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ORIGINAL ARTICLE

Transcript analysis reveals the involvement of NF-κB transcription factors for the activation of TGF-β signaling in nematode-infected *Drosophila*

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Abstract

The common fruit fly *Drosophila melanogaster* is a powerful model for studying signaling pathway regulation. Conserved signaling pathways underlying physiological processes signify evolutionary relationship between organisms and the nature of the mechanisms they control. This study explores the cross-talk between the well-characterized nuclear factor kappa B (NF-κB) innate immune signaling pathways and transforming growth factor beta (TGF-β) signaling pathway in response to parasitic nematode infection in *Drosophila*. To understand the link between signaling pathways, we followed on our previous studies by performing a transcript-level analysis of different TGF-β signaling components following infection of immune-compromised *Drosophila* adult flies with the nematode parasites *Heterorhabditis gerrardi* and *H. bacteriophora*. Our findings demonstrate the requirement of NF-κB transcription factors for activation of TGF-β signaling pathway in *Drosophila* in the context of parasitic nematode infection. We observe significant decrease in transcript level of glass bottom boat (gbb) and screw (scw), components of the bone morphogenic protein (BMP) branch, as well as Activinβ (actβ) which is a component of the Activin branch of the TGF-β signaling pathway. These results are observed only in *H. gerrardi* nematode-infected flies compared to uninfected control. Also, this significant decrease in transcript level is found only for extracellular ligands. Future research examining the mechanisms regulating the interaction of these signaling pathways could provide further insight into *Drosophila* anti-nematode immune function against infection with potent parasitic nematodes.

Keywords *Drosophila* · *Heterorhabditis* · Innate immunity · TGF-β · NF-κB · Imd pathway

Introduction

The common fruit fly, *Drosophila melanogaster*, is a powerful organism for modeling the molecular basis of evolutionary conserved signaling pathways. The genetic similarities between pathway components provide us with insight into how basic biological processes of vertebrate organisms' function (Buchon et al. 2014; Kimbrell and Beutler, 2001; Lemaitre and Hoffmann, 2007). The ability to detect microbial invasions and activate defense responses has been extensively studied in *Drosophila*, highlighting the contribution of this model system to infection and innate immunity research.

Drosophila is able to discriminate between different pathogenic infections and respond by employing diverse immune signaling pathways. Conserved signaling pathways (immune deficiency pathway (Imd) and Toll pathway) are activated through the NF-κB transcription factors *Relish*, *Dorsal*, and the Rel-containing gene *Dif* (*dorsal-related immunity factor*) mainly against Gram-negative bacteria, and Gram-positive bacteria, and fungi, respectively (Corbo and Levine, 1996; Ganesan et al., 2011; Kleino and Silverman, 2014; Lemaitre et al., 1995; Rutschmann et al., 2000; Valanne et al., 2011). However, *Dorsal* is activated during embryonic development, whereas *Dif* is activated during the immune response of adult flies (Tauszig et al., 2000). In addition, *Dif* without *Dorsal* is sufficient to mediate the induction of a subset of antimicrobial peptide genes, such as *drosomycin* and *defensin* in *Drosophila* (Meng et al., 1999). Other conserved signaling pathways,

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namely the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and c-Jun N-terminal kinase (JNK), also participate in immune responses in *Drosophila* (Agaisse et al. 2004; Myllymäki and Rämet, 2014; Rämet et al. 2002). Recent studies have indicated the involvement of the conserved transforming growth factor beta (TGF-β) signaling pathway in response to bacterial infection and wounding (Clark et al. 2011; Patrnogic et al. 2018a), but also in response to challenge with parasitic nematodes (Castillo et al. 2013; Eleftherianos et al. 2016; Patrnogic et al. 2018a, 2018b).

The TGF-β pathway in *Drosophila* includes fewer signaling components than in vertebrate counterparts, and it consists of the bone morphogenic protein (BMP) and the Activin pathway, which are characterized by extracellular ligands binding to type I and type II receptors, intracellular transducers, and nuclear readout genes (Peterson and O'Connor, 2014; Raftery and Sutherland, 1999). The extracellular ligands of the BMP pathway include decapentaplegic (dpp), glass bottom boat (gbb) and screw (scw), while the extracellular ligands of the Activin pathway include Activinβ (actβ), dawdle (daw), maverick (mav), and myoglianin (myo). Type I receptors include saxophone (sax) and thick veins (tkv) in the BMP pathway, and baboon (babo) in the Activin pathway, whereas type II receptors include punt (put) or wishful thinking (wit). Receptor binding leads to signal transduction, and in *Drosophila*, this is mediated by Mothers against dpp (Mad) in the BMP pathway, and intracellular protein Smad on X (smox) in the Activin pathway (Shi and Massagué, 2003; Zi et al. 2012). In addition, Mad activation can be achieved via signaling through receptor babo (Gesualdi and Haerry, 2007).

The TGF-β signaling pathway is involved in inflammation and tissue repair in mammals (Li et al. 2006). In particular, TGF-β is predominantly considered an immunosuppressive cytokine because the lack of TGF-β signaling is linked to increased proliferation and effector function of immune cells (Wan and Flavell, 2008). However, previous evidence indicates that TGF-β signaling also stimulates diverse immune processes including the proliferation of mouse $CD8^+$, the production of TNF-α by both $CD4^+$ and $CD8^+$ cells, the death of T cells, the induction of IL-17-producing cells, the differentiation of Th-17 cells, the development and survival of CD1d-dependent natural killer T cells, and $CD8^+$ T cells, and it further possesses anti-apoptotic properties (Johnston et al. 2015). Previous research has shown that it also modulates the *Drosophila* immune response following wounding through NF-κB regulation of *dpp*. More specifically, *dpp* is activated through wounding and it represses the activation of antimicrobial peptides (Clark et al. 2011).

