Vol 463|25 February 2010|doi:10.1038/nature08762

LETTERS

An essential role for XBP-1 in host protection against immune activation in *C. elegans*

Claire E. Richardson[1], Tristan Kooistra[1] & Dennis H. Kim[1]

The detection and compensatory response to the accumulation of unfolded proteins in the endoplasmic reticulum (ER), termed the unfolded protein response (UPR), represents a conserved cellular homeostatic mechanism with important roles in normal development and in the pathogenesis of disease[1]. The IRE1–XBP1/Hac1 pathway is a major branch of the UPR that has been conserved from yeast to human[2–6]. X-box binding protein 1 (XBP1) is required for the differentiation of the highly secretory plasma cells of the mammalian adaptive immune system[7,8], but recent work also points to reciprocal interactions between the UPR and other aspects of immunity and inflammation[9–11]. We have been studying innate immunity in the nematode *Caenorhabditis elegans*, having established a principal role for a conserved PMK-1 p38 mitogen-activated protein kinase (MAPK) pathway in mediating resistance to microbial pathogens[12]. Here we show that during *C. elegans* development, XBP-1 has an essential role in protecting the host during activation of innate immunity. Activation of the PMK-1-mediated response to infection with *Pseudomonas aeruginosa* induces the XBP-1-dependent UPR. Whereas a loss-of-function *xbp-1* mutant develops normally in the presence of relatively non-pathogenic bacteria, infection of the *xbp-1* mutant with *P. aeruginosa* leads to disruption of ER morphology and larval lethality. Unexpectedly, the larval lethality phenotype on pathogenic *P. aeruginosa* is suppressed by loss of PMK-1-mediated immunity. Furthermore, hyperactivation of PMK-1 causes larval lethality in the *xbp-1* mutant even in the absence of pathogenic bacteria. Our data establish innate immunity as a physiologically relevant inducer of ER stress during *C. elegans* development and indicate that an ancient, conserved role for XBP-1 may be to protect the host organism from the detrimental effects of mounting an innate immune response to microbes.

We began our analysis of the role of the UPR in *C. elegans* immunity by asking if the UPR is activated upon exposure of *C. elegans* larvae to *P. aeruginosa* strain PA14, a human opportunistic pathogen that can also infect and kill *C. elegans*[13]. First, we assessed expression of the transgenic transcriptional reporter *Phsp-4::GFP(zcIs4)* (the *C. elegans hsp-4* gene encodes a homologue of mammalian BiP/GRP78), which reflects activation of the IRE-1–XBP-1 branch of the UPR[5]. After exposure of *C. elegans* larvae to *P. aeruginosa*, green fluorescent protein (GFP) expression was induced in the intestine, the site of infection (Fig. 1a). We confirmed the pathogen-induced activation of the endogenous *hsp-4* gene by quantitative polymerase chain reaction with reverse transcription (qRT–PCR) (Fig. 1b).

The mechanism by which IRE-1 activates XBP-1 in response to ER stress is conserved from yeast to mammals[2,4–6]. Upon accumulation of unfolded proteins in the ER, the integral ER-membrane protein IRE-1 activates XBP-1 by alternative splicing of the *xbp-1* mRNA, thereby changing the reading frame. The activated 'spliced form' of XBP-1 regulates expression of genes involved in ER homeostasis, such as those encoding chaperones. Using qRT–PCR to quantify the IRE-1-mediated splicing of *xbp-1* messenger RNA after exposure to *P. aeruginosa*, we found that there was a marked induction of IRE-1-spliced *xbp-1* transcript within 4 h of exposure to pathogen (Fig. 1c).

We hypothesized that the rapid activation of the IRE-1–XBP-1 pathway after *P. aeruginosa* exposure might be caused by induction of the host immune response. A conserved p38 MAPK pathway mediates innate immunity in organisms ranging from *C. elegans* to

