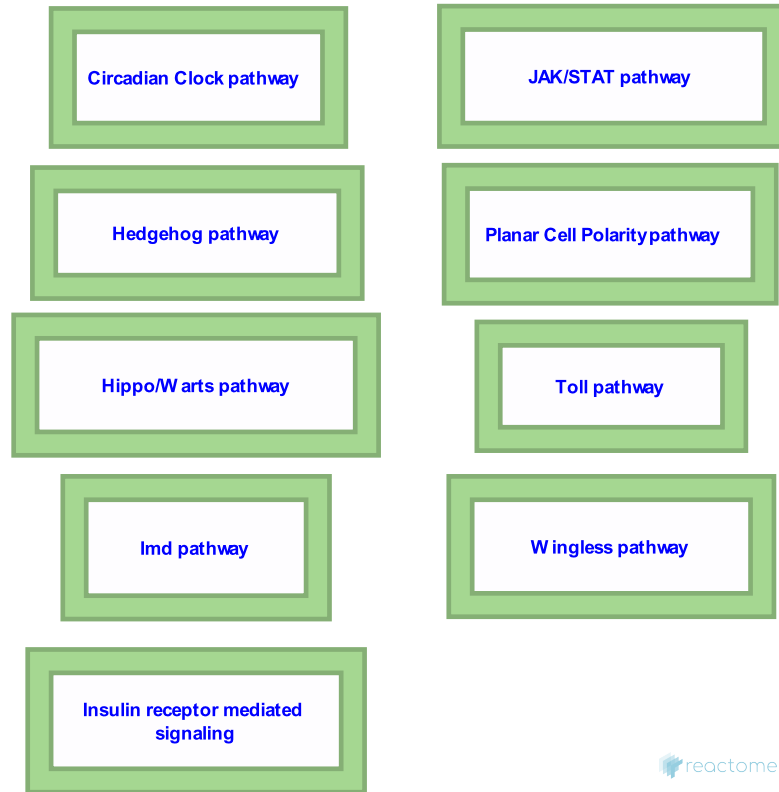


# Drosophila signaling pathways



Axelrod, JD., Broughton, S., D'Eustachio, P., Edery, I., Gianakou, M., Irvine, KD., Jassal, B., Kalderon, D., Leervers, SJ., Lemaitre, B., Nusse, R., Partridge, L., Perrimon, N., Piper, M., Silverman, N., Tatar, M., Williams, MG.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of [Creative Commons Attribution 4.0 International \(CC BY 4.0\) License](https://creativecommons.org/licenses/by/4.0/). For more information see our [license](https://reactome.org/licenses/).

This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the [Reactome Textbook](https://reactome.org/textbook/).

26/04/2024

## Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

## Literature references

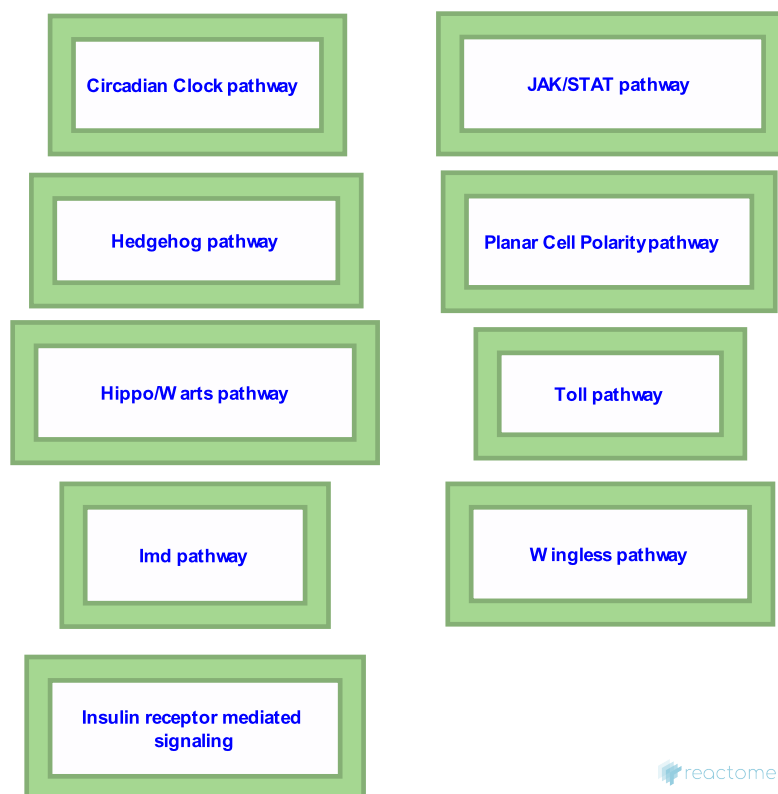
- Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics*, 18, 142. [↗](#)
- Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics*, 33, 3461-3467. [↗](#)
- Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res*, 46, D649-D655. [↗](#)
- Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology*, 14, e1005968. [↗](#)

Reactome database release: 88

This document contains 10 pathways ([see Table of Contents](#))

## Drosophila signaling pathways ↗

Stable identifier: R-DME-5252538



Between 2008 and 2010 the Flybase project annotated a series of Drosophila signaling processes in the Reactome format and released them at fly.reactome.org. These process annotations, edited for compatibility with Reactome's current web display, data analysis, and overlay features, are now accessible here.

