Title
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Highlights

- CEBP-2, the C. elegans ortholog of mammalian C/EBP-γ, promotes resistance to infection
- CEBP-2, like the ZIP-2 transcription factor, defends against translational block
- CEBP-2 mediates a response to perturbation in histone and mitochondrial function
- CEBP-2 and ZIP-2 are potential heterodimeric partners in surveillance immunity

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In Brief

Reddy et al. show that CEBP-2, the C. elegans ortholog of C/EBP-γ, acts together with the bZIP transcription factor ZIP-2 to promote host response against perturbation of core processes like mRNA translation. CEBP-2/ZIP-2 comprise a potential heterodimeric transcription factor that functions in surveillance immunity, a key aspect of epithelial defense.
The C. elegans CCAAT-Enhancer-Binding Protein Gamma Is Required for Surveillance Immunity

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SUMMARY

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 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 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 Barbier, 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., 2009). Translation-blocking toxins are made by diverse bacterial pathogens including P. aeruginosa (Iglewski et al., 1977), Corynebacterium diphtheriae (Pappenheimer, 1977), Vibrio cholera (Jørgensen et al., 2008), Legionella pneumophila (Belyi et al., 2006), Shigella spp, and Shiga toxin-producing E. coli (Pacheco and Sparrando, 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 relative 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 C/EBP gamma 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 (Figures 1A–1F). 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 (Figures 1G and 1H). 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 whether 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).

Next, we 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 (Djnović et al., 2013) in animals deficient for either zip-2 or cebp-2 at 16 hr post-infection and found that zip-2- and cebp-2-deficient animals accumulated...
significantly more intestinal PA14-dsRed than control animals (Figures 2C–2F and S1A–S1D). 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.

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 and 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. The scale bar represents 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 and **p < 0.05 with a two-tailed t test.
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

**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 hr 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. The scale bar represents 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 hr 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.
Localization in intestinal cells when feeding on E. coli expressing ToxA (Figures S2A, S2B, and S2E).

Next, we 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 (Figures S2C–S2E). 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, then 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 (Figures 3C and 3D).

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.
**P. aeruginosa infection**

- ToxA-induced translational inhibition in the intestine
- Block in histone or mitochondrial function
- Increase in ZIP-2 protein levels
- ZIP-2/CEBP-2 activation of gene expression
- Transcription of *irg-1* and other infection response genes
- Reduction of pathogen load
- Increased survival

(legend on next page)
Furthermore, there was not a change in cebp-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 cebp-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, whereas zip-2(tm4248) mutants had no reduction in brood size (Figure S3B). These differences in overall health between cebp-2 and zip-2 mutant animals 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 Increases 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-1::GFP reporter. In both cebp-2 RNAi-treated animals and cebp-2(tm5421) mutant animals, we found greatly reduced induction of irg-1::GFP after exposure to ToxA as compared to control, similar to that seen in worms lacking zip-2 function (Figures 4A–4H). 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 genes, 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 irg-1, oac-32, and F11D11.3 after ToxA-mediated translational inhibition (Figures 4I and 4J). 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 (Figures 4I and 4J).

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-1::GFP in a ZIP-2-dependent manner.

Figure 4. cebp-2 Is Required for Induction of Gene Expression and Survival upon Translational Inhibition by ToxA

(A and 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 and D) zip-2 RNAi (C) and cebp-2 RNAi (D) treated irg-1p::GFP animals after exposure to E. coli expressing ToxA.
(E and F) Wild-type irg-1p::GFP animals after exposure to E. coli expressing either (E) the empty vector control or (F) ToxA.
(G and 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 myo-2::mCherry expression in the pharynx as a marker for presence of the transgene. The scale bar represents 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, and *p < 0.05 with a two-tailed t-test.
(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 lifespan when fed E. coli expressing either ToxA or the vector control (p = 0.7), whereas zip-2(tm4248), cebp-2(tm5421), and cebp-2(tm5421); zip-2(tm4248) animals had significantly shorter lifespans 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.
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 ig-1p::GFP in cebp-2 mutants (Figures S4A–S4F). 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 (Figures S4G–S4L). 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 lifespan 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 (Figure 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 (Figure 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 (Figure 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-gamma 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 2 or 3 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 the 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 RNAi plates and incubated at 37°C for 24 hr. Synchronized L1 stage animals were fed RNAi for 2 or 3 days at 20°C. Animals at the L4 stage were washed onto plates and were harvested 4 hr later for qRT-PCR experiments or viewed 16–20 hr 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 hr 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 1 mM carbenicillin and used immediately. Animals at the L4 stage were washed onto assay plates and were harvested 24 hr later for qRT-PCR experiments or viewed 18–24 hr 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.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.01.055.
AUTHOR CONTRIBUTIONS

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