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## GPCRs in invertebrate innate immunity

Jerome Reboul, Jonathan J Ewbank

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2 **GPCRs in invertebrate innate immunity**  
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7 Jerome Reboul and Jonathan J. Ewbank  
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12 Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Inserm,  
13

14 U1104, CNRS UMR7280, 13288 Marseille, France  
15  
16

17 Corresponding author: Jonathan Ewbank  
18

19 Centre d'Immunologie de Marseille-Luminy,  
20

21 163 avenue de Luminy  
22  
23

24 13009 Marseille  
25

26 FRANCE  
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28 ewbank@ciml.univ-mrs.fr  
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## ABSTRACT

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5 G-protein coupled receptors (GPCRs) represent a privileged point of contact between  
6  
7 cells and their surrounding environment. They have been widely adopted in  
8  
9 vertebrates as mediators of signals involved in both innate and adaptive immunity.  
10  
11 Invertebrates rely on innate immune defences to resist infection. We review here  
12  
13 evidence from a number of different species, principally the genetically tractable  
14  
15 *Caenorhabditis elegans* and *Drosophila melanogaster* that points to an important role  
16  
17 for GPCRs in modulating innate immunity in invertebrates too. In addition to  
18  
19 examples of GPCRs involved in regulating the expression of defence genes, we  
20  
21 discuss studies in *C. elegans* addressing the role of GPCR signalling in pathogen  
22  
23 aversive behaviour. Despite the many lacunae in our current knowledge, it is clear  
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25 that GPCR signalling contributes to host defence across the animal kingdom.  
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## 1. Introduction

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5 Compared to the extensive literature linking G-protein coupled receptors (GPCRs) to  
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7 immunity in vertebrates, the subject has been relatively poorly explored in  
8  
9 invertebrate animals. In this review, we will cover early biochemical studies using the  
10  
11 horseshoe crab, then more recent work with the genetically tractable models  
12  
13 *Caenorhabditis elegans* and *Drosophila melanogaster*, and lastly a brief overview of  
14  
15 research with diverse non-model systems. For the sake of restricting this review to a  
16  
17 single phylogenetic group of animals, we will not include any studies on chordates,  
18  
19 such as Ascidians.  
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23  
24 Lacking adaptive immunity, invertebrates rely on their innate immune system to  
25  
26 defend themselves against infection. The first step in triggering an immune response  
27  
28 involves recognition of stranger/danger signals. Stranger signals are molecular  
29  
30 hallmarks of a particular group of microbial species, hence their alternative name of  
31  
32 microbe/pathogen-associated molecular patterns (MAMP/PAMP). The archetypal  
33  
34 MAMP is lipopolysaccharide, an indispensable component of the outer membrane of  
35  
36 Gram-negative bacteria. MAMPs are recognized by dedicated receptors, such as Toll-  
37  
38 like receptor (TLR) 4 in vertebrates [1]. Danger signals, also known as damage-  
39  
40 associated molecular patterns (DAMPs), on the other hand, can be endogenous  
41  
42 molecules, like mitochondrial proteins or ATP, aberrantly released into the  
43  
44 extracellular milieu [2]. The molecular architecture of GPCRs is particularly well  
45  
46 suited to allow binding of diverse chemical structures, from small organic molecules  
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48 to neuropeptides (reviewed in [3]). They are thus good candidates for mediating  
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50 perception of diverse danger signals in host cells.  
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## 2. GPCRs in horseshoe crab immunity

### 2.1. LPS-triggered signalling

The first indications of a role for GPCRs in defence came from work with horseshoe crabs. These are large, evolutionary ancient, marine arthropods, with a predicted origin some 450 million years ago. Of the 4 known species, primarily *Limulus polyphemus* and *Tachypleus tridentatus* have been used in research. Their blood contains motile cells called hemocytes or amebocytes, which are thought to play a similar role in host defence to macrophages in vertebrates. More than half a century ago, it was shown that exposing *Limulus* blood to Gram-negative bacteria provoked a rapid coagulation [4]. This response requires exocytosis of the clotting factor coagulogen, and is triggered by recognition of bacterial lipopolysaccharide (LPS) [5]. Interestingly, coagulogen is structurally similar to the *Drosophila* protein spaetzle [6]. In both species these proteins are ligands for immune receptors of the Toll family required for activation of the key transcription factor NFκB. This has led to the suggestion of an evolutionary ancient origin for NFκB signalling in defence throughout animals [7]. LPS recognition itself relies on the zymogen factor C [8]. Binding of LPS triggers an auto-activation of factor C to give rise to an active serine protease. By analogy with the activation of vertebrate GPCRs of the protease-activated receptor family (PARs) by thrombin, it is believed that the factor C protease would cleave the N-terminus of an as yet unidentified GPCR leading to the activation of diverse cellular responses (Figure 1; reviewed in [9]). There are clear parallels between this system and the PAR-dependent activation of NFκB in vertebrates [10].

