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Logical modelling of Drosophila signalling pathways

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Supplementary Material

Documentation of the logical models for the nine signalling pathways and for the processing of spatzle (ligand of Toll pathway) in Drosophila melanogaster.

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Logical model of Drosophila Wingless signaling pathway

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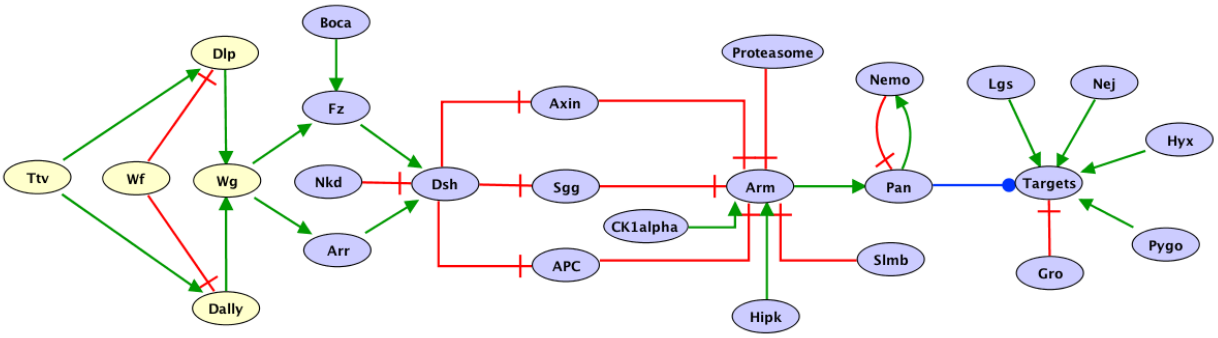

Regulatory graph for Drosophila Wingless (WG/WNT) pathway, displayed from components acting at the membrane on the left, to the main downstream effectors and a generic target node, along with inhibitory and activatory partners on the right. Blunt red and normal green arrows denote activatory and inhibitory interactions, respectively. The blue ball arrow denotes the fact that Pangolin (PAN) can activate or inhibit different sets of target genes.

Overview

In the absence of WG, the protein complex composed by Axin, Shaggy (SGG or ZW3) and APC sequesters and ubiquitinilates Armadillo, leading to a Slmb-dependant degradation by the proteasome.
In the absence of ARM, PAN binds to GRO to repress WG targets.
Binding of Wingless to Arrow (ARR) or Frizzled (FZ) triggers a set of reactions, starting with the activation of Dishevelled, which in turn inhibits the AXN-SGG-APC complex.
This leads (with the help of HIPK) to the accumulation and the stabilisation of ARM.
Next, ARM translocates into the nucleus and binds Pangolin (PAN). Then, the ARM/PAN complex with the help of other cofactors (LGS, Nej, Pygo and Hyx) activates the transcription of WG targets.
During some patterning processes as in wing disc, Nemo can inhibit PAN and thereby controls the level of WG signalling.
To study dynamically the WG signalling pathway, we define two initial states corresponding to the binding of WG ligand and to the absence of binding condition. From these two initial states, we compute the resulting stable states recapitulating the activation or the non activation of the pathway, respectively.

Selected references

- PMID:7813765
- PMID:12881613
- PMID:22855721
- PMID:22535229
- PMID:22371299

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Wg		input	• PMID:7781903 • PMID:8985186 • PMID:9435291 • PMID:9869644 • PMID:10508697 • PMID:10355030 • PMID:11076769 • PMID:11783990 • PMID:12490551 • http://flybase.org/reports/FBgn0004009.html WG level is provided as an initial self-sustained condition, which represents the presence of external WG signal. WG expression is limited to the anterior domain (Azpiazu et al. 1996). Ectopic WG causes only a small reduction in *bap* expression. Ectopic WG in HH mutant results in almost no *bap* expression. Ventral FB needs WG for its specification and loss of WG leads to the expansion of dorsolateral FB, with an ectopic *srp* expression (Riechmann et al. 1998). WG is needed for the induction of heart and dorsal muscle progenitors (Wu et al., 1995; Azpiazu et al., 1996; Carmena et al., 1998). WG acts negatively on *bap*, because *bap* expression is expanded in *wg* mutant embryo. WG suppresses the induction of trunk visceral mesoderm at different positions (Azpiazu et al., 1996). WG is a positive regulator of *tin* in VM development via PAN (Hosono et al., 2003). In H, WG activates *slp* via PAN (Azpiazu et al., 1996). WG is required for the development of SM because in *wg* mutants have no SM (Frasch et al., 1999).
Fz	1	Wg:1 & Boca	• PMID:8717036 • doi:10.1038/382225a0 • http://flybase.org/reports/FBgn0001085.html WG can associate with members of the Frizzled family of seven transmembrane-domain receptors. Frizzled proteins are receptors for WG and functions upstream of Dishevelled, recruiting this cytoplasmic protein to the cell membrane (Bhanot et al., 1996).
Arr	1	Wg:1	• PMID:11029007 • doi:10.1038/35035110 • PMID:14729180 • http://flybase.org/reports/FBgn0000119.html Arrow encodes an LDL-receptor related protein essential for Wingless signalling. FZ and Arrow have similar roles in the reception of WG signal and they act together as in a receptor complex. Secreted Wingless initiates signaling through the Frizzled family of receptors and Arrow. This leads to the phosphorylation of Dishevelled. (Wehrli et al., 2000; Tamai et al., 2000, Seto et al., 2004)
Dsh	1	(Arr:1 | Fz:1) & !Nkd	• PMID:7744250 • PMID:7813765 • PMID:14729180 • http://flybase.org/reports/FBgn0000499.html

			The binding of WG to its receptors induces the phosphorylation of the cytoplasmic protein Dishevelled (DSH). DSH, an essential protein for WG signalling, inhibits constitutive repressors of WG signalling. Phosphorylated DSH inhibits a multiprotein complex that includes Axin, Zeste-White-3, and Adenomatous Polyposis Coli (APC). In the presence of WG ligand, DSH inhibits the phosphorylation of Armadillo, thereby stabilizing it. Accumulation of Armadillo mediates the induction of WG target genes. (Klingensmith et al., 1994, Yanagawa et al., 1995, Seto et al., 2004).
Axin	1	!Dsh:1	• PMID:9482734 • PMID:14729180 • http://flybase.org/reports/FBgn0026597.html Axin knockout produces phenotypes that are similar to over-expression of the Drosophila WG. Over-expression of Axin produces phenotypes similar to loss of WG. Together, Axin, APC and Zeste-white 3 form a protein complex, which signals ARM for destruction by the proteasome. Axin is proposed to function as a scaffold that brings Zeste-White 3 and Armadillo together. This facilitates the phosphorylation of Armadillo, which is subsequently ubiquitinilated and degraded. In the presence of WG ligand, DSH inhibits the phosphorylation of ARM by the complex APC/SGG/Axin, thereby stabilizing it. (Ikeda et al., 1998 and Seto et al., 2004)
Sgg	1	!Dsh:1	• PMID:9233789 • PMID:9501208 • PMID:14729180 • PMID:17881495 • http://flybase.org/reports/FBgn0003371.html Shaggy phosphorylates ARM in the absence of WG signalling (Aberle et al., 1997, Sakanaka et al., 1998, Seto et al., 2004).
APC	1	!Dsh:1	• PMID:14729180 • http://flybase.org/reports/FBgn0015589.html APC is a component of the WG pathway functioning as a negative regulator in signal transduction. The cytoplasmic localization of ARM is regulated by phosphorylation. SGG, a serine threonine kinase, lies upstream of ARM, and positively regulates the phosphorylation of ARM, while APC is a member of this complex. This complex leads to the degradation of ARM in the absence of WG signalling (Seto et al., 2004).
Arm	1	!Sgg:1 & !APC:1 & !Axin:1 & !(Proteasome & Slmb) & Hipk & CK1alpha	• PMID:14729180 • PMID:17881495 • http://flybase.org/reports/FBgn0000117.html ARM mediates the induction of WG target genes. Upon WG signaling, ARM is stabilized and accumulates in the cytoplasm, serving as a measure of WG activity (Seto et al., 2004).
Proteasome		input	Proteasome complex (26 S in mammalian).
Slmb		input	• PMID:12442174

			• PMID:16386907 • PMID:17925225 • PMID:17881495 • http://flybase.org/reports/FBgn0023423.html The protein Supernumerary limbs (SLMB) is a subunit of a multi-protein complex that targets proteins for degradation by the ubiquitin-proteasome pathway (Ko et al., 2002, Smelkinson et al., 2006, 2007).
Hipk		input	• PMID:10391247 • PMID:17881495 • http://flybase.org/reports/FBgn0035142.html Loss and gain of functions of *hipk* demonstrates that HIPK plays a positive role in transmission of the WG signal. HIPK can promote ARM stabilization in regions of the wing disc receiving lower levels of WG signalling. Stabilized ARM can induce gene expression of WG targets. HIPK can form a complex with ARM and PAN. HIPK phosphorylates ARM. (Ishitani et al., 1999, Xu et al., 2007).
Pan	1	Arm:1 & !Nemo:1	• PMID:8985186 • PMID:11783990 • PMID:12490551 • PMID:17881495 • http://flybase.org/reports/FBgn0085432.html PAN is the WG effector. It is expressed in heart at stages 10-11. PAN activates *eve* (Knirr et al., 2001). WG activates *slp*via PAN, thereby contributing to the specification of FB (Reichmann, 1998) and VM (Azpiazu et al., 1996). The effect of WG on VM specification takes place after stage 10. (Azpiazu et al., 1996; Riechmann et al; 1998; Knirr et al., 2001; Hosono et al., 2003; Xu et al., 2007)
Nemo	1	Pan:1	• PMID:15169756 • http://flybase.org/reports/FBgn0011817.html Nemo inhibits the activity of WG in the wing disc patterning (Zeng et al., 2004).
Pygo		input	• PMID:18451032 • PMID:19493659 • http://flybase.org/reports/FBgn0043900.html Pygopus (PYGO) is essential for the transcriptional activity of ARM during Drosophila development. PYGO is recruited to Drosophila PAN target genes via the LGS-ARM adaptor chain. Drosophila Hyrax is required for nuclear transduction of WG signal and binds directly to the C-terminal region of ARM. Moreover, the transactivation potential of Hyrax depends on the recruitment of Pygopus to ARM. (Carrera et al., 2008, Kessler et al., 2009).
Nej		input	• PMID:18404694 • http://flybase.org/reports/FBgn0004396.html Nejire (NEJ) prevents ARM/PAN complex formation, thereby inhibiting transcription (Waltzer and Bienz, 1998).
Lgs		input	• PMID:17113272 • PMID:18404694 • PMID:18451032 • PMID:8985186 • http://flybase.org/reports/FBgn0039907.html The adaptor protein Legless (LGS) links ARM N-terminal

			homology domain (NHD) and PYGO, is essential for WG-regulated transcriptional activation (Hoffmans et al. 2007, Carrera et al., 2008, Jessen et al., 2008).
Hyx		input	• PMID:16630820 • http://flybase.org/reports/FBgn0037657.html The N-terminal region of Hyrax (HYX) directly interacts with the C-terminal region of ARM. HYX plays a key role in mediating the transcriptional output of ARM in response to WG pathway activation. Moreover, the transactivation potential of HYX depends on the recruitment of Pygopus to ARM (Mosimann et al., 2006).
Gro		input	• PMID:18404694 • http://flybase.org/reports/FBgn0001139.html PAN protein interacts with co-repressors such as Groucho to keep WG target genes silent in the absence of an active signal (Jessen et al., 2008).
Wf		input	• PMID:10421372 • PMID:11092814 • PMID:10421371 • PMID:12000788 • http://flybase.org/reports/FBgn0044028.html Wingful or Notum is a secreted extracellular antagonist that regulates patterning processes depending on long-range WG signaling. Loss of WF function causes a gain of WG activity. A gain of function causes a loss of WG signaling. WF presumably inhibits the activity of a co-receptor component, such as Dally or Dally-like (DLP) proteoglycans that appear to participate in WG reception (Lin and Perrimon 1999; Tsuda et al., 1999; Baeg et al., 2001). WF may inhibit such receptor components via its presumptive esterase activity, by modifying Dally glycosaminoglycan chains. (Gerlitz et al., 2002).
Nkd		input	• PMID:11274052 • PMID:10693810 • http://flybase.org/reports/FBgn0002945.html NKD regulates embryonic WG activity by acting as an inducible antagonist of the WG transduction component DSH (Rousset et al., 2001). Unexpectedly, NKD plays no discernible role at later stages of development, such as during the patterning of imaginal discs (Zeng et al., 2000). NKD is also used during embryonic patterning as the intracellular feedback antagonist, where WG functions at short range.
Boca		input	• PMID:9875856 • PMID:10457026 • PMID:10556068 • PMID:11029006 • PMID:9716412 • http://flybase.org/reports/FBgn0004132.html WG requires two transmembrane receptors, Arrow, a member of the LDLR family, and either Frizzled or Fz2 (Bhat, 1998; Bhanot et al., 1999; Chen and Struhl, 1999; Wehrli et al., 2000). Experimental localization of Boca and epistasis experiments suggest a potential role for Boca in the processing and/or transport of one of these receptors. (Axelrod et al., 1998).

CK1alpha		input	• PMID:11927557 • PMID:14966281 • http://flybase.org/reports/FBgn0015024.html RNAi knockdown of CKI alpha inhibits ARM phosphorylation and degradation, and induces PAN-mediated luciferase expression (Matsubayashi et al., 2004; Yanagawa et al., 2002). CKI alpha RNAi in Drosophila embryos results in a naked cuticle, while CKI alpha ectopic expression phenotype is consistent with ectopic WG signalling (Yanagawa et al., 2002). CKI alpha may positively regulate WG signalling by phosphorylating DSH (Matsubayashi et al., 2004; McKay et al., 2001). Alternatively, CKI alpha could exert a positive influence on WG pathway by phosphorylating ARR.
Dlp	1	Ttv & !Wf	• PMID:2699855 • PMID:8945511 • PMID:9043068 • PMID:8909553 • http://flybase.org/reports/FBgn0041604.html The heparan sulfate proteoglycan Dally and DLP are the substrates for TTV and are involved in WG movement signalling. In the wing imaginal discs, WG acts as a morphogen because it is organized in an extracellular protein gradient and activates the expression of target genes in a dose-dependent manner (Zecca et al., 1996; Neumann and Cohen, 1997). WG binds tightly to glycosaminoglycans and appears to interact with DLP, raising the possibility that DLP is also involved in shaping the gradient of extracellular WG. Experimental observations shows a high level of WG accumulation in wing discs over-expressing DLP, suggesting that DLP may have a high capacity to bind WG *in vivo*.
Ttv		input	• PMID:15056609 • PMID:14998928 • PMID:10963990 • http://flybase.org/reports/FBgn0020245.html The production of heparan sulfate proteoglycans (HSPG) involved in WG movement, such as Dally and Dally-like (DLP), requires Tout-velu (TTV), a heparan sulphate copolymerase. In the absence of TTV activity, WG is seen only in expressing cells, indicating that WG does not move beyond its production site. HSPGs are needed for WG to reach distant target cells, while TTV is required for the proper diffusion of the cholesterol-modified, membrane-associated HH-Np (Bornemann et al., 2004; Han et al., 2004; Lin et Perrimon, 2000).
Dally	1	Ttv & !Wf	• PMID:2699855 • PMID:8945511 • PMID:9043068 • PMID:8909553 • http://flybase.org/reports/FBgn0263930.html In Drosophila, there are two heparan sulfate proteoglycan (HSPG), division abnormally delayed DALLY and DLP. DALLY and DLP are the substrates for TTV and are involved in WG signalling. In the wing imaginal discs, WG acts as a morphogen. It is organized in an extracellular protein gradient and activates

			the expression of target genes in a dose-dependent manner (Zecca et al., 1996; Neumann and Cohen, 1997). WG binds tightly to glycosaminoglycans (Riechmann et al., 1996).
Targets	1	Pygo:1 & Lgs:1 & CBP:1 & PAN:1 & Hyx:1 & !Gro	• PMID:8985186 • PMID:12858517 • PMID:16221729 Targets or WG pathway in the mesoderm include: - *srp* in fat body domain, - *tin, slp, doc* in the heart domain.

Logical model of Drosophila Hh signaling pathway

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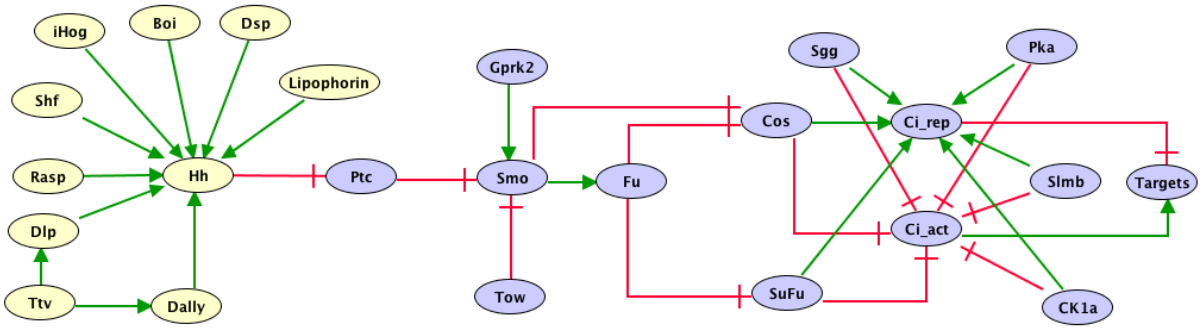


Regulatory graph for drosophila Hedgehog (HH) pathway, displayed from components acting at the membrane on the left to the main downstream effectors and a generic target node, along with inhibitory and activatory partners on the right. Blunt red and normal green arrows denote activatory and inhibitory interactions, respectively.

