BMP signaling to pharyngeal muscle in the *C. elegans* immune response to bacterial pathogen regulates anti-microbial peptide expression and pharyngeal pumping

Emma Jo Ciccarelli [1,2], Moshe Bendelstein [1], Cathy Savage-Dunn [1,2 *]

[1] Department of Biology, Queens College, CUNY, Flushing NY

[2] PhD Program in Biology, The Graduate Center, CUNY, New York NY

* Corresponding author

E-mail: cathy.savagedunn@qc.cuny.edu

Short title: Tissue specificity in *C. elegans* host-pathogen response

Abstract

Host response to pathogens recruits multiple tissues in part through conserved cell signaling pathways. In *C. elegans*, the bone morphogenetic protein (BMP) like DBL-1 signaling pathway has a role in the response to infection in addition to other roles in development and post-developmental functions. In the regulation of body size and fat storage, the DBL-1 pathway acts through cell autonomous and non-autonomous signaling in the epidermis (hypodermis). We have now elucidated the tissues that respond to DBL-1 signaling upon exposure to bacterial pathogens. The receptors and Smad signal transducers for DBL-1 are expressed in pharyngeal muscle, intestine, and epidermis. We demonstrate that expression of receptor-regulated Smad (R-Smad) gene *sma-3* in the pharynx is sufficient to improve the impaired survival phenotype of *sma-3* mutants and that expression of *sma-3* in the intestine has no effect when exposing worms to bacterial infection of the intestine. We also show that two antimicrobial peptide (AMP) genes – *abf-2* and *cnc-2* – are regulated by DBL-1 signaling through R-Smad SMA-3 activity in the pharynx. Finally, we show that pharyngeal pumping activity is reduced in *sma-3* mutants and that other pharynx-defective mutants also have reduced survival on bacterial pathogens. Our results identify the pharynx as a tissue that responds to BMP signaling to coordinate a systemic response to bacterial pathogens.

Author Summary

Animals exposed to infection mount a defense through immune activation. Innate immune responses are regulated by conserved cell signaling pathways. One conserved signaling pathway involved in the *C. elegans* immune response is the BMP-like DBL-1 pathway. Here we demonstrate that cell non-autonomous signaling through DBL-1 mediator SMA-3 plays a significant role in the response to bacterial intestinal infection. We also identify two antimicrobial peptides regulated by this signaling mechanism in response to bacterial infection. Our work provides insight into the way in which the BMP-like signaling triggers a systemic response to regulate immunity.

Introduction

Animals encounter a diverse range of microorganisms in their environments, many of which can pose a risk for infection. This exposure has resulted in the development of immune networks to protect the host from pathogenic threats. Innate immunity provides an immediate response to infection and functions through highly conserved mechanisms. In humans, innate immunity involves physical barriers such as the skin, recruitment of phagocytic cells, and the release of antimicrobial peptides. Expression of these antimicrobial peptides is regulated by a number of signaling pathways and these mechanisms are conserved across metazoan species [1-9].

The soil-dwelling nematode *Caenorhabditis elegans* naturally encounters many pathogens, thus necessitating a functioning immune system to provide adequate protection. *C. elegans* lacks an antibody-based acquired immunity, so response is limited to innate immunity including the release of antimicrobial peptides, however the signaling pathways responsible for their regulation

are highly conserved, even in mammals. For this reason, *C. elegans* has served as a useful organism in which to study many of the signaling pathways that are involved in mammalian innate immunity and allows for the study of how they regulate an antimicrobial response [3-9].

One pathway involved in the *C. elegans* immune response is the BMP-like DBL-1 signaling pathway [10]. BMPs are members of the large conserved Transforming Growth Factor beta (TGF-β) family of secreted peptide growth factors [11]. Members of the TGF-β family play critical roles in adaptive and innate immunity [12]. In *C. elegans*, the DBL-1 pathway is initiated through the neuronally expressed ligand DBL-1 [13 and 14]. The receptors DAF-4 and SMA-6 along with the signaling mediators SMA-2, SMA-3, and SMA-4 are endogenously expressed in the epidermis (also known as hypodermis in *C. elegans*), the intestine, and the pharynx [10, 14-19]. DBL-1 signaling plays a significant role in body size regulation, male tail development, mesodermal patterning, and lipid accumulation [14-26] in addition to its role in the *C. elegans* immune response [10]. This pathway has been well studied in the *C. elegans* response to the Gram-negative bacterium *Serratia marcescens* [10]. Our interest in this study was to determine the way in which DBL-1 signaling plays a broader role in the immune response against bacterial pathogens and the mechanisms through which this specific function is carried out.

Here we show that DBL-1 signaling through the receptor-regulated R-Smad SMA-3 is required for protection against bacterial pathogen in *C. elegans*. We demonstrate that expression of *sma-3* in pharyngeal muscle is sufficient to restore survival of *sma-3* deficient animals. DBL-1 signaling regulates expression of antimicrobial peptides ABF-2 (antibacterial factor related) [27] and CNC-2 (Caenacin) [28] and this regulation is through R-Smad SMA-3. We further show that pharyngeal expression of SMA-3 regulates ABF-2 and CNC-2 expression following pathogen

exposure. Finally, we demonstrate that *sma-3* mutants suffer a reduced pharyngeal pumping rate that could increase sensitivity to pathogens and that expression of *sma-3* in pharyngeal muscle restores the pumping rate to control levels.