### 2.2. Potentiation by tachyplestin

1 Hemocytes can also be activated in the absence of LPS by host-derived peptides  
2 including tachyplesin [11], originally described as an antimicrobial peptide (AMP)  
3  
4 [12]. Tachyplesin can interact *in vitro* with G<sub>o</sub> $\alpha$  and G<sub>i</sub> $\alpha$  proteins from bovine brain,  
5  
6 and both U-73122, an inhibitor of phospholipase C, and pertussis toxin, a G protein  
7  
8 inhibitor, strongly inhibit hemocyte exocytosis. Although tachyplesin has not been  
9  
10 demonstrated to activate G proteins, these results provide further evidence for a role  
11  
12 of GPCR signalling in horseshoe crab defence and suggest that a positive feedback  
13  
14 mechanism for hemocyte secretion exists [11].  
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### 22 **3. GPCRs in worm immunity**

#### 23 24 3.1. Defence via CO<sub>2</sub>/O<sub>2</sub> detection and chemical sensing

25  
26 Nematodes live in a microbe-rich environment and eat bacteria. They need to be able  
27  
28 to distinguish innocuous bacteria from potential pathogens. Data from a broad range  
29  
30 of species indicates that the Toll-like receptor (TLR) - NF $\kappa$ B signalling axis  
31  
32 mentioned above has been lost from nematodes [13]. There are no obvious NF $\kappa$ B  
33  
34 orthologs in worms and although *C. elegans* has one TLR, TOL-1 [14], it appears not  
35  
36 to have a direct role in host defence (reviewed in [15]). It does, however, have the  
37  
38 potential to influence the interaction between *C. elegans* and bacteria, including  
39  
40 pathogens [14] since *tol-1* is required for the terminal differentiation of a class of  
41  
42 neurons required for detection of CO<sub>2</sub> [16]. Like any metabolically active bacteria,  
43  
44 pathogens will produce CO<sub>2</sub>, and locally depress O<sub>2</sub> levels. *C. elegans* is believed to  
45  
46 measure gradients of O<sub>2</sub> and CO<sub>2</sub> to guide it to bacteria. It also integrates a variety of  
47  
48 other sensory cues, both attractive and repulsive, via GPCR chemoreceptors [17], to  
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50 distinguish good from bad food. *C. elegans* thus has the capacity to recognise  
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1 different foods and make choices based on the CO<sub>2</sub>/O<sub>2</sub> balance in combination with  
2 several other factors, including via chemosensation [18].  
3

### 4 3.1.1. G<sub>i</sub>α-like protein ODR-3 and the GPCR kinase GRK-2 in *Serratia marcescens* 5 avoidance 6 7

8  
9 As an example, *C. elegans* detects the *Serratia marcescens*-derived surfactant  
10 serrawettin W2 via the AWB chemosensory neurons and is repelled by it, even in the  
11 presence of an attractive CO<sub>2</sub>/O<sub>2</sub> environment. Thus worms will avoid a bacterial  
12 lawn that contains serrawettin W2 [19]. Since *S. marcescens* is a pathogen of  
13 *C. elegans* [20], this clearly has the potential to contribute to host survival. The AWB  
14 neurons express a range of GPCRs that all require the G<sub>i</sub>α-like protein ODR-3 and the  
15 GPCR kinase GRK-2 for their function in chemosensation [21, 22]. Phosphorylation  
16 by GRKs allows GPCRs to bind the negative regulatory protein arrestin. This blocks  
17 further G protein-mediated signalling and targets GPCRs for internalization. Mutants  
18 in either *odr-3* or *grk-2* are defective in their avoidance of lawns containing  
19 serrawettin W2, suggesting a role for one or more GPCR in this type of pathogen  
20 avoidance behaviour [19].  
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### 38 3.1.2. GPCR signalling in *Pseudomonas aeruginosa* avoidance 39

