ISJ 6: 32-43, 2009 ISSN 1824-307X

REVIEW

Signaling pathways in invertebrate immune and stress response

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Accepted March 4, 2009

Abstract

A wide variety of signaling pathways regulate immune and stress response in invertebrates and vertebrates. The fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* are extensively utilized model organisms for studies of such signaling pathways in invertebrates. Intriguingly, major signaling pathways in immune response in *Drosophila* and *C. elegans*, as represented by the Toll and Imd pathways and the DBL-1 and DAF-2/DAF-16 pathways, respectively, are different from each other. On the other hand, the mitogen-activated protein kinase (MAPK) pathways function in common in these organisms not only in immune response but also in response to various abiotic stressors such as heat shock, ultraviolet (UV) irradiation, oxidative stress and osmotic shock. Given that all of the above pathways are highly conserved and play diverse roles in vertebrates, particularly in mammals, *Drosophila* and *C. elegans* are important invertebrate models that facilitate the elucidation of evolutionarily conserved mechanisms of immune and stress response. We therefore focus on signaling pathways that regulate immune and stress response in *Drosophila* and *C. elegans* in this review.

Key Words: innate immunity; stress; MAP kinase; Toll; Imd; DAF-2

Introduction

To cope with pathogenic microorganisms, invertebrates rely solely on innate immunity because they do not have adaptive immunity. The well-characterized strategy against pathogens in invertebrate innate immunity is the induction of antimicrobial peptide (AMP) genes, the regulatory mechanisms of which have been extensively explored using model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*. In vertebrates, on the other hand, it has long been recognized that adaptive immunity is the main strategy used in the fight against various pathogens. However, a large body of recent evidence has demonstrated that innate immunity also plays a critical role in vertebrate immunity. Therefore, in order to understand the highly complex immune systems in vertebrates, and in mammals in particular, it will be of great importance to elucidate the regulatory mechanisms of innate immunity in invertebrate models, with a particular focus on signaling molecules that are conserved among species and involved in such mechanisms.

Invertebrate models also provide important information on the mechanisms that regulate the response to various abiotic stressors, such as heat shock, ultraviolet (UV) irradiation, oxidative stress and osmotic shock. The mitogen-activated protein kinase (MAPK) pathways, especially those converging on two subgroups of stress-responsive MAPKs, JNK and p38, are the major players in a wide variety of response to these stressors (Widmann *et al.*, 1999; Kyriakis and Avruch, 2001). Although these pathways are highly conserved from invertebrates to mammals and many physiological functions of these pathways have been revealed using mammalian models such as gene targeting mice, a great deal has also been revealed by studying these pathways as a primitive and efficient defense system in *Drosophila* and *C. elegans* (Stronach and Perrimon, 1999; Sakaguchi *et al.*, 2004). In fact, the genetic evidence that the stress-responsive MAPK pathways play pivotal roles not only in stress response but also in innate immunity was first revealed in analyses using these more rudimentary animals models (Boutros *et al.*, 2002; Kim *et al.*, 2002).

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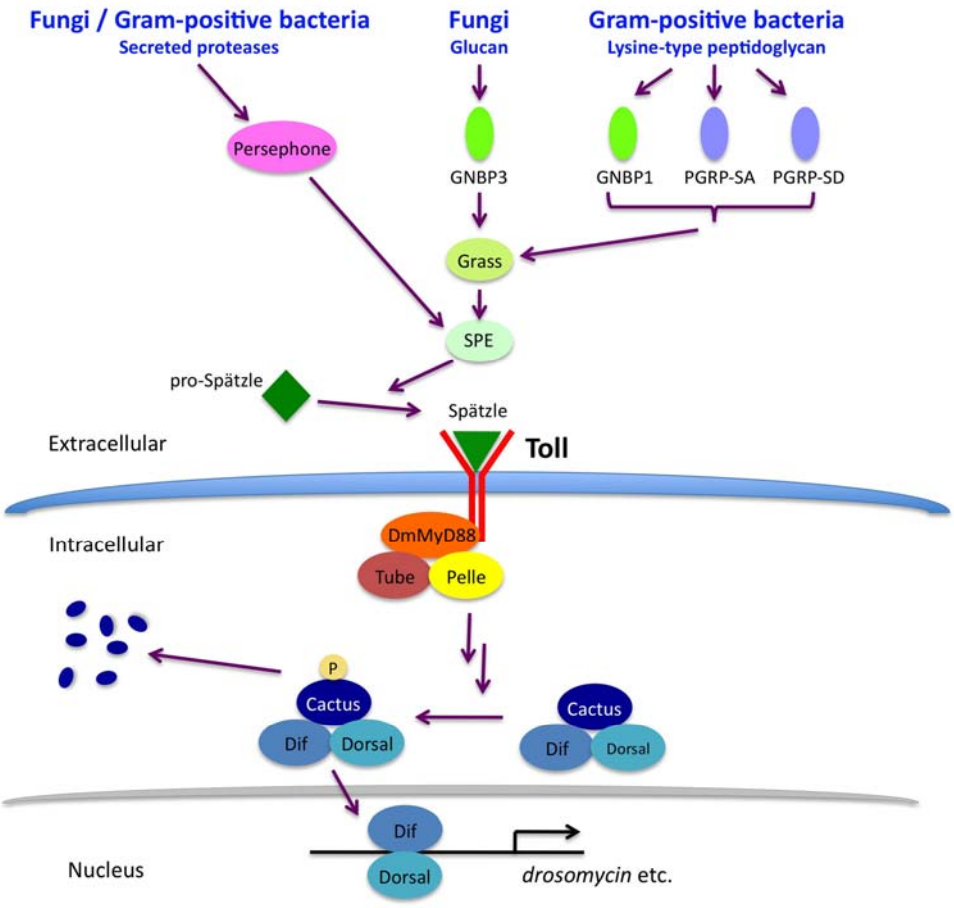