Results

DBL-1 Pathway R-Smad SMA-3 is required for survival against bacterial pathogen

Canonical TGF-β signaling employs a Co-Smad that forms a heterotrimer with R-Smads [11]. In response to fungal infection with *D. coniospora*, DBL-1/BMP signaling provides host protection through a non-canonical signaling cascade that does not include a Co-Smad [28]. DBL-1 signaling activity is also required for *C. elegans* survival against infection by the Gram-negative bacterium *S. marcescens* [10], which signaling components are required for this response was not determined. We therefore analyzed the survival phenotype of R-Smad mutant *sma-3* on *S. marcescens*. Animals were transferred from *E. coli* food to pathogenic bacteria at the fourth larval (L4) stage and survival on pathogen was monitored through adulthood until death. When we compare the survival of *sma-3* mutant animals, we see that they display a significantly reduced survival compared to control animals when exposed to *S. marcescens* infection, thus indicating that SMA-3 is involved in the response to *S. marcescens* infection (Fig 1B). We similarly observed that *sma-3* mutants have reduced survival when infected with the Gram-negative bacterium *P. luminescens* (Fig 1C), a pathogenic bacterium that is more virulent than *S. marcescens* against *C. elegans*. We conclude that SMA-3 plays a significant role in *C. elegans* survival against different bacterial pathogens.

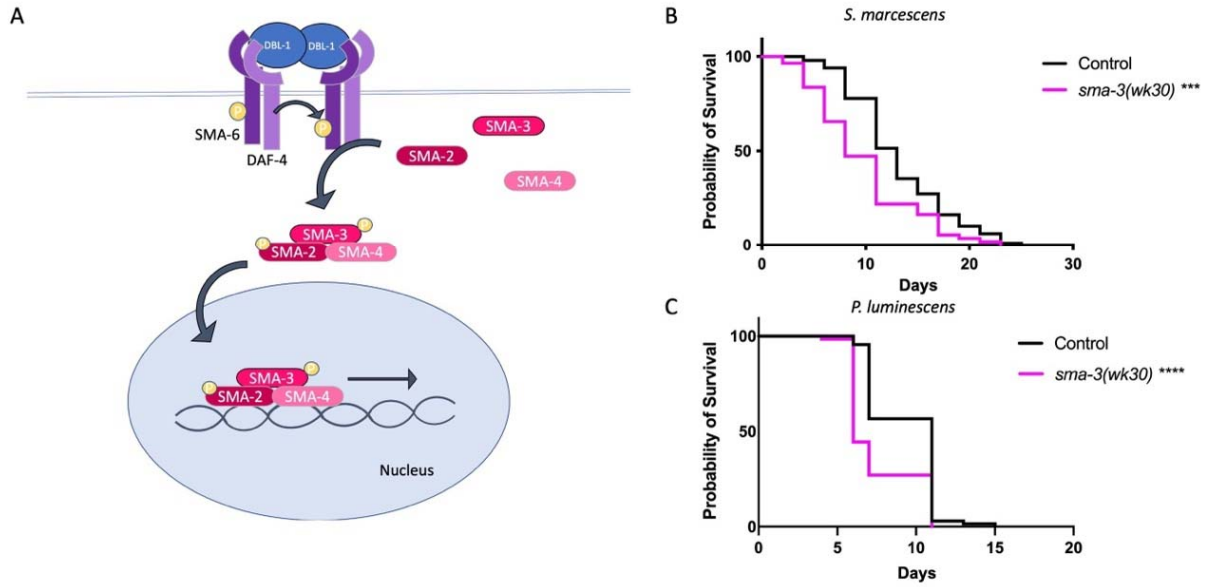

Figure 1. DBL-1 Pathway R-Smad SMA-3 is required for survival on bacterial pathogen. **A.** BMP signaling in *C. elegans* through the DBL-1 pathway. **B.** Survival of *sma-3(wk30)* on *S. marcescens*. n values: Control (99), *sma-3* (55). **C.** Survival of *sma-3(wk30)* on *P. luminescens*. n values: Control (67), *sma-3* (63). Survival analyses were repeated. Statistical analysis done using Log-rank (Mantel-Cox) Test. *** $P \leq 0.001$; **** $P < 0.0001$.

Expression of *sma-3(+)* in pharyngeal muscle rescues survival of *sma-3* mutants

As previously noted, DBL-1 pathway Smads – SMA-2, SMA-3, and SMA-4 – are endogenously expressed in the epidermis, the intestine, and the pharynx [10 and 14-19]. *sma-3* mutants have a small body size phenotype compared to control animals [14, 16-26]. Previous work has shown that expression of *sma-3* in the epidermis alone is sufficient to rescue the body size phenotype in a *sma-3* mutant background [17]. Additionally, DBL-1 signaling is cell non-autonomous in its effect on regulation of intestinal lipid stores, as expression of *sma-3* in the epidermis is sufficient to rescue the low-fat phenotype of DBL-1 pathway mutants [26]. We now sought to address in which tissue(s) SMA-3 activity is required to provide protection against bacterial infection. We used transgenic *C. elegans* strains in a *sma-3* mutant background to conduct these experiments. These strains have *sma-3* expressed under the control of tissue-specific promoters (epidermal expression using *vha-7p*; intestinal expression using *vha-6p*; pharyngeal muscle expression using *myo-2p*; control using endogenous *sma-3p* promoter), allowing for expression of *sma-3* in a tissue-specific manner. Upon exposure to *S. marcescens* we found that expression of *sma-3* in either epidermal or pharyngeal tissue resulted in rescue of the *sma-3* mutant reduced survival phenotype. Pharynx-specific expression of *sma-3* provided the most significant rescue of the *sma-3* phenotype (Fig 2A). We repeated this experiment using *P. luminescens* and similarly found that pharyngeal expression of *sma-3* alone was sufficient to rescue the *sma-3* mutant survival phenotype (Fig 2B). Interestingly, on this pathogen, intestinal expression of *sma-3* resulted in a worse survival outcome compared to *sma-3* mutants. From these results we conclude that the pharynx plays a significant role for DBL-1 pathway activity in response to bacterial infection in *C. elegans*.