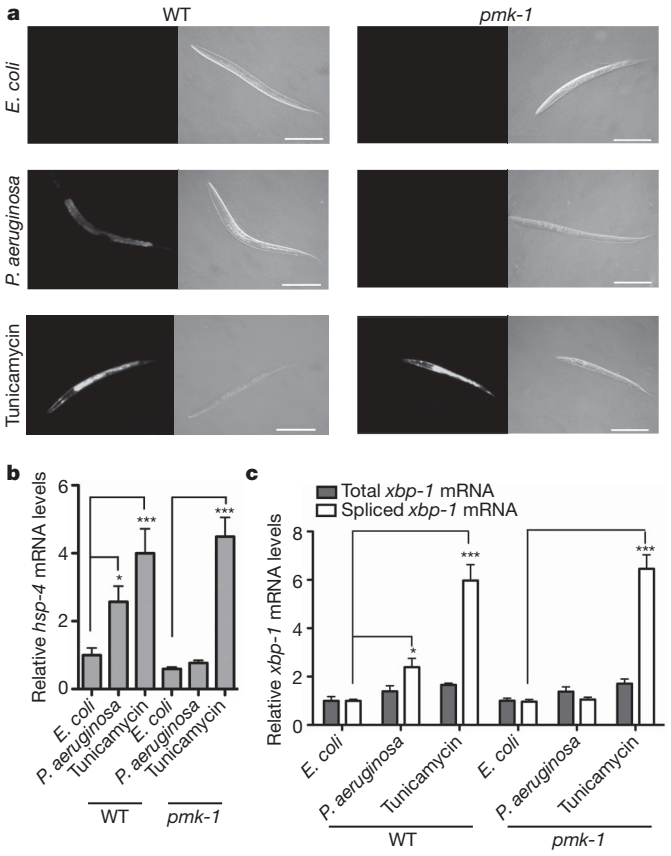


Figure 1 | PMK-1 p38 MAPK-dependent activation of the IRE-1–XBP-1-dependent UPR by *P. aeruginosa* infection. **a**, Fluorescence and differential interference contrast (DIC) microscopy of wild-type (WT) and mutant larvae carrying the *Phsp-4::GFP(zcIs4)* transgene 24 h after egg laying on indicated treatments. Scale bars, 100 μm. **b**, qRT–PCR of endogenous *hsp-4* mRNA levels. **c**, qRT–PCR analysis of total and spliced *xbp-1* mRNA levels. Values represent fold change relative to wild type on OP50 ± s.e.m. ($n = 4$ independent experiments, $^{*}P < 0.05$ and $^{***}P < 0.001$, two-way ANOVA with Bonferroni post test).

[1]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

©2010 Macmillan Publishers Limited. All rights reserved

humans[12,14]. We have previously shown that loss-of-function of the p38 MAPK orthologue PMK-1 results in markedly enhanced susceptibility to killing of *C. elegans* by *P. aeruginosa*. In the *pmk-1(km25)* mutant, we found no increase in IRE-1-spliced *xbp-1* mRNA and no induction of the *Phsp-4::GFP* reporter in response to *P. aeruginosa* (Fig. 1). RNA interference (RNAi) of *sek-1*, encoding a MAPKK required for PMK-1 activation[12], also abrogated induction of *Phsp-4::GFP* expression in response to *P. aeruginosa* infection (Supplementary Fig. 1a).

Consistent with the induction of XBP-1 splicing arising as a consequence of a transcriptional response to infection, we found that a mutant, *atf-7(qd22 qd130)*, carrying a mutation in a conserved transcription factor that abrogates the pathogen-induced expression of genes regulated by the PMK-1 pathway (R. Shivers *et al.*, manuscript submitted) also blocked induction of the *Phsp-4::GFP* reporter in response to *P. aeruginosa* PA14 (Supplementary Fig. 1b). Activation of the IRE-1–XBP-1 pathway in response to tunicamycin (an *N*-glycosylation inhibitor), however, occurred in the *pmk-1* mutant as in the wild-type strain N2 (hereafter referred to as wild type), as reported in a study of PMK-1-dependent activation of the IRE-1–XBP-1 pathway in the *C. elegans* response to a pore-forming toxin from *Bacillus thuringiensis*[15]. Whereas our data show that activation of the IRE-1–XBP-1 pathway by *P. aeruginosa* infection is dependent on the PMK-1 pathway, we did not observe a reciprocal dependence of activation of the PMK-1 pathway on XBP-1 (Supplementary Fig. 2).

We next asked if XBP-1 is required for resistance to *P. aeruginosa*, focusing on its role during larval development. Synchronized eggs of wild-type and mutants deficient in each of the three branches of the UPR were propagated on plates with *P. aeruginosa* PA14 as the only food source and development was monitored over time. Nearly all the eggs of the wild-type strain developed to at least the fourth larval stage (L4) in this assay, comparable to their growth and development on the relatively non-pathogenic bacterial strain and standard laboratory food source *Escherichia coli* OP50 (Fig. 2a). In contrast, the *xbp-1(zc12)* mutant on *P. aeruginosa* exhibited severely attenuated larval development and growth, as measured by the rate of progression between moults, size of larvae, and ability of larvae to reach the L4 stage by 72 h (Fig. 2a), eventually resulting in larval lethality. Loss-of-function mutations in each of the other two major branches of the UPR, *atf-6(ok551)* and *pek-1(ok275)*[4,16], did not affect larval development in the presence of *P. aeruginosa* (Fig. 2a). These data implicate a specific and essential role for the IRE-1–XBP-1 branch of the UPR in *C. elegans* development in the presence of *P. aeruginosa*.