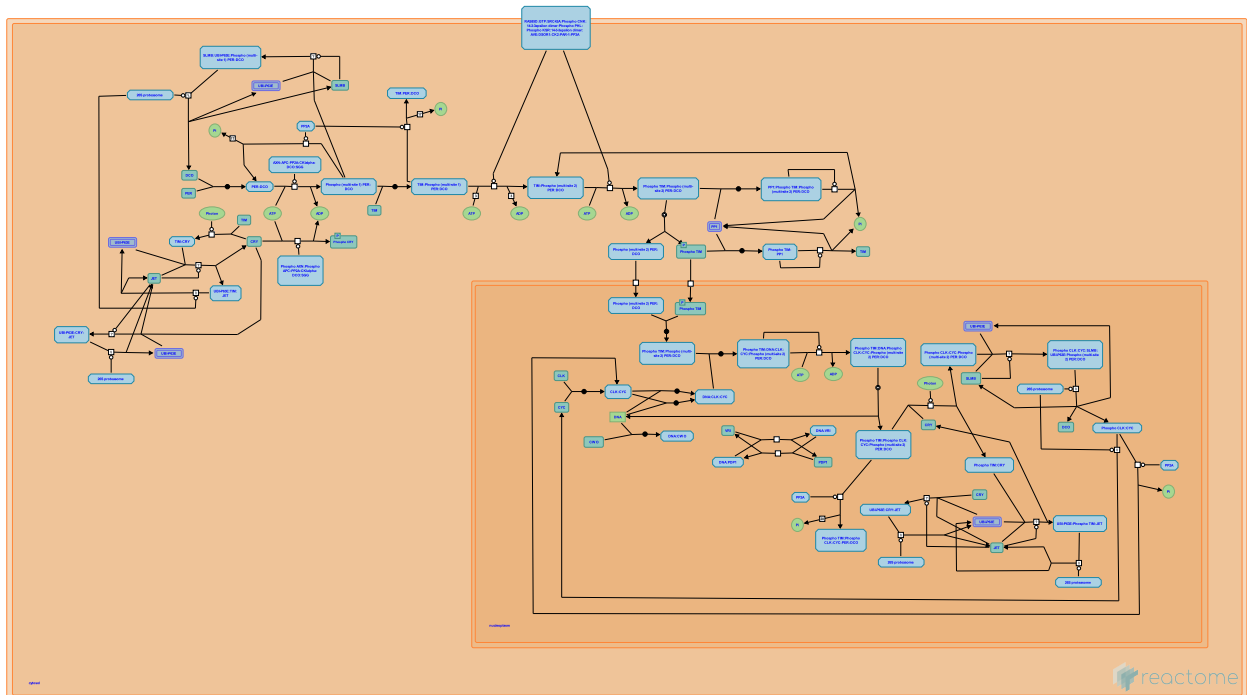
### Editions

2005-06-14	Authored, Reviewed	Jassal, B., Leervers, SJ., Piper, M., Gianakou, M., Partridge, L., Broughton, S.
2010-03-08	Authored	Williams, MG.
2014-02-05	Edited	D'Eustachio, P.

## Circadian Clock pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-432626



The *Drosophila* molecular circadian clock consists of two main interlocked sets of transcription/translation feedback loops. These both contain protein and mRNA components that cycle in abundance and subcellular localisation with a near 24 hour period. Within the core components of the clock pathway, both loops drive rhythmic transcription in the opposite phase to each other.

The pathways shown below are most representative of those occurring in key clock neurons within the brains of adult flies kept under standard 12 hour light:12 hour dark cycles at 25°C. Differences in one or more of the biochemical steps shown here and/or reactions not shown here can occur in different brain clock neurons (about 150 total cells per adult brain) and within the same clock cells as a function of changes in environmental conditions, such as day-length or temperature.

Starting from midday and continuing through to early night, the *per/tim* feedback loop is initiated when the basic-helix-loop-helix/PAS (PER-ARNT-SIM) domain transcription factors, Clock (CLK) and Cycle (CYC), form heterodimers and bind E-box regulatory elements to activate transcription of target genes *per*, *tim*, *vri*, and *Pdp1*. The levels of *per* and *tim* mRNA transcripts peak early in the night, whereas Period (PER) and Timeless (TIM) proteins do not reach peak abundance levels until mid-to-late evening. TIM stays at low levels at this point because it is destabilised by light. PER is phosphorylated by Discs overgrown (DCO) kinase aka Double-time (DBT) and Casein kinase II (CK2). Without TIM, which stabilises it, PER is targeted for degradation by the 26S proteasome via an interaction with the F-box containing protein, supernumerary limbs (SLMB). In addition, PER is stabilised by the protein phosphatases PP2A and PP1 while TIM is similarly stabilised by PP1 phosphatase. The coordinated action of both kinases and phosphatases, during this part of the day, keep PER at low levels and in a hypophosphorylated state, as hyperphosphorylated PER is removed due to low TIM levels.

After dusk during the early night, *per* and *tim* mRNA transcripts reach peak levels. TIM abundance begins to increase in the dark and forms a complex with PER. This, along with PP1 phosphatase, stabilises PER despite its continued phosphorylation by kinases. As a result PER and TIM accumulate to high levels during the middle of the night. TIM is then phosphorylated by CK2. PER and TIM dissociate, PER (in complex with DCO) enters the nucleus followed by TIM, a few hours later. Once in the nucleus PER and TIM reform their heterodimer and bind to the CLK:CYC heterodimer, so repressing the transcription of *per*, *tim*, *vri*, and *Pdp1*. The CLK:CYC heterodimer is removed from the E-boxes and sequestered in a stable complex with PER and TIM to reinforce the transcription inhibition. In addition, DCO bound to PER phosphorylates CLK. Towards the end of the night PER and TIM proteins are at their peak level.

At dawn, the introduction of light recalibrates the circadian clock. The blue-light photoreceptor cryptochrome (CRY) experiences a light-induced conformational change which forms a complex with hyperphosphorylated TIM, which

leads to both TIM and CRY degradation by the 26S proteasome via an interaction with the F-box containing protein Jetlag (JET). PER and CLK, hyperphosphorylated by DCO, the former no longer protected by TIM, are also degraded during the early morning. PER levels fall to their lowest level around midday, however, CLK levels remain constant since hypophosphorylated CLK is generated from new CLK protein expression or dephosphorylation of hyperphosphorylated CLK by PP2A protein phosphatase. Hypophosphorylated CLK then forms a heterodimer with CYC, binds to E-boxes of its target genes and a new cycle of *per*, *tim*, *vri*, and *Pdp1* transcription is initiated. There is an alternate view that CLK binds to CYC then CLK is phosphorylated in the cytosol. This promotes nuclear entry followed by further CLK phosphorylation. However, this string of events at present remain controversial and are not included in the current model.

There is a second feedback loop, the *Clk* loop, interlocked with the *per/tim* feedback loop. As mentioned above, at midday CLK:CYC heterodimers bind to the E-boxes and activate transcription of the target genes *vri* and *Pdp1*, which lead to the production of the proteins Vriille (VRI) and PAR domain protein-1 (PDP1) respectively. PDP1 activates transcription of *Clk* and *cry* genes, while VRI represses it, by competitively binding to regulatory sequences called VRI/PDP1 (V/P) boxes. Although *vri* mRNA accumulates in phase with *per* and *tim* mRNAs, *Pdp1* accumulation is delayed by several hours. In contrast to the delayed accumulation of PER and TIM protein levels, VRI protein levels rise in sync with *vri* mRNA. Between midday and dusk, VRI levels rise, and the superior VRI/PDP1 ratio means it predominantly binds to the V/P boxes to repress *Clk* and *cry* transcription. VRI levels reach their peak during the early night after dusk coinciding with minimal levels of *Clk* and *cry* mRNAs. However, CRY protein levels build slowly as it is relatively stable in the dark.

PDP1 levels reach their peak between mid to late night. VRI levels decline during this time due to TIM:PER influenced repression of *vri* transcription. The decreasing ratio of VRI/PDP1 levels favours binding of PDP1 to the V/P boxes resulting in activation of *Clk* and *cry* transcription. PDP1 protein levels start to decline during the late night and are low by the early morning. However, despite this, PDP1 continues to activate *Clk* and *cry* transcription until midday when VRI protein levels are sufficient, after the next cycle of CLK:CYC mediated transcription, to take over the binding of the V/P boxes and repress transcription.

Recently, CLK:CYC transcription of some new target genes and their associated feedback loops have come to light such as the *cwo* gene and its protein product clockwork orange (CWO). However, there is confusion as to whether CWO, which binds to the E-boxes of *per*, *tim*, *vri*, and *Pdp1* activates or represses their transcription. There are likely to be many more of these secondary transcription loops connected to the core clock mechanism to add yet more modes of regulation.

