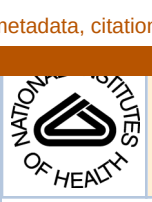

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Natural Polymorphisms in *C. elegans* HECW-1 E3 Ligase Affect Pathogen Avoidance Behaviour

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Abstract

Heritable variation in behavioural traits generally has a complex genetic basis[1], and thus naturally occurring polymorphisms that influence behaviour have been defined in only rare instances[2,3]. The isolation of wild strains of *Caenorhabditis elegans* has facilitated the study of natural genetic variation in this species[4] and provided insights into its diverse microbial ecology[5]. *C. elegans* responds to bacterial infection with conserved innate immune responses[6-8] and, while lacking the immunological memory of vertebrate adaptive immunity, exhibits an aversive learning response to pathogenic bacteria[9]. Here, we report the molecular characterization of naturally occurring coding polymorphisms in a *C. elegans* gene encoding a conserved HECT domain-containing E3 ubiquitin ligase, HECW-1. We show that two distinct polymorphisms in neighbouring residues of HECW-1 each affect *C. elegans* behavioural avoidance of a lawn of *Pseudomonas aeruginosa*. Neuron-specific rescue and ablation experiments, and genetic interaction analysis suggest that HECW-1 functions in a pair of sensory neurons to inhibit *P. aeruginosa* lawn avoidance behaviour through inhibition of the neuropeptide receptor NPR-1[10], which we have previously shown promotes *P. aeruginosa* lawn avoidance behaviour[11]. Our data establish a molecular basis for natural variation in a *C. elegans* behaviour that may undergo adaptive changes in response to microbial pathogens.

C. elegans is initially attracted to pathogenic *P. aeruginosa* PA14, but within a few hours develops an avoidance response to *P. aeruginosa* (Supplementary Fig. 1). *P. aeruginosa* lawn avoidance behaviour confers increased survival in the presence of a lawn of pathogenic bacteria[11-14]. We followed the kinetics of *P. aeruginosa* lawn avoidance behaviour of the laboratory wild type strain N2 (Bristol, England) after transfer from *E. coli* OP50 (Fig. 1a). We observed that over 50% of the N2 population vacated the lawn of *P. aeruginosa* by t = 8 h, and by t = 24 h, over 90% of the animals were found outside of the lawn of *P. aeruginosa* (Fig. 1b and Supplementary Fig. 1).

Characterization of the *npr-1* gene has shown that the 215V allele of *npr-1* found in the N2 strain, which has likely been derived in the laboratory[15,16], has increased activity compared with the ancestral 215F allele of *npr-1*, modifying multiple behavioural phenotypes including aggregation, aerotaxis, and locomotion specifically in the presence of bacterial food[10,17-19]. In addition, we have shown previously that the 215V allele of *npr-1* confers N2 with enhanced behavioural avoidance of a lawn of *P. aeruginosa* compared with wild isolates such as CB4856 (Hawaii, USA) that carry the 215F allele of *npr-1*[11]. Consistent with these prior observations, we observed that the wild isolates CB4856 and RC301

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(Freiburg, Germany) that carry the 215F allele of *npr-1* exhibited markedly delayed avoidance times from a lawn of *P. aeruginosa* compared with the *P. aeruginosa* avoidance times observed for N2 (Fig. 1b and Supplementary Fig. 1).

We observed that *P. aeruginosa* lawn avoidance behaviour by the CB4856 strain was also diminished relative to RC301, with approximately 75% of CB4856 animals remaining inside the lawn of *P. aeruginosa* after t = 24 h, compared with 25% lawn occupancy by RC301 animals after t = 24 h (Fig. 1b and Supplementary Fig. 1). We observed that the DA650 strain[10], which was generated by backcrossing the 215F *npr-1(g320)* allele of the RC301 strain into the N2 genetic background, exhibited kinetics of *P. aeruginosa* lawn avoidance comparable to RC301 (Fig. 1b and Supplementary Fig. 1). These observations confirmed that the *npr-1* polymorphism is a major determinant of behavioural avoidance of the *P. aeruginosa* lawn, but also revealed that additional genetic differences among wild isolates of *C. elegans* influence the behavioural avoidance of pathogenic bacteria.

We sought to identify these additional genetic loci that modulate the behavioural avoidance of pathogenic bacteria. We found a substantial contribution from a single locus in determining the different pathogen avoidance behaviours observed in CB4856 and DA650 (Supplementary Fig. 2). We performed positional mapping and transgenic rescue to narrow the genomic interval containing causative genetic differences to an 8 kb fragment (genetic analysis and positional mapping detailed in Supplementary Fig. 2). This 8 kb genomic interval included a single gene, F45H7.6 (Fig. 1c), which encodes an E3 ubiquitin ligase with two WW domains and a HECT domain (Fig. 1d), with strong homology to the human HECT, C2, and WW domain containing E3 ubiquitin ligase 1 (HECW1/NEDL1)[20]. Thus, we named the *C. elegans* F45H7.6 gene *hecw-1*. We identified two polymorphisms in *hecw-1* (Fig. 1c), including one coding polymorphism in exon 8 of *hecw-1* that results in a Q325P change in HECW-1 in CB4856 (Fig. 1d). Consistent with our mapping data implicating *hecw-1* as a determinant of *P. aeruginosa* lawn avoidance behaviour, we found that a deletion allele of *hecw-1*, *ok1347* (Fig. 1c), exhibited enhanced *P. aeruginosa* lawn avoidance behaviour (Supplementary Fig. 3).