The *xbp-1* developmental phenotype was alleviated in the presence of the *gacA* mutant of *P. aeruginosa* PA14 (Supplementary Fig. 3a), which has diminished pathogenicity in *C. elegans* and mammals[13,17]. On a lawn of wild-type *P. aeruginosa* PA14 mixed with relatively non-pathogenic *E. coli*, the *xbp-1* mutant again exhibited the severely attenuated developmental phenotype that was observed in the presence of *P. aeruginosa* alone, indicating that the pathogenicity of *P. aeruginosa*, not a nutritional deficiency, caused the *xbp-1* developmental phenotype (Fig. 2b and Supplementary Fig. 3b). Behavioural avoidance of pathogenic bacteria[18,19] can influence survival[20–22], but we found that the development of wild type was unaffected by experimental conditions in which the *P. aeruginosa* could not be avoided, demonstrating that the inability of the *xbp-1* mutant to develop on *P. aeruginosa* was not due to a defect in behavioural avoidance (C.E.R. and D.H.K., unpublished data). In addition, the attenuated development and death of the *xbp-1* mutant on *P. aeruginosa* was also unaffected by the blocking of cell necrosis (Supplementary Table 1).

Wild-type and *xbp-1* mutant larvae show characteristic ribosome-studded tubular structures of the ER by transmission electron microscopy when propagated on relatively non-pathogenic *E. coli* OP50 (Fig. 2c). The ER architecture of wild-type worms was not markedly perturbed upon exposure to *P. aeruginosa* (Fig. 2c). In contrast, *xbp-1(zc12)* larvae propagated on *P. aeruginosa* PA14 revealed disruption in ER morphology of *xbp-1* mutant worms upon pathogen exposure

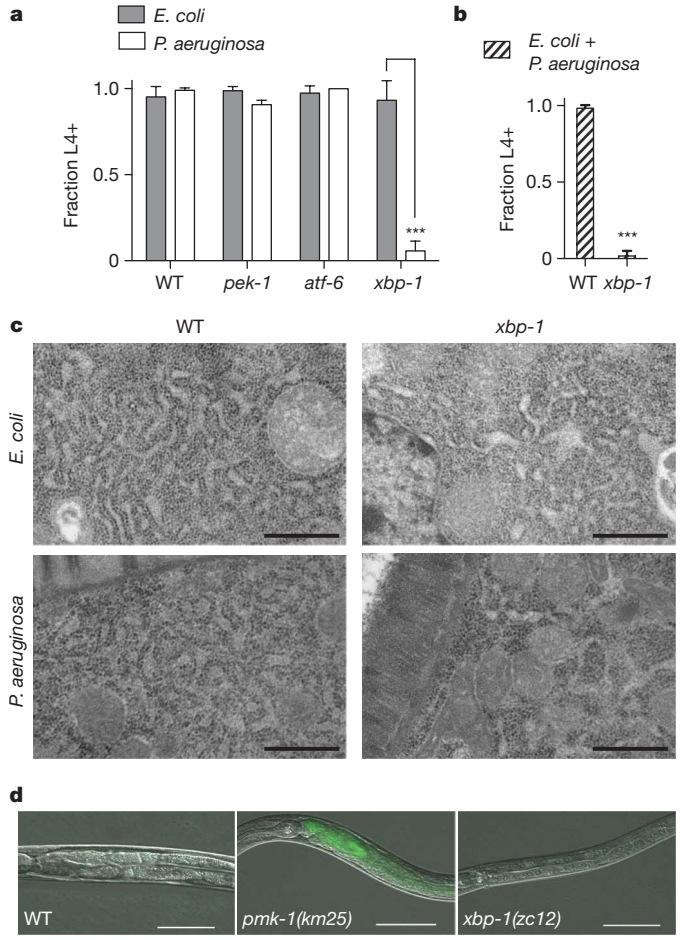


Figure 2 | XBP-1 is required for *C. elegans* development and survival on *P. aeruginosa*. **a**, **b**, Development of indicated mutants to the L4 larval stage or older after 3 days at 25 °C on either *P. aeruginosa* PA14 or *E. coli* OP50 (**a**), or a mixture of PA14 and *E. coli* HB101 (**b**). Plotted is mean ± s.d. ($n = 3$–4 plates, ***$P < 0.001$, two-way ANOVA with Bonferroni post test in **a**; $n = 4$ plates, *** $P < 0.001$ two-tailed *t*-test in **b**). Results in **a** and **b** are representative of three independent experiments. **c**, Transmission electron microscopy of L3 larvae at ×60,000 magnification. Scale bars, 500 nm. **d**, Fluorescence and DIC microscopy overlay of larvae 40 h after egg laying on *P. aeruginosa* PA14 expressing GFP.