## Literature references

- Weber, F. (2009). Remodeling the clock: coactivators and signal transduction in the circadian clockworks. *Naturwissenschaften*, 96, 321-37. [↗](#)
- Hardin, PE. (2005). The circadian timekeeping system of Drosophila. *Curr Biol*, 15, R714-22. [↗](#)
- Virshup, DM., Gallego, M. (2007). Post-translational modifications regulate the ticking of the circadian clock. *Nat Rev Mol Cell Biol*, 8, 139-48. [↗](#)
- Bae, K., Edery, I. (2006). Regulating a circadian clock's period, phase and amplitude by phosphorylation: insights from Drosophila. *J Biochem*, 140, 609-17. [↗](#)
- Hardin, PE. (2004). Transcription regulation within the circadian clock: the E-box and beyond. *J Biol Rhythms*, 19, 348-60. [↗](#)

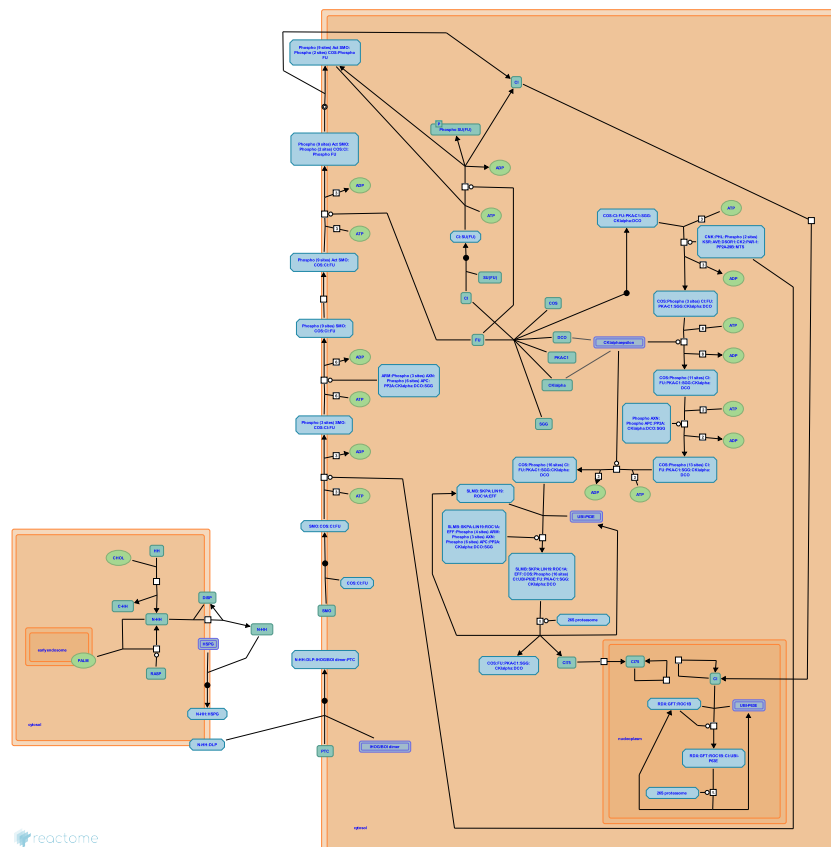
## Editions

2009-08-13	Authored	Williams, MG.
2009-08-14	Edited	Williams, MG.
2010-07-06	Reviewed	Edery, I.

# Hedgehog pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-209392



In the Hedgehog signalling pathway, as in the Wingless pathway, there are two main sets of signalling events. One comes into play when the Hedgehog (N-HH) ligand is bound to its cell surface receptor, Patched (PTC) and the other set occurs when the ligand isn't bound to it.

In Hedgehog sending cells, full-length Hedgehog ligand (HH) undergoes an autoprocessing event culminating in cleavage of itself into an N-terminal fragment (N-HH) modified by cholesterol and a C-terminal fragment (C-HH) which is no longer used in the pathway. N-HH is further processed through the action of the protein-cysteine N-palmitoyltransferase, Rasp (RASP), which palmitoylates the ligand. N-HH is secreted extracellularly with the assistance of the membrane protein, Dispatched (DISP). Efficient movement of N-HH requires heparan sulphate proteoglycans (HSPGs) such as Dally (DALLY) and Dally-like protein (DLP), which can aid the accumulation of N-HH at the cell surface and facilitate intercellular transport of ligand.

In cells not exposed to HH, the transmembrane protein PTC inhibits the transmembrane residing Smoothened (SMO). In the cytosol, full-length Cubitus Interruptus protein (CI) forms a complex with the Suppressor of Fused protein (SU(FU)) and the large kinesin-like scaffold protein, Costal2 (COS), which also binds the Ser/Thr protein kinase, Fused (FU). COS recruits the Ser/Thr kinases: protein kinase A (PKA-C1); Shaggy (SGG); casein kinase I alpha (CKIalpha); and casein kinase I epsilon (DCO). These phosphorylate CI which is now recognised by Slimb (SLMB) which is part of the SCF ubiquitin ligase complex. CI is ubiquitinated and partially degraded by the proteasome resulting in a truncated CI (CI75) which transports to the nucleus where it acts as a repressor of transcription.

However, if N-HH ligand is in the vicinity of the Hedgehog receiving cell, it binds to PTC, reducing the inhibiting effect PTC has on SMO. This leads to increased phosphorylation of SMO by PKA-C1 and CKIalpha, accompanied by a conformational change, increased stability and enhanced surface accumulation. CI associates with SMO via COS and is no longer efficiently phosphorylated and proteolysed after SMO activation. FU, COS and SU(FU) are phosphorylated and full-length CI gains greater access to the nucleus, where it activates transcription.

## Literature references

Kalderon, D. (2005). The mechanism of hedgehog signal transduction. *Biochem Soc Trans*, 33, 1509-12. ↗

- Beachy, PA., Lum, L. (2004). The Hedgehog response network: sensors, switches, and routers. *Science*, 304, 1755-9. [↗](#)
- Nybakken, K., Perrimon, N. (2002). Hedgehog signal transduction: recent findings. *Curr Opin Genet Dev*, 12, 503-11. [↗](#)
- Robbins, DJ., Ascano M, Jr., Ogden, SK., Stegman, MA. (2004). Regulation of Hedgehog signaling: a complex story. *Biochem Pharmacol*, 67, 805-14. [↗](#)
- Cohen MM, Jr. (2003). The hedgehog signaling network. *Am J Med Genet A*, 123, 5-28. [↗](#)

