THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 40, pp. 33191–33197, September 28, 2012

Endoplasmic Reticulum Stress Pathway Required for Immune Homeostasis Is Neurally Controlled by Arrestin-1[S]

Received for publication, July 5, 2012, and in revised form, July 30, 2012 Published, JBC Papers in Press, August 8, 2012, DOI 10.1074/jbc.M112.398362

Varsha Singh and Alejandro Aballay[1]

From the Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710

Background: GPCRs function in the *C. elegans* nervous system to control immunity.
Results: Arrestin-1, the only GPCR adaptor in *C. elegans*, functions in the nervous system to control immunity.
Conclusion: Neural arrestin-1 signal regulates immunity.
Significance: Our data underscore the importance of the nervous system in the control of longevity and immune homeostasis and suggest that overlapping and distinct neural circuits control these processes.

In response to pathogen infection, the host innate immune system activates microbial killing pathways and cellular stress pathways that need to be balanced because insufficient or excessive immune responses have deleterious consequences. Recent studies demonstrate that two G protein-coupled receptors (GPCRs) in the nervous system of *Caenorhabditis elegans* control immune homeostasis. To investigate further how GPCR signaling controls immune homeostasis at the organismal level, we studied arrestin-1 (ARR-1), which is the only GPCR adaptor protein in *C. elegans*. The results indicate that ARR-1 is required for GPCR signaling in ASH, ASI, AQR, PQR, and URX neurons, which control the unfolded protein response and a p38 mitogen-activated protein kinase signaling pathway required for innate immunity. ARR-1 activity also controlled immunity through ADF chemosensory and AFD thermosensory neurons that regulate longevity. Furthermore, we found that although ARR-1 played a key role in the control of immunity by AFD thermosensory neurons, it did not control longevity through these cells. However, ARR-1 partially controlled longevity through ADF neurons.

Increasing evidence indicates that activation of the innate immune system accounts for the major physiological, metabolic, and pathological responses to infections (1). The response to microbial infection is accompanied by increased demand for protein folding in the endoplasmic reticulum (ER),[2] which must be alleviated by UPR pathways (2–8). The nervous system, which can respond in milliseconds to different environmental stimuli, has several characteristics that make it an ideal partner with the innate immune system to regulate nonspecific host defenses (9–11).

To provide insights into the neural mechanisms that regulate innate immunity, we have taken advantage of the simple immune system and the very well characterized nervous system of *Caenorhabditis elegans*. A unique advantage to the study of neural mechanisms involved in the control of immune responses in *C. elegans* is the unparalleled characterization of its nervous system at the cellular level. Each of the 302 neurons of an adult animal has a precise identity, and, most uniquely, *C. elegans* is the only animal for which the wiring diagram of the nervous system has been established based on anatomical reconstructions. NPR-1, a GPCR similar to mammalian neuropeptide Y receptors, participates in a neural circuit that controls the p38/PMK-1 MAPK pathway required for innate immunity (12). In addition, OCTR-1, which is a catecholamine GPCR for octopamine, controls not only the p38/PMK-1 MAPK pathway but also a canonical and a noncanonical UPR pathway that may play a key role to alleviate ER stress during immune responses against microbial pathogens (7, 8).

The discovery that individual GPCRs function in the nervous system of *C. elegans* to control the immune system (7, 8, 12) offers a unique opportunity to analyze the role of the entire nervous system in immune homeostasis. To study the role of neural GPCR signaling in the regulation of UPR genes and its role in immune defense against bacterial infections, we took advantage of a unique component of GPCR signaling, the GPCR adaptor protein arrestin-1 (ARR-1). Arrestins block G protein-mediated signaling and can also function as signal transducers in their own right (13). The only member of the arrestin family in *C. elegans*, ARR-1, is expressed almost exclusively within the *C. elegans* nervous system (14), making it easy to manipulate neural ARR-1 signaling and to study its effect on innate immune responses against pathogen infection.

Here we show that ARR-1 functions in a number of sensory neurons that regulate UPR genes that are expressed in nonneuronal tissues and required for immune defense. ARR-1 also partially controls longevity through ADF neurons. The results highlight the importance of ARR-1 signaling in a subset of sensory neurons that control longevity and immune homeostasis during response to pathogen infection.

EXPERIMENTAL PROCEDURES

Bacterial Strains—The following bacterial strains were used: *Escherichia coli* OP50 (15), *Pseudomonas aeruginosa* PA14 (16), *Salmonella enterica* serovar *Typhimurium* 1344 (17), *Yersinia*

[S] This article contains supplemental Figs. S1–S4.

[1] To whom correspondence should be addressed: 268 Jones, Box 3054, Duke University Medical Center, Durham, NC 27710. Fax: 919-684-2790; E-mail: a.aballay@duke.edu.

[2] The abbreviations used are: ER, endoplasmic reticulum; ARR-1, arrestin-1; ERP, enhanced resistance to *P. aeruginosa*; GPCR, G protein-coupled receptor; NGM, nematode growth medium; qRT-PCR, quantitative real-time PCR; UPR, unfolded protein response.

pestis KIM5 (18), *P. aeruginosa* expressing GFP (16). *E. coli*, *P. aeruginosa*, and *S. enterica* cultures were grown in Luria-Bertani (LB) broth at 37 °C. *Y. pestis* culture was grown in LB broth at 25 °C.

Nematode Strains—The following *C. elegans* strains were cultured under standard conditions and fed *E. coli* OP50 (15). The Bristol N2 strain was used as wild type. N2, *arr-1 (ok401)*, *daf-16(mu86)*, *npr-1(ad609)*, and *octr-1(ok371)* strains were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis) and outcrossed at least three times. *arr-1(ok401);daf-16(mu86)*, *arr-1(ok401);npr-1(ad609)*, and *octr-1(ok371);arr-1(ok401)* were constructed using standard genetic techniques.

