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The *C. elegans* CCAAT-enhancer binding protein gamma is required for surveillance immunity

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Abstract

Pathogens attack host cells by deploying toxins that perturb core host processes. Recent findings from the nematode *C. elegans* and other metazoans indicate that surveillance or 'effector-triggered' pathways monitor functioning of these core processes and mount protective responses when they are perturbed. Despite a growing number of examples of surveillance immunity, the signaling components remain poorly defined. Here we show that CEBP-2, the *C. elegans* ortholog of mammalian CCAAT-enhancer binding protein gamma, is a key player in surveillance immunity. We show that CEBP-2 acts together with the bZIP transcription factor ZIP-2 in the protective response to translational block by *P. aeruginosa* Exotoxin A, as well as to perturbations of other processes. CEBP-2 serves to limit pathogen burden, promote survival upon *P. aeruginosa* infection, and also promote survival upon Exotoxin A exposure. These findings may have broad implications for the mechanisms by which animals sense pathogenic attack and mount protective responses.

Introduction

The innate immune system serves to defend hosts against pathogen infection, without the need for prior exposure to these pathogens (Kumar et al., 2011). A key component of the innate immune system is the detection of molecules characteristic of pathogens, so-called Pathogen-Associated Molecular Patterns or PAMPs. Hosts use pattern recognition receptors that are tuned to detect these PAMPs and trigger defense, often through upregulation of

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Author Contributions

KCR, TLD, AMN, CMH and ERT designed the experiments. KCR, TLD, and AMN performed the experiments. KCR and ERT wrote the manuscript. CMH and ERT secured funding.

Supplemental Information

Supplemental Information contains four figures, and Supplemental Experimental Procedures.

immune response gene expression. However, PAMPs are usually molecules found in broad classes of microbes and do not necessarily represent the presence of a pathogenic microbe. For example, lipopolysaccharide is a PAMP found in Gram-negative bacterial species, both pathogenic and non-pathogenic alike. Thus, PAMPs may be more accurately defined as Microbe-Associated Molecular Patterns, or MAMPs. MAMPs provide hosts information about the presence of microbes, but not necessarily whether those microbes are pathogenic (Ausubel, 2005; Sanabria et al., 2010).

A growing theme in animal immunity is that hosts specifically detect pathogen attack with surveillance or 'effector-triggered' immune pathways, which detect the effects of pathogen-delivered toxins and virulence factors, rather than recognizing the molecular structure of the factors themselves (Cohen and Troemel, 2015; Rajamuthiah and Mylonakis, 2014; Spoel and Dong, 2012; Stuart et al., 2013). For example, many bacterial toxins inhibit host mRNA translation elongation (Beddoe et al., 2010; Lee et al., 2013; Lemaitre and Girardin, 2013; Lemichez and Barbieri, 2013; Mohr and Sonenberg, 2012), and these toxins are quite prevalent in the environment, with up to 29% of soil samples in one study harboring DNA for translation-blocking Shiga toxin (Casas et al., 2006). Translation-blocking toxins are made by diverse bacterial pathogens including *P. aeruginosa* (Iglewski et al., 1977) *Corynebacterium diphtheriae* (Pappenheimer, 1977), *Vibrio cholera* (Jorgensen et al., 2008), *Legionella pneumophila* (Belyi et al., 2006) *Shigella* spp, and Shiga toxin-producing *E. coli* (Pacheco and Sperandio, 2012). Because these toxins are diverse in structure, it is arguably an efficient defense strategy for hosts to detect the common block in translation elongation caused by these toxins to trigger defense.

Recent findings indicate that *C. elegans* uses surveillance pathways for defense against toxins delivered by the bacterial pathogen *P. aeruginosa* that block not only mRNA translation, but also mitochondria, the proteasome and histones (Dunbar et al., 2012; Liu et al., 2014; McEwan et al., 2012; Melo and Ruvkun, 2012; Pellegrino et al., 2014). *P. aeruginosa* causes a lethal intestinal infection in its nematode host, and in the early response to infection *C. elegans* upregulates mRNA expression of many defense genes, including candidate anti-microbial peptides, detoxifying enzymes and efflux pumps (Shapira et al., 2006; Troemel et al., 2006). We identified the bZIP transcription factor ZIP-2 as a key mediator of this infection-induced gene expression, and showed that it promotes a defense response (Estes et al., 2010). The transcriptional response to *P. aeruginosa* infection appears to be predominantly a response to pathogenicity, triggered in part by the translation-blocking Exotoxin A (ToxA) (Dunbar et al., 2012; Estes et al., 2010; McEwan et al., 2012). In previous studies we showed that *C. elegans* intestinal cells appear to endocytose ToxA, which blocks mRNA translation specifically in the intestine, and this block is sensed by the host to upregulate defense gene expression. Surprisingly, this translational block appears to trigger an increase in protein levels of ZIP-2, apparently through regulation in cis by an upstream open reading frame (Dunbar et al., 2012). Thus, ZIP-2 appears to function in effector-triggered immunity in *C. elegans* to respond to the translational block caused by *P. aeruginosa*-delivered ToxA. However, ZIP-2 does not have an obvious mammalian ortholog, which made it unclear which transcription factor might be involved in this type of immunity in mammals.

Here we show that ZIP-2 acts together with another bZIP transcription factor called CEBP-2 in *C. elegans* surveillance immunity. Intriguingly, CEBP-2 is the *C. elegans* ortholog of mammalian CCAAT-enhancer binding protein gamma (C/EBP-gamma), which plays a role in the acute response to infection and inflammation in mammals, together with other C/EBP transcription factors (Gao et al., 2002; Parkin et al., 2002; Tsukada et al., 2011), although its role in effector-triggered defense has not been shown. We show that CEBP-2 is required to upregulate a transcriptional response to ToxA in *C. elegans*, and promote defense against insult by this toxin as well as against pathogen infection. We also show that CEBP-2 is required for upregulation of defense gene expression in response to RNAi against genes that function in the mitochondria and transcription-related processes, and that perturbation of these processes increases levels of ZIP-2 protein, similar to perturbation of translation (Dunbar et al., 2012). Thus, ZIP-2/CEBP-2 appears to be a key transcription factor in *C. elegans* surveillance immunity that promotes defense against pathogenic microbes.

