Role of GATA transcription factor ELT-2 and p38 MAPK PMK-1 in recovery from acute *P. aeruginosa* infection in *C. elegans*

Brian P. Head, Abiola O. Olaitan, and Alejandro Aballay

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA

**ABSTRACT**

Infectious diseases caused by bacterial pathogens reduce the fitness of their associated host but are generally limited in duration. In order for the diseased host to regain any lost fitness upon recovery, a variety of molecular, cellular, and physiological processes must be employed. To better understand mechanisms underlying the recovery process, we have modeled an acute *Pseudomonas aeruginosa* infection in *C. elegans* using brief exposures to this pathogen and subsequent antibiotic treatment. To identify host genes altered during recovery from *P. aeruginosa* infection, we performed whole genome expression profiling. The analysis of this dataset indicated that the activity of the host immune system is down-regulated upon recovery and revealed shared and pathogen-specific host responses during recovery. We determined that the GATA transcription factor ELT-2 and the p38 MAP kinase PMK-1 are necessary for animals to successfully recover from an acute *P. aeruginosa* infection. In addition, we found that ELT-2 plays a more prominent and earlier role than PMK-1 during recovery. Our data sheds further light on the molecular mechanisms and transcriptional programs involved in recovery from an acute bacterial infection, which provides a better understanding of the entire infectious disease process.

**Introduction**

Certain microbes, including bacteria, are capable of causing infectious disease—either acute or chronic—in their hosts. In humans, acute bacterial infections are controlled by a combination of immune responses, physiological alterations, and, if necessary, supplementary antibiotic treatment. The combined actions of these measures usually leads to the elimination of the disease-causing bacteria and the restoration of host health. The return to a healthy set point, or recovery, is crucial to avoid entering a state of reduced fitness that manifests in various pathogenic forms including recurrent infections, fibrosis, autoimmune disease, and chronic inflammatory disorders.

Recovery is a set of 3 loosely overlapping processes: resolution of inflammation, elimination of any residual harmful molecules, and repair of damaged tissue. Specific molecules that govern one or more of these processes have been revealed through various *in vitro* studies and, more recently, through a handful of *in vivo* studies.\(^1,5\) Signaling from a variety of molecules, including TGF-β, IL-10, arachidonic acid (AA)-derived lipid mediators, and resolution-associated molecular patterns (RAMPs), enhances host recovery through the induction of anti-inflammatory or pro-resolving pathways.\(^6,10\) Although many of these processes have been described and dissected, the full complement of resolution and repair pathways and the coordination of these pathways in the context of the host-microbe holobiont is not fully understood.

The nematode *Caenorhabditis elegans* has been utilized to study various aspects of the infectious disease process upon exposure to gram negative and gram positive bacteria.\(^11-17\) Many of these bacterial species, including *Pseudomonas aeruginosa*, *Salmonella enterica*, *Serratia marcescens*, *Staphylococcus aureus*, and *Enterococcus faecalis*, have a significant impact on human morbidity and mortality. Studies in *C. elegans* have identified the mechanistic details of conserved host pathways that protect the host from infection by these pathogens, from pathogen sensing to cellular surveillance to tolerance.\(^18-22\) Moreover, using the *C. elegans*-pathogen model, a variety of
microbial virulence factors and processes, which could potentially be targeted to disrupt the infectious disease process, have been identified. Other recent studies have characterized the extent of host damage caused by these pathogens in *C. elegans*, further adding to our understanding of bacterial pathogenesis in this model system. Overall, these studies have enabled us to better understand both conserved and novel pathways that could be modulated in human patients to parry the effects of bacterial pathogens.

Two of the most well-characterized infectious disease processes in *C. elegans* include those caused by *P. aeruginosa* and *S. enterica*. The opportunistic pathogen *P. aeruginosa* can kill *C. elegans* in a “slow” colonization-dependent manner or “fast” colonization-independent manner. Both of these killing modalities depend on the secretion of various virulence factors—including lipopolysaccharides, pyoverdins, elastases, exotoxin A, HCN, and NO— that contribute to pathogenesis in the host. In slow killing, specific *P. aeruginosa*-secreted virulence factors interfere with host proteins and pathways leading to the induction of a robust immune response in *C. elegans* in as little as 4 hours. In an uncontrolled infection, readily apparent damage at the tissue level is evident 8–24 hours after exposure and death of the animal comes shortly thereafter. *S. enterica* kills *C. elegans* in a colonization-dependent manner. Like *P. aeruginosa*, *S. enterica* also uses a wide variety of virulence factors—including LPS, SptP, KdpD, and RamA—to colonize and kill *C. elegans*. One major mechanism of pathogen-dependent killing is disseminated oxidative stress, a byproduct of host immune system activation, although other pathogenic mechanisms are surely involved. Studies of these and other infectious disease processes should enable the development of interventions that ameliorate the effects of bacterial pathogenesis in humans.

Recently, we extended the use of the *C. elegans*-pathogen model to study pathways that facilitate host recovery from an acute infection. By studying acute *S. enterica* infection of *C. elegans*, we demonstrated that, upon recovery, the innate immune response is downregulated and various cytoprotective pathways are up-regulated. We also identified a role for the GATA transcription factor ELT-2 in controlling the expression of genes that are markers of recovery from *S. enterica* infection. In this study, we modeled acute *P. aeruginosa* infection and recovery in *C. elegans* and characterized host whole genome expression profiles across multiple phases of the acute infection disease process, from exposure to early recovery to late recovery. We compared and contrasted transcriptional programs that respond to *P. aeruginosa* and *S. enterica* recovery to elucidate shared and pathogen-specific recovery pathways. We also determined that recovery from acute *P. aeruginosa* infection requires the action of ELT-2, as is the case with recovery from *S. enterica*. Finally, we showed that p38-MAPK PMK-1 is required for recovery from both *P. aeruginosa* and *S. enterica* infections. In contrast to ELT-2, PMK-1 seems to be involved at later stages of recovery. In summary, our results help to elucidate potentially conserved pathways that contribute to the re-establishment of host homeostasis, or recovery, after acute infection by bacterial pathogens.

### Results

**Modeling acute *P. aeruginosa* infection and recovery in *C. elegans***

We have previously utilized *C. elegans* as a model to investigate host transcriptional networks and pathways that are involved in recovery from acute *S. enterica* infection. Upon recovery from acute *S. enterica* infection, genes that are markers of innate immunity are down-regulated, while genes involved in xenobiotic detoxification, redox regulation, and cellular homeostasis are upregulated. Here, we examined host recovery after acute infection by *P. aeruginosa* to learn more about shared and pathogen-specific recovery pathways.