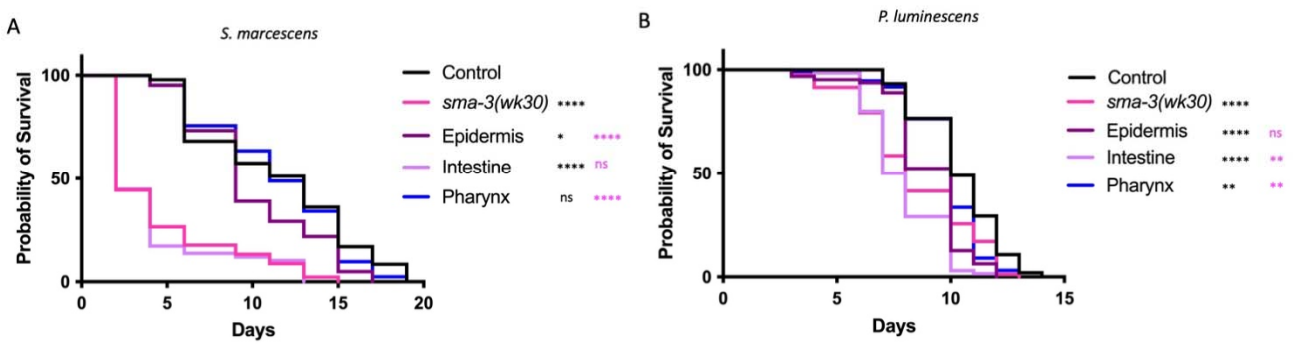

Figure 2. Expression of *sma-3(+)* in pharyngeal muscle rescues survival of *sma-3* mutants. **A.** Survival analysis of tissue-specific *sma-3* expressing strains on *S. marcescens*. Tissue-specific strains have *sma-3* expressed under regulation of tissue-specific promotors in a *sma-3(wk30)* mutant background. n values: Control (47), *sma-3* (45) Epidermis (41), Intestine (58), Pharynx (41). **B.** Survival of tissue-specific *sma-3* expressing strains on *P. luminescens*. n values: Control (102), *sma-3* (82) Epidermis (63), Intestine (134), Pharynx (131). Survival analyses were repeated. Statistical analysis done using Log-rank (Mantel-Cox) Test. ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P < 0.0001$. Black asterisks show significance relative to control; magenta shows significance relative to *sma-3* mutant.

DBL-1 regulates expression of AMPs *abf-2* and *cnc-2* through SMA-3

We next tested whether the protective nature of DBL-1 signaling against bacterial infection is due to induction of antimicrobial defense genes. We tested a panel of 12 immune response genes via qRT-PCR to determine whether DBL-1 signaling regulates their expression when animals are exposed to *P. luminescens* (Supplemental Figure S1). L4 animals were transferred from control plates to pathogen and collected as Day 1 adults following 24-hour exposure to pathogen. From this analysis we found two genes that demonstrated the expression pattern we expected if their regulation was mediated through DBL-1. Both *abf-2* and *cnc-2* were significantly upregulated in control animals upon 24-hour exposure to bacterial pathogen. Neither of these genes had a significant increase in expression in *dbl-1* mutant animals under the same infection conditions (Fig 3A).

ABF-2 is an antibacterial factor related protein that has been shown to be expressed in the pharynx of *C. elegans* and is involved in the response against bacterial infection [26]. *cnc-2* encodes a Caenacin protein previously shown to be involved in the *C. elegans* immune response to fungal infection, where it is regulated in epidermal tissue [28]. Our results suggest that both *abf-2* and *cnc-2* are regulated by DBL-1 signaling during the response to bacterial pathogen.
We then used qRT-PCR to test whether *sma-3* mutants demonstrate any change in *abf-2* levels when exposed to bacterial pathogen. Following 24-hour exposure to *P. luminescens* bacteria, *sma-3* mutants demonstrated no increase in *abf-2* levels as compared to control conditions. Additionally, *sma-3* mutants expressed overall lower levels of *abf-2* than control animals even in control conditions (Fig 3B). From these data we conclude that DBL-1 signaling does regulate

expression of known immune response genes and that loss of the signaling effector SMA-3 prevents induction of *abf-2* expression. This observation indicates that SMA-3 plays a role in the regulation of antimicrobial peptide ABF-2. When we use qRT-PCR to assess the expression pattern of *cnc-2* in *sma-3* mutants, we find a similar pattern for *cnc-2* expression. Control animals have a significant increase in *cnc-2* expression under infection conditions while *sma-3* mutants demonstrate no significant change in *cnc-2* expression when exposed to bacterial pathogen (Fig 3B).

As previously noted, earlier work showed that *cnc-2* is involved in the *C. elegans* response to fungal infection [28]. We therefore followed up these experiments with a survival analysis of a *cnc-2* mutant on *P. luminescens* to determine whether *cnc-2* plays a role in bacterial infection as well as fungal infection. Our results demonstrate that *cnc-2* loss of function mutant animals have a significantly reduced survival rate when exposed to *P. luminescens* as compared to control animals (Fig 3C). We therefore conclude that CNC-2 plays a role in bacterial infection, in addition to its known role against fungal pathogen [28].