Plasmid Constructs and Generation of Transgenic Lines—A 3.2-kb genomic fragment containing the *arr-1* gene was cloned into the BamHI and SmaI sites of pPD95_77 vector (Fire Lab *C. elegans* Vector kit; Addgene, Cambridge, MA) creating a translational fusion between ARR-1 and GFP. Plasmid *pVS3* was constructed by inserting 1924 bp of the *arr-1* promoter upstream of *arr-1::gfp* in pPD97_77. Plasmid *pVS4* was constructed by inserting 4685 bp of the *unc-119* promoter upstream of *arr-1::gfp* to confer pan-neuronal expression (19). Plasmid *pVS5* was constructed by inserting 3045 bp of the *sra-6* promoter upstream of *arr-1::gfp* to confer expression in ASH and ASI neurons (20). Plasmid *pVS6* was constructed by inserting 1095 bp of the *gcy-32* promoter upstream of *arr-1::gfp* to confer expression in AQR, PQR, and URX neurons (21). Plasmid *pVS7* was constructed by inserting 1472 bp of the *srh-142* promoter upstream of *arr-1::gfp* to confer expression in ADF neurons (20). Plasmid *pVS8* was constructed by inserting 940 bp of the *gcy-8* promoter upstream of *arr-1::gfp* to confer expression in AFD neurons (22). *arr-1(ok401)* animals were microinjected with the plasmids to generate transgenic strains AY103 *arr-1(ok401)*[*pVS3(*p*arr-1::arr-1::gfp):pRF4(rol-6(su1006))*], AY104 *arr-1(ok401)*[*pVS4(*p*unc-119::arr-1::gfp):pRF4(rol-6(su1006))*], AY105 *arr-1(ok401)*[*pVS5(*p*sra-6::arr-1::gfp):pRF4(rol-6(su1006))*], AY106 *arr-1(ok401)*[*pVS6(*p*gcy-32::arr-1::gfp):pRF4(rol-6(su1006))*], AY107 *arr-1(ok401)*[*pVS7(*p*srh-142::arr-1::gfp):pRF4(rol-6(su1006))*], and AY108 *arr-1(ok401)*[*pVS8(*p*gcy-8::arr-1::gfp):pRF4(rol-6(su1006))*]. The plasmids were maintained as extrachromosomal arrays. The expression pattern of neuron-specific promoters was confirmed for all of the transgenic strains.

C. elegans Killing Assay—*C. elegans* wild-type N2 animals and mutants were maintained as hermaphrodites at 20 °C, grown on modified nematode growth medium (NGM) agar plates (0.35% instead of 0.25% peptone), and fed with *E. coli* OP50 as described. The bacterial lawns used for *C. elegans* killing assays were prepared by placing a 15-μl drop of an overnight culture of the bacterial strains on modified NGM agar on plates 3.5 cm in diameter. Full lawn plates used for *C. elegans* killing assays were prepared by spreading a 25-μl drop of an overnight culture grown at 37 °C of *P. aeruginosa* on the complete surface of modified NGM agar in 3.5-cm-diameter Petri plates. Plates were incubated at 37 °C for 12~16 h. Plates were cooled down at room temperature for at least 1 h before seeding with synchronized young adult animals. The killing assays were performed at 25 °C, and live animals were transferred daily to fresh plates. Animals were scored at the times indicated and were considered dead when they failed to respond to touch.

C. elegans Lifespan Assay—*E. coli* OP50 was grown as described above. A 50-μl drop of the bacteria was plated on a 6-cm plate of modified NGM agar containing 40 μg/ml fluorodeoxyuridine from Sigma. The assays were performed at 20 °C.

Profile of Bacterial Accumulation in the Nematode Intestine—To determine the profiles of bacterial accumulation in the intestine, wild-type, *arr-1(ok401)*, and *octr-1(ok371)* animals were synchronized. Synchronized L1 larvae were grown on *E. coli* OP50 at 20 °C until they had reached the young adult stage. Wild-type, *arr-1(ok401)*, and *octr-1(ok371)* animals were then transferred to plates seeded with *P. aeruginosa* expressing GFP (*P. aeruginosa*/GFP) cultured for 24–40 h at 25 °C. Animals were transferred to an NGM plate seeded with *E. coli* for 15 min and transferred again to a new NGM plate seeded with *E. coli* for 30 min to eliminate *P. aeruginosa*/GFP stuck to the body of the nematodes. Animals were visualized and imaged using a Leica MZ FLIII fluorescence stereomicroscope.

Quantification of Intestinal Bacterial Loads—For the quantification of cfu, wild-type, *arr-1(ok401)*, AY103, AY104, AY105, AY106, AY107, and AY108 animals were synchronized by treatment of gravid adults with sodium hydroxide and bleach. Synchronized L1 larvae were grown on *E. coli* OP50 at 20 °C until they had reached the young adult stage. Wild-type, *arr-1(ok401)*, and transgenic animals were then transferred to plates seeded with *P. aeruginosa*/GFP for 24 h at 25 °C. Animals were transferred to an NGM plate seeded with *E. coli* for 15 min to eliminate *P. aeruginosa*/GFP stuck to the body of the worms. Animals were transferred to a new NGM plate seeded with *E. coli* for 30 min to further eliminate external *P. aeruginosa*/GFP. Ten nematodes/condition were transferred into 50 μl of PBS plus 0.1% Triton X-100 and ground. Serial dilutions of the lysates (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were plated onto LB/kanamycin to select for *P. aeruginosa*/GFP cells and grown overnight at 37 °C. Single colonies were counted next day and represented as the number of bacterial cells or cfu per worm.

Confocal Microscopy—AY103 and AY104 worm strains were imaged using the 63× lens of a Leica TCS SL confocal microscope. To construct the whole worm, the individual images were layered and flattened in Photoshop CS5.

RNA Isolation—Gravid adult wild-type, *arr-1(ok401)*, and the transgenic animals were lysed using a solution of sodium hydroxide and bleach (ratio of 5:2) and washed, and the eggs were synchronized for 22 h in S basal liquid medium at room temperature. Synchronized L1 larval animals were placed onto NGM plates seeded with *E. coli* OP50 and grown at 20 °C until the animals had reached the L4 larval stage. Animals were collected and washed with M9 buffer before transferring to NGM plates containing *P. aeruginosa* PA14 for 4 h at 25 °C. After 4 h, animals were collected and washed with M9 buffer, and RNA was extracted using TRIzol reagent (Invitrogen). Residual genomic DNA was removed by DNase treatment (Ambion, Austin, TX).

Quantitative Real-time PCR (qRT-PCR)—Total RNA was obtained as described above. qRT-PCR was conducted using the Applied Biosystems One-Step Real-time PCR protocol using gene-specific Taqman assays on an Applied Biosystems

7900HT real-time PCR machine in 96-well plate format. Twenty five nanograms of RNA was used for each replicate. Relative -fold changes for transcripts were calculated using the comparative *CT*(2-ΔΔ*CT*) method (23) and normalized to actin-4. Cycle thresholds of amplification were determined by StepOnePlus software (Applied Biosystems). All samples were run in triplicate. Taqman assay information is available upon request and on the Applied Biosystems website.

