

Long-Lived *C. elegans* *daf-2* Mutants Are Resistant to Bacterial Pathogens

Danielle A. Garsin,^{1,3} Jacinto M. Villanueva,^{1,3} Jakob Begun,^{1,3}
Dennis H. Kim,^{1,3} Costi D. Sifri,^{2,4} Stephen B. Calderwood,^{2,4}
Gary Ruvkun,^{1,3} Frederick M. Ausubel^{1,3*}

Our laboratories have studied the mechanisms of aging (1, 2) and immune function (3) in *Caenorhabditis elegans*. Herein we show that the mechanisms that govern these two processes may be interrelated.

The human Gram-negative bacterial pathogens *Pseudomonas aeruginosa* and *Salmonella enterica* and the Gram-positive pathogens *Enterococcus faecalis* and *Staphylococcus aureus* kill *C. elegans* by an infection-like process with remarkable overlap between the bacterial factors required for virulence in mammals and killing in nematodes (4, 5). Additionally, a p38 MAPK (mitogen-activated protein kinase) signaling cascade is a key component of the *C. elegans* innate immune response, as it is in mammals (3). These experiments establish *C. elegans* as a useful model for studying bacterial pathogenicity and host immunity. Here we show that certain long-lived *C. elegans* mutants are highly resistant to killing by bacterial pathogens.

To investigate the general relation between longevity and pathogen resistance, we tested whether *C. elegans* *daf-2* and *age-1* mutants exhibit enhanced resistance to *E. faecalis*, *S. aureus*, and *P. aeruginosa*. *daf-2* encodes an insulin-like receptor that functions upstream of the phosphatidylinositol 3-kinase (PI 3-kinase) encoded by *age-1*, and partial loss of function mutations in *daf-2* or *age-1* result in a long-lived phenotype (1). Both *daf-2* and *age-1* mutants were resistant to killing by *E. faecalis*, *S. aureus*, and *P. aeruginosa* (Fig. 1A; table S1). Most dramatic was the five- and sixfold increased survival of the *daf-2(e1370)* mutants relative to wild-type *C. elegans* when exposed to the Gram-positive pathogens *E. faecalis* and *S. aureus*, respectively (Fig. 1A; table S1).

Life-span extension through the DAF-2 insulin-signaling pathway in *C. elegans* occurs by de-repression of the forkhead transcription factor DAF-16, which is normally under negative regulation by DAF-2. Therefore, strong loss-of-function alleles of *daf-16* such as *mgDf47* suppress the long-lived phenotype of *daf-2* mutants (2). *daf-16(mgDf47)* also suppressed the pathogen-resistant phenotype of *daf-2(e1370)* (Fig. 1A; table S1). Interestingly, the *daf-16* mutant exhibited a comparable degree of susceptibility to pathogen-mediated killing as wild-type worms (Fig. 1A;

table S1) under the experimental conditions assayed. In these experiments, the *daf-2(e1370)* allele was more resistant to bacterial pathogens than the *daf-2(1368)* allele and the *age-1(hx546)* allele (Fig. 1A; table S1). This is most likely a consequence of differences in allele strengths of the *daf-2* and *age-1* mutants, all of which are partial loss-of-function mutants.

Because *C. elegans* *daf-2* and *age-1* mutants were identified in screens using *Escherichia coli* strain OP50 as the food source and because *E. coli* may also be pathogenic to *C. elegans* (5–7), the enhanced longevity phenotype of *daf-2* and *age-1* could reflect acquired resistance to *E. coli*-mediated killing. Indeed, nematodes lived considerably longer when feeding on the Gram-positive bacterium *Bacillus subtilis*, a common soil bacterium that *C. elegans* is likely to feed on in the wild (Fig. 1B) (8). It is not likely that *B. subtilis* is simply more nutritious or more readily digestible than *E. coli* because the rate of growth, egg to egg generation time, and number of eggs laid were the same whether feeding on *B. subtilis* or *E. coli* (fig. S1).

