NF- κ B in the Immune Response of *Drosophila*

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The nuclear factor κ B (NF- κ B) pathways play a major role in *Drosophila* host defense. Two recognition and signaling cascades control this immune response. The Toll pathway is activated by Gram-positive bacteria and by fungi, whereas the immune deficiency (Imd) pathway responds to Gram-negative bacterial infection. The basic mechanisms of recognition of these various types of microbial infections by the adult fly are now globally understood. Even though some elements are missing in the intracellular pathways, numerous proteins and interactions have been identified. In this article, we present a general picture of the immune functions of NF- κ B in *Drosophila* with all the partners involved in recognition and in the signaling cascades.

The paramount roles of NF- κ B family members in *Drosophila* development and host defense are now relatively well established and have been the subject of several in-depth reviews in recent years, including some from this laboratory (e.g., Hoffmann 2003; Minakhina and Steward 2006; Ferrandon et al. 2007; Lemaitre and Hoffmann 2007; Aggarwal and Silverman 2008). To avoid excessive duplication, we limit this text to the general picture that has evolved over nearly two decades—since the initial demonstration that the *dorsal* gene plays a role in dorsoventral patterning in embryogenesis of *Drosophila* and that it encodes a member of the NF- κ B family of inducible transactivators (Nüsslein-Volhard et al. 1980; Steward 1987; Roth et al. 1989). In the early nineties, it became apparent that NF- κ B also plays a role in the antimicrobial host

defense of *Drosophila* (Engström et al. 1993; Ip et al. 1993; Kappler et al. 1993; Reichhart et al. 1993). We focus in this article on the immune functions of NF- κ B and refer the reader to recent reviews for the roles of NF- κ B in development (Roth 2003; Brennan and Anderson 2004; Moussian and Roth 2005; Minakhina and Steward 2006).

The *Drosophila* genome codes for three NF- κ B family members (Fig. 1). Dorsal and DIF (for dorsal-related immunity factor) are 70 kDa proteins, with a typical Rel homology domain, which is 45% identical to that of the mammalian counterparts c-Rel, Rel A, and Rel B. Dorsal and DIF lie some 10 kbp apart on the second chromosome and probably arose from a recent duplication (Meng et al. 1999). Both proteins are retained in the cytoplasm by binding to the same 54-kDa inhibitor

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Figure 1. The NF- κ B and I κ B proteins in *Drosophila*. The length in amino acids is indicated by numbers. REL, Rel-homology domain; NLS, nuclear localization sequence; PEST, proline, glutamic acid, serine, and threonine-rich segment; Ac, acidic domain.

protein Cactus, which is homologous to mammalian I κ Bs (Schüpbach and Wieshaus 1989; Geisler et al. 1992). The single *Drosophila* Cactus gene is closest to mammalian I κ B α (Huguet et al. 1997). The third member of the family in *Drosophila*, Relish, is a 100-kDa protein with an amino-terminal Rel domain and a carboxy-terminal extension with typical ankyrin repeats, as found in Cactus and mammalian I κ Bs. Relish is similar to mammalian p100 and p105 and its activation requires proteolytic cleavage as in the case for these mammalian counterparts (reviewed in Hultmark 2003).

Put in simple terms, NF- κ B family members function in the host defense of *Drosophila* to control the expression of genes encoding immune-responsive peptides and proteins. Prominent among the induced genes are those encoding peptides with direct antimicrobial activity. To exert this function, Dorsal and DIF are translocated to the nucleus following stimulus-induced degradation of the inhibitor Cactus, whereas Relish requires stimulus-induced proteolytic cleavage for nuclear translocation of its amino-terminal Rel domain. This paradigm is similar to that observed in mammalian immunity. Again, for the sake of simplicity, we may say that the stimulus-induced degradation of Cactus, and the concomitant release of Dorsal or DIF, is primarily observed during Gram-positive bacterial and fungal infections and mediated by the Toll signaling pathway. In contrast, stimulus-induced