(Fig. 2c), with localized regions of dilated ER lumen, comparable to that induced by tunicamycin treatment of *xbp-1(zc12)* worms (Supplementary Fig. 4). Such changes in ER morphology are consistent with ER stress and perturbation[23], and indicate that a disruption in ER homeostasis is induced by *P. aeruginosa* PA14 infection in the *xbp-1* mutant.

We considered whether the developmental lethality of the *xbp-1* mutant might be attributable to the toxicity of PA14 arising from accelerated infection due to diminished resistance to *P. aeruginosa* in the *xbp-1* mutant. Because activation of the PMK-1 pathway results in the splicing of XBP-1 upon *P. aeruginosa* exposure, we first asked whether XBP-1 confers a survival benefit against *P. aeruginosa* by facilitating resistance to intestinal infection. Diminished activation of PMK-1-dependent innate immunity is accompanied by an increased rate of *P. aeruginosa* accumulation in the intestine[12](Fig. 2d). If XBP-1 functioned downstream of PMK-1 to promote the immune response to *P. aeruginosa*, we would anticipate that *xbp-1* deficiency would lead to an increased rate of *P. aeruginosa* accumulation relative to wild type. Interestingly, in spite of the severely attenuated development and survival of the *xbp-1* mutant larvae on *P. aeruginosa*, the kinetics of accumulation of a PA14-derived strain of *P. aeruginosa* expressing GFP in the *xbp-1(zc12)* mutant were comparable to that observed for wild type (Fig. 2d). Although the *pmk-1(km25)* mutant exhibited an increased

©2010 Macmillan Publishers Limited. All rights reserved

rate of accumulation of *P. aeruginosa* (Fig. 2d), larval development and survival was markedly greater than that observed for the *xbp-1* mutant (Fig. 3a, b and Supplementary Fig. 5). These data decouple enhanced susceptibility to *P. aeruginosa* infection from the attenuated larval development phenotype of the *xbp-1* mutant and indicate that although activation of XBP-1 by *P. aeruginosa* infection is PMK-1-dependent, the primary protective role of XBP-1 is not to facilitate the killing of *P. aeruginosa*.

In view of these data, we considered whether the XBP-1-dependent UPR functions in this setting to protect against the ER stress induced by the immune response itself. If this were the case, then diminishing the immune response might actually improve the survival of the *xbp-1* mutant on *P. aeruginosa*. To test this hypothesis, we examined the larval development of a *xbp-1(zc12);pmk-1(km25)* double mutant. Notably, the *xbp-1;pmk-1* double mutant showed markedly increased development and survival relative to the *xbp-1* mutant, to a degree that was comparable to the development and survival of the *pmk-1* mutant (Fig. 3a, b). RNAi of *sek-1* and RNAi of *atf-7* in the *xbp-1(zc12)* mutant each alleviated the attenuated development phenotype in the presence of *P. aeruginosa* (Supplementary Fig 6a, b). These data indicate that activation of the PMK-1-dependent transcriptional innate immune response to pathogen is indeed detrimental to survival during development in the absence of the IRE-1–XBP-1 pathway, and that the principal mechanism by which XBP-1 promotes development and survival during infection with *P. aeruginosa* is by protecting against the innate immune response.

To separate further the effect of toxicity caused by pathogenic *P. aeruginosa* from that caused specifically by immune activation on the *xbp-1* developmental phenotype, we next asked if the *xbp-1* mutant would be compromised in the setting of hyperactivation of the PMK-1-mediated immune response in the absence of *P. aeruginosa*. RNAi against *vhp-1*, which encodes a MAPK phosphatase that negatively regulates PMK-1, results in hyperactivation of PMK-1 (ref. 24). We observed that RNAi of *vhp-1* resulted in sustained induction of the *Phsp-4::GFP(zcIs4)* reporter with the relatively non-pathogenic, standard laboratory food source *E. coli* OP50 as the only bacterium present in the assay (Fig. 4a). RNAi-mediated knockdown of *vhp-1* had little effect on development of wild-type worms (Fig. 4b). In contrast, RNAi of *vhp-1* severely impaired development of the *xbp-1(zc12)* mutant in the absence of pathogenic bacteria (Fig. 4b) in a manner similar to that observed for the *xbp-1* mutant when propagated on *P. aeruginosa* (Fig. 2a). Both the induction of *hsp-4* and the larval developmental phenotype of the *xbp-1* mutant resulting from RNAi of *vhp-1* were suppressed by *pmk-1* (Fig. 4a, b), demonstrating that the observed effects of RNAi of *vhp-1* were mediated by the PMK-1-dependent immune response.

