General Overview 1

General Rules **2**

Pathway specific guidance **4**

Canonical Wnt pathway/Wnt-TCF Signaling Pathway 4

JAK-STAT Signaling Pathway 5

Insulin-like Receptor Signaling Pathway 6

Fibroblast Growth Factor Receptor Signaling Pathway 8

Platelet-Derived Growth Factor-Vascular Endothelial Growth Factor Receptor-Related Signaling Pathway 9

Sevenless Signaling Pathway 100

Epidermal Growth Factor Receptor Signaling Pathway 111

Torso Signaling Pathway 122

Hedgehog Signaling Pathway 133

Toll Signaling Pathway 14

Imd Signaling Pathway 165

Notch Signaling Pathway 176

Hippo Signaling Pathway 18

BMP 19

Activin 210

TNFα-Eiger Signaling Pathway 22

Pathway Curation in FlyBase

General Overview

Pathway components must be curated with particular care as they are used to populate **pathway pages** as follows:

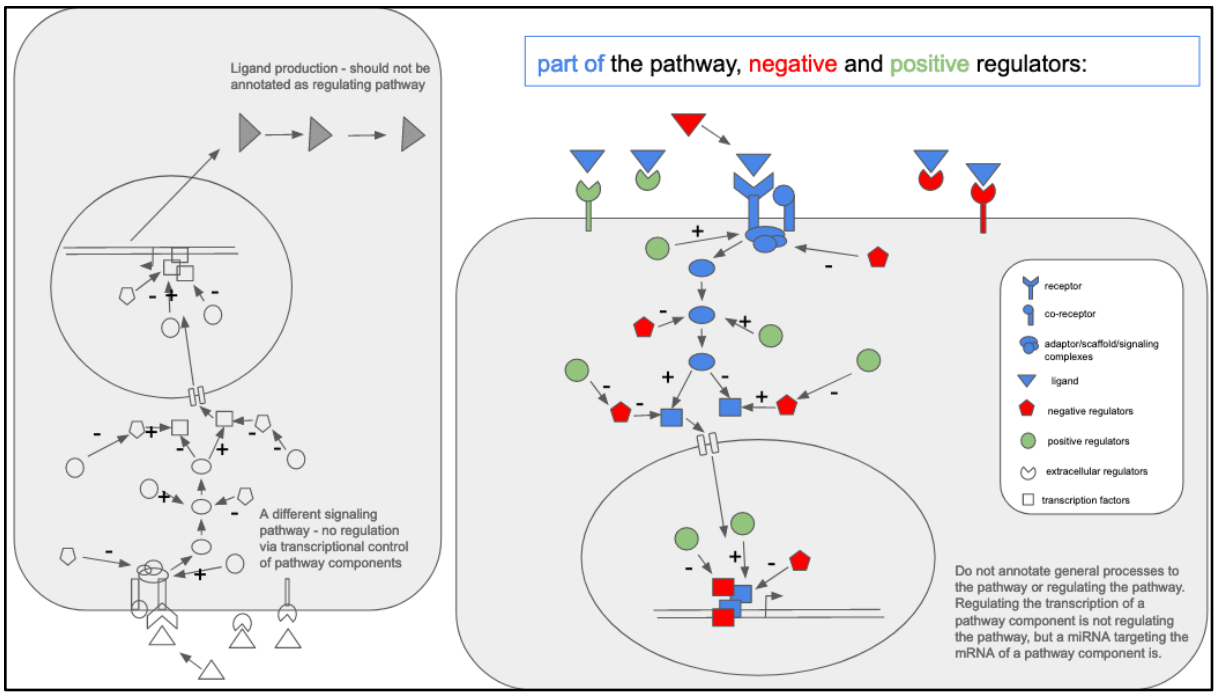
Core: The genes that lie within a pathway, required for executing the defined end-point of the pathway, should be annotated using the GO process term for pathway. Such genes include ligands, receptors and transcription factors that are specific for that pathway. General process entities, such as general chromatin modifying proteins, should not be labelled as part of the pathway.