To study recovery from acute *P. aeruginosa* infection, we employed *fer-1(b232ts)* animals, which are fertilization defective at the restrictive temperature. The use of this strain, rather than the wild-type N2 strain, prevents losing track of the initially exposed animals among their progeny. To model acute *P. aeruginosa* infection and recovery, we exposed larval stage 4 (L4) or adult *fer-1* (*b232ts*) animals to lawns of *P. aeruginosa* for 12 or 18 hours. After the initial exposure, animals were rinsed with M9 containing the antibiotic Neomycin (Neo) and then transferred to plates seeded with *P. aeruginosa* or to plates containing the antibiotic Streptomycin (Sm) seeded with Sm* Escherichia coli*. We found that the survival of adult animals exposed to *P. aeruginosa* for 12 or 18 hours and then treated with Neo/Sm is significantly higher than that of animals continuously exposed to *P. aeruginosa* (Fig. 1, yellow vs red lines) over a 3 day survival analysis period. Nearly all adult animals exposed to *P. aeruginosa* for 12 hours and then treated with Neo/Sm were alive at Day 3 (Fig. 1, yellow open squares). For adult animals exposed to *P. aeruginosa* for 18 hours and then treated with Neo/Sm, a smaller but still large percentage (~80%) of animals were alive at Day 3 (Fig. 1, yellow filled squares). In addition, the survival of adult animals exposed to *P. aeruginosa* for 12 hours and then treated with Neo/Sm was not statistically different
(p < 0.5637) from the survival of adult animals feeding on their normal E. coli food source (Fig. 1, yellow open squares vs black closed circles). Exposing L4 animals to P. aeruginosa for 12 or 18 hours followed by treatment with Neo/Sm did not lead to a shortening of lifespans relative to animals feeding on E. coli over the 3 day survival analysis period (Fig. S1). This data confirms previous results and indicates that we are capable of modeling acute P. aeruginosa infection in C. elegans using a combination of antibiotic treatment and a shift in bacterial food source.

For subsequent acute infection and recovery experiments, we decided to use adult fer-1(b232ts) animals exposed to P. aeruginosa-GFP for 12 or fewer (see below) hours. We used adult animals to avoid the effects of C. elegans larval development on recovery. For the length of exposure, 12 hours was the longest exposure time tested that did not alter survival over the 3 day survival analysis period (Fig. 1). Importantly, adult animals exposed to P. aeruginosa for 12 hours and then treated with Neo/Sm were not visibly colonized by P. aeruginosa-GFP at any point during the 3 day survival analysis period (data not shown). Exposure times shorter than 12 hours in duration should not alter host survival nor lead to P. aeruginosa intestinal colonization. In summary, we have established an acute P. aeruginosa infection and recovery system that allows us to study host processes leading to the restoration of homeostasis.

**Profi ling gene expression changes in C. elegans upon P. aeruginosa acute infection and recovery**

To investigate pathways that mediate C. elegans recovery from P. aeruginosa infection, we utilized Agilent microarrays to identify changes in gene expression over the entire time course of acute infection and recovery. We utilized hypochlorite treatment of gravid fer-1(b232ts) adults to developmentally synchronize a suitable number of animals for the gene expression profiling experiments. A 4 hour exposure to P. aeruginosa is sufficient for virulence factor-induced host cell alterations and for C. elegans to generate a robust immune response. Thus, for the gene expression profiling experiments, we utilized a 4 hour exposure of adult animals to P. aeruginosa. We collected cohorts of fer-1(b232ts) animals grown on E. coli for 72 hours (72 h), immediately following a 4 hour exposure to P. aeruginosa (76 h), and at 3 times during the recovery period (R1, R2, and R3) (Fig. 2A). As a control, we collected animals that were not exposed to P. aeruginosa but were exposed to the antibiotics Neo/Sm.

Log2-transformed data from the microarray is given in Table S1. A total of 84 genes are regulated by exposure to Neo/Sm antibiotics alone (Table S2); these genes were excluded from subsequent analyses. Overall, the expression profiles of 1,323 genes, or 6.35% of the genome, were altered more than 2-fold (p < 0.05 with FDR) in one or more relevant comparisons over the entire course of acute infection and recovery. These comparisons are infected versus uninfected (76 h vs 72 h) and recovered vs. infected animals (R1 vs 76 h, R2 vs 76 h, and R3 vs 76 h) (Table S3). A hierarchical clustering analysis of transcript abundance over time for these 1,323 genes is depicted in Figure 2B. The clustering analysis indicates that there are 4 major groupings of genes that respond to acute infection and recovery. One group of genes is strongly up-regulated after 4 hours of exposure to P. aeruginosa and then generally downregulated close to pre-exposure levels by R3 (Fig. 2B, cluster a). One group of genes is weakly upregulated after 4 hours of exposure to P. aeruginosa and then strongly down-regulated by R3 (Fig. 2B, cluster b). A third group of genes is strongly downregulated after 4 hours of exposure to P. aeruginosa and then generally upregulated close to pre-exposure levels by R3 (Fig. 2B, cluster c). The final group of genes
do not change upon exposure to *P. aeruginosa* but are upregulated during the recovery process (Fig. 2B, cluster d). The identity of each gene in clusters a-d is given in Table S4. In conclusion, we have identified more than 1,300 *C. elegans* genes that respond to acute *P. aeruginosa* infection and recovery.

A subset of the genes in our acute infection and recovery gene set are down- or up-regulated upon *P. aeruginosa* exposure, independent of their behavior during recovery. A total of 542 genes – 268 down-regulated and 276 upregulated – were altered upon *P. aeruginosa* exposure relative to animals feeding on *E. coli* (p < 0.05 with FDR) (Table S3). Comparison of our *P. aeruginosa*-regulated gene set to a *P. aeruginosa*-regulated gene set described in a previous study reveals a high degree of overlap between the 2 data sets (Fig. S2, Table S5). The representation factor, a measure of the correlation between 2 populations, is significantly higher than 1, indicating a high degree of overlap (Fig. S2). Thus, as previously noted, *C. elegans* has a highly stereotypic transcriptional response to *P. aeruginosa*.

We next assessed the transcriptional activity of these 542 pathogen-regulated genes during the recovery period. We found that 60% (156/268) of the genes downregulated upon pathogen exposure, were conversely up-regulated 24 hours post-treatment, at the R3 time point. Similarly, we found that 36% (98/276) of genes upregulated upon pathogen exposure, were down-regulated post-treatment. We therefore conclude that the rapid host transcriptional response that occurs after a 4 hour exposure to *P. aeruginosa* is quickly dampened upon recovery.