Overview

Processing of HH ligand

The precursor of HH is auto-catalytically cleaved to produce an N-terminal (HH-N) and a C-terminal (HH-C) fragments (Lee et al, 1994; Porter et al, 1996b).
A cholesterol moiety is covalently attached to the last amino acid of HH-N to create HH-Np, that is responsible for the biological activities of HH proteins (Ingham and McMahon, 2001; Lee et al, 1994; Porter et al, 1996b).
The N-terminal region of HH-Np is further modified by addition of palmitate that is essential for its signalling activity (Pepinsky et al, 1998, Wang et al, 2000; Amanai and Jiang, 2001; Chamoun et al, 2001; Lee and Treisman, 2001; Micchelli et al, 2002).
We model these aspects by an AND rule (combining inputs from DLP, IHOG, Rasp, DISP, SHF, Lipophorin, BOI and DALLY) attached to the component representing the secreted HH molecule, denoted Hh in our model.

HH Signalling

Two integral membrane proteins are involved in HH signal reception: Patched and Smoothened. HH binding to its receptor Patched (PTC) relieves PTC-mediated repression of Smoothened (SMO), a serpentine-like membrane protein required for HH signalling (Alcedo et al., 1996; Chen and Struhl, 1996).
This allows SMO stabilisation, activation, and phosphorylation by Shaggy (SGG), and downstream signalling through the formation of a protein complex including the serine threonine kinase Fused (FU), the kinesin-like protein Costa (COS), and the protein Suppressor of Fused (SU(FU)), ultimately controlling the post-translational processing of the protein Cubitus interruptus (CI) (Lum et al, 2003).
In the absence of HH, COS binds CI directly and sequesters it in the cytoplasm with the help of SUFU.
The recruitment of different kinases (Casein kinase 1 alpha, Shaggy, Protein kinase A) then leads to the phosphorylation of CI and to its proteolysis by SLMB.
The resulting truncated protein (CI_rep) is released and enters the nucleus, where it has a transcriptional repressing activity.
Recent evidence further indicates that SMO is inhibited by TOW, which tentatively mediates the

effect of PTC on SMO (Ayers et al, 2008 and 2009).
Following SMO activation, the transcription factor CI is phosphorylated and translocated into the nucleus in its entire form, which plays a transcriptional activatory role (CI_act).
In the model, a cascade of inhibitions, from HH on PTC, and from PTC on SMO, implements the indirect positive action of HH on SMO.
A protein complex including CI, COS, and FU, phosphorylates and thereby inhibits SU(FU), ultimately favouring the CI activatory form and its translocation into the nucleus.
We model the roles of the kinases (SGG, PKA, and CK1a), COS and SU(FU) (both needed to recruit the kinases) in the processing of CI in terms of inhibitory interactions on CI_act and activatory interactions on CI_rep.
Complexes are represented implicitly (they are formed as soon as the components are synthesised or activated), while logical rules define component activity requirements to form CI_act versus CI_rep forms.
To explore the dynamic of the pathway, we define two initial states to simulate the presence and the absence of signalling. On one hand, the non binding of HH (level expression 0) triggers a series of signalling cascades that lead to the activation of several kinases (for example SGG, PKA, CK1a, ...) at level of expression 1, which will permit the formation of CI repressor (expressed at level 1), which in turn will inhibit the targets. On the other hand, the presence of HH (level of expression 1) leads to a stable state corresponding to the signalling conditions leading to the formation of CI activator that will activate the targets node (level of expression 1).

Selected references

- PMID:16678090
- PMID:7985023
- PMID:11731473
- PMID:11290291
- PMID:18379584
- PMID:19285058

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Hh	1	Dlp:1 & iHog:1 & Rasp:1 & Disp:1 & Shf:1 & Lipophorin:1 & Boi:1 & Dally:1	• PMID:6776413 • PMID:1394430 • PMID:7985023 • PMID:8689684 • PMID:12372301 • PMID:14602684 • PMID:14729575 • PMID:9409685 • PMID:8898207 • PMID:8906794 • http://flybase.org/reports/FBgn0004644.html Hedgehog (HH) is a segment polarity gene (Nusslein-Volhard and Wieschaus, 1980; Lee et al, 1992) encoding a 47-kD protein that undergoes an intramolecular cleavage to yield a mature, N-terminal signaling polypeptide (Lee et al, 1994). This signaling protein is modified by cholesterol on the C-terminus and palmitoylated on a cysteine near the N-terminus (Porter et al, 1996). This dually lipidated molecule requires the action of Dispatched (Disp) for release from the cell membrane (Ma et al, 2002). Movement to neighboring cells likely involves the glypican family member Dally-like (Dlp) (Desbordes and Sanson, 2003; Han et al, 2004). HH modifications restrain its diffusion, while Dlp promotes its movement, contributing to the formation of a gradient that confers different cell-fate outcomes in a dose-dependent manner (Strigini and Cohen, 1997). Binding of HH to Patched (PTC) initiates pathway response (Chen and Struhl, 1996; Marigo et al,1996).
Smo	1	Gprk2:1 & !Ptc:1 & !Tow:1	• PMID:17483466 • PMID:12192414 • PMID:14636583 • PMID:14597665 • PMID:14614827 • PMID:12874118 • PMID:9244297 • PMID:14523402 • PMID:9244298 • PMID:11090136 • PMID:15691767 • PMID:15616566 • PMID:15592457 • PMID:15598741 • http://flybase.org/reports/FBgn0003444.html The activity of Smoothened (SMO) is regulated by the transmembrane protein Patched (PTC) (Taipale et al, 2002) and the Hedgehog (HH) signalling molecule that binds PTC. In the absence of HH, SMO activity is inhibited. Upon inactivation of PTC by HH binding, SMO recruits a large cytoplasmic complex to its cytoplasmic tail that contains COS (Lum et al, 2003; Jia et al, 2003; Ogden et al, 2003; Hooper, 2003), FU (Robbins et al, 1997; Lum et al, 2003; Ruel et al, 2003), and CI (Sisson et al, 1997;

			Wang et al, 2000, Zhang et al, 2005). This complex is required for CI-act formation (Zhang et al. 2005) and is dependent on the phosphorylation of SMO cytoplasmic tail by the kinases PKA, CK1 and SGG (Jia et al, 2004; Apionishev et al, 2005; Zhang et al.,2004).
Pka		input	• PMID:15616566 • PMID:15592457 • PMID:15598741 • PMID:14636583 • PMID:14597665 • PMID:14614827 • PMID:12874118 • PMID:9244297 • PMID:14523402 • PMID:9244298 • PMID:11090136 • PMID:15691767 • PMID:9482888 • PMID:10477300 • http://flybase.org/reports/FBgn0000273.html Protein kinase A (PKA) acts at two levels in HH pathway. Phosphorylation of SMO cytoplasmic tail by PKA is important for SMO-mediated recruitment of the cytoplasmic regulatory complex (COS, FU, and CI) (Robbins et al, 1997; Sisson et al, 1997; Wang et al. 2000; Lum et al, 2003; Jia et al, 2003 and 2004; Ogden et al. 2003; Hooper 2003; Ruel et al, 2003; Zhang et al, 2004 and 2005; Apionishev et al, 2005). Phosphorylation of CI by PKA ultimately targets CI for proteolytic processing into a transcriptional repressor CI-rep (Chen et al. 1998; Price and Kalderon 1999).
Ptc	1	!Hh	• PMID:8049467 • PMID:8595881 • PMID:8906794 • PMID:8898207 • PMID:12192414 • PMID:19285058 • http://flybase.org/reports/FBgn0003892.html Patched (PTC) is a receptor for HH (Chen and Struhl, 1996; Marigo et al, 1996). In the absence of HH binding, PTC suppresses the function of the transmembrane protein SMO (Taipale et al, 2002). PTC may also contributes to the control of the processing of lipophorin (Alyers et al, 2009). In the embryo imaginal discs, the distribution of HH in receiving cells is regulated by the receptor PTC, which is expressed in all anterior compartment cells and is up-regulated by HH signalling (Forbes et al, 1993; Goodrich et al, 1996; Marigo et al, 1996). Up-regulated PTC protein in HH-responding wing cells sequesters HH and thereby restricts further HH diffusion (Chen and Struhl, 1996).
Tow		input	• PMID:19285058 • http://flybase.org/reports/FBgn0035719.html The effect of Target of Wingless (TOW) over-expression on HH signalling occurs specifically in HH receiving cells. TOW apparently acts downstream of PTC, upstream of SMO and independently of COS, to

			destabilize positive pathway members and conversely stabilize negative members, resulting in a repression of all levels of HH signalling. Both ectopic PTC and TOW cause lipophorin accumulation in imaginal discs. Loss of TOW leads to sensitized HH signalling, with a phenotype resembling SU(FU) loss of function. PTC repression of SMO is tentatively mediated by TOW, possibly through regulation of the degradation or homeostasis of lipophorin particles, thereby controlling the availability of SMO ligand(s) (Alyers et al, 2009).
Cos	1	!Fu:1 & !Smo:1	• PMID:9244298 • PMID:11912487 • PMID:14636583 • PMID:14597665 • PMID:14614827 • PMID:12874118 • PMID:11090136 • PMID:15691767 • PMID:11934882 • http://flybase.org/reports/FBgn0000352.html Costa (COS) is a kinesin-like molecule with no predicted motor function (Sisson et al, 1997; Robbins et al, 1997). In the presence of HH, COS forms a complex with SMO (Lum et al, 2003; Jia et al. 2003; Odgen et al, 2003; Hooper, 2003), and recruits FU and CI with SMO (Sisson et al, 1997; Robbins et al, 1997; Wang et al, 2000; Lum et al, 2003; Zhang et al, 2005). This complex allows the dissociation of CI, leading to its activation and translocation to the nucleus. In the absence of HH binding, FU and COS are basally phosphorylated and, together with CI, form a complex sequestered on microtubules. In the presence of HH binding, FU and COS are hyper-phosphorylated, thereby weakening their binding to microtubules (Nybakken et al, 2002). In the absence of HH signalling, a similar complex is formed, involving SU(FU), PKA and CI.
SuFu	1	!Fu:1	• PMID:1468628 • PMID:7498739 • PMID:9601642 • PMID:14636583 • PMID:10952898 • PMID:11090136 • PMID:9874371 • http://flybase.org/reports/FBgn0005355.html Suppressor of Fused directly binds Cubitus interruptus (CI) (Monnier et al, 1998; Lum et al, 2003) and reduces the abundance (Ohlmeyer and Kalderon, 1998) and nuclear accumulation of CI (Methot and Basler ,2000; Wang et al, 2000). SU(FU) is phosphorylated by FU in response to HH signalling (Lum et al, 2003). In the absence of HH signaling, non phosphorylated SU(FU) binds to CI and thereby prevents nuclear accumulation of CI-act, the activated form of CI. Upon reception of the HH signal, FU is activated and counteracts SU(FU), favouring the activatory CI-act form (Monnier, 1998).

Fu	1	Smo:1	• PMID:6776413 • PMID:1468628 • PMID:11934882 • PMID:9244298 • PMID:9244297 • PMID:14636583 • http://flybase.org/reports/FBgn0001079.html Fused (FU) is a segment polarity gene (Nusslein-Volhard and Wieschaus 1980) that encodes a putative serine-threonine kinase (Preat et al, 1990; Nybakken et al, 2002). FU is a partner for the kinesin-like protein Costa (COS) (Sisson et al, 1997; Robbins et al, 1997; Lum et al., 2003) and participates in the Hedgehog (HH) signal transduction pathway. In the absence of HH stimulation, FU and COS are basally phosphorylated and, together with CI, form a high molecular weight protein complex that binds to microtubules. In the presence of HH stimulation, FU and COS are hyperphosphorylated, and the complex only weakly binds to microtubules (Nybakken et al, 2002).
Rasp		input	• PMID:11486055 • DOI:10.1126/science.1064437 • http://flybase.org/reports/FBgn0024194.html Skinny Hedgehog or Rasp likely palmitoylates Hedgehog (HH), a modification essential for its activity. Rasp is a 12-transmembrane protein with similarities to acyltransferases, encoded by a segment-polarity gene. Reduction or loss of Rasp function thus reduces the hydrophobicity of HH, consistent with a loss of NH2-terminal palmitoylation and with a role for Rasp function in palmitate transfer (Chamoun et al, 2001).
Shf		input	• DOI 10.1016/j.devcel.2005.01.003 • PMID:15691766 • PMID:15691765 • doi:10.1016/j.devcel.2004.12.018 • http://flybase.org/reports/FBgn0003390.html *shifted (shf)* mutations decrease the range and level of HH signaling (Glise et al, 2005; Gorfinkiel et al, 2005) SHF acts upstream of CI stabilization, while PTC acts downstream from SHF in HH pathway. SHF is required for normal range of movement of cholesterol modified HH in the wing disc. Secreted SHF controls the stability (accumulation) and movement of HH through direct interaction with HH.
Disp		input	• PMID:10619433 • PMID:12372301 • http://flybase.org/reports/FBgn0029088.html Dispatched (DISP) is a multi-span transmembrane protein essential for HH trafficking in producing cells. DISP appears to control the release of HH-Np from the produciIng cells. In the absence of DISP function, HH-Np accumulates in the producing cells and fails to move towards receiving cells (Burke et al,, 1999, Ma et al, 2002).
Slmb		input	• PMID:9990853 • PMID:16326393 • PMID:9693144

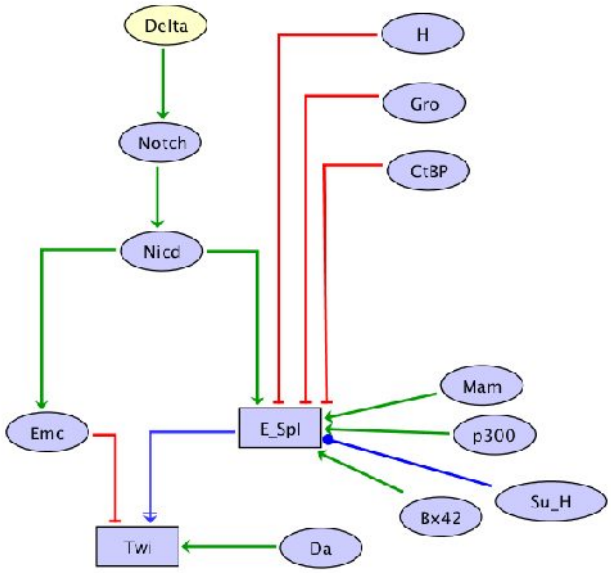
			• PMID:9461217 • http://flybase.org/reports/FBgn0023423.html The F-box protein supernumerary limbs (SLMB) functions as an adaptor protein between the ubiquitin ligase complex and substrates to be targeted for ubiquitinylation (Spencer et al, 1998). In the absence of HH, CI phosphorylation enables the recruitment of SLMB(Jia et al, 2005), resulting in CI ubiquitinylation and its proteasome-mediated proteolytic processing into a repressor (Theodosiou et al, 1998; Jiang and Struhl, 1998).
Ttv		input	• PMID:9756849 • PMID:10549295 • PMID:14729575 • PMID:15563523 • http://flybase.org/reports/FBgn0020245.html Production of heparan sulfate proteoglycans (HSPG) involved in HH movement, such as Dally and Dally-like (DLP), requires Tout-velu (TTV), a heparan sulphate copolymerase. In the absence of TTV activity, HH is only seen in HH-expressing cells, indicating that HH does not move beyond its site of production. HSPGs are needed for HH to reach target cells, while TTV is required for proper diffusion of the cholesterol-modified, membrane-associated HH-Np (Lind et al, 1998; The et al, 1999, Bellaiche et al, 1999, Han et al, 2004, Lin et al, 2004).
Dally	1	Ttv:1	• PMID:8985186 • PMID:14729575 • PMID:17609110 • DOI 10.1016/j.devcel.2007.04.019 • http://flybase.org/reports/FBgn0263930.html The heparan sulfate proteoglycanDivision Abnormally Delayed (DALLY) and Dally-like (DLP) are the substrates for TTV and are involved in HH signalling (Han et al, 2003). Activated by HH signalling in the wild type, *bap* expression is strikingly reduced in *dally* or *dlp* knockout embryos (Han et al., 2003). In wing development, loss of function of DALLY and DLP is similar to a typical loss of HH function. Both DALLY and DLP are required for full-strength HH signaling, but do not affect the range over which HH spreads (Eugster et al, 2007).
Lipophorin		Input	• doi:10.1016/j.devcel.2007.04.019 • PMID:17609110 Lipophorins are lipid-transporting particles that are important for Hedgehog (HH) movement. Lipophorins form a complex with HH and membrane associated and solubilized glypican family members. Interactions between lipophorins and the glypicans are mediated by the heparan sulfate moieties found on glypicans, and these interactions are likely important to HH movement. Lipophorins remain associated with glypicans when they are released from the plasma membrane. The released form of Dally is found in endosomes containing Lipophorin, HH, and the HH receptor PTC (in receiving cells).