Previous studies have established that insect parasitic nematodes, particularly from the genus *Heterorhabditis*, are able to infect and kill *Drosophila* larvae and adult flies (Arefin et al., 2015; Castillo et al., 2012; Dobes et al., 2012; Hallem et al., 2007; Hyrsl et al., 2011; Kucerova et al., 2016; Peña et al., 2015). Infection with these nematode parasites leads to transcriptional changes in Toll and Imd immune signaling as well as in the conserved TGF-β signaling pathway in *Drosophila* (Arefin et al., 2014; Castillo et al., 2013; Hallem et al., 2007). More recently, we have extended these findings to reveal a novel role for the TGF-β signaling branches in the fly anti-nematode immune response. We have shown that inactivation of *daw* or *dpp* regulates the survival of *Drosophila* flies to infection by *Heterorhabditis* nematodes and their mutualistic bacteria, whereas inactivation of *daw* reduces the persistence of the parasites in the mutant flies (Eleftherianos et al. 2016). In addition, inactivation of *Mad* or *dpp* promotes fly survival and increases antimicrobial peptide gene expression levels upon sterile injury or nematode infection, respectively, but not upon bacterial challenge (Patrnogic et al. 2018a). Furthermore, extracellular ligand *scw* and type I receptor *sax* in the BMP pathway as well as the type I receptor *babo* in the Activin pathway are substantially upregulated following *H. gerrardi* infection, which leads to upregulation of the intracellular component *Mad* (Patrnogic et al. 2018b).

In this study, we aimed to elucidate whether immune-related NF-κB transcription factors are playing a role in the activation of TGF-β signaling in response to parasitic nematode infection with *H. gerrardi* and *H. bacteriophora*. The main difference between the two nematode parasites is that they harbor distinct species of *Photorhabdus* mutualistic bacteria in their gut; *H. gerrardi* nematodes carry *P. asymbiotica*, whereas *H. bacteriophora* nematodes contain *P. luminescens* (Waterfield et al., 2009). For this, we examined changes in transcript levels of TGF-β extracellular and intracellular signaling components in immune defective mutant flies. Our results reveal the requirement of immune related NF-κB transcription factors to activate TGF-β signaling pathway in nematode-infected flies. This requirement is parasite-specific, as we only observe changes in transcript levels after infection with *H. gerrardi*, but not *H. bacteriophora*. Strikingly, these changes occur at the level of extracellular ligands, in both branches of TGF-β signaling, BMP (*gbb*, *scw*) and Activin (*Actβ*). Further studies using other insect or human parasitic nematode species could uncover the specificity of this activation requirement, as well as expose the molecular basis of certain mechanisms underlying host-parasite interactions.

Materials and methods

Fly stocks

Fly stocks were raised on standard cornmeal-soy–based food (Cat. No. 101-NV, Meidi laboratories) with few granules of dry baker's yeast, at 25 °C at 12:12 light:dark photoperiod,

60% humidity. Fly lines *Dif*^{1}, *Rel*^{e20} (Hedengren et al., 1999), and *Dif cn bw;Rel*^{e20}*/TM6c*, *Sb1* were obtained from Dr. Jean-Marc Reichhart's lab (National Center for Scientific Research, Strasbourg, France). Fly line used as background control in experiments was *cn bw* that was obtained as gift from Dr. Louisa Wu's lab (University of Maryland, College Park, USA). Both male and female adult flies aged 5–7 days old were used in infection experiments.

Nematode stocks

Entomopathogenic nematodes used in the experiments were *Heterorhabditis gerrardi* and *Heterorhabditis bacteriophora* TT01, amplified in the fourth instar larvae of the wax moth, *Galleria mellonella*, using the water trap technique (White 1927). *Heterorhabditis* infective juveniles (IJs) used in all infection experiments were 1–5 weeks old.

Infection

Infection of *D. melanogaster* adult flies with the infective juveniles of entomopathogenic nematodes *H. gerrardi* and *H. bacteriophora* was performed in the following way: IJs were washed in sterile water and diluted to the final density that was previously adjusted to approximately 1000 worms/fly for *H. gerrardi* and 500 worms/fly for *H. bacteriophora*. Nematode suspension in water (250 μl) was added to vials containing four filter papers (Whatman Grade 1, 20 mm). Additional 250 μl of 1% sucrose was added to vials to provide nutrients for the flies. For uninfected controls, 250 μl of water was added to vials with 250 μl of 1% sucrose.

Flies were anesthetized, and a mix of five males and females was added into each vial. The plug was pushed down to limit the movement and increase the probability of infection. Vials were placed in the incubator at 25 °C. Survival was quantified twice per day and additional 50 μl of 1% sucrose were added each time.

Transcriptional expression analysis

To analyze the transcriptional regulation of TGF-β genes upon infection with entomopathogenic nematodes, flies were infected as previously described and based on survival curves previously obtained, collected at 48, 96, and 120 h following nematode infection. Total RNA was extracted from at least five adult flies per replicate using TRIzol Reagent (Ambion, Life Technologies). Reverse transcription was performed using iScript™ cDNA Synthesis Kit (BIO-RAD). iTaq™ Universal SYBR® Green Supermix (Bio-Rad) was used for quantitative RT-PCR using CFX96™ Real-Time System, C1000™ Thermal Cycler with following conditions: 95 °C for 2 min, 40 cycles of 95 °C for 15 s and 61 °C for 30 s, 95 °C for 15 s, 65 °C for 5 s, and 95 °C for 5 s. CFX Manager 3.1 (Bio-Rad) was used for data analysis. Primers used to quantify mRNA levels are listed in Table 1.

Statistical analysis

For data plotting and statistical analyses, we used GraphPad Prism (v8.0.2). Three independent survival experiments were performed, and the results were analyzed statistically using Log-rank (Mantel-Cox). Experiments for transcriptional analysis were repeated three times and unpaired *t* test was used for statistics.