To validate the results of the microarray, we performed quantitative real-time PCR (qRT-PCR) on a subset of the 1,323 genes altered during recovery. This subset includes 5 downregulated genes (Fig. 3A) and 10 up-regulated genes (Fig. 3B) that spanned early to late responders and a variety of time course expression profiles (Fig. 3, white bars). We performed qRT-PCR on RNA harvested from *C. elegans* that were subjected to the same conditions as in the microarray experiments. Changes in gene expression as assessed by qRT-PCR were comparable to those observed by microarray profiling after 4 hours of exposure to *P. aeruginosa* (76 h vs 72 h), at recovery time point 1 (R1 vs 76 h), and at recovery time point 3 (R3 vs 76 h) (Fig. 3, colored vs white bars). Statistical analysis indicated that the gene expression changes determined by qRT-PCR and microarray
profiling were not statistically different in 39 out of 45 measurements ($p > 0.05$) (Fig. 3). Thus, the microarray data accurately reflects the majority of gene expression differences over the acute *P. aeruginosa* infection and recovery period.

**Shared and pathogen-specific host pathways regulate recovery from acute *P. aeruginosa* and *S. enterica* infections**

One of the goals of our study was to identify shared and pathogen-specific host responses during recovery from acute bacterial infection. To do this, we compared sets of genes regulated during *P. aeruginosa* recovery (Table S3) to sets of genes regulated during *S. enterica* recovery. In our previous analysis using recovery from acute *S. enterica* infection, we analyzed gene expression profiles 24 hours post-treatment to determine genes regulated during recovery. Thus, we began by focusing on *P. aeruginosa* recovery genes that have significantly altered expression 24 hours post-treatment (R3 vs 76 h). This time point also showed the greatest number of genes with altered transcription among all *P. aeruginosa* recovery gene sets (Table S3). The overlap of the *P. aeruginosa* and *S. enterica* recovery gene sets is shown in Figure 4A. The representation factors are 6.2 and 6.0 for down-regulated and upregulated gene sets, respectively (Fig. 4A). These values are less than that for the overlap between 2 studies of the transcriptional response of *C. elegans* exposed to *P. aeruginosa* for 4 hours (Fig. S2) but more than 1 indicating both common and distinct transcriptional responses of *C. elegans* to *P. aeruginosa* and *S. enterica* infections.
Shared and pathogen-specific recovery genes are given in Table S6. A similar comparative analysis at 6 hours post-treatment (R1 vs 76 h), also revealed shared and distinct host responses during recovery (Fig. S3A). Taken together, our data indicate that a small subset of genes are important for recovery from both of these 2 bacterial pathogens while a larger subset of genes are pathogen-specific.

To provide further insight into the biological functions of the genes that are transcriptionally altered during recovery from *P. aeruginosa*, we performed gene ontology (GO) analyses using the DAVID Bioinformatics Resource (DAVID, http://david.abcc.ncifcrf.gov/). The 10 gene ontology clusters with the highest enrichment score for down- and up-regulated *P. aeruginosa* recovery genes at 24 hours post-treatment (R3 vs 76 h) are shown in Fig. 4B (dark red, dark green). For the downregulated gene set, the highest scoring cluster is C-type lectins, a class of genes that is involved in *C. elegans* pathogen defense. Other high scoring down-regulated clusters include genes with F-box, cytochrome P450, CHK kinase, and cell wall metabolism ontologies. For the upregulated gene set, the highest scoring ontology cluster is collagen, a class of genes important for extracellular matrix integrity, tissue repair, and longevity responses. Other up-regulated clusters include genes with DUF-19, SCP-like extracellular domains, and UDP-glucuronosyltransferase (UGT) ontologies. A GO analysis of genes regulated 6 hours post-treatment (R1 vs 76 h) yields similar lists of high-scoring clusters (Fig. S3B). Our analysis indicates that, upon recovery from *P. aeruginosa* infection, *C. elegans* down-regulates genes that are important for controlling pathogen abundance and up-regulates a variety of other genes that are potentially involved in inflammation resolution, damage control, or tissue repair.

A comparison between GO clusters that are altered during *P. aeruginosa* recovery to GO clusters that are altered during *S. enterica* recovery, confirms the idea that there are both shared and pathogen-specific recovery pathways (Fig. 4B, dark red vs red, dark green vs green). The highest scoring cluster in both *P. aeruginosa* and *S. enterica* downregulated gene sets is C-type lectins, indicating that *C. elegans* dampen the inflammatory response.

Figure 4. Shared and pathogen-specific responses during recovery from acute bacterial infection. (A) Proportional Venn diagrams showing the overlap of *P. aeruginosa* R3 and *S. enterica* downregulated recovery genes, left, and overlap of *P. aeruginosa* R3 and *S. enterica* upregulated recovery genes, right. (B) Gene ontology analysis of *P. aeruginosa* R3 and *S. enterica* recovery genes using the DAVID Bioinformatics Database. Enrichment scores of the down-regulated clusters are shown in the left panel. Enrichment scores of the up-regulated clusters are shown in the right panel. The top 10 *P. aeruginosa* recovery clusters are shown. Only the top 10 *S. enterica* recovery clusters that overlap with one of the top 10 *P. aeruginosa* recovery clusters are shown.
response upon recovery (Fig. 4B, left panel). Comparison of the other top 10 GO clusters for the down-regulated gene set reveals distinct functions of genes that are downregulated upon recovery from the 2 bacterial pathogens (Fig. 4B, left panel). This would be expected as both *P. aeruginosa* and *S. enterica* provoke distinct *C. elegans* immune responses, outside of C-type lectin induction.\(^{15,30,31,41,42}\) For the upregulated gene sets, the highest scoring clusters are collagens for *P. aeruginosa* and UGTs for *S. enterica* (Fig. 4B, right panel). Collagen genes are not up-regulated during recovery from *S. enterica* recovery. However, UGTs, Phase II detoxification enzymes, are a key component of recovery from both acute infections. As with the down-regulated comparison, the GO data reveals a distinct set of functions for upregulated genes, outside of the UGT genes, during recovery from *P. aeruginosa* and *S. enterica*. We conclude that *C. elegans* uses both shared and distinct pathways during recovery from acute bacterial infection.