Results and Discussion

CEBP-2 is required for induction of ZIP-2-dependent genes in response to *P. aeruginosa* infection

Previously, we demonstrated that the bZIP protein ZIP-2 mediates induction of candidate defense genes and promotes survival upon *P. aeruginosa* infection (Estes et al., 2010). As bZIP proteins canonically act as dimers, we were interested in identifying a heterodimeric partner that could act together with ZIP-2 in *C. elegans* host defense. A comprehensive study of bZIP transcription factor protein-protein interactions *in vitro* identified *C. elegans C48E7.11* as the highest affinity binding partner for ZIP-2 (Reinke et al., 2013). *C48E7.11* is the top BLAST hit in the *C. elegans* genome for human CCAAT/enhancer binding protein gamma (C/EBPγ) transcription factor (NP_001797). It shares 37% amino acid identity with the human protein and has a similar domain structure, with most of the protein being composed of a bZIP domain. Therefore, we renamed *C48E7.11* CEBP-2, for CCAAT/enhancer binding protein 2.

We investigated whether CEBP-2 mediates a protective response to *P. aeruginosa* strain PA14 infection of *C. elegans*, which would support the hypothesis that CEBP-2 acts together with ZIP-2 as a heterodimeric transcription factor in mediating defense against infection *in vivo*. First, we examined whether *cebp-2* regulates expression of target genes known to be induced by *zip-2* upon infection. In particular, we examined *P. aeruginosa*-induced expression of a GFP reporter for infection response gene-1 (*irg-1p::GFP)* in *cebp-2*-deficient animals, using either *cebp-2* RNAi-treated animals or *cebp-2(tm5421)* mutant animals, and found greatly reduced induction of GFP compared to control, similar to that seen in worms lacking *zip-2* function (Figure 1A-F). This result indicates that *cebp-2*, like *zip-2*, regulates *irg-1* induction in response to *P. aeruginosa* infection.

We next confirmed that *cebp-2* controls pathogen induction of endogenous *irg-1* mRNA expression by using qRT-PCR to measure RNA levels in *cebp-2*-deficient animals. We found that *cebp-2* RNAi-treated animals had decreased induction of *irg-1* in response to *P. aeruginosa*, as compared to control, as well as decreased induction of two other *zip-2*-dependent genes, *F11D11.3* and *oac-32* (Figure 1G). However, induction of another *zip-2*-

dependent gene, infection response gene 2, *irg-2*, was not decreased in *cebp-2* RNAi-treated animals (Figure 1G). *cebp-2(tm5421)* mutant animals had a stronger phenotype, with greatly reduced mRNA induction of *irg-1*, *irg-2 F11D11.3* and *oac-32* (Figure 1H). We also tested a panel of infection response genes whose induction in response to *P. aeruginosa* infection does not require *zip-2* and found that most of these genes were induced normally in *cebp-2*-deficient animals (Figure 1G-H). The fact that *cebp-2* is required for induction of the infection response genes that also require *zip-2* for their induction supports the model that CEBP-2 and ZIP-2 work together as a transcription factor to mediate a transcriptional response to *P. aeruginosa* infection in *C. elegans*.

CEBP-2 and ZIP-2 promote resistance against *P. aeruginosa* infection

Our previous studies indicated that *zip-2*-mediated gene expression promotes a defense response, as *zip-2*-defective animals have modestly decreased survival upon infection with *P. aeruginosa* (Estes et al., 2010). To determine if *cebp-2* is also important for defense against killing by *P. aeruginosa*, we tested the survival of *cebp-2*-defective animals upon infection with *P. aeruginosa.* Indeed, we found that *cebp-2* RNAi-treated animals, like *zip-2* RNAi-treated animals, had a modest but significant decrease in survival upon infection (Figure 2A), indicating that *cebp-2*, like *zip-2*, promotes host defense. In addition, we found that *cebp-2* and *zip-2* mutants had a modest decrease in survival upon PA14 infection (Figure 2B). *cebp-2* mutants had slightly decreased survival compared to *zip-2* mutants, perhaps due to the decreased overall health of these animals (see results below). If however, *cebp-2* and *zip-2* were working together to regulate gene expression that promotes survival upon PA14 infection, then a *cebp-2;zip-2* mutant should not have a further decrease in survival compared to the *cebp-2* single mutant alone. Consistent with this model, we found that *cebp-2;zip-2* mutants did not have a greater decrease in survival compared to the single *cebp-2* mutant (Figure 2B).

We next investigated whether *zip-2* and *cebp-2* promote increased survival upon infection with *P. aeruginosa* by restricting pathogen accumulation in the intestine, or by improving tolerance of the pathogen (Medzhitov et al., 2012). To distinguish between these possibilities we measured fluorescence levels of *P. aeruginosa* PA14-dsRed (Djonovic et al., 2013) in animals deficient for either *zip-2* or *cebp-2* at 16 hours post-infection and found that *zip-2* and *cebp-2*-deficient animals accumulated significantly more intestinal PA14-dsRed than control animals (Figure 2C-F, S1A-D). This result indicates that both *zip-2* and *cebp-2* likely contribute to defense against killing by *P. aeruginosa* in part by controlling pathogen burden in the intestine. In addition, we found that the *cebp-2;zip-2* double mutant had a similar increase in PA14-dsRed levels in the intestine as the single mutants (Figure 2F). Together these results indicate that *zip-2* and *cebp-2* act to promote resistance to the pathogen *P. aeruginosa*, perhaps functioning together as a heterodimeric transcription factor to induce genes that limit pathogen accumulation in the intestine and promote survival upon infection.