Results

The requirement for NF-κB transcription factors to activate TGF-β signaling is parasite-specific

Using transcriptional analysis, we investigated whether the immune-related NF-κB transcriptional factors are necessary for the activation of the BMP and Activin TGF-β signaling pathways, following infection with the parasitic nematodes *H. gerrardi* and *H. bacteriophora* in *D. melanogaster* adult flies. We first analyzed the changes in expression of the extracellular ligands in both BMP and the Activin signaling pathways at specific time points following nematode infection (Fig. 1). The time points were chosen based on survival curves (Fig. 3) and demonstrate early, mid, and late time point during the course of the infection. We show here only expression at 48 h post infection, as later time points did not reveal any significant differences (Figs. S1 and S2). We did not observe any significant differences in gene transcript levels of extracellular ligands between the two nematode infections. However, only *H. gerrardi* leads to observable changes, specifically in expression of extracellular ligands *gbb* (Fig. 1b), *scw* (Fig. 1c), and *Actβ* (Fig. 1d). We found significantly lower transcript levels of *gbb* in *Dif* mutant flies following *H. gerrardi* infection. This implies NF-κB transcriptional requirement for activation of the BMP pathway following this specific type of infection. Similarly, we found significantly lower transcript levels of extracellular ligand *scw* in *Dif;rel* double mutants, implying the requirement of this ligand for regulating both immune pathways. Interestingly, in our previous studies, we observed significantly higher levels of extracellular ligand *scw*, in wild-type background *D. melanogaster* larvae specifically following infection with *H. gerrardi* nematodes (Patrnogic et al. 2018b). Another study performed in *D. melanogaster* larvae has shown that a mitochondrial perturbation leads to increased *Actβ* expression in the larval muscle via the NF-κB signaling cascade (Song et al. 2017). This suggests that blocking the NF-κB signaling cascade would

Table 1 List of primers and sequences used in quantitative RT-PCR experiments

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
rp49	GATGACCATCCGCCCAGCA	CGGACCGACAGCTGCTTGGC
drosomycin	GACTTGTTCGCCCTCTTCG	CTTGCACACACGACGACAG
diptericin	GCTGCGCAATCGCTTCTACT	TGGTGGAGTTGGGCTTCATG
actβ	CCATTCAAAGGCAGCAGGTG	AGCGGGTTGTGGAAATGACT
babo	CGCTCCATCTGGTGTAACGA	TCTGGTCCTTCGTCTTTGGC
daw	CGAGGAGGACGATGTACCGAT	GTGCTGCCTCTTGTGGATGA
dpp	TGGCGACTTTTCAAACGATTGT	CAGCGGAATATGAGCGGCAA
gbb	GGGACTCGGAATGGTTCTGC	CGTTGTCTATGTAAATCCCCGAC
mad	GACGAAGAGGAGAAGTGGGC	TAGATCACATGCGGCAGACC
mav	GTGATTTCAGCCCGTCAACAC	TTCAGCGTTCCGTGTCCAAT
myo	ATGCTGCGGTTGGAGAAAATA	CGTGACATATCGAGTTACACGG
sax	ACCCACACCTGCCAGAATG	CTTCCCCGTATTGCGTTTACT
smox	CGCCTATCAACAGCAACAGC	TGCCCACACTAAGCACACTC
scw	GCATCCTGGGCTCTGTGAAT	ACCGCAGCGTATCTGTCAAA

decrease the expression of *Actβ*. Indeed, here, we observed a significant decrease in *Actβ* expression in both *Dif*, and *Dif;rel* immune mutants. However, this is specifically in response to infection with *H. gerrardi*. Taken together, these results suggest a potential regulation of TGF-β signaling pathway via NF-κB signaling pathways; however, this effect is specific to infection with the parasitic nematode *H. gerrardi*.

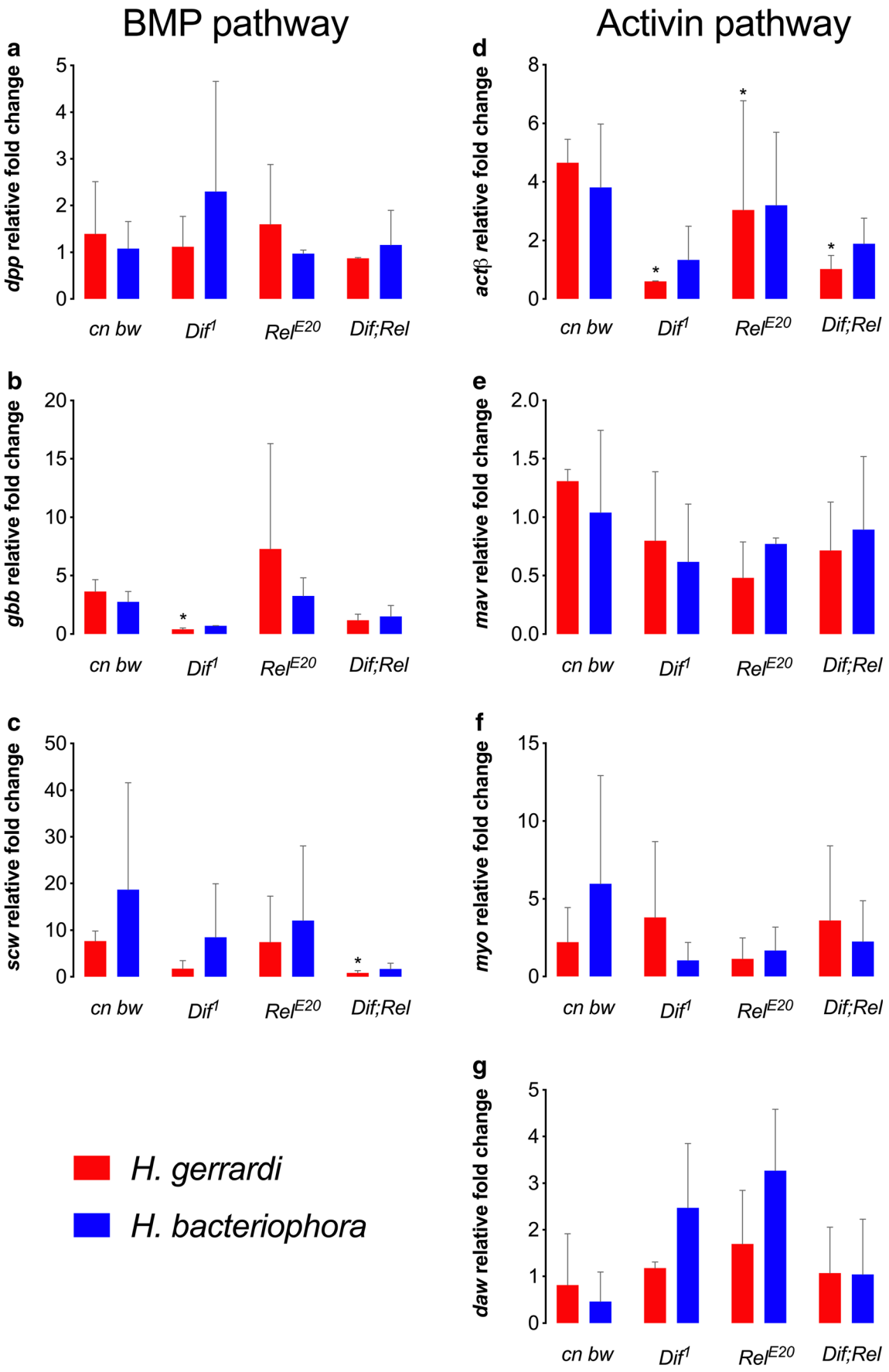
NF-κB transcriptional factors induce changes only in extracellular ligands

