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Do the Genes of the Innate Immune Response Contribute to Neuroprotection in *Drosophila*?

Rafael Cantera[a, b] Rosa Barrio[c]

[a] Zoology Department, Stockholm University, Stockholm, Sweden; [b] Developmental Neurobiology, IIBCE, Montevideo, Uruguay; [c] Functional Genomics, CIC bioGUNE, Derio, Spain

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Drosophila · Genome-wide analysis · Innate immune response · Neurodegeneration

Abstract

A profound debate exists on the relationship between neurodegeneration and the innate immune response in humans. Although it is clear that such a relation exists, the causes and consequences of this complex association remain to be determined in detail. *Drosophila* is being used to investigate the mechanisms involved in neurodegeneration, and all genomic studies on this issue have generated gene catalogues enriched in genes of the innate immune response. We review the data reported in these publications and propose that the abundance of immune genes in studies of neurodegeneration reflects at least two phenomena: (i) some proteins have functions in both immune and nervous systems, and (ii) immune genes might also be of neuroprotective value in *Drosophila*. This review opens this debate in *Drosophila*, which could thus be used as an instrumental model to elucidate this question.

Introduction

Neurodegenerative diseases are often correlated with the activation of the innate immune response in humans but the causes or consequences of this complex relationship remain to be determined in detail [1–3]. The fly *Drosophila melanogaster* is an eminent model organism for the study of the innate immune response, of which many aspects are shared by other organisms, including humans [4, 5], as well as for the study of cellular and molecular mechanisms responsible for neurodegeneration [6–8]. Compared to mammals, *Drosophila* has better genetic tools, less complex nervous and immune systems and no blood capillaries or chronic inflammation. Hence, the primary conditions responsible for nervous tissue deterioration should be more easily distinguished from secondary processes. Genetic links between neurodegeneration and the innate immune response have recently been established in *Drosophila*, opening the perspective for rapid advances in this field of biological and medical relevance.

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E-Mail karger@karger.com
www.karger.com/jin

Dr. Rafael Cantera
Zoology Department, Stockholm University
Svante Arrheniusväg 18B
SE–106 91 Stockholm (Sweden)
E-Mail rcantera@zoologi.su.se

Genes of the Immune Response Are Experimentally Associated with Neurodegeneration

Genes known to function in *Drosophila* innate immune response have been experimentally assigned a causative role in neurodegenerative processes [9–13]. In a review of these studies, Petersen and Wassarman [14] propose that activation of the immune response promotes neurodegeneration and two subsequent publications reinforce this view [12, 13].

Flies in which human Aβ42 is expressed in the retina suffer from a type of retinal degeneration that is diminished or even suppressed by mutations in genes that encode proteins of fundamental importance for the activation of immune response along the Toll pathway, including the receptors Toll, Tube and Pelle and the transcription factors homologues of human NF-κB, Dif and Dorsal [9]. However, the data also indicate that in this case degeneration is not mediated by the canonical Toll pathway. The two principal discrepancies are, perhaps, that mutations in the genes that encode Spatzle, a ligand of Toll, or Cactus (homologue of human I-κB), an important inhibitor of the Toll pathway, have no consequences for the retinal phenotype [9]. However, *cactus* seems to be upregulated in these flies, as is also the case in flies infected with pathogens [15, 16]. Furthermore, the operation of the Toll pathway during the immune response is based on the nuclear accumulation of the transcription factors Dif and Dorsal [17], but whether this happens in Aβ42-expressing flies is not known.

An association has been also established between neurodegenerative phenotypes and genes from the Imd pathway, the other main signaling pathway in the fly's immune response. The key transcription factor for the activation of this pathway is Relish, a homologue of mammalian NF-κB [5, 18]. Flies with mutations in *dnr1*, a gene that encodes a repressor of *Relish,* exhibit a neurodegenerative phenotype associated with increased transcripts of *Relish* target genes encoding Cecropin A1, Diptericin B, Attacin A and other antibacterial peptides [13]. Transgenic overexpression in nervous tissue of some (but not all of those tested) antibacterial peptides also causes neurodegeneration as does the introduction of bacteria in the brain using a fine needle [13]. However, it is not known whether *Relish* itself is upregulated in these flies and whether the Relish protein accumulates in the nuclei of brain cells as expected for an activation of the immune response via Relish [18, 19].