We sequenced the region of the *hecw-1* gene region encompassing amino acid 325 from a total of 162 *C. elegans* strains (Supplementary Table 1), and we found that only CB4856 carries the *hecw-1 325P* polymorphism. Unexpectedly, we identified a second polymorphism, which results in a Y322C substitution in HECW-1 (Fig. 1d) in 11 wild strains, including AB2 (Adelaide, Australia) and CB3198 (Pasadena, California, USA). Comparison of the *C. elegans* amino acid sequence flanking and including the polymorphisms with the sequences from other *Caenorhabditis* species and mammalian *hecw-1* orthologues suggested that these polymorphisms lie in a conserved, but relatively rapidly evolving region (Supplementary Fig. 4a).

Whereas the phenotypic effect of the *hecw-1 325P* polymorphism in CB4856 was suggested by mapping and rescue experiments, the *hecw-1 322C* polymorphism was detected by sequencing. To determine the effect of Y322C substitution on pathogen avoidance behaviour, we crossed the *hecw-1 322C* polymorphism in AB2 and CB3198 strains into the CB4856 background (thus substituting the *hecw-1 322C 325Q* alleles for the *hecw-1 322Y 325P* alleles of CB4856). We also crossed the *hecw-1 322Y 325Q* allele of N2 into the CB4856 background. After six backcrosses to the CB4856 strain, we observed that each of the two strains carrying the *hecw-1 322C 325Q* alleles exhibited *P. aeruginosa* lawn avoidance behaviour that was equivalent to that observed for the CB4856 strain (Fig. 1e). These data indicated that the *hecw-1 322C* (in AB2 and CB3198) and *hecw-1 325P* (in CB4856) polymorphisms confer comparable effects on *P. aeruginosa* lawn avoidance

behaviour, with delayed avoidance relative to the *hecw-1 322Y 325Q* allele crossed into the CB4856 background (Fig. 1e).

Although the strains carrying the *322C* and *325Q* alleles of *hecw-1* in the CB4856 background were generated from two independent strains, AB2 and CB3198, we considered that these strains may nevertheless carry linked loci that could modulate pathogen avoidance behaviour. Thus, we sought to determine the relative activities of the naturally occurring alleles of *hecw-1* in an otherwise isogenic strain background. We generated single-copy insertions each carrying an 8 kb *hecw-1* genomic sequence fragment in the N2-derived *hecw-1*(*ok1347*) background, with the transgenes differing only at HECW-1 amino acid positions 322 (Y/C) and/or 325 (Q/P). Consistent with our observations of strains carrying the naturally-occurring *hecw-1* polymorphisms in the CB4856 background, we observed that animals carrying either the *hecw-1 322C* or the *hecw-1 325P* substitutions rescued the *hecw-1*(*ok1347*) *P. aeruginosa* lawn avoidance phenotype with a delayed avoidance of the *P. aeruginosa* lawn compared with animals carrying the *hecw-1 322Y 325Q* allele (Fig. 1f). These data show that both the *hecw-1 322C 325Q* and *322Y 325P* polymorphisms result in increased HECW-1 activity relative to the *hecw-1 322Y 325Q* allele. Structural modeling of HECW-1, based on the crystal structure of the corresponding domain of human HECW1, suggested that the *hecw-1 325P* and *hecw-1 322C* polymorphisms may alter the same protein-protein interaction interface on the surface of HECW-1 without a radical disruption in overall structure (Supplementary Fig. 4b).

To determine the cells in which HECW-1 is expressed, we generated a transcriptional reporter consisting of Green Fluorescent Protein (GFP) under the control of the *hecw-1* promoter, which contains 0.9 kb sequence upstream of the first exon of *hecw-1* that was sufficient to rescue the *hecw-1(ok1347)* mutant. GFP fluorescence was observed in the nervous system throughout the body, but in the anterior ganglion was conspicuously limited to two neurons located posterior to the anterior bulb of the pharynx, each of which have projections extending anteriorly (Fig. 2a). We confirmed that these two neurons are the OLLL and OLLR neurons by co-localization experiments using the OLL-specific reporter *ser-2dp*::*gfp*[21] and the *hecw-1p*::*mCherry* transgene (Figs. 2b-2d).