			Lipophorins thereby increase HH signaling efficiency (Eugster et al, 2007).
Boi		input	• PMID:16630821 • DOI 10.1016/j.cell.2006.04.016 • http://flybase.org/reports/FBgn0040388.html Brother of iHog (BOI), like IHOG, functions as a receptor for Hedgehog (HH). BOI is a member of a larger family of HH-binding proteins that includes IHOG (Yao et al, 2006; Wilson et al, 2006).
iHog		input	• PMID:17077139 • PMID:16630821 • DOI 10.1016/j.cell.2006.04.016 • http://flybase.org/reports/FBgn0031872.html Interference Hog (IHOG) is a receptor for Hedgehog (HH) (McLellan et al, 2006) that facilitates the binding of HH to Patched (PTC). IHOG is a type I transmembrane glycoprotein, containing immunoglobulin (Ig) domains and fibronectin type III (FNIII) domains on its N-terminus (Yao et al, 2006, Wilson, 2006).
Gprk2		input	• PMID:17483466 • http://flybase.org/reports/FBgn0261988.html G protein receptor-coupled kinase 2 (GPRK2) participates in the Hedgehog (HH) pathway, likely by contributing to SMO protein phosphorylation in response to HH (Molnar et al, 2007).
CK1a		input	• PMID:15616566 • PMID:15598741 • PMID:15592457 • PMID:11955435 • PMID:11912487 • http://flybase.org/reports/FBgn0015024.html Casein kinase 1a (CK1a) phosphorylates both SMO (Jia et al, 2004; Apionishev et al, 2005; Zhang et al, 2004) and CI (Price and Kalderon, 2002; Jia et al, 2002). Phosphorylation by PKA primes both SMO and CI for subsequent CK1a phosphorylation.
Sgg		input	• PMID:11955435 • PMID:11912487 • http://flybase.org/reports/FBgn0003371.html Zeste white 3 or Shaggy (Sgg), functions in concert with the protein kinase A (PKA) and casein kinase 1a (CK1a) to promote the phorphorylation of CI (Price and Kalderon 2002; Jia et al. 2002) and its subsequent proteolytic processing into a truncated repressive form. Sgg is scaffolded by COS.
Targets	1	Ci_act:1 & !Ci_rep:1	HH pathway target genes participate in embryo segmentation, in mesoderm specification, as well as in cardiac cell diversification.
Dlp	1	Ttv:1	• PMID:8985186 • PMID:14729575 • http://flybase.org/reports/FBgn0041604.html The heparan sulfate proteoglycan Dally and DLP are the substrates for TTV and are involved in HH movement signalling (Han et al, 2003). During mesoderm specification, Bap expression is strikingly reduced in *dally* or *dlp* knockout embryos (Han

			et al., 2003). DLP is required for full-strength HH signalling, but does not affect the range over which HH spreads (Eugster et al, 2007).
Ci_rep	1	CK1a:1 & Slmb:1 & Sgg:1 & SuFu:1 & Pka:1 & Cos:1	• PMID:2166702 • PMID:8049467 • PMID:8769644 • PMID:9215627 • PMID:9482888 • PMID:10477300 • PMID:11955435 • PMID:11912487 • PMID:9244298 • PMID:9244297 • PMID:11090136 • PMID:15691767 • PMID:9601642 • PMID:14636583 • http://flybase.org/reports/FBgn0004859.html Cubitus Interruptus (CI) is the transcriptional effector of the HH pathway (Forbes et al, 1993; Alexandre et al, 1996). Repressor CI activity is mediated by an N-terminal protein fragment CI-rep lacking the carboxy regulatory domain (Aza-Blanc et al. 1997). Proteolysis of CI is initiated by phosphorylation by the protein kinases PKA, SGG and CK1a (Chen et al, 1998; Price and Kalderon, 2002; Jia et al, 2002). Activation of the Hedgehog (HH) pathway abrogates this processing (Aza-Blanc et al. 1997). CI directly binds to the other HH pathway components COS (Sisson et al, 1997; Robbins et al, 1997; Wang et al, 2000, Zhang et al, 2005) and Su(FU) (Monnier et al. 1998; Lum et al. 2003).
Ci_act	1	!((CK1a | Sgg | Pka:1) & Cos & SuFu & Slmb)	• PMID:2166702 • PMID:8049467 • PMID:8769644 • PMID:9215627 • PMID:9482888 • PMID:10477300 • PMID:11955435 • PMID:11912487 • PMID:9244298 • PMID:9244297 • PMID:11090136 • PMID:15691767 • PMID:9601642 • PMID:14636583 • http://flybase.org/reports/FBgn0004859.html Cubitus Interruptus (CI) is the transcriptional effector of the HH pathway (Forbes et al, 1993; Alexandre et al, 1996). Pathway activation is mediated by the full-length CI molecule CI-act (Aza-Blanc et al. 1997). CI directly binds to the other HH pathway components COS (Sisson et al, 1997; Robbins et al, 1997; Wang et al, 2000, Zhang et al., 2005) and SU(FU) (Monnier et al. 1998; Lum et al. 2003).

Logical model of Drosophila Notch signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila Notch pathway, displayed from ligand and receptor at the top to the main downstream effectors and an example of target node, along with inhibitory and activatory partners at the bottom. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively. The blue arrows denote dual interactions (i.e. activatory or inhibitory depending on the presence of co-factors or of the level of the regulator).

Overview

Notch signaling is involved in the modulation of Twist expression and the subdivision of the mesoderm into high and low domain of Twist. The binding of Delta leads to the cleavage and the release of the Notch intracellular domain NICD.

During mesoderm specification, NICD can inhibit Twist by forming a complex with EMC, or in combination with Enhancer of split and Suppressor of hairless proteins.

In this regard, we modeled the effect of Notch pathway on Twist expression. Our defined initial states reproduce biological data during mesoderm specification. When Delta is ON (high or medium signaling), the level of Twist expression can decrease from 2 to 1 or 0. When Delta is OFF (no signaling), Twist is expressed at its maximal level 2.

Selected references

- PMID:8633240
- PMID:15128668
- PMID:9733574
- PMID:8076205
- PMID:19896355

Description of regulatory graph components

Components	Values	Logical rules	Annotations
H		input	• PMID:16287856 • PMID:17362357 • PMID:22074602 • PMID:21756252 • http://flybase.org/reports/FBgn0001169.html Hairless antagonizes Notch signalling by recruiting GRO and CtBP (Nagel et al., 2005, Maier et al., 2008, Johnson et al., 2011, Nagel et al., 2011).
Notch	1	Delta:1	• PMID:15128668 • PMID:1690605 • http://flybase.org/reports/FBgn0004647.html Notch is ubiquitously expressed during gastrulation. It is activated by Delta (DL) and Serrate (SER). Notch null embryos, lacking both maternally contributed and zygotically expressed Notch, fail to modulate Twist expression into low and high mesoderm domains at stage 10, resulting in maintained uniform high Twist levels. Notch signaling acts as a transcriptional switch that alleviates Su(H)-mediated repression and converts Su(H) from a transcriptional repressor into a transcriptional activator. Furthermore, Su(H) could affect Twist expression through a multi-layer mechanism that includes direct, as well as indirect, transcriptional regulation (Tapanes-Castillo et al., 2004).
Delta		input	• PMID:8330521 • PMID:22571430 • PMID:22868267 • http://flybase.org/reports/FBgn0000463.html The Notch ligand Delta is expressed throughout the mesoderm at late stage 9 and stage 10 (Kooh et al., 1993, Majumder et al., 2012; Guruharsha et al., 2012).
E_Spl	1	!(Nicd & Mam) | !(Su_H_CSL | H) | !(Gro | CtBP) & H	• PMID:10370119 • PMID:15128668 • PMID:20458100 • PMID:22949507 • PMID:23213460 • http://flybase.org/reports/FBgn0000591.html Enhancer of split (E(SPL)) bHLH proteins are Notch-regulated transcriptional repressors. They directly bind promoters, recruit co-repressors, and repress transcription. In addition, these proteins interact with other promoter-bound bHLH proteins expressed throughout the mesoderm at uniform low levels. At stage 10, Df(3R)E(SPL) mutant embryos maintained uniform high Twist expression throughout the mesoderm. Like Notch null mutants, Df(3R)E(SPL) mutant embryos do not modulate Twist into low and high domains. Mesodermally expressed E(SPL) genes repress Twist at stage 10.
	2	Su_H & Nicd & Mam & !H & !Gro & !CtBP	
CtBP		input	• PMID:16287856 • PMID:18031354

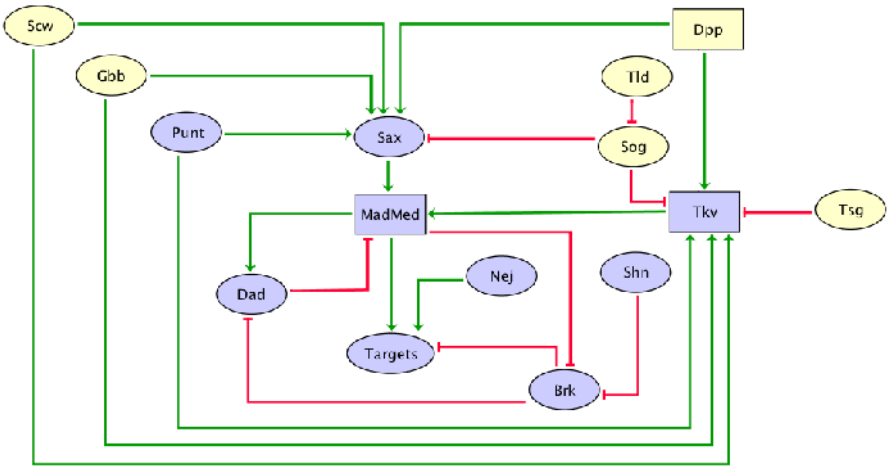
			• PMID:22125648 • PMID:21756252 • http://flybase.org/reports/FBgn0020496.html In the absence of Notch activity, CSL proteins (CBF1, Su(H) and LAG-1) recruit co-repressors. The adaptor Hairless (H) tethers the more global repressors Groucho and C-terminal binding protein (CtBP), which recruit histone deacetylases. Nagel et al., 2005; Nagel et al., 2007, Nagel et al., 2011; Kurth et al., 2011.
Nicd	1	Notch:1	A proteolytic processing mediates the release of the Notch intracellular domain (NICD), which enters the nucleus and interacts with the DNA-binding CSL protein (Su(H) in the model). Pan-mesodermal expression of a constitutively activated form of Notch (Nintra) has an effect opposite to that of a complete loss of Notch function: fewer cells express high Twist levels. Mastermind (MAM) and other transcription factors are recruited to the CSL complex, whereas co-repressors are released (probably GRO, CtTB). The assembly of the co-activator complex results in turnover of NICD. In the absence of Notch activity, CSL proteins recruit co-repressors. The adaptor Hairless tethers the more global repressors Groucho and CtBP, which recruit histone deacetylases. The rapidly changing levels of pathway activity require that the nuclear effectors have a short half-life. This is achieved by recruiting factors such as cyclin-dependent kinase-8 (CDK8), which phosphorylates NICD, turning it into a substrate of the nuclear ubiquitin ligase.
Gro		input	• PMID:18031354 • PMID:22125648 • PMID:21756252 • PMID:22305159 • http://flybase.org/reports/FBgn0001139.html Groucho is a co-repressor recruited by CSL proteins in the absence of Notch signalling. Nagel et al., 2007, Nagel et al., 2011, Kurth et al., 2011; Turki-Judeh et al., 2012.
Mam		input	• PMID:10545243 • PMID:11378391 • PMID:18930034 • PMID:10545243 • http://flybase.org/reports/FBgn0002643.html The co-activator Mastermind (MAM) is required to activate transcription. MAM proteins from different species share little sequence homology apart from a N-terminal region that forms an extended, helical domain that contacts CSL and the Ank domain of NICD, thereby enabling the formation of a trimeric complex. This complex triggers the transcription of Notch target genes. Helms et al., 1999; Morel et al., 2001; Cave et al., 2008.

Da	1	(basal value)	• PMID:8217842 • PMID:11688563 • PMID:15128668 • http://flybase.org/reports/FBgn0000413.html Daughterless (DA) is ubiquitously expressed throughout development (Cronmiller et al., 1993) and is required to maintain high Twist expression throughout the mesoderm during gastrulation. Loss- and gain-of-function experiments indicated that DA is a critical regulator of Twist in the early mesoderm and that inhibition of DA activity is required for proper Twist modulation (Castanon et al., 2001). Expressed at high levels in the early mesoderm, EMC has been shown to genetically and biochemically interact with DA, thereby providing a mechanism for inhibiting DA activity (Tapanes-Castillo et al., 2004).
Su_H		input	• PMID:15128668 • http://flybase.org/reports/FBgn0004837.html Supressor of Hairless (Su(H)) null mutant embryos modulate Twist levels properly and exhibit the low and high Twist pattern characteristic of wild-type embryos at stage 10. The pan-mesodermal expression of a constitutively trans-activating form of Su(H), Su(H)-VP16, leads to an expansion of the high Twist domain, a process involving Su(H). The Notch null phenotype results from the loss of a transcriptional switch that converts Su(H) from a constitutive repressor into an activator.
Emc	1	Nicd:1	• PMID:1690604 • http://flybase.org/reports/FBgn0000575.html In the embryonic mesoderm, Extra Macrochaetae (EMC) is expressed uniformly during gastrulation until stage 10.
Twi	1	Da & !Emc & !E_Spl:2	• PMID:1982429 • PMID:9362473 • PMID:11076769 • PMID:9435291 • PMID:8633240 • PMID:10355030 • http://flybase.org/reports/FBgn0003900.html Twist mutant embryo develop no mesoderm (Furlong, 2001). Wingless and Sloppy paired are required to generate higher levels of *twist* expression (Bodmer et al 1990; Baylies et al 1996; Reichmann et al 1997). Twist activity is required for the formation of most body wall muscle.
Bx42		input	• PMID:12204255 • http://flybase.org/reports/FBgn0004856.html Bx42 (SKIP (Ski-interacting protein) in mammals) is a transcriptional co-regulator and component of spliceosome that is recruited to promoters regulated by NICD. Mam in turn recruits the histone acetylase P300, which promotes assembly of initiation and elongation complexes (Negeri et al., 2002).
P300		input	• PMID:15128668 • PMID:14500836 • http://flybase.org/reports/FBgn0064148.html

			The histone acetylase P300 promotes the assembly of initiation and elongation complexes, once it is recruited by MAM on NICD regulated promotors. Takizawa et al., 2003, Tapanes-Castillo et al., 2004

Logical model of Drosophila Dpp signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila Dpp pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Rectangular and ellipsoid nodes denote ternary and Boolean components, respectively. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

Drosophila DPP (TGF-beta homolog) signalling pathway is triggered by ligand-induced formation of heterotetrameric complexes consisting of two type II receptors and two type I receptors with intrinsic serine/threonine kinase activity.
The type I receptor (SAX or TKV) is phosphorylated by the constitutively active type II receptor kinase (Punt). Consequently, the complex becomes active and phosphorylates the receptor-regulated Smads (R-Smads).
Phosphorylated R-Smads (MAD and Smox) form complexes with a common-mediator Smad (Medea) and translocate into the nucleus, where they regulate the transcription of target genes in co-operation with other transcription factors (*nejire*, *schnurri*).
DPP is a morphogen, *i.e.* a molecule distributed in a concentration gradient that elicits different cell fates as a function of its concentration, thereby organizing a series of cell types in a defined spatial array.
In response to DPP gradient, cells adopt different fates.
The establishment of dpp gradient involves the proteins SOG and TSG.
These proteins together capture the DPP ligand and prevent its binding to the receptor (Punt).
The heteromeric complex (SOG, DPP, TSG) then release the DPP ligand, a process involving the cleavage of SOG by Tolloid (a metalloprotease).
Other TGF-beta signals, Glass-bottom-boa (GBB) and Screw (SCW), help DPP to potentiate cells to respond.
SCW and GBB are never expressed together in the same region and affect different cells during:
i) early D/V patterning of the embryo and specification/differentiation of dorsal cells (if there is no screw, dpp alone is unable to establish the D/V pattern and embryo lack amnioserosa);
ii) the development of adult structures such as the wing.
GBB or SCW form heterodimeric complexes with DPP. These heterodimers can only signal through TKV, while SCW/SCW and GBB/GBB signals trough SAX, and DPP/DPP trough TKV and SAX.
To model DPP signalling and the formation of the gradient, we have considered three different levels for the TKV receptor (0, 1, 2) and the MADMED effector (0, 1, 2). The regulatory graph also accounts for the potentiation of responding cells due to association of DPP and SCW, or of DPP and GBB.

Activated by MADMED, DAD is a pathway inhibitor that can modulate the pathway activity from high to low signalling. DAD works by abrogating the phosphorylation of the MADMED complex by TKV or SAX, thus involving a negative circuit between DAD and the MADMED complex.
In addition, BRK another inhibitor of the DPP pathway can block the transcription of *dad*.
Our model reproduces the formation of the DPP signalling gradient and accounts for the role of the heterodimers signalling in cell potentiation.
To simulate DPP signalling, we start from an initial state corresponding to non differentiated cell, that can receives high or low level of DPP signal.
The use of ternary nodes enables us to account for differential effects of different DPP levels (gradient).
The cells receiving high level expression display the hetero-dimers SCW/DPP or GBB/DPP and correspond to Tld expression area, which promotes DPP gradient formation.
In presence of medium DPP, TSG and SOG but no TLD are initially needed to capture homo- or hetero-dimer, diminishing pathway signalling intensity (expression level 1 for TKV and MADMED).
In presence of high pathway signalling, two situations occur:
i) in cells potentiated by SCW: a sequestering complex (SOG/TSG/ DPP/SCW) will release the signalling molecule upon TLD clivage, in addition to normal DPP signalling. This leads to a higher signal transduction.
ii) in cells potentiated by GBB, the situation is similar but involve a different heterodimer (GBB/DPP).
These situations correspond to two different stable states with high TKV and MADMED (level 2), denoting that more receptors are required to enable a higher level of nuclear MADMED.
We consider five different initial states:
i) the first one corresponds to the absence of signalling, i.e. absence of DPP;
ii) the second one corresponds to medium signalling, characterized by the presence of Dpp at level 1 and of SCW;
iii) the third one corresponds to medium signalling, characterized by the presence of Dpp at level 1 and of GBB);
iv) the fourth one corresponds to the presence of DPP at level 2 and of SCW;
v) the last one corresponds to the presence of DPP at level 2 and of GBB.
These set of initial states enable the simulation of five situations. No signalling, two medium and two high signalling that characterize the behavior of the pathway. The stable state obtained with the no signalling simulation shows the absence of binding of the ligands to the receptors TKV and Punt (level of expression 0) and the non activation of target nodes. These medium signals simulations in presence of DPP, show the activation of the receptors (level of expression 1) and subsequent signalling cascade leading to the activation of pathway's targets. These medium signal are defined by the level of expression 1 for DPP, MADMED and TKV while in the high signalling sets, these nodes are expressed at level 2.
The node Tkv is multi-valued because the high signalling is characterized by the binding of hetero dimers (DPP/SCW or DPP/GBB) signalling through TKV. Note that GBB and SCW don't have the same expression pattern.