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41 Worms also avoid the pathogen *Pseudomonas aeruginosa*. This was shown recently  
42 to depend on detection of the bacterial secondary metabolites phenazine-1-  
43 carboxamide and pyochelin. These are recognised by an as yet undefined GPCR that  
44 acts via the Gα proteins GPA-2 and GPA-3. One consequence of this chemosensation  
45 is an increased neuronal secretion of the TGFβ orthologue DAF-7. This cytokine  
46 modifies how worms respond to ambient oxygen levels, so that they leave a lawn of  
47 *P. aeruginosa* in spite of an oxygen concentration that would normally be attractive  
48 [23].  
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There is plasticity in the behavioural response to pathogenic bacteria. *P. aeruginosa* induces aversive olfactory learning so that given a choice between *P. aeruginosa* and non-pathogenic *E. coli*, worms prefer *E. coli* [24]. For this, the AWB neurons act together with a second pair, called AWC, again requiring the function of the G<sub>i</sub>α-like protein ODR-3. Food preferences require the neuropeptide NLP-9 produced in AWB and its putative receptor, the GPCR NPR-18, as well as NLP-1 produced in AWC [25]. AWB and AWC act upstream of ADL sensory neurons to control the preference behaviour that can limit exposure to bacterial pathogens. In this context, recent work has suggested a role for several other neuropeptides, including NLP-10, for which the receptor is not known, and FLP-4 released from ADL, acting through its receptor, the GPCR NPR-4, in AIB interneurons. It also indicated a possible role in ADL for the orphan GPCR SRH-220. Loss of function *srh-220* mutants show a substantially reduced preference for *E. coli* when given a choice between it and pathogenic *P. aeruginosa*. This putative role for a GPCR is consistent with the similar deficit in odour preference observed in mutants for the unique arrestin orthologue ARR-1. This phenotype was rescued when ARR-1 was specifically expressed in the ADL sensory neurons [26]. A full understanding of these mechanisms will require the identification of the ligand for SRH-220.

### 3.1.3. The role of the neuropeptide receptor NPR-1 in immune responses

The GPCR neuropeptide receptor NPR-1 had been proposed to play a direct role in modulating innate immune gene expression upon *P. aeruginosa* infection. This has been challenged by other studies that suggested an indirect role for NPR-1 in defence (reviewed in [27, 28]). Indeed, *npr-1* mutants exhibit a broad range of behavioural phenotypes [29], including a change in CO<sub>2</sub> and O<sub>2</sub> sensation [30] that impact on its



1 capacity to avoid pathogenic bacteria. These could explain the observed alterations in  
2 the interaction between *npr-1* mutants and pathogens.  
3

#### 4 3.1.4. A broad role for a GPCR-LRR protein FSHR-1 in defence 5

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7 Another GPCR, the follicle stimulating hormone receptor homologue FSHR-1, is also  
8 required for the worm's capacity to recognise and avoid pathogenic bacteria.  
9  
10 Moreover it is needed for the expression of defence genes in the gut of worms  
11 infected with *P. aeruginosa* [31]. FSHR-1 additionally acts in the intestine to regulate  
12 genes required to resist heavy metal and oxidative stress, thereby contributing to the  
13 maintenance or re-establishment of homeostasis following infection [31]. Finally,  
14 FSHR-1 antagonizes the capacity of *C. elegans* to resist low temperatures; mutants  
15 lacking *fshr-1* function survive cold stress better than wild-type worms [32]. FSHR-1  
16 was originally studied because of its structure; it combines GPCR and leucine-rich  
17 repeat (LRR) domains [33]. LRR domains are frequently found in innate immune  
18 receptors, including TLRs and most NOD-like receptors (NLRs). In vertebrate TLRs,  
19 the LRRs mediate direct recognition with the appropriate MAMP. In invertebrates,  
20 the LRRs do not necessarily play such a role. Rather, as mentioned above, they bind  
21 endogenous cytokine-like proteins [34, 35]. Given its pleiotropic function, it is  
22 unlikely that FSHR-1 acts as a MAMP receptor in worms. Nevertheless, it will be  
23 very interesting to discover its physiological ligand.  
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#### 46 3.2. The DAMP receptor, DCAR-1 47