**Recovery from acute *P. aeruginosa* infection requires early ELT-2 activity and late PMK-1 activity**

To further explore pathways that mediate the expression of genes involved in recovery from *P. aeruginosa* infection, we compared the set of genes regulated during recovery from *P. aeruginosa* infection to previously identified gene sets that are known to be controlled by various signaling pathways, transcription factors, or treatments (Table S7). We found a significant overlap between *P. aeruginosa* recovery genes and gene sets regulated by ELT-2/GATA, PMK-1/p38 MAPK, DAF-16/FOXO, or SKN-1/Nrf (Table S7). Gene sets for HIF-1, hypoxia, and SMA-6/TGF-β did not have a significant overlap with *P. aeruginosa* recovery genes. Our analysis suggests that ELT-2, PMK-1, DAF-16, and SKN-1 may be involved in orchestrating gene expression programs that mediate *C. elegans* recovery from acute *P. aeruginosa* infection.

The GATA transcription factor ELT-2 is required for full recovery from acute *S. enterica* infection\(^4\) and may also be involved in recovery from acute *P. aeruginosa* infection (Table S7). Thus, we chose to examine the survival of *fer-1(b232ts) elt-2(RNAi)* animals during recovery and upon continuous exposure to *P. aeruginosa* or *E. coli*. Because ELT-2 is essential for *C. elegans* larval development\(^43\) we performed RNAi on L4 animals. This approach has been used successfully to inhibit elt-2 expression for at least 6 days.\(^{31,44}\) As with acute *S. enterica* infection, RNAi of elt-2 completely prevented the recovery of animals acutely exposed to *P. aeruginosa* for 12 hours over the 3 day analysis period (Fig. 5A, yellow lines, Fig. S4). RNAi of elt-2 also reduced the survival of animals continuously exposed to *P. aeruginosa* (Fig. 5A, red lines, Fig. S4), which is in line with ELT-2s role in the generation of an early transcriptional response to *P. aeruginosa* infection.\(^{31,45}\) RNAi of elt-2 did not alter the survival of animals growing on live *E. coli* over the 3 day analysis period, confirming that elt-2 RNAi at the L4 stage does not simply induce a general debilitation of the animals (Fig. 5A, black lines, Fig. S4).\(^{43,45}\) We conclude that, similar to the requirement of ELT-2 for *S. enterica* recovery, the function of ELT-2 is necessary for *C. elegans* to recover from an acute *P. aeruginosa* infection.

ELT-2 is known to regulate the transcription of genes in the intestine via the TGATAA (extended GATA) cis-regulatory motif.\(^{43,46}\) We used an *in silico* approach to examine the prevalence of TGATAA sites in our acute infection and recovery gene sets – *P. aeruginosa* exposure, 76 h, and 3 sequential recovery time points, R1, R2, and R3 (Table S8). For genes regulated upon exposure to *P. aeruginosa*, 64% have at least 1 TGATAA site in the 1.5 kb region upstream of their transcriptional start site. The same percentage, 64%, of R1 genes contain at least 1 TGATAA site in the upstream region. In subsequent time points, the percentage of genes with at least 1 TGATAA declines to 60%. This data also correlates to an overall decrease in the number of TGATAA sites per gene over the course of the acute infection and recovery time course (Table S8). A randomly selected set of genes from the *C. elegans* genome exhibited a lower enrichment of TGATAA sites by either percentage of genes or number of sites per gene (Table S8). This *in silico* analysis highlights the fact that ELT-2 plays a role at multiple phases of the acute infectious disease process, but is more important for early gene expression programs.

We next examined the effects of RNAi inhibition of the stress response kinase and innate immunity regulator PMK-1 during recovery. Using the same L4 RNAi treatment protocol employed in the elt-2 RNAi experiments above, we found that RNAi of pmk-1 significantly impaired but did not completely prevent the recovery of *fer-1(b232ts)* animals acutely exposed to *P. aeruginosa* for 12 hours (Fig. 5B, yellow lines, Fig. S4). RNAi of pmk-1 significantly reduced the survival of animals continuously exposed to *P. aeruginosa* but did not affect survival of animals on *E. coli*, as previously reported (Fig. 5B, red and black lines, Fig. S4).\(^{47}\) We found a similar effect on recovery using pmk-1 mutant animals (Fig. S5A). We conclude that host PMK-1 activity is required to both generate an immune response against *P. aeruginosa* and, also, to orchestrate recovery from an acute infection by this pathogen.

We also observed a significant enrichment of DAF-16- and SKN-1-regulated targets in the set of genes altered during recovery from *P. aeruginosa* infection.
Consequently, we explored whether the activity of these 2 transcription factors, in addition to PMK-1 and ELT-2, is required for recovery from acute \textit{P. aeruginosa} infection.

Neither RNAi of \textit{daf-16} nor that of \textit{skn-1} prevented the full recovery of \textit{fer-1(b232ts)} animals from acute \textit{P. aeruginosa} infection (Fig. S6, yellow lines). Importantly, the \textit{daf-16} RNAi and \textit{skn-1} RNAi protocols used for the survival analysis completely eliminated visible GFP fluorescence in animals expressing DAF-16::GFP and SKN-1::GFP translational reporters, respectively, indicating that the RNAi treatments were effective in reducing levels of DAF-16 and SKN-1 (data not shown). The \textit{daf-16} RNAi and \textit{skn-1} RNAi results also demonstrate the specificity of \textit{elt-2} RNAi and \textit{pmk-1} RNAi treatments in preventing recovery from \textit{P. aeruginosa} infection. We conclude that, in contrast to ELT-2 and PMK-1, neither DAF-16 nor SKN-1 activity are required for recovery from acute \textit{P. aeruginosa} infection.

The role of PMK-1 in recovery from \textit{P. aeruginosa} infection was unexpected. We previously found that \textit{pmk-1} RNAi at the L4 stage does not affect recovery from acute \textit{S. enterica} infection.\textsuperscript{4} Thus, we chose to reexamine the role of PMK-1 in recovery from acute \textit{S. enterica} infection using \textit{pmk-1} mutant animals. Mutant \textit{pmk-1} animals were unable to recover from acute \textit{S. enterica} infection (Fig. S5B), showing a stronger recovery defect relative to \textit{pmk-1(RNAi)} animals.\textsuperscript{4} To examine whether genes required for immunity are generally required for recovery from acute \textit{S. enterica} infection,
we tested animals with a loss of function mutation in fshr-1. We used fshr-1 mutants because they are more sensitive than wild-type animals to killing by both Gram-negative and Gram-positive bacteria.48 We found that while fshr-1 mutants are susceptible to S. enterica infection they are capable of fully recovering from infection by this pathogen (Fig. S5B-C). In summary, PMK-1 is involved in recovery from both P. aeruginosa and S. enterica, though its role in P. aeruginosa recovery is more prominent.

