MECHANISMS OF DEVELOPMENT XXX (2010) XXX–XXX

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

MECHANISMS OF DEVELOPMENT

Pellino enhances innate immunity in *Drosophila*

Amirhossein Haghayeghi [a], *Amila Sarac* [a], *Stefan Czerniecki* [a], *Jörg Grosshans* [b], *Frieder Schöck* [a,*]

[a] *Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, Que., Canada H3A 1B1*
[b] *Zentrum für Biochemie und Molekulare Zellbiologie Universitätsmedizin, Justus-von-Liebig-Weg 11, 37099 Göttingen, Germany*

ARTICLE INFO

Article history:
Received 19 November 2009
Received in revised form
19 January 2010
Accepted 25 January 2010
Available online xxxx

Keywords:
Pellino
Toll
Innate immunity
Pelle
IRAK

ABSTRACT

The innate immune response is a defense mechanism against infectious agents in both vertebrates and invertebrates, and is in part mediated by the Toll pathway. Toll receptor activation upon exposure to bacteria causes stimulation of Pelle/IRAK kinase, eventually resulting in translocation of the transcription factor NF-κB to the nucleus. Here we show that Pellino, a highly conserved protein interacting with activated Pelle/IRAK, acts as a positive regulator of innate immunity in *Drosophila*.

1. Introduction

The innate immune response is the first line of defense against infections in both vertebrates and invertebrates. In flies the Toll and IMD pathways mediate innate immunity. Gram-negative bacteria activate the IMD pathway resulting in Relish (a NF-κB homolog) translocation to the nucleus and induction of Diptericin and other antimicrobial peptides. Some Gram-positive bacteria and fungi activate the Toll pathway resulting in Dorsal or DIF (NF-κB homologs) translocation to the nucleus and activation of *Drosomycin* and other genes (Lemaitre and Hoffmann, 2007; Tanji and Ip, 2005). Exposure to bacteria results in proteolytic processing of Spätzle, which is the Toll receptor ligand. Activated Toll recruits three cytoplasmic proteins, dMyD88, Tube and Pelle, the serine/threonine kinase ortholog of IRAK. Recruitment causes phosphorylation and stimulation of Pelle/IRAK kinase. This results, in an as yet unknown manner, in degradation of the I-κB ortholog Cactus, which allows Dorsal or DIF, to which Cactus is bound in the cytoplasm, to translocate to the nucleus, and activate gene transcription (Lemaitre and Hoffmann, 2007; Tanji and Ip, 2005).

Pellino is a highly conserved component of the Toll pathway, which binds phosphorylated Pelle/IRAK (Grosshans et al., 1999). The vertebrate homologs of the Toll pathway are the Toll-like receptor (TLR) and Interleukin-1 receptor (IL-1R) pathways, where mammalian Pellinos (Pellino-1, -2, and -3) have also been shown to bind phosphorylated IRAK (Lin et al., 2008; Moynagh, 2009). All Pellinos contain a CHC2CHC2 RING E3 ubiquitin ligase domain, and it was recently shown that all three human Pellinos mediate polyubiquitination of IRAK (Schauvliege et al., 2006). In vitro, Pellinos can mediate both lysine 48- and lysine 63-linked polyubiquitination of IRAK (Butler et al., 2007; Ordureau et al., 2008).

* Corresponding author. Tel.: +1 514 398 6434; fax: +1 514 398 8051.
E-mail address: frieder.schoeck@mcgill.ca (F. Schöck).
0925-4773/$ - see front matter
doi:10.1016/j.mod.2010.01.004

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004

However, in a tissue culture model, Pellinos mainly mediate lysine 63-linked polyubiquitination (Xiao et al., 2008). While lysine 48-linked polyubiquitination results in recognition by proteasomes and degradation, lysine 63 polyubiquitination is used for many purposes, among them protein kinase activation, which plays an important role in NF-κB regulation (Skaug et al., 2009). Functional studies on the role of Pellinos in TLR/IL-1R signaling were conducted in tissue culture cells using RNAi-mediated depletion of Pellino. In such assays Pellino-3b negatively regulates IL-1R signaling, whereas Pellino-2, the closest homolog of *Drosophila* Pellino, positively regulates TLR/IL-1R-dependent NF-κB activation (Xiao et al., 2008; Yu et al., 2002). Pellino-1 can act both positively and negatively on the TLR/IL-1R pathway (Choi et al., 2006; Jiang et al., 2003).

Here we identify a chemically induced mutation in *Drosophila* Pellino and show that *Drosomycin* induction and innate immunity is severely compromised in this mutant.

2. Materials and methods

2.1. Fly genetics

We used the following stocks: Df(3R)06624, TM3 Sb twi-Gal4 UAS-2xEGFP, TM3 Sb hs-hid, hs-Gal4, tubP-Gal4, arm-Gal4 UAS-αcateninGFP, hs-Flp22, FRT82B ovo^{D1}, FRT82B 7T2, and TM3 hb8-lacZ. We made Pli^{1} hs-Gal4 and Pli^{1} UAS-Pli recombinants by standard crosses.