48 One GPCR that acts as a DAMP receptor has been identified in *C. elegans*. DCAR-1  
49 was originally described as being expressed in chemosensory neurons and to be a  
50 putative receptor for the DOPA-derived small molecule dihydrocaffeic acid (DHCA)  
51 [36]. It is also expressed in the nematode epidermis where it can be activated by  
52 hydroxyphenyllactic acid (HPLA), a derivative of tyrosine that accumulates when  
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1 worms are wounded or infected by the fungus *Drechmeria coniospora* [37]. The  
2 signal transduction pathway downstream of DCAR-1 has been extensively studied  
3  
4 through genetic and biochemical approaches (Figure 2). DCAR-1 acts upstream of the  
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6  $G_{12\alpha}$  protein GPA-12 [37] that in turn is upstream of 2 phospholipase C $\beta$  (PLC $\beta$ )  
7  
8 enzymes (PLC-3 and EGL-8). These hydrolyse phosphatidylinositol 4,5-bisphosphate  
9  
10 (PIP2) to produce inositol trisphosphate (IP3) and diacylglycerol (DAG). DAG then  
11  
12 activates a protein kinase C (TPA-1; [38]) that switches on a conserved p38 MAPK  
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14 cascade [39, 40], ultimately leading to the activation of a STAT-like transcription  
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16 factor and the expression of antimicrobial peptide genes in the epidermis [41].  
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### 21 3.3. Multiple roles for the $G_q\alpha$ EGL-30 in host defence

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23 A second  $G\alpha$  protein (the  $G_q\alpha$  EGL-30) functions in a cell autonomous manner within  
24  
25 the intestine via the PLC $\beta$  EGL-8 to regulate the activity of the p38 MAPK pathway  
26  
27 and thereby the expression of intestinal immune effectors upon infection with  
28  
29 *P. aeruginosa*. EGL-30 and EGL-8 additionally act in a cell non-autonomous manner,  
30  
31 by modulating the level of insulin/IGF1 signalling from neurons [42]. Secretion of  
32  
33 insulin has a wide-ranging impact on the physiology of the worm, influencing lifespan  
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35 as well as stress and pathogen resistance (reviewed in [28]).  
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41 The G proteins GPA-12 and EGL-30 are also involved in the interaction between  
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43 *C. elegans* and *Microbacterium nematophilum*, a Gram-positive bacterium [43] that  
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45 worms avoid. EGL-30 intervenes at two different levels, influencing both the aversive  
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47 behavioural response to *M. nematophilum* mediated by neuronal activity, and an  
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49 epithelial defence mechanism. Thus GPA-12 and EGL-30 are both positive regulators  
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51 of neurotransmitter release in cholinergic motor neurons, required for the changes in  
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53 locomotion behaviour that underlie pathogen avoidance [44]. At the same time, EGL-  
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55 30 acts in the rectal epithelium to modulate the immune response, involving changes  
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in gene expression and alteration of cellular morphology. The GPCR that acts upstream of EGL-30 has recently been identified. Like DCAR-1, it appears to recognise an amino acid derivative (R. McMullan, personal communication).

The EGL-30-dependent pathway functions in cooperation with a Ras signalling pathway [45]. Serotonin, released from ADF chemosensory neurons, acting directly or indirectly via serotonin receptors, the GPCRs SER-1 and SER-7, and possibly others, activates the G<sub>o</sub>α protein GOA-1 that in turn triggers a downstream protective signal transduction cascade in the rectal epithelium. The system is complex, since GOA-1 acts antagonistically to EGL-30 both in neurons and the rectal epithelium [46]. Further, the level of serotonin production in ADF depends on the expression of *tph-1*, corresponding to a rate-limiting tryptophan hydroxylase. It is modulated indirectly by a GPCR pathway in olfactory sensory neurons involving the G<sub>q</sub>α protein EGL-30 [47]. The identity of the putative GPCR that acts upstream of EGL-30 in the AWB and AWC sensory neurons for the detection of pathogens is not yet known. But it is noteworthy that the arrestin ARR-1 plays a broad role in immune regulation [48].

### 3.3. Other GPCRs in *C. elegans* defence

DCAR-1 was identified through a genome-wide RNAi screen for genes required for antimicrobial peptide gene expression [49]. Several other GPCRs also emerged as candidates in this screen, but their precise role in innate immunity is currently unclear [37]. In different species, GPCR families have undergone lineage-specific expansions. In the case of *C. elegans* there are more than 1500 predicted GPCRs, many involved in chemosensation [50-52]. Before any functional studies had been undertaken, *in silico* analyses of sequence evolution and polymorphisms in natural isolates suggested a role for certain GPCR families in mediating interactions with microbes. Among them, the SRH family stands out because of its very high functional polymorphism