proteolytic cleavage of Relish, and concomitant nuclear translocation of its amino-terminal Rel domain, is the hallmark of the response to Gram-negative bacterial infection and mediated by the Imd signaling pathway. Whether these pathways are also involved in the multifaceted defense against viruses remains an open question (Zamboni et al. 2005). The Toll pathway was further shown to be involved in hematopoiesis of flies (Qiu et al. 1998). Of note, the Cactus-NF- κ B module also plays a central role in the elimination of *Plasmodium* parasites in infected mosquitoes (Frolet et al. 2006). In the following, we review our information of the two established signaling pathways, Toll and Imd, which lead to gene reprogramming through NF- κ B in response to bacterial and fungal infections. We first consider the upstream mechanisms that mediate the recognition of infection and allow for a certain level of discrimination between invading microorganisms. Gene reprogramming in this context is best illustrated by the induction of the antimicrobial peptide genes, which serve as the most convenient readouts of the antimicrobial defense of *Drosophila* (see Samakovlis et al. 1990; Reichhart et al. 1992; Ferrandon et al. 1998). Flies produce at least seven families of mostly cationic, small-sized, membrane-active peptides, with spectra variously directed against Gram-positive (defensins) and Gram-negative (dipterocins, attacins, and drosocin) bacteria, and against fungi (drosomycins and metchnikowins), or with overlapping spectra

(cecropins) (reviewed in Bulet et al. 1999; Hetru et al. 2003). The primary site of biosynthesis of these peptides is the fat body, a functional equivalent of the mammalian liver. Blood cells also participate in the production of antimicrobial peptides. As a rule, these molecules are secreted into the hemolymph where they reach remarkably high concentrations to oppose invading microorganisms (Hetru et al. 2003). This facet of the antimicrobial host defense is generally referred to as systemic immune response. Of note, the gut and the tracheae also produce antimicrobial peptides in response to microbes (see Tzou et al. 2000; Onfelt Tingvall et al. 2001; Liehl et al. 2006; Nehme et al. 2007).

During infection, the Toll and Imd pathways control the expression of hundreds of genes. In addition to the antimicrobial peptides, these genes encode proteases, putative cytokines, cytoskeletal proteins, and many peptides and proteins whose function in the host defense are still not understood (De Gregorio et al. 2001; Irving et al. 2001).

ACTIVATION OF NF- κ B VIA THE TOLL SIGNALING CASCADE

The Toll signaling cascade is activated by Gram-positive bacterial peptidoglycan and by fungal β -(1,3)-glucan (Ochiai and Ashida 2000; Michel et al. 2001; Leulier et al. 2003; Gottar et al. 2006). In contrast to initial assumptions, these microbial inducers do not directly interact with the Toll transmembrane receptor, but with circulating proteins belonging to two distinct families: The peptidoglycan recognition proteins (PGRPs) (Kang et al. 1998; Werner et al. 2000; Steiner 2004) and the glucan-binding proteins (Lee et al. 1996; Gobert et al. 2003; Gottar et al. 2006; Wang et al. 2006) (GNBPs, formerly referred to as Gram-negative binding proteins—the acronym is preserved here for consistency in the literature and now noted as glucan binding proteins). Binding of the microbial inducers to these proteins results in the activation of proteolytic cascades, which culminate in the cleavage of the cytokine Spaetzle

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and binding of cleaved Spaetzle to the Toll receptor, thereby triggering the intracytoplasmic signaling cascade.

The *Drosophila* genome encodes 13 members of the peptidoglycan recognition protein family (Fig. 2A), and one of these can give rise to three splice isoforms (PGRP-LC, see later) (Werner et al. 2000; Kaneko et al. 2004; Piao et al. 2005). They all share an evolutionary conserved domain related to bacteriophage type II amidases. This amidase function is retained in the majority of PGRPs (Mellroth et al. 2003; Steiner 2004), whereas some have lost the amino acids required for amidase activity and function as recognition PGRPs (Kim et al. 2003). Recognition PGRPs can discriminate between the predominant PGN of Gram-positive bacteria, which is characterized by a lysine residue in position three of the stem peptide, and the PGN of Gram-negative bacteria (and of Gram-positive bacilli), which carries a diaminopimelic acid (DAP) in the same position of the stem peptide (Fig. 2B) (Leulier et al. 2003). It is remarkable that this small difference, in fact the addition in DAP-PGN of a carboxyl in α -position of an amine of Lysine, is sufficient to discriminate between two large groups of pathogens and to trigger distinct signaling cascades and gene expression programs. Prototypical PGRPs recognizing Gram-positive bacterial peptidoglycan are the circulating PGRP-SA (Michel et al. 2001) and PGRP-SD (Bischoff et al. 2004), whereas an essential recognition protein for Gram-negative peptidoglycan is the transmembrane protein PGRP-LC (Choe et al. 2002; Gottar et al. 2002; Ramet et al. 2002). Three GNBPs are present in the *Drosophila* genome (Fig. 3). They have in common an amino-terminal domain, which in GGBP-3 mediates recognition of polymeric glucans, and a carboxy-terminal glucanase-like domain, which has lost its catalytic activity (Wang et al. 2006; A. Roussel, pers. comm.).