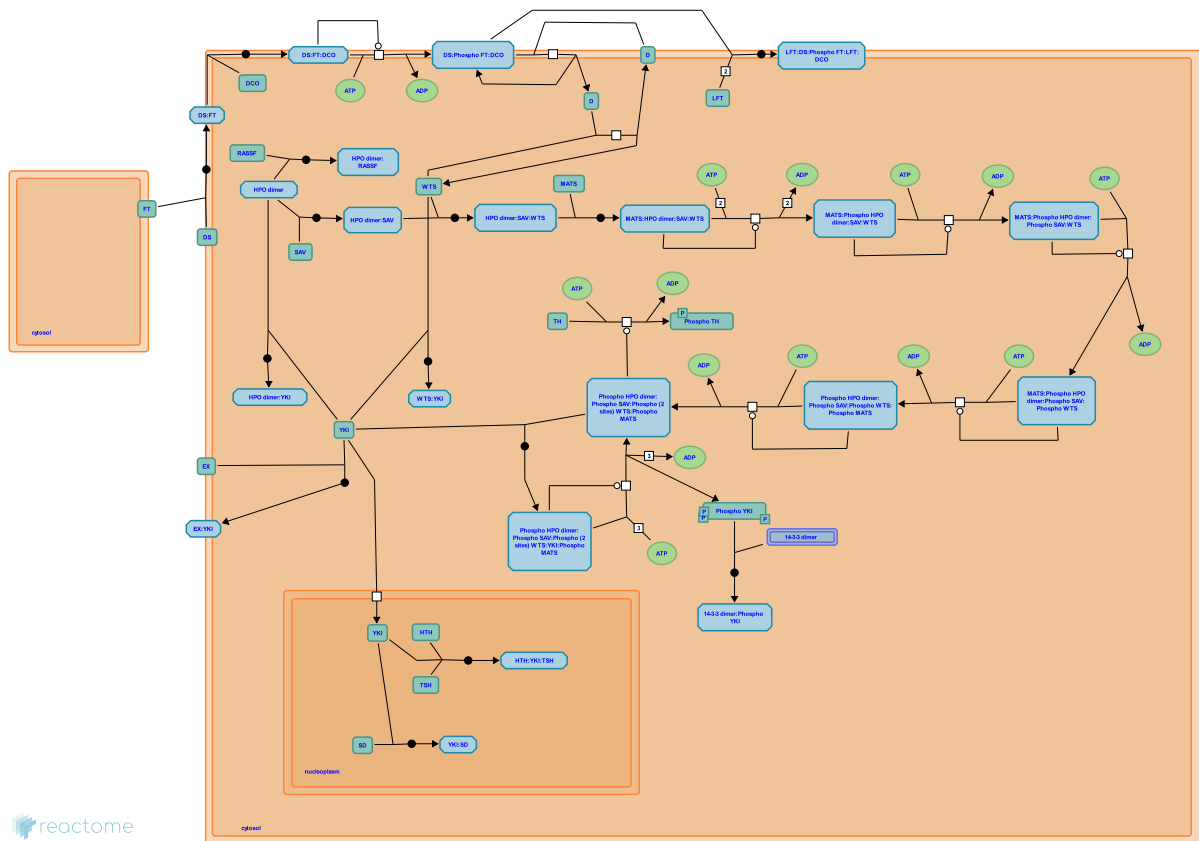
## **Editions**

2006-12-07	Authored, Edited	Williams, MG.
2008-03-17	Reviewed	Kalderon, D.

## Hippo/Warts pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-390146



The Hippo/Warts signalling pathway has been shown to be crucial in control of organ size in both *Drosophila* and mammals. Such regulation is achieved by transcriptional activation of target genes such as cyclins A, B, and E; E2F1; Diap1; and bantam microRNA.

The cadherin domain containing transmembrane protein Fat (FT) interacts with another cadherin domain containing protein Dachsous (DS). FT inhibits the unconventional myosin, Dachs (D), by reducing its accumulation at the plasma membrane. So, D fails to get access to and interact with the serine/threonine kinase Warts (WTS). WTS is a key component in the Hippo kinase cassette. In this, the active Ser/Thr kinase Hippo (HPO) homodimer interacts and activates the scaffolding protein Salvador (SAV). This in turn recruits WTS which correspondingly recruits its protein Mob as tumor suppressor (MATS) which activates WTS. Active WTS recruits the transcriptional co-activator Yorkie (YKI) to this complex assembly. YKI is phosphorylated and binds to a 14-3-3 dimer so that it is retained in the cytosol and unable to enter the nucleus and activate its target genes and consequently growth can be stopped. Recently, phosphorylation-independent negative regulation of YKI has been observed. Binding of its WW domains to PPXY sequence motifs found in HPO, WTS, and the FERM domain containing Expanded (EX) means that YKI remains in the cytosol and does not enter the nucleus to activate its target genes.

In the absence of the FT:DS interaction, D accumulates at the plasma membrane where it interacts with WTS and inhibits it. D also reduces levels of EX at the plasma membrane. Merlin (MER) is in its inactive phosphorylated state. HPO homodimer, SAV, and WTS remain unphosphorylated and inactive. YKI is not phosphorylated and translocates to the nucleus where it complexes with Scalloped (SD) or Homothorax (HTH) and Teashirt (TSH) to promote the transcription of its target genes.

Two cytoplasmic Band 4.1 superfamily members, Merlin (MER) and EX which contain a FERM (Four-point one, Ezrin, Radixin, Moesin) domain are believed to regulate HPO by promoting its phosphorylation, however, the precise mechanism of this and how MER and EX are themselves activated remains unknown. Additionally, unactivated unphosphorylated HPO associates with Ras association family member (RASSF) where it remains in its inactive state.

Two upstream components of the Hippo/Warts pathway are important target genes involved in feedback regulation. These target genes encode the proteins EX and Four-jointed (FJ), a golgi Ser/Thr kinase involved in regulating FT.



HPO has been reported as phosphorylating Thread (TH) aka Diap1 affecting its stability. These show that feedback regulation is present in this signalling pathway.

Another example of feedback regulation in this pathway involves the small protein Lowfat (LFT) which binds to and influences levels of both FT and DS, which in turn have influence on levels of LFT.

## Literature references

Edgar, BA. (2006). From cell structure to transcription: Hippo forges a new path. *Cell*, 124, 267-73. [↗](#)

Irvine, KD., Reddy, BV. (2008). The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. *Development*, 135, 2827-38. [↗](#)

Edgar, BA., Saucedo, LJ. (2007). Filling out the Hippo pathway. *Nat Rev Mol Cell Biol*, 8, 613-21. [↗](#)

Tapon, N., Harvey, K. (2007). The Salvador-Warts-Hippo pathway - an emerging tumour-suppressor network. *Nat Rev Cancer*, 7, 182-91. [↗](#)