We used viable molecularly mapped P elements carrying a mini-white marker that were evenly spaced on chromosome arm 3R for recombination mapping (Zhai et al., 2003). Briefly, we crossed P/*7T2* virgin females to *7T2*/TM3 *Sb, hs-hid* males. After heat shocking, the only survivors are red-eyed P/*7T2* and white-eyed recombinants (+/*7T2*). Recombination frequency and projected molecular distance can then easily be calculated (Zhai et al., 2003).

To generate germ line clones, *hs-Flp22*; *FRT82B ovo^{D1}* males were crossed to *FRT82B Pli^{1}* females and heat-shocked at third instar for 2 h. *FRT82B ovo^{D1}*/*FRT82B Pli^{1}* females with germ line clones were then mated to either *Pli^{1}*/TM3, *Sb twi-Gal4 UAS-2xEGFP* for the JNK experiments or to *Pli^{1}*/TM3, *hb8-lacZ* stained with anti-β galactosidase antibody for the Twist experiments to distinguish maternal and zygotic *Pli^{1}* mutants from paternally rescued mutants.

2.2. Molecular biology

Pellino cDNAs were isolated from an ovarian cDNA library cloned in λ phages by hybridisation with the *Pellino* plasmid JG-Pli70 isolated in a two-hybrid screen for interactors of Pelle (Grosshans et al., 1999; Stroumbakis et al., 1994). The longest cDNA of 2900 nucleotides with the coding sequence at nucleotide 389–1663 was excised as an EcoRI–NotI fragment and cloned into pUAST. Transgenic flies were produced by injection into *y w* flies.

Genomic DNA preparation and PCR, as well as Western blotting were performed using standard protocols using the TM3, *Sb twi-Gal4 UAS-2xEGFP* balancer to select homozygously mutant embryos.

For RT-PCR, RNA was isolated from embryos using Trizol according to the manufacturer's instructions (Invitrogen). cDNAs were then synthesized using SuperScript II Reverse Transcriptase (Invitrogen). 1155 bp of the 3′ end of *Pellino* cDNA was amplified with primers CGGAGTCACCCATTGACTTT and GCAGCCAGAGACACAACAAA.

2.3. Histochemistry and antibodies

Cuticle preparations and antibody stainings were performed as previously described (Jani and Schöck, 2007; Schöck and Perrimon, 2002). The following primary antibodies were used: rabbit anti-Pellino (1:2000 (Grosshans et al., 1999)), goat anti-Pelle (1:500; sc-15769; Santa Cruz Biotechnology), rabbit anti-JNK1 (1:300; sc-571; Santa Cruz Biotechnology), rabbit anti-phospho-JNK (1:5000, Promega), rabbit anti-Twist (1:2500 (Thisse et al., 1988)), rabbit anti-β galactosidase (1:2000; Cappel), mouse anti-α tubulin (1:10,000; DM1a; Sigma), and mouse anti-dJUN (1:1000 (Kockel et al., 1997)). As secondary antibody for Western blots we used horseradish peroxidase-linked IgG (1:2500; GE Healthcare), and for immunostainings antibodies of the AlexaFluor series (1:400; Invitrogen).

2.4. Infections and mRNA analysis

For septic injury we used *Drosophila* adults. Homozygous *Pli^{1}* adults were generated by heat shocking the progeny of *Pli^{1}* hs-Gal4/TM3 and *Pli^{1}* UAS-Pli/TM3 at 37 °C for 45 min for 3 d. These adults were aged between 2 and 4 d; one-half was heat-shocked again at 37 °C for 20 min for 4 consecutive days; the other half was aged without heat shocking. Adults were pricked on the lateral side of the thorax with a 0.2 mm diameter tungsten needle dipped into a 10× concentrated culture of *Escherichia coli* or *Micrococcus luteus* bacterial LB cultures (Romeo and Lemaitre, 2008). Infected adults were incubated at 25 °C and allowed to recover for 24 and 12 h following *M. luteus* and *E. coli* infection, respectively; then they were used for RNA extraction using Trizol (Invitrogen) according to the manufacturer's instructions. We purified the extracted total RNA with DNase I (Invitrogen). We used SuperScript™ II Reverse Transcriptase (Invitrogen) for cDNA synthesis (Leulier et al., 2003; Romeo and Lemaitre, 2008).