Similarly, we examined changes in the transcript levels of genes encoding the intracellular components of the two branches of TGF-β signaling. In our previous studies, we have observed transcriptional upregulation of the intracellular components *sax* and *mad* following *H. gerrardi* infection, albeit in larvae, and following wounding in adult flies (Patrnogic et al., 2018a, 2018b). However, no significant changes in gene expression were observed between the two nematode infections performed in this study, nor between background controls and immune pathway mutants within each type of nematode infection (Figs. 2, S3, and S4). This could suggest that the requirement for NF-κB transcriptional factors to activate the TGF-β signaling pathway is at the level of extracellular ligands.

NF-κB immune signaling pathways promote survival against parasitic nematodes

Since *D. melanogaster* loss-of-function immune pathway mutants are more susceptible to infections when challenged with different pathogens (Lemaitre et al. 1995; Rutschmann et al. 2002), we investigated the survival rate of immune pathway mutant adult flies following infection with either species of *Heterorhabditis* nematodes. Indeed, we observe significant decrease in survival rate in the immune pathway mutants following these infections (Fig. 3). More specifically, *Dif* (Fig. 3a), *rel* (Fig. 3b), and *Dif;rel* double mutants (Fig. 3c) are more susceptible to infection with *H. gerrardi* compared to wild-type background controls. Similarly, *rel* (Fig. 3e) and *Dif;rel* double mutants (Fig. 3f) show increased susceptibility to infection with *H. bacteriophora* compared to wild-type background control. This infection however does not lead to significant decrease in survival rate in *Dif* mutants (Fig. 3d) when compared to background controls, although we do observe susceptibility to it. In addition, we looked at the expression of antimicrobial peptides following these parasitic infections. As expected, there is no significant increase in the expression of antimicrobial peptides, namely *diptericin* and *drosomycin*, that are used as readouts for these pathways (Fig. 4). In a previous study, significant induction in antimicrobial peptide gene expression was observed following infection with *H. bacteriophora* nematodes (Hallem et al. 2007). However, this study was performed with *Drosophila*

Fig. 1 Gene transcript levels of extracellular TGF-β signaling components in immune pathway mutants. Transcript levels of *dpp*, *gbb*, *scw* extracellular ligands in the BMP pathway (**a**, **b**, **c**) and *actβ*, *mav*, *myo*, *daw* in the Activin pathway (**d**, **e**, **f**, **g**) following infection with the parasitic nematodes *Heterorhabditis gerrardi* or *H. bacteriophora*. *Drosophila melanogaster* adult flies were infected with *H. gerrardi* or *H. bacteriophora* infective juveniles, and mRNA expression of various TGF-β pathway components was quantified by real-time RT-PCR 48 h following infection. Gene transcript levels are normalized to non-infected controls at the corresponding hour. Graphs represent the mean ± SD of at least two independent experiments. Statistical significance was determined using unpaired *t* test comparing the background control line to the corresponding immune pathway mutant. $*p < 0.05$


BMP pathway
Activin pathway
a
dpp relative fold change
b
gbb relative fold change
c
scw relative fold change
d
actβ relative fold change
e
mav relative fold change
f
myo relative fold change
g
daw relative fold change
cn bw
Dif1
RelE20
Dif;Rel
H. gerrardi
H. bacteriophora