Our data indicate that the XBP-1-mediated UPR has an essential role during *C. elegans* larval development by protecting against the activation of innate immunity. Transcriptional profiling of the *C. elegans* response to infection with *P. aeruginosa*[25,26] has revealed the induction of at least 300 genes[25], nearly half of which are predicted to be trafficked through the ER. Although this innate immune response promotes pathogen resistance, retarding the intestinal accumulation of *P. aeruginosa* and substantially increasing the fraction of the population that survives through larval development (as shown by the survival of wild type relative to the *pmk-1(km25)* mutant (Fig. 3 and Supplementary Fig. 5)), our data suggest that innate immunity also constitutes a physiologically relevant source of ER stress that

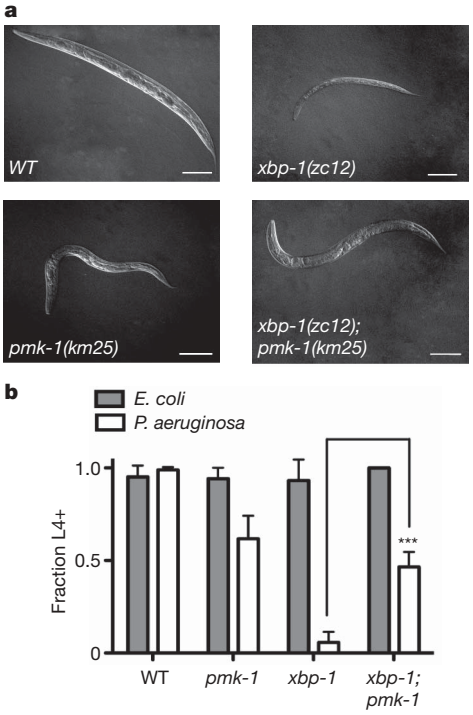


Figure 3 | Suppression of the *P. aeruginosa*-induced larval lethality phenotype of *xbp-1(zc12)* by *pmk-1(km25)*. **a**, DIC microscopy of representative worms of each genotype 3 days after egg laying on *P. aeruginosa* PA14. Scale bars, 100 μm. **b**, Development of indicated mutants to the L4 larval stage or older after 3 days at 25 °C on either *P. aeruginosa* PA14 or *E. coli* OP50. Plotted is the mean ± s.d. ($n = 3$–4 plates, $^{***}P < 0.001$, two-way ANOVA with Bonferroni post test). Representative results of three independent experiments are shown.

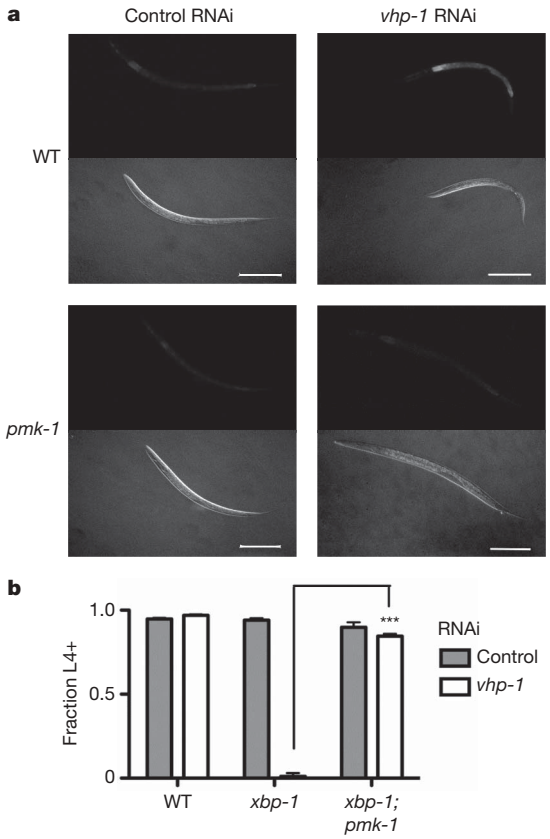


Figure 4 | XBP-1 is required for development and survival during pathogen-independent constitutive activation of PMK-1. **a**, Fluorescence and DIC microscopy of wild-type and *xbp-1* larvae carrying the *Phsp-4::GFP(zcIs4)* transgene, which have been treated with control (empty vector) or *vhp-1* RNAi, 24 h after egg laying. Scale bars, 100 μm. **b**, Development to the L4 larval stage or older after 3 days at 25 °C of wild-type, *xbp-1(zc12)* and *xbp-1(zc12);pmk-1(km25)* mutants that have been subjected to RNAi of *vhp-1* and propagated on *E. coli* OP50. Plotted is mean ± s.e.m. ($n = 3$ independent experiments, $^{***}P < 0.001$, two-way ANOVA with Bonferroni post test).