To determine whether expression of HECW-1 in the OLL neuron pair is sufficient to regulate pathogen avoidance behaviour, we performed rescue experiments utilizing transgenes comprised of *hecw-1* cDNA fused to GFP under the control of neuron-specific promoters. Expression of a *hecw-1* cDNA::*gfp* transgene under the control of either its endogenous *hecw-1* promoter (Supplementary Fig. 5) or the OLL specific *ser-2d* promoter[21] (Fig. 2e) was sufficient to rescue the *hecw-1(ok1347) P. aeruginosa* lawn avoidance phenotype (Fig. 2f). Although the *ser-2d* promoter directs additional expression in PVD neurons[21], heterologous expression of *hecw-1::gfp* in PVD did not rescue the *P. aeruginosa* lawn avoidance phenotype of *hecw-1* (Supplementary Fig. 6a). In addition, an alternative promoter directing *hecw-1::gfp* expression in neurons including the OLL neuron pair, but not PVD neurons, rescues the *hecw-1* mutant phenotype (Supplementary Fig. 6b). These data suggest that HECW-1 activity in the OLL neuron pair is sufficient to rescue the *P. aeruginosa* lawn avoidance phenotype of *hecw-1(ok1347)*.

To determine whether the OLL neuron pair is necessary for the negative regulation of pathogen avoidance behaviour, we carried out laser ablation of the OLL neuron pair using transgenic animals carrying the *ser-2dp::gfp* reporter to mark the OLL neurons for ablation. We ablated and mock ablated the OLL neuron pair and allowed the animals to recover overnight on plates seeded with *E. coli* OP50. To confirm that the OLL neurons were successfully ablated, we checked the OLL GFP signals of the ablated animals before we transferred them to *P. aeruginosa* plates. We found that animals with the OLL neuron pair

ablated exhibited markedly enhanced *P. aeruginosa* lawn avoidance behaviour relative to mock-ablated animals as determined by diminished occupancy of the *P. aeruginosa* lawn after 5 h (Fig. 3a). These data suggest a role for the OLL neuron pair in the negative regulation of pathogen avoidance behaviour in *C. elegans*.

We also generated transgenic animals in the N2 background expressing the caspase *csp-1b* cDNA (Denning, D. and Horvitz, H.R. unpublished results), which induces cell death, under the control of the OLL-specific promoter, *ser-2dp*. Using two transgenic lines with more than 90% of the animals lacking the OLL neuron pair (Figs. 3b and 3c), we found that animals lacking the OLL neuron pair exhibited an enhanced pathogen avoidance behaviour phenotype as was observed in OLL laser-ablated animals (Fig. 3d). The enhanced *P. aeruginosa* lawn avoidance behaviour of the *hecw-1(ok1347)* mutant and OLL-ablated strains led us to ask whether the lawn avoidance behaviour might be observed even in the presence of nonpathogenic *E. coli* OP50. However, in contrast to the marked avoidance behaviour observed in the presence of a lawn of pathogenic *P. aeruginosa*, we found that neither the *hecw-1(ok1347)* deletion, nor genetic ablation of OLL mediated by *ser-2dp::csp-1b* conferred a discernible lawn avoidance behavioural phenotype in the presence of *E. coli* OP50 (Fig. 3e).

Ultrastructural studies suggest a mechanosensory function for the OLL neuron pair[22]. Consistent with these data, we found that OLL-ablated and *hecw-1(ok1347)* mutant animals exhibited a defective withdrawal response of *C. elegans* to a light touch on the tip of its nose, the Nose Touch phenotype[23] (Supplementary Fig. 7a and 7b). The involvement of the OLL neuron pair in *P. aeruginosa* lawn avoidance behaviour suggests that the mechanosensory detection of bacteria may contribute to HECW-1-regulated pathogen avoidance behaviour. A role for mechanosensation in *C. elegans*-bacteria interactions has been previously implicated in studies of dopamine-dependent mechanosensory signaling in the *C. elegans* basal slowing response when encountering a lawn of bacteria[24].

The observed *P. aeruginosa* lawn avoidance phenotype likely results from the integration of multiple attractive and repulsive behaviours induced by nutritional, metabolic, and pathogenic aspects of the bacterial lawn. We have previously shown that *P. aeruginosa* lawn avoidance behaviour of the *npr-1* mutant contributes to enhanced survival in a pathogen killing assay with *P. aeruginosa*[11,14]. Consistent with these prior studies, we observed that the *hecw-1(ok1347)* mutant exhibited enhanced survival compared with N2 (Fig. 4a). We proceeded to ask whether HECW-1 might act through NPR-1 to modulate behavioural avoidance of pathogenic bacteria. Whereas the *hecw-1(ok1347)* mutation conferred an enhanced *P. aeruginosa* lawn avoidance phenotype in the presence of the *npr-1 215V* allele and *npr-1(g320) 215F* alleles, we found that the putative null alleles, *npr-1(ad609)* and *npr-1(ky13)*, suppressed the enhanced *P. aeruginosa* avoidance phenotype conferred by the *hecw-1(ok1347)* mutation (Fig. 4b and Supplementary Fig. 8a). Consistent with the observed genetic interaction between *hecw-1* and *npr-1* with regard to *P. aeruginosa* lawn avoidance behaviour, we observed that both the pathogenesis survival and Nose Touch phenotypes conferred by the *hecw-1* loss-of-function in the presence of *P. aeruginosa* were also suppressed by null mutations in *npr-1* (Fig. 4a and Supplementary Fig. 7b). The OLL neurons may connect through the IL2 and CEP neurons to the RMG neurons, in which NPR-1 activity exerts its diverse influence over *C. elegans* behaviour[25]. Partial rescue of the sensitivity of the *npr-1(ky13)* mutant to the effect of the *hecw-1(ok1347)* mutation on pathogen avoidance was observed using an *npr-1* cDNA transgene under the control of the *flp-5* promoter, which directs expression in RMG and ASE neurons (Supplementary Fig. 8b). These data support a model in which HECW-1 in the OLL neuron pair functions to inhibit the activity of NPR-1 in the RMG inter/motor neuron (Fig. 4c).

