Mitochondrial UPR repression during *Pseudomonas aeruginosa* infection requires the bZIP protein ZIP-3

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Mitochondria generate most cellular energy and are targeted by multiple pathogens during infection. In turn, metazoans employ surveillance mechanisms such as the mitochondrial unfolded protein response (UPR^{mt}) to detect and respond to mitochondrial dysfunction as an indicator of infection. The UPR^{mt} is an adaptive transcriptional program regulated by the transcription factor ATFS-1, which induces genes that promote mitochondrial recovery and innate immunity. The bacterial pathogen *Pseudomonas aeruginosa* produces toxins that disrupt oxidative phosphorylation (OXPHOS), resulting in UPR^{mt} activation. Here, we demonstrate that *Pseudomonas aeruginosa* exploits an intrinsic negative regulatory mechanism mediated by the *Caenorhabditis elegans* bZIP protein ZIP-3 to repress UPR^{mt} activation. Strikingly, worms lacking *zip-3* were impervious to *Pseudomonas aeruginosa*-mediated UPR^{mt} repression and resistant to infection. Pathogen-secreted phenazines perturbed mitochondrial function and were the primary cause of UPR^{mt} activation, consistent with these molecules being electron shuttles and virulence determinants. Surprisingly, *Pseudomonas aeruginosa* unable to produce phenazines and thus elicit UPR^{mt} activation were hypertoxic in *zip-3*–deletion worms. These data emphasize the significance of virulence-mediated UPR^{mt} repression and the potency of the UPR^{mt} as an antibacterial response.

mitochondrial UPR | ZIP-3 | ATFS-1 | UPR^{mt} | immunity

Metazoans differentiate pathogenic and commensal bacteria in part by monitoring the integrity of essential intracellular activities. For example, the opportunistic pathogen *Pseudomonas aeruginosa* secretes multiple toxins that perturb host protein synthesis and mitochondrial function (1–3). Interestingly, disruption of either process, independent of bacterial infection, elicits immune response activation (4–7). *P. aeruginosa* secretes multiple toxins capable of perturbing oxidative phosphorylation (OXPHOS), including cyanide, siderophores, and phenazines, which impair OXPHOS complex IV, host iron acquisition, and electron transport, respectively (2, 3, 8, 9).

One mechanism by which cells respond to mitochondrial dysfunction is by activating the mitochondrial unfolded protein response (UPR^{mt}), which is regulated by the bZIP protein ATFS-1, a unique transcription factor that harbors both a mitochondrial targeting (MTS) and nuclear localization (NLS) sequence. ATFS-1 is efficiently imported into healthy mitochondria via its MTS and degraded in the mitochondrial matrix. However, mitochondrial perturbations such as perturbed proteostasis and OXPHOS impairment reduce mitochondrial protein import, which in turn causes the accumulation of ATFS-1 in the cytosol. ATFS-1 can then traffic to the nucleus via its NLS. In the nucleus, ATFS-1 induces a transcriptional program that includes a mitochondrial recovery program, as well as an antibacterial response involving peptides and secreted lysozymes that defend against bacterial pathogens (10, 11). The presence of both the MTS and a NLS allows ATFS-1 to respond to mitochondrial import deficiency as a surrogate for OXPHOS function and also serves as a mechanism to detect and defend against pathogens that perturb mitochondrial function (12).

Results

***P. aeruginosa* Perturbs Mitochondrial Function and Impairs UPR^{mt} Activation.** Using the UPR^{mt} transcriptional reporter strains *hsp-6$_{pr}$::gfp* and *hsp-60$_{pr}$::gfp*, in which GFP expression is regulated by mitochondrial chaperone promoters, we sought to better understand the relationship between *P. aeruginosa* exposure, mitochondrial dysfunction, and UPR^{mt} activation. Exposure of *Caenorhabditis elegans* to *P. aeruginosa* has been shown to result in modest activation of *hsp-6$_{pr}$::gfp* (12, 13). Consistent with the pathogen causing mitochondrial dysfunction, mitochondrial membrane potential was depleted within 3 h of exposure to *P. aeruginosa,* and oxygen consumption was also impaired (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S1*A*).

Diverse forms of mitochondrial stress are known to cause UPR^{mt} activation. For example, when worms were raised on the nonpathogenic food source *Escherichia coli* (OP50), impairment of mitochondrial genome replication due to ethidium bromide (EtBr) exposure resulted in *hsp-6$_{pr}$::gfp* activation (Fig. 1*C*) (4). Surprisingly, when worms were exposed to both *P. aeruginosa*

Significance

Mitochondria are the compartments in animal cells that produce the most energy and are often targets of bacterial toxins during infection. In response, hosts employ an adaptive transcriptional response known as the mitochondrial unfolded protein response (UPR^{mt}) to maintain mitochondrial function and eliminate the toxic bacteria. Here, we demonstrate that the pathogen *Pseudomonas aeruginosa* exploits a negative regulatory mechanism built into the UPR^{mt} to prevent activation of the antibacterial response. Impressively, if the negative regulator ZIP-3 is inhibited, worms are resistant to infection as they are able to effectively activate the UPR^{mt}. The pathogen potentially evolved means to impair the UPR^{mt} because a virulence determinant that ordinarily maintains biofilm metabolism perturbs mitochondrial function eliciting the antibacterial response.