1 [53, 54]. For example, there is at least one naturally occurring deletion allele of *srh-*  
2 *220*, a gene involved in pathogen discrimination as described above, present in the  
3  
4 Germany isolate MY1 (see [http://www.wormbase.org/species/c\\_elegans/strain/MY1](http://www.wormbase.org/species/c_elegans/strain/MY1)).  
5  
6 Thus GPCRs are central to diverse aspects of host-pathogen relationships in the  
7  
8 worm, but whether SRH-220 or other GPCRs are *bona fide* MAMP receptors remains  
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10 to be determined.  
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#### 16 **4. GPCRs in fly immunity**

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21 The best-characterised aspect of innate immune defences in *Drosophila* is the  
22 regulation by NF $\kappa$ B of antimicrobial gene expression in the fat body following  
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24 activation of Toll (by the cytokine-like protein Spaetzle) or via the peptidoglycan-  
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26 triggered IMD pathway [35]. Flies additionally have the capacity to produce reactive  
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28 oxygen species in the intestine through the activity of DUOX enzymes. These  
29  
30 contribute to protect flies from gut infection. In contrast to peptidoglycan-induced  
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32 IMD activation, acute DUOX activation requires membrane-localised PLC $\beta$  and was  
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34 therefore proposed to be principally via GPCR signalling [55]. Subsequent work  
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36 revealed uracil, excreted by non-commensal bacteria in the gut, to be a trigger for  
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38 increasing DUOX activity [56]. The identity of the putative uracil-binding GPCR has  
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40 yet to be reported (Figure 3).  
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48 The other evidence for a role of GPCR signalling in flies is also indirect. One  
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50 significant hit in a genome-wide cell-based RNAi screen for genes required for Toll  
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52 and IMD pathway corresponded to the *Drosophila* G protein-coupled receptor kinase  
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54 Gprk2 [57]. The immune function for this GRK was suggested to be potentially  
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56 related to its established role in regulating hedgehog signalling [58, 59], perhaps  
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1 through an effect on cell surface receptor recycling [60]. Interestingly, uracil-  
2 dependent DUOX activation in the fly gut also requires hedgehog, which is needed  
3  
4 for the formation of a subset of endosomes that act as signalling platforms [61].  
5

6  
7 There is circumstantial evidence for a link between GPCR signalling and defence  
8  
9 against viral infection in *Drosophila*. Thus, the G $\beta$ -like protein RACK1 (Receptor for  
10 Activated C Kinase 1) that acts downstream of the GPCR DCAR-1 in *C. elegans* [37,  
11 38] is required for the translation of Cricket Paralysis Virus (CrPV) proteins in fly  
12 cells. It should be noted, however, that RACK1 is also a ribosomal protein and its role  
13 in viral replication, which is linked to IRES-dependent translation, may be totally  
14 independent of GPCRs [62]. As a further example, *Drosophila* can be parasitized by  
15 wasps that deposit their eggs in larvae. Wasp secretions, transferred into the host with  
16 the egg, can contain symbiotic viruses that suppress the normal host defences that  
17 lead to encapsulation and killing of wasp eggs [63-65]. One viral effector, CrV2, has  
18 been demonstrated to bind directly to a host G $\alpha$  protein *in vitro* [66], raising the  
19 possibility of a wasp block of GPCR signalling important in host defence.  
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### 39 **5. GPCRs in immunity in other invertebrate species**

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43 If the literature on GPCRs in defence in the 2 model organisms *C. elegans* and  
44 *D. melanogaster* is sparse, in other invertebrates it is even more patchy. There are  
45 occasional reports of a role for GPCRs in immunity for a diverse mix of species.  
46  
47 Thus, for example, in crayfish, exposure to dead Gram-negative bacteria provokes up-  
48 regulation of the GPCR-encoding gene HP1R. Knocking down HP1R expression  
49 renders animals more susceptible to infection, supporting a role for GPCR signalling  
50 in defence [67]. The response to LPS involves production of astakines, the crayfish  
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1 equivalent of prokineticins (PROKs). In vertebrates, these cytokines signal through  
2 GPCRs, and this is probably the case in crayfish too [68]. In shrimp, arrestins are  
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4 required for defence against Gram-positive bacteria, but this has been suggested to be  
5  
6 via a direct effect on the Cactus-Dorsal complex in the Toll signalling pathway rather  
7  
8 than a modulation of GPCR signalling [69]. More examples are likely to be found in  
9  
10 the future.  
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## 17 **6. Concluding remarks**