To monitor expression levels by quantitative real-time PCR we used the iCycler iQ system using 1× iQ SYBR Green Supermix (Bio-Rad) with the following primers: *Drosomycin*, CGTGAGAACCTTTTCCAATATGATG and TCCCAGGACCAC CAGCAT; *Diptericin*, GCTGCGCAATCGCTTCTACT and TGGTGGA GTGGGCTTCATG. We normalized all values relative to *RpL32* expression levels as reference gene (primers GACGCTTCA AGGGACAGTATCTG and AAACGCGGTTCTGCATGAG) using the Q-Gene software package provided by Bio-Rad, which calculates normalized expression levels according to the formula $NE = E_{reference}\exp(Ct_{reference})/E_{target}\exp(Ct_{target})$, where NE stands for normalized expression level, E for amplification efficiency and Ct for threshold cycle (Simon, 2003). We used the amplification efficiencies previously published for the primers and protocol employed by us (Romeo and Lemaitre, 2008). Afterwards we set wild type induction to 100%.

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004

For survival experiments, embryos were collected from the cross of Pli^1 hs-Gal4/TM3 with Pli^1 UAS-Pli/TM3; the embryos were then sterilized by dechorionation in 50% bleach for 3 min, and grown on regular fly food, or fly food autoclaved for 50 min, and dispensed into sterilized bottles (Tang et al., 2008). Adults were scored for the absence of *Stubble* marking the balancer chromosome. For survival curves, homozygous Pli^1 mutant adults or wild type adults were pricked as described in the previous paragraph. Log-rank analysis was used to establish statistical significance between survival curves.

3. Results and discussion

We uncovered the mutant *7T2* in a collection of zygotic lethal mutations while screening for mutants with maternal-effect germ band retraction defects (Schöck and Perrimon, 2003). *7T2* zygotic mutants are pupal lethal, whereas *7T2* germ line clones have germ band retraction defects, but also twisted embryos and embryos with head involution defects (Fig. S1 and Table S1). We mapped *7T2* by recombination with molecularly mapped P elements (Zhai et al., 2003). The third round of P element-mediated recombination narrowed the area to a small region between 19675990 and 19756853 basepairs containing eight genes (Table S2). The small deletion *Df(3R)06624* within that region did not complement *7T2*, and *7T2* as well as *7T2/Df(3R)06624* zygotic mutants are pupal lethal. Mapping this deficiency by PCR indicates that it only deletes *Pellino* (*Pli*) in addition to *TF-IIAS* (Grosshans et al., 1999; Zeidler et al., 1996) (Fig. S2). *7T2* germ line clones crossed to *Df(3R)06624* show the same frequency of defects as crossed to *7T2*. In contrast, *7T2* germ line clones crossed to paternally wild type flies show very few defects, indicating that the mutation causing the observed maternal and zygotic phenotypes lies within *Df(3R)06624* (Table S1). As lethal mutations in *TF-IIAS* complement *7T2*, we assayed by RT-PCR for the presence of *Pellino* mRNA and by Western blotting for the presence of Pellino protein in *7T2* mutants. Both *Pellino* mRNA and Pellino protein are absent in *7T2* and *Df(3R)06624* (Fig. 1A and B). We finally generated UAS-Pli transgenic flies to test for rescue of *7T2* by ubiquitous expression of *Pellino* using the UAS/Gal4 system (Brand and Perrimon, 1993). Expressing Pellino with hs-Gal4 using 1-h heat shocks every day from first-instar onwards rescues *7T2* homozygous mutants to viability (31% homozygous adults with heat-shock [$n = 548$], compared to 3% homozygous adults without heat-shock [$n = 491$]; see also Fig. 4A). We therefore conclude that *7T2* disrupts *Pli* and is a null allele we refer to as Pli^1 from now on.