Flies with mutations in *ATM (telomere fusion, tefu)*, a gene associated with the neurodegenerative disease ataxia-telangiectasia in humans, show significantly reduced ATM kinase activity and elevated levels of gene transcripts encoding antimicrobial peptides [11] but not of *spatzle*, *Toll*, *Dorsal*, *Dif*, *Relish, imd* and other genes important for the activation of immune responses induced in *Drosophila* by bacterial or fungal pathogens [15, 16]. The neurodegenerative process caused by mutated *ATM* is inhibited by loss-of-function mutations in *Relish*, but not in *imd*, demonstrating that *Relish* is necessary for the development of this pathology although not acting through the canonical Imd pathway. A similar relationship was observed in flies with light-dependent retinal degeneration caused by mutations in *norpA* [10]. This phenotype is associated with an increment in the antimicrobial peptide Diptericin, encoded by one of the genes activated by the Imd pathway during a response to pathogens. Both Diptericin upregulation and retinal degeneration are blocked by mutations in two elements central for the Imd pathway *(dredd* and *Relish)* but not by the equally important gene *imd*.

In summary, functional relationships between genes of the innate immune response and neurodegeneration have been firmly demonstrated in *Drosophila*, but the data indicate that they are not mediated via activation of canonical immune response pathways. It may well be that the discrepancies reflect tissue-specific differences in immune response pathways or that in the brain some immune response genes have nonimmune functions.

Regulation of Immune Response Genes Is a General Feature across Genomic Studies of Neurodegeneration

Genes annotated as integral to the immune response are also associated with neurodegeneration because they have abnormally high or low transcripts in genome-wide studies in several neurodegenerative models [14, 20–26] (table 1). The gene catalogues generated by these studies are clearly enriched in genes from the immune response and, in at least 2 cases, the immune response genes constituted the predominant functional group [11, 20]. Also, the catalogues published in other studies appear enriched in immune response genes, although this was not explicitly recognized in the corresponding publications [27, 28] (table 1). When considering the disproportionate presence of immune response genes among the hits of genomic studies of flies with neurodegeneration, it is important to notice that the majority of the genes annotated as part of the immune response in *Drosophila* do so because they exhibited abnormally high or low transcripts in microar-

Table 1. Genes related to the innate immune response in *Drosophila* misregulated during neurodegeneration