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22 Evidence has accumulated for an important role for GPCRs in invertebrate innate  
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24 immunity. Despite the many examples of functions for downstream components of  
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26 GPCR signalling pathways, perhaps because of functional redundancy, there are  
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28 extremely few cases where a single GPCR has been assigned an unambiguous and  
29  
30 specific role in defence. Identifying innate immune GPCRs that acts as DAMP or  
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32 MAMP receptors therefore remains a major challenge for the future.  
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## 39 **Footnote**

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3 **Figure Legends**  
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7 Figure 1. A model for activation of effector secretion by LPS in horseshoe crab.  
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9 Binding of LPS to factor C activates its proteolytic domain leading to cleavage of the  
10 putative GPCR and triggering of downstream signaling. In addition to IP<sub>3</sub>-dependent  
11 exocytosis, there is the potential for DAG-dependent activation of p38 signaling via a  
12 PKC. Secretion of the host defense molecule tachyplesin has been proposed to  
13 potentiate heterotrimeric G protein signaling, providing a positive feedback  
14 mechanism. Figure adapted from [9].  
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26 Figure 2. Simplified model of the regulation of p38 signaling by the GPCR DCAR-1  
27 in the epidermis of *C. elegans*. Fungal infection or physical injury leads to the  
28 production of hydrophenyllactic acid (HPLA) that activates DCAR-1 and downstream  
29 elements. The name of each nematode protein and its vertebrate orthologue is given.  
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66 Figure 3. Model of DUOX activation in *Drosophila*. Uracil produced by pathogenic  
67 bacteria activates an as yet unidentified GPCR. This triggers calcium release from the  
68 endoplasmic reticulum via a PLC, and subsequent ROS production by Duox.  
69 Infection also drives expression of Duox via PLC-dependent activation of p38  
70 MAPK.  
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Figure 1

