Supplemental Information

Supplemental Material and Methods

General methods and strains

C. elegans strains were handled, maintained and crossed following standard protocols [1]. *P. aeruginosa* strains were cultivated for 12 h at 37ºC in King's B broth under constant shaking. Other bacterial strains were cultivated in LB broth (see Supplemental Table 1 for details on *C. elegans* and bacterial strains).

Infection and toxicity assays

To avoid confusing progeny that grew and blend with the originally infected cohort, and the confounding effect of progeny hatching within the worm, on survival analysis in the infection assay, we used either N2 hermaphrodites with inactivated sperm (Fig. 2C, D), conditional sterile *fer-15(b26)* (Fig. 4A) or worms rendered sterile by exposure to double stranded RNA (dsRNA) directed against *cdc-25.1*, a gene important for germline proliferation (Fig. 4B). *fer-15* worms have a normal lifespan and resistance to bacterial infection [2]. N2 animals with inactivated sperm that lay unfertilized oocytes were obtained by exposing L3 larvae for 24 to 36 h at 27ºC. Curves in Figure 2 (C and D) and Figure 4 are representative of at least two independent trials, each with 120 - 150 hermaphrodites.

The toxic compounds used were cadmium chloride (Sigma, 287652), copper sulfate (EM Science, CX2185-1), zinc sulfate (EM Science, ZX0105-10), sodium arsenite (Fisher, S225-100), colchicine (Sigma, 9754) and chloroquine (Sigma, 6628).

qRT-PCR

RNA samples were obtained following Trizol (Invitrogen)/chloroform extraction, DNAse treated (Ambion), and then diluted in DEPC-treated water to a final concentration of 100 ng/ml. RNA levels were quantified by qRT-PCR using specific primer sets, the One-Step RT-PCR Kit with SYBR Green (Biorad) in combination with the iCycler (Biorad). In addition to having the same mRNA concentration for every sample, data from the qRT-PCR experiments were normalized to levels of F44B9.5 mRNA. This mRNA showed a constant level in the tested conditions. The level of a specific mRNA was calculated relative to

a reference obtained with animals fed on *E. coli* (OP50) at the same time. Primer sequences are given in Supplemental Materials.

Fluorescence quantification

For assays of the reporter gene expression, treated animals were analyzed with the Union Biometrica COPAS automated sorter following the manufacturer's instructions. The time of flight is a measurement of length. The fluorescence and TOF are measured in arbitrary, but constant units.

Microscopy

Animals were deposited in a drop of M9 (25 mM Levamisole) on a glass slide with a 2% agar pad. They were examined using fluorescence microscopy with a Leica DMRXA2 microscope, and photographs taken using a Leica DC500 camera.

Image manipulation

All photomicrographs were treated in the same way using Adobe Photoshop CS2 (version 9.0). Briefly, the yellow signal was replaced by blue color to simplify the discrimination between the green fluorescence (GFP) and the yellow fluorescence corresponding to autofluorescence of intestinal vesicles.

Supplemental RNAi constructions

The majority of the dsRNA-expressing bacteria came from the Ahringer library [3]; in some cases, more specific constructs were made. For *sek-1* dsRNA expressing HT115, we cloned a *sek-1* genomic fragment (primers GCT TCT CAT TTC TTG CCT GC and CCG AGA CTT AAG CAA ATC GC and genomic N2 DNA as a template) in pPD129.36.

The same procedure was used for bacteria expressing dsRNA against *tir-1b* isoform (primers ATG TGT GCC CTA GTC TCA TC and CTT TGC ATA CGG GTG GAA GAA T, N2 genomic DNA as template), *tir-1a, c, e* isoform (primers GCAT TCT TCG AGT TGG ACG ATG ATG ATG ATC TGT CC and AAA TGA AAT GCG GCG ACA CTT CGA GCT TCC CG and N2 genomic DNA as template) and against *gfp* (primers ATG AGT AAA GGA GAA GAA CT and CTA TTT GTA TAG TTC ATC CA and Fire Vector pPD95.75 as a template).

qRT-PCR primers

The primers used were: GGA AAA TCA GAA TGG GAC GA and TTG ATT GCC ATG AAT GGT GT for *pgp-5*; CGT TGG AGA CAA CAC GAC AT and CAA ATG TCC AGT CGA CAC GC for *pgp-6*; ACC CTG CCG ATC TCA AAA AAG and CGC AAA TGT AAA ATA TGC GCA T for *pgp-7*; GAT TTC GAG AGG CGT TCA ACG and GAA GCA AGT GTC CAG CTT GT for *pgp-8*; CAA TGC GTG TGC TTC GTG TC and ATA CCA CCA GCA AAC GTG GC for *pgp-1*; C TAT GGA TGG AAC ATG GCAC and CTT GCC TCA ATT GCG GAA TCC for *pgp-3*; GCG ATT GAT CAC CGA CTC GC and CAA TTG GTG CCA TAC TCC AGC for *pgp-4*; AAT CTG CTG CTT TGA GAA TG and GCT GAT TCA TCT CTG TGA CC for F44B9.5.

