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# A Polymorphism in *npr-1* Is a Behavioral Determinant of Pathogen Susceptibility in *C. elegans*

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The nematode *Caenorhabditis elegans* responds to pathogenic bacteria with conserved innate immune responses and pathogen avoidance behaviors. We investigated natural variation in *C. elegans* resistance to pathogen infection. With the use of quantitative genetic analysis, we determined that the pathogen susceptibility difference between the laboratory wild-type strain N2 and the wild isolate CB4856 is caused by a polymorphism in the *npr-1* gene, which encodes a homolog of the mammalian neuropeptide Y receptor. We show that the mechanism of NPR-1–mediated pathogen resistance is through oxygen-dependent behavioral avoidance rather than direct regulation of innate immunity. For *C. elegans*, bacteria represent food but also a potential source of infection. Our data underscore the importance of behavioral responses to oxygen levels in finding an optimal balance between these potentially conflicting cues.

Microbes, including commensal organisms and pathogens, profoundly influence the immune and metabolic physiology of host organisms (*1*). We used the nematode *Caenorhabditis elegans* as an experimental host to dissect the molecular basis of interactions between host species and microorganisms. *C. elegans* exhibits diverse behaviors in response to bacteria provided as a nutrient source (*2*–*4*). Feeding behavior can be modulated by environmental conditions, including oxygen concentration (*5*). Some bacterial species are pathogenic to *C. elegans* (*6*), and *C. elegans* responds by activating conserved innate immune pathways (*7*–*9*) and avoiding pathogens (*10*–*12*).

We found that the standard laboratory strain N2 (isolated in Bristol, England) and strain CB4856 (isolated in Hawaii, USA) exhibited a marked difference in susceptibility to the human opportunistic pathogen *Pseudomonas aeruginosa* strain PA14 (Fig. 1A) (*13*). The mean time to 50% lethality (LT50) for CB4856 was shorter (50 ± 7.8 hours) than that for N2 (90 ± 13 hours). Using a collection of recombinant inbred lines (*14*), we mapped the pathogen susceptibility trait to a 774-kb region of the X chromosome (LGX) containing *npr-1*, which encodes a G protein–coupled receptor related to the mammalian neuropeptide Y receptor (Fig. 1B). The 215V *npr-1* allele in N2 has increased NPR-1 activity relative to the 215F *npr-1* allele in CB4856, and the 215V allele confers behavioral differences that are dominant to those conferred by the 215F allele (*15*). To test the possibility that *npr-1* causes the difference in pathogen susceptibility between the N2 and CB4856 strains, we used *npr-1* loss-of-function mutants isolated in the N2 background. Like the CB4856 strain, the *npr-1* presumptive null alleles *ad609* and *ky13*, along with the reduction-of-function alleles *ur89* and *n1353*, had enhanced susceptibility to killing by PA14 (Fig. 1C and fig. S1). The enhanced susceptibility of *npr-1(ky13)* mutants was rescued by a transgene containing N2 wild-type (WT) copies of *npr-1*, and an N2 *npr-1* null mutation failed to complement the pathogen susceptibility phenotype of CB4856 (Fig. 1C). Thus, the enhanced susceptibility to pathogen of CB4856 is caused by the ancestral 215F allele of *npr-1*. This finding is consistent with a recent report by Styer *et al.* (*16*) that showed that loss-of-function mutations in the *npr-1* gene in the N2 background result in enhanced susceptibility to pathogen killing.

The 215F *npr-1* allele in CB4856 and loss-of-function mutations in *npr-1* confer a constellation of related behavioral phenotypes that have been termed "social feeding"—the animals associate together in groups (clumping) and are often found at the edge of the bacterial lawn (bordering) (*15*). The characterization of aerotaxis behavior in *C. elegans* revealed that CB4856 and *npr-1* loss-of-function mutants prefer the decreased oxygen concentrations found at the edge of the live bacterial lawn, which drives the bordering phenotype (*5*, *17*, *18*). We hypothesized that differences in behavior, instead of in innate immune responses as recently proposed (*16*), might underlie the observed pathogen susceptibility differences caused by the *npr-1* polymorphism. By spending more time on the bacterial lawn, CB4856 and *npr-1* mutants would receive an increased dose of the pathogenic bacteria, leading to higher mortality. Multiple independent experiments support our hypothesis.

First, mutations in the oxygen-sensing guanylate cyclase *gcy-35* and the neuronal signaling genes *ocr-2* and *osm-9*, which are necessary for *npr-1*–mediated bordering and aerotaxis behaviors (*5*, *19*, *20*), also suppressed the pathogen susceptibility of *npr-1* mutants (fig. S2, A and B). These data suggest that the clumping and bordering behaviors mediated by responses to oxygen concentration are necessary for the enhanced susceptibility to the pathogen.