The ecological and evolutionary impact that microbes may have on host organisms has been increasingly appreciated to extend beyond the immune system, encompassing diverse aspects of host physiology[26]. Our work provides a molecular basis for how natural variation can lead to changes in behaviour that may facilitate adaptation of *C. elegans* to microbes.

Methods Summary

C. elegans were cultivated and strains constructed using standard methods[27]. Transgenic strains were generated using indicated constructs and standard microinjection methods[28]. MosSCI single-copy insertions were generated as described[29]. Assays measuring *C. elegans* pathogen avoidance behaviour utilized *P. aeruginosa* PA14 plates prepared as follows: a 100 mL solution of LB was inoculated with a single colony of *P. aeruginosa* PA14 and grown overnight without shaking at 37°C (OD=0.2-0.3). 30 μL of this culture was used to seed the center of each 100-mm NGM plate, and the seeded plates were incubated for 24 h at room temperature (22.5°C). Approximately 30 Larval Stage 4 (L4) animals were transferred onto plates containing the *P. aeruginosa* PA14 lawn at 22.5°C, and occupancy was determined at the indicated times. Survival assays were carried out on 35 mm Slow-Killing Assay plates[30] supplemented with 5-fluorodeoxyuridine (0.05 mg/mL) and seeded with *P. aeruginosa* PA14 prepared as above and maintained at 22.5°C. Laser ablations were performed on L3-stage larvae as described in text. Microscopy and image analysis was carried out on an AxioImager Z1 fluorescence microscope fitted with CCD camera (AxioCam) and processed with Axioplan image processor software (Zeiss). The statistical analyses were performed using GraphPad Prism software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank D. Denning and H. R. Horvitz for the *csp-1b* cDNA. We thank Y. Kohara for the *hecw-1* and *npr-1* cDNAs. We thank J. Meisel for sequencing of *hecw-1* polymorphisms. We thank D. Ma for technical advice on laser ablation. We thank T. Schwartz for expert advice with the structural modeling of HECW-1. The *hecw-1(ok1347)* deletion allele was generated by the *C. elegans* Knockout Consortium and obtained, along with other strains used in this study, from the *Caenorhabditis* Genetics Center (CGC), which is supported by the NIH—National Center for Research Resources. This work was supported by NIH Grant GM084477 (to D.H.K.).

METHODS

Strains

C. elegans were maintained at 20°C using standard methods[27]. Strains were maintained at 20°C then shifted to 22.5°C for *P. aeruginosa* PA14 lawn avoidance assays and pathogenesis survival assays. The *hecw-1(ok1347)* mutant strain was derived from the RB1265 strain (generated by the *C. elegans* Gene Knockout Consortium and obtained from the *Caenorhabditis* Genetics Center) by backcrossing six times to N2. The *npr-1(g320)*; *hecw-1(ok1347)* double mutant was generated by crossing *hecw-1(ok1347)* into DA650 three times. ZD641, ZD642, ZD643 were generated by backcrossing the *hecw-1* alleles in N2, AB2 and CB3198 into CB4856 six times. MT15623 (a gift from H.R. Horvitz) carries *npr-1(ky13)*; *lin-15AB(n765ts)* X; *nEx1252* (*npr-1* genomic; *lin-15AB* rescue) were used and crossed into *hecw-1(ok1347)*; *npr-1(ky13)*; *lin-15AB(n765ts)* for rescue experiments. Transgenic strains were isolated by microinjecting various plasmids[28], (typically at 50-100 μg/ml) together with either of the following coinjecting markers, *rol-6dm*, *myo-2p*::*mCherry* (a gift from S. Nakano), and *mec-4p*::*gfp* (a gift from M. Driscoll) in wild type or mutant animals.

