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Review

Large-scale RNAi screens add both clarity and complexity to *Drosophila* NF-κB signaling

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ABSTRACT

NF-κB signaling is an immune response mechanism remarkably conserved through phylogeny. The genetically tractable model animal *Drosophila melanogaster* is an important model organism for studying NF-κB signaling in the immune response. Fruit flies have two NF-κB signaling pathways: the Toll and the Imd pathway. Traditional genetic screens have revealed many important aspects about the regulation of *Drosophila* NF-κB signaling and have helped us to also understand the immune response in humans. For example, the discovery that Toll like receptors are the main immune signaling molecules in mammals was based on work in flies. During the past decade high throughput RNA interference (RNAi)-based screening in cultured *Drosophila* cells has become a common method for identifying novel genes required for numerous cellular processes including NF-κB signaling. These screens have identified many novel positive and negative regulators of *Drosophila* NF-κB signaling thus enhancing our understanding of these signaling cascades.

Contents

Abbreviations: RNAi, RNA interference; Dif, Dorsal-related immunity factor; AMP, antimicrobial peptide; imd, immune deficiency; JNK pathway, c-Jun N-terminal kinase pathway; Jak–STAT pathway, Janus kinase–Signal Transducer and Activator of Transcription pathway; dsRNA, double-stranded RNA; siRNA, small interfering RNA; *Drs*, *Drosomycin*; TLR, Toll-like receptor; TIR domain, Toll/IL-1R domain; IRAK, IL-1R-associated kinase; DD, death domain; IκB, inhibitor of κB; Gprk2, G protein-coupled receptor kinase 2; Dredd, death-related ced-3/Nedd2-like protein; PGRP, peptidoglycan recognition protein; PGN, peptidoglycan.

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1. RNAi screening – history

RNA interference (RNAi) is an ancient gene silencing mechanism for host defense against parasitic genes such as viral genes and transposons. RNAi was first observed by plant scientists attempting to alter flower colors in petunias (Napoli et al., 1990), but the phenomenon became better understood when Andrew Fire and Craig C. Mello published their work on RNAi in *Caenorhabditis elegans* in 1998 (Fire et al., 1998). The RNAi machinery detects dsRNA as foreign and activates a complex RNAi pathway. Signaling through the RNAi pathway results in the degradation of the corresponding

mRNA and causes the silencing of the target gene expression. Soon after the work in *C. elegans* it was demonstrated that RNAi works efficiently also in *Drosophila* (Hammond et al., 2000; Kennerdell and Carthew, 1998). The enormous potential of this mechanism for research became evident, and applications for large-scale gene silencing were developed in *Drosophila* cells (Rämet et al., 2002). Consequently, within the past decade, large-scale RNAi-based *in vitro* screening has become a commonly used method for identifying gene products involved in a variety of biological processes (e.g., Boutros et al., 2004; Cherry et al., 2005; Dasgupta et al., 2005; Lum et al., 2003; Rämet et al., 2002).

2. NF-κB signaling in *Drosophila* immune response

NF-κB is a transcription factor that regulates the expression of multiple target genes involved in various cellular processes. NF-κB is essential for the proper functioning of the immune system (Ghosh et al., 1998; Li and Verma, 2002), and NF-κB pathways are remarkably well conserved in evolution. *Drosophila* has three NF-κB factors, Dorsal, Dorsal-related immunity factor (Dif), and Relish (Dushay et al., 1996), which are targets for two signaling pathways: Dif and/or Dorsal are mediators of Toll signaling (Valanne et al., 2011), and Relish mediates the induction of most of the humoral antimicrobial peptides (AMPs) (Hedengren et al., 1999), mainly via the Imd signaling pathway. Activation of the Toll pathway and/or the Imd pathway leads to the expression of AMPs and other target genes (Boutros et al., 2002; De Gregorio et al., 2001; Irving et al., 2001; Kallio et al., 2005; Lemaitre et al., 1995a,b; Tanji et al., 2010; Valanne et al., 2007). The *Drosophila* Toll pathway is involved in innate immunity and developmental processes (Belvin and Anderson, 1996; Halfon et al., 1995; Qiu et al., 1998), whereas the Imd pathway appears to function mainly in immune defenses (Lemaitre and Hoffmann, 2007; Valanne et al., 2011). An overview of the *Drosophila* immune response, including NF-κB signaling, is shown in Fig. 1. *Drosophila* immune response can be divided into local, cellular and humoral responses, all in which NF-κB signaling plays an important role.