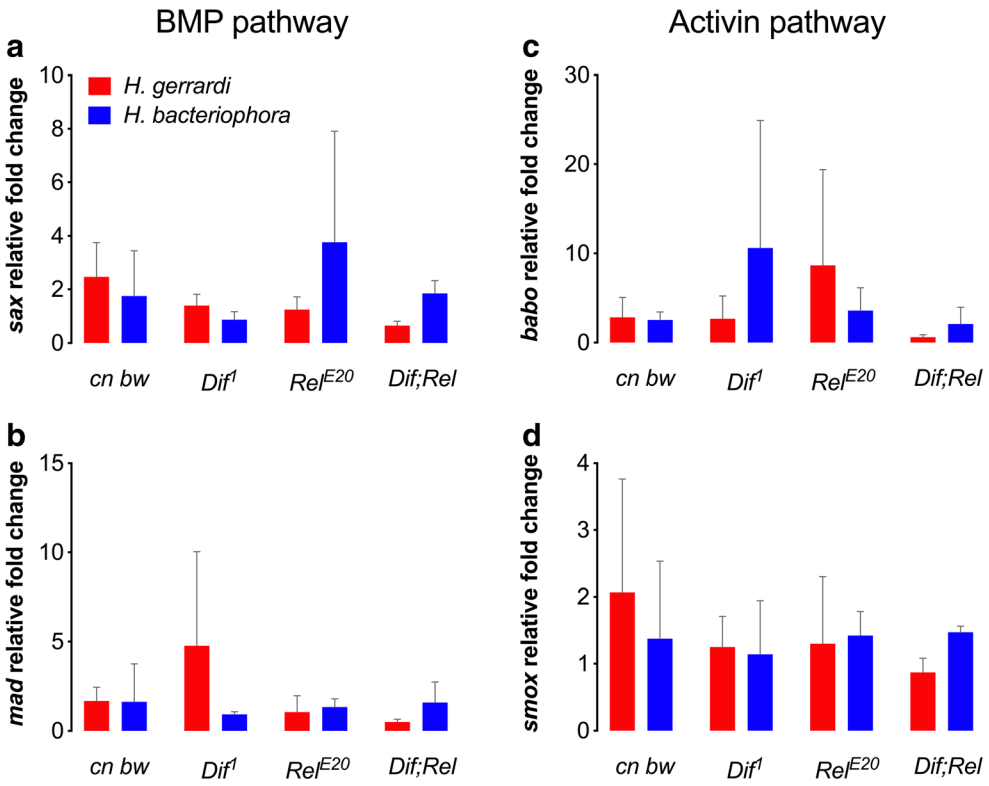


Fig. 2 Gene transcript levels of various intracellular TGF-β signaling components in immune pathway mutants. Transcript levels of *sax* and *mad* intracellular components in the BMP pathway (**a**, **b**) and *babo* and *smox* in the Activin pathway (**c**, **d**) following infection with the parasitic nematodes *Heterorhabditis gerrardi* or *H. bacteriophora*. *Drosophila melanogaster* adult flies were infected with *H. gerrardi* or *H. bacteriophora*, and mRNA expression of the TGF-β components was quantified by real-time RT-PCR 48 h following infection. Gene transcript levels are normalized to non-infected controls at the corresponding hour. Graphs represent the mean ± SD of at least two independent experiments. Statistical significance was determined using unpaired *t* test comparing background controls to corresponding immune pathway mutants

larvae instead of adult flies and examined an earlier time point of infection. It is also important to stress that immune responses are highly variable in larvae because of the fluctuating concentrations of ecdysone (the steroid hormone that triggers metamorphosis) (Fellous and Lazzaro, 2010). In addition, the IJ stage of entomopathogenic nematodes is the only stage that is capable of infecting insects (Dillman et al., 2012); therefore, we have standardized our experiments by using specific age IJs for infections. Taken together, these results suggest that the expression of immune-related genes through the activation of NF-κB signaling pathways might promote the survival of *D. melanogaster* adult flies following infection with the nematode parasites *H. gerrardi* and *H. bacteriophora*; however, lack of immune signaling activation leads to increased fly sensitivity to parasitic nematode infection.

Discussion

Previous studies have demonstrated the importance of *Drosophila* as model to study conserved signaling pathways and their implication in the immune response against microbial infections. Following on our previous transcript analysis studies of TGF-β signaling pathway activated in response to parasitic nematodes, here, we investigated a requirement of NF-κB transcriptional factors to activate the TGF-β signaling pathway. In the current study, we have examined the transcript-level changes of TGF-β signaling components in NF-κB immune signaling pathway mutants following infection with the potent parasitic nematodes, *H. gerrardi* and *H. bacteriophora*.

Our results demonstrate that the requirement for NF-κB transcriptional factors to activate the TGF-β signaling is parasite-specific. The nematode parasites used in this study are *H. gerrardi* and *H. bacteriophora*, which form a mutualistic relationship with their respective gut bacteria, *Photorhabdus asymbiotica* and *P. luminescens*. *Photorhabdus* bacteria are Gram-negative insect pathogens that produce a wide variety of toxins and virulence factors in order to infect their hosts efficiently. Following nematode infection, the bacterial cells are released into the fly body cavity where they are able to multiply rapidly, colonize different tissues, and kill the host (Ciche 2007; Waterfield et al. 2009). The parasite-specific response that we observe might stem from the difference in the pathogenicity between the mutualistic bacteria residing in