©2010 Macmillan Publishers Limited. All rights reserved

necessitates the compensatory activity of the UPR in *C. elegans* larval growth and development. An ancient role for the UPR may be to facilitate the development and survival of metazoan cell types that are exposed to the environment and mount a defensive, secretory response to microbial pathogens and abiotic toxins[4,27]. Our data not only support this hypothesis, but more specifically indicate that in fact the essential role of XBP-1 lies not in the neutralization of these environmental insults, but instead in conferring protection against the potentially lethal ER stress that is induced by this response. Our findings support the idea that the microbiota of multicellular organisms may represent the most commonly encountered and physiologically relevant inducer of such ER stress. Consistent with this hypothesis is the Paneth cell death observed in *Xbp1* intestinal knockout mice[10]. Evolutionary conservation of the function of XBP-1 that we have defined in *C. elegans* would implicate the UPR in protection against ER toxicity caused by activation of immune and inflammatory pathways in mammals, where the UPR may have a critical role in the pathogenesis of infectious and inflammatory autoimmune diseases.

METHODS SUMMARY

Caenorhabditis elegans strains were maintained as described[28]. Strains used in this study were N2 Bristol wild-type strain, SJ4005 *Phsp-4::GFP(zcIs4)*, ZD305 *pmk-1(km25);Phsp-4::GFP(zcIs4)*, ZD441 *atf-7(qd22 qd130);Phsp-4::GFP(zcIs4)*, ZD361 *xbp-1(zc12)*, RB545 *pek-1(ok275)*, RB772 *atf-6(ok551)*, ZD363 *xbp-1(zc12);pmk-1(km25)*, ZD416 *xbp-1(tm2457)*, ZD417 *xbp-1(tm2457);pmk-1(km25)*, ZD418 *xbp-1(tm2482)* and ZD419 *xbp-1(tm2482);pmk-1(km25)*. Strain construction is detailed in Methods. All *P. aeruginosa* (strain PA14) plates were prepared as described[13]; exceptions are detailed in Methods. For tunicamycin treatments, tunicamycin was added to *E. coli* plates at 5 $\mu g\,ml^{-1}$ final concentration. Images were acquired with an Axioimager Z1 microscope using worms anaesthetized in 0.1% sodium azide. Transmission electron microscopy was performed as described in Methods. For RNA analysis, L1 larvae were synchronized by hypochlorite treatment, washed onto OP50 plates and grown at 20 °C for 23 h on OP50, then washed in M9 onto treatment plates. After 4 h, worms were washed off plates and frozen in liquid nitrogen. RNA and qRT–PCR methods are detailed in Methods. For development assays, *E. coli* OP50 plates were prepared in parallel with PA14 plates. Strains were egg laid in parallel onto four plates each of PA14 and OP50 (at least 80 eggs for each strain and treatment), and the fraction of worms growing to at least the L4 larval stage (L4+) between the plates was averaged. The L4 stage of development was chosen owing to the ease of scoring vulval development. RNAi by feeding of bacteria[29] was performed as described by propagating stains on *E. coli* HT115 carrying the RNAi clones as indicated at 20 °C, and adult progeny were used to lay eggs in experiments, which were performed at 25 °C. Details regarding RNAi clones are in Methods. Statistical analysis was performed using GraphPad Prism (GraphPad Software).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 2 June; accepted 15 December 2009.