2.1. Local response

When *Drosophila* epithelial cells encounter microbes, the activation of complex signaling pathways leads to the induction of potent AMP expression in these cells. This kind of a direct response against microbes in the form of the local production of AMPs is also found in other animals, including mammals, and is considered an essential ancestral antimicrobial defense mechanism (Brennan and Anderson, 2004; Ferrandon et al., 1998; Tzou et al., 2000). In *Drosophila* larvae, AMPs have mostly been detected in the tracheal epithelia, the oral region and the pharynx, whereas in adults most tissue-specific AMPs are expressed in the reproductive tract, suggesting that it too could serve as an entry site for pathogens. In addition, AMP expression has been detected in the labelar glands, in the malpighian tubules and in the gut. Importantly, the tissue-specific expression of

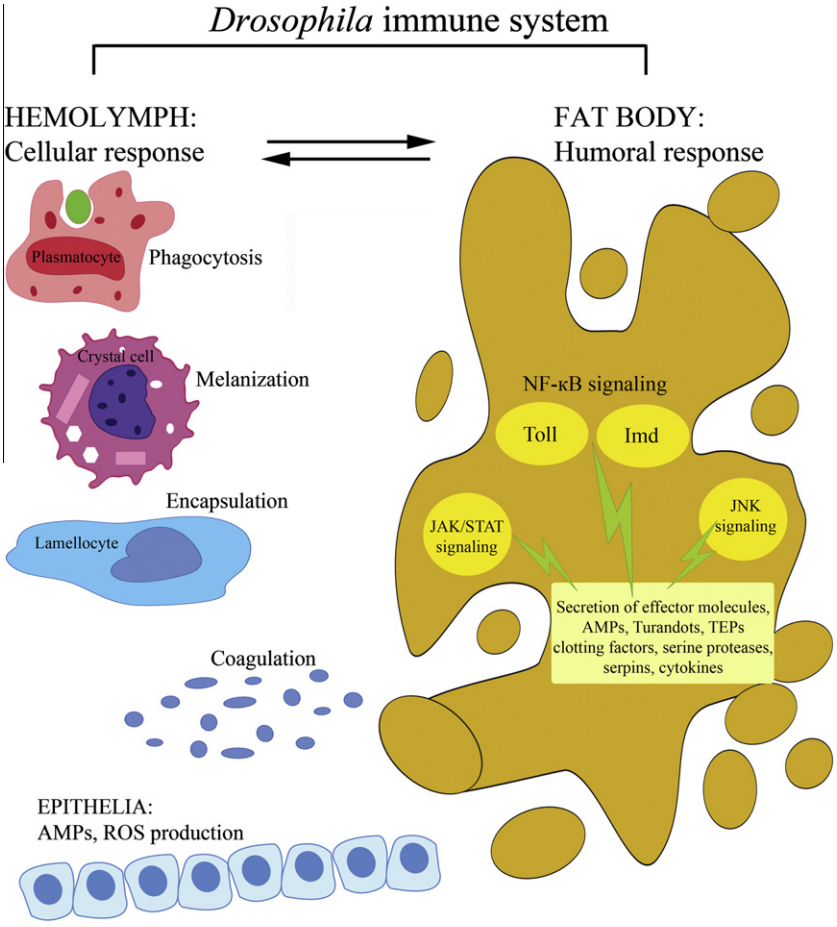

Fig. 1. The overview of the *Drosophila* immune system. The *Drosophila* immune system can be divided into local, cellular and humoral responses. Local response is mediated by epithelial cells expressing potent AMPs against invading microbes. In the *Drosophila* gut, the local response is also regulated by different levels of ROS production. *Drosophila* cellular response, phagocytosis, melanization and encapsulation are orchestrated by hemocytes, i.e., plasmatocytes, crystal cells and lamellocytes, respectively. The *Drosophila* humoral response is based on complex signaling pathways regulating the production of AMPs and several other molecules.