Selected references

- PMID:12239569
- PMID:18588885
- PMID:21385708
- PMID:22710168
- PMID:22257639

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Dpp		input	• PMID:1423606 • PMID:1765005 • PMID:18506030 • PMID:8330541 • PMID:10769238 • PMID:7997266 • PMID:8086336 • PMID:7913899 • PMID:7700357 • PMID:9409686 • http://flybase.org/reports/FBgn0000490.html Decapentaplegic (DPP) is the morphogen responsible of DV polarity (Ferguson et al., 1992). Its primary pattern is established in the dorsal ectoderm by repression in more ventral regions by Dorsal (Ray et al., 1991). DPP regulates target genes through two mechanisms: directly by activating gene expression, or indirectly by SHN-dependent repression of BRK (Yao et al., 2008). During embryonic development, DPP is essential for formation of the dorsal-ventral (D/V) axis (Wharton et al., 1993), the subdivision of the mesoderm into somatic versus visceral or cardiac components (Yu et al., 2000), the induction of endoderm in the developing gut (Staehling-Hampton et al., 1994; Frasch et al., 1995), and the formation of trachea (Wappner et al., 1997).
Scw		input	• PMID:7958918 • PMID:9827802 • PMID:12239569 • http://flybase.org/reports/FBgn0005590.html Screw (SCW) potentiates DPP signaling during early D/V patterning of the embryo (Arora et al., 1994). In the absence of SCW function, DPP alone is unable to establish two distinct thresholds at their normal position (dorsal ectoderm and amnioserosa) (Nguyen et al., 1998; Eldar et al., 2002).
Gbb	1	input	• PMID:9521913 • PMID:9636086 • PMID:12239569 • http://flybase.org/reports/FBgn0024234.html Glass-bottom-boat (GBB) potentiates DPP signalling during the development of adult structures such as the wing (Chen et al., 1998; Khalsa et al., 1998; Haerry et al., 1998).
Punt		input	• PMID:7697720 • PMID:9636086 • http://flybase.org/reports/FBgn0003169.html Punt is a transmembrane serine/threonine kinases type II receptor for DPP. Upon ligand binding, Punt phosphorylates the Type I receptor (TKV or SAX) by forming a heteromeric complex, leading to the phosphorylation of Smad proteins (Letsou et

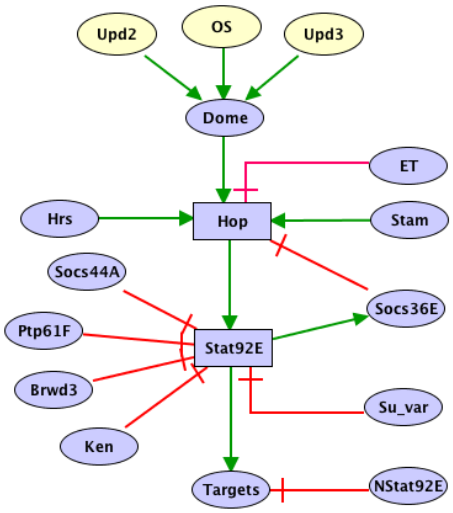
			al., 1995; Yu et al., 2000).
Tkv	1	Punt & (Dpp:1 | Scw | Gbb) & !(Sog | Tsg) & !Dpp:2	• PMID:9694800 • PMID:8044837 • PMID:8001784 • PMID:8903352 • http://flybase.org/reports/FBgn0003716.html DPP signaling is initiated by binding of the ligand to a complex of the type I and type II serine/threonine kinase receptors, Thickveins (TKV) and Punt, respectively (Xu et al., 1998). Activated TKV phosphorylates the BMP-specific Smad Mothers against DPP (MAD), leading to its association with the co-Smad Medea (MED) and accumulation of the MADMED complex in the nucleus. Loss of TKV in the embryo and in imaginal discs mimics the loss of DPP function (Nellen et al., 1994; Terracol and Lengyel, 1994; Burke and Basler, 1996).
	2	Punt & Dpp:2 & !(Sog | Tsg)	
Sax	1	Punt & (Gbb:1 | Scw:1 | Dpp) & !Sog	• PMID:9694800 • PMID:12239569 • http://flybase.org/reports/FBgn0003317.html Screw/Screw (SCW/SCW) dimer signals through SAX, while TKV is required for DPP/DPP and DPP/Screw dimers signalling (Nguyen et al. 1998, Eldar et al., 2002).
Tsg		input	• PMID:9232597 • PMID:12239569 • http://flybase.org/reports/FBgn0003865.html TSG (Twisted-gastrulation) protein is a secreted protein required to specify the fate of the dorsal midline cells (Mason et al., 1997; Eldar et al., 2002). TSG participates in the formation of the gradient expression of DPP, by forming a complex with SOG, which sequesters DPP/DPP, DPP/SCW or DPP/GBB dimers.
Sog	1	!Tld	• PMID:8752215 • PMID:10769238 • PMID:12239569 • http://flybase.org/reports/FBgn0003463.html SOG (Short gastrulation) is a secreted factor generating an activity gradient of DPP involved in the specification of dorsal embryonic cell fates by preventing its interaction with TKV. SOG diffuses dorsally to transport and limit DPP activity by preventing ligand binding (Holley et al., 1996; Yu et al., 2000; Eldar et al., 2002).
Tld		input	• PMID:1840509 • PMID:10769238 • PMID:10769238: • PMID:12239569 • http://flybase.org/reports/FBgn0003719.html *tolloid (tld)* transcripts are detected in precellular cleavage cycle 10 embryos in a broad dorsal-on/ventral-off pattern of expression. This temporal and spatial pattern is very similar to that seen for DPP. TLD encodes the Drosophila homolog of the vertebrate

			BMP-1 metalloprotease (Shimell et al., 1991). It may thus be involved in the activation of SCW through the cleaving of SOG, leading to release the heterodimer DPP/SCW by the SOG/DPP/SCW or Sog/DPP/SCW/TSG complexes.TLD cleavage is stimulated by DPP binding. (Aurora et al.,1994; Yu et al., 2000; Eldar et al., 2002)
MadMed	1	[(Tkv:1 | Sax:1) & !Dad:1 & !Tkv:2] | (Tkv:2 & Dad:1)	• PMID:18506030 • PMID:9694800 • http://flybase.org/reports/FBgn0011648.html • http://flybase.org/reports/FBgn0011655.html Activated TKV phosphorylates the mad (MP-specific Smad Mothers against DPP), leading to its association with MED (co-Smad Medea) and to the accumulation of the MADMED complex in the nucleus. MAD and MED-binding sites have been found in the promoters of many DPP responsive genes (Yao et al., 2008). All MED sites can also bind MAD, but some MAD sites do not bind MED sites (Xu et al., 1998).
	2	Tkv:2 & !Dad:1	
Brk	1	!MadMed:1 & !Shn:1	• PMID:11262410 • PMID:11159914 • PMID:11306550 • PMID:16829514 • PMID:16109720 • PMID:12705870 • PMID:15296719 • PMID:9694800 • http://flybase.org/reports/FBgn0024250.html BRK (Brinker) plays essential roles in the regulation of most DPP targets. BRK binds to the enhancers of *dpp* target genes and functions as a constitutive repressor (Kirkpatrick et al., 2001; Rushlow et al., 2001; Saller and Bienz, 2001). This repression is controlled by a SHN/MADMED (SMM) complex that antagonizes transcriptional activation by binding to *brinker* regulatory regions (Gao and Laughon, 2006; Gao et al., 2005; Muller et al., 2003; Pyrowolakis et al., 2004). Thus, DPP regulates its target genes through two mechanisms: (I) directly by activating gene expression; (ii) indirectly by SHN-dependent repression of *brk*. Repression by DPP results in an inverse gradient of BRK throughout development (Yao et al., 2008).
Shn		input	• PMID:12705870 • PMID:16829514 • PMID:16109720 • PMID:15296719 • http://flybase.org/reports/FBgn0003396.html SHN encodes a large protein containing eight zinc fingers (Arora et al., 1995; Grieder et al., 1995; Staehling-Hampton et al., 1995). The C-terminal 600 amino of SHN, including zinc fingers six to eight, is sufficient to repress *brk* transcription in vivo upon DPP signaling (Muller et al., 2003). This repression is mediated by a SHN/MADMED (SMM) complex, which antagonizes transcriptional activation by binding to a GRCGNC(N5)GTCTG motif (Gao and Laughon, 2006; Gao et al., 2005; Muller et al., 2003; Pyrowolakis et al., 2004).

Dad		Input	• PMID:18588885 • PMID:7697720 • http://flybase.org/reports/FBgn0020493.html Daughters against DPP (DAD) is an I-Smad that exclusively inhibits DPP signaling mediated through MAD by blocking its phosphorylation by SAX and TKV. DAD interact with TKV and SAX physically to mediate its inhibition. (Kamiya et al., 2008)
Nej		input	• PMID:14550792 • doi:10.1016/j.ydbio.2007.01.036 • PMID:17336283 • http://flybase.org/reports/FBgn0004396.html Nejire (NEJ) is a transcriptional co-activator that is conserved in metazoans. It acts as a co-activator by bridging interactions between DNA- binding transcription factors and the basal transcription machinery and by affecting the access of factors to DNA through their intrinsic acetyltransferase (AT) activity (Lilja et al., 2003, 2007).
Targets	1	MadMed & Nej:1 & !Brk:1	DPP targets include *optomotor blind* and *spalt* in the imaginal wing disc (Yu et al., 2000), as well as *dorsocross*, *tinman*, *bagpipe* and *eve* in the dorsal mesoderm during specification and diversification.

Logical model of Drosophila JAK/STAT signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for the Drosophila JAK/STAT pathway, displayed from the ligand and receptor at the top, to the main downstream effectors and a generic target node, along with inhibitory and activatory partners at the bottom. Rectangular and ellipsoid nodes denote ternary and Boolean components, respectively. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

In Drosophila, three secreted ligands (OS, UPD2 and UPD3) have been identified for the JAK/STAT pathway. Their binding to the receptor dome induces its homo-dimerization, enabling hop to phosphorylate specific tyrosine residues of the receptor. Consequently, STAT92E is also phosphorylated by HOP, leading to his homo-dimerization and nuclear translocation.
In the nucleus, STAT92E binds to target DNA sequences and acts as an activator of transcription of several target genes (Hou and Perrimon, 1997).
During Drosophila development, the JAK/STAT pathway is involved in embryonic segmentation, eye development, cell growth, haematopoiesis, and sex determination (Luo and Dearolf, 2001; Aaron et al, 2011).
JAK/STAT signalling also plays important roles during spermatogenesis (Kiger et al, 2001) and oogenesis (Denef and Schupbach, 2003; Xi et al, 2003, Aaron et al, 2011).
To study the dynamic of the pathway, we define a set of initial states representative of in vivo situations during JAK/STAT signalling.
More precisely, we define a three initial states corresponding to pathway signalling (binding of OS or UPD2 or UPD3) and two initial states corresponding to pathway signalling in the presence of an inhibitor (SOCS44A or BRWD3) and one initial state corresponding to non signalling conditions (no binding of ligands).

Selected references

- PMID:9066269
- PMID:11746233
- PMID:21965617
- PMID:11752574
- PMID:12747848
- PMID:12586061

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Stat92E	1	Hop:1 & !Su_var & !Ptp61F & !Ken & !Brwd3 & !Socs44A	• PMID:8608595 • PMID:8608596 • http://flybase.org/reports/FBgn0016917.html Signal-transducer and activator of transcription protein at 92E (STAT92E) is the transcriptional effector of the JAK/STAT pathway. Phosphorylated STAT92E homodimers translocate to the nucleus to regulate the expression of specific target genes. STAT92E encodes a 761 amino acid STAT and is the only known STAT protein encoded protein in Drosophila (Hou et al, 1996; Yan et al, 1996).
	2	Hop:2 & !Su_var & !Ptp61F & !Ken & !Brwd3 & !Socs44A	
Dome	1	Upd2 | OS | Upd3	• PMID:11696329 • PMID:12429573 • http://flybase.org/reports/FBgn0043903.html Domeless (or Master Of Marelle) encodes the transmembrane receptor of the JAK/STAT pathway (Brown et al, 2001; Chen et al, 2002). Physical interactions between DOME and OS, as well as the ability of DOME to activate HOP have been demonstrated (Chen et al, 2002).
Socs36E	1	Stat92E:2	• PMID:12101419 • PMID:12204282 • PMID:15488148 • PMID:16055650 • http://flybase.org/reports/FBgn0041184.html SOCS (Suppressors of cytokine signalling) proteins act as negative regulators of the JAK/STAT pathway. Three *socs* genes have been identified in the Drosophila genome (*socs36e*, *socs44a* and *socs16d*). Suppressor of cytokine signaling at 36E (SOCS36E) was shown to suppress the activity of both HOP and STAT92E. *socs36E* gene is itself activated by STAT92E, thus forming a negative feedback loop down-regulating the pathway activity (Callus and Mathey-Prevot, 2002; Karsten et al, 2002; Rawlings et al, 2004; Baeg et al, 2005).
OS		input	• PMID:9784499 • PMID:10346822 • PMID:12967563 • PMID:16277982 • http://flybase.org/reports/FBgn0004956.html Outstretched (OS) is a secreted glycoprotein of 47 kDa, released by heparin (Harrison et al, 1998). Besides OS, two additional ligands (UPD2 and UPD3) have been identified (Castelli-Gair Hombrıa and Brown, 2002). The Drosophila JAK/STAT pathway activity is mediated exclusively by the ligands OS, UPD2 and UPD3 (Harrison et al, 1998; Zeidler et al, 1999b; Agaisse et al, 2003). *os* and *upd2* have overlapping segmental expression patterns during mesoderm development. OS and UPD2 function in a redundant manner (Hombria et al, 2005).

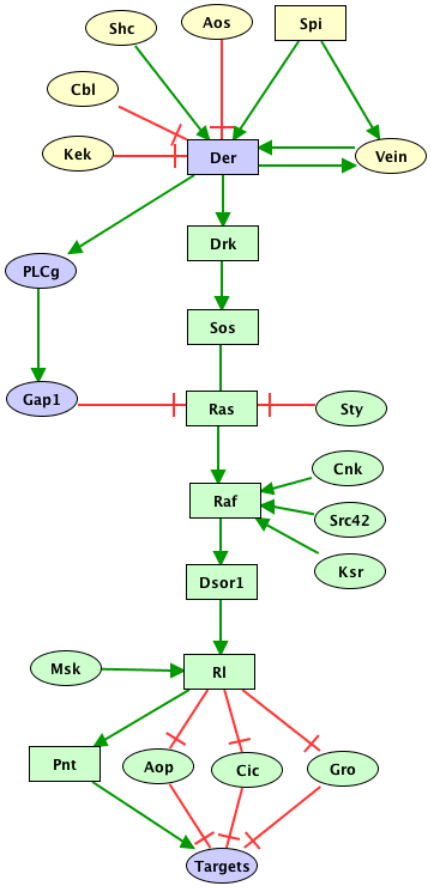
Hop	1	Dome & !ET & [(Stam & Hrs & Socs36E) | !(Stam & Hrs) & !Socs36E)]	• PMID:8626752 • PMID:8608596 • http://flybase.org/reports/FBgn0004864.html The gene *hopscotch (hop)* encodes a receptor-associated to JAK (Binari and Perrimon, 1994). Upon ligand binding, DOME activates HOP, which in turn, phosphorylates STAT92E dimers (Stancato et al, 1996). HOP can phosphorylate STAT92E at tyrosine residue 711, which is required for STAT92E DNA binding activity (Yan et al, 1996).
	2	Dome & !ET & !Socs36E & Stam & Hrs	
Upd2		input	• PMID:16277982 • PMID:15925495 • http://flybase.org/reports/FBgn0030904.html Unpaired 2 (UPD2) is a second pathway activator that functions redundantly to OS (Castelli-Gair Hombria et al, 2005; Gilbert et al, 2005).
Upd3		input	• PMID:16277982 • PMID:12967563 • http://flybase.org/reports/FBgn0053542.html Unpaired 3 (UPD 3) is expressed in the developing gonads (Castelli-Gair Hombría et al, 2005), the larval lymph gland, and in circulating haemocytes following septic injury (Agaisse et al, 2003). UPD3 is the third JAK/STAT pathway activator.
Su_var		input	• PMID:12077349 • PMID:12855578 • PMID:14607831 • PMID:11390354 • PMID:20616536 • http://flybase.org/reports/FBgn0003585.html PIAS proteins (protein inhibitors of activated STAT) represent another well-characterized group of pathway suppressors (like SOCS) that bind to STATs and target them for degradation via sumoylation (Kotaja et al, 2002; Ungureanu et al, 2003; Wormald and Hilton, 2004; Gronholm et al, 2010). A single Drosophila PIAS gene (*pias*), also called *su(var)2-10* (*suppressor of variegation 2-10*) and *zimp*, has been identified. Drosophila PIAS is found in the cytoplasm and nucleus (Hari et al, 2001).
Brdw3		input	• PMID:16094372 • http://flybase.org/reports/FBgn0011785.html D40- and Bromo-domain-containing protein (BRWD3) is a large protein that strongly suppresses the transcription of *stat92e* (Muller et al, 2005).
Socs44A		input	• PMID:12101419 • PMID:12204282 • PMID:15488148 • PMID:16055650 • http://flybase.org/reports/FBgn0033266.html SOCS (Suppressors of Cytokine signalling) factors act as negative regulators of the JAK/STAT pathway. Three SOCSs genes have been identified in the Drosophila genome (*socs36e*, *socs44a* and *socs16d*). Although SOCS44A (Suppressor of cytokine signaling at 44a) does not show JAK/STAT-dependent expression, it can inhibit pathway activity (Rawlings et al, 2004).