Supplemental Results

TGF-beta nor insulin pathways do not affect pgp-5 induction during infection. We also determined the requirement for two additional immunity pathways, the TGF-beta and insulin pathway, for *pgp-5* induction upon infection. Abrogation of neither the TGF-beta nor insulin pathways, as represented by *sma-6(e1482)* and *daf-2(e1370)*, respectively, affected *pgp-5* induction upon PA14 infection. The fold increase in *pgp-5* mRNA in *sma-6* and *daf-2* animals, as determined by qRT-PCR, were not statistically different from wild type; they were 37 ± 10 and 67 ± 35 compare to 51 ± 6 (Student's t-test, $p > 0.54$ and $p > 0.26$, respectively).

Supplemental Table 1

Strain	Description	Source or reference
C. elegans		
N2	Wild-type	*Caenorhabditis* Genetic Center
BC10030	Promoter fusion *pgp-5::gfp* strain. *dpy-5(e907)* I; sEx864 [rCes-C05A9.1::GFP + pCes361(*dpy-5*(+))]	This study
WE5172	*dpy-5(e907)* I; *ajIs1*[pCes361(*dpy-5*(+)) + sEX864] X. sEx864 from the BC10030 strain integrated by gamma irradiation into the X chromosome of BC10030 and out-crossed 4 times with N2 animals.	This study
WE5173	*pgp-5(ok856)* X. Obtained from the CGC. Prior to the phenotypic analyses presented below, the strain was backcrossed 6 times with N2 animals to remove exogenous mutations.	*Caenorhabditis* Genetic Center
WE5179	*tir-1(ok1052)*, outcrossed 2 times	[4]
DH26	*fer-15(b26)* II	*Caenorhabditis* Genetic Center
Bacterial Strains		
OP50	*Escherichia coli*	*Caenorhabditis* Genetic Center
PA14	*P. aeruginosa*	[5]
SL1344	*S. enterica serovar. Typhimurium*	[6]
Db11	Non-pigmented strain of *Serratia marcescens*	[7]
8325-4	Wild-type *Staphylococcus aureus*	H. Ingmer (RVAU, Frederiksberg, Denmark)

Supplemental Table 2

Bacterial species tested

E. coli	*B. subtilis*	*P. aeruginosa* FK	*P. aeruginosa* SK	*S. marcescens*	*S. typhimurium*	*S. aureus*
-	-	-	+++	+	++	+

Conditions tested[1]

cadmium	copper	zinc	colchicine	chloroquine	arsenite	heat shock	starvation	dauer larvae
++	++	+	+	-	-	-	-	-

Biotic and abiotic conditions triggering the p*pgp-5::gfp* transcriptional induction.

+ indicates fluorescence detectable with a low magnification microscope (25X), while – corresponds to no visible transgene expression with a low magnification microscope (25X) with +++ for very strong induction in the intestine, ++ for medium and + for low.

FK corresponds to condition in which PA14 kills, in part by secreted phenazine toxins [8], while SK represents PA14 infection-mediate killing [9]. *pgp-1* and *pgp-3* are required for resistance to rapid killing by phenazines produced by PA14 [8]. We asked if *pgp-5* is required for resistance to phenazines by comparing the mean time-to-death (TD_{mean}) of *pgp-5* and wild type animals under conditions in which phenazines are important toxic components [8]. *pgp-5* mutants and wild type died with similar kinetics, with a TD_{mean} of 3.88 ± 0.29 hours and 3.80 ± 0.13 hours, respectively (Log rank test, $p > 0.05$), indicating that *pgp-5* is not necessary to protect *C. elegans* from phenazine toxicity.

[1] Cadmium chloride (50-200 µM), copper sulfate (50-200 µM), zinc sulfate (0.5-2 mM), colchicine (1-3 mM), chloroquine (1-3 mM), sodium arsenite (0.5-2 mM), heat shock (2 h at 30°C), starvation (worms without food for 1 h or 24 h) and transgene expression during the dauer stage. At least 2 independent experiments were performed, each with more than 50 animals.

References

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