*. The GO term 'transforming growth factor beta receptor signaling pathway' (GO:0007179) should not be used as a generic term - it is not a parent term for these pathways in GO and represents a class of ligands that do not exist in flies.

Pathway Page Terms:

GO:0032924 activin receptor signaling pathway
GO:0032926 negative regulation of activin receptor signaling pathway
GO:0032927 positive regulation of activin receptor signaling pathway

There are common components used in activin and BMP signaling: e.g. co-SMAD, Med and the type II receptors (put/wit). These pathways can be differentiated by the downstream SMAD (Mad for BMP signaling and Smox for activin signaling) and the type I receptors (sax/tkv for BMP signaling and babo for activin signaling). The receptor complexes bind different sets of ligands. The various combinations of these specific pathway components can be used to distinguish between BMP and activin signaling when combined with an assay which reports on any TGF-β-type signaling pathway.

The activin receptor consists of a babo (type I receptor) isoform with either put or wit (type II receptor). babo has three different isoforms:

Isoform	**length (aa)**	**UniProtKB**	
babo-A	601	A1Z7L9	
babo-B	622	A1Z7L8	(ref proteome)
babo-C	595	Q7YU60	

FBrf0194818 suggests that babo isoforms A and B can bind daw
FBrf0066967 suggests that babo isoforms A and B can bind Actβ
FBrf0209265 suggests that daw only uses put, not wit and preferentially acts with babo-C

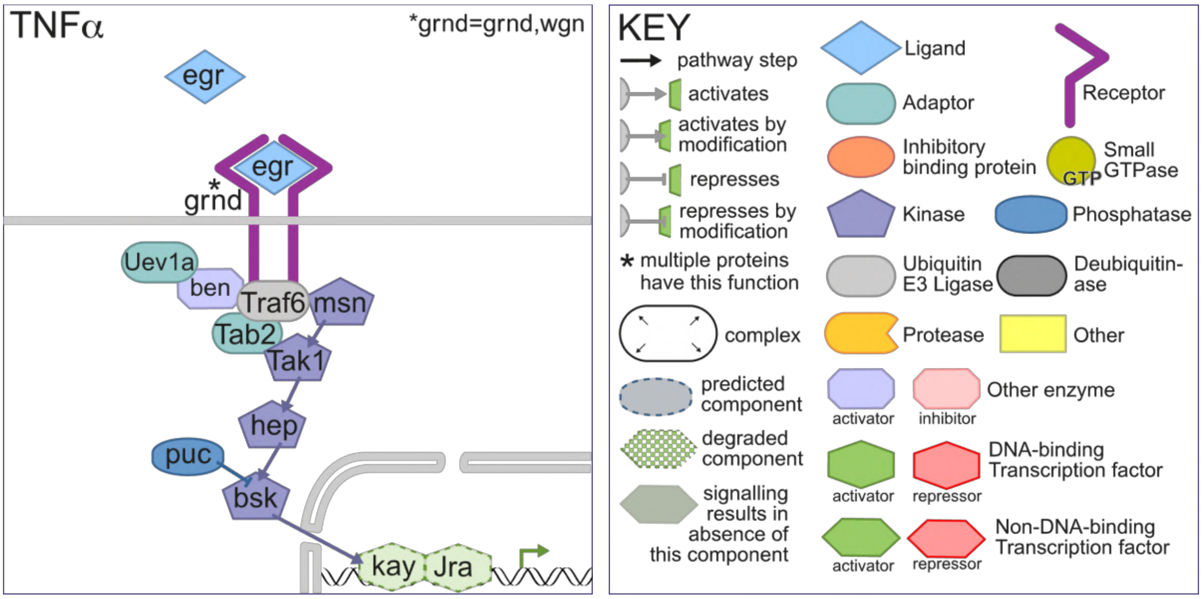
If the isoform is specified, annotate to that particular isoform in Protein2GO and add a comment to the annotation to explain why isoform was chosen. If no isoform was used, use the reference proteome isoform (A1Z7L8) and then note that this was chosen as no isoform was specified.

Assays used for the activin signaling pathway

I. Phosphorylation of Smox (FBrf0106271, FBrf0194818)

II. 3TP-Lux luciferase reporter in cell culture (note, that this is probably also responsive to BMP pathway activation but we have only seen this used with the activin pathway so far, FBrf0187566)

16. TNFα-Eiger Signaling Pathway



The Tumor Necrosis Factor α (TNFα) signaling pathway is activated by egr binding to a member of the TNF receptor superfamily. Activation of the pathway leads to activation of the Jun N-terminal kinase (JNK) cascade and cell death (FBrf0225608.)
The two TNF receptors in *D.mel* are wgn and grnd. While egr is usually TM-bound, it can be shed by Tace to circulate in the blood, acting remotely through grnd (FBrf0232008).
To promote apoptosis, the pathway activates transcription of hid, rpr and grim (not to be annotated to the pathway), which block Diap1 (inhibitor of apoptosis).