NStat92E		input	• PMID:8608596 • PMID:12231627 • PMID:16129580 STAT92E (Signal-transducer and activator of transcription protein at 92E) effects can be counteracted by naturally occurring N-terminally truncated STAT92E protein (Yan et al, 1996; Henriksen et al, 2002). When ectopically expressed, NSTAT92E exerts a dominant-negative effect on the expression of the JAK/STAT target genes, such as *even-skipped* or *trachealess* (Henriksen et al, 2002; Karsten et al, 2005).
Targets	1	Stat92E & !NStat92E	• PMID:8314084 • PMID:9066269 • PMID:19217429 Embryos mutant for HOP, STAT92E, DOME or OS exhibit characteristic segmentation and gastrulation defects. Gap genes, pair-rule genes, and segment polarity genes are targets of JAK/STAT pathway (*eve, runt,...*) (Binari and Perrimon, 1994; Hou et al, 1997). *tinman* is also a target gene of JAK/STAT pathway (Liu et al, 2009).
Ptp61F		input	• PMID:16055650 • PMID:16094372 • PMID:15710397 • http://flybase.org/reports/FBgn0003138.html Protein tyrosine phosphatase 61F (PTP61F) (homologue of human PTPB1, for phospho-Tyr phosphatase B1) acts as a suppressor of STAT92E-dependent transcription (Baeg et al, 2005; Müller et al, 2005). PTP61F is expressed in a pattern complementary to that of OS. A nuclear spliced form of PTP61F (PTP61FC) can affect pathway activity in vivo downstream of HOP (Muller et al, 2005). PTP61FC probably acts at the level of STAT92E. (Zi et al, 2005).
Ken		input	• PMID:16401426 • http://flybase.org/reports/FBgn0011236.html Ken and Barbie *(*KEN) (homolog of human BCL6, for B-cell lymphoma 6) belongs to the family of BTB/POZ domain containing transcriptional repressors and functions as a transcriptional repressor in competition with STAT92E. During Drosophila development, KEN is sufficient to down-regulate the expression of a subset of putative JAK/STAT target genes (incomplete overlap between consensus sequences bound by KEN and STAT92E) (Arbouzova et al, 2006).
ET		input	• PMID:20624926 • http://flybase.org/reports/FBgn0031055.html Eye Transformer (ET) is a negative regulator of the JAK/STAT pathway in Drosophila. It functions as a regulator of STAT92E phosphorylation upstream of the receptor dome. It has putative cytokine binding motifs and could function as a receptor that captures OS, UPD2 and UPD3 ligand from DOME. It presumably inhibits DOME activation by forming a

			non-signalling heterodimer with dome, or by inhibiting DOME homodimer/HOP signalosome. Et is involved in STAT92E phosphorylation and co-precipitates with DOME and HOP (Kallio et al, 2010).
Stam		input	• PMID:10231582 • PMID:10851045 • PMID:11728436 • PMID:12972556 • http://flybase.org/reports/FBgn0027363.html Signal transducing adaptor molecule (STAM) associates with Hrs (Hepatocyte growth factor regulated tyrosine kinase substrate) protein. The resulting complex increases JAK/STAT signalling (Mesilaty- Gross et al, 1999; Bromberg and Darnell, 2000; Lohi and Lehto, 2001; Mizuno et al, 2003).
Hrs		input	• PMID:10231582 • PMID:10851045 • PMID:11728436 • http://flybase.org/reports/FBgn0031450.html HRS (Hepatocyte growth factor regulated tyrosine kinase substrate) protein associates with the protein STAM to increase JAK/STAT pathway signalling (Mesilaty- Gross et al, 1999; Bromberg and Darnell, 2000; Lohi and Lehto, 2001; Mizuno et al, 2003).

Logical model of Drosophila EGF signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila EGF pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Rectangular and ellipsoid nodes denote ternary and Boolean components, respectively. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

Four activating ligands, Spitz, Keren, Gurken and Vein have been identified for drosophila EGF receptors, called DER. Spitz (SPI) is the major ligand and is involved in most situations where the pathway is activated. Keren plays a minor, redundant role, while Gurken is used exclusively during oogenesis.

These ligands are produced as inactive transmembrane precursors, which are retained in the endoplasmic reticulum and needed to processed by the chaperone protein Star. Processed ligands are directed into another compartment where they encounter Rhomboid (RHO) serine proteases, which cleave the ligand precursors within the transmembrane domain to release the active, secreted ligand form. RHO also cleaves and inactivates Star, attenuating the level of cleaved ligand that is produced. The fourth ligand, Vein, is produced as a secreted molecule, which is a weaker activating ligand used either to enhance signalling by other ligands or in specific situations such as muscle patterning. Binding of ligands to DER leads to dimerization and triggering of the canonical DRK/SOS/RAS/RAF/DSOR1/Rolled pathway. DRK (SH2-domain-containing protein) recruits

SOS (Son of sevenless, a guanine nucleotide exchange factor) to catalyze the exchange of RAS bound GDP for GTP exchange, thereby activating RAS. RAS then promotes the activation of RAF, leading to DSOR1 activation, and eventually to Rolled (RL) activation. RL inactivates transcriptional co-repressors, such as Aop, and activates transcription factors, such as Pointed (PNT) (O'Neill et al., 1994; Brunner et al., 1994). The transcriptional activator PNT is a the major effector of the pathway. The protein Anterior open (AOP) is a constitutive repressor, which competes for PNT binding sites and can be removed from the nucleus and degraded upon phosphorylation by MAP kinases.

AOS (Argos), STY (Sprouty) and KEK (Kekkon) are inducible repressive elements involved in negative feedbacks. AOS is a secreted molecule, which sequesters the ligand SPI (Spitz). STY acts downstream of DER, but upstream of RAS and RAF, by recruiting GAP1 and blocking the ability of DRK to bind to its positive effector. KEK is a transmembrane protein forming a non-functional heterodimer with the receptor.

Constitutively expressed, CBL (E3 ligase) modulates DER signalling by recognizing activated, internalized receptor molecules and inducing their ubiquitination and degradation. CBL may also enhance the endocytosis of DER, following ligand binding. Modulation of DER signalling by CBL has been reported only in the follicle cells, which receive the Gurken signal from the oocyte (Levkowitz et al., 1999; Yokouchi et al., 1999; Waterman et al., 2000; Pai et al., 2000).

Our logical model represents a cell receiving different combinations of ligands binding (SPI or Vein or both) and express/receive different levels of inhibitory inputs (Aos, Sty, Cbl, Kek). The signalling pathway is characterized by no signalling, medium or high signalling process designed by multi-valued nodes.

We consider five main wild-type cellular situations:

i. Cells secreting ligands but lacking Der activation (inhibition of Der), leading to no signalling.
ii. Cells receiving medium signal with SPI expressed at level 1 and/or Vein expressed also at level 1, leading to medium signalling.
iii. Cells receiving SPI at level of expression 1 (and/or Vein expressed at level 1) and in presence of an inhibitor (e.g. STY, AOS, or KEK), leading to no signalling.
iv. Cells receiving SPI at level of expression 2 in the absence of inhibitors, leading to high signalling.
v. Cells receiving SPI at level of expression 2 (and/or Vein expressed at level 1) in presence of an inhibitor (e.g. STY, AOS, or KEK,...), leading only to medium signalling.

Selected references

- PMID:7559490
- PMID:9182757
- PMID:9809073
- PMID:8033205
- PMID:8047146

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Aos		input	• PMID:1606617 • PMID:7651519 • PMID:1576953 • PMID:8565833 • PMID:9367443 • PMID:12648473 • PMID:16123311 • PMID:18369317 • PMID:8026629 • PMID:8812109 • http://flybase.org/reports/FBgn0004569.html AOS (Argos) is a secreted protein containing an EGF-like domain (Freeman et al., 1992), which inhibits DER stimulation by activating ligands such as SPI (Schweitzer et al 1995; Wasserman and Freeman, 1998). AOS is induced in response to DER signalling in the cells receiving high levels of DER activation, and plays a major role in restricting the activation range of the activating ligands (Golembo et al., 1996; Queenan et al., 1997). AOS is secreted and reaches several cell rows away from the site of production. It maintains a steady-state level of signalling such that the cells receiving maximal levels of SPI maintain DER activation, in spite of the production of AOS, while in the cells further away from the source, AOS attenuates activation by SPI (Shiloh et al., 2003, 2005; Yogev et al., 2008). AOS and Kekkon (another inhibitor), are expressed in exactly the same pattern (Sawamoto et al., 1994; Musacchio and Perrimon, 1996).
Cbl		input	• PMID:11051547 • PMID:15282549 • http://flybase.org/reports/FBgn0020224.html CBL is an E3 ligase that recognizes the activated, endocytosed DER and induces its ubiquitination and degradation. CBL may also enhance the endocytosis of DER, following ligand binding. Although CBL is broadly expressed, it only modulates DER signalling in the follicle cells, which receive the Gurken (another ligand) signal from the oocyte. In *cbl* mutant cells, DER is hyper-activated, leading to the repression of genes such as *pipe* (Pai et al., 2000; Gur et al., 2004).
Cnk		input	• PMID:9814705 • PMID:10860999 • PMID:16326394 • PMID:14517245 • PMID:15660123 • PMID:16600911 CNK (Connector Enhancer of KSR) is required upstream of RAF (Therrien et al., 1998) for the control various processes, including cell proliferation/survival, differentiation and migration (Therrien et al., 1998; Baonza et al., 2000; Cabernard and Affolter, 2005).

			CNK directly associates with the kinase domain of RAF via a short amino-acid sequence, called the RAF-interacting motif (RIM), and modulates RAF activity according to the EGF signalling status (Douziech et al., 2003; Laberge et al., 2005). When there is no signalling, the binding of CNK to RAF is inhibited by a second motif adjacent to the RIM, called the inhibitory sequence (IS). When the pathway is activated, CNK integrates SRC42 and RAS activities, leading to RAF activation (Laberge et al., 2005).
Der	1	[(Spi:1 | Vein) & !Aos:1 & !Kek:1 & !Cbl & !Spi:2 & Shc] | [Spi:2 & Shc & (Kek:1 | Aos:1 | Cbl)]	• PMID:2515109 • PMID:1820687 • PMID:1576953 • PMID:18369317 • http://flybase.org/reports/FBgn0003731.html DER (Drosophila Epidermal growth factor Receptor homolog), has been shown to fulfill multiple roles during development. In the embryo, it plays a role in the establishment of ventral ectodermal fates and differentiation of the midline glial cells (Raz and Shilo, 1991, 1992), Malpighian tubule development (Baumann and Skaer, 1993), germ-band retraction, and head development (Schejter and Shilo, 1989; Clifford and Schiipbach 1990). During imaginal disc development, DER was shown to be essential for the proliferation of disc cells (Clifford and Schfpbach, 1990), vein and bristle formation in the wing disc (Diaz-Benjumea and Garcia-Bellido, 1990), and the differentiation of photoreceptors in the eye disc (Baker and Rubin, 1989; Xu and Rubin, 1993). Four activating ligands (Spitz, Keren, Gurken, Vein) and one inhibitory ligand (Kekkon) allow versatile combinations of DER activation (Shiloh et al., 2003).
	2	Spi:2 & !Kek:1 & !Aos:1 & !Cbl & Shc	
Drk	1	Der:1 & !Der:2	• PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0004638.html DRK is an adaptor molecule. Drk recruits the guanine nucleotide exchange factor SOS to catalyze the exchange of GDP bound to RAS for GTP, thereby creating active RAS (Cabrita et al., 2003; Yan et al., 2009).
	2	Der:2	
Dsor1	1	Raf:1 & !Raf:2	• PMID:16600911 • http://flybase.org/reports/FBgn0010269.html Dsor1 is known to phosphorylate RL. Phosphorylated RL can then enter in the nucleus.
	2	Raf:2	
Gap1	1	PLCg:1 | PLCg:2	• PMID:1898771 • PMID:10089881 • http://flybase.org/reports/FBgn0004390.html GAP proteins stimulate the hydrolysis of GTP bound to RAS, thereby converting RAS into the GDP-bound, inactive state (Bourne et al., 1991). By recruiting GAP1 and/or blocking the ability of DRK to bind to its positive effectors, Sprouty prevents the formation of functional signalling complexes associated with the cytoplasmic domain of receptor tyrosine kinases (Casci et al., 1999).
Kek		input	• PMID:12648473 • PMID:10102272

			• PMID:8026629 • PMID:8812109 • http://flybase.org/reports/FBgn0015399.html • http://flybase.org/reports/FBgn0015400.html • http://flybase.org/reports/FBgn0028370.html KEK (Kekkon) is a transmembrane inhibitory protein that binds DER extracellular domain and attenuates receptor dimerization (Ghiglione et al., 1999; Shiloh et al., 2003). AOS and KEK, are expressed in exactly the same pattern (Sawamoto et al., 1994; Musacchio and Perrimon, 1996).
Ksr		input	• PMID:8521512 • PMID:11141565 • PMID:16326394 KSR facilitates the phosphorylation of RAS and RL by RAF and thereby enhance RL activity, which can target both nuclear and non-nuclear substrates.
Msk		input	• PMID:9214382 • PMID:10228156 • PMID:11262240 Moleskin (Msk) is a member of the importin superfamily of nuclear importers, which can bind directly to the nuclear pore complex (Gorlich et al., 1997; Jakel et al., 1999). Msk physically binds Drosophila RL (Lorenzen et al., 2001).
PLCg	1	Der:1	• PMID:9811587 • PMID:19884307 • http://flybase.org/reports/FBgn0003416.html PLCg (enzyme Phospholipase C) plays a crucial, inhibitory role in the transduction of DER signalling (Thackeray et al., 1998; Salzer et al., 2010).
Pnt	1	Rl:1	• PMID:8223245 • PMID:8047146 • PMID:8033205 • PMID:9154002 • PMID:12648473 • PMID:16123311 • PMID:18369317 • PMID:16600911 • PMID:19884307 • http://flybase.org/reports/FBgn0003118.html The *pnt* locus encodes two distinct protein isoforms: PntP1 and PntP2, here represented by a single component (PNT). The *pnt* gene is a nuclear target of the signalling cascade acting downstream of RL (Rolled). Both isoforms act as effectors of the Ras/MAP kinase pathway in multiple developmental contexts (e.g. eye, neural cells and the midline glial cells). (Klambt et al., 1993; Brunner et al., 1994; O'Neill et al., 1994; Roignant et al., 2006; Yogev et al., 2008; Salzer et al., 2010).
	2	Rl:2	
Raf	1	Ras:1 & !Ras:2 & Cnk & Src42 & Ksr	• PMID:16600911 • http://flybase.org/reports/FBgn0003079.html RAF is a critical effector of the RAS. The phosphorylation and activation of RAF by RAS
	2	Ras:2 & Cnk & Src42 & Ksr	

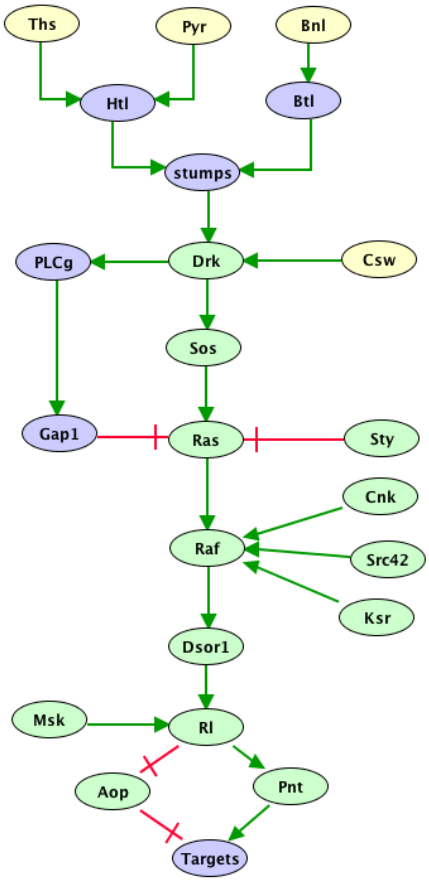
			occurs in the presence of the scaffold protein KSR (Kinase suppressor of RAS).
Ras	1	Sos:1 & !(Sty:1 & Gap1) Gap1:1 & Sty:1 & Sos:2	• PMID:8978043 • PMID:8951053 • PMID:8824589 • PMID:7749324 • PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0003204.html Raspberry (RAS) functions downstream of DER to establish follicular cell fate during oogenesis (Schnorr et al., 1996; Golembo et al., 1996; Schnepp et al., 1996). RAS is a molecular switch, cycling between an inactive GDP-bound and active GTP-bound form. RAS is activated by upstream receptor tyrosine kinases (RTKs) upon ligand binding following the recruitment of SOS by DRK. RAS promotes the activation of RAF (Pole hole), DSOR1 and eventually of RL (Cabrita et al., 2003; Yan et al., 2009).
	2	Sos:2 & !(Sty:1 & Gap1)	
Rl	1	Dsor1:1 & !Dsor1:2 & Msk	• PMID:16600911 • http://flybase.org/reports/FBgn0003256.html • http://www.sdbonline.org/fly/torstoll/mapkin1.htm Rolled (RL) is essential to the proper functioning of the RAS signalling pathway. After phosphorylation by DSOR1 and translocation in the nucleus, RL phosphorylates PNT, enabling it to activate pathway target genes.
	2	Dsor1:2 & Msk	
Shc		input	• PMID:7651398 • PMID:10882065 SHC is required for DER signalling in the eye, wing, and ovary. In the absence of SHC protein, DER signalling is only partially reduced. *shc* is widely expressed throughout the embryo and specifically binds to DER (Lai et al., 1995; Luschnig, 2000).
Sos	1	Drk:1 & !Drk:2	• PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0001965.html SOS (Son of sevenless) activates RAS. Upon ligand binding, DER auto-phosphorylates and recruit adaptor molecules, such as DRK, which in turn recruits SOS to the signalling complex (Cabrita et al., 2003; Han et al., 2009).
	2	Drk:2	
Spi		input	• PMID:9154002 • PMID:12648473 • PMID:16123311 • PMID:18369317 • http://flybase.org/reports/FBgn0005672.html The primary DER activating ligand is SPI (Spitz). SPI is responsible for DER activation in most tissues. SPI is produced as an inactive membrane precursor and is ubiquitously expressed. The active secreted form of Spitz is produced by tightly regulated cleavage of the membrane-bound precursor.