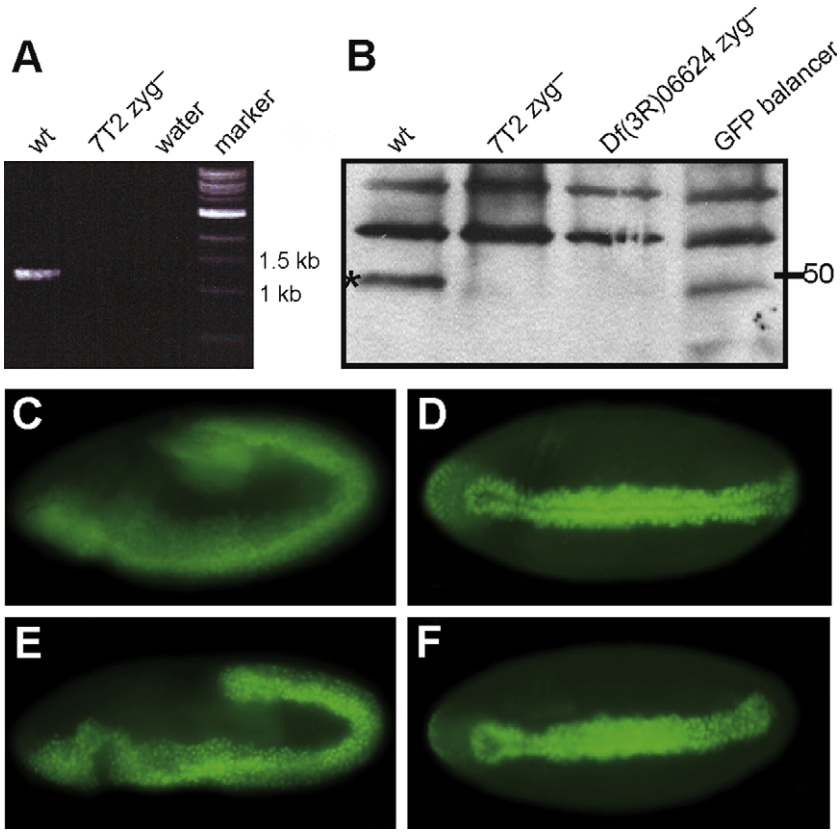

Fig. 1 – Pli^1 does not affect Twist expression in the ventral furrow. (A) RT-PCR with primers amplifying exons 4, 5 and 6 of *Pellino* show amplification of 1155 basepair product only in mRNA from wild type, but not from late-stage homozygous *7T2* embryos. (B) Western blot with rabbit anti-Pellino antibody shows absence of a 47 kDa band corresponding to Pellino (marked with an asterisk) in late-stage homozygous *7T2* and *Df(3R)06624* embryos. Unspecific slower migrating bands serve as loading control. (C and D) Anti-Twist antibody staining of wild type embryos. A lateral view of a germ band-extending embryo, and a slightly earlier ventral view is shown. (E and F) Pli^1 germ line clones at the same stage show no difference in width of the Twist expression domain.

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004

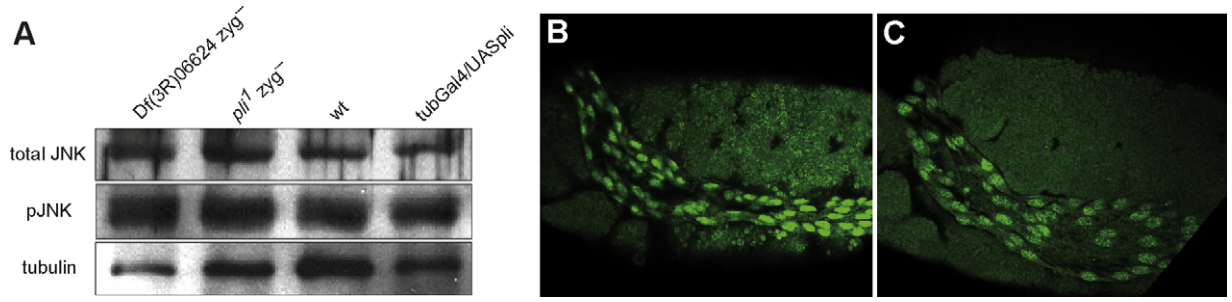

Fig. 2 – Pli^1 does not affect JNK signaling. (A) Comparison of total and phosphorylated JNK levels shows no difference between wild type and late-stage homozygous Pli^1, *Df(3R)06624* or Pellino-overexpressing embryos. (B) Anti-dJUN antibody staining of wild type embryo at the onset of germ band retraction shows nuclear dJUN localization. (C) Anti-dJUN antibody staining of Pli^1 germ line clone at the same stage shows no difference in dJUN localization.

Pellino was first identified as a biochemical interaction partner of Pelle in the Toll pathway (Grosshans et al., 1999). Pellino is a 424 amino acid protein, which shares 58% identical amino acids with human Pellino-2. As all Pellino proteins described to date interact with Pelle/IRAK, one may expect dorsal–ventral patterning defects. As mentioned previously, Pli^1 germ line clones exhibit 19% twisted embryos similar to phenotypes observed in *twi* and *sna* mutants. To determine if Twist expression, a read-out of dorsoventral signaling mediated by the Toll pathway, is reduced, we stained Pli^1 germ line clone and wild type embryos with anti-Twist antibody. We could not detect a difference in the width of the Twist expression domain, suggesting that Pli^1 either does not affect dorsoventral signaling or the phenotype and penetrance is too weak to be detected (Fig. 1C–F). Better quantitative methods for analyzing the Dorsal gradient or the Twist expression domain, which were recently established, may resolve this issue in the future (Kanodia et al., 2009).

We next wanted to know if *Pli* plays a role in germ band retraction. Vertebrate Pellino-2 and -3 have been implicated in JNK signaling (Schauvliege et al., 2007). Time-lapse analysis of Pli^1 mutants marked by *arm-Gal4 UAS-αcateninGFP* showed that Pli^1 mutants look very similar to βPS integrin (mys^{XG43}) null mutant embryos (data not shown) (Schöck and Perrimon, 2003). It was recently shown that JNK signaling upregulates integrin expression at the leading edge during dorsal closure (Homsy et al., 2006). Furthermore, JNK signaling is active in the amnioserosa during germ band retraction, and is restricted to the leading edge later on during dorsal closure (Reed et al., 2001). Finally, strong mutants in the JNK pathway have also germ band retraction defects (Bellotto et al., 2002). We therefore, explored if Pli^1 can modulate the JNK pathway by testing for the presence of nuclear dJUN staining in the amnioserosa at the onset of germ band retraction. We first compared phospho-JNK to total JNK levels in late-stage Pli^1 mutants and wild type by Western blotting. We also compared nuclear localization of dJUN in the amnioserosa at the onset of germ band retraction in wild type and Pli^1 germ line clones (Fig. 2). We could not detect any differences in JNK signaling by these two readouts, suggesting that *Drosophila* Pellino does not modulate JNK signaling.