Statistical Analysis—Animal survival was plotted as a nonlinear regression curve using the PRISM (version 4.00) computer program. Survival curves were considered different from the appropriate control indicated in the main text when p values were <0.05. Prism uses the product limit or Kaplan-Meier method to calculate survival fractions and the log rank test, which is equivalent to the Mantel-Heanszel test, to compare survival curves. A two-sample t test for independent samples was used to analyze cfu and qRT-PCR results; p values <0.05 are considered significant. All experiments were repeated at least three times unless otherwise indicated.

RESULTS

ARR-1 Signaling Regulates Pathogen Resistance and Lifespan Extension by Targeting Different Pathways—We studied whether ARR-1 is required for *C. elegans* defense against bacterial infections by exposing *arr-1(ok401)* mutant animals to *P. aeruginosa* and by comparing their survival with that of wild-type animals. *arr-1(ok401)* animals exhibited an enhanced resistance to *P. aeruginosa* (ERP) phenotype (Fig. 1*A*), suggesting that the lack of inhibition of GPCR signaling in the entire nervous system due to a lack of ARR-1 activity enhances immunity. Wild-type and *arr-1(ok401)* animals exhibited similar pumping rates on *P. aeruginosa*, indicating that they are exposed to a comparable dose of pathogen (data not shown). To determine whether the enhanced immune response caused by mutation in the *arr-1* gene is specific to *P. aeruginosa*, we exposed *arr-1(ok401)* animals to *S. enterica* and *Y. pestis*, two Gram-negative pathogens known to kill *C. elegans* (24, 25). As shown in Fig. 1, *C* and *D*, *arr-1(ok401)* animals also exhibited an enhanced resistance to these pathogens.

Because pathogen avoidance is part of the *C. elegans* defense response to *P. aeruginosa* that is controlled by the nervous system (12), we examined the ERP phenotype of *arr-1(ok401)* animals on agar plates that were completely covered in bacteria, a condition that eliminates pathogen avoidance. *arr-1(ok401)* animals died at a slower rate than wild-type animals (Fig. 1*B*), indicating that pathogen avoidance does not play a role in the ERP phenotype of *arr-1(ok401)* animals. Consistent with this conclusion, *arr-1(ok401)* animals are resistant to *S. enterica* (Fig. 1*C*), a pathogen that does not elicit an avoidance behavior (26).

The extended lifespan of *arr-1(ok401)* animals grown on bacterial pathogens may simply be a consequence of their extended lifespan when grown on nonpathogenic *E. coli* (27). However, *arr-1(ok401)* mutants were more susceptible to the Gram-positive bacterium *Enterococcus faecalis* than wild-type animals (data not shown), making this possibility unlikely. In addition, although inhibition of the FOXO family transcription factor DAF-16 that controls longevity fully suppressed the extended

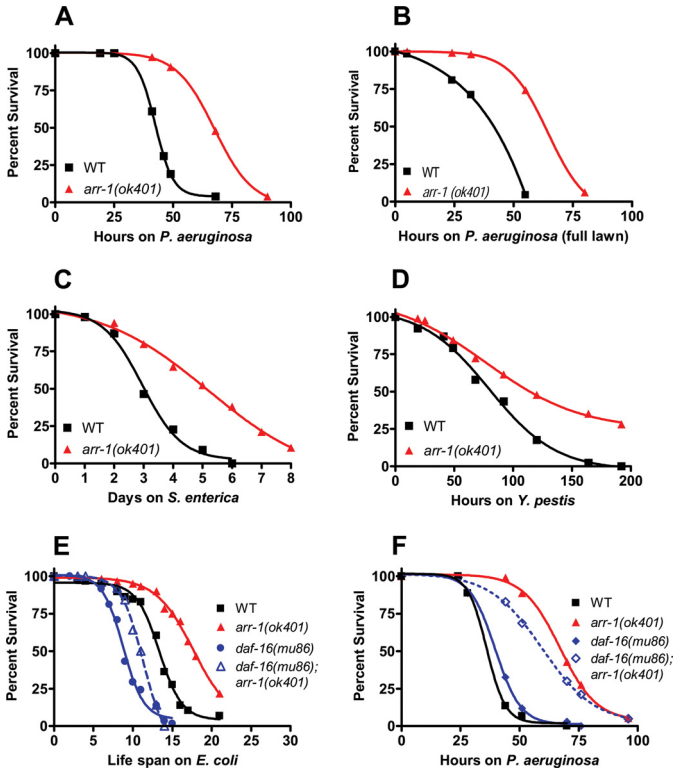

FIGURE 1. **ARR-1 regulates both pathogen resistance and lifespan extension.** *A–D*, wild type (WT) and *arr-1(ok401)* animals were exposed to *P. aeruginosa* (*A*), full lawn of *P. aeruginosa* (*B*), *S. enterica* (*C*), and *Y. pestis* (*D*) and scored for survival. *E*, wild-type, *arr-1(ok401)*, *daf-16(mu86)*, and *daf-16(mu86); arr-1(ok401)* mutant animals were scored for lifespan. *F*, wild-type, *arr-1(ok401)*, *daf-16(mu86)*, and *daf-16(mu86);arr-1(ok401)* mutant animals were exposed to *P. aeruginosa* and scored for survival. *A–D*, differences were statistically significant in all cases (p values <0.0001). *E*, WT *versus arr-1(ok401)* ($p < 0.0001$), *arr-1(ok401) versus daf-16(mu86);arr-1(ok401)* ($p < 0.0001$). *F*, *arr-1(ok401) versus daf-16(mu86);arr-1(ok401)* ($p = 0.0205$) are shown. Results represent assays of three to five independent experiments; $n = 100–120$ animals/strain.

lifespan of *arr-1(ok401)* animals (Fig. 1*E*), it only slightly suppressed the ERP phenotype of *arr-1(ok401)* animals (Fig. 1*F*). These results suggest that ARR-1 regulates different pathways involved in pathogen resistance and lifespan extension.