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 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. GO:0000122 negative regulation of transcription by RNA polymerase II

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. Pathways components can be targeted by gene products that also target other pathways e.g. Med is a core component of 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 another larger process term in GO (i.e. not single-step processes such as phosphorylation or sub-processes such as pathway cassettes, such as MAPK signaling) 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.
   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 endocytotic

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, epidermal growth factor receptor ligand maturation, patched ligand maturation).
   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. Canonical Wnt pathway/Wnt-TCF Signaling Pathway

The canonical Wnt signaling (known as the Wnt-TCF signaling pathway in FlyBase) 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 canonical wnt 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 canonical Wnt 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. Beta-catenin/arm translocation into nucleus (FBrf0158859)
iv. Assembly of destruction complex (FBrf0245515)
v. LOF Phenotypic assay (if supported by other evidence):
    1. cuticle/segmentation phenotypes e.g. lawn-of-denticles (FBrf0223299).
    2. Wing/wing disc phenotypes (FBrf0072872) e.g. loss of wing margin bristles and the appearance of notches along the wing margin.

Other frequently used & other useful terms associated with canonical Wnt pathway components:

Molecular Function

GO:0042813 Wnt-activated receptor activity
GO:0016015 morphogen activity
GO:0015026 coreceptor activity
GO:0005109 frizzled binding
GO:0048018 receptor ligand activity
GO:0060090 molecular adaptor activity
GO:0008013 beta-catenin binding
GO:0003713 transcription coactivator activity
GO:0017147 Wnt-protein binding

Biological Process

GO:0061357 positive regulation of Wnt protein secretion
GO:0061358 negative regulation of Wnt protein secretion
GO:0007367 segment polarity determination
GO:0032436 positive regulation of proteasomal ubiquitin-dependent protein catabolic process
GO:0008587 imaginal disc-derived wing margin morphogenesis
GO:0035293 chitin-based larval cuticle pattern formation
GO:0048190 wing disc dorsal/ventral pattern formation
GO:0007476 imaginal disc-derived wing morphogenesis
GO:0007480 imaginal disc-derived leg morphogenesis
GO:0031146 SCF-dependent proteasomal ubiquitin-dependent protein catabolic process

Cellular Component

GO:1990907 beta-catenin-TCF complex
GO:0030877 beta-catenin destruction complex
GO:0019005 SCF ubiquitin ligase complex

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

Other frequently used & other useful terms associated with JAK-STAT pathway components:
Molecular function
GO:0004896 cytokine receptor activity
GO:0005126 cytokine receptor binding
GO:0005125 cytokine activity
GO:0097677 STAT family protein binding
Biological Process
GO:0008284 positive regulation of cell population proliferation

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 (GO:0014065 phosphatidylinositol 3-kinase signaling).
b This activates the 3-phosphoinositide-dependent protein kinase, Pdk1 that phosphorylates and activates Akt1 (PKB) (GO:0051897 positive regulation of protein kinase B signaling).

c. This is opposed by Pten that converts PIP3 to PIP2 (GO:0014067 negative regulation of phosphatidylinositol 3-kinase signaling).
d. Akt1 kinase phosphorylates many components in the pathway including foxo, sgg and Tsc1/Tsc2(gig) (GO:0043491 protein kinase B signaling).
e. Akt1 inhibits the activity of the TSC1-TSC2 complex (GO:0033596 TSC1-TSC2 complex), a Rheb GTPase that stimulates Torc1 signaling (GO:1904263 positive regulation of TORC1 signaling) and therefore the TSC1-TSC2 complex (GO:1904262 negative regulation of TORC1 signaling).
f. The TORC1 complex (GO:0031931 TORC1 complex) is a Tor kinase-containing complex, inhibited by rapamycin, that phosphorylates many downstream targets of the insulin pathway including S6k and Thor (GO:0038202 TORC1 signaling). The TORC1 complex is also activated by amino acids and stress signaling.
g. The TORC2 complex (GO:0031932 TORC2 complex, GO:0038203 TORC2 signaling) phosphorylates Akt1, enhancing Pdk1 phosphorylation of the Akt1 T-loop and therefore supporting full activation of Akt1 (GO:0051897 positive regulation of protein kinase B signaling)

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.