Second, we altered the standard slow-killing pathogenesis assay (*21*) by spreading the PA14 lawn to the edges of the agar plate. In this "big lawn" assay, there is no region of the plate in which the animals can avoid pathogen. Under these conditions, N2 displayed increased susceptibility equivalent to both CB4856 and *npr-1(ad609)*, whereas the susceptibilities of *npr-1(ad609)* and *npr-1(ky13)* were equivalent in both assays (Fig. 2A and figs. S3 and S4). These data demonstrate that the pathogen susceptibility difference arises not from differential activation of immune pathways, but rather from the aberrant aerotaxis behavior of the N2 strain in the presence of the pathogenic lawn that results in lower exposure to the pathogen.

Third, we carried out the standard pathogenesis assay at 10% oxygen concentration, which suppresses bordering and aerotaxis behaviors in CB4856 and *npr-1* mutants (*5*, *18*). We observed that this reduced oxygen concentration also suppressed the pathogen susceptibility phenotypes of CB4856 and *npr-1(ad609)*. Allowing CB4856 and *npr-1* mutants to disperse off of the bacterial lawn results in survival that is equivalent to that observed for N2.

In addition, we found that three dauer-defective mutants that weakly aggregate and bor-

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der on the bacterial lawn in an *npr-1*–independent manner (*20*) were more susceptible to PA14 killing than N2 (fig. S6), indicating that social feeding behaviors are sufficient to cause increased susceptibility. Also, we examined five transcriptional targets of the *pmk-1* p38 MAPK immune signaling pathway (*22*) and observed no differences between the WT and *npr-1* mutant strains (fig. S7). The behavioral differences between N2 and *npr-1* mutant strains precluded the analysis of gene expression changes on worms exposed to the pathogen because different degrees of pathogen exposure, and not direct NPR-1–mediated regulation of innate immunity, control infection- and immunity-related transcriptional changes.

We analyzed the kinetics of pathogen accumulation using green fluorescent protein (GFP)–labeled PA14 and red fluorescent beads, showing that CB4856 and *npr-1* mutants have a greater intake in the standard slow-killing assay as compared with N2 (Fig. 3, A to F). Whereas the increased accumulation of PA14-GFP could be attributed either to increased exposure and intake or to diminished intestinal immune responses, the concomitant accumulation of fluorescent-labeled beads suggests that the increased accumulation is due to increased exposure. We also observed that N2, CB4856, and *npr-1* mutants have equivalent rates of PA14-GFP accumulation under big lawn assay conditions (Fig. 3, G to I), fully consistent with the equivalent survival that we observe under these assay conditions (Fig. 2A).

These experiments show that the increased exposure to pathogen, mediated by the behavior of the animals, is necessary and sufficient for susceptibility to *P. aeruginosa* PA14. Our data contradict the claim of the recent report by Styer *et al*. (*16*) that the pathogen susceptibility phenotype of *npr-1* mutants results from direct neuronal regulation of the intestinal immune response. Styer *et al*. also observed that N2 worms died faster on plates completely covered with PA14 and that *npr-1(ad609)* was more resistant to PA14 at a lower oxygen concentration. However, they reported residual differences in survival between N2 worms and *npr-1(ad609)* in both of these assays and interpreted these residual differences as evidence of direct neuronal regulation of innate immunity. We see similar survival among N2, CB4856, and *npr-1* mutant worms on the big lawn and at lower oxygen concentrations. Small residual effects are difficult to rule out due to possible differences between strains and assay conditions, but we would interpret any such effects as more likely to represent subtle uncontrolled variation in behavior than the presence of a nonbehavioral resistance mechanism. Our results are internally consistent between two laboratories (fig. S3). Additionally, our interpretation of a behaviorally mediated difference in susceptibility is supported by our observations of faster accumulation of both GFP-labeled PA14 and fluorescent beads by CB4856 and *npr-1* mutants in the standard assay but equal accumulation of PA14 by these strains and N2 on the big lawn.