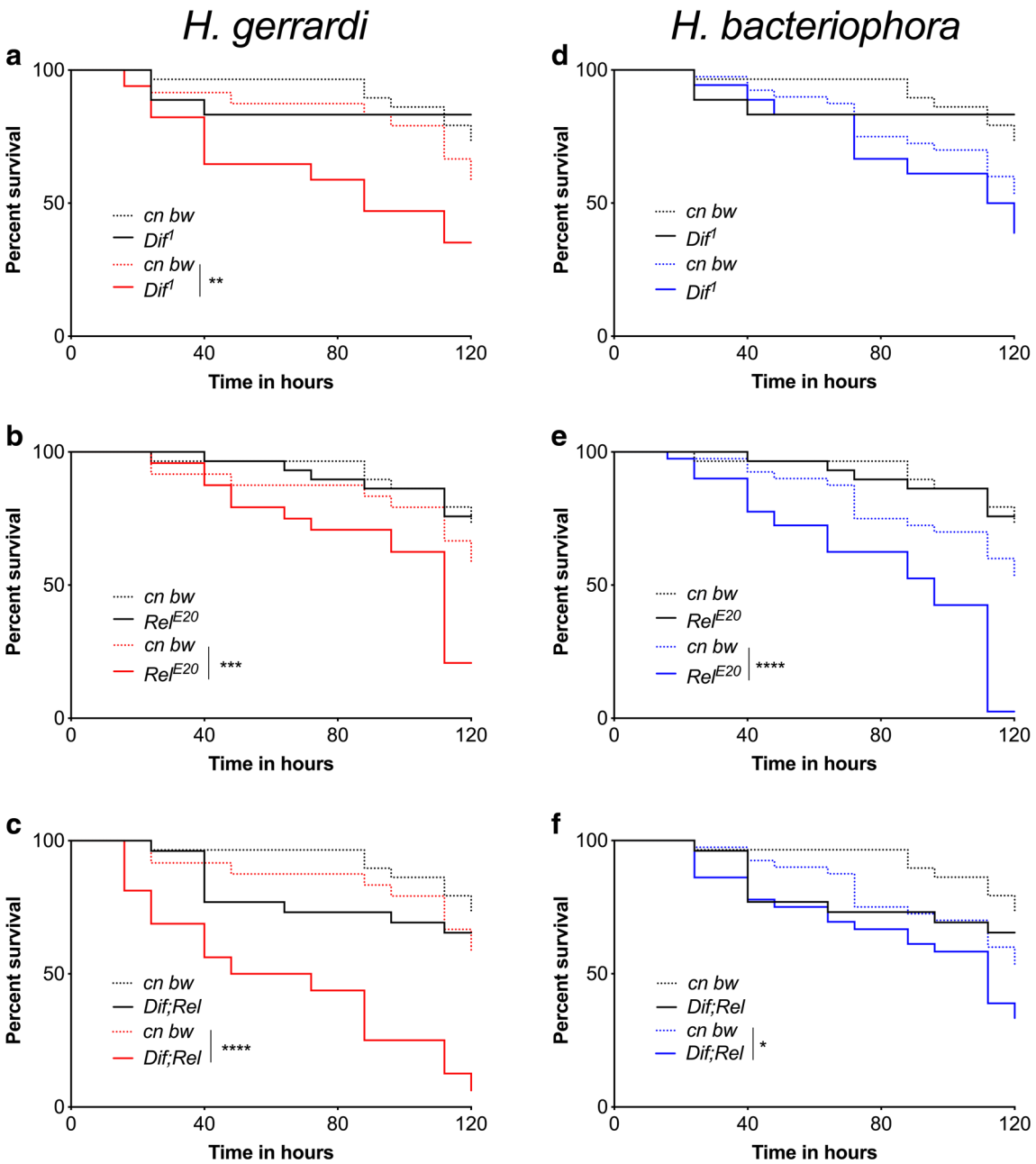


Fig. 3 Survival of immune pathway mutants following infection with the parasitic nematodes *Heterorhabditis gerrardi* (**a**, **b**, **c**) or *H. bacteriophora* (**d**, **e**, **f**). *Drosophila melanogaster* adult flies were infected with *H. gerrardi* or *H. bacteriophora*, and survival was monitored twice per day. Graphs represent at least three independent experiments. Statistical significance was determined using log-rank (Mantel-Cox) test comparing infected background control flies to immune pathway mutants (cn bw vs Dif^1 $^{**}p = 0.0066$; cn bw vs Rel $^{***}p = 0.0007$; cn bw vs dif;rel $^{****}p < 0.0001$; cn bw vs rel **** $p < 0.0001$; cn bw vs dif;rel $^{*}p = 0.0467$)

the gut of their nematode vectors, and their interaction with the *Drosophila* immune response.

Interestingly, repression in transcript levels of different *Drosophila* BMP and Activin signaling components was observed only upon infection with the parasitic nematode *H. gerrardi*. In addition, as the regulation of TGF-β signaling is achieved in the extracellular space, the cell membrane, and the intracellular region, we examined transcript levels of TGF-β gene expressed in different parts of the cell. Our results demonstrate that the transcriptional changes occur only at the extracellular level and the only distinct changes in gene expression are those of the extracellular ligands *gbb*, *scw*, and *Actβ*. In our previous study, albeit in background control larvae, extracellular ligand *scw* was induced significantly following infection with *H. gerrardi* nematodes (Patrnogic et al. 2018b). Here, we find a reverse effect, where in mutant flies

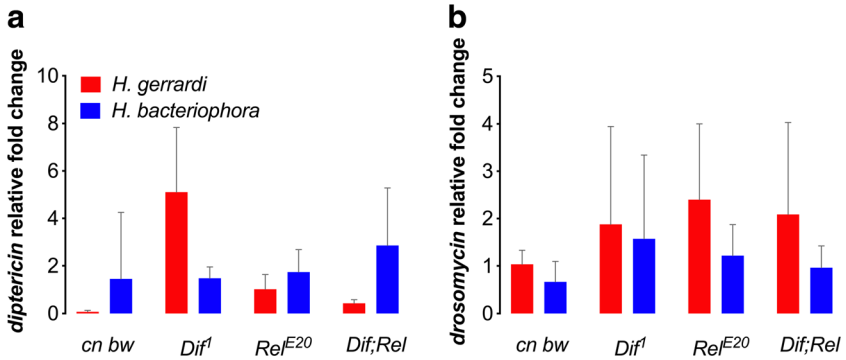


Fig. 4 Transcript levels of antimicrobial peptides following infection with the parasitic nematodes *Heterorhabditis gerrardi* or *H. bacteriophora*. *Drosophila melanogaster* adult flies were infected with *H. gerrardi* or *H. bacteriophora* and gene transcript levels of *diptericin* (**a**) and *drosomycin* (**b**) were quantified using real-time RT-PCT 48 h following infection. Gene transcript levels are normalized to non-infected controls at the corresponding hour. Graphs represent the mean ± SD of at least two independent experiments were performed. Statistical significance was determined using unpaired *t* test comparing background controls to immune pathway mutants

with deficient both NF-κB immune signaling pathways, expression of *scw* is reduced. This implies that if NF-κB immune signaling activity is impaired, it leads to repression of certain TGF-β signaling components. In contrast, functional NF-κB immune signaling leads to induction of TGF-β signaling components. Taken together, these findings suggest that a molecular mechanism can link TGF-β and NF-κB signaling pathways in *Drosophila* during immune response to potent nematode parasites.