1. Schröder, M. & Kaufman, R. J. The mammalian Unfolded Protein Response. *Annu. Rev. Biochem.* **74,** 739–789 (2005).
2. Cox, J. S. & Walter, P. A novel mechanism for regulating activity of a transcription factor that controls the Unfolded Protein Response. *Cell* **87,** 391–404 (1996).
3. Mori, K., Kawahara, T., Yoshida, H., Yanagi, H. & Yura, T. Signalling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the Unfolded Protein Response pathway. *Genes Cells* **1,** 803–817 (1996).
4. Shen, X. *et al.* Complementary signaling pathways regulate the Unfolded Protein Response and are required for *C. elegans* development. *Cell* **107,** 893–903 (2001).
5. Calfon, M. *et al.* IRE1 couples endoplasmic reticulum load to secretory capacity by processing the *xbp-1* mRNA. *Nature* **415,** 92–96 (2002).
6. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. & Mori, K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107,** 881–891 (2001).
7. Reimold, A. M. *et al.* Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **412,** 300–307 (2001).
8. Iwakoshi, N. N. *et al.* Plasma cell differentiation and the Unfolded Protein Response intersect at the transcription factor XBP-1. *Nature Immunol.* **4,** 321–329 (2003).
9. Todd, D. J., Lee, A.-H. & Glimcher, L. H. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nature Rev. Immunol.* **8,** 663–674 (2008).
10. Zhang, K. *et al.* Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* **124,** 587–599 (2006).
11. Kaser, A. *et al.* XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* **134,** 743–756 (2008).
12. Kim, D. H. *et al.* A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297,** 623–626 (2002).
13. Tan, M.-W., Mahajan-Miklos, S. & Ausubel, F. M. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl Acad. Sci. USA* **96,** 715–720 (1999).
14. Dong, C., Davis, R. J. & Flavell, R. A. MAP kinases in the immune response. *Annu. Rev. Immunol.* **20,** 55–72 (2002).
15. Bischof, L. J. *et al.* Activation of the Unfolded Protein Response is required for defenses against bacterial pore-forming toxin *in vivo*. *PLoS Pathog.* **4,** e1000176 (2008).
16. Shen, X., Ellis, R. E., Sakaki, K. & Kaufman, R. J. Genetic interactions due to constitutive and inducible gene regulation mediated by the Unfolded Protein Response in *C. elegans*. *PLoS Genet.* **1,** e37 (2005).
17. Rahme, L. G. *et al.* Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268,** 1899–1902 (1995).
18. Zhang, Y., Lu, H. & Bargmann, C. I. Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438,** 179–184 (2005).
19. Pujol, N. *et al.* A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11,** 809–821 (2001).
20. Reddy, K. C., Anderson, E. C., Kruglyak, L. & Kim, D. H. A polymorphism in *npr-1* is a behavioral determinant of pathogen susceptibility in *C. elegans*. *Science* **323,** 382–384 (2009).
21. Shivers, R. P., Kooistra, T., Chu, S. W., Pagano, D. J. & Kim, D. H. Tissue-specific activities of an immune signaling pathway regulate physiological responses to pathogenic and nutritional bacteria in *C. elegans*. *Cell Host Microbe* **6,** 321–330 (2009).
22. Styer, K. L. *et al.* Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science* **322,** 460–464 (2008).
23. Rutkowski, D. T. *et al.* Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. *PLoS Biol.* **4,** e374 (2006).
24. Kim, D. H. *et al.* Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. *Proc. Natl Acad. Sci. USA* **101,** 10990–10994 (2004).
25. Troemel, E. R. *et al.* p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet.* **2,** e183, doi:10.1371/journal.pgen.0020183 (2006).
26. Shapira, M. *et al.* A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc. Natl Acad. Sci. USA* **103,** 14086–14091 (2006).
27. Kaufman, R. J. *et al.* The Unfolded Protein Response in nutrient sensing and differentiation. *Nature Rev. Mol. Cell Biol.* **3,** 411–421 (2002).
28. Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77,** 71–94 (1974).
29. Timmons, L. & Fire, A. Specific interference by ingested dsRNA. *Nature* **395,** 854 (1998).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Electron microscopy was performed by M. McKee in the Microscopy Core of the Center for Systems Biology/Program in Membrane Biology at Massachusetts General Hospital (supported by NIH grants DK43351 and DK57521). We thank E. Hartwieg and G. Voeltz for discussions regarding the interpretation of electron microscopy images. We thank T. Stiernagle and the Caenorhabditis Genetics Center (supported by the NIH), and S. Mitani and the National Bioresource Project of Japan for strains. T.K. was supported by summer research fellowships from the Howard Hughes Medical Institute. This work was supported by NIH grant R01-GM084477, a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund, and an Ellison Medical Foundation New Scholar Award (to D.H.K.).

Author Contributions C.E.R. and D.H.K. conceived and planned experiments. C.E.R. and T.K. performed experiments. C.E.R. and D.H.K. analysed the data and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.H.K. (dhkim@mit.edu).