1. Phosphorylation of:
    a. Akt1 (PI3K branch)
    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)
2. tGFP (PH domain-GFP fusion protein; FBrf0144797) marker of PI3K activation.
3. Exclusion of foxo from nucleus (PI3K branch)

Other frequently used & other useful terms associated with insulin receptor pathway components:

Molecular function

GO:0043560 insulin receptor substrate binding
GO:0005158 insulin receptor binding
GO:0005068 transmembrane receptor protein tyrosine kinase adaptor activity
GO:0005009 insulin-activated receptor activity

Biological Process

GO:0014065 phosphatidylinositol 3-kinase signaling
GO:0043491 protein kinase B signaling
GO:0040018 positive regulation of multicellular organism growth
GO:0042593 glucose homeostasis

GO:0032869 cellular response to insulin stimulus
GO:0030307 positive regulation of cell growth
GO:0038202 TORC1 signaling
GO:0014065 phosphatidylinositol 3-kinase signaling
GO:0008284 positive regulation of cell population proliferation
GO:0038203 TORC2 signaling
GO:1903940 negative regulation of TORC2 signaling
GO:0070371 ERK1 and ERK2 cascade
GO:0008340 determination of adult lifespan
GO:0070328 triglyceride homeostasis
GO:0045793 positive regulation of cell size
GO:0009267 cellular response to starvation
GO:0046622 positive regulation of organ growth
GO:0007568 aging

Cellular Component

GO:0033596 TSC1-TSC2 complex
GO:0031932 TORC2 complex
GO:0031931 TORC1 complex
GO:0000159 protein phosphatase type 2A complex
GO:0005943 phosphatidylinositol 3-kinase complex, class IA

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 differential btl or 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)

Other frequently used & other useful terms associated with FGFR pathway components:

Molecular Function

GO:0005104 fibroblast growth factor receptor binding

GO:0005007 fibroblast growth factor-activated receptor activity
GO:0005068 transmembrane receptor protein tyrosine kinase adaptor activity
GO:0048018 receptor ligand activity
GO:0042056 chemoattractant activity
MAPK/Erk1 Cascade terms:
GO:0004707 MAP kinase activity
GO:0004708 MAP kinase kinase activity
GO:0004709 MAP kinase kinase kinase activity
GO:0005078 MAP-kinase scaffold activity
Biological Process
GO:0007426 tracheal outgrowth, open tracheal system
GO:0007427 epithelial cell migration, open tracheal system
GO:0007426 tracheal outgrowth, open tracheal system
GO:0007430 terminal branching, open tracheal system
GO:0007498 mesoderm development
GO:0008078 mesodermal cell migration
GO:0001710 mesodermal cell fate commitment
GO:0021782 glial cell development
GO:0070371 ERK1 and ERK2 cascade

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:
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!
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

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:

a. Phosphorylation of:
    i. Pvr tyrosine
    ii. Jun kinase (bsk) (for the JNK branch)
    iii. rl (for Erk branch)
    iv. Akt1 (PI3K branch)
    v. S6k (PI3K branch - it's downstream of TORC1, so could also be a marker for TOR pathway)
b. tGFP (PH domain-GFP fusion protein; FBrf0144797) marker of PI3K activation.
c. 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, Rac and Cdc42 proteins (FBrf0180198)
d. Cell size - in cell culture (FBrf0209753)
e. Border cell migration (FBrf0187480)
f. Hemocyte number (FBrf0180198)