Expression of the extracellular ligand of the Activin pathway *Actβ* has previously been shown to decrease in larval muscle cells with perturbed mitochondrial function. Further investigation revealed that decreases in *Actβ* expression was due to blocking of the NF-κB/Rel signaling pathway (Song et al. 2017). Similarly, we observe reduction in extracellular ligand *Actβ* expression following *H. gerrardi* nematode infection in flies lacking functional NF-κB signaling pathway components. More specifically, flies lacking transcription factor Dif (functions in the Toll signaling pathway), as well as flies lacking both transcription factors Dif and Rel together (affecting both Toll and Imd pathways), demonstrate reduction in extracellular ligand *Actβ* expression. Taken together, the current results along with previous findings in *Drosophila* larvae showing decrease in *Actβ* expression by preventing activation of the NF-κB/Rel signaling pathway suggest that NF-κB signaling can interfere with the expression of *Actβ*.

In our previous studies, we established the involvement of the *Drosophila* TGF-β signaling pathway in the response against parasitic nematodes. The current study extends our previous findings by demonstrating parasite-specific requirement NF-κB transcriptional factors for the activation of TGF-β signaling pathway. Future research could be focusing on the detailed characterization of the relationship between these two conserved signaling pathways by elucidating the epistasis between specific signaling components and exploring the responses to parasitic nematode infection when both pathways are impaired. In addition, overexpression of TGF-β extracellular ligands in immune pathway–impaired mutant flies would be a useful tool to understand the role of these signaling components in the *Drosophila* anti-nematode response. Furthermore, we could look whether infections with axenic nematodes in order to establish whether nematodes deficient of their mutualistic bacteria are able to confer a similar effect. In addition, infections with other parasite nematode species might provide more insight in the role of TGF-β signaling pathway in anti-nematode responses. Along those lines, functional immunity studies in the *Drosophila* model following infection with different parasitic nematodes could further reveal whether interaction between these two evolutionary conserved pathways at the signaling level also reflect changes in innate immune anti-nematode activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Agaisse H, Perrimon N, Agaisse H, Perrimon N (2004) The roles of JAK/STAT signaling in *Drosophila* immune responses. Immunol Rev 198:72–82. https://doi.org/10.1111/j.0105-2896.2004.0133.x

Arefin B, Kucerova L, Dobes P, Markus R, Strnad H, Wang Z, Hyrsl P, Zurovec M, Theopold U (2014) Genome-wide transcriptional analysis of *Drosophila* larvae infected by entomopathogenic

nematodes shows involvement of complement, recognition and extracellular matrix proteins. J. Innate Immun. 6:192–204. https://doi.org/10.1159/000353734

Arefin B, Kucerova L, Krautz R, Kranenburg H, Parvin F, Theopold U (2015) Apoptosis in hemocytes induces a shift in effector mechanisms in the *Drosophila* immune system and leads to a pro-inflammatory state. PLoS One 10:e0136593. https://doi.org/10.1371/journal.pone.0136593

Buchon N, Silverman N, Cherry S (2014) Immunity in *Drosophila melanogaster* - from microbial recognition to whole-organism physiology. Nat Rev Immunol 14:796–810. https://doi.org/10.1038/nri3763

Castillo JC, Shokal U, Eleftherianos I (2013) Immune gene transcription in *Drosophila* adult flies infected by entomopathogenic nematodes and their mutualistic bacteria. J Insect Physiol 59:179–185. https://doi.org/10.1016/j.jinsphys.2012.08.003

Castillo JC, Shokal U, Eleftherianos I (2012) A novel method for infecting *Drosophila* adult flies with insect pathogenic nematodes. Virulence 3:339–347. https://doi.org/10.4161/viru.20244

Ciche T (2007) The biology and genome of *Heterorhabditis bacteriophora*. WormBook 1(9). https://doi.org/10.1895/wormbook.1.135.1

Clark RI, Woodcock KJ, Geissmann F, Trouillet C, Dionne MS (2011) Multiple TGF-β superfamily signals modulate the adult *Drosophila* immune response. Curr Biol 21:1672–1677. https://doi.org/10.1016/j.cub.2011.08.048

Corbo JC, Levine M (1996) Characterization of an immunodeficiency mutant in *Drosophila*. Mech Dev 55:211–220. https://doi.org/10.1016/0925-4773(96)00506-0

Dillman AR, Chaston JM, Adams BJ, Ciche T a, Goodrich-Blair H, Stock SP, Sternberg PW (2012) An entomopathogenic nematode by any other name. PLoS Pathog 8:8–11. https://doi.org/10.1371/journal.ppat.1002527

Dobes P, Wang Z, Markus R, Theopold U, Hyrs P (2012) An improved method for nematode infection assays in *Drosophila* larvae. Fly (Austin) 6:75–79. https://doi.org/10.4161/fly.19553

Eleftherianos I, Castillo JCJC, Patrnogic J (2016) TGF-β signaling regulates resistance to parasitic nematode infection in *Drosophila melanogaster*. Immunobiology 221:1362–1368. https://doi.org/10.1016/j.imbio.2016.07.011

Fellous S, Lazzaro BP (2010) Larval food quality affects adult (but not larval) immune gene expression independent of effects on general condition. Mol Ecol 19:1462–1468. https://doi.org/10.1111/j.1365-294X.2010.04567.x

Ganesan, S, Aggarwal, K, Paquette, N, Silverman, N, 2011. NF-κB/Rel proteins and the humoral immune responses of *Drosophila melanogaster*. 25–60. https://doi.org/10.1007/82_2010_107

Gesualdi SC, Haerry TE (2007) Distinct signaling of *Drosophila* Activin/TGF- β family members. Fly (Austin). 1:212–221. https://doi.org/10.4161/fly.5116

Hallem E a, Rengarajan M, Ciche TA, Sternberg PW (2007) Nematodes, bacteria, and flies: a tripartite model for nematode parasitism. Curr Biol 17:898–904. https://doi.org/10.1016/j.cub.2007.04.027