Our data underscore that behavioral avoidance can confer dramatic effects on survival in the presence of pathogenic bacteria. The physiological interplay between mechanisms of aerotaxis and pathogen survival is intriguing given the molecular connection between responses to changes in oxygen and innate immunity in mammals (*23*). The N2 allele of *npr-1* causes animals to spend less time in regions of low oxygen concentration in the presence of bacteria, thereby

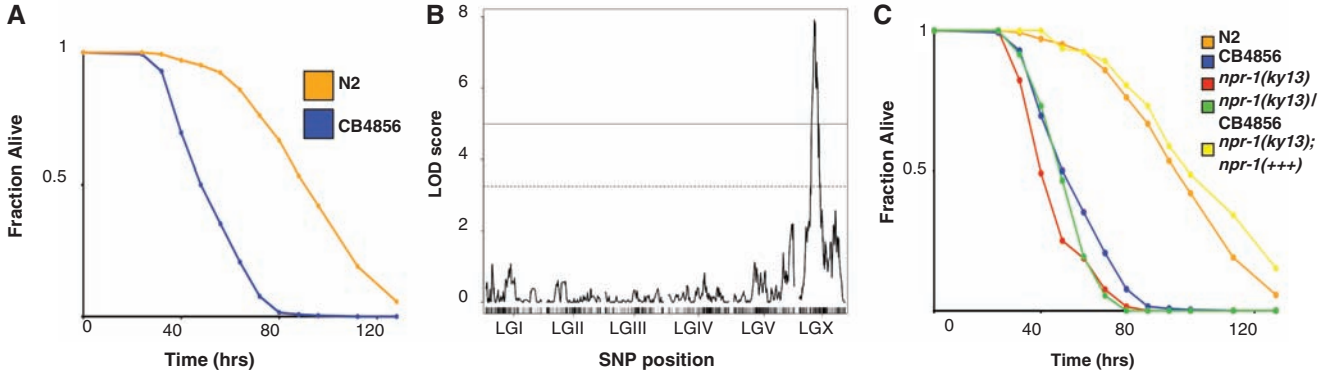


**Fig. 1.** The enhanced pathogen susceptibility of CB4856 is caused by the 215F allele of *npr-1*. (**A**) Fraction alive versus time for N2 (orange) and CB4856 (blue) fed PA14 using standard assays (*19*). Means from 27 independent experiments for each strain are shown. (**B**) Logarithm of the odds ratio (LOD) score for linkage between LT50 and 1454 single-nucleotide polymorphisms (SNPs) in 126 recombinant inbred lines showing one major quantitative trait locus on LGX. Dotted and solid lines show 5 and 0.1% genome-wide significance levels, respectively, determined from 1000 permutations. (**C**) Fraction alive versus time for N2 (orange), CB4856 (blue), the *npr-1* loss-of-function mutant *ky13* (red), a complementation test between *npr-1(ky13)* derived from the N2 background and *npr-1* derived from the CB4856 background (green), and rescue of the *npr-1(ky13)* allele (yellow).

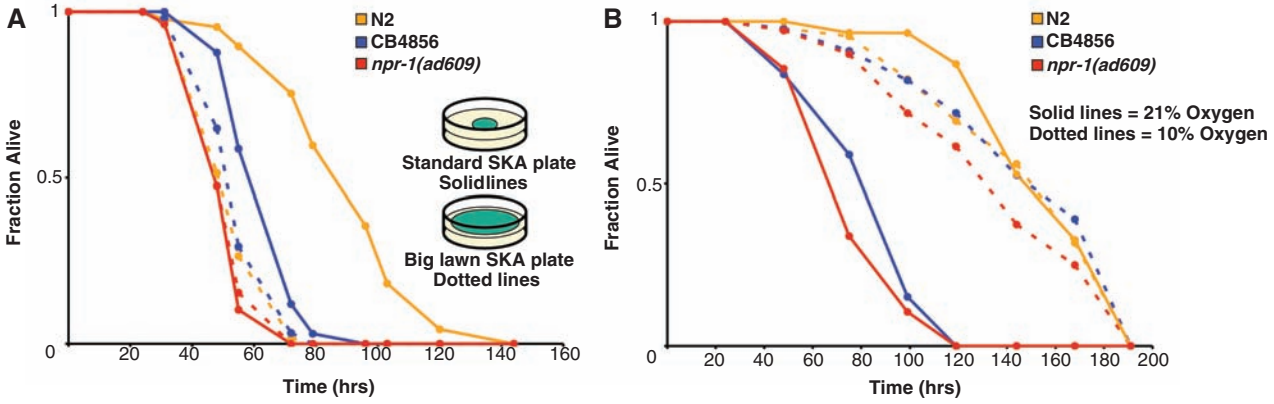


**Fig. 2.** *npr-1*–mediated behaviors determine the susceptibility of *C. elegans* to PA14. (**A**) Fraction alive versus time for N2 (orange) and CB4856 (blue) fed PA14 on standard plates (solid lines) and big lawn plates (dotted lines). (**B**) Fraction alive versus time for N2 (orange), CB4856 (blue), and the *npr-1* loss-of-function allele *ad609* (red) at 21% oxygen (solid lines) and 10% oxygen (dotted lines).