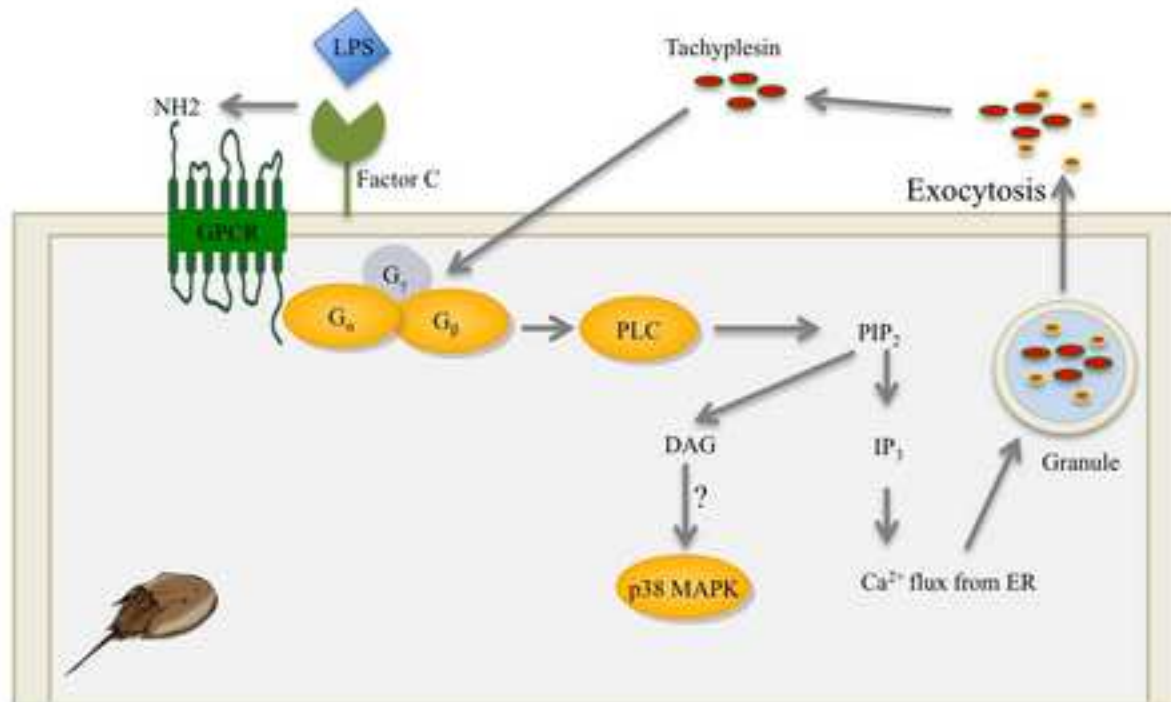


Figure 2

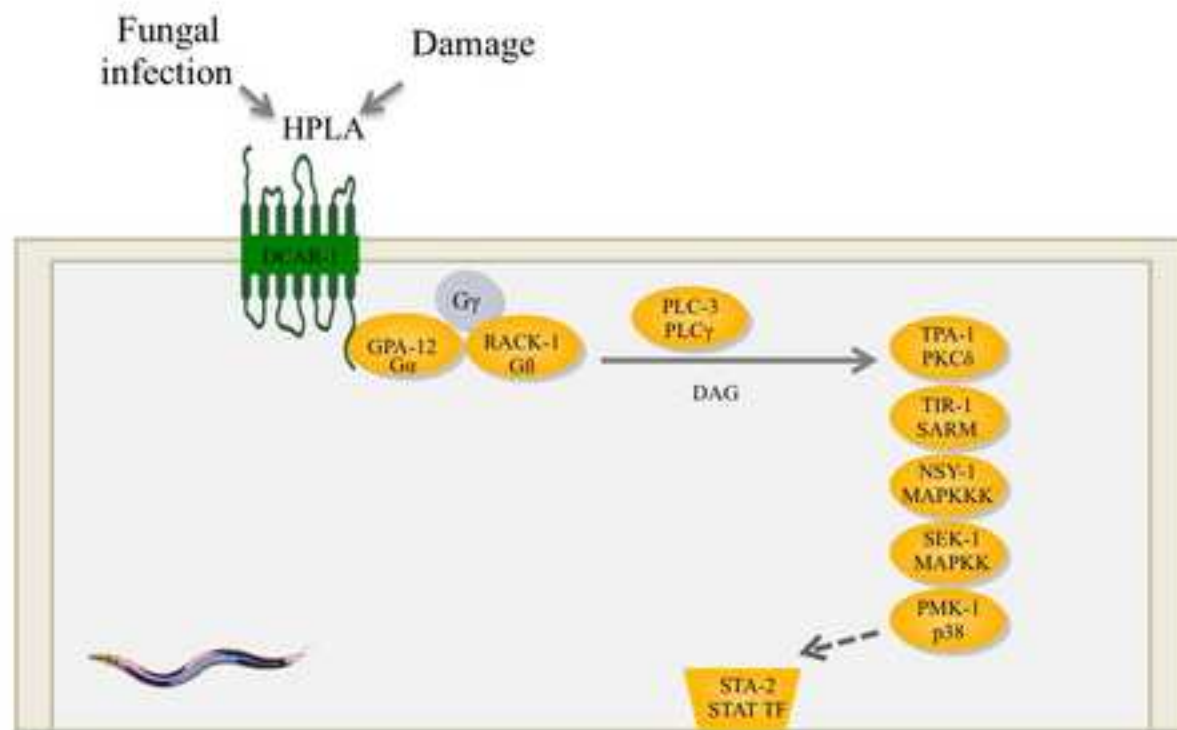
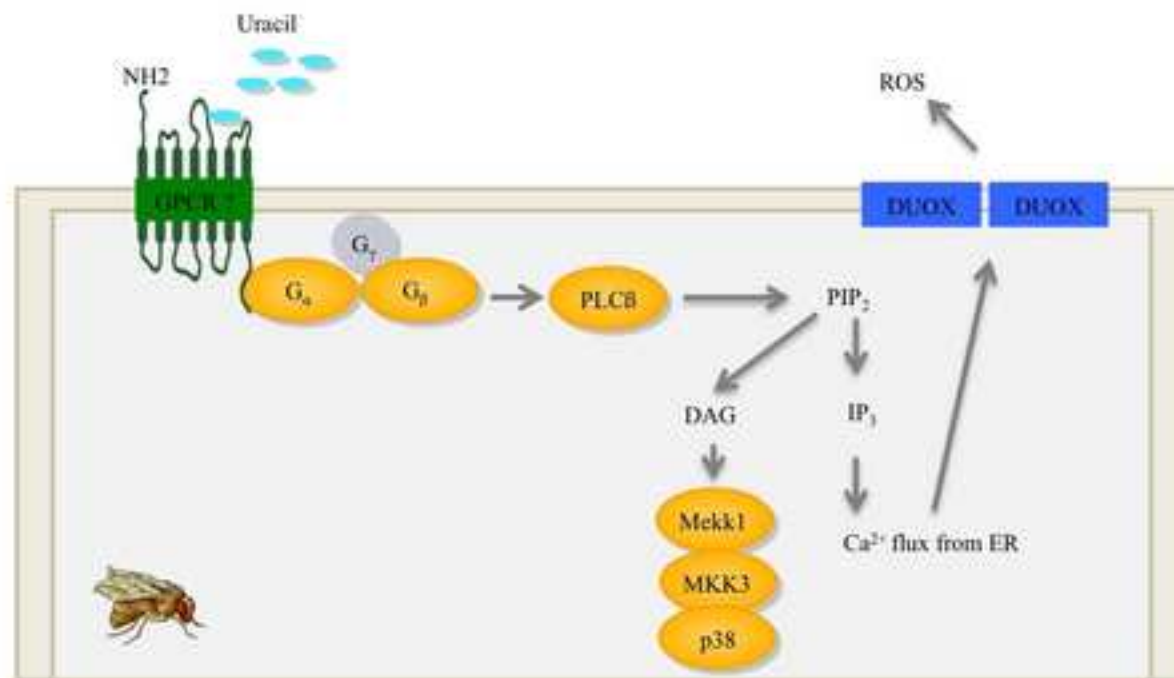
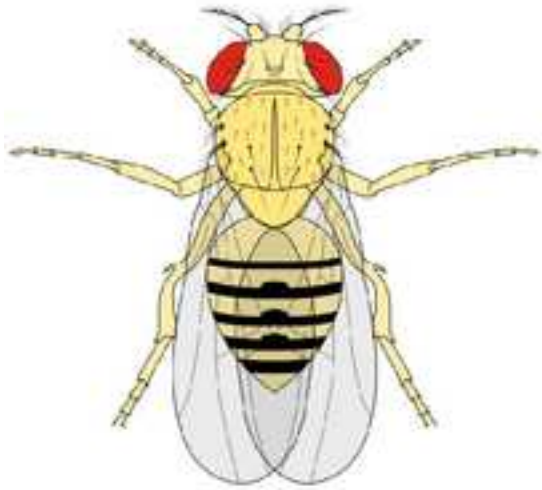