Another important function of the Toll pathway lies in innate immunity. We analyzed the induction of *Drosomycin*, a Toll pathway target gene, and *Diptericin*, an IMD pathway target gene, 24 h after pricking adults with *M. luteus* or 12 h after pricking adults with *E. coli*, respectively. We employed quantitative real-time RT-PCR (qPCR) to compare induction levels in Pli^1 mutant flies rescued to adulthood and continuously expressing Pellino (Pli^+) with Pli^1 mutant flies rescued to adulthood without any further Pellino expression (Pli^-). *Drosomycin* expression levels are strongly reduced in Pli^- flies (Fig. 3A), whereas *Diptericin* induction is largely unaffected (Fig. 3B), demonstrating that Pellino is required for *Drosomycin* induction in Pli^1 mutant *Drosophila*. We also observe a reduction of *Drosomycin* induction in Pli^1 mutant larvae assayed by semiquantitative RT-PCR (Fig. S3). We did not observe a difference in Pelle/IRAK protein levels in Pli^1 mutants compared to wild type (Fig. S3). If Pellino functions mostly to modulate Toll signaling by enhancing it, this could explain why we did not observe a phenotype in dorsoventral patterning. Toll pathway signaling in innate immunity is more sensitive to perturbations than in dorsoventral patterning, which can be observed for example in hypomorphic mutations of *dMyd88*, which show a strong phenotype in innate immunity but no phenotype in dorsoventral patterning (Tauszig-Delamasure et al., 2002).

We then asked if overexpression of Pellino causes a stronger induction of *Drosomycin*. We therefore, overexpressed UAS-Pli with the strong and ubiquitous βtub-Gal4 driver and analyzed adult flies by qPCR. *M. luteus* infection of flies overexpressing Pellino results in a threefold stronger induction of *Drosomycin* compared to wild type flies. Importantly, Pellino overexpression cannot induce *Drosomycin* expression on its own, it can only enhance expression already induced by *M. luteus* infection (Fig. 3C). These data indicate that Pellino is a limiting factor in Toll signaling, but that it cannot activate the pathway by itself as for example dMyd88 (Tauszig-Delamasure et al., 2002).

We also tested if Pellino overexpression affects Pelle/IRAK stability upon *M. luteus* infection, but we could not detect any differences in the level of Pelle protein (Fig. 3D). This is consistent with recent data on mammalian Pellino suggesting a role in regulating Pelle/IRAK activity rather than stability (Xiao et al., 2008).

Lastly, we asked if Pellino is required for innate immunity in vivo. We therefore, tested survival of Pli^1 UAS-Pli/Pli^1 hs-Gal4 flies in a germ-free environment. These flies have some baseline survival presumably owing to leaky expression of