Binding of Lys-PGN to PGRP-SA in circulation activates a cascade of CLIP-domain zymogens (Piao et al. 2005), which includes the serine proteases Grass and Spirit (Kambris et al. 2006; El Chamy et al. 2008), and leads

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Figure 3. GNBP1 and GNBP3.

to the activation of the zymogen spaetzle-processing enzyme (SPE) (Jang et al. 2006). SPE in turn cleaves Spaetzle, a 37-kDa polypeptide with nine cysteines, engaged in four intramolecular disulfide bridges folded in a typical cystine-knot array (Fig. 4) (Mizuguchi et al. 1998). The additional cysteine residue allows for covalent dimerization of Spaetzle (DeLotto and DeLotto 1998). Cleavage of Spaetzle by SPE occurs amino-terminally outside the cystine-knot domain and the dimeric carboxy-terminal fragment binds to Toll, inducing the dimerization of the transmembrane receptor, thereby triggering activation of the downstream signaling cascade (Mizuguchi et al. 1998; Weber et al. 2003, 2005).

For reasons that are not fully understood, Lys-PGN activation of the PGRP-SA-dependent zymogen cascade requires the concomitant presence of one of the members of the GGBP family (GNBP-1) (Gobert et al. 2003; Pili-Floury et al. 2004; Wang et al. 2006). Further, PGRP-SD, another circulating member of the Lys-PGN recognition proteins, can substitute for PGRP-SA or potentiate its role (Bischoff et al. 2004). How recognition of the microbial ligands to the cognate binding proteins translates into activation of the downstream zymogen cascades remains to be established (Fig. 4).

Fungal or yeast glucans, which are also potent activators of the Toll pathway, interact with GNBP-3 and in turn trigger a zymogen cascade, which activates the serine protease SPE, and leads to the cleavage of Spaetzle and to Toll activation. This zymogen cascade shares some of the serine proteases identified downstream of PGRP-SA.

Of major potential interest are recent observations that fungal proteases can activate the circulating zymogen Persephone (Ligoxygakis et al. 2002) during the process of infection, mediating activation of SPE and of Toll. This pathogen-induced activation of Toll is not directly dependent on recognition of microbial cell wall components, but rather on microbial virulence factors (Gottar et al. 2006). It can be mimicked, again in a Persephone-dependent way, through injection of exogenous subtilisin, hence the proposal that Persephone is responsive to a danger signal (Fig. 4) (El Chamy et al. 2008).

The *Drosophila* transmembrane receptor Toll is a member of a family of nine receptors, which are involved in developmental processes during embryogenesis and probably later in development (see Eldon et al. 1994; Tauszig et al. 2000; Kambris et al. 2002; Gay and Gangloff 2007). Toll has an extracytoplasmic domain with numerous leucine-rich repeats (LRR) of which several contain cysteines. Of note, Toll9 has a single cysteine-containing LRR in proximity of the plasma membrane, as is the case in mammalian Toll-like receptors (see Fig. 5). The intracytoplasmic domain of Toll is homologous to the intracytoplasmic signaling domain of the mammalian interleukin-1 receptor and of all mammalian TLRs (and is referred to as TIR domain [Hashimoto et al. 1988]). Interaction of dimeric, cleaved Spaetzle leads to dimerization of Toll and its TIR domains, which in turn interact with a platform of three distinct death domain containing proteins, dMyD88, Tube, and Pelle (Fig. 6) (Lemaitre et al. 1996; Tauszig-Delemasure et al. 2002). dMyD88 is homologous to mammalian MyD88, and interacts through