Hedengren, M, Bengt, A, Dushay, MS, Ando, I, Ekengren, S, Wihlborg, M, Hultmark, D, 1999. Relish, a central factor in the control of humoral but not cellular immunity in *Drosophila* 4, 827–837

Hyrsl P, Dobes P, Wang Z, Hauling T, Wilhelmsson C, Theopold U (2011) Clotting factors and eicosanoids protect against nematode infections. J. Innate Immun. 3:65–70. https://doi.org/10.1159/000320634

Johnston CJC, Smyth DJ, Dresser DW, Maizels RM (2015) TGF-β in tolerance, development and regulation of immunity. Cell Immunol 299:14–22. https://doi.org/10.1016/j.cellimm.2015.10.006

Kimbrell D a, Beutler B (2001) The evolution and genetics of innate immunity. Nat Rev Genet 2:256–267. https://doi.org/10.1038/35066006

Kleino A, Silverman N (2014) The *Drosophila* IMD pathway in the activation of the humoral immune response. Dev Comp Immunol 42:25–35. https://doi.org/10.1016/j.dci.2013.05.014

Kucerova L, Broz V, Arefin B, Maaroufi HO, Hurychova J, Strnad H, Zurovec M, Theopold U (2016) The *Drosophila* chitinase-like protein IDGF3 is involved in protection against nematodes and in wound healing. J Innate Immun 8:199–210. https://doi.org/10.1159/000442351

Lemaitre B, Hoffmann J (2007) The host defense of *Drosophila melanogaster*. Annu Rev Immunol 25:697–743. https://doi.org/10.1146/annurev.immunol.25.022106.141615

Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann J a (1995) A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. Proc Natl Acad Sci U S A 92:9465–9469. https://doi.org/10.1073/pnas.92.21.9465

Li MO, Wan YY, Sanjabi S, Robertson A-KL, Flavell RA (2006) Transforming growth factor- β regulation of immune responses. Annu Rev Immunol 24:99–146. https://doi.org/10.1146/annurev.immunol.24.021605.090737

Meng X, Khanuja BS, Ip YT (1999) Toll receptor-mediated *Drosophila* immune response requires Dif, an NF- kB factor. Genes Dev 13:792–797. https://doi.org/10.1101/gad.13.7.792

Myllymäki H, Rämet M (2014) JAK/STAT pathway in *Drosophila* immunity. Scand J Immunol 79:377–385. https://doi.org/10.1111/sji.12170

Patrnogic J, Heryanto C, Eleftherianos I (2018a) Wounding-induced upregulation of the bone morphogenic protein signaling pathway in *Drosophila* promotes survival against parasitic nematode infection. Gene 673:112–118. https://doi.org/10.1016/j.gene.2018.06.052

Patrnogic J, Heryanto C, Eleftherianos I (2018b) Transcriptional upregulation of the TGF-β intracellular signaling transducer Mad of *Drosophila* larvae in response to parasitic nematode infection. Innate Immun 175342591879066:349–356. https://doi.org/10.1177/1753425918790663

Peña JM, Carrillo Ma, Hallem Ea (2015) Variation in the susceptibility of *Drosophila* to different entomopathogenic nematodes. Infect Immun:83. https://doi.org/10.1128/IAI.02740-14

Peterson AJ, O'Connor MB (2014) Strategies for exploring TGF-β signaling in *Drosophila*. Methods 68:183–193. https://doi.org/10.1016/j.ymeth.2014.03.016

Raftery L a, Sutherland DJ (1999) TGF-β family signal transduction in *Drosophila* development: from Mad to Smads. Dev Biol 210:251–268. https://doi.org/10.1006/dbio.1999.9282

Rämet M, Lanot R, Zachary D, Manfruelli P (2002) JNK signaling pathway is required for efficient wound healing in *Drosophila*. Dev Biol 241:145–156. https://doi.org/10.1006/dbio.2001.0502

Rutschmann, S, Jung, a. C., Hetru, C., Reichhart, JM, Hoffmann, J. a, Ferrandon, D., 2000. The Rel protein DIF mediates the antifungal but not the antibacterial host defense in *Drosophila*. Immunity 12, 569–580. https://doi.org/10.1016/S1074-7613(00)80208-3

Rutschmann S, Kilinc A, Ferrandon D (2002) Cutting edge: the toll pathway is required for resistance to gram-positive bacterial infections in *Drosophila*. J Immunol 168:1542–1546. https://doi.org/10.4049/jimmunol.168.4.1542

Shi Y, Massagué J (2003) Mechanisms of TGF- β signaling from cell membrane to the nucleus. Cell 113:685–700. https://doi.org/10.1016/S0092-8674(03)00432-X

Song W, Owusu-Ansah E, Hu Y, Cheng D, Ni X, Zirin J, Perrimon N (2017) Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-associated obesity model. Proc Natl Acad Sci 114:8596–8601. https://doi.org/10.1073/pnas.1708037114

Tauszig S, Jouanguy E, Hoffmann J a, Imler JL (2000) Toll-related receptors and the control of antimicrobial peptide expression

in *Drosophila*. Proc Natl Acad Sci U S A 97:10520–10525. https://doi.org/10.1073/pnas.180130797

Valanne S, Wang J-H, Rämet M (2011) The *Drosophila* toll signaling pathway. J Immunol 186:649–656. https://doi.org/10.4049/jimmunol.1002302

Wan, YY, Flavell, RA, 2008. TGF-β and regulatory T cell in immunity and autoimmunity 28, 647–659. https://doi.org/10.1007/s10875-008-9251-y

Waterfield NR, Ciche T, Clarke D (2009) *Photorhabdus* and a host of hosts. Annu Rev Microbiol 63:557–574. https://doi.org/10.1146/annurev.micro.091208.073507

White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302–303. https://doi.org/10.1089/dna.1998.17.321

Zi Z, Chapnick DA, Liu X (2012) Dynamics of TGF-β/Smad signaling. FEBS Lett 586:1921–1928. https://doi.org/10.1016/j.febslet.2012.03.063