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004

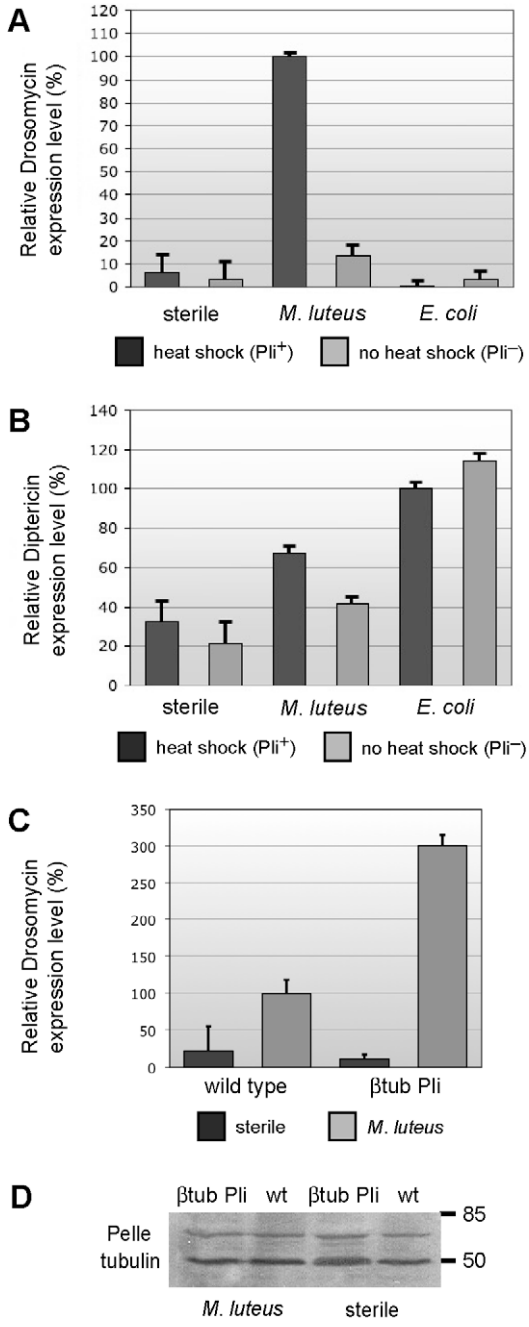

Fig. 3 – *Drosomycin* induction is reduced in Pli^1. (A–C) Quantitative real-time RT-PCR (qPCR) was used to determine expression levels of innate immunity target genes. All values are normalized to *RpL32*. Error bars represent standard error of the mean of two independent experiments each done in triplicate. (A) qPCR of *Drosomycin* expression levels in Pli^1 mutant and rescued flies. (B) qPCR of *Diptericin* expression levels in Pli^1 mutant and rescued flies. (C) qPCR of *Drosomycin* expression levels in wild type flies and flies overexpressing Pellino. (D) Western blot with anti-Pelle antibody of flies from (C) shows that levels of Pelle/IRAK protein are unchanged. Anti-α tubulin antibody detects tubulin as a loading control. Two molecular weight markers are indicated on the right in kDa.

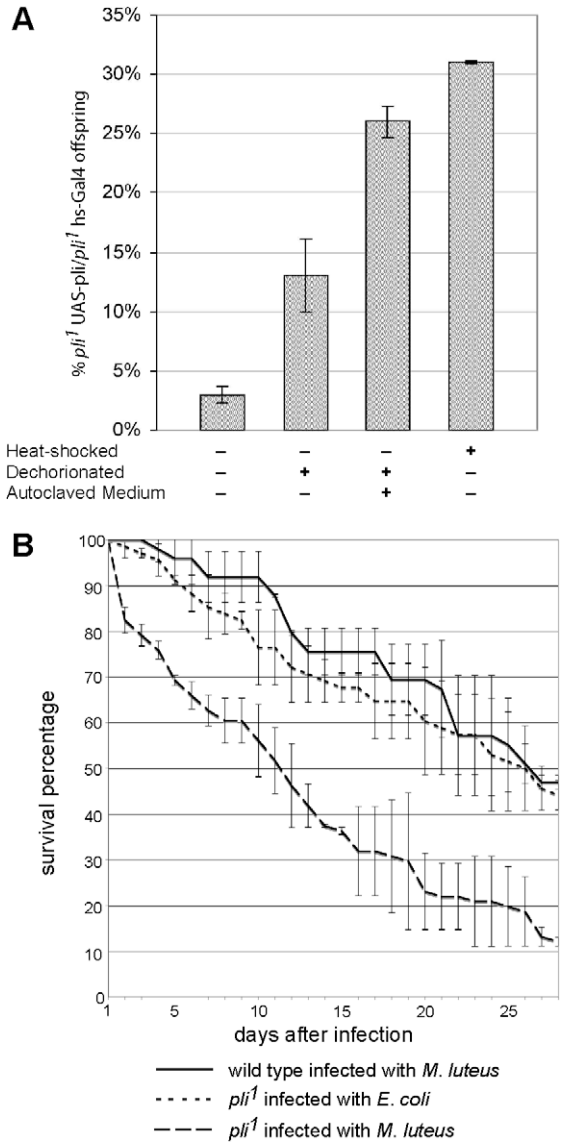

Fig. 4 – Innate immunity is severely compromised in Pli^1. (A) Percent homozygous survivors of Pli^1 UAS-Pli/TM3 crossed to Pli^1 hs-Gal4/TM3 flies. 33% corresponds to the Mendelian ratio expected for full viability. Dechorionation with bleach removes germs from the egg, medium was sterilized by autoclaving. (B) Survival curves of non-heat-shocked Pli^1 UAS-Pli/Pli^1 hs-Gal4 flies infected with *M. luteus* (total $n = 91$) or *E. coli* (total $n = 68$) or wild type flies infected with *M. luteus* (total $n = 49$). Log-rank analysis was used to compare controls (wild type infected with *M. luteus* and Pli^1 infected with *E. coli*) to Pli^1 infected with *M. luteus*. Statistical significance between combined control curves and Pli^1 infected with *M. luteus* is $p < 0.0001$. We also performed log-rank analysis to compare the two controls, and found no statistical significance ($p = 0.58$). Error bars, standard deviation of three experiments.

UAS-Pli in the absence of heat-shock. We can increase the survival of "hypomorphic" Pli^1 flies (Pli^1 null plus potentially leaky transgene) to wild type levels by removing infectious

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004

agents from their environment (Fig. 4A). We finally determined if these *Pli*^{1} mutant adults are more susceptible to *M. luteus* infection. Infection of *Pli*^{1} mutants with *M. luteus* strongly reduces viability, resulting in 50% lethality approximately 15 days earlier than in wild type flies infected with *M. luteus* or in *Pli*^{1} mutant flies infected with *E. coli* (Fig. 4B).