Further analysis of the role of ELT-2 and PMK-1 in recovery from P. aeruginosa reveals additional details about the sequence of molecular events that may take place during recovery. The effects of elt-2 RNAi on recovery are evident at the earliest time points indicating that ELT-2 is required early in the recovery process (Fig. 5A, yellow lines, Fig. S4). In contrast, the effects of pmk-1 RNAi on recovery are evident at later time points (Fig. 5B, yellow lines, Fig. S4). As shown in Figure 5, 50% of elt-2 RNAi animals failed to recover at 48 hours while more than 50% of pmk-1 RNAi animals remain alive at 72 hours. Our in silico analysis of overlaps between recovery and published gene sets (Table S7) supports this sequential requirement for ELT-2 followed by PMK-1 during recovery (Fig. 5C). We conclude that although both ELT-2 and PMK-1 are both required for recovery from acute P. aeruginosa infection, ELT-2 plays a more prominent and earlier role than PMK-1.

Discussion

We have utilized C. elegans to investigate host pathways that contribute to recovery from acute P. aeruginosa infection. Gene expression profiling of animals at multiple time points during the recovery phase shows that a broad class of genes, some expected and others novel, are altered during this phase. Further analysis of P. aeruginosa recovery genes using an in silico approach enabled the identification of 2 intracellular factors that regulate gene expression during recovery. We show that these factors, the GATA transcription factor ELT-2 and the p38 MAP kinase PMK-1, are required for recovery from acute P. aeruginosa infection, though ELT-2 plays a more prominent and early role than PMK-1.

In our study, we employed a slow killing protocol to investigate P. aeruginosa pathogenesis, as opposed to fast or liquid killing protocols.29 Slow killing involves accumulation of P. aeruginosa in the intestine of C. elegans and requires several virulence factors, including the secreted Exotoxin A molecule.23,49-51 Importantly, these virulence factors are utilized by P. aeruginosa to cause disease in other organisms, including in mammals.21 In fast and liquid killing, P. aeruginosa has different modes of killing that involve molecules of the pyocyanin-phenazine class or siderophores, respectively. These molecules contribute to the rapid killing of C. elegans in part by eliciting oxidative stress or a hypoxic response, respectively.28,52 In this study, there is no evidence of a transcriptional signature indicating oxidative stress or hypoxia upon exposure or during recovery (Table S7), confirming the differences between slow, fast, and liquid killing. Further studies are necessary to determine whether C. elegans could recover from other modes of P. aeruginosa pathogenesis and what host pathways might be involved in those instances.

We previously investigated pathways that mediate C. elegans recovery from acute S. enterica infection.4 We have taken advantage of our previously published results by comparing and contrasting P. aeruginosa and S. enterica recovery processes to learn about shared and pathogen-specific recovery pathways. The recovery phase for both infections is characterized by the down-regulation of C-type lectins and the up-regulation of Phase II detoxification genes (Fig. 4B). Outside of these 2 shared signatures, most genes regulated during recovery from P. aeruginosa and S. enterica are pathogen-specific. This is not surprising as both the immune response and the type of damage that P. aeruginosa and S. enterica pathogens produce during an infection is quite different. As mentioned above, P. aeruginosa modulates host biology through translation inhibition, oxidative stress, hypoxia, mitochondrial toxicity, and through other known and unknown mechanisms.23,27,28,50-53 S. enterica, on the other hand, has been shown to induce organism-wide oxidative stress in C. elegans.52 Our data indicates a complex cellular and physiological response of C. elegans during recovery from either of these 2 pathogens.

In the context of P. aeruginosa infection, multiple cuticular collagen genes are up-regulated during recovery (Fig. 4B). As P. aeruginosa slow killing is known to involve pathogen accumulation in the lumen of the intestine and subsequent damage to intestinal epithelial cells,11,25 it is interesting to note the upregulation of collagen genes in the cuticle, or skin, of C. elegans. It is likely that during the slow killing process, P. aeruginosa also induces damage to the cuticle, either directly or indirectly. It is also possible that many of these collagens are components of both the cuticle and the intestinal extracellular matrix. Further studies on these collagen genes would help to determine the exact mechanisms underlying slow killing pathogenicity and the repair processes important for recovery. No matter the location, rebuilding the extracellular matrix and restoration of tissue integrity is likely to be an important for recovery. Recently, collagen remodeling by SKN-1 has been shown to be important for longevity.40 Although we have ruled out the action of
SKN-1 in recovery from *P. aeruginosa* acute infection, perhaps infection resolution signals feed into the collagen remodeling pathway downstream of SKN-1 and facilitate tissue repair.

The GATA transcription factor ELT-2 contributes to recovery from acute pathogen infection (Fig. 5A). ELT-2-mediated transcription also specifies the formation of the *C. elegans* intestine and it participates in the generation of a robust immune response upon exposure of *C. elegans* to various pathogens. For ELT-2 to specify these diverse transcriptional outputs, it must act on the genome with other nuclear factors. We previously observed an up-regulation of many DAF-16- and SKN-1-regulated genes, in addition to ELT-2-regulated genes, during recovery from acute *S. enterica* infection. This suggested that ELT-2 may modulate transcription during recovery in parallel to or in sequence with these transcription factors. We find that these factors do not play a role in recovery from acute *P. aeruginosa* infection (Fig. S6), although they may still be involved in recovery from *S. enterica* infection. Other as yet unknown factors must therefore be involved in specifying ELT-2 transcriptional activity during recovery from acute pathogen infection.

Our study shows that *C. elegans* initiates shared and distinct transcriptional responses that mediate recovery from different pathogens. By virtue of their molecular nature, many of the genes we have identified are likely to act in a local or paracrine manner to facilitate recovery. Other recovery genes, including a litany of genes encoding secreted proteins or proteins that are capable of synthesising a long-range response, may influence recovery in a systemic or endocrine manner. Future studies should seek to ascertain the mechanism by which these genes influence recovery. Combining these studies with the data presented here will help to shed light on the complex nature by which a host returns to homeostasis following an acute infection.