Figure 3





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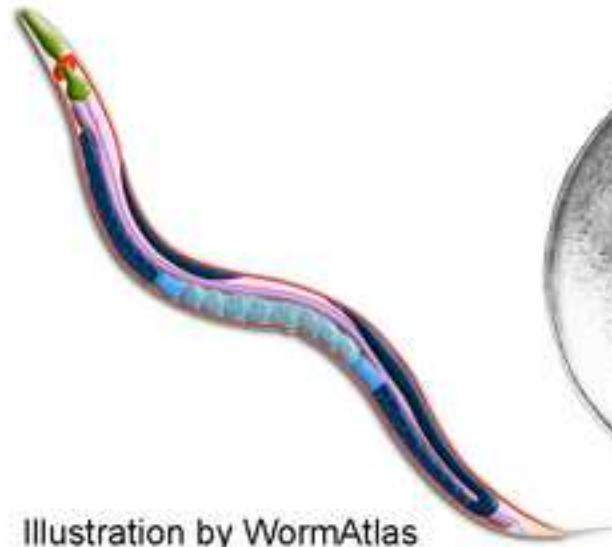


Illustration by WormAtlas

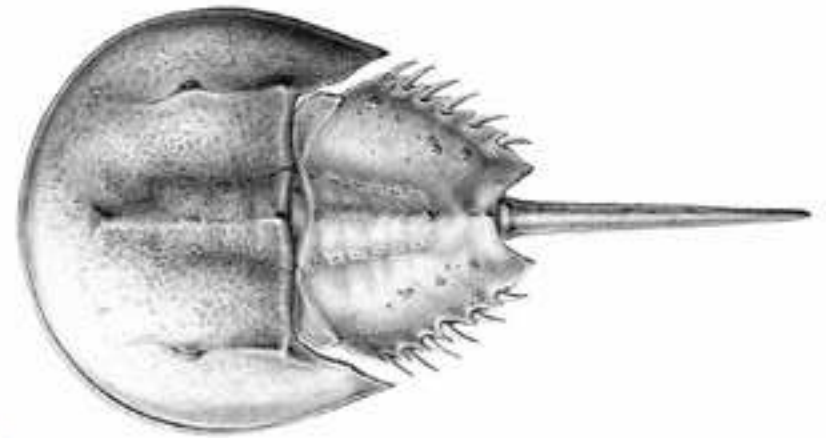


Illustration by Andrew Leach