Other frequently used & other useful terms associated with insulin receptor pathway components

Molecular function

GO:0005068 transmembrane receptor protein tyrosine kinase adaptor activity
GO:0005172 vascular endothelial growth factor receptor binding
GO:0035591 signaling adaptor activity

MAPK/Erk1 Cascade terms:

GO:0004707 MAP kinase activity
GO:0004708 MAP kinase kinase activity
GO:0004709 MAP kinase kinase kinase activity
GO:0005078 MAP-kinase scaffold activity

Biological Process

GO:0035099 hemocyte migration
GO:0007298 border follicle cell migration
GO:0046330 positive regulation of JNK cascade
GO:1904263 positive regulation of TORC1 signaling
GO:0070371 ERK1 and ERK2 cascade

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.

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).

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.

Pathway Page Terms:

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

Other frequently used & other useful terms associated with insulin receptor pathway components

Molecular function

GO:0008288 boss receptor activity
GO:0005118 sevenless binding
GO:0035591 signaling adaptor activity
GO:0005068 transmembrane receptor protein tyrosine kinase adaptor activity

Biological process

GO:0007465 R7 cell fate commitment
GO:0070371 ERK1 and ERK2 cascade

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

1. Activation of rl (pErk) (e.g. FBrf0223725, FBrf0098244, FBrf0210285)
2. 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 are in the EGFR pathway and not another RTK.

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

Other frequently used & other useful terms associated with Egfr pathway components
Molecular function
GO:0005154 epidermal growth factor receptor binding
Biological Process
GO:0038005 peptide bond cleavage involved in epidermal growth factor receptor ligand maturation
GO:0007474 imaginal disc-derived wing vein specification
GO:0001751 compound eye photoreceptor cell differentiation
GO:0007426 tracheal outgrowth, open tracheal system
GO:0070371 ERK1 and ERK2 cascade

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.)

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.:

1. Activation of rl (pErk) (FBrf0157176)
2. cic excluded from nucleus (FBrf0157176)
3. Expression of tll and hkb (FBrf0157176)
4. Mutant phenotype: lack of embryonic terminal structures (FBrf0135732)

Pathway Page Terms:

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

Other frequently used & other useful terms associated with insulin receptor pathway components

Molecular function

GO:0005122 torso binding

Biological Process

GO:0007362 terminal region determination
GO:0070371 ERK1 and ERK2 cascade

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 present 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

1. ci nuclear accumulation of the full length version.
2. Cleavage of ci to the repressor form, Ci(R) (for negative regulation)
3. Phosphorylation of downstream components e.g. cos phosphorylation at Ser-57 (FBrf0211312), smo phosphorylation.
4. 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)
5. Width wing disc, the width of the Ci(A)//kn domain is often used as a readout of activity.
6. ci cleavage state: e.g. antibodies which recognize the full-length but not the truncated form of ci (FBrf0211312, FBrf0123234).
7. *ptc-luc* reporter assay in cell culture (FBrf0245753)

Other frequently used & other useful terms associated with hedgehog pathway components:

Molecular function

GO:0097108 hedgehog family protein binding
GO:0005113 patched binding
GO:0005119 smoothened binding
GO:0008158 hedgehog receptor activity

Biological Process

GO:0035222 wing disc pattern formation
GO:0048100 wing disc anterior/posterior pattern formation
GO:0007367 segment polarity determination

Cellular Component

GO:0035301 Hedgehog signaling complex

Useful notes:
There are two common reagents used when looking at PkA signaling in the hh pathway:
UAS-mC* or C* (Mmus\PrkacamC.UAS, FBal0058457) - 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 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

Assays used for the Toll signaling pathway

1. Production of antimicrobial peptides - Drs (also induced by Imd, but to a much less extent), BomS1,
2. NFκB luciferase reporter (cell culture, also a reporter for Imd signaling FBrf0234632)
3. Susceptibility to fungal and gram-positive bacterial infections (FBrf0190205)
4. 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.
5. Nuclear localization of dl, FBrf0217797.
6. Cleavage/activation of components of the zymogen cascade (FBrf0135928).