**Materials and methods**

**Strains and reagents**

*C. elegans* strains HH142 [fer-1(b232ts)], N2 [wild-type], KU25 [pmk-1(km25)], RB911 [shhr-1(0k778)], LD1 [SKN-1::GFP], and TJ356 [DAF-16::GFP] were provided by the Caenorhabditis Genetics Center. *C. elegans* strains were maintained at 15°C on NGM-OP50 plates without antibiotics. The following bacterial strains were used for maintenance or experiments: *E. coli* strain OP50-1 (OP50) [SmR],1,4 *E. coli* strain HT115 (HT115) [TeR],4 *E. coli* strain HT115 pL4440-RNAi (HT115-RNAi) [AmpR, TeR],4 *P. aeruginosa* strain PA14 (PA14) [RiR],1 P. aeruginosa strain PA14 expressing GFP (PA14-GFP) [KanR RifR],11 *S. enterica* Typhimurium strain 1344 (S. enterica) [SmR].4 Bacteria were grown overnight for 13-15 hours in 3 ml LB broth at 37°C except for HT115-RNAi bacteria (see below). Ampicillin (Merck, #171254-25GM), Carbenicillin (Cellgro, #46-100-RG), Neomycin (Sigma, #N1876-25G), Streptomycin (Sigma, S6501-100G), and isopropyl-β-D-thiogalactopyranoside (Anatrace, I1003) stocks were kept frozen at −20°C until needed.

**P. aeruginosa survival assays**

**Plate preparation:** HT115, OP50, or PA14-GFP were grown overnight for 13-15 hours in 3 ml LB broth at 37°C. A total of 200 μl of the resulting HT115 culture was seeded on 6.0 cm modified (0.35% peptone) NGM plates without antibiotics and allowed to grow for 1-2 d at 25°C. A total of 50 μl (exposure) or 30 μl (scoring) of the resulting PA14-GFP culture was seeded on 3.5 cm modified (0.35% peptone) NGM plates without antibiotics and allowed to grow overnight at 37°C. A total of 30 μl of the resulting OP50 culture was seeded on 3.5 cm modified (0.35% peptone) NGM plates with 50 μg/ml Streptomycin and allowed to grow for 1-2 d at 25°C.

**Survival assay:** Gravid *fer-1(b232ts)* animals were allowed to lay eggs for 4 hours at 25°C on 6.0 cm HT115 plates. Gravid animals were removed and the eggs/plates were incubated for 36 or 72 hours at 25°C. Synchronized L4 or adult animals were transferred to full lawn 3.5 cm OP50 or PA14-GFP plates and exposed to these bacteria for 12 or 18 hours at 25°C. After the designated amount of time, animals were washed off OP50 or PA14-GFP plates using M9, rinsed with several changes of M9, treated with 200 μg/ml Neomycin in M9 for 20 minutes, and subsequently transferred to 3.5 cm OP50 or PA14-GFP plates. The animals/plates were incubated at 25°C. Animals were scored every day and were considered dead when they failed to respond to touch. Animals were transferred to fresh plates every other day for the entire length of the experiment. Survival was plotted using Kaplan-Meier survival curves and analyzed by the log-rank test using GraphPad Prism (GraphPad Software, Inc.). Survival curves resulting in P values of < 0.05 were considered significantly different. A total of 25-75 (uninfected = 25, continuously infected = 75, recovered = 75) animals per condition were used.

**RNAi-coupled *P. aeruginosa* survival assays**

**Plate preparation:** HT115, OP50, and PA14-GFP plates were prepared as described above. HT115-RNAi strains were grown for 9 hours in 5 ml LB broth containing 50 μg/ml Ampicillin at 37°C. A total of 250 μl of the
Assays were performed as previously described. S. enterica condition were used. All animals were transferred to full lawn 10.0 cm OP50 plates and incubated for 72 hours at 25°C. At 76 hours, animals were washed off OP50 or PA14-GFP plates. The animals/plates were incubated at 25°C. At designated harvest time points (72, 76, 82, 88, and 100 hours), animals were washed off plates using M9, rinsed with several changes of M9, and transferred to full lawn 10.0 cm PA14-GFP plates. At 76 hours, animals were washed off PA14-GFP plates using M9, rinsed with several changes of M9, treated with 200 µg/ml Neomycin in M9 for 20 minutes, and subsequently transferred to 10.0 cm OP50 or PA14-GFP plates. The animals/plates were incubated at 25°C. At designated harvest time points, the microarray data was deposited in the NCBI Gene Expression Omnibus database: GSE81592.

Microarray analysis

Amplification, hybridization, and scanning were performed as previously described. Scanned data was log2 transformed and quantile normalized using Partek Genomics Suite (Partek Inc.). P value and fold-change calculations were performed using Partek Genomics Suite (Partek Inc.). A P value of < 0.05 using the FDR linear step-up procedure was considered significant. Hierarchical clustering (Euclidean, average linkage) was performed on the 1,323 genes that were altered during the acute infection and recovery time course using Partek Genomics Suite (Partek Inc.). For each of the 5 acute infection and recovery time points, 3 biological replicates were assed. For the antibiotic exposure control time points, 82 hOP and 82 hOPC, 2 or 3 biological replicates were assessed. The microarray data was deposited in the NCBI Gene Expression Omnibus database: GSE81592.

RNA isolation for qRT-PCR

Total RNA was extracted from animals that were subjected to the same conditions as those described in the RNA isolation for microarray analysis section above.

Quantitative real-time PCR (qRT-PCR)

Quantitative real-time PCR assays were performed as previously described. To compare qPCR and microarray data, a one sample, 2-tailed t-test was performed.

Bioinformatics

All gene lists, including those culled from the literature, were passed through WormBase Converter using the WS220 genome release as the output (references are noted in Table S7). A total of 20,834 WS220 genes are represented by 1 or more probes in the Agilent C. elegans V2 array (Agilent Technologies).
was performed using the DAVID Bioinformatics Database (david.abcc.ncifcrf.gov).\textsuperscript{56} Overlap of 2 gene sets was performed using Partek Genomics Suite (Partek Inc.). Statistical significance of the overlap between 2 gene sets was calculated using the following on-line program: nemates.org/MA/progs/overlap_stats.html. The representation factor (RF) represents the number of overlapping genes divided by the expected number of overlapping genes drawn from 2 independent groups. A background gene list of 20,834 was used for the calculation. P values were calculated using the exact hypergeometric probability. 1.5 kb cis-regulatory sequences were identified using WormMart (wormbase.org). Detailed bioinformatics protocols are available upon request.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

Acknowledgments

C. elegans strains were provided by the Caenorhabditis Genetics Center (University of Minnesota), which is funded by the NIH-ORIP. We thank Dr. Danielle Ferraz Mello for technical support and helpful discussions and the Duke GCB Microarray Facility for technical advice.

