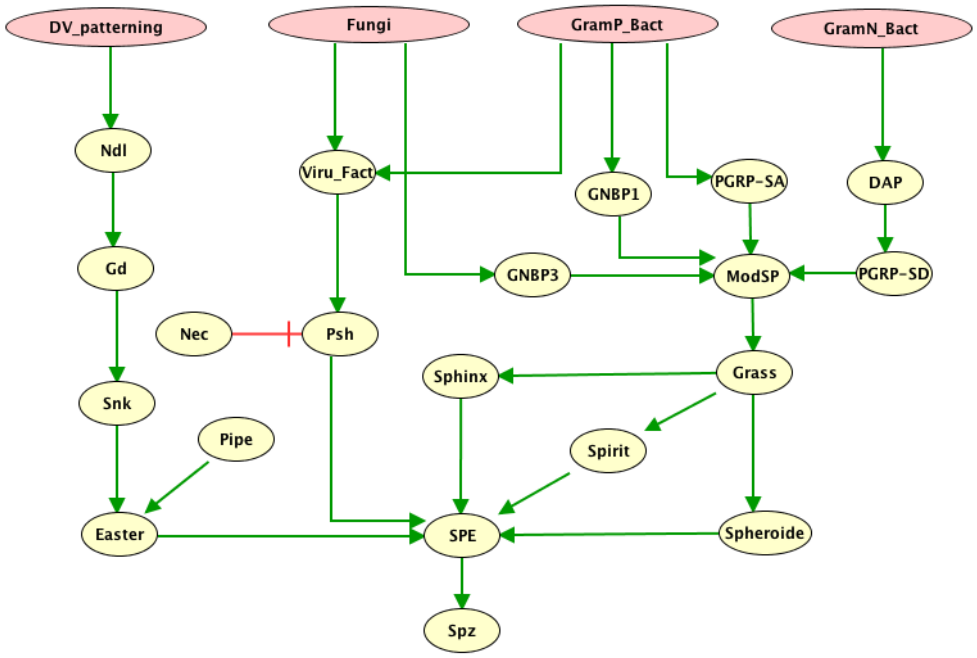
Logical model of Drosophila Spz processing

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).