Other frequently used & other useful terms associated with Toll Signaling pathway components:

Molecular function

GO:0004252 serine-type endopeptidase activity
GO:0005121 Toll binding
GO:0042834 peptidoglycan binding
GO:0038187 pattern recognition receptor activity
GO:0008745 N-acetylmuramoyl-L-alanine amidase activity

Biological Process

GO:0009950 dorsal/ventral axis specification
GO:0045087 innate immune response and child terms that give pathogen responded to)
GO:0002225 positive regulation of antimicrobial peptide production
GO:0050830 defense response to Gram-positive bacterium
GO:0050832 defense response to fungus
GO:0061760 antifungal innate immune response
GO:0031638 zymogen activation

Special note for the Toll pathway pages:

As GO:0008063 Toll signaling pathway is defined as “A series of molecular signals initiated by the binding of an extracellular ligand to the receptor Toll on the surface of a target cell, and ending with

regulation of a downstream cellular process, e.g. transcription." This does not include the proteolytic activation of spatzle ligand, which for insects is a crucial part of this pathway, we need to resolve this disparity with the GO. Also, GO:0008063 Toll signaling pathway is just applicable to Drosophila and is not related to GO:0002224 toll-like receptor signaling pathway in what we would think of as a meaningful way. This has not been resolved - it is difficult to accommodate the other species wanting to nest this term under GO:0002221 'pattern recognition receptor signaling pathway' - which excludes its use for Drosophila DV pattern formation. This will need more work on our part to find a solution. In the interim we will use these pages with the mapping to the GO terms as indicated for the proteolytic activation of spatzle ligand (there are no positive regulators of this cascade that we have found):
Extracellular Spatzle Activating Pathway Core Components - GO:0045752 positive regulation of Toll signaling pathwa
Negative Regulators of Spatzle Activating Pathway - GO:0045751 negative regulation of Toll signaling pathway

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

1. Production of antimicrobial peptides - DptA, DptB, AttA-D (FBrf0234632).
2. NFκB luciferase reporter (cell culture, also a reporter for Toll-mediated signaling, FBrf0234632).

3. AttA-Luc reporter gene in cell culture (FBrf0227121)
4. Cleavage and/or nuclear localization of Rel (FBrf0190362).
5. Survival rates/bacterial levels after infection with gram negative bacteria infection are also used to report on the integrity of the pathway, but should not be used as an assay in isolation (FBrf0234032).
6. JNK pathway activation e.g. transcription of puc and Sulf1 (FBrf0204914).

Other frequently used & other useful terms associated with insulin receptor pathway components

Molecular function

GO:0016019 peptidoglycan receptor activity
GO:0051059 NF-kappaB binding

Biological Process

GO:0050829 defense response to Gram-negative bacterium
GO:0045087 innate immune response
GO:0006964 positive regulation of biosynthetic process of antibacterial peptides active against Gram-negative bacteria
GO:0038061 NIK/NF-kappaB signaling

Cellular Component

GO:0033256 I-kappaB/NF-kappaB complex

12. Notch Signaling Pathway

The Notch receptor signaling pathway is activated by the binding of the transmembrane receptor Notch (N) to transmembrane ligands, Dl 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)

1. Cleavage of Notch.
2. 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).
3. 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 adjecent to cells presenting ligand in trans).
4. Phenotypes: wing margin notching, thickened veins, ectopic sensory bristles, misorientation of ommatidia (FBrf0237921).