CEBP-2 and ZIP-2 are both expressed in intestinal nuclei during *P. aeruginosa* infection

Previous studies indicated that much of the *P. aeruginosa*-mediated induction of infection response genes such as *irg-1* was due to pathogen-induced perturbation of core processes,

including inhibition of mRNA translation (Dunbar et al., 2012; McEwan et al., 2012). Indeed, a key trigger of *irg-1* induction appears to be the *P. aeruginosa* translational inhibitor Exotoxin A (ToxA), because heterologous expression of ToxA in non-pathogenic *E. coli* is sufficient to induce *irg-1* mRNA expression in *C. elegans*, in a *zip-2*-dependent manner (McEwan et al., 2012). The induction of *irg-1* mRNA upon infection is likely mediated by an increase in ZIP-2 protein levels, which could then serve to increase *irg-1* transcription. Indeed, ZIP-2 protein levels increase upon *P. aeruginosa* infection and also with pharmacological inhibition of translation by the elongation inhibitor cycloheximide (Dunbar et al., 2012). Consistent with this model, we show here that ZIP-2 protein levels increase upon exposure to ToxA. Animals carrying a *ZIP-2::GFP* transgene had virtually no GFP expression when feeding on *E. coli* carrying the empty expression vector, but had strong GFP expression with nuclear localization in intestinal cells when feeding on *E. coli* expressing ToxA (Figure S2A-B,E).

We next investigated whether *cebp-2* was required for the increased ZIP-2 protein levels seen after exposure to ToxA, because one possible explanation for the similar defects seen in *zip-2* and *cebp-2*-deficient animals in response to *P. aeruginosa* infection is that *cebp-2* is required for ZIP-2 protein expression. However, we found that *cebp-2* was not required for ZIP-2 protein expression in response to ToxA, as *cebp-2* RNAi-treated animals had robust induction of *ZIP-2::GFP* in intestinal nuclei after feeding on *E. coli* expressing ToxA (Figure S2C-E). This result indicates that the similar phenotypes of *zip-2* and *cebp-2*-deficient animals are not due to regulation of ZIP-2 expression by CEBP-2.

If CEBP-2 and ZIP-2 function together in the response to *P. aeruginosa* infection, these proteins should be expressed at the same time and in the same location. To test this model, we generated a transgene that contains 1.1 kb of genomic DNA upstream of the predicted *cebp-2* ATG start site followed by the *cebp-2* genomic coding region with GFP fused to the C terminus. We found that animals carrying this *CEBP-2::GFP* transgene express GFP broadly in somatic tissues including the intestine, with strong nuclear localization (Figure 3A). We did not see any change in *CEBP-2::GFP* expression or localization in animals infected with *P. aeruginosa* (Figure 3B), indicating that CEBP-2 is constitutively expressed, unlike ZIP-2. In addition, we did not find that *zip-2* was required for CEBP-2 expression, as *CEBP-2::GFP* expression did not change after *zip-2* RNAi treatment (Figure 3C-D). Furthermore, there was not a change in *cepb-2* mRNA expression (or other genes in the *cebp-2* operon) after *zip-2* RNAi (Figure 3E), further supporting the conclusion that *zip-2* is not required for *cebp-2* expression.

To confirm that the *CEBP-2::GFP* expression construct was functional, and thus likely to reflect endogenous expression of CEBP-2 protein, we analyzed whether it could rescue the *cebp-2* mutant phenotype. Indeed, we found that this CEBP-2::GFP construct could rescue the defects in gene induction in response to PA14 in the *cebp-2(tm5421)* mutant (Figure 3F). This result also confirms that the *cepb-2* gene induction phenotype in the *cebp-2(tm5421)* mutant strain is not due to a background mutation, and rather due to a mutation in the *cebp-2* gene itself.

Taken together these results indicate that ZIP-2 and CEBP-2 do not function to regulate expression or localization of each other, and are both present in intestinal nuclei during infection with *P. aeruginosa* when there is robust gene induction of *irg-1* and other infection response genes. These results are consistent with the model that ZIP-2 and CEBP-2 function together as a heterodimeric transcription factor to induce genes in the context of pathogen infection.

cebp-2 mutants have a decrease in body size and reproductive output

Although *zip-2* and *cebp-2* mutants appear to have nearly identical phenotypes in terms of their response to pathogen infection, they do differ in terms of overall health and vigor. In particular, *cebp-2(tm5421)* mutants had reduced body size compared to wild-type animals during normal well-fed conditions (Figure S3A), a defect that was rescued by the *CEBP-2::GFP* transgene. In contrast, *zip-2(tm4248)* mutants had no decrease in body size compared to wild-type animals. We also found that *cebp-2(tm5421)* mutants had a significantly reduced brood size compared to wild-type animals, while *zip-2(tm4248)* mutants had no reduction in brood size (Figure S3B). These differences in overall health between *cebp-2* and *zip-2* mutants may be due to CEBP-2 acting in a dimer with a different bZIP transcription factor to regulate growth and reproduction. Notably, mammalian C/EBP-gamma does not appear able to regulate transcription on its own, but rather partners with several different C/EBP factors to regulate distinct outputs (Tsukada et al., 2011). Indeed, CEBP-2 has been shown in vitro to interact with several other binding partners (Reinke et al., 2013), and recently was shown to have a role in fat metabolism as well (Xu et al., 2015), which may explain its effects on body size and reproduction.