Fig. 1 The Toll pathway. Cell wall components of Gram-positive bacteria and fungi (lysine-type peptidoglycans and glucans, respectively) are recognized by pattern recognition proteins and activate protease cascades including such proteases as the serine protease Grass. Proteases secreted from fungi and Gram-positive bacteria activate the serine protease Persephone and its downstream protease cascade. These cascades finally activate SPE, which cleaves pro-Spätzle to form mature Spätzle. Upon the binding of Spätzle to Toll, DmMyD88, Tube and Pelle are recruited to Toll. Following the phosphorylation and degradation of Cactus, Dif and Dorsal are released from Cactus, translocate to the nucleus and induce AMP genes such as *drosomycin.*

In the first and second parts of this review, we focus on major signaling pathways in immune response in *Drosophila* and *C. elegans*, as represented by the Toll and Imd pathways and the DBL-1 and DAF-2/DAF-16 pathways, respectively. In the last part we examine the functions of the stress-responsive MAPK pathways as a common signaling system regulating immune and stress response in *Drosophila* and *C. elegans*.

Signaling pathways in immune response in *Drosophila*

The Toll and Imd pathways have been extensively investigated as major signaling pathways in immune response in *Drosophila*. These pathways are known to be functionally and molecularly conserved among other insects, such as the mosquitoes *Aedes aegypti* and *Anopheles gambiae* (Christophides *et al.*, 2002; Waterhouse *et al.*, 2007), the beetles *Tribolium castaneum* (Zou *et al.*, 2007), the honey bees *Apis mellifera* (Evans *et al.*, 2006) and the silkworms *Bombyx mori* (Tanaka *et al.*, 2008), indicating that these pathways play central roles in immune response in insects.

The Toll pathway

When flies are infected with Gram-positive bacteria or fungi, AMP gene expression is enhanced via the Toll Pathway in the fat body cells (Lemaitre *et al.*, 1996). The *Toll* gene was first found to be required for the establishment of dorsal-ventral polarity in *Drosophila* embryos (Anderson *et al.*, 1985). Toll is a founder member of the highly conserved Toll-like receptor (TLR) family, which is composed of type I transmembrane proteins with an ectodomain characterized by several repeats of leucine-rich motifs. Although most members of this family recognize and directly bind to pathogen-associated molecular patterns (PAMPs) as host pattern recognition proteins, Toll does not directly bind to pathogens or PAMPs but instead

binds to the mature form of the extracellular protein Spätzle as an endogenous ligand (Weber *et al.*, 2003).

Upon infection with Gram-positive bacteria, *Drosophila* recognizes a bacterial cell wall component, lysine-type peptidoglycan (PGN), by means of pattern recognition proteins such as peptidoglycan-recognition protein (PGRP) short-form A (PGRP-SA), PGRP short-form D (PGRP-SD) and Gram-negative binding protein 1 (GNBP1) in the hemolymph (Werner *et al.*, 2000; Michel *et al.*, 2001; Gobert *et al.*, 2003; Bischoff *et al.*, 2004) (Fig. 1). Results from genetic and biochemical analyses suggest that the ternary complex of all these proteins and the complex formed between PGRP-SA and GNBP1 differentially bind to PGNs in a manner dependent on the bacterial strains from which PGNs are purified (Wang *et al.*, 2008). These recognition proteins thereafter activate serine protease cascades, resulting in cleavage of pro-Spätzle by the Spätzle processing enzyme (SPE) (Jang *et al.*, 2006). Although the proteases that are engaged in SPE activation have not been fully revealed, a recent study has clarified that the serine protease Grass functions upstream of SPE (El Chamy *et al.*, 2008).

Fungal infection also activates the Toll pathway via protease cascades, and is dually detected by a pattern recognition protein GNBP3 and a serine protease Persephone (Psh) (Gottar *et al.*, 2006). Fungal cell wall components such as β-(1,3)-glucans are recognized by GNBP3. Proteases and chitinases secreted from fungi, which perforate the cuticle barrier and allow entry of fungi into the body cavity (Clarkson and Charnley, 1996), are sensed by Psh. These recognition systems activate SPE through protease cascades, leading to cleavage of pro-Spätzle. Recently, Psh has also been shown to be required for sensing Gram-positive bacterial proteases (El Chamy *et al.*, 2008).