Other frequently used & other useful terms associated with insulin receptor pathway components:

Molecular function
GO:0005112 Notch binding
GO:0048018 receptor ligand activity
Biological Process
GO:0007423 sensory organ development
GO:0008587 imaginal disc-derived wing margin morphogenesis
GO:0016360 sensory organ precursor cell fate determination
GO:0048190 wing disc dorsal/ventral pattern formation
GO:0007220 Notch receptor processing
GO:0006509 membrane protein ectodomain proteolysis
GO:0046331 lateral inhibition
GO:0035333 Notch receptor processing, ligand-dependent
Cellular Component
GO:0070765 gamma-secretase complex
GO:1990433 CSL-Notch-Mastermind transcription factor complex

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.

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

Other frequently used & other useful terms associated with insulin receptor pathway components:

Biological Process

GO:0046621 negative regulation of organ growth
GO:0008285 negative regulation of cell population proliferation
GO:0043065 positive regulation of apoptotic process (this should really be causally upstream, fix when doing apoptotic pathway)

Cellular Component

GO:0090443 FAR/SIN/STRIPAK complex
GO:0036375 Kibra-Ex-Mer complex
GO:0045179 apical cortex
GO:0098592 cytoplasmic side of apical plasma membrane
GO:0016327 apicolateral plasma membrane

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-beta-type signaling pathway.

1, dSmad2 Mad (FBrf0240051)
2. Dpp target genes:
Positive regulation: bi (FBrf0098897, FBrf0240051, FBrf0087626), Dad (FBrf0098897), lab (FBrf0051544), salm (FBrf0220378)
Negative regulation: brk (FBrf0107889, FBrf0158763)
3. Phenotypes: Wing development: LOF - diminished wing size and lack of crossveins (FBrf0187398)

Other frequently used & other useful terms associated with BMP receptor pathway components:

Molecular function
GO:0036122 BMP binding
GO:0098821 BMP receptor activity
GO:0070700 BMP receptor binding
GO:0048018 receptor ligand activity

Biological Process
GO:0060395 SMAD protein signal transduction
GO:0007476 imaginal disc-derived wing morphogenesis
GO:0008586 imaginal disc-derived wing vein morphogenesis
GO:0007474 imaginal disc-derived wing vein specification

GO:0009953 dorsal/ventral pattern formation
GO:0007378 amnioserosa formation
GO:0007391 dorsal closure
GO:0001745 compound eye morphogenesis
Cellular Component
GO:0070724 BMP receptor complex
GO:0071144 heteromeric SMAD protein complex

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-beta-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

1. Phosphorylation of Smox (FBrf0106271, FBrf0194818)
2. 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)

Other frequently used & other useful terms associated with insulin receptor pathway components:

Molecular function

GO:0017002	activin-activated receptor activity
GO:0070697	activin receptor binding
GO:0048185	activin binding
GO:0048018	receptor ligand activity

Biological Process

GO:0060395	SMAD protein signal transduction
GO:0007411	axon guidance
GO:0016319	mushroom body development
GO:0002052	positive regulation of neuroblast proliferation

Cellular Component

GO:0071144	heteromeric SMAD protein complex
GO:0048179	activin receptor complex

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 Dmel 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

When possible, annotate core members of the pathways also to **upstream_of positive regulation of cell death (GO:0010942)**. Same for positive regulators of the pathway, while negative regulators should be annotated to **upstream_of negative regulation of cell death (GO:0060548)**.

Assays used for the TNFα signaling pathway:

1. 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).
2. Phenotypes: small eye phenotype, necrosis tissue in the eye.

Other frequently used & other useful terms associated with TNFα pathway components:

Molecular function:

GO:0032813 tumor necrosis factor receptor superfamily binding
GO:0005031 tumor necrosis factor-activated receptor activity

Biological Process

GO:0010942 positive regulation of cell death
GO:0060548 negative regulation of cell death
GO:0007254 JNK cascade
GO:0046330 positive regulation of JNK cascade
GO:0046329 negative regulation of JNK cascade

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.