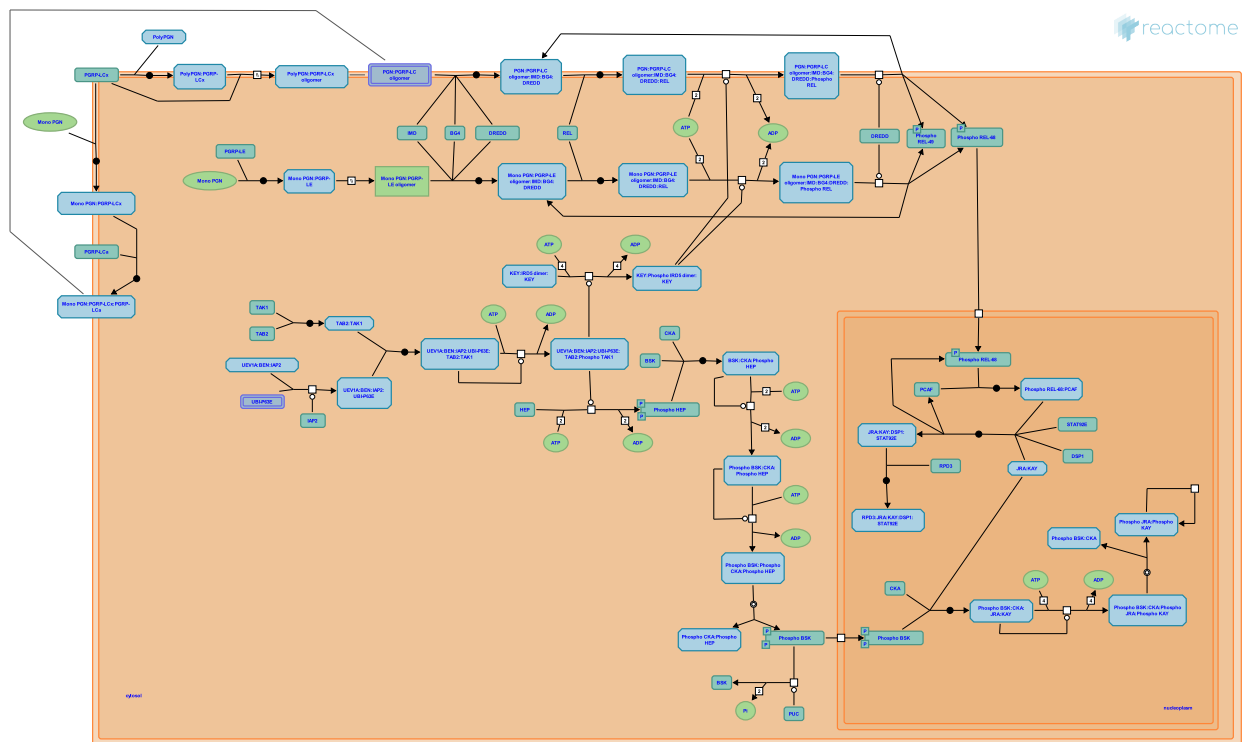
## Editions

2009-01-23	Authored, Edited	Williams, MG.
2009-11-25	Reviewed	Irvine, KD.

## Imd pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-209459



The Imd pathway mediates the response of *Drosophila* to the presence of diaminopimelic acid-type peptidoglycan (DAP-PGN) found in all Gram negative and many Gram positive bacteria, over lysine-type PGN found in Gram positive bacteria. It operates in the fat-body and hemocytes in response to a systemic infection and is activated upon recognition of DAP-PGN by the PGRP-LC/LE receptors, which leads to activation of the NFkappaB-like transactivator Relish (REL). Elements regulated by the pathway during a systemic infection have been identified by microarray and include a large set of antibacterial peptides genes (De Gregorio et al., 2002; Boutros et al., 2002). In addition, the Imd pathway plays an important role in the relationship many epithelia have with the external world, where it mediates the local inducible immune response (Tzou et al., 2000; Zaidman-Remy et al., 2006). The canonical component of the Imd pathway contains: PGRP-LC (Gottar et al., 2002; Choe et al., 2002; Ramet et al., 2002), IMD (Georgel et al., 2001), DFADD (BG4) (Leulier et al., 2002; Naitza et al., 2002), DREDD (Leulier et al., 2000), REL (Hedengren et al., 1999), Kenny (KEY) and IRD5 (Silverman et al., 2000; Rutschmann et al., 2000; Lu et al., 2001), TAK1 (Vidal et al., 2001; Silverman et al., 2003), TAB2 (Gesellchen et al., 2005; Kleino et al., 2005; Zhuang et al., 2006), Inhibitor of apoptosis 2 (IAP2) (Gesellchen et al., 2005; Kleino et al., 2005; Leulier et al., 2006). Flies lacking these genes are viable but are highly susceptible to Gram negative bacterial infection (Lemaitre et al., 1995). It is not clear if this pathway plays another role beside immunity but overactivation of the Imd pathway induces strong lethality due to apoptosis (Georgel et al., 2001). It should also be noted that the Imd pathway is present and functional in almost all epithelial cells. However, the responses in these tissues are not identical to those observed in the fat body (or in cell culture). In particular, the outputs of Imd signalling are significantly modified in the gut. During Imd signalling in the gut, gene targets producing PGN-digesting PGRP-LB and -SC proteins are expressed but other REL target genes, especially the AMP genes are mostly silent.

In response to infection by Gram-negative bacteria the Imd pathway of the innate immunity response is activated. DAP-PGNs, found on Gram-negative bacteria, are recognised and bind to the PGRP-LC receptor at the plasma membrane or intracellularly bind to the PGRP-LE receptor. This causes the receptor to dimerise/multimerise and activate resulting in the recruitment of the adaptor proteins IMD and DFADD (BG4) along with the caspase 8 orthologue DREDD. Meanwhile, the Ser/Thr MAPK kinase kinase, TAK1 and its partner TAB2 are activated possibly through the IAP2:Bendless (BEN):UEV1a ubiquitin E3 ligase complex. TAK1 and TAB2, in turn phosphorylate the IKKbeta orthologue IRD5. Activated IRD5, in complex with IKKgamma orthologue Kenny (KEY), phosphorylates the NFkappaB orthologue Relish (REL). REL consists of an N-terminal nuclear factor containing domain (REL-68) and an inhibitory C-terminal domain (REL-49) responsible for anchoring REL in the cytoplasm. REL is then cleaved by the caspase, DREDD, releasing the N-terminal domain REL-68. This translocates to the nucleus where it is able to activate transcription of genes encoding antimicrobial peptides.

In addition to being a key component of the Imd pathway, TAK1 kinase is involved in triggering a JNK kinase signalling cascade, starting with the phosphorylation of the JNKK, Hemipterous (HEP). This binds to a scaffolding protein, Connector of kinase to AP-1 (CKA), bringing into close proximity the JNK protein, Basket (BSK) which is phosphorylated by HEP. CKA is also phosphorylated which results in the dissociation of BSK which translocates to the nucleus. Here it binds with a nuclear-residing CKA molecule which additionally recruits the AP-1 transcription factor consisting of the c-Jun orthologue, JRA, and the c-Fos orthologue, Kayak (KAY). BSK may phosphorylate both JRA and KAY which dissociate from CKA and activate transcription of genes involved in the early immune response thought to be involved in wound repair and stress mechanisms. Additionally, the gene responsible for encoding the phosphatase Puckered (PUC) is activated which is responsible for dephosphorylating BSK, an example of a negative regulatory loop.

The Imd pathway mediates the immune response against Gram-negative bacteria infection. During immune challenge, the JNK pathway is activated prior to the activation of the Imd pathway. This early immune response does not require REL activity, in fact, it has been proposed that the main target of the JNK pathway activation, the AP-1 transcription factor, inhibits the activation of REL dependent genes. However, once REL is activated the early response is terminated and a sustained immune response of the Imd pathway is active.