Upon binding of Spätzle to Toll, DmMyD88, the *Drosophila* ortholog of mammalian MyD88, and the death domain proteins Tube and Pelle are recruited to the intracellular domain of Toll, where they form a complex (Horng and Medzhitov, 2001; Tauszig-Delamasure *et al.*, 2002). Pelle is the *Drosophila* ortholog of IL-1 receptor-associated kinase (IRAK), while the counterpart of Tube has not been found in mammals. This protein complex formation induces phosphorylation of Cactus, the *Drosophila* homolog of mammalian IκB (Nicolas *et al.*, 1998), although it has not yet been revealed whether the kinase Pelle directly phosphorylates Cactus. Under unstimulated conditions, Cactus retains and thus inhibits the NF-κB-like transcription factors Dif and Dorsal in the cytoplasm. Once phosphorylated, Cactus is degraded by the ubiquitin-proteasome system, and thereby Dif and Dorsal are released from Cactus and translocate to the nucleus (Ip *et al.*, 1993), where they eventually induce the AMP genes such as *drosomycin* (Engström, 1999).

The Imd pathway

Another major immune control system in *Drosophila* is the Imd pathway (Fig. 2). The *Imd* gene was originally discovered in a survey of immune deficient mutant flies that exhibited lower viability than wild type flies with reduced expression of some AMP genes in response to bacterial challenges (Lemaitre *et al.*, 1995).

Much as in the Toll pathway, PGN recognition by PGRPs is the initial step in the Imd pathway. In this pathway, PGRP-LC recognizes diaminopimelic acid (DAP)-type PGNs, which are components of Gram-negative bacteria and some Gram-positive bacteria (Choe *et al.*, 2002; Gottar *et al.*, 2002; Rämet *et al.*, 2002). Three alternatively spliced isoforms of PGRP-LC, LCa, LCx and LCy, have been identified, and all of them are putative type II transmembrane proteins with common N-terminal cytoplasmic and transmembrane domains but different extracellular domains (Werner *et al.*, 2003; Kaneko *et al.*, 2004). Among these isoforms, PGRP-LCa and PGRP-LCx have been shown to form the LCa-LCx heterodimer and LCx-LCx homodimer, which function in the signal transduction induced by monomeric and polymeric PGNs, respectively (Mellroth *et al.*, 2005). Recent analyses have revealed that PGRP-LE is another player in the recognition of DAP-type PGNs and functions synergistically with PGRP-LC (Takehana *et al.*, 2004; Kaneko *et al.*, 2006). Consistent with the fact that PGRP-LE possesses no predicted transmembrane domain or signal sequence, PGRP-LE is detected in the cytosol and functions as an intracellular receptor for the monomeric DAP-type PGN tracheal cytotoxin (TCT). On the other hand, a version of PGRP-LE (PGRP-LE^{PG}) containing only the PGRP domain that is conserved among all PGRPs is found outside the cell and enhances PGRP-LC-mediated PGN recognition on the cell surface. These findings suggest that *Drosophila* also employs conserved mechanisms for PAMPs recognition using both extracellular and intracellular receptors as in mammals (Sansonetti, 2006).

After the recognition of PGNs, PGRP-LC and PGRP-LE activate intracellular signaling. Imd, a protein with a death domain similar to that of mammalian receptor interacting protein (RIP), appears to be most proximal to the PGRPs, since Imd has been shown to physically interact with PGRP-LC and to a lesser extent with PGRP-LE (Georgel *et al.*, 2001; Choe *et al.*, 2005; Kaneko *et al.*, 2006). Although it remains elusive whether these interactions are indeed required for downstream signaling, Imd subsequently binds to the *Drosophila* Fas-associated death domain-containing protein (dFADD). The Imd-dFADD complex further recruits Dredd, the *Drosophila* ortholog of mammalian caspase-8, and elicits caspase activity of Dredd (Hu and Yang, 2000; Leulier *et al.*, 2000; Leulier *et al.*, 2002; Naitza *et al.*, 2002).

Downstream of the formation of this active protein complex, *Drosophila* TGF-β-activated kinase 1 (DTAK1) is activated by a mechanism not fully understood but involving the *Drosophila* homologs of human ubiquitin-conjugating enzymes Ubc13 and UEV1a (Zhou *et al.*, 2005). DTAK1 then activates the *Drosophila* IκB kinase (DmIKK) complex (Lu *et al.*, 2001; Vidal *et al.*, 2001; Silverman *et al.*, 2003), which in turn conceivably directly phosphorylates the NF-κB-like transcription factor Relish (Silverman *et al.*, 2000). Relish possesses a DNA-binding Rel homology domain but