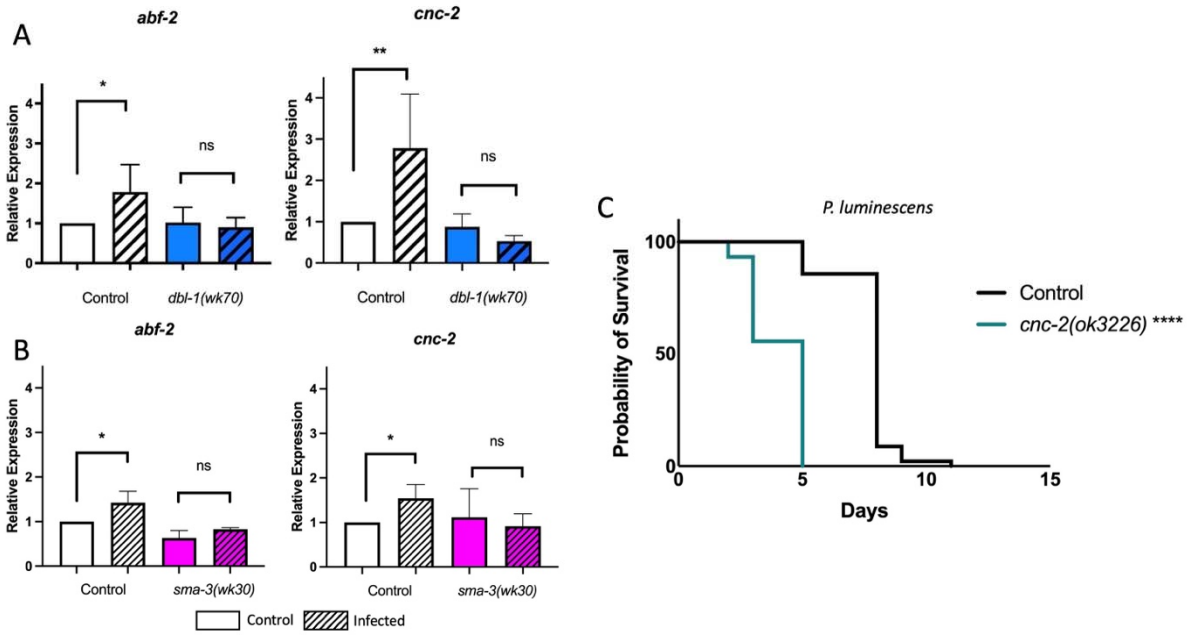

Figure 3. DBL-1 regulates expression of AMPs *abf-2* and *cnc-2* through SMA-3.

A. qRT-PCR results for *abf-2* and *cnc-2* expression in *dbl-1(wk70)* animals. **B.** qRT-PCR results for *abf-2* and *cnc-2* in *sma-3(wk30)* animals. **C.** Survival analysis of *cnc-2(ok3226)* animals on *P. luminescens* pathogenic bacteria. n values: Control (91), *cnc-2* (74). Survival analysis was repeated. Data for qRT-PCR represents repeated analyses from two biological replicates. Statistical analysis for qRT-PCR was done using One-way ANOVA with multiple comparison test. Error bars represent standard deviation. Statistical analysis for survival was done using Log-rank (Mantel-Cox) Test. ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; **** $P < 0.0001$.

DBL-1 pathway mediated regulation of AMP expression requires pharyngeal SMA-3 activity

Bacterial infection such as that caused by *S. marcescens* or *P. luminescens* results in colonization and infection in the intestine of the worm [10, 29-32]. As our survival analyses demonstrated that intestine-specific *sma-3* expression was not sufficient to ameliorate the *sma-3* reduced survival phenotype, we next tested whether DBL-1 mediated regulation of antimicrobial factors is cell autonomous or cell non-autonomous. We performed qRT-PCR analysis using the tissue-specific *sma-3* expressing strains to establish the expression patterns for *abf-2* and *cnc-2* after a 24-hour exposure to *P. luminescens* bacteria. Consistent with our survival analysis results, which indicated significance for pharyngeal *sma-3* expression in *C. elegans* survival, *sma-3* expression in the pharynx alone was sufficient to cause an induction in *abf-2* and *cnc-2* transcript levels following *P. luminescens* exposure (Fig 4AB). Interestingly, *sma-3* expressed in the epidermis also resulted in significant *abf-2* and *cnc-2* induction, although less significantly for *abf-2* than the increase seen in the pharynx expressing strain.

We were surprised that *sma-3* expression in the epidermis would rescue induction of *abf-2*, which is reported to be expressed in the pharynx [27]. We decided to re-examine GFP::SMA-3 expression in our transgenic strain. Upon analysis, we noted that our epidermal expressing strain demonstrated some fluorescence in the pharynx in addition to the anticipated epidermal GFP signal (Supplemental Figure S2). Therefore, low levels of pharyngeal SMA-3 may contribute to the phenotypes seen in this strain. However, the pharyngeal *sma-3* expressing strain showed GFP fluorescence localized to the pharynx alone, as expected. Therefore, we conclude from these

results that *sma-3* signaling in the pharynx has both cell autonomous and cell non-autonomous effects on AMP expression of the pharyngeal *abf-2* and the epidermal *cnc-2*.