Reference	Type of assay	Genes identified, total n	Genes related to immune response[a], n (%)	Genes[b]
Ferreiro et al. [24], 2012	*spalt* homozygous mutant vs. wild type at stages 16 and 17 of embryogenesis	482	48 (9.9)	**CG10912**, CG11842, CG13323, CG1358, CG14219, **CG15784**, CG16772, **CG17107**, CG18067, **CG18179**, CG18180, CG18301, CG30080, **CG5778**, CG5791, **CG6639**, CG7296, CG8562, CG9360, CG9468, CG9649, CG9989, **Cpr49Ae**, Ctr1B, **deltaTry**, **Drsl5**, Ect3, Hf, Hsp70Bc, **IM1**, **IM10**, IM14, **IM2**, **IM23**, **IM3**, **IM4**, Jon25Bi, Jon25Bii, Jon25Biii, Jon44E, **Jon65Ai**, Jon99Ci, **Jon99Fi**, Listericin, **LysX**, PebIII, [Tg], **Tsf1**
Greene et al. [20], 2005	*parkin* homozygous mutant vs. heterozygous in 1-day-old adults	26	8 (30.8)	**AttA**, **CG11459**, CG12505, **CG3604**, **Dpt**, **IM4**, **LysE**, LysS
Kumimoto et al. [25], 2013	Expression of mutated human SOD1	124[c]	34 (27.4)	**AttA**, **AttC**, AttD, **CecC**, **CG10912**, CG13947, CG14500, CG15043, CG15263, **CG15282**, CG15829, **CG18179**, **CG33109**, **CG4269**, **CG4757**, CG9080, CG9463, **CG9733**, **Def**, **DptB**, **Drs**, **Drsl3**, **Drsl4**, fit, **Jon65Ai**, **Jon99Fi**, **LysP**, **Mtk**, **Obp99b**, **PGRP-SB1**, **PGRP-SC1b**, **PGRP-SD**, **pirk**, Prx2540-1
Nelson et al. [28], 2005	Overexpression of a polyQ repeat in the eye using GMR-GAL4	49	4 (8.2)	CG10641, crq, **mus209**, puc
Palgi et al. [23], 2012	*Manf* homozygous mutant embryos and larvae vs. wild type	1,243[d]	65 (5.2)	AGO2, **AttA**, cactin, Cec2, **CecA1**, **CecA2**, **CecB**, CG11159, **CG13422**, CG1887, **CG9733**, **Drs**, Drsl1, **Drsl2**, **Drsl3**, **Drsl5**, GNBP1, GNBP2, GNBP3, He, Hml, ik2, **IM1**, **IM10**, **IM23**, **IM3**, **IM4**, kay, key, **LysX**, lz, **Mtk**, os, PGRP-LA, PGRP-LB, **PGRP-LC**, PGRP-LF, **PGRP-SA**, **PGRP-SD**, **Phk-3**, **pirk**, Pli, pll, psh, Pvr, Rab11, Rala, Rel, Sp7, SPE, spirit, spz, Sr-CI, Sr-CII, **Tep1**, Tep3, Tep4, Tollo, TotA, TotC, TotX, **Tsf3**, upd3, vir-1, zfh1
Petersen et al. [11], 2012	Overexpression of human TBP protein with an expanded polyQ tract in the eye using GMR-GAL4	163	51 (31.3)	**AttA**, AttB, **AttC**, Cdc6, **CecA1**, **CecA2**, **CecB**, **CecC**, **CG11459**, **CG13422**, CG16978, **CG17107**, CG17760, **CG33109**, **CG3699**, **CG4269**, CG42807, **CG6639**, CG6788, CG7738, CG9616, **CG9733**, Cyp316a1, **Cyp6g1**, **Def**, **Dpt**, **DptB**, **Drs**, **Drsl4**, GADD45, grass, GstD5, **IM10**, **IM23**, **Lsp1beta**, Lsp2, **LysE**, **LysP**, mthl2, **Mtk**, **PGRP-SA**, **PGRP-SB1**, PGRP-SC2, **PGRP-SD**, **pirk**, **Spn88Eb**, Sr-CIV, **Tep1**, TotM, **Tsf3**, yellow-f
Ren et al. [22], 2011	Overexpression of human TBP protein with an expanded polyQ tract in the eye using GMR-GAL4	352	31 (8.8)	Cbs, **CecB**, CG10467, CG10514, CG10621, CG11891, CG11892, CG13641, **CG15282**, **CG15784**, CG16718, CG34370, **CG3604**, **CG3699**, **CG42351**, **CG4269**, **CG4757**, CG8129, Cyp28d1, **deltaTry**, kn, **Lsp1beta**, **mus209**, **PGRP-LC**, **PGRP-SD**, **Phk-3**, **Prat2**, smp-30, Toll-6, **Tsf1**, Vago
Scherzer et al. [27], 2003	Overexpression of human α-synuclein in neurons using elav-GAL4	94	20 (21.3)	**Acp1**, CG10383, CG15065, CG4019, **CG5778**, CG7203, Cyp4e2, **Cyp6g1**, Glt, Got2, Irc, **Obp99b**, PGRP-SC1a, **PGRP-SC1b**, **Prat2**, Rfabg, Spat, **Spn88Eb**, Thor, vkg
Shieh and Bonini [21], 2011	Overexpression of CAG repeat in neurons using elav-GAL4	152	11 (7.2)	**AttA**, CG11413, CG13325, **CG42351**, CG5493, CG9119, CG9837, CG9935, Idgf3, **IM2**, **Mtk**
Vanden Broeck et al. [26], 2013	Loss of function and overexpression of TDP-43	100[e]	10 (10.0)	**Acp1**, CG15021, CG15293, **CG18179**, CG7778, Cpr49Ab, **Cpr49Ae**, **Drsl2**, **Jon65Ai**, Jon65aiv

[a] Percentage of immune genes versus total number of genes is shown in parenthesis.
[b] Genes in bold appear in more than one study. Underlined genes have human homologues.
[c] 124 genes upregulated at 5 or 45 days (from table 1 in Kumimoto et al. [25]).
[d] Only upregulated genes are considered.
[e] The 100 genes listed in figure 4 in Vanden Broeck et al. [26] (the top 50 down- and top 50 upregulated in both LOF and GOF conditions).