Neural ARR-1 Regulates Innate Immunity against Bacterial Pathogens—Expression of ARR-1::GFP under its own promoter restored ARR-1 expression in the nervous system (Fig. 2*C*) and rescued the ERP phenotype of *arr-1(ok401)* animals (Fig. 2*A*), providing evidence for the role of neuronal ARR-1 signaling in the regulation of innate immunity. ARR-1::GFP expressed under the regulation of a pan-neuronal promoter *unc-119* (Fig. 2*F*) also fully rescued the ERP phenotype of *arr-1(ok401)* animals (Fig. 2*D*). Native or pan-neuronal expression of ARR-1 also rescued the reduced accumulation of *P. aeruginosa* phenotype exhibited by *arr-1(ok401)* animals (Fig. 2, *B* and *E*).

Role of ARR-1 Function in Neurons Expressing GPCRs That Control Innate Immunity—OCTR-1, a putative octopamine GPCR, functions in sensory neurons designated ASH and ASI to actively suppress immune defense against *P. aeruginosa* responses by down-regulating the expression of *abu* genes (7). These genes belong to a family of *pqn* (prion-like glutamine[Q]/asparagine[N]-rich domain-bearing protein) genes that have been named *abu* (activated in blocked unfolded protein

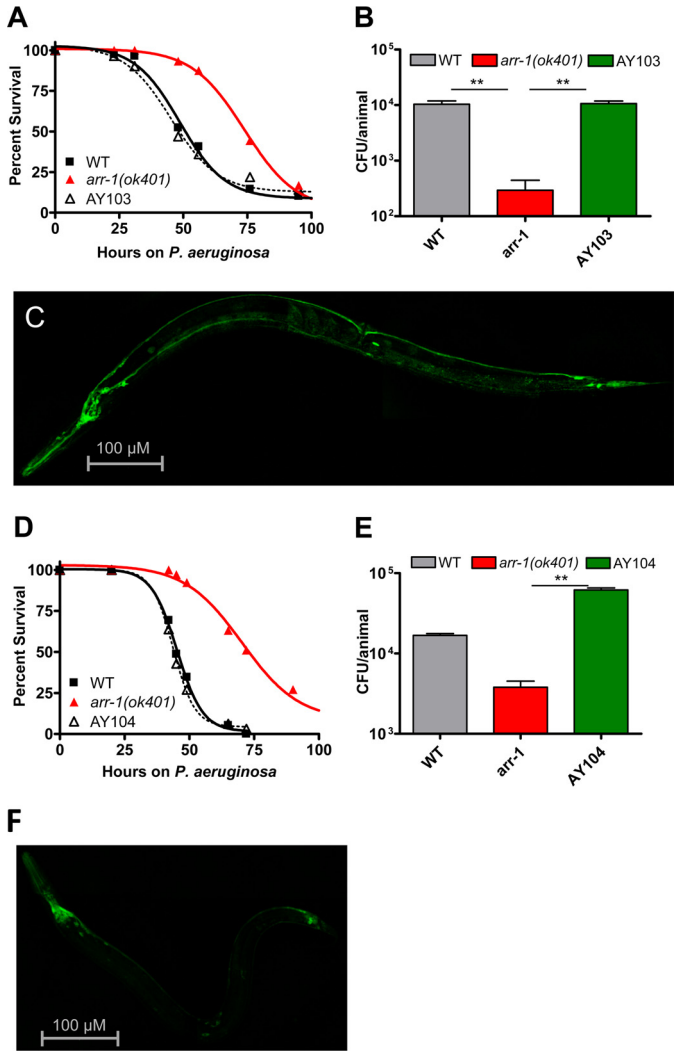

FIGURE 2. **ARR-1 functions in the nervous system to regulate innate immunity.** *A*, wild type (WT), *arr-1(ok401)*, and *arr-1(ok401)*[*pVS3*(p*arr-1::arr-1::gfp*):*pRF4(rol-6(su1006))*] (AY103) animals exposed to *P. aeruginosa* and scored for survival. *B*, WT, *arr-1(ok401)*, and AY103 animals exposed to *P. aeruginosa*/GFP for 24 h, and the cfu were quantified. *C*, confocal image of AY103 animal expressing ARR-1::GFP. *D*, WT, *arr-1(ok401)*, and *arr-1-(ok401)*[*pVS4*(p*unc-119::arr-1::gfp*):*pRF4(rol-6(su1006))*] (AY104) animals exposed to *P. aeruginosa* and scored for survival. *E*, WT, *arr-1(ok401)*, and AY104 animals exposed to *P. aeruginosa*/GFP for 24 h and the cfu quantified. *F*, confocal image of AY104 animals expressing ARR-1::GFP in the nervous system. *A*, WT *versus* *arr-1(ok401)* ($p < 0.0001$), WT *versus* AY103 ($p = 0.7384$), *arr-1(ok401) versus* AY103 ($p < 0.0001$). *D*, WT *versus* AY104 ($p = 0.4647$), *arr-1(ok401) versus* AY104 ($p < 0.0001$). *A* and *D*, representative assays of at least three independent experiments; $n = 100–120$ animals/strain. *B* and *E*, mean ± S.E. (*error bars*). **, $p < 0.005$; ***, $p < 0.0005$; $n = 4$ independent experiments.

response) as they are activated in *xbp-1* mutant animals when ER stress is induced (7, 28). They are also up-regulated in response to *P. aeruginosa* infection and strongly expressed in nonneuronal tissues such as the pharynx and the intestine (7, 28), primary interfaces between host cells involved in immune responses and bacterial pathogens. We found that five *abu* genes were up-regulated in *arr-1 (ok401)* animals when exposed to *P. aeruginosa*. The up-regulation of *abu* genes was rescued by ARR-1 expression in the nervous system (supplemental Fig. S1), indicating that neural ARR-1 regulates this noncanonical UPR pathway.