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Figure 4. Induction of Toll and Imd pathway by pathogens. Gram-positive bacteria are recognized by PGRP-SA with the cooperation of GNBP1 and PGRP-SD. This recognition results in activation of a proteolytic cascade culminating in the cleavage of Spaetzle via the serine protease Spaetzle Processing Enzyme (SPE). Dimeric cleaved Spaetzle binds to the Toll receptor, activating the intracellular signaling cascade shown in Figure 6. Fungi are recognized by GNBP3 and induce a proteolytic cascade, which also activates SPE. Entomopathogenic fungi have been shown to also activate the Toll pathway through secreted proteases, which activate the circulating zymogen Persephone (virulence factor). Gram-negative bacteria are directly detected by the transmembrane receptor PGRP-LC, which recognizes the DAP-type peptidoglycan. The amidase PGRP-LB and PGRP-SC degrade PGN to nonimmunogenic moieties (Fig. 2).

its TIR domain with the TIR domain of Toll. Through its death domain, it associates with the death domain of Tube. Tube has a bifunctional death domain, which allows it to also interact with the death domain of Pelle (Sun et al. 2004), which is a member of the IL-1R associated kinase (IRAK) family of serine-threonine kinases. In the process of activation of the Toll signaling cascade, Cactus is phosphorylated by an as yet unidentified kinase; Pelle does apparently not fulfill this role. Phosphorylated Cactus undergoes K48 ubiquitination and is degraded by the proteasome (Belvin et al. 1995; Fernandez et al. 2001).

Dorsal and/or DIF are thus relieved from their inhibition and translocate to the nucleus where they bind to κ B-response elements and transactivate a specific set of genes (Bergmann et al. 1996; Reach et al. 1996), namely the drosomycin gene, which is often used as a readout for Toll pathway activation. Genetic evidence points to DIF as the major transactivator in Toll-dependent defenses in adults, whereas Dorsal can substitute for DIF in larvae (Rutschmann et al. 2000a).

NF- κ B activation by Toll has relatively slow kinetics, with maximum levels at 24–48 h (if transcript levels of the drosomycin gene are

Figure 5. Representatives of the Toll and the TLR families.

used as readouts) (Lemaitre et al. 1997). Negative regulation of this response during infection has only been marginally investigated. The serpin necrotic, which blocks the serine protease Persephone upstream of Spaetzle, is strongly induced during Toll pathway activation, potentially contributing to down-regulation of the pathway (Irving et al. 2001).

This short overview of NF- κ B activation by the Toll pathway in response to microbial infections calls for several comments: (1) the parallels between some developmental regulations and this immune response are remarkable. We now know that Spaetzle is a member of a family of several neurotrophins, some of which (including Spaetzle) have been shown to play neurotrophic functions (Parker et al. 2001; Zhu et al. 2008). Of the five presently established members of the *Drosophila* neurotrophin-Spaetzle family, and of the nine Tolls,

which are functional in flies, apparently only the Spaetzle-Toll couple was recruited to serve an immune function, in addition to its developmental roles; (2) neurotrophins (including Spaetzle) require, like many cytokines and growth factors, proteolytic cleavage to become active ligands. In the embryonic zymogen cascade (not discussed here, but see Moussian and Roth 2005, for instance), a small molecular weight compound initiates the cascade, whereas in the immune response, small bacterial or cell wall components similarly trigger a comparable cascade; (3) as regards the intracytoplasmic signaling cascade, the various players appear mostly identical, but it is obvious that our information here is still fragmentary. For one, the actual Cactus-kinase has not yet been identified and there are reasons to expect that K63 ubiquitination plays a major role in stabilization/scaffolding of the activating intracytoplasmic

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Figure 6. Toll pathway in adult *Drosophila melanogaster*. Signaling through Toll involves the receptor/adaptor complex, composed of three death-domain-containing proteins, MyD88, Tube, and Pelle. The trimeric complex assembles around the bipartite DD of Tube. It is unclear how the signal is transduced from the complex to Cactus, a homolog of I κ B. Phosphorylated Cactus is degraded after polyubiquitination by the proteasome. The liberated DIF is translocated to the nucleus and binds to NF- κ B response elements, inducing the expression of hundreds of genes, namely those encoding antimicrobial peptides, such as drosomycin.

complexes. Direct phosphorylation has also been proposed to confer transactivating potential to Dorsal, independently of the release from Cactus (Drier et al. 2000). In essence, however, our view remains still superficial and much additional work will be required to fully understand how this pathway functions, both in immunity and development.