Toll pathway mutants are typically female sterile (Charatsi et al., 2003), therefore, the zygotic lethality of *Pli*^{1} is surprising. Pellino may either act in an additional pathway, or *Pli*^{1} still carries a second-site mutation that reduces its viability. The baseline survival of *Pli*^{1} UAS-Pli recombinants points in that direction, as well as the occasional adult escapers we observe in *7T2/Df(3R)06624*. Importantly, this possibility does not affect our conclusions, because we demonstrate germ line clone defects with *7T2/Df(3R)06624* (Table S1), and we observe normal *Drosomycin* induction upon expression of UAS-Pli in *Pli*^{1} mutants (Fig. 3).

Overall our data indicate that Pellino is required to ensure survival in the presence of certain Gram-positive infectious agents, and that postembryonic Pellino functions predominantly as a positive regulator of innate immunity. In mammalians Pellino-1 and -3 play a predominantly negative role in innate immunity, whereas there is evidence for a positive role for Pellino-2 (Moynagh, 2009; Yu et al., 2002). The evolutionarily conserved role of Pellino therefore, may be that of a positive regulator of innate immunity.

Acknowledgements

We particularly thank Norbert Perrimon for providing the *7T2* mutant, Dirk Bohmann for the anti-dJUN antibody, Laura Nilson for the anti-Twist antibody, and Beili Hu for injections. This work was supported by the New Opportunities Grant 9607 (F.S.) from the Canadian Foundation for Innovation, and by operating Grant 298534-04 (F.S.) from the Natural Sciences and Engineering Research Council of Canada. F.S. is a Canadian Institutes of Health Research New Investigator (MSH-76596).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mod.2010.01.004.

REFERENCES

Bellotto, M., Bopp, D., Senti, K.A., Burke, R., Deak, P., Maroy, P., Dickson, B., Basler, K., Hafen, E., 2002. Maternal-effect loci involved in Drosophila oogenesis and embryogenesis: P element-induced mutations on the third chromosome. Int. J. Dev. Biol. 46, 149–157.

Brand, A., Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415.

Butler, M.P., Hanly, J.A., Moynagh, P.N., 2007. Kinase-active interleukin-1 receptor-associated kinases promote polyubiquitination and degradation of the Pellino family: direct evidence for PELLINO proteins being ubiquitin-protein isopeptide ligases. J. Biol. Chem. 282, 29729–29737.

Charatsi, I., Luschnig, S., Bartoszewski, S., Nüsslein-Volhard, C., Moussian, B., 2003. Krapfen/dMyd88 is required for the establishment of dorsoventral pattern in the Drosophila embryo. Mech. Dev. 120, 219–226.

Choi, K.C., Lee, Y.S., Lim, S., Choi, H.K., Lee, C.H., Lee, E.K., Hong, S., Kim, I.H., Kim, S.J., Park, S.H., 2006. Smad6 negatively regulates interleukin 1-receptor-toll-like receptor signaling through direct interaction with the adaptor Pellino-1. Nat. Immunol. 7, 1057–1065.

Grosshans, J., Schnorrer, F., Nüsslein-Volhard, C., 1999. Oligomerisation of Tube and Pelle leads to nuclear localisation of dorsal. Mech. Dev. 81, 127–138.

Homsy, J.G., Jasper, H., Peralta, X.G., Wu, H., Kiehart, D.P., Bohmann, D., 2006. JNK signaling coordinates integrin and actin functions during Drosophila embryogenesis. Dev. Dyn. 235, 427–434.

Jani, K., Schöck, F., 2007. Zasp is required for the assembly of functional integrin adhesion sites. J. Cell Biol. 179, 1583–1597.

Jiang, Z., Johnson, H.J., Nie, H., Qin, J., Bird, T.A., Li, X., 2003. Pellino 1 is required for interleukin-1 (IL-1)-mediated signaling through its interaction with the IL-1 receptor-associated kinase 4 (IRAK4)-IRAK-tumor necrosis factor receptor-associated factor 6 (TRAF6) complex. J. Biol. Chem. 278, 10952–10956.

Kanodia, J.S., Rikhy, R., Kim, Y., Lund, V.K., Delotto, R., Lippincott-Schwartz, J., Shvartsman, S.Y., 2009. Dynamics of the Dorsal morphogen gradient. Proc. Natl. Acad. Sci. USA 106, 21707–21712.

Kockel, L., Zeitlinger, J., Staszewski, L.M., Mlodzik, M., Bohmann, D., 1997. Jun in Drosophila development: redundant and nonredundant functions and regulation by two MAPK signal transduction pathways. Genes Dev. 11, 1748–1758.

Lemaitre, B., Hoffmann, J., 2007. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. 25, 697–743.