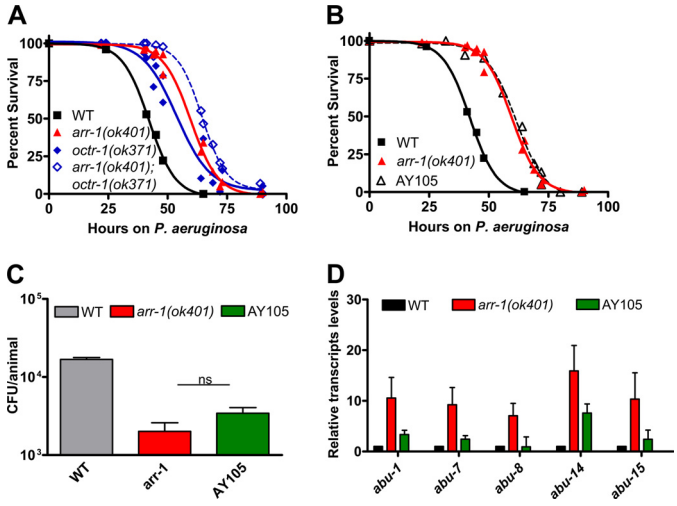

FIGURE 3. **The ERP phenotype of *arr-1(ok401)* animals is independent of OCTR-1 in ASH and ASI neurons.** *A*, WT, *arr-1(ok401)*, *octr-1(ok371)*, and *arr-1(ok401);octr-1(ok371)* animals were exposed to *P. aeruginosa* and scored for survival. *B*, WT, *arr-1(ok401)*, and *arr-1(ok401)*[*pVS5*(p*sra-6::arr-1::gfp*):*pRF4(rol-6(su1006))*] (AY105) animals were exposed to *P. aeruginosa* and scored for survival. *C*, WT, *arr-1(ok401)*, and AY105 animals were exposed to *P. aeruginosa*/GFP for 24 h, and cfu were quantified. *D*, histogram shows qRT-PCR analysis of *abu* genes in WT, *arr-1(ok401)*, and AY105 animals exposed to *P. aeruginosa* for 4 h. *A*, WT *versus* *octr-1(ok371)* ($p < 0.0001$), *arr-1(ok401) versus* *arr-1(ok401);octr-1(ok371)* ($p = 0.0006$), *octr-1(ok371) versus* *arr-1(ok401);octr-1(ok371)* ($p < 0.0001$). *B*, representative assays of at least three independent experiments show WT *versus* AY105 ($p < 0.0001$), *arr-1(ok401) versus* AY105 ($p = 0.0925$). Shown are representative assays of at least three independent experiments; $n = 100$ animals/strain. *C* shows mean ± S.E. (*error bars*); $n = 4$ independent experiments. *D* shows mean ± S.E.; $n = 3$ independent experiments.

To study the role of ARR-1 in ASH and ASI neurons to regulate immune homeostasis, we studied *arr-1(ok401)* animals carrying a deletion in *octr-1*. The ERP phenotype of *arr-1(ok401);octr-1(ok371)* animals was stronger than that of *arr-1(ok401)* or *octr-1(ok371)* animals (Fig. 3*A*). In addition, unlike *octr-1(ok371)* animals, *arr-1(ok401)* animals are more resistant to bacterial accumulation (Fig. 3*C* and supplemental Fig. S2), indicating that the ERP phenotype of *arr-1(ok401)* animals is mediated through GPCRs other than OCTR-1. If OCTR-1 were the only GPCR blocked by ARR-1 in ASH and ASI neurons, the rescue of ARR-1 expression in these neurons would further enhance the ERP phenotype of *arr-1(ok401)* animals and the up-regulation of *abu* genes. However, no differences in survival or bacterial accumulation were observed between *arr-1(ok401)* and *arr-1(ok401)* animals expressing ARR-1 under the regulation of the *sra-6* promoter (Fig. 3, *B* and *C*), which drives ARR-1 expression to OCTR-1-expressing neurons ASH and ASI. Neither did the expression of ARR-1 further enhance the up-regulation of *abu* genes in *arr-1(ok401)* animals (Fig. 3*D*). These results suggest that there may be another GPCR in ASH and ASI neurons whose signaling promotes immune homeostasis.

Another GPCR, NPR-1, which is related to mammalian neuropeptide Y receptors, functions in a neural circuit involving AQR, PQR, and URX sensory neurons to enhance immune responses against *P. aeruginosa* (12). If ARR-1 were responsible for blocking NPR-1 signaling in the nervous system, we would expect *npr-1* mutation or expression of ARR-1 in NPR-1-expressing neurons to rescue the ERP phenotype of *arr-1(ok401)* animals. Indeed, the ERP phenotype of *arr-1(ok401)* was par-

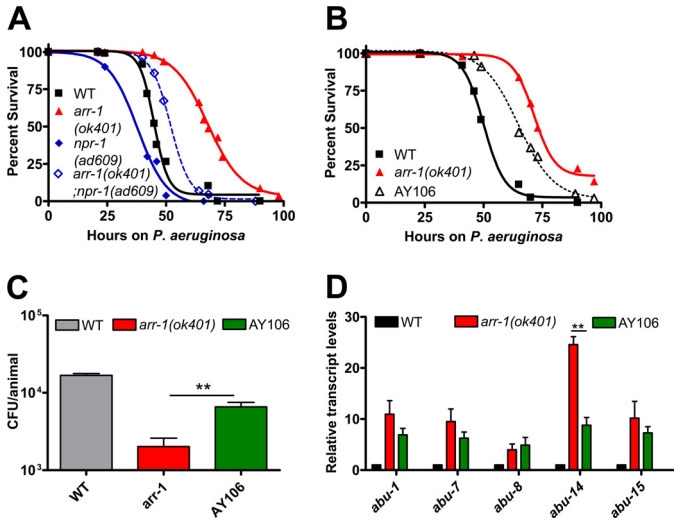

FIGURE 4. **NPR-1 partially controls innate immunity through ARR-1 signaling in AQR, PQR, and URX neurons.** *A*, WT, *arr-1(ok401)*, *npr-1(ad609)*, and *arr-1(ok401);npr-1(ad609)* animals were exposed to *P. aeruginosa* and scored for survival. *B*, WT, *arr-1(ok401)*, and *arr-1(ok401)*[*pVS6*(p*gcy-32*::*arr-1*::*gfp*):*pRF4*(*rol-6*(*su1006*))] (AY106) animals were exposed to *P. aeruginosa* and scored for survival. *C*, WT, *arr-1(ok401)* and AY106 animals were exposed to *P. aeruginosa*/GFP for 24 h, and the cfu were quantified. *D*, histogram shows qRT-PCR analysis of *abu* genes in WT, *arr-1(ok401)* and AY106 animals exposed to *P. aeruginosa* for 4 h. *A*, WT *versus npr-1(ad609)* ($p = 0.001$), WT *versus arr-1(ok401);npr-1(ad609)* ($p < 0.0001$), *arr-1(ok401) versus arr-1(ok401); npr-1(ad609)* ($p < 0.0001$). *B*, WT *versus* AY106 ($p < 0.0001$), *arr-1(ok401) versus* AY106 ($p < 0.0001$). *A* and *B*, representative assays of at least three independent experiments; $n = 100$ animals/strain. *C*, mean ± S.E. (*error bars*); $n = 4$ independent experiments. *D*, mean ± S.E.; $n = 3$ independent experiments. **, $p < 0.005$.