Pathway Page Terms:
GO:0033209 tumor necrosis factor-mediated signaling pathway
GO:0010804 negative regulation of tumor necrosis factor-mediated signaling pathway
GO:1903265 positive regulation of tumor necrosis factor-mediated signaling pathway

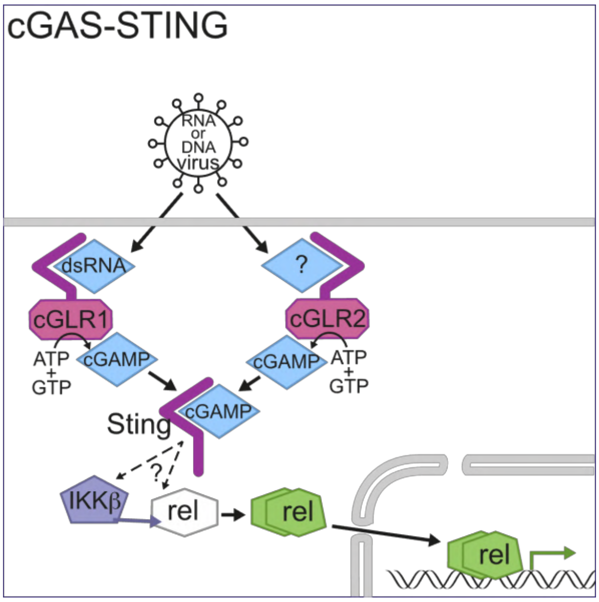
Assays used for the TNFα signaling pathway:

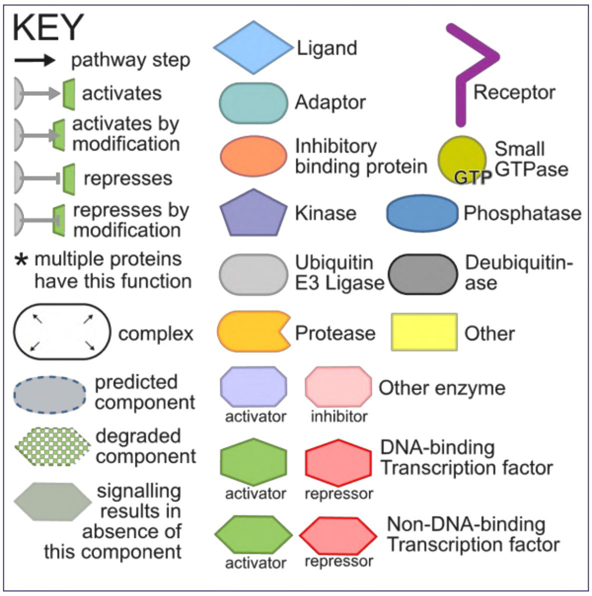
I. LacZ enhancer-trap allele for puc. This assay is usually used to check activation of JNK cascade. To confirm that the JNK cascade was activated by egr, puc expression level is assessed in the eye disc of GMR>regg1GS9830 flies (FBrf0148977).

II. Phenotypes: small eye phenotype, necrosis tissue in the eye.

Notes:
While in other models [Traf4](#) orthologs have a role in the TNFα signalling pathway, in *D.mel* it has been shown that this gene is not involved ([FBrf0200559](#)).
[kay](#) and [Jra](#) are known targets of the JNK cascade, so we would expect to see evidence of them being targets of the TNFα signaling pathway too. There seems to be no experimental evidence showing a direct effect of [egr](#) signalling on these two genes, though, and [FBrf0148977](#) even shows that [Jra](#) shows no genetic interaction with [egr](#).

17. cGAS/STING Signaling Pathway





The [cGAS/STING Signaling Pathway](#) (cyclic GMP-AMP synthase-stimulator of interferon genes pathway) is an anti-viral pattern recognition pathway. In Drosophila, cyclic GMP-AMP (cGAMP) synthases are activated by viral infection, cGAMP is in turn detected by STING ultimately resulting in the activation the NF-κB-like factor [Rel](#) and its translocation into the nucleus to activate the transcription of anti-viral genes ([FBrf0252868](#)). As this is a relatively recent discovery in flies, there is currently very little data associated with this pathway.