P. aeruginosa PA14 avoidance assay

A 100 mL solution of LB was inoculated with a single colony of *P. aeruginosa* PA14 and grown overnight without shaking at 37°C (OD=0.2-0.3). 30 °L of this culture was used to seed the center of each 100-mm NGM plate, and the seeded plates were incubated for 24 h at room temperature (22.5°C). Approximately 30 Larval Stage 4 (L4) animals were transferred onto plates containing the *P. aeruginosa* PA14 lawn at 22.5°C, and lawn occupancy was measured at the indicated times. Two plates of each genotype were performed in each experiment and all experiments were performed at least three times. Upon being transferred to the *P. aeruginosa* PA14-containing plates, animals explore the plate for about 10-15 min until they find the bacterial lawn and then remain in the lawn. Subsequently, lawn occupancy is measured over time as the lawn avoidance behaviour is observed.

P. aeruginosa pathogenesis survival assay

C. elegans survival assays on *P. aeruginosa* PA14 were set up as described previously[30], with the following modifications. 3 μL of the aforementioned *P. aeruginosa* PA14 culture was used to seed 35-mm Slow-Killing Assay plates[30] modified by the addition of 5-fluorodeoxyuridine (0.05 mg/mL), used to prevent eggs from hatching. Seeded plates were incubated for 24 h at 22.5°C. L4 stage animals were then transferred to the *P. aeruginosa* PA14 plates, which were maintained at 22.5°C throughout the survival assay. Animals were scored as dead when they no longer responded to the repeated touch of a platinum wire.

Mapping of *hecw-1*

Single nucleotide polymorphisms (SNPs) between N2 and CB4856 were used to determine the genetic loci responsible for the difference in pathogen avoidance behaviour between DA650 and CB4856. We found a strong linkage in the area between -7.2 to -5.5 map units (cM) on chromosome III. Further positional SNP walking defined a 25 kb interval between SNPs haw42334 (-7.18 cM) and haw42350 (-7.07 cM) on chromosome III, which included the major molecular determinant of the *P. aeruginosa* PA14 lawn avoidance behaviour between CB4856 and DA650 (Supplementary Figure 2). An 8kb genomic region from CB4856 encompassing *hecw-1* and depicted in Fig. 1c rescued the DA650 avoidance phenotype.

Microscopy

Animals were mounted in M9 with levamisole (10 mM) onto slides with a 2% agarose pad. The slides were viewed using an AxioImager Z1 fluorescence microscope (Zeiss) with 20x/0.8, 40x/1.3 (oil) and 63x/1.4 (oil) objectives. The fluorescence signals were recorded by a CCD camera (AxioCam) in a 12 bit format without saturation. The images were captured and processed using Axioplan image processor software.

Molecular cloning

The genomic region of *hecw-1* was amplified by PCR using primers 5'-ATCGTGGTT TCGCACTTTGTTTTGGAAAC-3' and 5'-AACCAGTCGCCGTGAGGATGCG-3'. The 8 kb genomic fragment from either N2 or CB4856 was subsequently cloned into a Topo®(Invitrogen) vector. The *hecw-1* promoter region was amplified by using a 5' primer containing 5'-ATCGTGGTTTCGCACTTTGTTTTGGAAAC-3' and 3' primer containing 5'-TCTCCGAAGCGTGATCATTCTAAAAACC-3'. The 900 bp fragment was subsequently cloned into the pPD95.75 vector (Fire Lab Vector Kit, Addgene) to generate

the *hecw-1* promoter GFP reporter. *hecw-1* and *npr-1* cDNAs were gifts from Y. Kohara. The *ser-2d* promoter was generated using primers as described previously. *csp-1b* cDNA is a gift from D. Denning (D. Denning and H.R. Horvitz unpublished results). *flp-5*, *flp-3* and F49H12.4 promoters were cloned from genomic DNA as described previously. Detailed primer sets and methods used for cloning are available upon request.

Generation of MosSCI single insertion lines

Single-copy insertion of transgenes was performed using the direct MosSCI technique targeting the *ttTi5605* Mos allele on chromosome II, as described[29]. The *hecw-1* genomic sequences varying only in the coding polymorphisms in exon 8 were cloned into the pCFJ151 targeting vector using the BsiWI and XhoI restriction sites. The 8kb N2 *hecw-1* genomic sequences were modified by site directed mutagenesis (QuikChange II XL Site-Directed Mutagenesis Kit. Agilent Technologies) using the following primers to introduce the Q325P change: CTTGATTCTCGCATAAGGCGGTTTTAAACTCGTCTCG and the Y322C change: CTGCATTGCATCTGGATTCTCGTATAAGGCGG. The modified pCFJ151 vectors, carrying the *hecw-1 322Y 325Q*, *hecw-1 322Y 325P*, or *hecw-1 322C 325Q* alleles, were then each injected with co-injection vectors pGH8, pCFJ90, pCFJ104 and pJL43.1 into at least 60 animals. After positive and negative selections, two integrated lines were then sequenced to confirming the correct sequences of *hecw-1*.