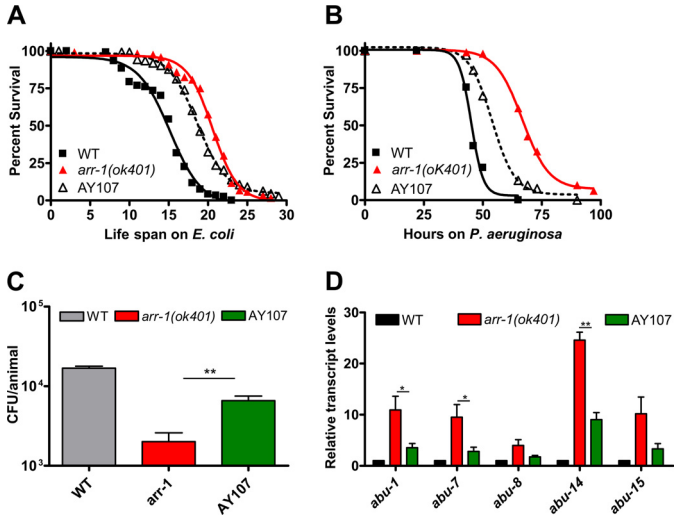

FIGURE 5. **ARR-1 signaling in ADF neurons suppresses innate immunity and longevity.** *A*, WT, *arr-1(ok401)*, and *arr-1(ok401)*[*pVS7*(p*srh-142*::*arr-1*::*gfp*):*pRF4*(*rol-6*(*su1006*))] (AY107) animals were scored for lifespan. *B*, WT, *arr-1(ok401)*, and AY107 animals were exposed to *P. aeruginosa* and scored for survival. *C*, WT, *arr-1(ok401)*, and AY107 animals were exposed to *P. aeruginosa*/GFP for 24 h, and cfu were quantified. *D*, histogram shows qRT-PCR analysis of *abu* genes in WT, *arr-1(ok401)*, and AY107 animals exposed to *P. aeruginosa* for 4 h. *A*, WT *versus* AY107 ($p < 0.0001$), *arr-1(ok401) versus* AY107 ($p = 0.0483$). *B*, WT *versus* AY107 ($p < 0.0001$), *arr-1(ok401) versus* AY107 ($p < 0.0001$). *A* and *B*, representative assays of three independent experiments; $n = 100$ animals per strain. *C*, mean ± S.E. (*error bars*); $n = 4$ independent experiments. *D*, mean ± S.E. *, $p < 0.05$; **, $p < 0.005$; $n = 3$ independent experiments.

tially rescued by *npr-1* mutation and by expression of ARR-1 under the control of the *gcy-32* promoter, which drives its expression to AQR, PQR, and URX neurons (Fig. 4, *A* and *B*). The reduced accumulation of *P. aeruginosa* phenotype exhibited by *arr-1(ok401)* animals was also partially rescued by expression of ARR-1 in the aforementioned neurons (Fig. 4*C*). ARR-1 expression in *arr-1(ok401)* animals rescued the up-regulation of only one of five *abu* genes (Fig. 4*D*), suggesting that ARR-1 function in AQR, PQR, and URX neurons only plays a small role in the control of *abu* genes. These results also indicate that ARR-1 signaling is required in additional neurons to control immune homeostasis globally.

ARR-1 Signaling in ADF Chemosensory Neurons Regulates Both Pathogen Resistance and Lifespan Extension—Because ARR-1 regulates DAF-16-mediated lifespan extension (27) and ADF sensory neurons control DAF-16 activation (29), we studied whether ARR-1 functions in ADF neurons to control either the lifespan extension or the ERP phenotype of *arr-1(ok401)* animals. We found that although ARR-1 expression under the regulation of the *srh-142* promoter, which drives ARR-1 expression to ADF neurons, slightly rescued the extended lifespan of *arr-1(ok401)* animals (Fig. 5*A*), it rescued the ERP phenotype of *arr-1(ok401)* animals more strongly (Fig. 5*B*). In contrast, we found that *daf-16* mutation fully suppressed the extended lifespan of *arr-1(ok401)* animals (Fig. 1*E*) and slightly suppressed the ERP phenotype of *arr-1(ok401)* animals (Fig. 1*F*). Thus, ARR-1 signaling in ADF neurons appears to play a more important role in the regulation of immunity against bacterial infections than in the control of lifespan. ARR-1 expression in ADF neurons also partially rescued the enhanced expression of *abu* genes and reduced bacterial accumulation of *arr-1(ok401)* animals (Fig. 5, *C* and *D*), further supporting the function of ARR-1 signaling in ADF neurons to control immune homeostasis.

Regulation of Pathogen Resistance via ARR-1 Signaling in AFD Thermosensory Neurons—We hypothesized that ARR-1 signaling might control longevity and immune response by acting in thermosensory AFD neurons, as these neurons have been implicated in the control of not only temperature sensation and thermotaxis but also longevity (30–32). We found that although ARR-1 activity driven to AFD neurons by the *gcy-8* promoter had no effect on the extended lifespan of *arr-1(ok401)* animals (Fig. 6*A*), it strongly rescued their ERP phenotype (Fig. 6*B*). In addition, ARR-1 activity in AFD neurons strongly rescued the enhanced expression of *abu* genes and reduced bacterial colonization of *arr-1(ok401)* animals (Fig. 6, *C* and *D*). These results indicate that ARR-1 signaling in these sensory neurons plays a crucial role in the control of immune homeostasis during bacterial infections.