In addition to these core components, many new gene products have been linked to the Imd pathway but their function have not been fully established:

- Amidase PGRPs: Recently, it has been shown that the Imd pathway is down-regulated by the amidase PGRPs, PGRP-LB and PGRP-SC1. They exert their action extracellularly by scavenging peptidoglycan into non-immune stimulatory fragments.
- PGRP-LE: PGRP-LE participates with PGRP-LC in the sensing of DAP-PGN and may play a role in the sensing of monomeric PGN in the cytosol.
- PGRP-LF: PGRP-LF appears to block PGRP-LC-mediated activation of the Imd/JNK pathway possibly by interaction with PGRP-LC at the plasma membrane or by sequestering PGN away from PGRP-LC (Maillet et al., 2008).
- Negative regulators of the Imd pathway: CASPAR, Plenty of SH3s (POSH).
- SCF complex components may be involved in the processing of REL: SKPA, Cullin1 (LIN19), and Slimb (SLMB) (Khush et al., 2002).
- Components of the ubiquitin E3 ligase complex: Bendless (BEN) and UEV1A (Zhou et al., 2005).
- JNK: The Imd pathway can activate the JNK pathway, through the JNKK Hemipterous (HEP), at the level of TAK1 (Boutros et al., 2002; Silverman et al., 2003).
- Nuclear factor Akirin (aka Bhringi (BHR)) (Goto et al., 2008) and GATA zinc finger transcription factor, Serpent (SRP) (Senger et al., 2004) synergize with Relish (REL) and activate transcription.

## Literature references

- Davis, RJ. (2000). Signal transduction by the JNK group of MAP kinases. *Cell*, 103, 239-52. [↗](#)
- Hoffmann, JA. (2003). The immune response of *Drosophila*. *Nature*, 426, 33-8. [↗](#)
- Blanot, D., Lemaitre, B., Pili-Floury, S., Poidevin, M., Zaidman-Rémy, A., Ueda, R. et al. (2006). The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity*, 24, 463-73. [↗](#)
- Perkins, ND. (2006). Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. *Oncogene*, 25, 6717-30. [↗](#)
- Valanne, S., Lemaitre, B., Poidevin, M., Enwald, H., Ulvila, J., Kallio, J. et al. (2005). Inhibitor of apoptosis 2 and TAK1-binding protein are components of the *Drosophila* Imd pathway. *EMBO J*, 24, 3423-34. [↗](#)

## Editions

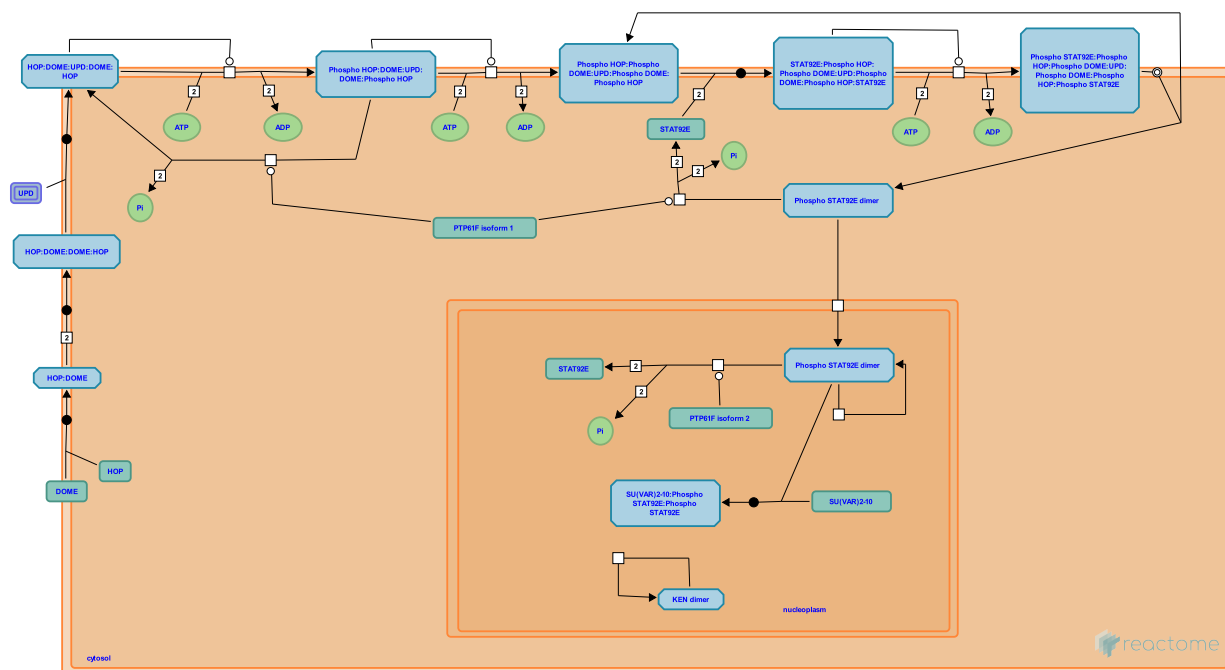
2007-07-11	Authored, Edited	Williams, MG.
2008-06-20	Reviewed	Lemaitre, B., Silverman, N.



## JAK/STAT pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-209405



The JAK/STAT pathway is one of the main eukaryotic signalling pathways. In vertebrates, there are several ligands, receptors, JAK kinases and STAT molecules making detailed study of the system very complex. In *Drosophila*, the JAK/STAT pathway is much less redundant, offering one set of related ligands UPD (OS, UPD2, UPD3), a receptor Domeless (DOME), a Janus Associated Kinase Hopscotch (HOP), and a STAT transcription factor (STAT92E).

The DOME receptor, like many cytokine receptors, contains no tyrosine kinase domain. However, it is constitutively associated with the kinase HOP. The DOME receptor dimerises at the plasma membrane whereupon it binds a UPD ligand. This activates the receptor-associated HOP which are now able to phosphorylate each other and also the cytoplasmic tail of the DOME receptor. This creates a docking site for monomeric cytoplasmic STAT92E proteins which can bind via their SH2 domains. Once bound to the receptor complex, STAT92E is itself phosphorylated. It dissociates from the receptor complex and dimerises, the interaction stabilised by the SH2 domain of one molecule binding to the phospho-Tyr of the other. The dimer translocates to the nucleus where it binds to a palindromic DNA sequence in the pathway target gene promoters to activate transcription.

There are also negative regulators present in this pathway. Some are present in the nucleus and, in the case of SU(VAR)2-10 which is a *Drosophila* PIAS or Zimp protein, act by binding to STAT92E dimer. Others, such as the BCL6 orthologue, Ken and Barbie (KEN), bind to DNA sequences on target genes which overlap with STAT92E binding sites. There is a protein tyrosine phosphatase, PTP61F, that exists in two differently spliced forms. One is active in the cytosol and dephosphorylates HOP and STAT92E while the other dephosphorylates STAT92E in the nucleus.

A truncated version of STAT92E, deltaNSTAT92E, that lacks the N-terminal 133 amino acids has also been found. It is believed this truncated STAT forms homodimers and heterodimers with full-length STAT92E. Increasing the deltaNSTAT92E to STAT92E ratio in overexpression and RNAi experiments results in the repression of target gene transcription. This may be due to the truncated protein failing to attract coactivators or failing to form tetramers, necessary for activation of certain genes and assembled by the N-terminal domains, when two STAT binding sites for dimers are close together (Henrikson et al, 2002; Yan et al, 1996).