Leulier, F., Parquet, C., Pili-Floury, S., Ryu, J.H., Caroff, M., Lee, W.J., Mengin-Lecreulx, D., Lemaitre, B., 2003. The Drosophila immune system detects bacteria through specific peptidoglycan recognition. Nat. Immunol. 4, 478–484.

Lin, C.C., Huoh, Y.S., Schmitz, K.R., Jensen, L.E., Ferguson, K.M., 2008. Pellino proteins contain a cryptic FHA domain that mediates interaction with phosphorylated IRAK1. Structure 16, 1806–1816.

Moynagh, P.N., 2009. The Pellino family: IRAK E3 ligases with emerging roles in innate immune signalling. Trends Immunol. 30, 33–42.

Ordureau, A., Smith, H., Windheim, M., Peggie, M., Carrick, E., Morrice, N., Cohen, P., 2008. The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. Biochem. J. 409, 43–52.

Reed, B.H., Wilk, R., Lipshitz, H.D., 2001. Downregulation of Jun kinase signaling in the amnioserosa is essential for dorsal closure of the *Drosophila* embryo. Curr. Biol. 11, 1098–1108.

Romeo, Y., Lemaitre, B., 2008. Drosophila immunity: methods for monitoring the activity of Toll and Imd signaling pathways. Methods Mol. Biol. 415, 379–394.

Schauvliege, R., Janssens, S., Beyaert, R., 2006. Pellino proteins are more than scaffold proteins in TLR/IL-1R signalling: a role as novel RING E3-ubiquitin-ligases. FEBS Lett. 580, 4697–4702.

Schauvliege, R., Janssens, S., Beyaert, R., 2007. Pellino proteins: novel players in TLR and IL-1R signalling. J. Cell. Mol. Med. 11, 453–461.

Schöck, F., Perrimon, N., 2002. Cellular processes associated with germ band retraction in *Drosophila*. Dev. Biol. 248, 29–39.

Schöck, F., Perrimon, N., 2003. Retraction of the Drosophila germ band requires cell–matrix interaction. Genes Dev. 17, 597–602.

Simon, P., 2003. Q-Gene: processing quantitative real-time RT-PCR data. Bioinformatics 19, 1439–1440.

Skaug, B., Jiang, X., Chen, Z.J., 2009. The role of ubiquitin in NF-kappaB regulatory pathways. Annu. Rev. Biochem. 78, 769–796.

Stroumbakis, N.D., Li, Z., Tolias, P.P., 1994. RNA- and single-stranded DNA-binding (SSB) proteins expressed during Drosophila melanogaster oogenesis: a homolog of bacterial and eukaryotic mitochondrial SSBs. Gene 143, 171–177.

Tang, H., Kambris, Z., Lemaitre, B., Hashimoto, C., 2008. A serpin that regulates immune melanization in the respiratory system of Drosophila. Dev. Cell 15, 617–626.

Tanji, T., Ip, Y.T., 2005. Regulators of the Toll and Imd pathways in the Drosophila innate immune response. Trends Immunol. 26, 193–198.

Tauszig-Delamasure, S., Bilak, H., Capovilla, M., Hoffmann, J.A., Imler, J.L., 2002. Drosophila MyD88 is required for the response to fungal and Gram-positive bacterial infections. Nat. Immunol. 3, 91–97.

Thisse, B., Stoetzel, C., Gorostiza-Thisse, C., Perrin-Schmitt, F., 1988. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early Drosophila embryos. Embo J. 7, 2175–2183.

Xiao, H., Qian, W., Staschke, K., Qian, Y., Cui, G., Deng, L., Ehsani, M., Wang, X., Qian, Y.W., Chen, Z.J., Gilmour, R., Jiang, Z., Li, X., 2008. Pellino 3b negatively regulates interleukin-1-induced TAK1-dependent NF kappaB activation. J. Biol. Chem. 283, 14654–14664.

Yu, K.Y., Kwon, H.J., Norman, D.A., Vig, E., Goebl, M.G., Harrington, M.A., 2002. Cutting edge: mouse pellino-2 modulates IL-1 and lipopolysaccharide signaling. J. Immunol. 169, 4075–4078.

Zeidler, M.P., Yokomori, K., Tjian, R., Mlodzik, M., 1996. Drosophila TFIIA-S is up-regulated and required during Ras-mediated photoreceptor determination. Genes Dev. 10, 50–59.

Zhai, R.G., Hiesinger, P.R., Koh, T.W., Verstreken, P., Schulze, K.L., Cao, Y., Jafar-Nejad, H., Norga, K.K., Pan, H., Bayat, V., Greenbaum, M.P., Bellen, H.J., 2003. Mapping Drosophila mutations with molecularly defined P element insertions. Proc. Natl. Acad. Sci. USA 100, 10860–10865.

Please cite this article in press as: Haghayeghi, A. et al, Pellino enhances innate immunity in Drosophila, Mech. Dev. (2010), doi:10.1016/j.mod.2010.01.004