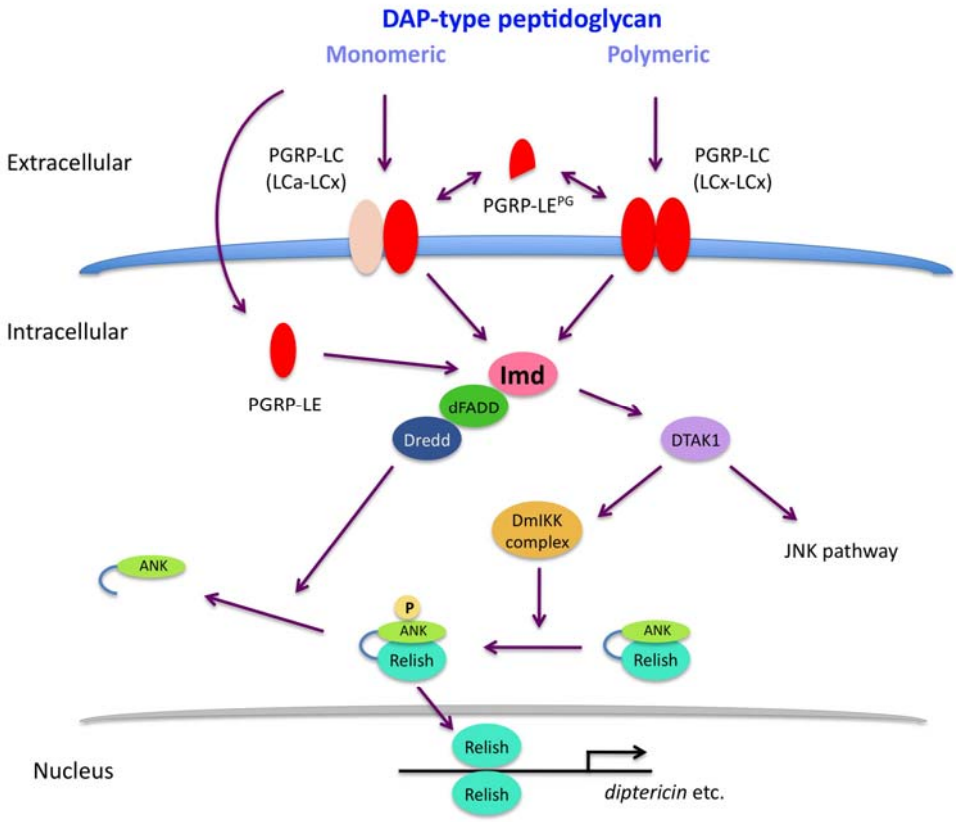

Fig. 2 The Imd pathway. Upon the recognition of DAP-type peptidoglycans of Gram-negative bacteria by pattern recognition proteins, Imd binds to dFADD and Dredd, and the caspase activity of Dredd is induced. DTAK1 is also activated, and activated TAK1 in turn activates the DmIKK complex. Subsequent DmIKK-induced phosphorylation and Dredd-mediated cleavage of Relish generate the mature form of Relish as a transcription factor, which translocates to the nucleus and induces AMP genes such as *diptericin*.

it is masked by an ankyrin repeats-containing IκB-like domain within the same molecule. Phosphorylation of Relish triggers endoproteolytic cleavage of the linker region between these two domains of Relish, probably via the activation of Dredd (Stoven *et al.*, 2003). Finally, Relish translocates to the nucleus and induces AMP genes such as *diptericin* (Cornwell and Kirkpatrick, 2001).

Signaling pathways in the immune response in *C. elegans*

Whereas *Drosophila* Toll is a major player in immune response, as described above, the sole *C. elegans* Toll homolog TOL-1 has been assumed not to function in immune response. Indeed, *tol-1* deletion mutants have been reported not to exhibit altered sensitivity to various pathogens (Pujol *et al.*, 2001). Recently, however, a possible role of TOL-1 in combating bacteria has been proposed, because *tol-1* mutants were shown to be sensitive to *Salmonella enterica* and *Escherichia coli*, and TOL-1 was needed to prevent *S. enterica* from invading into the pharynx (Tenor and Aballay, 2008). However, the downstream signaling remains unknown mainly due to a lack of functionally conserved NF-κB-like factors in *C. elegans*. In this section, therefore, we focus on the DBL-1 and DAF-2/DAF-16 pathways as the major immune control systems in *C. elegans*. Although these pathways are highly conserved from invertebrates to vertebrates, their direct immune functions have not been extensively investigated in other invertebrates than *C. elegans*, except for *Anopheles* mosquitoes, in which these pathways have been shown to be critically involved in innate immunity to malaria parasite infection (Lieber and Luckhart, 2004; Luckhart and Riehle, 2007).

The DBL-1 pathway

In *Drosophila*, induction of the AMP genes upon infection is the most prominent mechanism of defense against pathogens, as described above. Until recently, however, it was not understood whether such inducible anti-pathogen defense systems are also employed in *C. elegans*. Mallo et al. clearly addressed this issue (Mallo *et al.*, 2002). By using a high-density cDNA microarray, they showed that infection of *C. elegans* by *Serratia marcescens* induced an upregulation of the expression of many genes–including those encoding lectins and lysozymes, which are known to be involved in immune response in other organisms, thereby demonstrating