In mammalian systems, SOCS proteins negatively influence the JAK/STAT pathway. Activated STATs stimulate transcription of the SOCS genes and the resulting SOCS proteins bind phosphorylated JAK kinases and their receptors to turn off the pathway in a simple negative feedback loop. SOCS can affect their negative regulation in three ways. First, by binding phospho-Tyr on the receptors to physically block STAT recruitment. Second, by binding directly to JAKs or the receptors to specifically inhibit JAK kinase activity. Third, by interacting with elongin BC complex and Cullin 2, facilitating the ubiquitination of JAKs and, presumably, the receptors. Ubiquitination of these targets decreases their stability by targeting them for proteasomal degradation (Rawlings et

al, 2004).

Three SOCS proteins have been identified in *Drosophila*: SOCS36E, SOCS44A, and SOCS16D. At the present time, there is no evidence to demonstrate a physical interaction between *Drosophila* SOCS proteins and another protein such as HOP or a receptor.

## Literature references

Small, S., Darnell JE, Jr., Desplan, C., Dearolf, CR., Yan, R. (1996). Identification of a Stat gene that functions in *Drosophila* development. *Cell*, 84, 421-30. [↗](#)

Chen, X., Perrimon, N., Zheng, Z., Hou, SX. (2002). The Jak/STAT pathway in model organisms: emerging roles in cell movement. *Dev Cell*, 3, 765-78. [↗](#)

Darnell JE, Jr., Levy, DE. (2002). Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol*, 3, 651-62. [↗](#)

Luo, H., Dearolf, CR. (2001). The JAK/STAT pathway and *Drosophila* development. *Bioessays*, 23, 1138-47. [↗](#)

Brown, S., Hombria, JC. (2002). The fertile field of *Drosophila* Jak/STAT signalling. *Curr Biol*, 12, R569-75. [↗](#)

## Editions

2006-11-02	Authored	Williams, MG.
2006-11-03	Edited	Williams, MG.
2008-01-16	Reviewed	Perrimon, N.





potential post-translational modifications is difficult (Strutt et al., 2004).

In each cell, before the onset of PCP signalling, STAN, FZ, VANG, DS, and FT are uniformly distributed on the cell surface with PK, DSH, and DGO present in the cytoplasm. During PCP signalling, DS binds to FT in an adjacent cell (heterophilic protein-protein interaction) along with STAN binding to its neighbour in the adjacent cell (homophilic protein-protein interaction). Polarisation of the PCP components along the proximal-distal and R3-R4 cell boundaries now occurs as part of a bistable switch mechanism. FZ recruits DSH and DGO to the R3/Distal cell boundary while VANG recruits PK to the R4/Proximal cell boundary. It is believed that there is an interaction (mutual recruitment) between STAN:FZ:DSH:DGO complexes on one side and STAN:VANG:PK complexes on the adjacent cell surface. It is controversial to say whether communication is mediated via STAN homodimers, or through a direct contact between FZ and VANG. However, both these complexes antagonise each other, thus inhibiting formation of the oppositely orientated complex. This leads to asymmetric enrichment and distribution of the main PCP components. The FZ:DSH:DGO complex becomes enriched at the Distal/R3 cell boundary while the proteins that function as antagonists of FZ, the VANG:PK complex becomes enriched at the Proximal/R4 boundary. STAN becomes enriched on both sides, probably stabilising both complexes.

PK can also interact with DSH, causing a reduction of DSH membrane localisation. This can be seen as a negative feedback loop. However, DGO can compete with PK for DSH binding and so remove the inhibitory action of PK on PCP signalling. DGO stabilises the FZ:DSH complex and the signal now appears to branch with DSH interacting with both RHO1 and RAC1 small GTPases.

In the RHO1 branch, RHO1 binds to DSH via the Dishevelled Associated Activator of Morphogenesis bridging protein (DAAM) and becomes activated. Activated RHO1 (the GTP-bound form) binds Rho kinase (ROK) which becomes activated. Non-muscle Myosin II is a hexamer composed of two of each of the following: heavy chain Zipper (ZIP), regulatory light chain Spaghetti squash (SQH), and the essential light chain MLC-C. Myosin phosphatase is a heterotrimer composed of a protein phosphatase catalytic subunit Flapwing (FLW) or PP1-87B; a Myosin phosphatase targeting subunit, Myosin binding subunit (MBS) or MYPT-75D; and a small subunit of unknown function sometimes referred to as M20. Activated ROK phosphorylates SQH, this leads to a change in the conformation and increase in ATPase catalytic activity of ZIP. Multivalent bipolar filaments are more readily formed which are more capable of binding multiple actin filaments. However, Myosin phosphatase dephosphorylates SQH leading to the inactivation of non-muscle Myosin II. Myosin phosphatase is itself negatively regulated through phosphorylation of MBS by ROK. Thus, ROK doubly activates non-muscle Myosin II by direct phosphorylation of SQH and inactivation of Myosin phosphatase by phosphorylating MBS.

Additionally, in the wing, proximally localised VANG acts via the Planar Polarity Effector (PPE) proteins Inturned (IN), Fuzzy (FY), and Fritz (FRTZ) to stabilise the formin inhibitor protein, multiple wing hairs (MWH) resulting in the regulation of actin polymerisation and its inherent effect on hair formation.

In the RAC1 branch, after DSH binds to RAC1 there is genetic evidence to suggest that the JNK signalling cascade is activated by the STE20 kinase Misshapen (MSN). The MKK7 orthologue Hemipterous (HEP) is phosphorylated by components downstream of RAC1. Additionally, components from the RHO1 branch, FLW, MBS, and ZIP, appear to interact genetically with members of the JNK pathway, namely Basket (BSK), HEP, and Puckered (PUC). Nonmuscle myosin acts upstream, mediating an activating signal on JNK.

## Literature references

- Fanto, M., McNeill, H. (2004). Planar polarity from flies to vertebrates. *J Cell Sci*, 117, 527-33. [↗](#)
- Seifert, JR., Mlodzik, M. (2007). Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. *Nat Rev Genet*, 8, 126-38. [↗](#)
- Klein, TJ., Mlodzik, M. (2005). Planar cell polarization: an emerging model points in the right direction. *Annu Rev Cell Dev Biol*, 21, 155-76. [↗](#)
- Strutt, H., Strutt, D., Hofstra, K., Mundy, J. (2004). Cleavage and secretion is not required for Four-jointed function in *Drosophila* patterning. *Development*, 131, 881-90. [↗](#)
- Strutt, H., Strutt, D. (2005). Long-range coordination of planar polarity in *Drosophila*. *Bioessays*, 27, 1218-27. [↗](#)