©2010 Macmillan Publishers Limited. All rights reserved

METHODS

Strain construction. To make ZD361 and ZD363, SJ17 *xbp-1(zc12)* was outcrossed twice, removing *Phsp-4::GFP (zcIs4)*, then crossed to ZD39 *pT24B8.5::GFP(agIs9)III;pmk-1(km25)* to isolate a *xbp1(zc12);pmk-1(km25)* double mutant. This *xbp-1(zc12);pmk-1(km25)* double mutant was backcrossed to ZD39 to isolate 4× backcrossed *xbp-1(zc12);pmk-1(km25)* and *xbp-1(zc12)*. To make ZD416, ZD417, ZD418 and ZD419, strains carrying the *xbp-1(tm2457)* and *xbp-1(tm2482)* alleles were outcrossed four times, then crossed to ZD39 *pT24B8.5::GFP(agIs9)III;pmk1(km25)*, from which single *xbp-1* and double *xbp-1;pmk-1* strains were isolated.

Preparation of *P. aeruginosa* treatment plates. *P. aeruginosa* PA14 was grown in Luria Broth (LB). For RNA preparation and electron microscopy, 25 µl overnight culture was seeded onto 10-cm plates. To make plates with lawns of mixed bacteria, *E. coli* HB101 containing an ampicillin-resistant plasmid encoding GFP was grown in LB with 50 µg ml^{-1} ampicillin in parallel to *P. aeruginosa*, and the two cultures were mixed in equal parts before seeding 10 µl of the mix onto plates containing 25 µg ml^{-1} carbenicillin. The presence of *E. coli* in the lawns at the end of assays was confirmed by the expression of GFP. To visualize intestinal accumulation of *P. aeruginosa*, *P. aeruginosa* PA14 containing an ampicillin-resistant plasmid encoding GFP was grown in LB with 50 µg ml^{-1} ampicillin, and 10 µl of the culture was seeded onto plates containing 25 µg ml^{-1} carbenicillin and spread to cover the surface of the plate (to prevent the growth of patches of the lawn from which the GFP plasmid had been lost).

Transmission electron microscopy. Synchronized L1 larvae were grown on *E. coli* (OP50) or *P. aeruginosa* (PA14) at 25 °C to the L3 stage (Fig. 2c), or as described in the figure legend (Supplementary Fig. 4), then fixed as described[30]. The wild-type and *xbp-1(zc12)* strains were grown in parallel on *P. aeruginosa* and fixed twice in independent experiments with similar results.

RNA extraction, cDNA preparation and quantitative RT–PCR. Total RNA was isolated using Tri Reagent (Ambion), DNA contamination was removed with DNA-free treatment (Ambion). cDNA was synthesized with RETROscript kit (Ambion) using oligo(dT) primers. Quantitative real-time PCR reactions were performed in triplicate using FastStart SYBR green master mix (Roche) with a Realplex mastercycler (Eppendorf). Relative mRNA was determined with the $\Delta\Delta C_t$ method using expression of *snb-1* (Fig. 1b) or the mean expression of *snb-1*, *act-1* and *tba-1* as a normalization control (Fig. 1c and Supplementary Fig. 2)[25,31,32]. Biological replicates were compared as described[33]. Primer efficiencies were tested with dilution series, and primer sequences are provided in Supplementary Table 2.

RNAi methodology. The *vhp-1* RNAi clone and clones of genes required for necrosis were from the Ahringer library, and the *sek-1* RNAi clone was from the Vidal library[34,35]. A segment of the *atf-7* coding region, 771-bp region of *atf-7* corresponding to bases 11,532 to 12,303 with respect to cosmid C07G2, was amplified by PCR and subcloned into the Fire vector L4440 (R. Shivers *et al.*, manuscript submitted). The sequences of all clones were confirmed before use.

30. Troemel, E. R., Félix, M.-A., Whiteman, N. K., Barrière, A. & Ausubel, F. M. Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. *PLoS Biol.* **6,** e309 (2008).
31. Pocock, R. & Hobert, O. Oxygen levels affect axon guidance and neuronal migration in *Caenorhabditis elegans*. *Nature Neurosci.* **11,** 894–900 (2008).
32. Hoogewijs, D., Houthoofd, K., Matthijssens, F., Vandersompele, J. & Vanfleteren, J. R. Selection and validation of a set of reliable reference genes for quantitative *sod* gene expression analysis in *C. elegans*. *BMC Mol. Biol.* **9,** doi:10.1186/1471-2199-9-9 (2008).
33. Willems, E., Leyns, L. & Vandesompele, J. Standardization of real-time PCR gene expression data from independent biological replicates. *Anal. Biochem.* **379,** 127–129 (2008).
34. Kamath, R. S. *et al.* Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421,** 231–237 (2003).
35. Rual, J. F. *et al.* Toward improving *Caenorhabditis elegans* phenome mapping with an orfeome-based RNAi library. *Genome Res.* **14,** 2162–2168 (2004).

©2010 Macmillan Publishers Limited. All rights reserved