CEBP-2 mediates a transcriptional response to inhibition of mRNA translation and inhibition of other core processes, which increase ZIP-2 protein expression

As mentioned above, previous studies indicate that ToxA-mediated translational inhibition appears to be responsible for a subset of the *P. aeruginosa* infection-induced transcriptional response in *C. elegans*. This gene induction is partially dependent on *zip-2*, and the *zip-2* signaling pathway was shown to protect *C. elegans* from killing by ToxA. We therefore investigated whether *cebp-2* is similarly required for ToxA-mediated gene induction and for survival after exposure to ToxA. We first examined whether *cebp-2* is required for ToxA-induced expression of the *irg-1p::GFP* reporter. In both *cebp-2* RNAi-treated animals and *cebp-2(tm5421)* mutant animals we found greatly reduced induction of *irg-1p::GFP* after exposure to ToxA as compared to control, similar to that seen in worms lacking *zip-2* function (Figure 4A-H). This result indicates that *cebp-2*, like *zip-2*, regulates *irg-1* induction after ToxA treatment. We next tested whether *cebp-2* regulates endogenous *irg-1* induction as well as induction of two other *zip-2*-dependent gene, *oac-32* and *F11D11.3*, in response to ToxA exposure. We used qRT-PCR to measure RNA levels in *cebp-2*-deficient animals and found that *cebp-2*, like *zip-2*, is required for induction of both *irg-1*, *oac-32* and *F11D11.3* after ToxA-mediated translational inhibition (Figure 4I, J). We also tested an additional three genes whose induction in response to ToxA treatment does not require *zip-2* (McEwan et al., 2012) and found that these genes were induced normally in *cebp-2*-deficient animals (Figure 4I, J).

Surveillance pathways in *C. elegans* monitor not only mRNA translation, but also core processes mediated by mitochondria, the proteasome and transcriptional machinery (Bakowski et al., 2014; Dunbar et al., 2012; Liu et al., 2014; Melo and Ruvkun, 2012). Our previous screen found RNAi clones against not only translation factors, but also mitochondrial pathways and histones can induce *irg-1p::GFP* in a ZIP-2-dependent manner (Dunbar et al., 2012). Thus, we investigated whether *cebp-2* was required for surveillance of these processes. Indeed, we found that RNAi against the histone H2A *his-57* and the mitochondrial enzyme dihydrolipoamide dehydrogenase *dlat-1* no longer induced *irg-1p::GFP* in *cebp-2* mutants (Figure S4A-F). Thus, *cebp-2* appears to be important for gene induction upon perturbation of multiple core processes.

Previously, we had found that either genetic or chemical inhibition of mRNA translation caused an increase in ZIP-2 protein levels (Dunbar et al., 2012), explaining how translational inhibition could lead to an induction of ZIP-2-dependent gene expression. Here we extend those analyses to blockade of other core processes, such as mitochondrial function and histone function. In particular we found that RNAi against the histone H2A *his-57* and the mitochondrial enzyme dihydrolipoamide dehydrogenase *dlat-1* caused an increase in ZIP-2::GFP protein expression (Figure S4G-L). Thus, perturbation of several core processes appears to increase ZIP-2 protein expression, where it could act together with the constitutively expressed CEBP-2 to promote a transcriptional response to xenobiotic insults.

CEBP-2 mounts a protective response against ToxA-mediated killing

Previous studies found that wild-type animals mount a defense response against ToxA, as ToxA treatment does not compromise survival unless immune pathways are defective (McEwan et al., 2012). *zip-2* mutants have a substantially shorter life span when fed *E. coli* expressing ToxA as compared to control. To determine whether *cebp-2* is important for defense against killing by ToxA, we exposed *cebp-2(tm5421)* mutant animals to *E. coli* expressing either a vector control or ToxA. We found that *cebp-2* mutants, like *zip-2* mutants, have greatly decreased survival upon treatment with ToxA, with relatively normal survival on the vector control (Fig. 4K). Thus, *cebp-2* is required for the defense response against the pathogen-derived toxin ToxA. Furthermore, we found that the *cebp-2;zip-2* double mutant had a similar decrease in survival upon ToxA exposure as the *cebp-2* and *zip-2* single mutants (Fig. 4K). Together these results support the model that *cebp-2* is acting together with *zip-2* to mount a protective response against ToxA-mediated killing (Fig. 4L).

Concluding remarks: CEBP-2 and ZIP-2 act together in surveillance immunity in *C. elegans*

A growing theme in animal innate immunity is that hosts are able to discriminate pathogens from other microbes through the use of surveillance pathways that monitor disruption of host processes commonly targeted by pathogens. This ‘effector-triggered’ immunity is critical for epithelial cells that encounter a wide variety of microbial species. In addition to the responses to the bacterial pathogen *P. aeruginosa* described here and in other publications, recent findings suggest that defense against natural eukaryotic pathogens in *C. elegans* can also be triggered by perturbing core processes (Bakowski et al., 2014). Our discovery that CEBP-2 and ZIP-2 act as a potential heterodimeric transcription factor in

surveillance immunity against *P. aeruginosa* in *C. elegans* sheds light on this process and also provides a mammalian connection to be explored. CEBP-2 is the ortholog of C/EBP-gamma in mammals, which is a bZIP transcription factor that heterodimerizes with several other bZIP transcription factors to regulate upregulation of cytokines such as IL-6 and IL-8 in response to classic PAMPs like LPS (Gao et al., 2002), although it has not yet been shown to play a role in effector-triggered immunity. Interestingly, interindividual variation in C/EBP-γ transcript expression levels has been implicated as a risk factor for altered severity of lung disease in cystic fibrosis (Gu et al., 2009) – a genetic disease in which chronic *P. aeruginosa* pneumonia is a pathological hallmark. Future studies could investigate the role that C/EBP-gamma and its binding partners play in surveillance immunity in mammals in order to better understand how animals discriminate pathogens from other microbes to fight off infection.