ACTIVATION OF NF- κ B BY THE IMD PATHWAY

Whereas activation of the Toll pathway is initiated by interaction of microbial ligands with circulating proteins, the Imd pathway is triggered on direct interaction of the transmembrane receptor PGRP-LC (Gottar et al. 2002; Choe et al. 2002; Ramet et al. 2002; Choe et al. 2005) with Gram-negative bacterial peptidoglycan (diaminopimelic peptidoglycan-DAP-PGN) (Fig. 3). The 55 kDa PGRP-LC consists of an extracytoplasmic part that harbors the conserved peptidoglycan recognition domain,

a short transmembrane domain of 22 amino acids, and an intracytoplasmic signaling domain. The PGRP-LC gene can generate three distinct splice isoforms, giving rise to three types of receptors with identical transmembrane and intracytoplasmic sequences and slightly different exodomains, and referred to as LCa, LCx, and LCy (Werner et al. 2000). It is now understood that PGRP-LCx homodimers sense polymeric DAP-PGN and that LCx-LCa heterodimers detect short PGN end fragments (Kaneko et al. 2004). Such short PGN fragments are released by bacteria during growth and division (for a detailed structural study of the interaction of PGRP-LC isoforms and DAP-PGN, see Chang et al. 2006; Lim et al. 2006). Binding of monomeric and multimeric DAP-PGN to the cognate receptors induces their dimerization or oligomerization and leads to signaling (Mellroth et al. 2005; Chang et al. 2006; Lim et al. 2006).

In addition to PGRP-LC, other members of the PGRP family appear to play an upstream role in the Imd pathway. One of these is PGRP-

LE (Takehana et al. 2002), which can act as an intracellular receptor for monomeric peptidoglycan (Kaneko et al. 2006). A naturally truncated form of LE, containing only the PGRP domain, functions extracellularly to enhance PGRP-LC-mediated recognition on the cell surface (Takehana et al. 2004). The membrane-associated PGRP-LF acts as a specific negative regulator of the Imd pathway: Reduction of PGRP-LF levels, in the absence of infection, is sufficient to trigger Imd pathway activation (Maillet et al. 2008). Furthermore, normal development is impaired in the absence of functional PGRP-LF, a phenotype which is mediated by the JNK pathway (Maillet et al. 2008) (see the following).

The intracytoplasmic cascade (Fig. 7) of the Imd pathway starts with the recruitment of the 25 kDa death domain protein Imd (of note, the sequence of this particular death domain is closest to that of mammalian RIP1 that is TNF-receptor interacting protein) (Lemaitre

et al. 1995; Georgel et al. 2001). Both the intracytoplasmic domain of PGRP-LC and the adaptor protein Imd contain a so-called RHIM domain (for receptor interacting protein [RIP] homotypic interaction motif) required for signaling, but not for their respective interaction, which probably requires an additional as yet unidentified partner (Kaneko et al. 2006; Choe et al. 2005). Imd further associates with the mammalian homolog of FADD and with the caspase-8 homolog DREDD (Leulier et al. 2000, 2002). Through mechanisms not fully understood at present, but which are likely to involve K63 ubiquitination, this upstream receptor–adaptor complex activates the MAP3kinase TAK1 (Zhou et al. 2005). TAK1 is associated with the homolog of mammalian TAB2, which has a conserved sequence domain known to interact with K63 polyubiquitin chains (Boutros et al. 2002; Silverman et al. 2003; Gesellchen et al. 2005; Geuking et al. 2005; Kleino et al. 2005).