DISCUSSION

We sought to investigate the role of GPCR signaling in the entire nervous system in the regulation of immune response against bacterial infections taking advantage of a unique component of GPCR signaling, the adaptor protein ARR-1. Our findings suggest a broad role for neuronal signaling of ARR-1 in the control of organismal homeostasis. Neural expression of ARR-1 rescued both the extended lifespan and ERP phenotype of *arr-1 (ok401)* animals (Fig. 2*D* and supplemental Fig. S3), indicating that neuronal ARR-1 controls both of these pro-

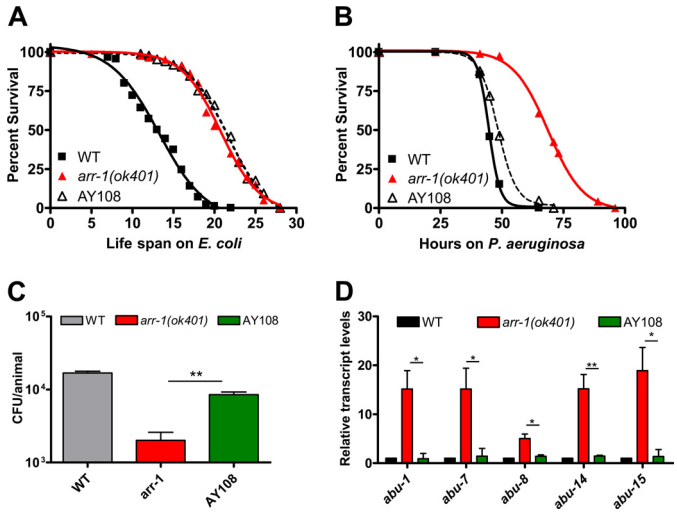

FIGURE 6. **ARR-1 signaling in AFD neurons suppresses innate immunity.** *A*, WT, *arr-1(ok401)*, and *arr-1(ok401)*[*pVS7*(p*srh-142::arr-1::gfp*):*pRF4(rol-6(su1006))*] (AY108) animals were scored for lifespan. *B*, WT, *arr-1(ok401)*, and AY108 animals were exposed to *P. aeruginosa* and scored for survival. *C*, histogram shows qRT-PCR analysis of *abu* genes in WT, *arr-1(ok401)*, and AY108 animals exposed to *P. aeruginosa* for 4 h. *D*, WT, *arr-1(ok401)*, and AY108 animals were exposed to *P. aeruginosa*/GFP for 24 h, and the cfu were quantified. *A*, WT *versus* AY108 ($p < 0.0001$), *arr-1(ok401) versus* AY108 ($p = 0.3224$). *B*, WT *versus* AY108 ($p = 0.0021$); *arr-1(ok401) versus* AY108 ($p < 0.0001$). *A* and *B*, representative assays of at least three independent experiments; $n = 100$ animals/strain. *C*, mean ± S.E. (*error bars*); $n = 3$ independent experiments. *D*, mean ± S.E.; $n = 4$ independent experiments. *, $p < 0.05$; **, $p < 0.005$.

cesses. The results indicate that ARR-1 functions in different sensory neurons to control certain aspects of innate immunity (Fig. 7). They also indicate that ARR-1 partially controls longevity through one of these neurons (Figs. 5*A* and Fig. 6*A* and supplemental Figs. S3 and S4), suggesting that overlapping and distinct neural circuits control longevity and immune homeostasis. It remains to be determined whether ARR-1 signaling participates in the control of the canonical UPR pathway that was recently found to be controlled by OCTR-1 in adult animals (8).

We found that, in addition to ASH and ASI, ADF and AFD sensory neurons controlled the noncanonical UPR pathway, highlighting the importance of the nervous system in the control of this pathogen-induced pathway that may help alleviate the ER stress caused by the increased demand for protein folding that takes place during immune activation. Notably, the noncanonical UPR genes are strongly expressed in the pharynx and the intestine, which are the primary interfaces between host cells involved in immune responses and bacterial pathogens (7). This is consistent with the idea that the nervous system acts as a master regulator of microbial killing pathways and cellular stress pathways that need to be balanced as insufficient or excessive immune responses have deleterious consequences in the infected organism.

The *C. elegans* nervous system transmits signals from thermosensory neurons AFD to control the activity of a steroid-signaling pathway that controls longevity (32). Our results show that ARR-1 signaling in AFD neurons did not control longevity (Fig. 6*A*), suggesting that GPCRs may not participate in thermosensory signaling cascades involved in the control of longevity. However, ARR-1 in AFD neurons appeared to play a crucial role in the control of immunity (Fig. 6, *B–D*), suggesting

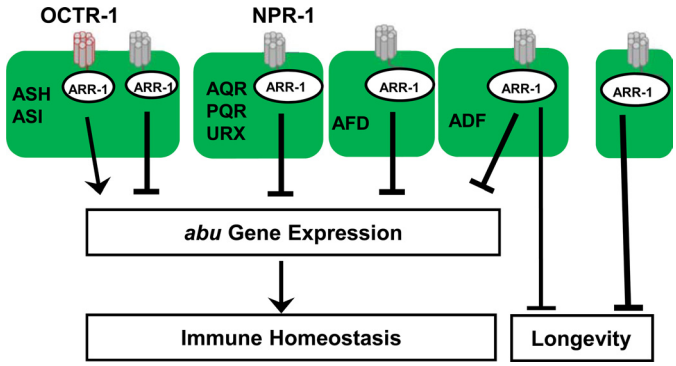

FIGURE 7. **Schematic of neural control of immune homeostasis by ARR-1 signaling.** In addition to OCTR-1, at least another receptor may signal through ARR-1 in ASH and ASI neurons to control *abu* gene expression. ARR-1 function in AQR, PQR, and URX neurons appears to play only a small role in the control of *abu* genes, whereas ARR-1 function in ADF and AFD neurons plays a more important role. ARR-1 signaling in ADF neurons also partially regulates longevity. Chemosensory ADF and thermosensory AFD neurons have unidentified receptors that may be regulated by ARR-1 to control *abu* gene expression and immune homeostasis.

that GPCR signaling and thermosensation in these cells may play an important role in response to pathogen infections. Fever is an ancient immune mechanism used by metazoans in response to microbial infections, and warm temperatures activate a conserved pathway involving HSF-1 that helps *C. elegans* fight bacterial infections (33). Whereas only homeotherms are capable of internally increasing the body temperature, both homeotherms and poikilotherms exhibit warmth-seeking behavior when infected. Our results raise the interesting possibility that AFD neurons may participate in circuits that integrate inflammatory cues and thermosensation to fight infections.

In summary, our results indicate that the *C. elegans* nervous system utilizes a network of sensory neurons that controls longevity and a stress-induced pathway that plays a key role in cellular homeostasis during responses against bacterial infections. Whereas ARR-1 signaling in both AFD and ADF neurons controls immunity, it only partially controls longevity through ADF neurons. The identification of GPCRs in these cells capable of sensing pathogens or the damage caused by infections or aging is an important area for further study.