Experimental Procedures

RNAi experiments

RNAi experiments were performed as described (Estes et al., 2010; Kamath et al., 2003). Overnight cultures of RNAi feeding clones were seeded onto RNAi plates and incubated at 25°C for 1 day. Synchronized L1 stage animals were fed RNAi for two or three days at 20°C. All experiments with feeding RNAi used an *unc-22* positive control RNAi clone, which resulted in twitching animals in all experiments. See Supplemental Experimental Procedures for further details.

Pathogen infection experiments

Pathogen infection experiments were performed as described (Troemel et al., 2006). Briefly, overnight cultures of *P. aeruginosa* strain PA14 were seeded onto SK plates, then incubated at 37°C for 24 h, followed by 25°C for 24 h. Animals at the L4 stage were washed onto plates and were harvested 4 h later for qRT-PCR experiments, or viewed 16-20 h later for GFP experiments.

ToxA assays

ToxA and vector control assay plates were prepared as described (McEwan et al., 2012). Overnight cultures of *E. coli* were diluted 1:20, grown for 2 h at 37°C, induced with 0.84 M IPTG, and grown another hour at 37°C. Concentrated bacteria (10×) were seeded on NGM plates containing 5 mM IPTG and 1mM carbenicillin and used immediately. Animals at the L4 stage were washed onto assay plates and were harvested 24 h later for qRT-PCR experiments, or viewed 18-24 h later for GFP experiments.

ToxA and P. aeruginosa survival assays

ToxA survival assays and *P. aeruginosa* slow-killing experiments were performed as described, with the addition of FUDR (100 μg/ml) to inhibit progeny formation (McEwan et al., 2012; Troemel et al., 2006). Thirty to fifty L4 stage animals were transferred to assay plates prepared as described above and incubated at 25°C, using three plates per strain in each experiment. Survival was monitored over time until all animals had died.

Gene expression analysis

RNA extraction, reverse transcription and qRT-PCR were performed as described (Troemel et al., 2006). qRT-PCR primer sequences are available upon request. For all qRT-PCR experiments, each biological replicate was measured in duplicate and normalized to the control gene *nhr-23*, which did not change expression upon infection or exposure to ToxA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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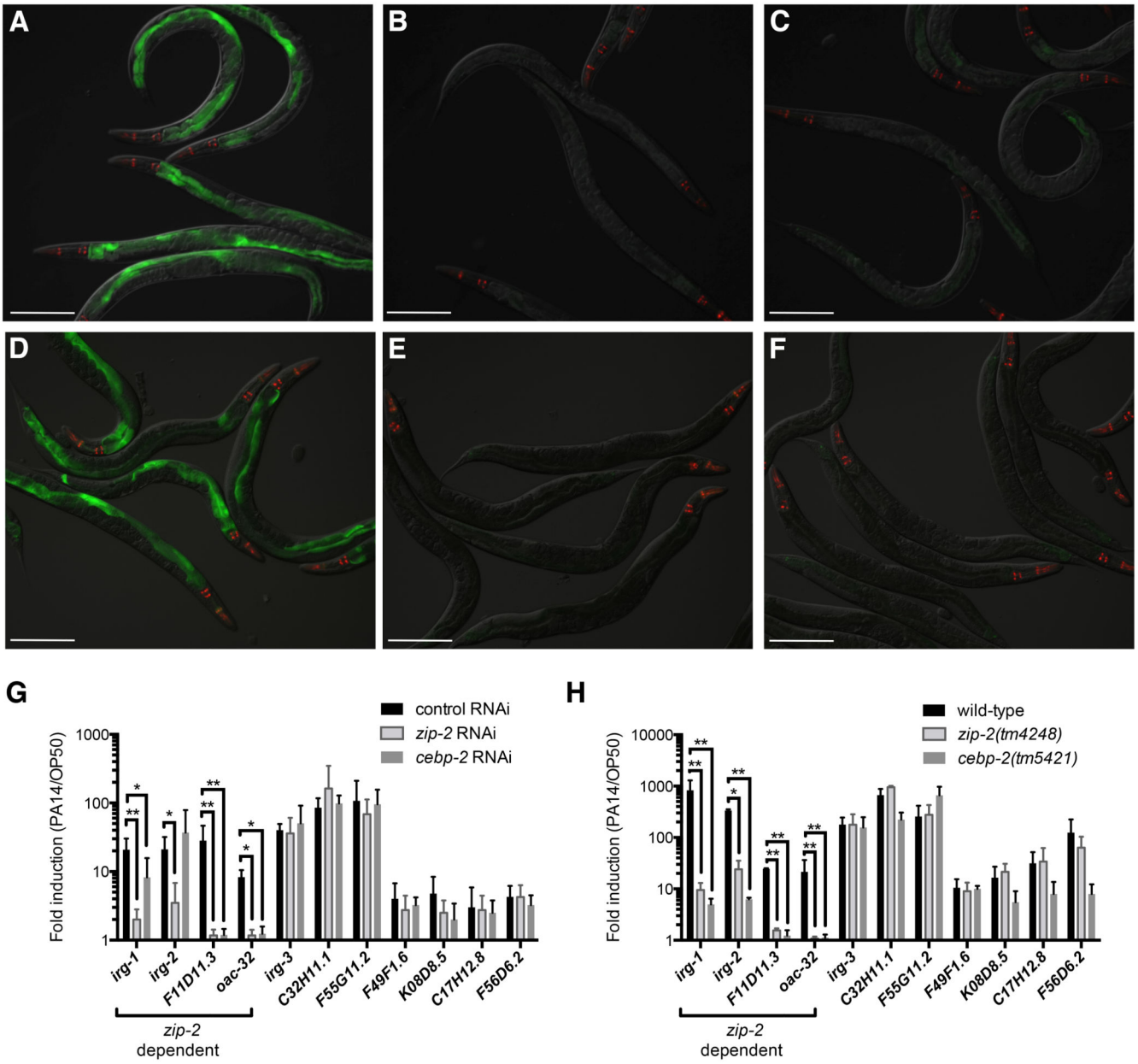