Figure 7. Imd pathway in adult *Drosophila melanogaster*. The transmembrane protein PGRP-LC senses the presence of DAP-type PGN and activates the Imd pathway. Imd has an essential role in controlling the phosphorylation of Relish through the activation of TAK1 and the IKK signalosome. Cleavage of phosphorylated Relish is dependent on the caspase Dredd. The activation of TAK1 and the IKK signalosome requires several proteins, including FADD and Dredd. Cleaved Relish is translocated in the nucleus and binds to NF- κ B response elements, inducing the expression of numerous genes encoding immune response proteins, namely antimicrobial peptides such as Diptericin. In addition to phosphorylating the IKK complex, TAK1 also acts as a JNK kinase and activates the JNK pathway.

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Downstream of the TAK1/TAB2 protein complex, the Imd pathway branches into a signaling cascade, leading to Relish activation and, a second, to JNK activation (Vidal et al. 2001; Zhuang et al. 2006). In contrast to the Toll pathway, the Imd-Relish cascade relies on an IKK complex, which consists of homologs of both IKK β (referred to as Ird5) (Wu and Anderson 1998; Silverman et al. 2000; Lu et al. 2001) and IKK γ /NEMO (referred to as kenny) (Rutschmann et al. 2000b). Once activated by TAK1, the IKK complex phosphorylates the NF- κ B protein Relish on specific serine residues and phosphorylated Relish is cleaved into an amino-terminal transcriptional regulatory domain, which translocates to the nucleus where it binds to Relish response elements and directs expression of dedicated genes (Stoven et al. 2000; De Gregorio et al. 2002). Interestingly, a κ B sequence code has been identified in the promoters of immune-responsive genes, which directs binding of the NF- κ B family members of *Drosophila* and thereby induces specialized programs of gene expression in the Toll and Imd pathway (Busse et al. 2007). The carboxy-terminal fragment remains in the cytoplasm. It is probable that the caspase-8 homolog DREDD, after its initial association with the death domain protein dFADD, is responsible for cleavage of Relish (Leulier et al. 2002; Stöven et al. 2003). A recent study has identified a 32 kDa highly conserved protein, Akirin, which acts in conjunction with Relish to control Imd pathway dependent gene transcription (Goto et al. 2008).

TAK1 activates the JNK branch of the Imd pathway by signaling to Hemipterous, a *Drosophila* homolog of JNKK, which phosphorylates basket (dJNK), resulting in the activation of *Drosophila* AP-1 (Boutros et al. 2002; Silverman et al. 2003; Park et al. 2004). The exact role of the JNK branch in the host defense of *Drosophila* is not firmly established. The gene set up-regulated by this branch leads to production of cytoskeletal proteins (probably involved in wound repair) and in proapoptotic signaling (Delaney et al. 2006). These genes exhibit transient induction kinetics, with peak values at 1 h postinduction.

Negative regulation of JNK can be mediated by Relish-dependent degradation of TAK1 (Park et al. 2004).

The kinetics of the Imd-Relish pathway are rapid as compared to those of the Toll pathway, and activation, to judge from the transcription profiles of antimicrobial peptide genes, is relatively short-lived (peak values at 6 h postinduction) (Lemaitre et al. 1997). Recent investigations point to several levels of negative control of the Imd pathway. For one, down-regulation of the highly immunogenic DAP-PGN has been documented for several of the PGRPs that have retained their amidase activity and cleave PGN between the glucan chains and the stem peptides to nonimmunogenic fragments (Mellroth et al. 2003; Mellroth and Steiner 2006; Zaidman-Remy et al. 2006). This is in particular the case for PGRP-LB, SB1, and SC1. As noted above, PGRP-LF also serves as a negative regulator of this pathway. Other levels of negative regulation are the recently identified proteins Pirk (Aggarwal et al. 2008; Kleino et al. 2008; Lhocine et al. 2008) and Caspar (Kim et al. 2006). Pirk is a cytoplasmic protein that co-immunoprecipitates with Imd and the cytoplasmic tail of PGRP-LC. Down-regulation of Pirk results in hyperactivation of the Imd pathway on infection with Gram-negative bacteria, whereas overexpression of Pirk reduces the Imd pathway responses in vitro and in vivo. Further, Pirk-overexpressing flies are more susceptible to Gram-negative bacterial infection than wild-type flies. Caspar is a cytoplasmic homologous to human Fas-associated factor 1 (hFAF-1), which associates with various components of the TNF pathway, namely FAS, FADD, caspase-8, and NF- κ B. Caspar mutants show constitutive activation of diptericin in the absence of infection (Kim et al. 2006). Overexpression of Caspar, on the other hand, inhibits the induction of antimicrobial peptides and it has been proposed that Caspar blocks Relish cleavage by interfering with the Caspase-8 homolog DREDD (Kim et al. 2006).