## Editions

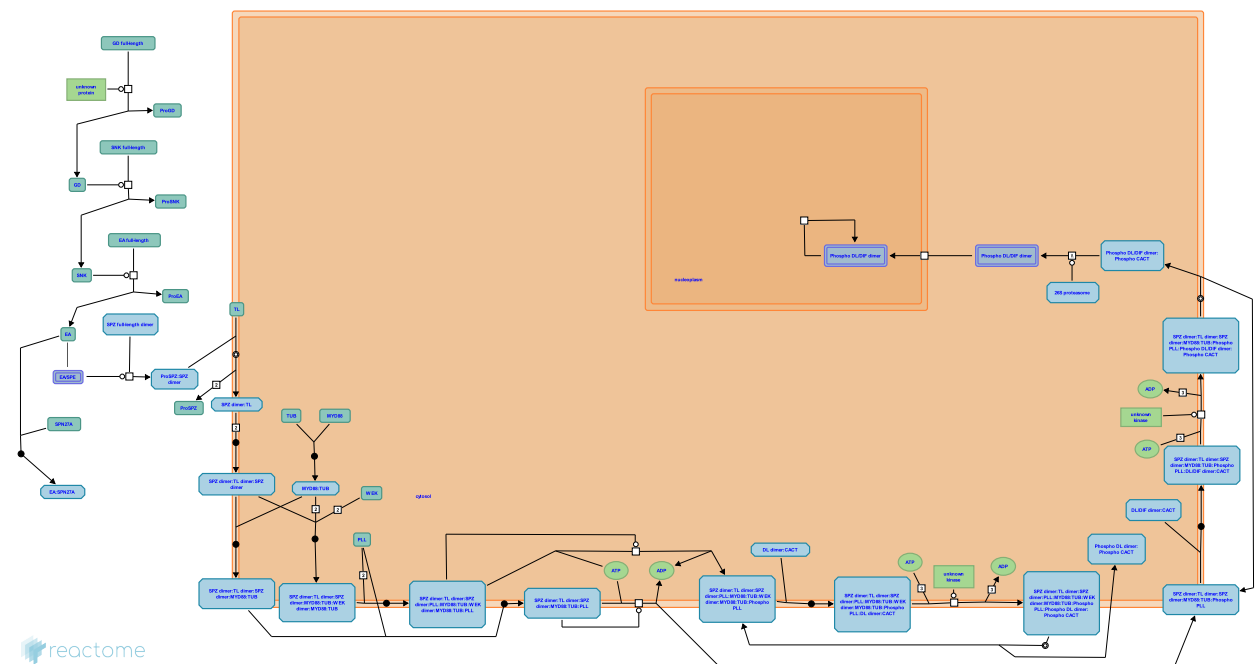
2008-05-19	Edited	Williams, MG.
2008-05-20	Authored	Williams, MG.
2009-11-19	Reviewed	Axelrod, JD.



## Toll pathway ↗

**Location:** [Drosophila signaling pathways](#)

**Stable identifier:** R-DME-209449



In *Drosophila*, the Toll pathway establishes the embryonic dorsoventral axis and triggers innate immune responses to infection, along with the Imd pathway.

The Toll pathway plays a key role in the response to Gram-positive cocci and Fungi by regulating a large set of genes (including antimicrobial peptide genes, many small peptides with unknown function as well as components of the melanization and clotting cascades) by the fat body and in hemocytes that circulate in the hemolymph (De Gregorio et al., 2002). The canonical component of the Toll pathway contains: Spatzle (SPZ), Toll (TL), Pelle (PLL), Tube (TUB), MYD88, Cactus (CACT), Dorsal (DL), Dorsal-related immunity factor (DIF) (Belvin et al., 1996; Tauszig-Delamasure et al., 2002). Many other functions have been proposed for the Toll pathway including hemocyte differentiation (Evans et al., 2003; Meister, 2004), muscle attachment and motoneuron defects (Halfon et al., 1995).

The Toll pathway is activated after the cleavage of SPZ by serine protease cascades. The proteolytic cascade activating SPZ during dorsoventral patterning has been well delineated and involves the serine proteases: Gastrulation defective (GD); Snake (SNK); and Easter (EA), that directly cleave the full-length Spatzle (SPZ) dimer ligand. This cascade is negatively regulated by the serpin SPN27A that acts at the level of EA (Dissing et al., 2001; LeMosy et al., 2001). The cascades activating SPZ during the immune response are much more complex with branches involved in the sensing of Glucan found in fungi (through GNB3) or Lysine-type peptidoglycans (PGNs) found in gram positive cocci (through PGRP-SA, GNBPI and PGRP-SD) and via the serine protease, Persephone (PSH), in the sensing of entomopathogenic fungus via the detection of protease. These proteolytic cascades which are not yet well characterised converge and lead to activation of Spatzle-processing enzyme (SPE) that cleave Spatzle in the hemolymph.

The processed SPZ dimer binds to the extracellular part of the Toll (TL) receptor at the plasma membrane. This causes TL to activate and dimerise through its cytoplasmic domains. In response to this activity, the adaptor proteins MYD88, Tube (TUB), and the Ser/Thr kinase Pelle (PLL) are recruited to the TL receptor cytoplasmic region to form the 'signalling complex'. In addition, during dorsoventral patterning in the embryo, the zinc-finger adaptor protein, Weckle (WEK) forms an extra link between MYD88 and the TL receptor (Chen et al., 2006). PLL is activated, autophosphorylates and recruits the DL/DIF dimer, complexed to the NF-kappaB inhibitor orthologue, Cactus (CACT) to the 'signalling complex'. CACT is complexed to NF-kappaB orthologue dimers of either Dorsal (DL) in dorsoventral polarity and larvae immune response or Dorsal-related immunity factor, Dif (DIF), the main transcription factor in the innate immunity response. The next stage is unclear but it is believed that PLL or an unknown kinase tentatively labelled the 'Cactus kinase' phosphorylate CACT and the DL/DIF dimer. CACT in complex with the DL/DIF dimer dissociates from the 'signalling complex'. The phosphorylated CACT is degraded probably by the 26S proteasome, and the now free phosphorylated DL/DIF dimer translocates to the nucleus to activate transcription of genes encoding a battery of antimicrobial peptides in the immune response or genes that

organise dorsoventral patterning.

## Literature references

- Hashimoto, C., Keshishian, H., Halfon, MS. (1995). The *Drosophila* toll gene functions zygotically and is necessary for proper motoneuron and muscle development. *Dev Biol*, 169, 151-67. [↗](#)
- Dissing, M., Giordano, H., DeLotto, R. (2001). Autoproteolysis and feedback in a protease cascade directing *Drosophila* dorsal-ventral cell fate. *EMBO J*, 20, 2387-93. [↗](#)
- Hoffmann, JA. (2003). The immune response of *Drosophila*. *Nature*, 426, 33-8. [↗](#)
- Banerjee, U., Hartenstein, V., Evans, CJ. (2003). Thicker than blood: conserved mechanisms in *Drosophila* and vertebrate hematopoiesis. *Dev Cell*, 5, 673-90. [↗](#)
- Wang, L., Ligoxygakis, P. (2006). Pathogen recognition and signalling in the *Drosophila* innate immune response. *Immunobiology*, 211, 251-61. [↗](#)

## Editions











2007-07-11	Authored	Williams, MG.
2007-07-12	Edited	Williams, MG.
2008-06-20	Reviewed	Lemaitre, B., Silverman, N.



## Editions

2006-06-28	Edited	Williams, MG.
2006-07-26	Authored	Williams, MG.
2008-01-19	Reviewed	Nusse, R.

# Table of Contents

Introduction	1
 Drosophila signaling pathways	2
 Circadian Clock pathway	3
 Hedgehog pathway	5
 Hippo/Warts pathway	7
 Imd pathway	9
 Insulin receptor mediated signaling	11
 JAK/STAT pathway	12
 Planar Cell Polarity pathway	14
 Toll pathway	16
 Wingless pathway	18
Table of Contents	20