			Even when expressed at high levels, the precursor form is inactive. The spatial and temporal pattern of DER activation thus depends on the regulated processing of SPI. SPI precursor is normally retained in the endoplasmic reticulum, before trafficking from the endoplasmic reticulum to the Golgi compartment. This step is carried out by STAR. Once reaching the Golgi, SPI encounters RHO (Rhomboid), which is essential for SPI cleavage. The catalytic domain of RHO resides within its conserved transmembrane domains, giving rise to regulated intra-membrane proteolysis. The cleavage of SPI by RHO presumably takes place within the Golgi. (Schweitzer and Shilo, 1997; Shiloh et al., 2003, 2005; Yogev et al., 2008)
Sty		input	• PMID:15173823 • PMID:10089881 • PMID:10457022 • PMID:14515177 • PMID:16123311 • http://flybase.org/reports/FBgn0014388.html STY (Sprouty) exerts its inhibitory effect on receptor tyrosine kinase (RTK) signalling by intercepting essential elements of the RAS/MAPK cascade through diverse mechanisms (Kim and Bar-Sagi, 2004). STY is an intracellular protein, associated with the inner surface of the plasma membrane. It acts downstream of DER, but upstream of RAS and RAF, by recruiting GAP1 and blocking the ability of DRK to bind to its positive effectors. An intriguing aspect of the inhibitory function of Sty is that its expression depends on pathway activity, implying a negative feedback loop. (Casci et al., 1999; Reich et al., 1999; Cabrita et al., 2003; Shilo et al., 2005).
Src42		input	• PMID:2996778 • PMID:8682295 • PMID:15660123 Src42 interacts with CNK and contribute to RAF activation (Simon et al., 1985; Takahashi et al., 1996, Laberge et al., 2005).
Vein	1	Spi & Der	• PMID:9925640 • PMID:12169631 • PMID:12648473 • pmid:18369317 • http://flybase.org/reports/FBgn0003984.html VN (Vein) is a secreted ligand with relatively weak activation capacity, used in tissues where low activation levels are required. Vein is expressed in a highly dynamic pattern in the embryo and larval imaginal discs (Golembo et al., 1999; Reich et al., 2002; Shiloh et al., 2003). Analysis of *vein* loss-of-function phenotypes reveals two different modes of activity. Vein functioning independently of Spi. For example, in the embryo, the migrating muscle fibers approach the ectodermal muscle attachment (EMA) cells, produce Vein, and activate DER on the EMA cells. Vein functions in synergy with SPI, such that the

			combined activity of both ligands gives rise to proper activation of the receptor. Vein can activate DER even in the presence of AOS, thus balancing the parallel negative-feedback circuit involving AOS (Golembo, 1999; Reich et al., 2002; Shiloh et al., 2003, 2005; Yogev et al., 2008).
Aop	1	!Rl	• PMID:7781063 Anterior open (Aop) inhibits RTK targets genes. Direct phosphorylation of the transcriptional co-repressor AOP by RL leads to its export from the nucleus and subsequent ubiquitin-mediated protein degradation (Rebay and Rubin, 1995).
Gro	1	!Rl	• PMID:15592470 *groucho* is a neurogenic gene and member of the Enhancer of split complex (E[spl]-C). During Drosophila development, GRO is ubiquitously expressed. In RTKs signalling, it has a repressor activity and functions in combination with CIC. However, the phosphorylation of GRO in response to EGF activation weakens its repressor capacity.
Cic	1	!Rl	• PMID:15592470 • PMID:10652276 • PMID:21270056 • PMID:22526417 Capicua (CIC) has been conserved widely during evolution (from C. elegans to humans). Several experimental studies suggest that CIC interacts with GRO through the formation of a protein complex. This CIC/GRO complex acts as a repressor of RTKs pathway (EGF and TOR pathway). It functions by repressing EGF targets. When phosphorylated by RL, CIC/GRO repression capacity is weakened.
Targets	1	(Pnt:1 | Pnt:2) & !Aop & !(Cic & Gro)	• PMID:20463031 • PMID:16963016 • PMID:15084467 • PMID:12485984 • PMID:12183378 The EGF pathway is involved in the development of the mesoderm, the specification of muscle, epithelial cell adhesion, in ventral epidermis development. The pathway is also implicated in the development of the wing.

Logical model of Drosophila FGF signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila FGF pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

Drosophila genome encodes two FGF receptors, HTL (Heartless) and BTL (Breathless), which are required for the morphogenesis of different tissues.
BTL is expressed in the tracheae, while HTL is expressed in embryonic mesoderm and was first identified because of its essential role in heart development.
BTL ligand, BNL is encoded by the *branchless* gene (Sutherland et al 1996).
THS (Thisbe) and PYR (Pyramus) function in a partially redundant fashion to activate *heartless (htl)*.
Upon ligand binding receptor dimerization triggers the canonical DRK/SOS/RAS/RAF/DSOR1/RL pathway.
In contrast with other RTKs, Stumps is needed to trigger signal transduction (see Klint et al, 1995; Kouhara et al, 1997).
Stumps is a cytoplasmic protein expressed in cells also expressing the FGF receptors.
The presence of an ankyrin repeat, a coiled-coil structure and many tyrosines suggests that

Stumps could bind SH2 domains of proteins such as DRK or CSW (Vincent et al 1998).
As a result, DRK recruits the guanine nucleotide exchange factor SOS (Son of sevenless), which catalyzes the exchange of GDP bound to RAS for GTP.
Activated RAS then promotes the activation of RAF (Pole hole), DSOR1, and eventually that of RL (Rolled).
RL can activate transcription through the inactivation of transcriptional co-repressors such as Anterior Open (AOP), as well as through the activation of transcriptional activators such as PNT (Pointed, with two forms denote by suffixes P1 and P2) (O'Neill et al, 1994; Brunner et al, 1994).
The negative regulator STY (Sprouty) acts downstream of SOS but upstream of RAS and RAF, by recruiting GAP1 and blocking the ability of DRK to bind to its positive effector.
Our model enables the simulation of pathway responses to different ligand combinations.
In this regard, we define four initial states to simulate different behavior of the pathway. The first initial state reproduces the signalling through the receptor HTL (bound by Pyr and Ths), the second initial state corresponds to the signalling through the receptor BTL, the third initial state corresponds to the involvement of the inhibitor Sprouty during signalling conditions and the fourth initial state corresponds to the absence of signalling (no ligands binding). Each of these initial states lead to a specific stable state representative of *in vivo* conditions.

Selected references

- PMID:8978613
- PMID:7559490
- PMID:7784079
- PMID:9809073
- PMID:8033205
- PMID:8047146

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Htl	1	Ths:1 | Pyr:1	• PMID:15075295 • PMID:15634694 • PMID:9342046 • PMID:9621429 • PMID:9187139 • http://flybase.org/reports/FBgn0010389.html HTL (Heartless, Drosophila FGF receptor) is initially expressed throughout the mesoderm of early embryos. Proper spreading of the mesoderm depends on FGF signalling. A signal from the ectoderm triggers the activity of HTL in the mesoderm, resulting in the activation of MAPK (Stathopoulos et al, 2004; Wilson et al, 2004; Gabay et al, 1997). Still in the mesoderm, FGF signalling provides a differentiation signal for heart cell precursors at the dorsal edge of the ectoderm (Michelson et al, 1998). In *htl* loss-of-funciton mutants, there is no spreading of the mesoderm (Shishido et al, 1997).
Bnl		input	• PMID:8978613 • PMID:12062107 • PMID:12194851 • http://flybase.org/reports/FBgn0014135.html BNL (Branchless) is an homolog of FGF essential for the morphogenesis of the trachea, air sacs, and male genital imaginal disc (Sutherland et al, 1996; Ahmad and Baker, 2002; Sato and Kornberg, 2002). During embryogenesis, BNL is expressed in a highly dynamic fashion in discrete epithelial cells of developing embryos.
Btl	1	Bnl:1	• PMID:8978613 • doi:10.1242/dev.01603 • PMID:14993266 • http://flybase.org/reports/FBgn0005592.html BNL binding activates drosophila BTL (Breathless) FGF receptor and thereby controls the movement (branching) of the trachea (Sutherland et al, 1996). BTL is also important for the specification of a subset of the tracheal cell types (Wilson et al, 2004).
Stumps	1	Btl:1 | Htl:1	• PMID:9778498 • PMID:9809073 • PMID:12767830 • PMID:15082772 • PMID:15634694 • http://flybase.org/reports/FBgn0020299.html The intracellular protein Stumps (also called Heartbroken or Stumps) is essential for signal transduction by the Drosophila FGF receptors (Michelson et al, 1998; Vincent et al, 1998). Stumps physically interacts with the receptor (Battersby et al, 2003; Petit et al, 2004; Wilson et al, 2004). Stumps protein is only present in cells that express FGF receptors.
Pyr		input	• PMID:15075295 • http://flybase.org/reports/FBgn0033649.html THS (Thisbe) and PYR (Pyramus) are two FGF signalling molecules that presumably function in a redundant fashion to activate Htl (Heartless).

			The two genes exhibit dynamic expression patterns in tissues that influence the development of different mesoderm lineages, including the neurogenic ectoderm (early mesoderm spreading), muscle precursors (dorsal muscles, visceral muscles, and heart), hindgut (visceral musculature), and neuroblasts. The combination of *ths* and *pyr* expression profiles may produce a dynamic FGF activity gradient within the neurogenic ectoderm, guiding the spreading of the mesoderm into the dorsal ectoderm. Mutant embryos lacking both *ths* and *pyr* exhibit defects that are quite similar to those seen in *htl* mutants, including a delay in mesoderm spreading during gastrulation, a reduction in dorsal mesoderm lineages, the loss of pericardial and cardial cells, the absence of hindgut musculature, and disruptions in the ventral oblique muscles. Finally, expression of activated HTL or THS can rescue the loss of dorsal mesoderm lineages in mutant embryos.
Ths		input	• PMID:15075295 • http://flybase.org/reports/FBgn0033652.html For biological information see annotations for PYR.
Aop	1	!Rl:1	• PMID:7781063 • http://flybase.org/reports/FBgn0000097.html AOP (Anterior open) inhibits RTK targets genes. Direct phosphorylation of the transcriptional co-repressor AOP leads to its export from the nucleus and subsequent ubiquitin-mediated protein degradation (Rebay and Rubin et al, 1995).
PLCg	1	Drk:1	• PMID:9811587 • PMID:19884307 • http://flybase.org/reports/FBgn0003416.html PLCg (enzyme Phospholipase C) plays a crucial, inhibitory role in the transduction of FGF signalling (Thackeray et al, 1998; Salzer et al, 2010).
Sos	1	Drk:1	• doi:10.1160/TH03-04-0217 • PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0001965.html SOS (Son of sevenless) is a guanine nucleotide-releasing factor that activates RAS, which subsequently recruits the protein kinase RAF to the plasma membrane (Cabrita et al, 2003; Han et al, 2009).
Pnt	1	Rl:1	• PMID:9154002 • PMID:12648473 • PMID:16123311 • PMID:18369317 • PMID:16600911 • PMID:19884307 • http://flybase.org/reports/FBgn0003118.html Drosophila FGF target genes are activated by phosphorylated PNTP2 Schweitzer and Shilo, 1997; Shiloh et al, 2003, 2005; Roignant et al, 2006; Yogev et al, 2008, Salzer et al, 2010.
Rl	1	Dsor1:1 & Msk	• PMID:16600911 • http://flybase.org/reports/FBgn0003256.html RL (Rolled) kinase is essential for the proper functioning of RAS signalling pathway. After phosphorylation by DSOR1 and translocation in the nucleus, RL phosphorylates PNT.

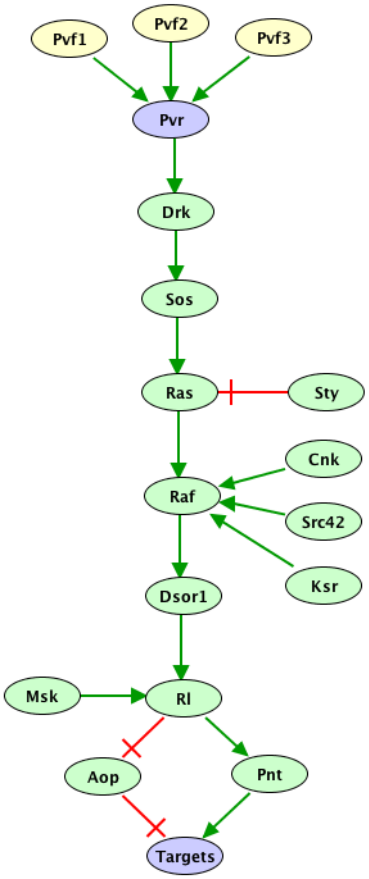
Drk	1	Stumps:1 & Csw	• PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0004638.html DRK (Downstream of receptor kinase) is the homolog the adaptor molecule of Grb2. Normally, RAS can be activated by upstream receptor tyrosine kinases (RTKs) upon ligand binding when DRK recruits the guanine nucleotide exchange factor SOS, which catalyzes the exchange of GDP bound to RAS for GTP, thereby creating active RAS (Cabrita et al, 2003; Yan et al, 2009).
Ras	1	Sos:1 & !Sty:1 & Gap1:1 Sos:1 & Sty:1 & !Gap1:1 Sos:1 & !Sty:1 & !Gap1:1	• PMID:8978043 • PMID:8951053 • PMID:8824589 • PMID:9778498 • PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0003204.html RAS is a molecular switch, cycling between an inactive GDP-bound and active GTP-bound form. RAS can be activated by upstream receptor tyrosine kinases (RTKs) upon ligand binding following the recruitment of SOS by DRK. RAS promotes the activation of RAF (Pole hole), DSOR1 and eventually of RL (Rolled). (Cabrita et al, 2003; Yan et al, 2009)
Targets	1	Pnt:1 & !Aop:1	• PMID:11832242 • PMID:11141565 An example of targets of FGF pathway during mesoderm development is the gene *eve*. BTL and Stumps are targets of PNT in Epithelial Branching Morphogenesis.
Gap1	1	PLCg:1 PLCg:2	• PMID:1898771 • PMID:10089881 • http://flybase.org/reports/FBgn0004390.html GTPase-activating protein 1 (GAP) protein stimulates the hydrolysis of GTP bound to RAS, thereby converting RAS into the GDP-bound, inactive state (Bourne et al, 1991). By recruiting GAP1 and/or blocking the ability of DRK to bind to its positive effectors, STY (Sprouty) prevents the formation of functional signalling complexes associated with the cytoplasmic domain of receptor tyrosine kinases (Casci et al, 1999).
Dsor1	1	Raf:1	• PMID:16600911 • http://flybase.org/reports/FBgn0010269.html Downstream of RAF1 (DSOR1) is the kinase which phosphorylates RL , which can then enters in the nucleus.
Sty		input	• PMID:15173823 • PMID:10089881 • PMID:10457022 • PMID:14515177 • PMID:16123311 • http://flybase.org/reports/FBgn0014388.html STY (Sprouty) inhibits receptor tyrosine kinase (RTK) signalling by intercepting essential elements of the RAS/RAF cascade through diverse mechanisms (Kim and Bar-Sagi, 2004). STY is an intracellular protein, associated with the inner surface of the plasma membrane.

Raf	1	Ras:1 & Src42 & Cnk & Ksr	• PMID:16600911 • http://flybase.org/reports/FBgn0003079.html Pole hole (RAF) is a critical effector of the small GTPase RAS in cells. GTP-RAS activates the kinase RAF. This initiates a kinase cascade in which RAF phosphorylates DSOR1 in the presence of KSR (scaffold protein Kinase Suppressor of RAS),
Csw		input	• PMID:15082772 • PMID:15634694 • http://flybase.org/reports/FBgn0000382.html CSW (Corkscrew) is important for the FGF-dependent formation of heart precursors and the development of the tracheal system (Gabay et al 1997; Johnson Hamlet and Perkins, 2001; Perkins et al, 1996). Stumps is likely involved in the recruitment of CSW to the signalling complex (Petit et al, 2004; Wilson et al, 2004).
Cnk		input	• PMID:9814705 • PMID:10860999 • PMID:16326394 • PMID:14517245 • PMID:15660123 • PMID:16600911 • http://flybase.org/reports/FBgn0021818.html CNK (Connector Enhancer of KSR) is required upstream of RAF (Therrien et al., 1998) for the control of various processes, including cell proliferation/survival, differentiation and migration (Therrien et al, 1998; Baonza et al, 2000; Cabernard and Affolter, 2005). CNK directly associates with the kinase domain of RAF via a short amino-acid sequence, called the RAF-interacting motif (RIM), and modulates RAF activity according to the FGF signalling status (Douziech et al., 2003; Laberge et al., 2005). Without FGF signal, CNK-bound RAF is inhibited by a second motif adjacent to the RIM, called the inhibitory sequence (IS). Upon FGF activation, CNK integrates SRC42 and RAS activities, which then lead to RAF activation.
Src42		input	• PMID:2996778 • PMID:8682295 • PMID:15660123 • http://flybase.org/reports/FBgn0264959.html SRC oncogene at 42A (SRC42) interacts with CNK and contributes to RAF activation (Simon et al, 1985; Takahashi et al, 1996, Laberge et al, 2005).
Msk		input	• PMID:9214382 • PMID:10228156 • PMID:11262240 • http://flybase.org/reports/FBgn0026252.html Moleskin (MSK) is a member of the importin superfamily of nuclear importers, which can bind directly to the nuclear pore complex (Gorlich et al, 1997; Jakel et al, 1999). MSK is tyrosine phosphorylated in response to growth factor stimulation of FGF signalling and physically binds Drosophila RL (Lorenzen et al, 2001). MSK is a General RL Nuclear Import Factor.
Ksr		input	• PMID:8521512 • PMID:11141565 • PMID:16326394

			• PMID:7559490 • PMID:9182757 • PMID:9809073 • PMID:8033205 • PMID:8047146 • http://flybase.org/reports/FBgn0015402.html KSR (Kinase suppressor of RAS) facilitates the phosphorylation of DSOR1 and RL by RAF, and thereby enhance RL activity, which can target both nuclear and non-nuclear substrates.