Pathway Page Terms:
GO:0140896 cGAS/STING signaling pathway

<u>Assays used for the cGAS/STING Signaling Pathway</u>
I. Measuring cGAMP levels (mass spectrometry, chromatography) ([FBrf0250901](#)).
II. STING-dependent expression of genes such as [nazo](#), [STING](#), [Srg1](#), [Srg2](#) and [Srg3](#) ([FBrf0252229](#)).

Gene Group to GO term mapping

Pathway Name (ID) _Sub-Group Name (ID)	GO Term Mapping (ID)
Activin Signaling Pathway (FBgg0001093)	
_Activin Signaling Pathway Core Components (FBgg0001092)	activin receptor signaling pathway (GO:0032924)
_Negative Regulators of Activin Signaling Pathway (FBgg0001091)	negative regulation of activin receptor signaling pathway (GO:0032926)
_Positive Regulators of Activin Signaling Pathway (FBgg0001095)	positive regulation of activin receptor signaling pathway (GO:0032927)
BMP Signaling Pathway (FBgg0001086)	
_BMP Signaling Pathway Core Components (FBgg0001085)	BMP signaling pathway (GO:0030509)
_Negative Regulators of BMP Signaling Pathway (FBgg0001084)	negative regulation of BMP signaling pathway (GO:0030514)
_Positive Regulators of BMP Signaling Pathway (FBgg0001094)	positive regulation of BMP signaling pathway (GO:0030513)
cGAS/STING Signaling Pathway (FBgg0001792)	
_cGAS/STING Signaling Pathway Core Components (FBgg0001793)	cGAS/STING signaling pathway (GO:0140896)
EGFR Signaling Pathway (FBgg0000958)	
_EGFR Ligand Production (FBgg0000953)	epidermal growth factor receptor ligand maturation (GO:0038004)
_EGFR Signaling Pathway Core Components (FBgg0000951)	epidermal growth factor receptor signaling pathway (GO:0007173)
_Negative Regulators of EGFR Signaling Pathway (FBgg0000950)	negative regulation of epidermal growth factor receptor signaling pathway (GO:0042059)
_Positive Regulators of EGFR Signaling Pathway (FBgg0000956)	positive regulation of epidermal growth factor receptor signaling pathway (GO:0045742)
FGFR Signaling Pathway (FBgg0000967)	
_FGFR Signaling Pathway Core Components (FBgg0000968)	fibroblast growth factor receptor signaling pathway (GO:0008543)
_Negative Regulators of FGFR Signaling Pathway (FBgg0000969)	negative regulation of fibroblast growth factor receptor signaling pathway (GO:0040037)
_Positive Regulators of FGFR Signaling Pathway (FBgg0000970)	positive regulation of fibroblast growth factor receptor signaling pathway (GO:0045743)
Hedgehog Signaling Pathway (FBgg0000978)	
_Hedgehog Production (FBgg0001018)	patched ligand maturation (GO:0007225)
_Hedgehog Signaling Pathway Core Components (FBgg0000979)	smoothened signaling pathway (GO:0007224)
_Negative Regulators of Hedgehog Signaling Pathway (FBgg0000980)	negative regulation of smoothened signaling pathway (GO:0045879)
_Positive Regulators of Hedgehog Signaling Pathway (FBgg0000981)	positive regulation of smoothened signaling pathway (GO:0045880)
Hippo Signaling Pathway (FBgg0000917)	
_Hippo Signaling Pathway Core Components (FBgg0000913)	hippo signaling (GO:0035329)
_Negative Regulators of Hippo Signaling Pathway (FBgg0000916)	negative regulation of hippo signaling (GO:0035331)
_Positive Regulators of Hippo Signaling Pathway (FBgg0000912)	positive regulation of hippo signaling (GO:0035332)
Insulin-like Receptor Signaling Pathway (FBgg0000910)	
_Insulin-like Receptor Signaling Pathway Core Components (FBgg0000904)	insulin receptor signaling pathway (GO:0008286)
_Negative Regulators of Insulin-like Receptor Signaling Pathway (FBgg0000900)	negative regulation of insulin receptor signaling pathway (GO:0046627)
_Positive Regulators of Insulin-like Receptor Signaling Pathway (FBgg0000898)	positive regulation of insulin receptor signaling pathway (GO:0046628)
Imd Signaling Pathway (FBgg0001194)	
_Imd Signaling Pathway Core Components (FBgg0001195)	peptidoglycan recognition protein signaling pathway (GO:0061057)
_Negative Regulators of Imd Signaling Pathway (FBgg0001196)	negative regulation of peptidoglycan recognition protein signaling pathway (GO:0061060)
_Positive Regulators of Imd Signaling Pathway (FBgg0001197)	positive regulation of peptidoglycan recognition protein signaling pathway (GO:0061059)
JAK-STAT Signaling Pathway (FBgg0000883)	
_JAK-STAT Signaling Pathway Core Components (FBgg0000881)	receptor signaling pathway via JAK-STAT (GO:0007259)
_Negative Regulators of JAK-STAT Signaling Pathway (FBgg0000884)	negative regulation of receptor signaling pathway via JAK-STAT (GO:0046426)
_Positive Regulators of JAK-STAT Signaling Pathway (FBgg0000882)	positive regulation of receptor signaling pathway via JAK-STAT (GO:0046427)
Notch Signaling Pathway (FBgg0001068)	
_Notch Signaling Pathway Core Components (FBgg0001064)	Notch signaling pathway (GO:0007219)
_Negative Regulators of Notch Signaling Pathway (FBgg0001063)	negative regulation of Notch signaling pathway (GO:0045746)
_Positive Regulators of Notch Signaling Pathway (FBgg0001069)	positive regulation of Notch signaling pathway (GO:0045747)
Pvr Signaling Pathway Core Components (FBgg0000973)	