Acknowledgments—We thank the Caenorhabditis Genetics Center (University of Minnesota) for strains used in this study and Jingru Sun in our laboratory for the arr-1(ok401);octr-1(ok371) animals.

REFERENCES

1. Tracey, K. J. (2011) Cell biology: ancient neurons regulate immunity. *Science* **332,** 673–674
2. Haskins, K. A., Russell, J. F., Gaddis, N., Dressman, H. K., and Aballay, A. (2008) Unfolded protein response genes regulated by CED-1 are required for *Caenorhabditis elegans* innate immunity. *Dev. Cell* **15,** 87–97
3. Richardson, C. E., Kooistra, T., and Kim, D. H. (2010) An essential role for XBP-1 in host protection against immune activation in *C. elegans*. *Nature* **463,** 1092–1095
4. Bischof, L. J., Kao, C. Y., Los, F. C., Gonzalez, M. R., Shen, Z., Briggs, S. P., van der Goot, F. G., and Aroian, R. V. (2008) Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin *in vivo*. *PLoS Pathog* **4,** e1000176
5. Martinon, F., Chen, X., Lee, A. H., and Glimcher, L. H. (2010) TLR acti-

vation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* **11,** 411–418
6. Woo, C. W., Cui, D., Arellano, J., Dorweiler, B., Harding, H., Fitzgerald, K. A., Ron, D., and Tabas, I. (2009) Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by Toll-like receptor signalling. *Nat. Cell Biol.* **11,** 1473–1480
7. Sun, J., Singh, V., Kajino-Sakamoto, R., and Aballay, A. (2011) Neuronal GPCR controls innate immunity by regulating noncanonical unfolded protein response genes. *Science* **332,** 729–732
8. Sun, J., Liu, Y., and Aballay, A. (July 13, 2012) Organismal regulation of XBP-1-mediated unfolded protein response during development and immune activation. *EMBO Rep.* 10.1038/embor. 2012.100
9. Sternberg, E. M. (2006) Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat. Rev. Immunol.* **6,** 318–328
10. Andersson, J. (2005) The inflammatory reflex: introduction. *J. Intern. Med.* **257,** 122–125
11. Tracey, K. J. (2002) The inflammatory reflex. *Nature* **420,** 853–859
12. Styer, K. L., Singh, V., Macosko, E., Steele, S. E., Bargmann, C. I., and Aballay, A. (2008) Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science* **322,** 460–464
13. Kovacs, J. J., Hara, M. R., Davenport, C. L., Kim, J., and Lefkowitz, R. J. (2009) Arrestin development: emerging roles for β-arrestins in developmental signaling pathways. *Dev. Cell* **17,** 443–458
14. Palmitessa, A., Hess, H. A., Bany, I. A., Kim, Y. M., Koelle, M. R., and Benovic, J. L. (2005) *Caenorhabditus elegans* arrestin regulates neural G protein signaling and olfactory adaptation and recovery. *J. Biol. Chem.* **280,** 24649–24662
15. Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* **77,** 71–94
16. Tan, M. W., Rahme, L. G., Sternberg, J. A., Tompkins, R. G., and Ausubel, F. M. (1999) *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 2408–2413
17. Wray, C., and Sojka, W. J. (1978) Experimental *Salmonella typhimurium* infection in calves. *Res. Vet. Sci.* **25,** 139–143
18. Une, T., and Brubaker, R. R. (1984) *In vivo* comparison of avirulent Vwa- and Pgm- or Pstr phenotypes of *Yersiniae*. *Infect. Immun.* **43,** 895–900
19. Maduro, M., and Pilgrim, D. (1996) Conservation of function and expression of unc-119 from two *Caenorhabditis* species despite divergence of non-coding DNA. *Gene* **183,** 77–85
20. Troemel, E. R., Chou, J. H., Dwyer, N. D., Colbert, H. A., and Bargmann, C. I. (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* **83,** 207–218
21. Yu, S., Avery, L., Baude, E., and Garbers, D. L. (1997) Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. *Proc. Natl. Acad. Sci. U.S.A.* **94,** 3384–3387
22. Inada, H., Ito, H., Satterlee, J., Sengupta, P., Matsumoto, K., and Mori, I. (2006) Identification of guanylyl cyclases that function in thermosensory neurons of *Caenorhabditis elegans*. *Genetics* **172,** 2239–2252
23. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C^{T}$ method. *Methods* **25,** 402–408
24. Aballay, A., Yorgey, P., and Ausubel, F. M. (2000) *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr. Biol.* **10,** 1539–1542
25. Styer, K. L., Hopkins, G. W., Bartra, S. S., Plano, G. V., Frothingham, R., and Aballay, A. (2005) *Yersinia pestis* kills *Caenorhabditis elegans* by a biofilm-independent process that involves novel virulence factors. *EMBO Rep.* **6,** 992–997
26. Tenor, J. L., and Aballay, A. (2008) A conserved Toll-like receptor is required for *Caenorhabditis elegans* innate immunity. *EMBO Rep.* **9,** 103–109
27. Palmitessa, A., and Benovic, J. L. (2010) Arrestin and the multi-PDZ domain-containing protein MPZ-1 interact with phosphatase and tensin homolog (PTEN) and regulate *Caenorhabditis elegans* longevity. *J. Biol. Chem.* **285,** 15187–15200
28. Urano, F., Calfon, M., Yoneda, T., Yun, C., Kiraly, M., Clark, S. G., and Ron, D. (2002) A survival pathway for *Caenorhabditis elegans* with a blocked unfolded protein response. *J. Cell Biol.* **158,** 639–646
29. Liang, B., Moussaif, M., Kuan, C. J., Gargus, J. J., and Sze, J. Y. (2006) Serotonin targets the DAF-16/FOXO signaling pathway to modulate stress responses. *Cell Metab.* **4,** 429–440
30. Mori, I., and Ohshima, Y. (1995) Neural regulation of thermotaxis in *Caenorhabditis elegans*. *Nature* **376,** 344–348
31. Sugi, T., Nishida, Y., and Mori, I. (2011) Regulation of behavioral plasticity by systemic temperature signaling in *Caenorhabditis elegans*. *Nat. Neurosci.* **14,** 984–992
32. Lee, S. J., and Kenyon, C. (2009) Regulation of the longevity response to temperature by thermosensory neurons in *Caenorhabditis elegans*. *Curr. Biol.* **19,** 715–722
33. Singh, V., and Aballay, A. (2006) Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 13092–13097