Laser ablation

Laser microsurgeries were performed on L3-stage larvae as described in text. Cell identifications were based on a *ser-2dp::gfp* and *sra-6p::gfp* reporter and cell morphology. The ablated animals were confirmed under a dissection scope to have lost the GFP signals of OLL and ASH neurons 16 hours later, before the *P. aeruginosa* PA14 lawn avoidance and Nose Touch assay were performed.

Nose Touch behavior assay

Nose Touch sensory responses were assayed as previously described[23] in an observer-blinded manner. Each animal was tested on food for reversal of locomotion after a forward collision with a hair. Each animal was tested ten times, and 20 or more animals were tested for each genotype or for neuronal laser ablations.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software.

Structure Modeling

Based on the deposited WW domain of the human orthologue HECW1 (PDB code 3L4H), we threaded the *C. elegans* sequence (29% identity over 109 aligned residues) and generated a model with MODELLER. The model in Supplementary Fig. 4b is rendered using PyMOL

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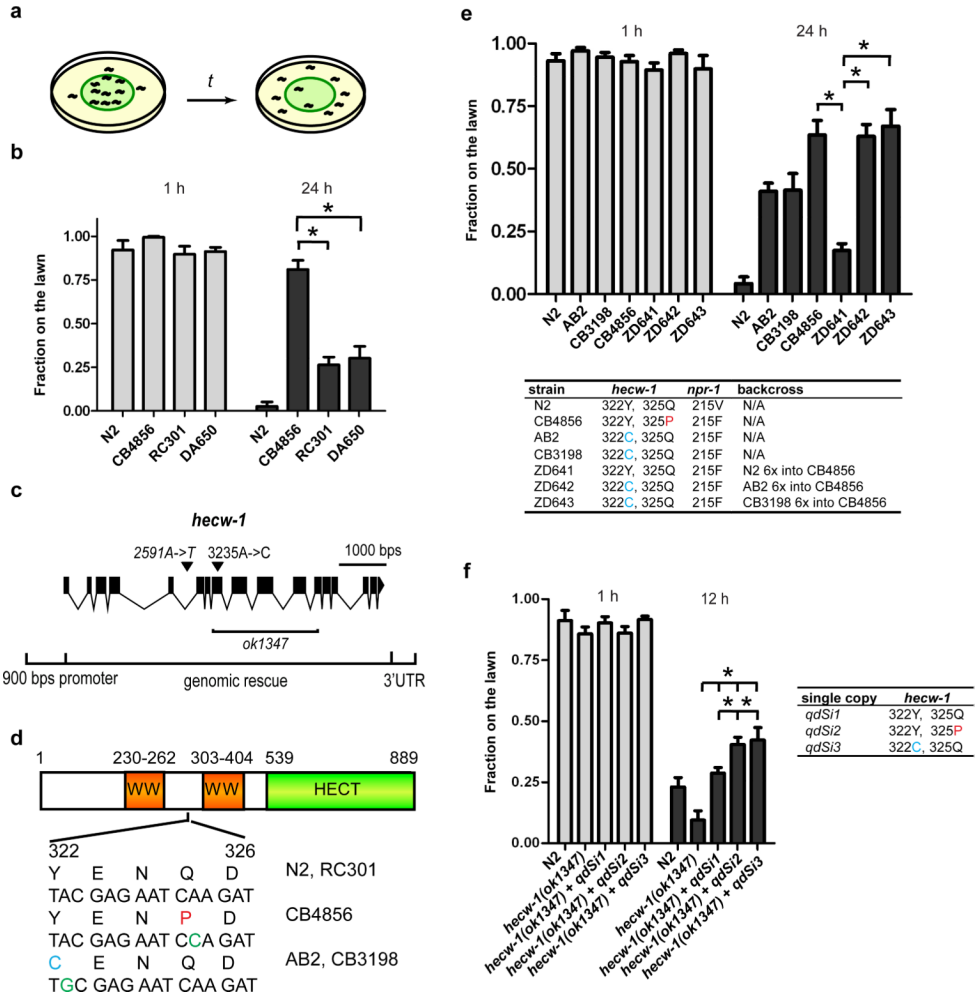

strain	*hecw-1*	*npr-1*	backcross
N2	322Y, 325Q	215V	N/A
CB4856	322Y, 325P	215F	N/A
AB2	322C, 325Q	215F	N/A
CB3198	322C, 325Q	215F	N/A
ZD641	322Y, 325Q	215F	N2 6x into CB4856
ZD642	322C, 325Q	215F	AB2 6x into CB4856
ZD643	322C, 325Q	215F	CB3198 6x into CB4856

single copy	*hecw-1*
qdSi1	322Y, 325Q
qdSi2	322Y, 325P
qdSi3	322C, 325Q

Figure 1. Natural variation in *C. elegans hecw-1* modulates behavioural avoidance of *P. aeruginosa*