Figure 1. *cebp-2* is required for infection response gene induction upon *P. aeruginosa* PA14 infection

(A-C) *irg-1p::GFP* animals treated with either (A) L4440 RNAi control, (B) *zip-2* RNAi, or (C) *cebp-2* RNAi and infected with PA14. (D-F) *irg-1p::GFP* expression in (D) wild-type, (E) *zip-2(tm4248)*, or (F) *cebp-2(tm5421)* animals infected with PA14. In (A)-(F), green is *irg-1p::GFP*, red is *myo-2::mCherry* expression in the pharynx as a marker for presence of the transgene. Images are overlays of green, red and Nomarski channels and were taken with the same camera exposure for all. Scale bar, 200 μm. (G) qRT-PCR comparison of PA14-induced gene expression in control RNAi (L4440), *zip-2* RNAi, and *cebp-2* RNAi treated animals. (H) qRT-PCR comparison of PA14-induced gene expression in wild-type, *zip-2(tm4248)*, and *cebp-2(tm5421)* animals. For (G) and (H), results shown are the average

of two independent biological replicates, error bars are SD. ** $p < 0.01$, * $p < 0.05$ with a two-tailed t test.

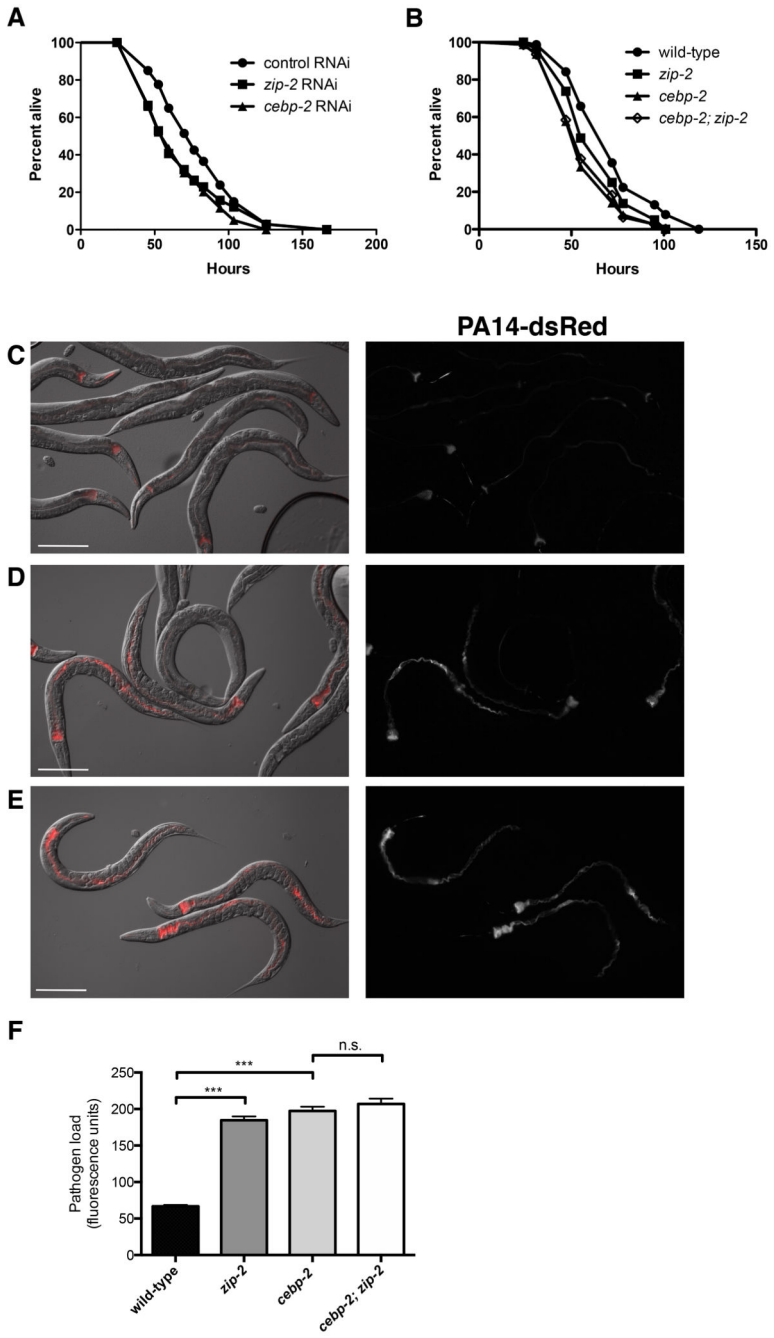

Figure 2. *cebp-2* and *zip-2* control pathogen burden and promote survival upon *P. aeruginosa* infection

(A) Survival of RNAi control (L4440), *zip-2* RNAi, and *cebp-2* RNAi treated animals on *P. aeruginosa* PA14. *zip-2* RNAi and *cebp-2* RNAi treated animals were more susceptible to killing by PA14 than control ($p < 0.0001$ for each). (B) Survival of wild-type, *zip-2(tm4248)*, *cebp-2(tm5421)*, and *cebp-2(tm5421);zip-2(tm4248)* animals on PA14. *zip-2(tm4248)*, *cebp-2(tm5421)*, and *cebp-2(tm5421);zip-2(tm4248)* animals were more susceptible to killing by PA14 than wild-type ($p < 0.001$ for each); there was not a

significant difference between *cebp-2(tm5421)*, and *cebp-2(tm5421);zip-2(tm4248)*, p=0.7. For (A) and (B), graph shows a representative assay of three independent replicates. (C-E) Images of (C) wild-type, (D) *zip-2(tm4248)* and (E) *cebp-2(tm5421)* animals after 16 hours of exposure to dsRed-expressing PA14. In each panel, the left image shows an overlay of Nomarski with red fluorescence and the right image shows red fluorescence alone. Scale bar, 200 μm. (F) Quantification of dsRed fluorescence levels in the intestine of wild-type, zip-2(tm4248), cebp-2(tm5421), and cebp-2(tm5421); zip-2(tm4248) animals after 16 hours of infection with dsRed-expressing PA14. Fluorescence was measured with a COPAS Biosort machine. Results shown are a representative assay of two independent replicates, with at least 500 animals measured for each sample. Error bars are SEM. ***, $p < 0.001$ with a two-tailed t test; n.s., not significant.

