Logical model of Drosophila VEGF signaling pathway

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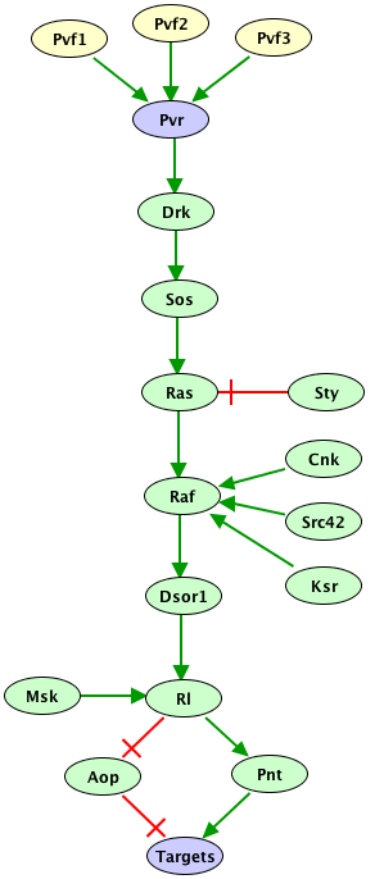

Regulatory graph for Drosophila VEGF pathway, displayed from ligand and receptor at the top to the main downstream effectors and a generic target node at the bottom. Red blunt and green normal arrows denote activatory and inhibitory interactions, respectively.

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 (Ducheck et al., 2001; Brückner et al., 2004; Wood et al., 2006; Harris et al., 2007; Sims et al., 2009).
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:11595182
- PMID:17462868
- PMID:9809073
- PMID:8033205
- PMID:8047146
- PMID:16651377
- PMID:15239955

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.