(a) Schematic of the assay of pathogen avoidance behaviour in which *C. elegans* were transferred to plates containing a lawn of *P. aeruginosa* PA14, and lawn occupancy was scored over time. (b) *P. aeruginosa* lawn occupancy of *C. elegans* strains assayed at t = 24 h. Full time course in Supplementary Fig. 1. (c) Schematic of the 8 kb rescuing genomic fragment encompassing *hecw-1* (Supplementary Fig. 2c). The two polymorphisms that vary between CB4856 and DA650 within this region are shown, as is the region deleted in the *hecw-1(ok1347)* mutant. (d) The coding polymorphisms in *hecw-1* result in a Q325P substitution in CB4856 and a Y322C substitution in the AB2 and CB3198 wild strains. (e) *P. aeruginosa* lawn avoidance behaviour of strains carrying *hecw-1 322Y 325Q*, *322Y 325P*, and *322C 325Q* alleles in the CB4856 background and constructed as indicated. (f) *P. aeruginosa* lawn avoidance behaviour of animals each carrying a single integrated copy of *hecw-1* genomic sequence with the naturally occurring *322Y 325Q*, *322C 325Q*, and *322Y 325P* alleles of *hecw-1* in the N2-derived *hecw-1(ok1347)* background. In (b) and (e) * $P <$ 0.001 was determined by the ANOVA multiple comparisons test. In (f) * $P < 0.001$ and ** $P < 0.02$ was determined by the Student's t test based on the results of four independent experiments. Error bars indicate s.e.m.

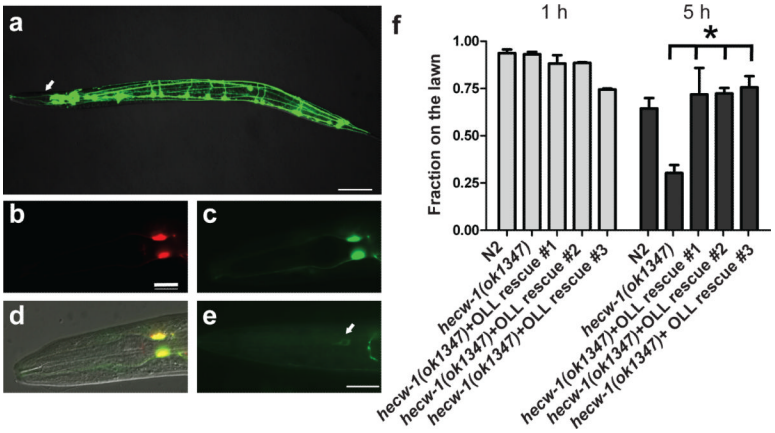

Figure 2. HECW-1 functions in the OLL sensory neuron pair to negatively regulatepathogen avoidance behaviour

(a) Expression of GFP under the control of a *hecw-1p* showing fluorescence throughout the nervous system of the body, but limited expression in the anterior ganglion (arrow points to single pair of head neurons showing expression). Scale bar indicates 50 μm. (b-d) HECW-1 is expressed in the OLL sensory neurons. (b) Anterior ganglion expression of *hecw-1*p::*mCherry*. (c) Expression of the OLL sensory neuron marker *ser-2dp*::*gfp*. (d) Merge of Nomarski, (b) and (c). Scale bar indicates 5 μm. (e) Expression of a *ser-2dp::hecw-1 cDNA::gfp* translational fusion protein in the OLL neuron pair (indicated by arrow). Scale bar indicates 10 μm. (f) *P. aeruginosa* lawn avoidance behaviour of N2, *hecw-1*(*ok1347*), and three independent transgenic lines of the *hecw-1*(*ok1347*) mutant expressing *ser-2p::hecw-1:gfp*. * $P < 0.001$ was determined by the ANOVA multiple comparisons test. Error bars indicate s.e.m.

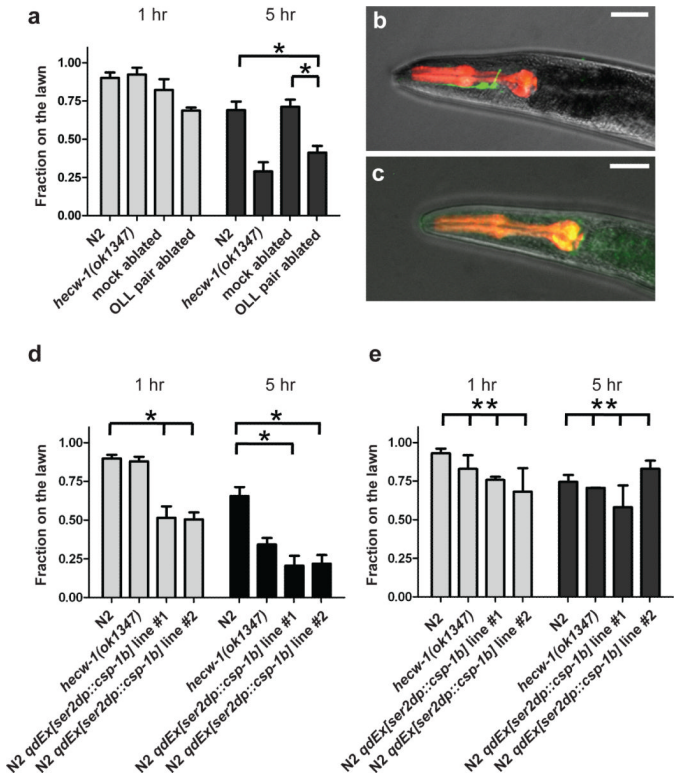

Figure 3. The OLL sensory neuron pair negatively regulates pathogen avoidance behaviour