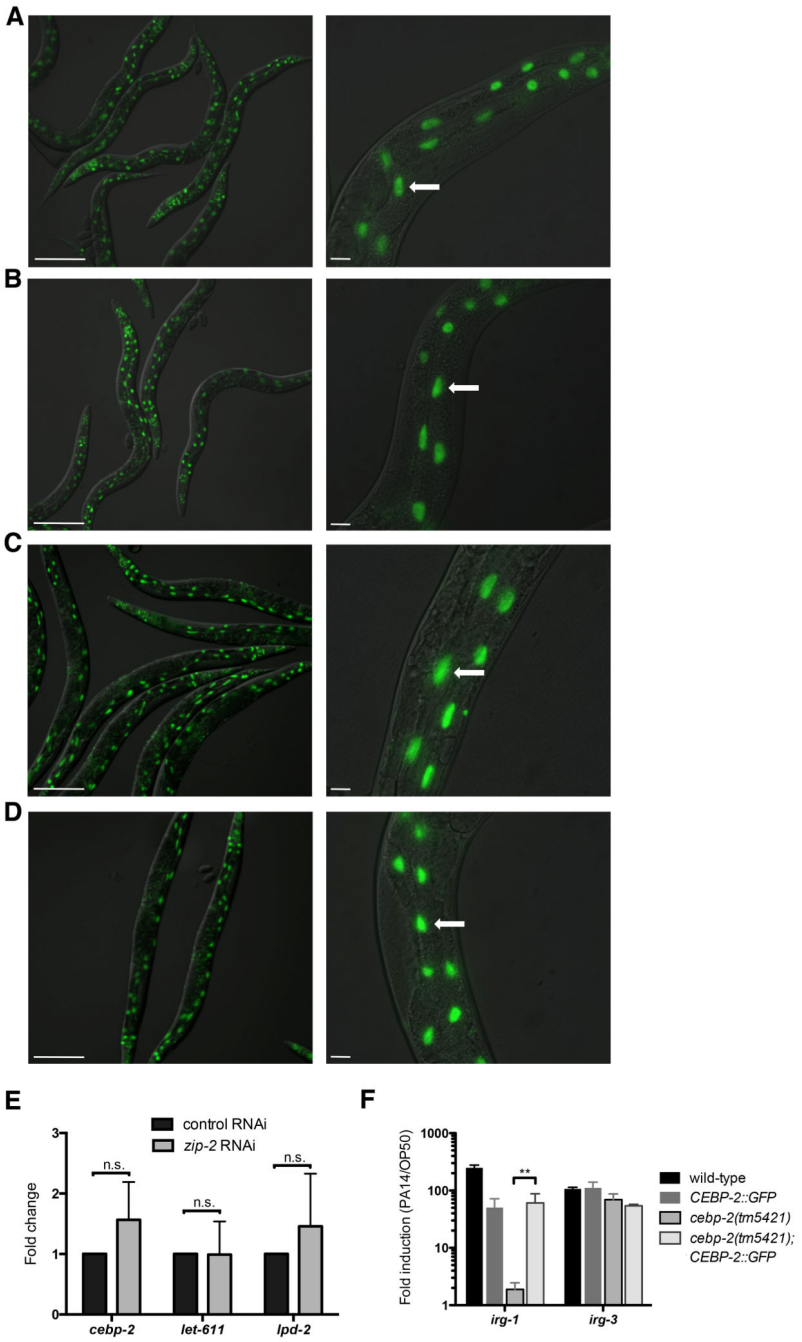

Figure 3. *CEBP-2::GFP* is expressed in intestinal nuclei independently of infection and independently of ZIP-2

(A) *CEBP-2::GFP* transgenic animals express GFP throughout the body with strong nuclear localization. (B) *CEBP-2::GFP* transgenic animals show nuclear GFP in the intestine when infected with *P. aeruginosa*. (C-D) *CEBP-2::GFP* transgenic animals have GFP in intestinal nuclei after treatment with (C) control RNAi or (D) *zip-2* RNAi. (A-D) Images are overlays of green and Nomarski channels, with left panels imaged at 10× (scale bar, 200 μm) and right panels imaged at 40× (scale bar, 20 μm). White arrows indicate intestinal nuclei. (E)

qRT-PCR comparison of gene expression in control RNAi (L4440) and *zip-2* RNAi treated animals, shown as the fold change relative to L4440. (F) qRT-PCR measurement of gene expression shows that the *CEBP-2::GFP* transgene rescues the *irg-1* induction defect of *cebp-2(tm5421)* mutants. For (E) and (F), results shown are the average of two independent biological replicates, error bars are SD. ** $p < 0.01$ with a two-tailed t test; n.s., not significant.