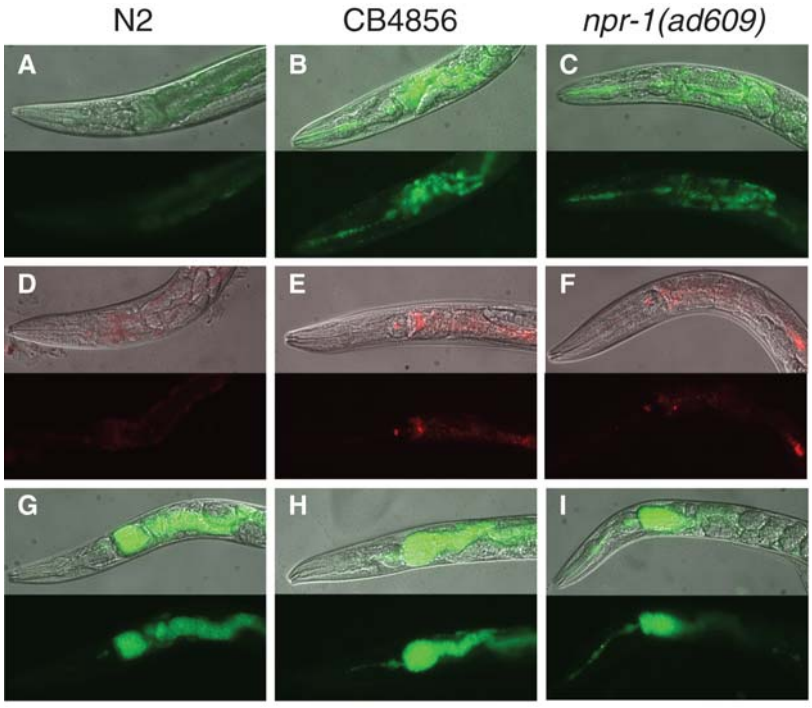


**Fig. 3.** *npr-1*–mediated behaviors cause increased exposure to bacteria. Differential interference contrast and fluorescence microscopy of N2, CB4856, and *npr-1(ad609)* animals fed GFP-labeled PA14 for 24 hours on a standard lawn (**A** to **C**) or fed a 50:1 mixture of unlabeled PA14 and 0.2-μm fluorescent beads (**D** to **F**) or on a big lawn of GFP-labeled PA14 (**G** to **I**).

decreasing their exposure to bacterial pathogens and leading to greater survival. Whether this trade-off would be beneficial in nature is debatable, as increased avoidance of potentially pathogenic bacteria may also decrease consumption of bacterial food. We speculate that the maintenance of the 215V allele of *npr-1* may have depended on laboratory propagation of N2 in plentiful sources of bacterial food that can be pathogenic, whereas in the wild, maximizing consumption of scarce food might be the preferred strategy.

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Figs. S1 to S7
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# The Structure of Rat Liver Vault at 3.5 Angstrom Resolution

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Vaults are among the largest cytoplasmic ribonucleoprotein particles and are found in numerous eukaryotic species. Roles in multidrug resistance and innate immunity have been suggested, but the cellular function remains unclear. We have determined the x-ray structure of rat liver vault at 3.5 angstrom resolution and show that the cage structure consists of a dimer of half-vaults, with each half-vault comprising 39 identical major vault protein (MVP) chains. Each MVP monomer folds into 12 domains: nine structural repeat domains, a shoulder domain, a cap-helix domain, and a cap-ring domain. Interactions between the 42-turn-long cap-helix domains are key to stabilizing the particle. The shoulder domain is structurally similar to a core domain of stomatin, a lipid-raft component in erythrocytes and epithelial cells.

Vaults are large barrel-shaped ribonucleoprotein particles that are highly conserved in a wide variety of eukaryotes (*1*). Although several functions have been proposed for vaults since their discovery in 1986 (*2*–*10*), including roles in multidrug resistance, cell signaling, and innate immunity, their cellular function remains unclear. Most vault particles are present in the cytoplasm, but a few of them localize to the nucleus (*11*,*12*). Rat liver vault comprises a small untranslated RNA consisting of 141 bases (vRNA) (*13*) and three proteins. The sequences of these proteins are known for the human vault. The 99-kD major vault protein (MVP, Swiss-Prot entry Q14764) is the major structural protein and can self-assemble to form vault-like particles (*14*); the 193-kD vault poly(adenosine diphosphate–ribose) polymerase (VPARP, Swiss-Prot entry Q9UKK3) presumably ribosylates substrates (*15*); and the

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