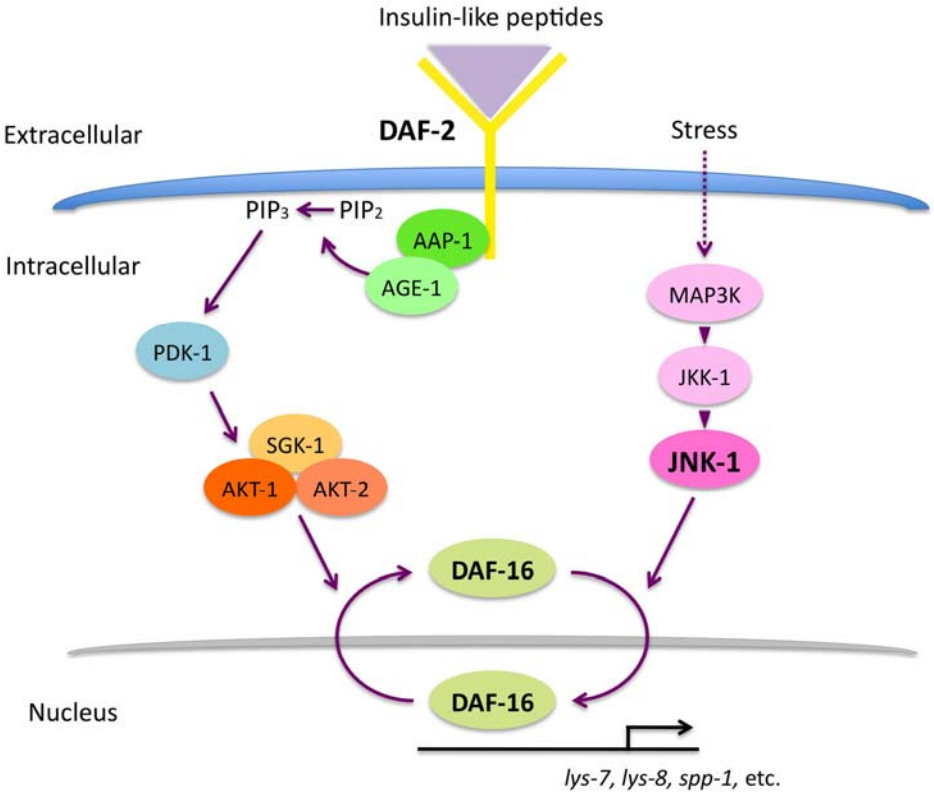

Fig. 3 Functional interaction between the DAF-2/DAF-16 and JNK pathways. The DAF-2/DAF16 pathway starts with the binding of insulin-like peptides, which triggers activation of the PI3 kinase-like complex formed between AGE-1 and AAP-1. AGE-1/AAP-1 then catalyzes the conversion of PI-4,5-P_2 (PIP_2) to PI-3,4,5-P_3 (PIP_3). PDK-1 activated by PIP_3 phosphorylates and thus activates the AKT-1–AKT-2–SGK-1 kinase complex. Finally, this complex phosphorylates and retains DAF-16 in the cytoplasm. In contrast, JNK-1 promotes the translocation of DAF-16 into the nucleus in response to environmental stressors, leading to the expression of numerous target genes to prevent damage to the cell.

for the first time the existence of inducible antibacterial defenses in *C. elegans*. These AMP genes in *C. elegans* have recently been shown to be preferentially expressed in the intestine, which is directly exposed to microbes (Alper *et al.*, 2007). Intriguingly, some of the infection-inducible genes found in this study overlapped with genes under control of the DBL-1 signaling pathway (Mochii *et al.*, 1999). DBL-1 is a transforming growth factor (TGF)-β-related ligand that was first found in the context of body size regulation and male tail patterning (Morita *et al.*, 1999; Suzuki *et al.*, 1999), in accordance with the functions of the TGF-β-family members in body patterning in both invertebrates and vertebrates. Consistent with these gene expression analyses, *dbl-1* mutants have been shown to exhibit increased susceptibility to *S. marcescens* (Mallo *et al.*, 2002). Recently, it has been demonstrated that neuronal expression of DBL-1 promotes expression of the Caenacin family antimicrobial peptides in the epidermis probably in a paracrine manner after infection with the fungus *Drechmeria coniospora*, suggesting the tissue-specific role of DBL-1 in immune response (Zugasti and Ewbank, 2009).

Although it remains to be clarified how the DBL-1 ligand is regulated in response to bacterial infection, it has been speculated that DBL-1 activates the following signaling pathway in its target cells (Nicholas and Hodgkin, 2004). In a manner analogous to the mammalian TGF-β receptor-Smad system (Kawabata and Miyazono, 1999), SMA-6 (type I receptor) and DAF-4 (type II receptor) form a heterodimer in response to DBL-1 stimulation, which induces phosphorylation of SMA-2 and SMA-3, the orthologs of the receptor-regulated Smad proteins, mammalian Smad1 and Smad5, respectively. Then SMA-2 and SMA-3 form a complex with SMA-4, the ortholog of the mammalian common-mediator Smad4, and translocate to the nucleus, leading to the expression of antimicrobial factors (Savage *et al.*, 1996; Krishna *et al.*, 1999). However, it has recently been shown that SMA-3 alone, but neither SMA-2 nor SMA-4, is required for AMP expression in response to *D. coniospora* infection, implying the existence of a non-canonical TGF-β signaling pathway in *C. elegans* (Zugasti and Ewbank, 2009).