Funding

B.H. is funded by NIH Grant GM103122. A.A. is funded by grants from NIGMS GM070977 and NIAID AI117911.

References

[1] Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. Nat Rev Immunol 2013; 13:59-66; PMID:23197111; http://dx.doi.org/10.1038/nri3362

[2] Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 2014; 40:315-27; PMID:24656045; http://dx.doi.org/10.1016/j.immuni.2014.02.009

[3] Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014; 510:92-101. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24899309; PMID:24899309; http://dx.doi.org/10.1038/nature13479

[4] Head B, Aballay A. Recovery from an acute infection in C. elegans requires the GATA transcription factor ELT-2. PLoS Genet 2014; 10:e1004609; PMID:25340560; http://dx.doi.org/10.1371/journal.pgen.1004609

[5] Torres BY, Oliveira JHM, Thomas Tate A, Rath P, Cumnock K, Schneider DS. Tracking Resilience to Infections by Mapping Disease Space. PLoS Biol 2016; 14:e1002436; PMID:27088359; http://dx.doi.org/10.1371/journal.pbio.1002436

[6] Wahl SM. TGF-beta in the evolution and resolution of inflammatory and immune processes. Introduction. Microbes Infect 1999; 1:1247-9; PMID:10611751; http://dx.doi.org/10.1016/S1286-4579(99)00261-0

[7] Filippi CM, von Herath MG. IL-10 and the resolution of infections. J Pathol 2008; 214:224-30; PMID:18161757; http://dx.doi.org/10.1002/path.2272

[8] Shields AM, Panayi GS, Corrigall VM. Resolution-associated molecular patterns (RAMP): RAMPs defending immunological homeostasis? Clin Exp Immunol 2011; 165:292-300; PMID:21671907; http://dx.doi.org/10.1111/j.1365-2249.2011.04433.x

[9] Laidlaw BJ, Cui W, Amezquita RA, Gray SM, Guan T, Lu Y, Kobayashi Y, Flavell RA, Kleinlein SH, Craft J, et al. Production of IL-10 by CD4(+) regulatory T cells during the resolution of infection promotes the maturation of memory CD8(+) T cells. Nat Immunol 2015; 16:871-9; PMID:26147684; http://dx.doi.org/10.1038/nri3224

[10] Serhan CN, Chiang N, Dalli J, Levy BD. Lipid mediators in the resolution of inflammation. Cold Spring Harb Perspect Biol 2015; 7:a016311; http://dx.doi.org/10.1101/cshperspect.a016311

[11] Tan MW, Mahajan-Miklos S, Ausubel FM. Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. Proc Natl Acad Sci U S A 1999; 96:715-20. PMID:9892699; http://dx.doi.org/10.1073/pnas.96.2.715

[12] Shivers RP, Youngman MJ, Kim DH. Transcriptional responses to pathogens in Caenorhabditis elegans. Curr Opin Microbiol 2008; 11:251-6. PMID:18567532; http://dx.doi.org/10.1016/j.mib.2008.05.014

[13] Partridge FA, Gravato-Nobre MJ, Hodgkin J. Signal transduction pathways that function in both development and innate immunity. Dev Dyn 2010; 239:1330-6; PMID:20131356

[14] Marsh EK, May RC. Caenorhabditis elegans, a model organism for investigating immunity. Appl Environ Microbiol 2012; 78:2075-81; PMID:22286994; http://dx.doi.org/10.1128/AEM.07486-11

[15] Simonsen KT, Gallego SF, Faergeman NJ, Kallipolitis BH. Strength in numbers: "omics" studies of C. elegans innate immunity. Virulence 2012; 3:477-84; PMID:23076279; http://dx.doi.org/10.4161/viru.21906

[16] Pukkila-Worley R, Ausubel FM. Immune defense mechanisms in the Caenorhabditis elegans intestinal epithelium. Curr Opin Immunol 2012; 24:3-9; PMID:22236697; http://dx.doi.org/10.1016/j.coi.2011.10.004

[17] Ewbank JJ, Pujol NL. Local and long-range activation of innate immunity by infection and damage in C. elegans. Curr Opin Immunol 2016; 38:1-7; PMID:26517153; http://dx.doi.org/10.1016/j.coi.2015.09.005

[18] Wong D, Bazopoulou D, Pujol N, Tavernarakis N, Ewbank JJ. Genome-wide investigation reveals pathogen-specific and shared signatures in the response of Caenorhabditis elegans to infection. Genome Biol 2007; 8:R194. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17875205; PMID:17875205; http://dx.doi.org/10.1186/gb-2007-8-9-r194

[19] Engelmann I, Griffon A, Tichit L, Montanana-Sanchis F, Wang G, Reinke V, Waterston RH, Hillier LW, Ewbank JJ. A comprehensive analysis of gene expression changes provoked by bacterial and fungal infection in C. elegans. PLoS One 2011; 6:e19055. Available from: http://www.
ncbi.nlm.nih.gov/pubmed/21602919; PMID:21602919; http://dx.doi.org/10.1371/journal.pone.0019055

[20] Melo JA, Ruvkun G. Inactivation of conserved C. eugens genes engages pathogen- and xenobiotic-associated defenses. Cell 2012; 149:452-66. PMID:22500807; http://dx.doi.org/10.1016/j.cell.2012.02.050

[21] Aballay A. Role of the nervous system in the control of proteostasis during innate immune activation: insights from C. eugens. PLoS Pathog 2013; 9: e1003433; PMID:23950707; http://dx.doi.org/10.1371/journal.ppat.1003433

[22] Cohen LB, Troemel ER. Microbial pathogenesis and host defense in the nematode C. eugens. Curr Opin Microbiol 2015; 23:94-101. PMID:25461579; http://dx.doi.org/10.1016/j.mib.2014.11.009

[23] Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM. Molecular mechanisms of bacterial virulence elucidated using a Pseudomonas aeruginosa-Caenorhabditis eugens pathogenesis model. Cell 1999; 96:47-56. PMID:9989496; http://dx.doi.org/10.1016/S0092-8674(00)80958-7

[24] Tenor JL, McCormick BA, Ausubel FM, Aballay A. Caenorhabditis eugens-based screen identifies Salmonella virulence factors required for conserved host-pathogen interactions. Curr Biol 2004; 14:1018-24. http://dx.doi.org/10.1016/j.cub.2004.05.050

[25] Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Czeairliyan BO, Ausubel FM. Distinct pathogenesis and host responses during infection of C. eugens by P. aeruginosa and S. aureus. PLoS Pathog 2010; 6:1-24. http://dx.doi.org/10.1371/journal.ppat.1000982