_Pvr Signaling Pathway Core Components (FBgg0000973)	vascular endothelial growth factor receptor signaling pathway (GO:0048010)
_Negative Regulators of Pvr Signaling Pathway (FBgg0000974)	negative regulation of vascular endothelial growth factor receptor signaling pathway (GO:0030948)
Sevenless Signaling Pathway (FBgg0000941)	
_Sevenless Signaling Pathway Core Components (FBgg0000935)	sevenless signaling pathway (GO:0045500)
_Negative Regulators of Sevenless Signaling Pathway (FBgg0000942)	negative regulation of sevenless signaling pathway (GO:0045873)
_Positive Regulators of Sevenless Signaling Pathway (FBgg0000938)	positive regulation of sevenless signaling pathway (GO:0045874)
Toll Signaling Pathway (FBgg0001059)	
_Extracellular Spatzle Activating Pathway Core Components (FBgg0001056)	Toll receptor ligand protein activation cascade (GO:0160032)
_Negative Regulators of Spatzle Activating Pathway (FBgg0001055)	negative regulation of Toll receptor ligand protein activation cascade (GO:0160035)
_Toll-NF-kappaB Signaling Pathway Core Components (FBgg0001058)	Toll signaling pathway (GO:0008063)
_Negative Regulators of Toll-NF-kappaB Signaling Pathway (FBgg0001053)	negative regulation of Toll signaling pathway (GO:0045751)
_Positive Regulators of Toll-NF-kappaB Signaling Pathway (FBgg0001057)	positive regulation of Toll signaling pathway (GO:0045752)
TNFalpha-Eiger Signaling Pathway (FBgg0001560)	
_TNFalpha-Eiger Signaling Pathway Core Components (FBgg0001561)	tumor necrosis factor-mediated signaling pathway (GO:0033209)
_Negative Regulators of TNFalpha-Eiger Signaling Pathway (FBgg0001562)	negative regulation of tumor necrosis factor-mediated signaling pathway (GO:0010804)
_Positive Regulators of TNFalpha-Eiger Signaling Pathway (FBgg0001563)	positive regulation of tumor necrosis factor-mediated signaling pathway (GO:1903265)
Torso Signaling Pathway (FBgg0000934)	
_Torso Signaling Pathway Core Components (FBgg0000937)	torso signaling pathway (GO:0008293)
_Negative Regulators of Torso Signaling Pathway (FBgg0000940)	negative regulation of torso signaling pathway (GO:0120177)
_Positive Regulators of Torso Signaling Pathway (FBgg0000936)	positive regulation of torso signaling pathway (GO:0120176)
Wnt-TCF Signaling Pathway (FBgg0000889)	
_Wnt Production (FBgg0000918)	Wnt protein secretion (GO:0061355)
_Wnt-TCF Signaling Pathway Core Components (FBgg0000890)	canonical Wnt signaling pathway (GO:0060070)
_Negative Regulators of Wnt-TCF Signaling Pathway (FBgg0000891)	negative regulation of canonical Wnt signaling pathway (GO:0090090)
_Positive Regulators of Wnt-TCF Signaling Pathway (FBgg0000892)	positive regulation of canonical Wnt signaling pathway (GO:0090263)