(a) *P. aeruginosa* lawn avoidance behaviour of N2 animals carrying the *ser-2dp::gfp* transgene and subjected to laser ablation of OLL. Mock animals were treated in parallel without laser treatment. Two independent experiments were performed, and 15 and 30 animals were ablated in each experiment. (b) Fluorescence microscopy of animals expressing the *ser-2dp::gfp* transgene in OLL, and (c) carrying the *ser-2dp::gfp* transgene along with the *csp-1b* caspase under the control of *ser-2d* promoter that results in genetic ablation of OLL. Pharyngeal red fluorescence signals arise from co-injection marker *myo-2p:mcherry*. Scale bar indicates 20 μm. Genetically ablated animals were then transferred to plates seeded with (d) *P. aeruginosa* PA14 and (e) *E. coli* OP50 respectively, and lawn occupancy was recorded at the indicated times. * $P < 0.001$ was determined by the ANOVA multiple comparisons test. ** $P = 0.23$ was determined by the ANOVA multiple comparisons test. Error bars indicate s.e.m.

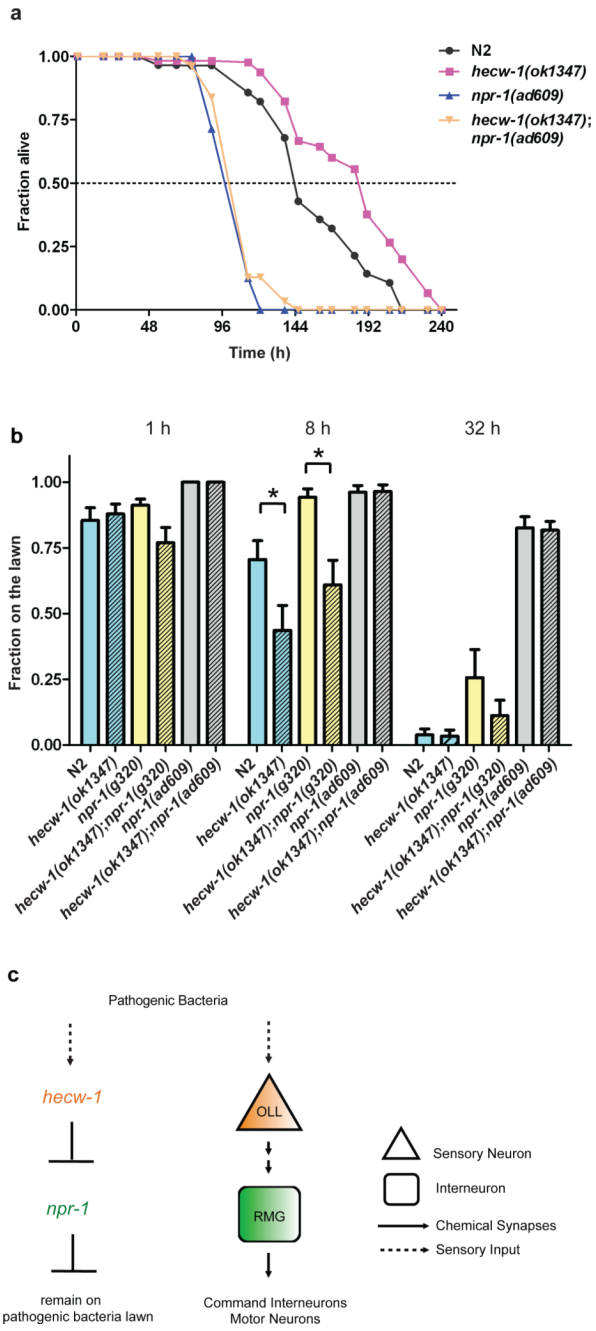

Figure 4. Regulation of pathogen avoidance behaviour and survival by HECW-1 is dependent on NPR-1

(a) Survival assay of indicated mutants on *P. aeruginosa* PA14 at 22.5°C. (b) *P. aeruginosa* lawn avoidance behaviour of double mutants in the N2 background carrying the *hecw-1*(*ok1347*) mutation in combination with three different *npr-1* alleles— the N2 wild type *npr-1 215V* allele, the 215F allele *npr-1(g320)*, and the putative null *npr-1*(*ad609*). *$P < 0.001$ was determined by the ANOVA multiple comparisons test. Error bars indicate s.e.m. (c) Model for the function of HECW-1 in the regulation of pathogen avoidance behaviour.