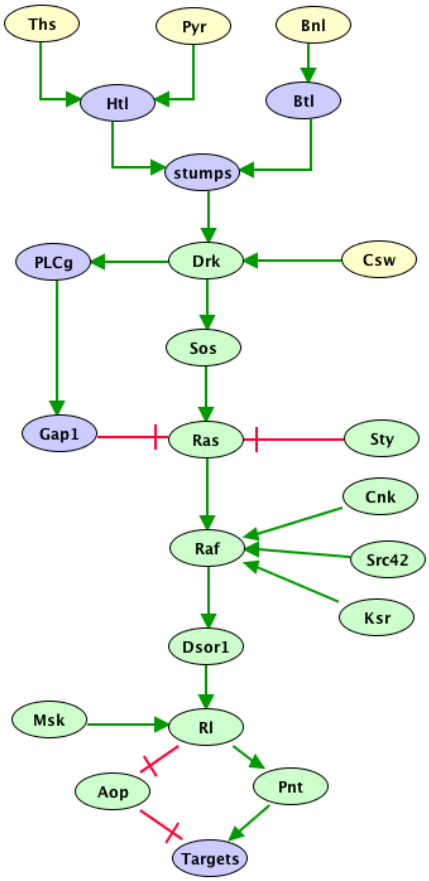
Logical model of Drosophila FGF signaling pathway

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.