Table 2. Innate immune genes are overrepresented among genes regulated during defense responses to various challenges

Reference	Stress condition	Stimulus	Percentage of innate immune response genes[a]
Gruenewald et al. [31], 2009	Hyperoxia	2 days on 99.5% oxygen	1.80
Azad et al. [38], 2009	Hypoxia	2.5 h on 1.5% oxygen	12.90
Stergiopoulos et al. [39], 2009	Osmotic stress	4 h on 4% NaCl	34[b]
Zimmerman et al. [32], 2006	Mechanical stress	4 h mechanical stimulation	50
Boltz and Carney [34], 2008	ER stress	Mutation in the p24 gene *loj*	20.40
Zinke et al. [35], 2002	Starvation, larva	4 h on PBS	8.80[c]
Fujikawa et al. [37], 2009	Starvation, adult	24 h on water only	18.60
Blanco et al. [40], 2010	Tissue regeneration	Wing disk fragmentation	n.s.
Seong et al. [41], 2011	Low-dose irradiation	Gamma irradiation of eggs 0.8 Gy/min	10.80
Karpac et al. [42], 2011	DNA damage	UV radiation	23.80

[a] Innate immune response group overrepresentation was reported by the authors, except for Zinke et al. [35], 2002.
[b] Calculated for the 100 top genes in supplementary table 1 from Stergioupoulos et al. [39], 2009.
[c] Calculated from 34 genes in figure 2 from Zinke et al. [35], 2002.

ray studies of flies challenged with pathogens [15, 16, 29], but whether they are all relevant for mounting an efficient immune response is not yet tested experimentally.

The data produced by the genome-wide studies of flies with neurodegenerative pathologies give rise to five intriguing observations: (i) none of the studies detected upregulation of *imd*, *Dif*, *dorsal* and other genes of great relevance for the immune response elicited by pathogens; (ii) at least some of the genes known to be upregulated during the activation of an immune response were indeed downregulated in flies with neurodegeneration; (iii) some immune response genes were upregulated in heterozygous mutants which do not develop a neurodegenerative pathology [24]; (iv) there is little overlap among the immune response genes detected across studies, and (v) the overrepresentation of immune response genes appears to be universal rather than associated with particular types of neurodegeneration.

These observations take us to reconsider the interpretation that the activation of the immune response is causative of neurodegeneration in all cases.

Immune Response Genes Are Overrepresented during the Responses to a Variety of Adverse Conditions

The immune response is a particular class of defense response. We were intrigued about whether immune response genes are also overrepresented in responses to adverse conditions other than neurodegeneration. Our review of published results led us to conclude that they are indeed overrepresented among the genes responding to oxidative stress [30, 31], mechanical stress [32], endoplasmic reticulum (ER) stress [33, 34], starvation [35–37], hypoxia [38], osmotic stress [39], wing disk regeneration [40], ionizing irradiation [41] or UV irradiation-mediated DNA damage [42] (table 2).

Each stress condition elicits a transcriptional response comprising the regulation of genes specific for the experimental protocol (for instance, genes of the Redox group are regulated when oxidative stress is induced in *Drosophila* flies by dietary administration of hydrogen peroxide or paraquat, or by exposing them to hyperoxia) and of genes that are also regulated by other challenges, showing that some genes respond to a variety of noxious stimuli. For example, the humoral factor Turandot, *TotA*, is induced by exposure to bacteria, heat stress, oxidative stress, mechanical stress, UV radiation and dehydration [43]. Among 449 genes with abnormally high transcripts in the head of flies exposed to hyperoxia, 68 are also upregulated in flies challenged by dietary administration of hydrogen peroxide or other protocols that cause oxidative stress [31]. Tens of genes are regulated by oxidative stress (regardless of whether this condition is caused by hyperoxia or hydrogen peroxide) and also by heat stress or ionizing radiation [44]. Some genes are regulated both by tunicamycin (which induces a form of ER stress) and by oxidative stress caused either by hydrogen peroxide or paraquat [33]. At least for some of the genes regulated

upon a variety of challenges it is reasonably well established that their regulation confers tolerance or protection against the experimental challenge [31, 33, 43, 44].