[26] Aballay A, Yorgey P, Ausubel FM. Salmonella typhimurium proliferates and establishes a persistent infection in the intestine of Caenorhabditis eugens. Curr Biol 2000; 10:1539-42. PMID:11114525; http://dx.doi.org/10.1016/0960-9822(00)00830-7

[27] Gallagher LA, Manoil C. Pseudomonas aeruginosa PAO1 kills Caenorhabditis eugens by cyanide poisoning. J Bacteriol 2001; 183:6207-14; PMID:11591663; http://dx.doi.org/10.1128/JB.183.21.6207-6214.2001

[28] Cezairliyan B, Vinayavekhin N, Grenfell-Lee D, Yuen GJ, Bailey AM, Ivens A, Kingsley R, Cottell JL, Wain J, Piddock LJ V. RamA, a member of the AraC/XylS family, influences both virulence and efflux in Salmonella enterica serovar Typhimurium. J Bacteriol 2010; 192:1607-16; PMID:20081028; http://dx.doi.org/10.1128/JB.01517-09

[29] Kirienko N V, Cezairliyan BO, Ausubel FM. Programmed cell death mediates Salmonella typhimurium colonization of Caenorhabditis eugens and survival in macrophages. Cell Microbiol 2011; 13:1618-37; PMID:21790938; http://dx.doi.org/10.1111/j.1462-5822.2011.01645.x

[30] Sem X, Rhen M. Pathogenicity of Salmonella enterica in Caenorhabditis eugens relies on disseminated oxidative stress in the infected host. PLoS One 2012; 7:e54517. PMID:23028994; http://dx.doi.org/10.1371/journal.pone.0045417

[31] Kramer JM. Basement membranes. WormBook 2005; 1-15; PMID:18050423; http://dx.doi.org/10.1895/wormbook.1.16.1

[32] Ewald CY, Landis JN, Abate JP, Murphy CT, Blackwell TK. Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity. Nature 2014; PMID:25517099; http://dx.doi.org/10.1038/nature14021

[33] Aballay A, Ausubel FM. Characterization of mediators of microbial virulence and innate immunity using the Caenorhabditis eugens host-pathogen model. Cell Microbiol 2003; 5:435-44. http://dx.doi.org/10.1046/j.1462-5822.2003.00287.x

[34] Aballay A, Tan MW. Resistance to antimicrobial peptides contributes to persistence of Salmonella typhimurium in the C. eugens intestine. Cell Microbiol 2008; 10:1259-73; Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18221392; PMID:18221392

[35] Alegado RA, Campbell MC, Chen WC, Slutz SS, Tan MW. Characterization of mediators of microbial virulence and innate immunity using the Caenorhabditis eugens host-pathogen model. Cell Microbiol 2003; 5:435-44. http://dx.doi.org/10.1046/j.1462-5822.2003.00287.x
Acad Sci U S A 2013; 110:15067-72; PMID:23980181; http://dx.doi.org/10.1073/pnas.1311725110

[45] Kerry S, TeKippe M, Gaddis NC, Aballay A. GATA transcription factor required for immunity to bacterial and fungal pathogens. PLoS One 2006; 1:e77. PMID:17183709; http://dx.doi.org/10.1371/journal.pone.0000077

[46] McGhee JD, Fukushige T, Krause MW, Minnema SE, Goszczynski B, Gaut J, Kohara Y, Bossinger O, Zhao Y, Khattri J, et al. ELT-2 is the predominant transcription factor controlling differentiation and function of the C. elegans intestine, from embryo to adult. Dev Biol 2009; 327:551-65. PMID:19111532; http://dx.doi.org/10.1016/j.ydbio.2008.11.034

[47] Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, Inoue H, Tanaka-Hino M, Hisamori N, Matsumoto K, Tan MW, et al. A conserved p38 MAP kinase pathway in Caenorhabditis elegans innate immunity. Science (80-) 2002; 297:623-6. Available from: http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=12142542; PMID:12142542

[48] Powell JR, Kim DH, Ausubel FM. The G protein-coupled receptor FSHR-1 is required for the Caenorhabditis elegans innate immune response. Proc Natl Acad Sci U S A 2009; 106:2782-7. PMID:19196974; http://dx.doi.org/10.1073/pnas.0813048106

[49] Tan MW, Rahme LG, Sternberg JA, Tompkins RG, Ausubel FM. Pseudomonas aeruginosa killing of Caenorhabditis elegans used to identify P. aeruginosa virulence factors. Proc Natl Acad Sci U S A 1999; 96:2408-13. PMID:10051655; http://dx.doi.org/10.1073/pnas.96.5.2408

[50] Dunbar TL, Yan Z, Balla KM, Smelkinson MG, Troemel ER. C. elegans detects pathogen-induced translational inhibition to activate immune signaling. Cell Host Microbe 2012; 11:375-86. PMID:22520465; http://dx.doi.org/10.1016/j.chom.2012.02.008

[51] McEwan DL, Kirienko N V, Ausubel FM. Host translational inhibition by Pseudomonas aeruginosa exotoxin A triggers an immune response in Caenorhabditis elegans. Cell Host Microbe 2012; 11:364-74; PMID:22520464; http://dx.doi.org/10.1016/j.chom.2012.02.007

[52] Kirienko NV, Kirienko DR, Larkins-Ford J, Wahlby C, Ruvkun G, Ausubel FM, Wahlby C, Ruvkun G, Ausubel FM. Pseudomonas aeruginosa disrupts Caenorhabditis elegans iron homeostasis, causing a hypoxic response and death. Cell Host Microbe 2013; 13:406-16. PMID:23601103; http://dx.doi.org/10.1016/j.chom.2013.03.003

[53] Kirienko N V, Ausubel FM, Ruvkun G. Mitophagy confers resistance to siderophore-mediated killing by Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 2015; 112:1821-6. PMID:25624506; http://dx.doi.org/10.1073/pnas.142495412

[54] Block DHS, Twumasi-Boateng K, Kang HS, Carlisle JA, Hangau A, Lai TY-J, Shapira M. The Developmental Intestinal Regulator ELT-2 Controls p38-Dependent Immune Responses in Adult C. elegans. PLoS Genet 2015; 11:e1005265; PMID:26016853; http://dx.doi.org/10.1371/journal.pgen.1005265

[55] Benjamini Y, Hochberg Y. Controlling the False Discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995; 57:289-300. Available from: http://www.jstor.org/stable/2346101

[56] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4:44-57. PMID:19131956; http://dx.doi.org/10.1038/nprot.2008.211