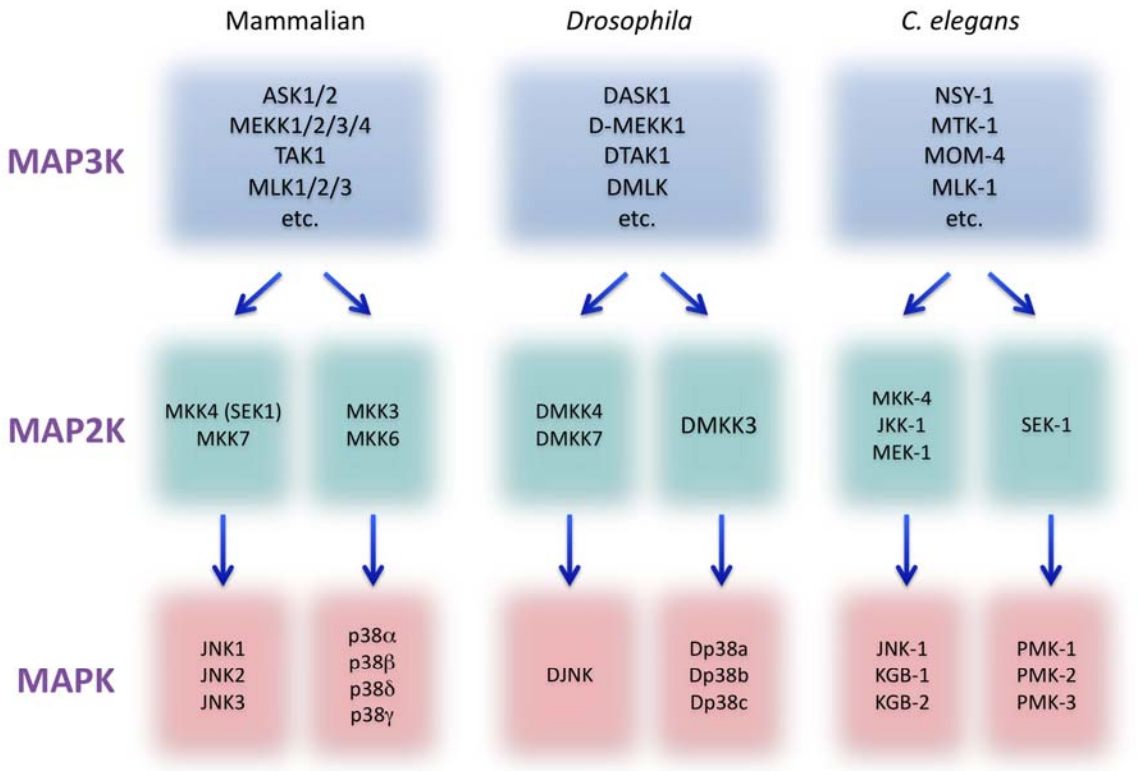

Fig. 4 The stress-responsive MAPK pathways. The stress-responsive MAPK pathways that converge on JNK and p38 in mammalian, *Drosophila* and *C. elegans* are shown. Each pathway consists of three classes of protein kinases: MAPK, MAP2K and MAP3K. MAP3K phosphorylates and thereby activates MAP2K, and activated MAP2K in turn phosphorylates and activates MAPK.

The DAF-2/DAF-16 pathway

DAF-2 and DAF-16 are *C. elegans* orthologs of the mammalian insulin/insulin-like growth factor (IGF)-I receptor and the FOXO family transcription factor, respectively (Kimura *et al.*, 1997; Lin *et al.*, 1997; Ogg *et al.*, 1997). The DAF-2/DAF-16 pathway has been investigated mainly from the viewpoint of metabolism, longevity, and dauer formation, which is an alternate larval stage that worms enter under unfavorable environmental conditions. The best-known phenotype of *daf-2* mutants is the marked increase in life span, but they also exhibit resistance to pathogenic bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Garsin *et al.*, 2003).

The DAF-2/DAF-16 pathway comprises signaling components highly homologous to those in well-characterized mammalian insulin/IGF-I signaling. This pathway starts with the binding of insulin-like peptides to DAF-2, which triggers the activation of AGE-1 and AAP-1, the orthologs of the p110 catalytic and p55 regulatory subunits of mammalian PI3 kinase, respectively (Morris *et al.*, 1996; Wolkow *et al.*, 2002) (Fig. 3). AGE-1/AAP-1 then catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) to phosphatidyl-3,4,5-trisphosphate (PIP_3) (Engelman *et al.*, 2006). PIP_3 activates the 3-phosphoinositide-dependent kinase-1 homolog PDK-1 (Paradis *et al.*, 1999), which in turn phosphorylates and thus activates the AKT-1–AKT-2–SGK-1 kinase complex (Hertweck *et al.*, 2004). Finally, this AKT complex phosphorylates and retains DAF-16 in the cytoplasm (Paradis and Ruvkun, 1998; Lin *et al.*, 2001).

In the presence of DAF-2 antagonists or perturbation of DAF-2 signaling, DAF-16 stays in the nucleus, which is a prerequisite for the induction of a wide variety of genes, including those regulating metabolism and cellular stress response (Murphy *et al.*, 2003). Importantly, antimicrobial genes, such as the antibacterial lysozyme genes *lys-7* and *lys-8*, which have been shown to be induced upon infection with *S. marcescens* (Mallo *et al.*, 2002), and the saposin-like gene *spp-1*, which has been shown to have antibacterial activity (Bányai and Patthy, 1998), are also the targets of DAF-16. This finding suggests that insulin-like signaling in *C. elegans* counteracts the antibacterial activity through DAF-16-dependent gene induction, and provides a reasonable explanation for the previous finding that *daf-2* mutants exhibited increased resistance to pathogenic bacteria (Garsin *et al.*, 2003). Consistent with these findings, *P. aeruginosa* was found to suppress DAF-16-dependent immune response by activating the DAF-2 pathway, probably through the induction of the insulin-like peptide INS-7 (Evans *et*

al., 2008), further demonstrating the importance of the DAF-2/DAF-16 pathway in the immune response in *C. elegans*. In most literature, DAF-16 regulation is mainly observed in the intestinal cells where it is feasible to detect nuclear translocation of DAF-16, whereas regulation in other tissues remains to be elucidated.