We would like to add several comments to this short overview of the activation of NF- κ B by the Imd pathway: (1) the intracytoplasmic

signaling cascade shows some remarkable similarities with that of the TNF-receptor pathway. This is in contrast to that of the Toll pathway previously discussed. Also in contrast to the Toll pathway, the Imd pathway has a transmembrane receptor with dedicated recognition domains for microbial cell wall components of Gram-negative bacterial origin. As a result, this pathway cannot rely on an amplification cascade similar to that of the zymogen cascade downstream of the circulating recognition PGRPs in Toll pathway activation. It has been proposed that the capacity of PGRP-LCx dimers to respond to DAP-PGN end chain fragments (monomeric PGN or tracheal cytotoxin) obviates the necessity of an amplification cascade, as these fragments are likely to be produced in high amounts during growth and division of infecting Gram-negative bacteria (Boneca 2005); (2) as in the TNF-receptor pathway, the Imd pathway branches downstream of the MAP3kinase TAK1 and also activates the JNKinase system, thereby leading to the remarkably rapid transcription of a gene set distinct from that induced by cleaved Relish. The detailed analysis of the recent literature, which is beyond the scope of the present review, points to a complex interplay between these two pathways, and namely to the possibility that gene products induced by one pathway tend to down-regulate the other pathway; (3) the Imd pathway was discovered through its role in the defense against Gram-negative bacterial infection (Lemaitre et al. 1995). However, several recent observations point to Imd pathway activation (as judged by induction of antimicrobial peptide genes) in the absence of infection. One striking example is the report by Mukae et al. (2002) that chromosomal DNA from apoptotic cells that escaped digestion by appropriate enzymes can activate the expression of diptericin and attacin (Imd pathway readouts). M. Lagueux (pers. comm.) had noted that during metamorphosis, significant transcription of these genes occurred in the absence of infection. In conclusion, we need to develop a better understanding of the Imd pathway, both regarding the signaling cascade itself and the roles and

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interactions of its various partners, and regarding the potential functions of this pathway in development and in inflammation.

CONCLUDING REMARKS

We have focused in this short overview on NF- κ B in antimicrobial defenses of *Drosophila*. Obviously, in addition to this central role, NF- κ B is centrally involved in embryonic development and differentiation in flies—as it is in other organisms (see Baldwin 1996). We now know that the Rel/NF- κ B family of inducible transactivators originated at the dawn of the Metazoa and are present in Sponges (*Amphimedon queenslandica*) (Gauthier and Degnan 2008), Cnidaria (*Nematostella vectensis*) (Sullivan et al. 2007), and in all Bilateria. Genome sequencing data from early phyla indicate that many of the genes discussed in this article are present in these groups and we may anticipate that future studies will unravel NF- κ B regulatory pathways that share essential characteristics with those described here.

Regarding the future of the studies related to NF- κ B in flies, essential questions remain unresolved. For one, our information on the identities and roles of the players in these pathways is too fragmentary. To give but a few examples: We do not understand the mechanisms by which binding of microbial cell components to the recognition proteins, be it PGRPs or GNBPs, activate the downstream events; further, we suspect that K63 polyubiquitination plays a role in the signaling cascades but have very little reliable information in this regard. We do not fully understand how the signaling pathways discussed here interact with each other and with a variety of other cellular signaling pathways—even our knowledge on the interaction of the Imd-Relish pathway with the JNK pathway is sketchy. Whereas the studies on the Toll pathway in immune defenses have been crucially dependent on its role in embryonic development, it is not yet understood whether the Imd pathway similarly plays a role in development, apoptosis, and inflammation (as TNF does in mammals). Much additional work and

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new approaches are required if we are to fully understand the activation and roles of NF- κ B in the flies. These are some of the frontiers in this field of research.

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