Hence, enrichment in immune response genes is a general feature in the gene catalogues generated by studies of the organism's transcriptional response to adverse conditions. The transcriptional activation of some immune genes is perhaps triggered by signals emanating from damaged or distressed cells, as proposed by the *danger* model [45]. This may have several explanations. One will be that the regulation of immune response genes confers protection against deterioration of cells and tissues regardless of whether it starts through mechanical damage, pathogen-induced alterations, heat shock, oxidative stress or other causes. The immune response gene GADD45, for example, is upregulated by experimental brain damage in the rat [46], by wounding in both flies and mice [47] and by tissue regeneration [40] or ER stress [34] in flies. Upregulation of GADD45 in the nervous system extends the life span of flies, pointing to a protective action [48]. Hence, its upregulation in flies suffering from neurodegeneration caused by a mutation in ATM [11] could reflect a neuroprotective rather than a neurotoxic action.

Another option could be that some immune response genes have additional functions and their regulation during neurodegenerative pathologies might contribute to a defense response in brain tissue via nonimmunogenic pathways. Genes of the Toll pathway, for instance, are involved in the development and maintenance of axons and neuronal synapses. Loss of function of *dorsal* protects against the pathology caused by transgenic expression of human Aβ42 [9], but is deleterious for the integrity of axons and neuromuscular synapses [49–51], and *dorsal* activation is necessary for nervous tissue recovery from injury, promoting glial proliferation [52]. Thus, the regulation of *dorsal* and other genes from the Toll pathway might have functional relevance for both neurodegeneration and neuroprotection independently of immune response pathways. Still, some immune response genes that appear in genomic studies of neurodegeneration may simply do so as a result of misregulation because of failures in signaling pathways caused by tissue deterioration.

A further option is the relationship between pleiotropy and the history of gene annotation, because genes that were first annotated as important for the immune response still might have completely different functions, including protection of nervous tissue integrity. An exciting example of this possibility (although with the opposite chronology in the discovery of a second, unexpected function) is *parkin*, a gene for which mutations were first associated with Parkinson's disease long before it was discovered that it also has an immune function, mediating protection against intracellular bacteria in humans [53].

These options can also be considered from an evolutionary perspective. Living organisms often confront adverse conditions in their external and internal environment in the form of sudden changes in temperature, nutrient and oxygen supplies, osmotic stress, DNA damage, pathogens or other challenges. Mounting defense responses specific for each of these situations should be of high adaptive value already for unicellular organisms early during evolution. Later on, during the evolution of multicellularity, some elements of unicellular defense responses were perhaps incorporated into new gene networks acting in specific cells or tissues and responding to more specific stimuli and some will serve more than one function.

Regulation of Immune Response Genes Might Reflect a Neuroprotective Response upon Neurodegeneration

Here, we hypothesize that the development of a neurodegenerative process in the fly can be regarded as a particular type of adverse condition and that it elicits a defense response. By analogy with the defense responses cited above, a neuroprotective response will share genes with other defense responses. This would explain, to some degree, why practically all genome-wide studies of gene expression in flies with neurodegeneration result in catalogues enriched with immune response genes. Therefore, some immune response genes would be associated with a response of the animal to combat the degeneration of nervous tissue rather than being causative of degeneration. At this point, it deserves to be mentioned that there is a dynamic functional relationship at several levels between neurodegeneration, the immune, endocrine and nervous systems and general metabolism [54]. Hence, the activation of some immune genes might reflect their contribution to the development and regulation of a neuroprotective response through genetic mechanisms acting not only in nervous tissue.

The participation in neuroprotection of genes normally activated during various defense responses is relatively well documented. For example, genes responding to heat shock (*Hsp70Bc* and other *Hsp* genes) [31, 55, 56] or oxidative challenge (*Sod*) [31, 57, 58] have neuroprotective function. Among 126 genes for which RNA interference

Table 3. Innate immune genes defined as suppressors or enhancers of neurodegeneration