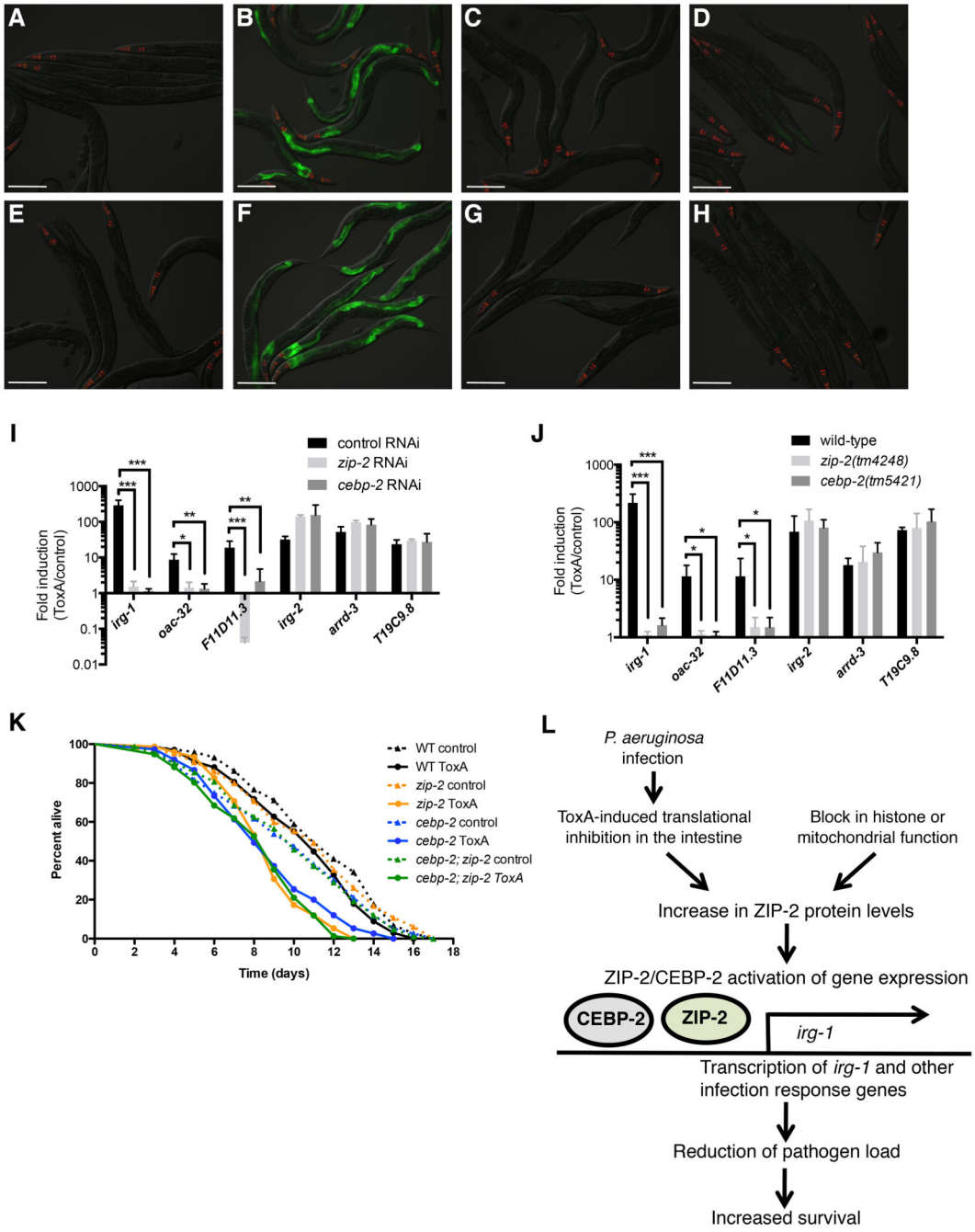

Figure 4. *cebp-2* is required for induction of gene expression and survival upon translational inhibition by ToxA

(A-B) RNAi control (L4440) treated *irg-1p::GFP* animals after exposure to *E. coli* expressing either (A) the empty vector control or (B) ToxA. (C) *zip-2* RNAi and (D) *cebp-2* RNAi treated *irg-1p::GFP* animals after exposure to *E. coli* expressing ToxA. (E-F) Wild-type *irg-1p::GFP* animals after exposure to *E. coli* expressing either (E) the empty vector control or (F) ToxA. (G-H) *irg-1p::GFP* expression in (G) *zip-2(tm4248)* and (H) *cebp-2(tm5421)* animals after exposure to *E. coli* expressing ToxA. (A-H) Images are

overlays of green, red, and Nomarski channels and were taken with the same camera exposure for all. Green is *irg-1p::GFP*, red is *myo-2::mCherry* expression in the pharynx as a marker for presence of the transgene. Scale bar, 200 μm. (I) qRT-PCR comparison of ToxA-induced gene expression in control RNAi (L4440), *zip-2* RNAi, and *cebp-2* RNAi treated animals. (J) qRT-PCR comparison of ToxA-induced gene expression in wild-type, *zip-2(tm4248)*, and *cebp-2(tm5421)* animals. For (I) and (J), results shown are the average of two independent biological replicates, error bars are SD. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ with a two-tailed t test. n.s., not significant. (K) Survival of wild-type N2, *zip-2(tm4248)*, *cebp-2(tm5421)*, and *cebp-2(tm5421); zip-2(tm4248)* animals on *E. coli* expressing either ToxA or an empty expression vector starting at the L4 stage. Graph shows a representative assay of three independent replicates. N2 worms had no difference in life span when fed *E. coli* expressing either ToxA or the vector control ($p = 0.7$), while *zip-2(tm4248)*, *cebp-2(tm5421)*, and *cebp-2(tm5421); zip-2(tm4248)* animals had significantly shorter life spans on *E. coli* expressing ToxA as compared to the vector control ($p < 0.001$ for each). (L) Model for ZIP-2/CEBP-2 activation of gene expression after *P. aeruginosa* infection.