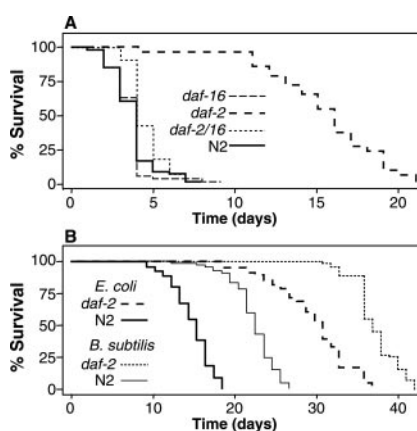


Fig. 1. Pathogen resistance of the *C. elegans* *daf-2(e1370)* mutant. (A) Survival of N2, *daf-2(e1370)*, *daf-16(mgDf47)*, and *daf-2;daf-16* *C. elegans* feeding on *E. faecalis* strain OG1RF. (B) Adult life-span of N2 and *daf-2(e1370)* *C. elegans* when feeding on *B. subtilis* (PY79) or *E. coli* (OP50) grown on NG (nematode growth) medium with FUdR (5-Fluoro-2-deoxyuridine). Assays were carried out as previously described (2, 5). STATA 6 statistical software (Stata, College Station, TX) was used to plot survival by the Kaplan-Meier method.

The *daf-2* mutant retained extended longevity relative to wild-type mutants when propagated on *B. subtilis* (Fig. 1B), but the fractional extension in life-span was modest ($76 \pm 9\%$) compared with the fractional extension observed on pathogenic bacteria [*E. faecalis*, $325 \pm 57\%$; *S. aureus*, $514 \pm 189\%$; *P. aeruginosa*, $118\% \pm 14\%$; and *E. coli*, $110 \pm 11\%$ (9)]. The *age-1* mutant also lived longer on *B. subtilis* (table S1). The extended survival of the *daf-2* and *age-1* mutants on the relatively innocuous *B. subtilis* suggests that additional factors beyond pathogen resistance are likely involved in life-span regulation. However, when feeding on pathogenic bacteria, especially Gram-positive ones, mechanisms underlying pathogen resistance appear to be the dominant contributor to the overall longevity of *daf-2* and *age-1* mutants.

Our data suggest that the insulin signaling pathway modulates both inherent longevity and pathogen resistance to affect overall survival in a manner dependent on the pathogenicity of the bacteria on which *C. elegans* is feeding. The linkage of longevity and pathogen resistance to the same signaling pathway may have general relevance to the observation that most organisms become more susceptible to infection as they age.

References and Notes

1. C. E. Finch, G. Ruvkun, *Annu. Rev. Genomics Hum. Genet.* **2**, 435 (2001).
2. C. A. Wolkow, K. D. Kimura, M.-S. Lee, G. Ruvkun, *Science* **290**, 147 (2000).
3. D. H. Kim *et al.*, *Science* **297**, 623 (2002).
4. A. Aballay, F. M. Ausubel, *Curr. Opin. Microbiol.* **5**, 97 (2002).
5. D. A. Garsin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10892 (2001).
6. D. Garigan *et al.*, *Genetics* **161**, 1101 (2002).
7. G. V. Mallo *et al.*, *Curr. Biol.* **12**, 1209 (2002).
8. The difference in life-span between feeding on *E. coli* OP50 and *B. subtilis* PY79 was particularly dramatic when the longevity assays were carried out at 27°C rather than at 25°C; the worms lived almost 100% longer on *B. subtilis* than they did on *E. coli* (table S1).
9. Fractional life-span extension was calculated based on the LT_{50} values (amount of time for 50% of worms to die) shown in table S1.
10. We thank S. Lee and R. Feinbaum for helpful discussions and critical reading of the manuscript. D.A.G. and J.M.V. are supported by postdoctoral fellowships from the Irvington Institute for Immunological Research. C.D.S. and D.H.K. are supported by postdoctoral fellowships from the Howard Hughes Medical Institute. This work was supported by NIH grant GM48707 (to F.M.A.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5627/1921/DC1

Fig. S1
Table S1

5 November 2002; accepted 28 February 2003

¹Department of Genetics, ²Department of Microbiology and Molecular Genetics, Harvard Medical School, ³Department of Molecular Biology, ⁴Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA 02114, USA.

*To whom correspondence should be addressed. E-mail: Ausubel@molbio.mgh.harvard.edu