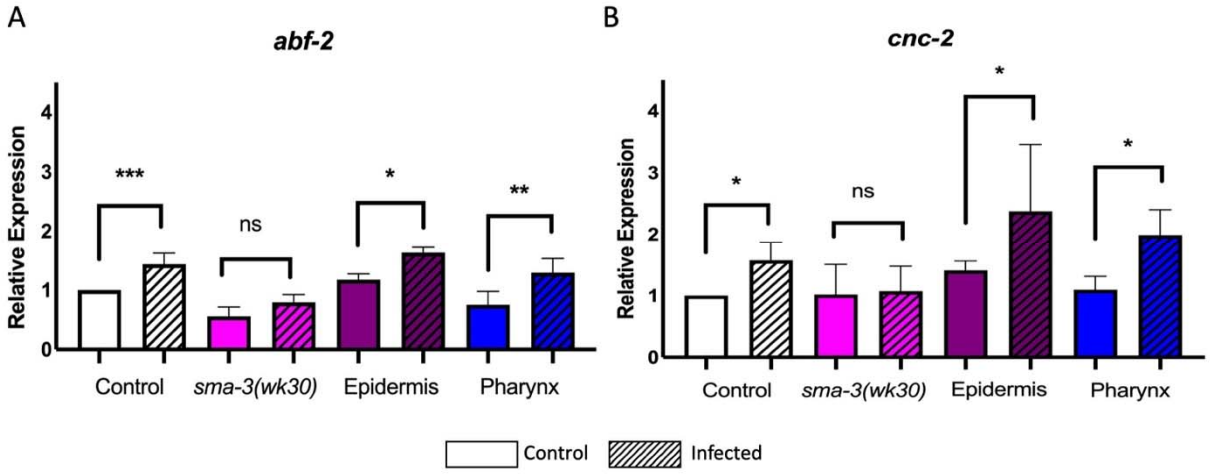

Figure 4. SMA-3 mediated regulation of AMP expression is cell non-autonomous.

A. qRT-PCR results for *abf-2* in transgenic *sma-3* expressing animals. **B.** qRT-PCR results for *cnc-2* in transgenic *sma-3* expressing animals. Data for qRT-PCR represents repeated analyses from two biological replicates. Statistical analysis for qRT-PCR was done using One-way ANOVA with multiple comparison test. Error bars represent standard deviation. ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$.

Expression of *sma-3* in pharyngeal muscle restores pumping rate in *sma-3* mutants

We next investigated whether *sma-3* expression in the pharynx resulted in any effect on the mechanical function of the pharynx, by assaying the pharyngeal pumping rate. To match the conditions of our survival and gene expression studies, we transferred L4 animals from control plates to plates seeded with pathogenic *P. luminescens* bacteria and measured pumping rates in Day 1 adults after 24 hours of exposure to pathogen. We found that *sma-3* mutants had a reduction in pumping rate as compared to control animals on pathogen. The pumping rate of *sma-3* mutants was restored when *sma-3* was expressed solely in the pharynx (Fig 5A), indicating potentially cell autonomous regulation of pumping rate by SMA-3.

To assess whether functionality of the pharynx contributes to survivability on pathogenic bacteria, we determined the survival patterns and pumping rates of two pharyngeal defect mutants. We used *eat-2* and *phm-2*, both involved in pharyngeal grinder function, for these experiments. Mutation of *eat-2* [33-36] or of *phm-2* [33, 37-38] results in reduced grinder function and a reduced rate of pharyngeal pumping. Following exposure of either strain to *P. luminescens* bacteria for 24 hours, there was a significant decrease in pharyngeal pumping rate (Fig 5B), consistent with our expectations. Furthermore, both *phm-2* and *eat-2* mutants have a significantly reduced rate of survival compared to control animals when exposed to *P. luminescens* as well (Fig 6). Other studies have also demonstrated that both mutations result in sensitivity to bacterial infection, due to live bacteria reaching the intestine for colonization [37-41]. Taking all of our results together with these prior studies suggests that disrupting pharyngeal function contributes to susceptibility to infection.

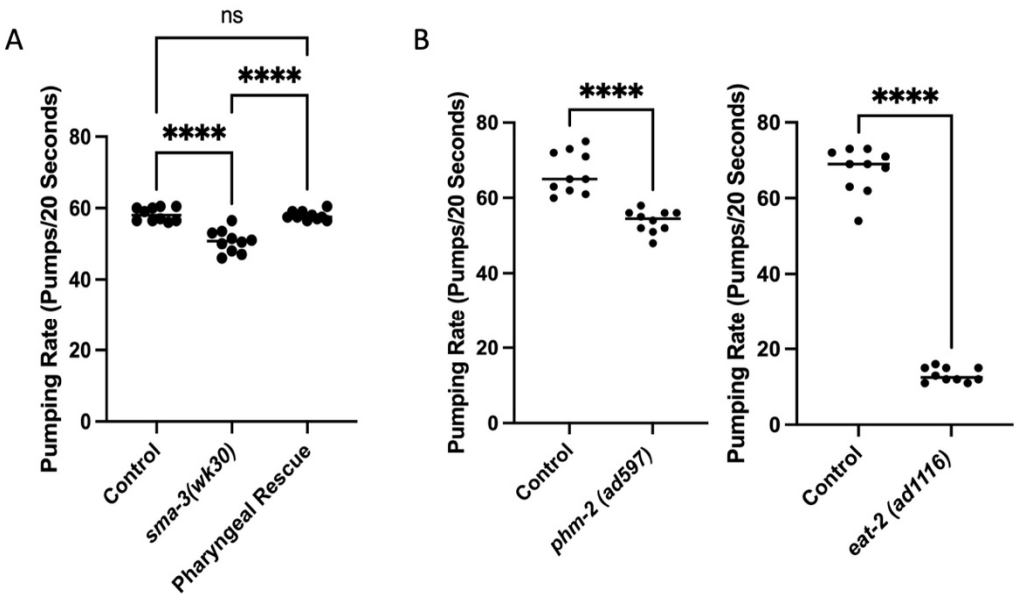

Figure 5. Expression of *sma-3* in pharyngeal muscle restores pumping rate from reduction in *sma-3* mutants.