Logical model of Drosophila VEGF signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila VEGF pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

VEGF (also called PDGF or PVF) pathway participates in different developmental processes, including border cell migration, hemocyte migration and survival, thorax closure during metamorphosis, the rotation and dorsal closure of the male terminalia. and embryonic salivary gland tissue migration.
The ability of PVR to activate the MAP-kinase pathway is important for control of cell growth and differentiation in other tissues.
Three genes in the Drosophila genome code for PVR ligands: PVF1, PVF2, and PVF3.
Binding of one of the ligands (PVF1, 2 or 3) to the receptor PVR triggers the canonical DRK/SOS/RAS/RAF/DSOR1/RL pathway (Klint et al, 1995; Kouhara et al, 1997).
DOF is needed to assemble the PVR receptor and allow it to auto-phosphorylate, likely as an adaptor that links the receptor to RAS pathway.
DOF is a cytoplasmic protein which is expressed ubiquitously only in cells that express the FGF receptors. It contains an ankyrin repeat, a coiled-coil structure and many tyrosines within

environments that suggest that if phosphorylated they act as binding sites for the SH2 domains of proteins such as DRK or CSW (Vincent et al, 1998).
The SH2-domain-containing protein DRK recruits the guanine nucleotide exchange factor, Son of sevenless (SOS), to catalyze the exchange of GDP bound to RAS for GTP, thereby activating RAS with the help of activated KSR.
RAS promotes the activation of RAF, leading to the activation of DSOR1, and ultimately to that of the MAP kinase Rolled (RL).
Rolled can activate transcription, both through inactivation of transcriptional co-repressors such as AOP, as well as through the activation of transcription factors such as the ETS-domain-containing protein Pointed (PNT) (ONeill et al, 1994; Brunner et al, 1994).
The activation of PNT is a major output of the pathway. It is either phosphorylated by MAP kinase to produce an active transcriptional activator (PointedP2), or transcriptionally induced by MAP kinase to produce a constitutive transcriptional activator (PointedP1).
Sprouty (STY) acts downstream of the receptor, but upstream of RAS1 and RAF, by recruiting GAP1 and blocking the ability of DRK to bind to its positive effector.
We have considered three typical initial states corresponding to

i. ligands binding in wild-type signalling enabling situation (VEGF_signalling),
ii. ligand binding in the presence of the inhibitor Sprouty (Sprouty_inhibition),
iii. absence of ligand (No_signalling).

Selected publications

- PMID:7559490
- PMID:9182757
- PMID:9809073
- PMID:8033205
- PMID:8047146

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Pvf1		input	• PMID:11595182 • PMID:12810594 • PMID:17462868 • http://flybase.org/reports/FBgn0030964.html Vascular endothelial growth factor 1. Three genes in the Drosophila genome code for PVR ligands: PVF1, PVF2, and PVF3. PVF1 contains a unique cysteine-rich CXCXC motifs not found in the other two ligands, and it has a distinct expression pattern. The developing salivary gland is the strongest site of PVF1 expression, beginning at stage 12 and persisting through stage 17. Duchek and collaborators (2001) further proposed that PVF1 is secreted from the oocyte where it acts as a guidance factor for the border cells (McDonald et al, 2004; Harris et al, 2007).
Pvf2		input	• PMID:11955438 • PMID:16651377 • PMID:19216764 • http://flybase.org/reports/FBgn0031888.html Vascular endothelial growth factor 2. Both PVF2 and PVF3 are expressed in the ventral midline, where they are thought to act in a partially redundant manner as attractive cues for hemocytes migrating out of the head (Cho et al, 2002; Wood et al, 2006). PVF2 and PVF3 have a similar expression pattern and may originate from a duplication, and are functionally similar to each other (Cho et al, 2002). Sims et al, 2009 further showed that PVF2 and PVF3 act redundantly to activate PVR.
Pvf3		input	• PMID:11955438 • PMID:16651377 • PMID:19216764 • http://flybase.org/reports/FBgn0085407.html Vascular endothelial growth factor 2. For more information, see annotations for PVF2.
Pvr	1	Pvf1:1 | Pvf2:1 | Pvf3:1	• PMID:15239955 • PMID:17462868 • http://flybase.org/reports/FBgn0032006.html Vascular endothelial growth receptor In the embryonic hematopoietic system, PVR mediates anti-apoptotic survival of blood cells throughout embryonic development. PVR is also required for invasion into/migration within the posterior end of the embryo (Bruckner et al, 2004). PVR is further required in the migration of the embryonic salivary gland tissue. Finally, PVR is necessary for cell survival and for migration of the hemocytes throughout the embryo (Bruckner et al, 2004 and Harris et al, 2007).
Sty		input	• PMID:15173823 • PMID:10089881 • PMID:10457022 • PMID:14515177 • PMID:16123311

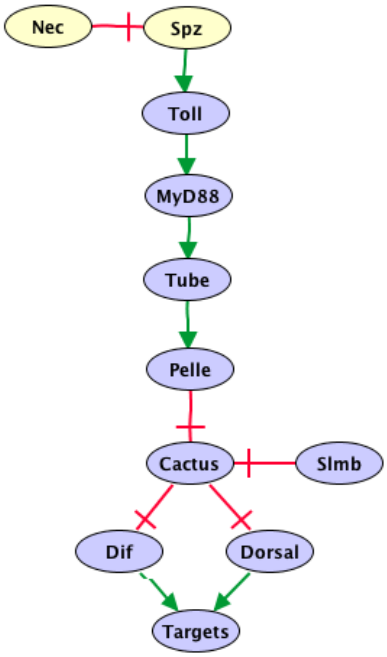
			• http://flybase.org/reports/FBgn0014388.html STY (Sprouty) protein has a conserved carboxy-terminal cysteine-rich domain that is necessary for their specific localization and function. Sprouty is an intracellular protein, associated with the inner surface of the plasma membrane. Sprouty exerts its inhibitory effect on receptor tyrosine kinase (RTK) signaling by intercepting essential elements of the RAS/MAPK cascade through diverse mechanisms (Kim and Bar-Sagi, 2004). Sprouty acts upstream of Ras1 and Raf, by recruiting Gap1 and blocking the ability of Drk to bind to its positive effectors. The expression of Sprouty is dependent on the pathway's activity, implying a negative feedback loop (Casci et al, 1999; Reich et al, 1999; Cabrita et al, 2003; shilo et al, 2005).
Drk	1	Pvr:1	• PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0004638.html Downstream of receptor kinase (DRK) is the homolog of the adaptor molecule GRB2. Upon ligand binding at receptor tyrosine kinases (RTKs), DRK recruits Son of sevenless (SOS), to activate RAS.
Aop	1	!Rl:1	• PMID:7781063 • http://flybase.org/reports/FBgn0000097.html Active RL (Rolled) down-regulates specific targets, including the transcriptional co-repressor Anterior Open (AOP or YAN). Direct phosphorylation of AOP leads to its export from the nucleus and subsequent ubiquitin-mediated protein degradation (Rebay and Rubin, 1995). AOP inhibits RTKs targets genes.
Rl	1	Dsor1:1 & msk	• PMID:16600911 • http://flybase.org/reports/FBgn0003256.html The RL (Rolled/MAPK) kinase is essential for the proper functioning of the RAS signaling pathway. After phosphorylation by DSOR1 and translocation in the nucleus, RL phosphorylates Pointed P2 (PNTP2).
Sos	1	Drk:1	• PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0001965.html Son of sevenless (SOS) is a guanine nucleotide-releasing factor that activates RAS by promoting the exchange of GDP for GTP.
Raf	1	Ras & Src42 & CNK & Ksr	• PMID:16600911 • http://flybase.org/reports/FBgn0003079.html RAF or Pole hole is a critical effector of the small GTPase RAS in cells. GTP-Ras activates the kinase RAF. This initiates a kinase cascade in which RAF phosphorylates DSOR1, in the presence of the scaffold Protein Kinase Suppressor of RAS (KSR).
Dsor1	1	Raf:1	• PMID:16600911 • http://flybase.org/reports/FBgn0010269.html Downstream of RAF1 (DSOR1) is the kinase responsible for the phosphorylation of Rolled, which can then enter the nucleus.
Targets	1	Pnt:1 & !Aop:1	• PMID:11832242

			• PMID:11141565
Ras	1	Sos:1 & !Sty:1	• PMID:8978043 • PMID:8951053 • PMID:8824589 • PMID:7749324 • PMID:14515177 • PMID:19366732 • http://flybase.org/reports/FBgn0003204.html Raspberry (RAS) is a molecular switch, cycling between an inactive GDP-bound and active GTP-bound form. RAS promotes the activation of RAF, leading to the activation of DSOR1 and eventually that of the RL (Cabrita et al, 2003; Yan et al, 2009).
Pnt (*pointed)*	1	Rl:1	• PMID:8223245 • PMID:8047146 • PMID:8033205 • PMID:9154002 • PMID:12648473 • PMID:16123311 • PMID:18369317 • PMID:16600911 • PMID:19884307 • http://flybase.org/reports/FBgn0003118.html The *pointed (pnt)* gene is a target of the signalling cascade acting downstream of Rolled/MAP Kinase. It encodes two distinct protein isoforms, PNTP1 and PNTP2. Both isoforms act as effectors of the Ras/MAP kinase pathway in multiple developmental contexts (eye, neural cells and the midline glial cells) (Klambt et al, 1993; Brunner et al, 1994; O'Neill et al, 1994; Roignant et al, 2006; Yogev et al, 2008; Salzer et al, 2010).
Ksr		input	• PMID:8521512 • PMID:11141565 • PMID:16326394 • http://flybase.org/reports/FBgn0015402.html KSR (Inactive kinase suppressor of Ras) activity is required downstream of Torso pathway during the development of the fly embryo extremities, as well as downstream of EGF and Sevenless pathways during eye development, suggesting that it is a general constituent of RTK pathways (Therrien et al, 1995). KSR facilitates the phosphorylation of DSOR1 and RL by RAF, and enhances the generation of activated RL.
Src42		input	• PMID:2996778 • PMID:8682295 • PMID:15660123 • http://flybase.org/reports/FBgn0264959.html SRC oncogene at 42A (SRC42) acts as an intermediate kinase linking activated RTKs to CNK tyrosine phosphorylation and suggested that this event is RAS-independent (Laberge et al, 2005).
Msk		input	• PMID:9214382 • PMID:10228156 • PMID:11262240 • http://flybase.org/reports/FBgn0026252.html The *moleskin* gene encodes Drosophila Importin-7 (DIM-7). MSK/DIM-7 is a member of the importin superfamily of nuclear importers, which can bind directly to the nuclear pore complex (Gorlich et al, 1997; Jakel et al, 1999).

			In Drosophila, MSK/DIM-7 is tyrosine phosphorylated in response to growth factor stimulation of RTKs, and it physically binds Rolled (Lorenzen et al, 2001). MSK is a General RL Nuclear Import Factor.
Cnk		input	• PMID:9814705 • PMID:10860999 • PMID:16326394 • PMID:14517245 • PMID:15660123 • PMID:16600911 • http://flybase.org/reports/FBgn0021818.html Connector Enhancer of KSR (CNK) activity is required for various RTK-mediated developmental events and affect cell proliferation/survival, differentiation and migration (Therrien et al, 1998; Baonza et al, 2000; Cabernard and Affolter, 2005). CNK directly associates with the kinase domain of RAF via a short amino-acid sequence, called the RAF-interacting motif (RIM), and modulates RAF activity according to the RTK signalling status (Douziech et al, 2003; Laberge et al, 2005). Without signalling, CNK binding is inhibited by a second motif adjacent to the RIM, called the inhibitory sequence (IS). Upon pathway activation, CNK integrates SRC42 and RAS activities, which then lead to RAF activation. The binding of SRC42 to an RTK-dependent phospho-tyrosine residue (pY1163) located at the C-terminal of the IS motif appears to release the inhibitory effect that the IS motif imposes on RAF catalytic function (Laberge et al, 2005). Functional analysis indicated that CNK acts downstream of RAS, but at a step upstream of RAF.

Logical model of Drosophila Toll signaling pathway

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila Toll pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

Toll was initially discovered as an essential component of the pathway that establishes the dorsal, ventral axis of the early Drosophila embryo. If any component in that genetic pathway is missing, no ventral or lateral cell types develop and the resulting embryos lack all mesoderm and the entire nervous system (Anderson et al., 2000).
Fungal and Gram-positive bacterial infections in Drosophila also stimulate the Toll pathway.
Activation of Toll leads to recruitment of three cytoplasmic proteins, which are MYD88, Tube and Pelle, to form the signalling complex underneath the cell membrane (Sun et al, 2004).
Subsequently, through interactions via death domains, assembly of the signalling complex containing MYD88, Tube and Pelle occurs (Sun et al, 2004; Tanji et al, 2005).
From this complex, signalling proceeds through the phosphorylation and degradation of the Drosophila IkB factor Cactus. In non signaling conditions, Cactus is bound to Dorsal or Dorsal-related immunity factor (DIF), inhibiting their activity and nuclear localization.
Pelle is the only kinase reported for Cactus phosphorylation. After phosphorylation, nuclear translocation of Dorsal/DIF leads to activation of transcription of several sets of target genes.
(Tanji et al, 2005; Minakhina et al, 2006; Valanne et al, 2011).
To reproduce pathway signalling dynamics, we define two initial states corresponding to no signalling conditions (no ligand binding) and to signalling conditions (binding of SPZ to the receptor Toll).

Selected references

- PMID:21576362
- PMID:10980426
- PMID:12524386
- PMID:21209287

Description of regulatory graph components

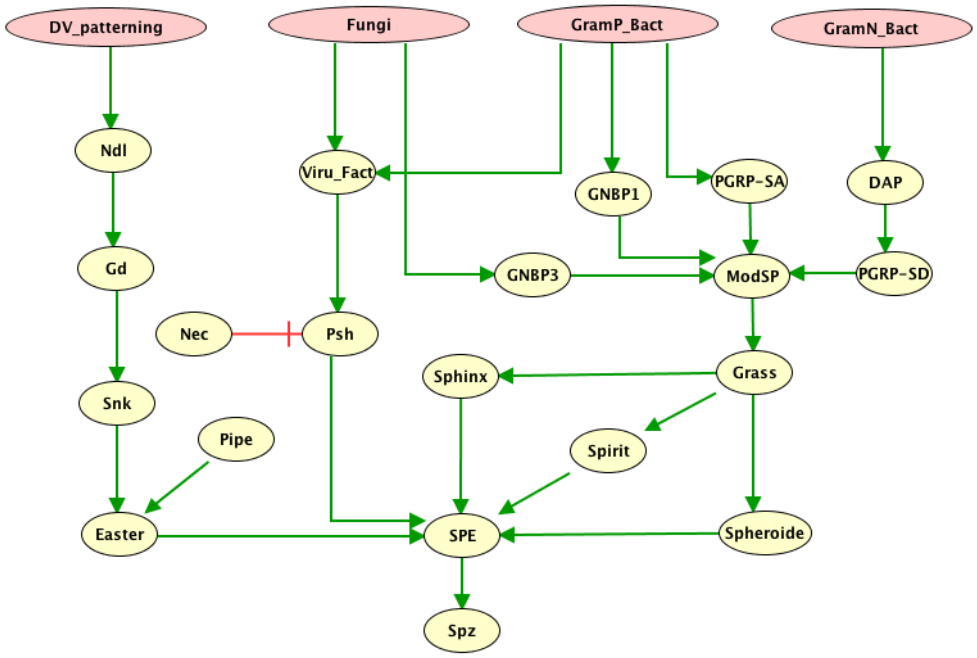
Components	Values	Logical rules	Annotations
Toll	1	Spz	• PMID:9598341 • PMID:12888566 • PMID:14966134 • http://flybase.org/reports/FBgn0262473.html Drosophila Toll receptor family comprises nine members. Their extracellular domain is composed of leucine-rich repeats (LRRs) flanked by small domains characterised by specific arrangement of cystein residues, whereas the intracytoplasmic domain shows striking similarities with the cytoplasmic tail of the interleukin-1 (IL-1) receptor. On the one hand, the function of Toll consisted in the regulation of the expression of antimicrobial peptides, which are strongly induced in the fat body in response to septic injury. On the other hand, Toll is involved in the establishment of the dorso-ventral (DV) axis of the Drosophila embryo. (Wu et al, 1997; Dunne et al, 2003 and Lee et al, 2004).
Spz	1	!Nec	• PMID:12872120 • PMID:15197269 • PMID:14751763 • http://flybase.org/reports/FBgn0003495.html Spatzle (SPZ) is synthesized and secreted as an inactive precursor consisting of a prodomain and a C-terminal region (C-106). In DV patterning, SPZ is processed into its active C-106 form by a serine protease cascade including Nudel, Gastrulation Defective, Snake, and Easter. In addition, sulfotransferase Pipe is required independently of the protease cascade to activate Easter. In microbe recognition, SPZ- processing enzyme (SPE) is responsible for SPZ cleavage. It has been showed that a truncated SPZ can bind to Toll and activate the Toll pathway, demonstrating that SPZ probably acts as a ligand for Toll in vivo. Extracellular recognition factors initiate protease cascades leading to the activation of the Toll receptor ligand Spatzle. This activation induces proteolysis, which causes a conformational change exposing determinants that are critical for binding of the Toll receptor. (Weber et al, 2003; Hu et al, 2004 and Ferrando et al, 2004)
MyD88	1	Toll	• PMID:12351681 • PMID:12888566 • PMID:14685264 • http://flybase.org/reports/FBgn0033402.html MyD88 and Tube act as adaptor proteins. The TIR interleukin-1 receptor domain of Toll binds to the TIR domain of MyD88, however, binding occurs only when the receptor is active. MyD88 and Pelle do not come into contact with each other. Instead, two distinct surfaces in the adaptor protein Tube separately bind MyD88 and Pelle. (Sun et al, 2002; Dunne et al, 2003; Sun et al. 2004)
Tube	1	MyD88	• PMID:17072326 • http://flybase.org/reports/FBgn0003882.html Tube is an adaptor that functions downstream of Toll and upstream of Pelle. Tube recruits the Pelle kinase to the complex formed with toll, thereby increasing the Pelle concentration. (Minakhina et al, 2006)
Pelle	1	Tube	• PMID:15797509 • PMID:17072326