The MAPK pathways

The MAPK pathways are signal transduction systems evolutionarily conserved in all eukaryotic organisms, and consist of three classes of protein kinases: MAPK, MAPK kinase (MAP2K) and MAP2K kinase (MAP3K) (Widmann *et al.*, 1999; Kyriakis and Avruch, 2001). MAP3K phosphorylates and thereby activates MAP2K, and activated MAP2K in turn phosphorylates and activates MAPK. Through these sequential biochemical events, the signals are amplified and diversified in a step-by-step manner (Fig. 4). The stress-responsive MAPK pathways that converge on the MAPKs, JNK and p38, are activated by various environmental stressors such as heat shock, UV irradiation, oxidative stress and osmotic shock. In response to these stressors, these pathways induce a wide variety of cellular responses such as DNA repair, cell cycle arrest and apoptosis. These pathways are also activated in response to pathogen challenges and mediate immune responses. In this section, the roles of the stress-responsive MAPK pathways in immune and stress response in *Drosophila* and *C. elegans* are reviewed, although such roles have been assigned to highly conserved MAPK pathways.

The MAPK pathways in immune response

DTAK1, which is the activator of the IKK complex in the *Drosophila* Imd pathway as mentioned above, is a member of the *Drosophila* MAP3K family and activates the *Drosophila* JNK (DJNK) pathway (Boutros *et al.*, 2002; Silverman *et al.*, 2003). When lipopolysaccharides (LPS), the principal cell wall components of Gram-negative bacteria, are applied to adult flies and cultured cells, DJNK is activated downstream of DTAK1 and induces immune-related genes such as those encoding cytoskeletal regulators and pro-apoptotic signaling (Sluss *et al.*, 1996; Boutros *et al.*, 2002). However, it is still controversial whether the DTAK1-DJNK axis directly induces the AMP genes (Silverman *et al.*, 2003; Kallio *et al.*, 2005; Delaney *et al.*, 2006).

In addition to this transcriptional control, it has recently been proposed that the DJNK pathway negatively controls the transcription of a group of genes mediated by the IKK-Relish axis, another branch downstream of DTAK1 (Kim *et al.*, 2005). Conversely, the DJNK pathway has been shown to be negatively regulated by Relish-induced proteasomal degradation of DTAK1 (Park *et al.*, 2004). This cross talk between two branches downstream of DTAK1 appears to be critical to determine the duration, *i.e.*, transient versus sustained, of gene induction in the immune response. Thus, DJNK is directly or indirectly involved in the control of a wide variety of genes, including the AMP genes, and therefore is a critical regulator of immune response in *Drosophila.*

The *Drosophila* p38 (Dp38) pathway is also involved in immune response. Among the three isoforms of Dp38 identified so far - Dp38a, Dp38b and Dp38c - Dp38a and Dp38b have mainly been examined in their role as mediators of immune and stress response in *Drosophila*. Treatment of *Drosophila* cultured cells with the p38 inhibitor SB203580, which inhibits both Dp38a and Dp38b, has been shown to enhance LPS-induced AMP gene expression, while overexpression of Dp38a reduced this expression in flies infected with bacteria (Han *et al.*, 1998b). However, the recent analysis of mutant flies deficient in Dp38a has revealed that Dp38a is not required for survival or for AMP gene induction following infection with several bacteria (Craig *et al.*, 2004). Nevertheless, the latter finding does not necessarily exclude the relevance of the Dp38 pathway in immune response, because Dp38b and/or Dp38c may compensate for the deficiency of Dp38a. Consistent with this hypothesis, a possible immune function of Dp38c was recently proposed. In response to bacterial septic injury, Dp38c has been shown to mediate the induction of the *Dopa decarboxylase (Ddc)* gene. Ddc catalyses the production of dopamine, which is metabolized to produce reactive quinones that kill invading microorganisms, and thus Dp38c may upregulate quinones via the *Ddc* gene (Davis *et al.*, 2008).