A. Pumping rate in Pumps/20 Seconds of *sma-3(wk30)* mutants and pharyngeal *sma-3* rescue animals on *P. luminescens* bacteria. **B.** Pharyngeal pumping rate in Pumps/20 Seconds for pharyngeal defect mutants *phm-2(ad597)* and *eat-2(ad1116)* on *P. luminescens* bacteria. 10 worms per strain were counted and experiments were repeated on two separate biological replicates. Statistical analysis was performed using One way ANOVA with multiple comparison test. ns $P > 0.05$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

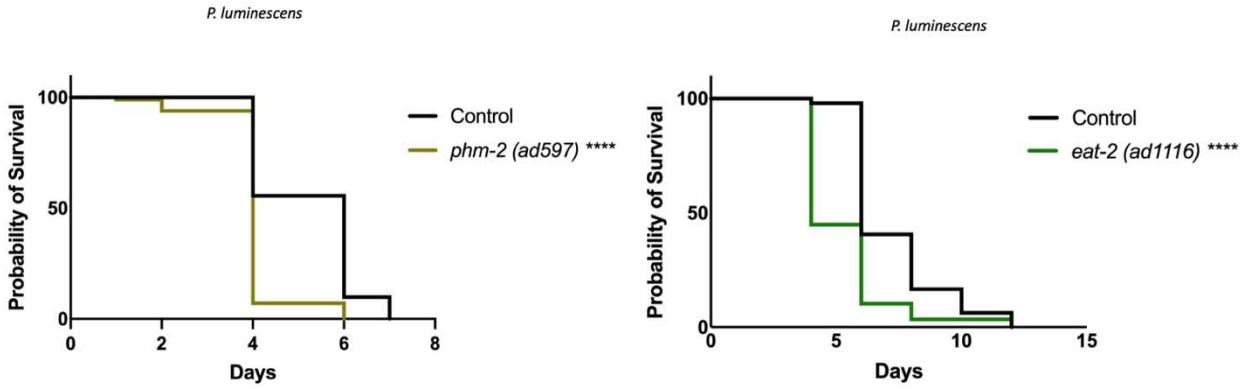

Figure 6. Mutants with pharyngeal defects have reduced survival on *P. luminescens* bacteria.

Survival analysis of *phm-2(ad597)* and *eat-2(ad1116)* on *P. luminescens* bacteria. n values: Control (81), *phm-2* (99), Control (96), *eat-2* (58). Statistical analysis was done using Log-rank (Mantel-Cox) Test. **** $P \leq 0.0001$.

Discussion

The BMP-like ligand DBL-1 has previously been shown to play a significant role in the *C. elegans* immune response to bacterial pathogen [10]. Additionally, it has been demonstrated that the DBL-1 pathway effector, R-Smad SMA-3 is involved in a non-canonical signaling response to fungal infection, in which it acts without SMA-2 or the Co-Smad SMA-4 [28]. A strength of the *C. elegans* system is the ability to ask questions about site of action in the context of the intact organism, shedding light on how systemic organismal responses may be generated. We had previously shown that *sma-3* expressed solely in the epidermis is sufficient to restore normal body size and lipid stores in adult animals [17 and 26]. In this study, we employed survival analyses to determine where SMA-3 acts in the *C. elegans* immune response to bacterial pathogens. We demonstrate that *sma-3* expression in the intestinal site of infection does not have any measurable effect on survival against bacterial pathogen. However, expression of *sma-3*, and therefore pathway activity, in the pharynx, does restore survival towards that of control animals. Improved survival of pharynx-specific expressing strains as compared to *sma-3* mutants suggests the cell non-autonomous activity of the DBL-1 pathway on immunity.

We also determined whether the effect of DBL-1 signaling on the immune response to bacterial pathogen is related to regulation of immune response related genes. Here we demonstrate through qRT-PCR analysis that DBL-1 signaling regulates the expression of *abf-2* and *cnc-2* transcripts upon exposure to pathogenic *P. luminescens*. We also show that this effect is regulated through R-Smad SMA-3 expression in the pharynx. This result supports the hypothesis that DBL-1 signaling to regulate AMP expression is both cell autonomous and cell non-autonomous. We note that expression levels in some of the *sma-3* transgenic strains are higher

than those seen in N2 control animals, which could be due to a higher level of expression of SMA-3 from the multi copy transgenes.

The antibacterial factor related peptide ABF-2 has previously been shown to play a role in the response to bacterial pathogen [27]. *abf-2* has a complex relationship with *abf-1:* the two genes are capable of being transcribed as a polycistronic operon transcript, but also as individual genes with different expression patterns. It will be interesting to determine whether SMA-3 acts at the level of transcription or post-transcriptionally to mediate regulation of these genes. In contrast, CNC-2 has been primarily correlated with fungal infection of the epidermis and has not previously been associated with induction in response to *P. luminescens* infection [28, 42]. Previous investigations utilizing microarray and RNA-seq have only found decreases in *cnc-2* upon *P. luminescens* infection, although this difference may relate to the different methodologies used [43, 44]. In light of this, we also looked to see how *cnc-2* mutants survive on *P. luminescens* and found that *cnc-2* mutant animals had a significantly reduced survival compared to control animals. This increased susceptibility is consistent with our findings by qRT-PCR and the results demonstrating that DBL-1 signaling regulates *cnc-2* expression levels in response to bacterial pathogen exposure as part of the *C. elegans* immune response to *P. luminescens* infection.