			• http://flybase.org/reports/FBgn0010441.html Pelle is a serine, threonine kinase. Increased local concentration of Pelle might lead to trans-phosphorylation and stimulation of the Pelle kinase activity. Ativated Pelle acts on cytoplasmic Dorsal, Cactus and DIF protein complexes. Pelle has been shown to auto-phosphorylate himself and to phosphorylate Toll and Tube. (Tanji et al, 2005; Minakhina et al, 2006)
Cactus	1	!(Pelle & Slmb)	• PMID:17072326 • PMID:21209287 • http://flybase.org/reports/FBgn0000250.html From the oligomeric MyD88-Tube-Pelle complex, Toll signalling proceeds to the phosphorylation and degradation of the Drosophila IkB factor Cactus. In non signaling conditions, Cactus is bound to Dorsal and/or DIF, inhibiting their activity and nuclear localization. So, the nuclear translocation of both Dorsal and DIF requires Cactus degradation. To be degraded, Cactus is phosphorylated by Pelle. After signal-induced degradation of Cactus, DIF and Dorsal translocate to the nucleus and activate the expression of antimicrobial peptide genes. (Tanji et al, 2005; Valanne et al, 2011)
Nec		input	• PMID:10489372 • PMID:18432983 • http://flybase.org/reports/FBgn0002930.html The *necrotic* (*nec*) gene encodes a proteinase inhibitor of the *serpin* family (serine proteinase inhibitor). NEC controls a proteolytic cascade which activates the innate immune response to fungal and Gram+ bacterial infections. In *nec* null mutants, the Toll-mediated immune response is constitutively activated (constitutive expression of cleaved SPZ and constitutive expression of Drosomycin), even in the absence of infection, implying that NEC continually restrains this immune response. (Levashina et al, 1999; Takeda et al, 2003).
Slmb		input	• PMID:12401167 • PMID:12432393 • PMID:12401167 • PMID:11500045 • PMID:10097128 • PMID:10022863 • http://flybase.org/reports/FBgn0023423.html Supernumary limbs (SLMB) defined a phosphopeptide motif to target proteins for ubiquitination and subsequent proteolysis (ubiquitin-proteasome pathway). Slmb is an important regulator of several developmental pathways, in particular the Wingless, Hedgehog and Toll pathways.
Dif	1	!Cactus	• PMID:12872120 • PMID:21209287 • http://flybase.org/reports/FBgn0011274.html DIF was identified as a dorsal-related immune responsive gene that does not participate in dorsal ventral patterning. Instead, it mediates an immune response in Drosophila larvae and interacts with Cactus. Dorsal and DIF are redundant in larvae and can form heterodimers. (Weber et al., 2003; Minakhina et al, 2006; Valanne et al, 2011).
Dorsal	1	!Cactus	• PMID:12872120 • PMID:21209287 • http://flybase.org/reports/FBgn0260632.html Threshold levels of Dorsal control the expression of zygotic genes. Dorsal-

			mediated gene expression represents the transition from maternal to zygotic control of dorsal ventral patterning in the Drosophila embryo. This asymmetry is transmitted to the embryo through the interaction of two groups of genes. One group is expressed specifically on the ventral side of the follicle cells that surround the oocyte and secrete the egg membranes. The other group is mainly made up of the genes *gastrulation defective*, *snake* and *easter*, all three encoding serine proteases. The proteases form an activation cascade that culminates in the maturation and cleavage of the ligand SPZ, which in turn activates the Toll-Dorsal signaling pathway. (Morisato and Anderson, 1994; Weber et al., 2003; Minakhina et al, 2006).
targets	1	Dorsal | Dif	• PMID:11832242 • PMID:11141565 • PMID:23083631 • PMID:22902989 • PMID:22902989 • PMID:22611248 The Toll pathway is involved in several developmental processes (dorso-ventral patterning, apoptosis, oogenesis, cardiac development) and in the immune response.

Logical model of Drosophila Spz processing

Mbodj, Junion, Brun, Furlong and Thieffry (2013). Logical modelling of drosophila signalling pathways. Submitted to *Molecular BioSystems*.



Regulatory graph for Drosophila SPZ processing pathway, in response to different types of immunological challenges. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

Overview

During DV patterning, a regulatory cascades composed by three dorsal group genes *gastrulation-defective*, *snake* and *easter*, encoding serine proteases, lead to the cleavage of Spatzle (SPZ), that in turn activates the Toll-dorsal signaling pathway (Morisato and Anderson, 1994; Weber et al., 2003).
Spatzle presumably forms a gradient in the perivitelline fluid.
Toll signaling is ultimately responsible for the formation of the embryonic dorsal nuclear gradient.
In the nucleus, dorsal controls the expression of zygotic genes in a concentration-dependent manner and this process results in the patterning of the dorsal–ventral embryonic axis.
twist is one of the earliest target genes controlled by the highest concentration of dorsal in the mesodermal cells. It is a transcriptional activator that cooperates with dorsal in activating *snail* in the mesoderm.
Dorsal and Twist also cooperate to activate the neurogenic gene, *sim* (*single minded*), expressed in the neurectoderm and repressed by Snail in the mesoderm.
Natural or experimentally induced infections by fungi or bacteria elicit a specific response in both adult flies and larvae.
The proteoglycans of Gram-positive and Gram-negative bacteria are sensed by distinct pattern recognition proteins called PGRPs (peptidoglycan recognition proteins (Royet et al., 2004).
Different PRGPs cooperate to activate the Toll pathway. The activation of PGRP-SA by Gram-positive bacteria leads to Spatzle cleavage (Gobert et al., 2003).
Fungal infection also leads to the cleavage of Spatzle, but the proteolytic cascade in this case

involves the circulating serine protease Persephone and its serine protease inhibitor, Necrotic (Ligoxygakis et al., 2002a and b; Pelte et al., 2006).
Circulating PGRP-SA receptor activates the Toll pathway upon detection of Lysine-type PGN which is a major component of the cell wall of many Gram-positive bacterial strains.
GNBP1 (Gram-Negative Binding Protein 1) associates with PGRP-SA and this complex activates a downstream proteolytic cascade that leads to the cleavage of Spatzle, which then activates the Toll transmembrane receptor.
In addition, four other serine proteases, namely Spirit, Spheroide, and Sphinx1 and 2, were identified in response to both fungi and Gram-positive bacteria infections.
Thus, PGRP-SA and GNBP1 define a Gram-positive-specific branch of Toll receptor activation.
PGRP-SD also belongs to this branch and is required for the detection of other Gram-positive and negative bacterial strains.
In short, the maturation of SPZ activates Toll in both early embryo and immune response and is controlled by different sets of proteases (Bischoff et al., 2004; Valanne et al., 2011).
To reproduce biological data during SPZ processing, we define four initial states corresponding the biological process involved. All these initial state lead to the formation of the active form of SPZ.

Selected references

- PMID:8124709
- PMID:12872120
- PMID:15004693
- PMID:14684822
- PMID:12456640
- PMID:10489372
- PMID:12098703
- PMID:15448690
- PMID:21209287

Description of regulatory graph components

Components	Values	Logical rules	Annotations
Ndl	1	DV_patterning	• PMID:1425342 • PMID:8139688 • http://flybase.org/reports/FBgn0002926.html NDL (Nudel) is localised within the perivitelline space and associates with the vitelline envelope. It acts as the scaffold of a zymogen activation complex containing GD, SNK and EA. Its own serine protease domain, perhaps autoactivated with the help of cofactors (not known yet), cleaves GD, thus initiating the protease cascade that ends with the proteolytic processing of SPZ to produce the Toll ligand. The sequential action of GD, SNK, EA, and SPZ is supported by genetic studies (Chasanet al., 1992; Smithand DeLotto,1994). NDL, GD, SNK, EA, PSH are serine proteases containing a Clip domain, exclusively found in insects and believed to play a regulatory role in the sequential activation of serine proteases.
Gd	1	Ndl	• PMID:1425342 • PMID:8139688 • PMID:7671306 • http://flybase.org/reports/FBgn0000808.html GD (Gastrulation defective) is a serine proteases containing a Clip domain.
Snk	1	Gd	• PMID:1425342 • PMID:8139688 • PMID:7671306 • http://flybase.org/reports/FBgn0003450.html SNK (Snake) is a serine protease containing a Clip domain N-terminal to the catalytic domain.
Easter	1	Snk & Pipe	• PMID:1425342 • PMID:7671306 • PMID:8139688 • http://flybase.org/reports/FBgn0000533.html Easter is a serine protease containing a Clip domain.
Pipe		input	• PMID:20605458 • http://flybase.org/reports/FBgn0003089.html The sulfotransferase Pipe is required independently of the NDL/GD/SNK protease cascade to activate Easter (Cho et al., 2010).
DV_patterning		input	Dorso-ventral patterning.
Spirit	1	Grass	• PMID:16631589 • http://flybase.org/reports/FBgn0030051.html Spirit is a functional chymotrypsin-like serine protease containing a Clip domain. (Kambris et al., 2006).
SPE	1	Easter | Psh | Sphinx | Spirit | Spd	• PMID:21209287 • http://flybase.org/reports/FBgn0039102.html In microbe recognition, the SPZ-processing enzyme (SPE) is responsible for SPZ cleavage. Spirit, Grass, and SPE, are functional chymotrypsin-like serine proteases containing a Clip domain N-terminal to the catalytic domain. The Clip domain is exclusively found in insect serine proteases and is believed to play a regulatory role in the sequential activation of SPZ (Valanne et al., 2011).
Grass	1	ModSP	• PMID:16631589

			• PMID:18724373 • PMID:19126860 • http://flybase.org/reports/FBgn0039494.html Grass (Gram-positive specific serine protease) was originally identified to be specifically involved in the recognition of Gram-positive bacteria, but was later shown to be important also for the recognition of fungal components. (Kambris et al., 2006; El Chamy et al., 2008; Ashok et al., 2009). Grass is a functional chymotrypsin-like serine protease containing a Clip domain.
Fungi		input	Fungi induces the Toll pathway.
GramP_Bact		input	Gram positive bacteria induces the Toll pathway.
Spd	1	Grass	• PMID:16631589 • PMID:16399077 • http://flybase.org/reports/FBgn0030774.html Grass can associated with the serine proteases Spirit, Sphinx 1 and 2 or Spheroid (Kambris et al., 2006), in a complex with PRRs (pattern-recognition receptors), directing Grass activity toward SPE (Kambris et al., 2006).
Sphinx	1	Grass	• PMID:16631589 • http://flybase.org/reports/FBgn0052383.html • http://flybase.org/reports/FBgn0052382.html Sphinx1 and 2 are serine proteases idendified in response to both fungi and Gram-positive bacteria (Kambris et al., 2006).
ModSP	1	(PGRP-SA | PGRP-SD) & (GNBP1 | GNBP3)	• PMID:19590012 • http://flybase.org/reports/FBgn0051217.html Upstream of Grass, the *modular serine protease* (*modSP*), is conserved in insect immune reactions, and plays an essential role in integrating signals from the recognition molecules Gram-negative binding protein (GNBP) 3 and PGN recognition protein (PGRP)-SA to the Grass-SPE-SPZ cascade. Survival and antimicrobial peptide gene expression analyses strongly suggest a role of ModSP in the activation of the Toll pathway by Gram-positive bacteria, these experiments demonstrate that ModSP is essential for Toll activation by Gram-positive bacteria and that over-expression of full-length ModSP is sufficient to activate the Toll pathway. Epistasis analysis indicates that ModSP functions downstream of PGRP-SA and GNBP1 and upstream of grass in the pathway that links Gram-positive bacterial recognition to Toll activation (Buchon et al., 2009).
GramN_Bact		input	Gram negative bacteria activates Toll pathway.
Psh	1	Viru_Fact & !Nec	• PMID:12098703 • PMID:17190605 • PMID:18724373 • http://flybase.org/reports/FBgn0030926.html A third protease cascade leading to the activation of SPE is mediated by the protease PSH (Persephone), which is proteolytically matured by the secreted fungal virulence factor PR1 and Gram-positive bacterial virulence factors. *psh* is activated by fungal virulence factors (substances that enhance the infectivity of the microbe) and detects proteases and chitinases secreted by spores of fungi that infect insects (entamopathogenic fungi). These virulence factors degrade the cuticle to enable the fungi to gain entry into the host. (Ligoxygakis et al., 2002; Gottar et al., 2006; El Chamy et al. 2008)

Viru_Fact	1	Fungi | GramP_Bact	• PMID:17190605 • PMID:14693381 • PMID:15890886 Many pathogens have adapted to their hosts and developed specific strategies to defeat their defenses. Fungi are able to infect insects following deposition of spores on the surface of the cuticle. To penetrate this physical barrier, they secrete several virulence factors such as chitinases and proteases. Virulence factors can be detected by the innate immune system. (Bagga et al., 2004; Wang et al., 2005).
PGRP-SA	1	GramP_Bact	• PMID:11106397 • PMID:15843462 GNBP1, PGRP-SA, and PGRP-SD, appear to mainly recognize Gram-positive bacteria. PGRP-SA recognizes peptidoglycans. PGRP-SA is a receptor of the Toll pathway, which shows elicitor specificity for bacteria with a peptidoglycan structure containing a Lys in the third position of the cross-linking tetrapeptide. PGRP-SA binds strongly to Lys-type peptidoglycan (examples: *M. luteus*, *S. aureus*, and *L. casei*). PGRP-SA has poor affinity for diaminopimelic acid (DAP)-containing peptidoglycan from *B. megaterium* but binds strongly to DAP-type peptidoglycan from *E. coli* and *L. plantarum*. Furthermore, PGRP-SA binds weakly to ornithine-containing peptidoglycan from *L. fermentum* (Bischoff et al., 2004; Mellroth et al., 2005).
GNBP1	1	GramP_Bact	• PMID:10827089 • PMID:10713054 • PMID:10671539 • PMID:19590012 • http://flybase.org/reports/FBgn0040323.html GNBP1 belongs to the family of GNBP Glucan Recognition Proteins (Kim et al., 2000). Members of this family have been reported to bind to (1,3)-glucan, a major component of the fungal cell wall (Ma and Kanost, 2000; Ochiai and Ashida, 2000). In Drosophila, three members of this family, GNBP1 to 3, have been described (Kim et al., 2000). Buchon et al., 2009, showed that full-length GNBP1 had no enzymatic activity. GNBP1 is suggested to be a linker between PGRP-SA and ModSP. GNBP1, PGRP-SA, and PGRP-SD appear to mainly recognize Gram-positive bacteria.
PGRP-SD	1	DAP	• PMID:18304640 PGRP- SD presumably recognizes Diaminopimelic acid (DAP)-type PGNs from Gram-negative bacteria, thereby activating the Toll pathway (Leone et al., 2008). In addition, flies with the PGRP-SA; PGRP-SD double mutation are highly susceptible to Gram-positive bacteria infection (Bischoff et al., 2004)
DAP	1	GramN_Bact	• PMID:18304640 PGRPs can discriminate between PGN containing DAP or lysine residue at the third position of the stem peptide. Diaminopimelic (DAP)-type peptidoglycans can activate both the Toll and Imd pathways. PGRP-SA has a poor affinity for Diaminopimelic acid (DAP)-containing peptidoglycans from *B. megaterium* but binds strongly to DAP-type peptidoglycans from *E. coli* and *L. plantarum.*

			(Leonne et al., 2008)
GNBP3	1	Fungi	• PMID:17190605 • PMID:19590012 • http://flybase.org/reports/FBgn0040321.html For fungi recognition, over-expression of GNBP3 triggers the Toll pathway, resulting in a constitutive expression of Drosomycin in the absence of an immune challenge (Gottar et al., 2006; Buchon et al., 2009). Among the GNBPGNBP3 shows the greatest degree of similarity to lepidopteran (1,3)- glucan recognition proteins and was therefore a good candidate for being a fungal-specific sensor.
Nec		input	• PMID:16360948 • PMID:10489372 • PMID:12456640 • PMID:12098703 • http://flybase.org/reports/FBgn0002930.html *nec* codes for the serine protease inhibitor (Serpin) SPN43Ac, which negatively regulates the Toll pathway, whereas *psh* encodes a secreted serine protease required for its activation in response to infection with the fungus *Beauveria bassiana* and gram positive bacteria. The Necrotic regulates Toll activation by inhibiting PSH, which is involved in the cleavage of SPZ. (Levashina et al., 1999). Serpins are characterized by a highly conserved tertiary structure and a dynamic mechanism of inhibition. The proteinase molecule is distorted and trapped in a covalently linked Serpin–proteinase complex, which is targeted for destruction (Gettins, 2002). NEC has an alanine-rich hinge region and its active site is characterized by leucine and serine in the P1–P01 positions. Following infection with a mixture of Gram-positive and Gram-negative bacteria, the necrotic protein is cleaved (Levashina et al., 1999). Following fungal infection, NEC N-terminal cleavage is blocked by mutations in the serine proteinase PSH, which is required for the fungal and gram-positive bacteria response. (Ligoxygakis et al., 2002; Pelte et al., 2006)
Spz	1	SPE	• PMID:1425342 • PMID:7671306 • PMID:16399077 • http://flybase.org/reports/FBgn0003495.html SPZ is the Toll pathway ligand. It is synthesized and secreted as an inactive precursor consisting of a prodomain and a C-terminal region (C-106). In DV patterning, SPZ is processed into its active C-106 form by a serine protease cascade including NDL, SNK, GD and EA. In addition, the sulfotransferase Pipe is required independently of the protease cascade to activate EA. .. In microb recognition, Spatzle-processing enzyme (SPE) is responsible for SPZ cleavage. (Chasan et al., 1992; Hong et al., 1995, Jang et al., 2006).