In *C. elegans*, it remains unknown whether the JNK pathway is directly involved in innate immune response. On the other hand, the p38 ortholog PMK-1 has received much attention as a regulator in innate immunity. PMK-1 has been characterized as a MAPK functioning downstream of the MAP2K SEK-1 and the MAP3K NSY-1, orthologs of mammalian MKK3/MKK6 and ASK1, respectively. NSY-1 and SEK-1 were first identified as critical regulators of cell fate determination in a subset of olfactory neurons (Sagasti *et al.*, 2001; Tanaka-Hino *et al.*, 2002). The NSY-1-SEK-1-PMK-1 pathway was later found to play a crucial role in innate immune response (Kim *et al.*, 2002). Loss-of-function mutation of or RNAi against these genes clearly increases susceptibility to *P. aeruginosa*. However, mutation of *unc-43*, which encodes a Ca^{2+}/calmodulin-dependent protein kinase acting upstream of NSY-1 in cell fate determination in neurons (Sagasti *et al.*, 2001), did not result in enhanced susceptibility. Instead, TIR-1, the *C. elegans* ortholog of mammalian Toll-interleukin 1 receptor (TIR) domain protein SARM, appears to regulate the NSY-1-SEK-1-PMK-1 pathway and to be required for survival and for the expression of AMP genes following bacterial infection (Couillault *et al.*, 2004; Liberati *et al.*, 2004). Moreover, TIR-1, NSY-1 and SEK-1 were found to physically interact with each other (Tanaka-Hino *et al.*, 2002; Chuang and Bargmann, 2005). These findings suggest that TIR-1 plays a pivotal role in innate immune response through activation of the NSY-1-SEK-1-PMK-1 pathway. Whereas the immune function of this pathway has been proposed to exert in the intestine (Sakaguchi *et al.*, 2004), this pathway has recently been shown to play a critical role in response to wounding as well as infection in the epidermis (Pujol *et al.*, 2008).

The MAPK pathways in stress response

Apart from biotic stressors such as the bacterial components, various abiotic stressors also activate the JNK and p38 pathways. In *Drosophila*, Dp38 is activated by heat shock, UV irradiation, osmotic shock and oxidative stress in the cultured cells, and *Dp38a* mutant flies are consistently susceptible to some of these stressors, indicating that Dp38 confers stress resistance to flies (Han *et al.*, 1998a; Han *et al.*, 1998b; Craig *et al.*, 2004; Zhuang *et al.*, 2006). DJNK is also activated by stressors similar to those that activate Dp38 in the cultured cells (Botella *et al.*, 2001; Chen *et al.*, 2002; Ryabinina *et al.*, 2006). It has been shown using genetically engineered flies that DJNK activity alleviates the toxic effects of reactive oxygen species (ROS), probably through the induction of various categories of protective genes, thereby reducing the accumulation of oxidative damage and prolonging the lifespan of flies (Wang *et al.*, 2003). Recently, it has been shown that DJNK suppresses the insulin/IGF-I signaling and activates DFoxo, the counterpart of *C. elegans* DAF-16, in response to oxidative stress. DJNK suppressed expression of insulin-like peptides in neuroendocrine cells in the brain, called the insulin-producing cells (IPCs), whereas DJNK induced expression of target genes of DFoxo in the cells of peripheral tissues. This functional interaction between DJNK and the insulin/IGF-I signaling appears to contribute to the protective role of DJNK against oxidative stress (Wang *et al.*, 2005).

Also in *C. elegans*, DAF-16 is an important target molecule of JNK-1, an isoform among three JNK-like kinases in *C. elegans* (Fig. 3). In response to environmental stressors such as heat shock, UV irradiation and excess ROS, JNK-1 was found to directly interact with and phosphorylate DAF-16 and promote the translocation of DAF-16 into the nucleus, leading to the expression of numerous target genes to prevent damage from harmful stressors (Oh *et al.*, 2005; Wolf *et al.*, 2008). Intriguingly, the JNK pathway acted in parallel with the DAF-2 pathway to regulate lifespan, and both pathways converged on DAF-16. Moreover, in mammals, the nuclear translocation and transcriptional activation of FOXO4, a member of the mammalian FOXO family, in response to low levels of oxidative stress have been shown to be mediated through phosphorylation of FOXO4 by JNK (Essers *et al.*, 2004). Thus, this interaction between the JNK and insulin/IGF-I pathways is functionally conserved among species and suggests that signaling pathways for stress response and lifespan are closely regulated by each other.

Heavy metals are well-studied stressors used in the analysis of stress response in *C. elegans*. Mutants of the components of the JNK pathway have been shown to display elevated sensitivity to copper and cadmium, although the tissue(s) where the JNK pathway functions is unclear (Koga *et al.*, 2000; Villanueva *et al.*, 2001; Mizuno *et al.*, 2004). Importantly, a *daf-2* mutant and an *age-1* mutant gained resistance to these metals, suggesting that the functional interaction between the JNK and DAF-2 pathways is critical for the response to heavy metals as well (Barsyte *et al.*, 2001).

Perspectives and conclusions

To take an overview of signaling pathways in invertebrate immune and stress response, we here focused on *Drosophila* and *C. elegans* as suitable model organisms. However, we must note that a large body of evidence from other useful invertebrate models supports the important findings obtained using *Drosophila* and *C. elegans.*

As the present review has shown, these two organisms rely on different signaling pathways for their immune response, *i.e.*, the Toll and Imd pathways versus the DBL-1 and DAF-2/DAF-16 pathways. This appears to depend at least in part on the existence or absence of the NF-κB-like factors and their regulatory systems. At the same time, the stress-responsive MAPK pathways are utilized by both *Drosophila* and *C. elegans* to regulate immune and stress response. Taken together with the highly conserved functional interaction between the MAPK and insulin/IGF-I pathways, this suggests that the MAPK pathways might play pivotal roles in integrating the other diverged and specialized signaling pathways with reference to the external and internal stress conditions. Further investigation of such signal networks in invertebrate models will surely shed new light on the highly complex systems regulating immune and stress response in mammals.

Acknowledgements

We thank all of the members of the Laboratory of Cell Signaling for their valuable input.

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