Because the focus of action of SMA-3 is the pharynx, we considered whether DBL-1 signaling activity in the pharynx influences the physical function of the pharynx. We used pharyngeal pumping rate as a method for measuring the effect of *sma-3* on pharyngeal mechanical action. We found that *sma-3* mutant animals have a reduced pharyngeal pumping rate as compared to control animals and that this reduction can be rescued with expression of *sma-3* in pharyngeal

muscle. Similarly, pharyngeal function mutants *phm-2* and *eat-2* have reduced survival on *P. luminescens.* We infer that reduced pharyngeal pumping may lead to diminished physical disruption of bacteria, so that increased levels of live bacteria enter the intestine causing pathological harm. In summary, we have identified the pharyngeal muscle as a critical responder to BMP signaling to generate a system organismal response to pathogenic bacteria. R-Smad SMA-3 in the pharynx is sufficient to rescue deficits in survival, induction of multiple AMPs, and physical function of the pharynx. Although most work on the *C. elegans* immune response to bacteria has focused on the intestine, the pharynx is an earlier point of contact for exposure to bacteria and may play a more important role than previously recognized.

Materials and Methods

Nematode Strains and Growth Conditions

C. elegans were maintained on *E. coli* (DA837) at 20°C on EZ worm plates containing streptomycin. The N2 strain is used as a control unless otherwise specified. In tissue-specific *sma-3* experiments, the strains used are: CS152 *qcIs6[sma-3::*GFP + *rol-6(su1006)]* , CS628 *sma-3(wk30); qcIs55[vha-7p::*GFP*::sma-3 + rol-6(su1006)]* ; CS640 *sma-3(wk30); qcIs53[myo2p::*GFP*::sma-3 + rol-6(su1006)]* ; CS619 *sma-3(wk30); qcIs59[vha6p::*GFP*::sma-3 + rol-6(su1006)]*; CS635 *sma-3(wk30); qcIs62[rol-6(su1006)]*. Remaining strains used in this study are*: sma-3(wk30), cnc-2(ok3226), sma-6(wk7), sma2(e502), dbl-1(wk70), phm-2(ad597), eat-2(ad1116).*

Bacteria

Control bacteria used in all experiments is *E. coli* strain DA837, cultured at 37°C. *S. marcescens* strain Db11 cultured at 37°C and *P. luminescens* cultured at 30°C were used for pathogenic bacteria exposure. *Serratia* (Db11) is seeded on EZ worm plates containing streptomycin and grown at 37°C. *P. luminescens* is seeded on EZ worm plates with no antibiotic and grown at 30°C.

Survival Analysis

Survival plates were prepared at least one day prior to plating worms. Each plate was seeded with 500 µl of pathogenic bacteria. FuDR (5-Fluoro-2'-deoxyuridine) was used to prevent reproduction, at a concentration of 50 µM per plate. This treatment was done to reduce the

incidence of matricide by internal hatching of embryos during survival analysis. All survival experiments were carried out at 20 °C. All survival analyses were repeated. All graphs were made using GraphPad Prism software and statistical analysis performed using Logrank/Mantel Cox test.

qRT-PCR Analysis

Worms were synchronized using overnight egg lay followed by four-hour synchronization. When animals reached L4 stage, they were washed and moved to experimental plates for 24-hour exposure to *P. luminescens*. Animals were collected and washed after 24-hour exposure and RNA was extracted using previously published protocol [45] followed by Qiagen RNeasy miniprep kit (Cat. No. 74104). cDNA was generated using Invitrogen SuperScript IV VILO Master Mix (Cat. No.11756050). qRT-PCR analysis was done using Applied Biosystems *Power* SYBR Green PCR Master Mix (Cat. No. 4367659). Delta delta Ct analysis was done using Applied Biosystems and StepOne software. All qRT-PCR analysis was repeated on separate biological replicates. All graphs were made using GraphPad Prism software and statistical analysis was performed using One-way ANOVA with Multiple Comparison Test.

Pumping Rate Experiments

Pumping rate experiments were done with animals exposed at L4 stage for 24 hours on *P. luminescens* bacteria. Pharyngeal pumps counted over 20 second timespan. All pumping experiments were repeated. Graph made using GraphPad Prism software and statistical analysis performed using One-way ANOVA with Multiple Comparisons Test.