Reference	Gene symbol	Gene effect	Protein function	Signaling vs. effectors
Ambegaokar and Jackson[65], 2011	mei-9	Suppressor	Protein binding	Unknown
Ambegaokar and Jackson [65], 2011	Meek1	Enhancer	Kinase	Signaling
Ambegaokar and Jackson [65], 2011	Nrg	Suppressor	Calcium ion binding	Effector
Ambegaokar and Jackson [65], 2011	RpS21	Enhancer	Ribosome binding	Unknown
Blard et al. [62], 2007	Myd88	Enhancer	Protein binding	Signaling
Blard et al. [62], 2007	E(bx)	Enhancer	DNA binding	Unknown
Blard et al. [62], 2007	Sodh-1	Enhancer	Enzyme	Unknown
Chan et al. [66], 2002; Zhang et al. [59], 2010	Uba2	Enhancer	SUMO activating enzyme	Signaling
Kaltenbach et al. [63], 2007	Eip75B	Suppressor	DNA binding	Signaling
Shulman and Feany [60], 2003	par-1	Suppressor	Protein kinase	Unknown
Zhang et al. [59], 2010	Hop	Suppressor	Protein binding	Signaling
Zhang et al. [59], 2010	brm	Enhancer	Protein binding	Signaling
Zhang et al. [59], 2010	lwr	Enhancer	Protein binding, SUMO ligase	Signaling
Zhang et al. [59], 2010	Rab11	Enhancer	Protein binding, GTPase	Signaling
Zhang et al. [59], 2010	Jra	Suppressor	Protein binding, DNA binding	Signaling
Zhang et al. [59], 2010	lic	Enhancer	Protein kinase	Signaling
Zhang et al. [59], 2010	Slu7	Enhancer	Zinc anion binding, nucleic acid binding	Unknown

either suppresses or enhances the neurodegenerative phenotype associated with Huntingtin aggregates, 8 genes (6.3%) belong to the immune response group [59]. Immune response genes are also well represented among genes for which down- or upregulation suppresses or enhances a variety of neurodegenerative traits [59–63] (table 3). Hence, elevated or decreased transcripts of some immune response genes could have neuroprotective function in some cases.

An important question is whether a neuroprotective response can be defined and studied with a genomic approach. Changes in the transcriptome caused by an immune challenge were defined by infecting flies with known pathogens and analyzing gene expression at different time points thereafter, usually at intervals of a few hours [15, 16, 29]. It is also relatively simple to define changes triggered in the transcriptome by oxidative stress, starvation, hyperoxia or other adverse conditions by switching the flies from standard to experimental conditions and comparing thereafter gene expression after a few hours (table 2).

The characterization of a neuroprotective response through genome-wide studies confronts a particular difficulty because neurodegenerative phenotypes develop often slowly, over intervals of days or even weeks, and we know relatively little about the temporal dynamics for the development of key pathological processes responsible for the final symptoms. Only in a few studies, samples were taken at two or three time points, including presymptomatic stages [22, 25, 27], and it seems that more time points are necessary to provide enough temporal resolution. A complementary way to obtain a better separation between neurodegenerative and neuroprotective traits will be to study changes in the transcriptome of flies in which a neurodegenerative process is attenuated or blocked by expression of a transgene ('rescue experiments'). Another option will be to include in the study heterozygous animals when they do not develop a neurodegenerative phenotype. This might have several potential benefits. It could increment the confidence of the hits identified in the homozygous mutant when heterozygosity correlates with intermediate levels of the neurodegenerative phenotype and gene transcripts [12, 24]. At the same time, when only homozygous mutants develop the neurodegenerative phenotype, the transcriptome of heterozygotes might provide information about genes that are regulated as part of a neuroprotective response. For instance, loss-of-function mutations in the genes of the *spalt* family of transcription factors cause neurodegeneration only in homozygosity [64]. However, heterozygous mutants also show regulation of immune response genes, suggesting that at least in this case the abnormal transcript levels of some immune response genes are probably rather associated with neuroprotection than with neurodegeneration [24].

Concluding Remarks

Although the debate on the relationship between neurodegeneration and the innate immune response is relatively poorly defined because the cause or consequence of this complex association is often not known, it has been clear for some years that elements of the innate immune response might mediate neuroprotection in humans [1, 3]. *Drosophila* could be used as an instrumental model to elucidate this question. The recognition that misregulation of immune response genes causes or aggravates neurodegenerative conditions in *Drosophila* is indubitably supported by experimental evidence [14]. However, it seems also probable that the transcriptional regulation of some immune response genes might reflect, instead, a contribution to a protective response. To distinguish between the two possibilities is not trivial. The idea that regulation of some immune response genes might have a neuroprotective function can be tested experimentally using the formidable tool kit offered by *Drosophila* genetics. Along this line of work, genome-wide studies of neurodegenerative processes in the fly will gain from denser temporal data series, the study of heterozygotes when they do not develop a neurodegenerative phenotype and the potential connection between NF-kB pathway activation and tissue homeostasis.

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