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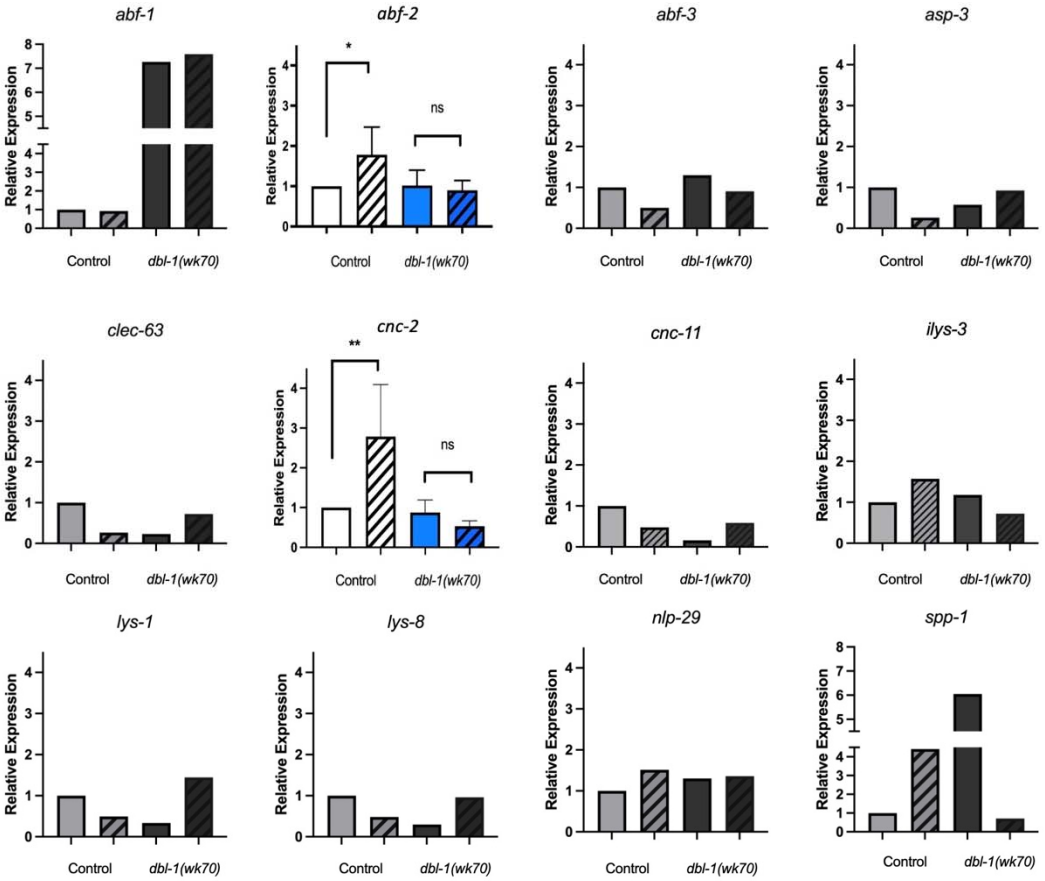
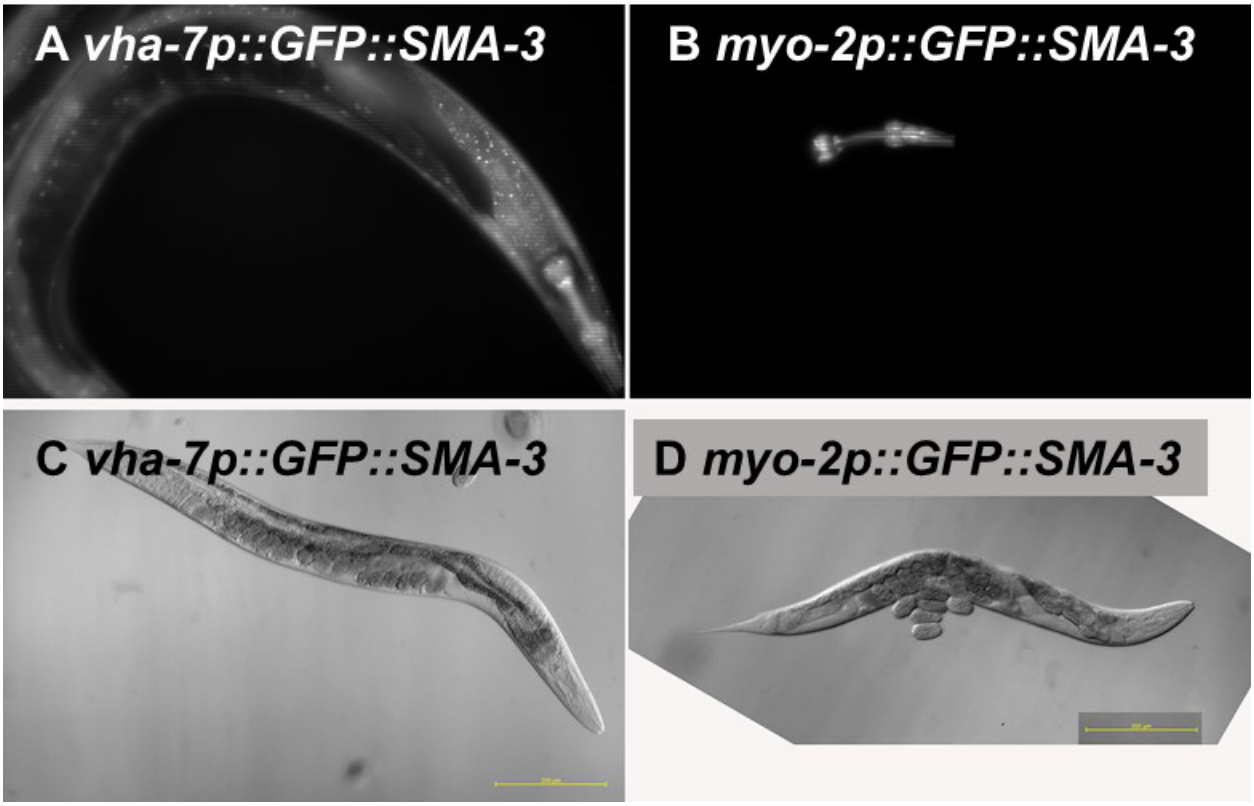
Supporting Information



S1Fig. qRT-PCR analysis panel of 12 immune response genes in *dbl-1(wk70)* mutants. qRT-PCR analysis of 12 immune response genes in control and *dbl-1(wk70)* mutant animals, comparing 24-hour bacterial pathogen exposure (diagonally hatched bars) to control conditions. *P. luminescens* used as bacterial pathogen. Data for *abf-2* and *cnc-2* represents repeated analyses for two biological replicates. Remaining genes were analyzed for one biological sample. Graph made using GraphPad Prism. Statistical analysis for *abf-2* and *cnc-2* done using One-way ANOVA with multiple comparison test. ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$.



S2Fig. Representative images of *sma-3* transgenic strains.

A. Fluorescence image of *vha7p::GFP::SMA-3* at 20X magnification demonstrating GFP::SMA-3 expression in epidermis and pharynx. **B.** Fluorescence image of *myo2p::GFP::SMA-3* at 20X magnification demonstrating specific GFP::SMA-3 expression in pharyngeal muscle. **C.** Nomarski image of *vha7p::GFP::SMA-3* demonstrates rescue of body size. Scale bar shows 200 µm. **D.** Nomarski image of *myo-2p::GFP::SMA-3* demonstrates that body size is not rescued by pharyngeal expression of SMA-3. Scale bar shows 200 µm.