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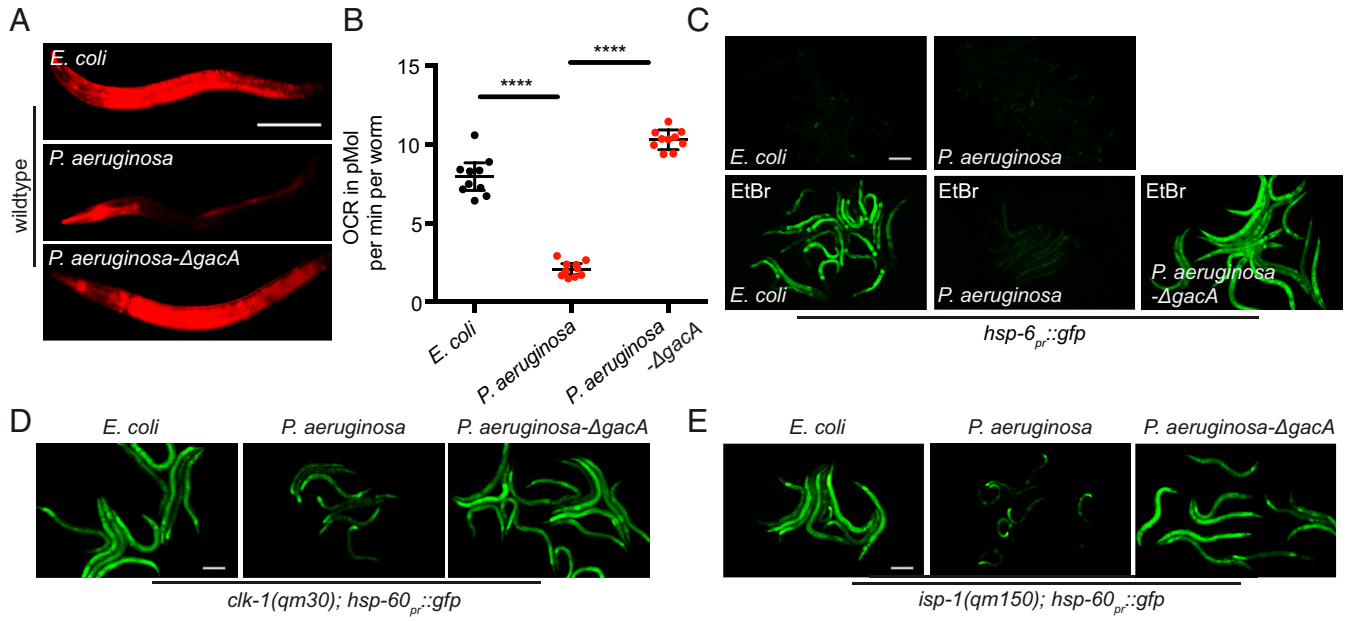

Fig. 1. *P. aeruginosa* perturbs mitochondrial function and represses the UPR^{mt}. (*A*) Images of TMRE-stained wild-type worms following 15 h of exposure to *E. coli*, *P. aeruginosa*, or *P. aeruginosa-ΔgacA*. (Scale bar, 0.1 mm.) (*B*) Oxygen consumption rates (OCRs) of wild-type worms following 12 h of exposure to *E. coli*, *P. aeruginosa*, or *P. aeruginosa-ΔgacA*. $n = 10$; error bars indicate mean ± SD; $^{****}P < 0.03$ (Student's *t* test). (*C*) *hsp-6$_{pr}$::gfp* worms on *E. coli*, *P. aeruginosa*, or *P. aeruginosa-ΔgacA* exposed to a control or 30 μg/mL ethidium bromide. Images were obtained 27 h after exposure. (Scale bar, 0.1 mm.) (*D*) *clk-1(qm30);hsp-60$_{pr}$::gfp* worms exposed to *E. coli*, *P. aeruginosa*, or *P. aeruginosa-ΔgacA* for 48 h. (Scale bar, 0.1 mm.) (*E*) *isp-1(qm150);hsp-60$_{pr}$::gfp* worms exposed to *E. coli*, *P. aeruginosa*, or *P. aeruginosa-ΔgacA* for 48 h. (Scale bar, 0.1 mm.)

and EtBr, *hsp-6$_{pr}$::gfp* was impaired (Fig. 1*C*). Similarly, *P. aeruginosa* infection impaired UPR^{mt} activation caused by the *clk-1(qm30)* or *isp-1(qm150)* mutant alleles, which impair ubiquinone biosynthesis and OXPHOS, respectively (14) (Fig. 1 *D* and *E* and *SI Appendix*, Fig. S1*B*). Interestingly, UPR^{mt} repression required the *P. aeruginosa* two-component regulator GacA (Fig. 1 *C*–*E*), which is required for the expression of most virulence genes in *P. aeruginosa* (15–17). Together, these data suggest that *P. aeruginosa* evolved a mechanism to impair the UPR^{mt} during infection.

ZIP-3 Negatively Regulates UPR^{mt} Activation. We next sought to identify the mechanism(s) by which the UPR^{mt} is inhibited during *P. aeruginosa* exposure by focusing on host factors. We previously found that ATFS-1 regulates expression of the gene encoding the bZIP transcription factor ZIP-3 by binding the *zip-3* promoter (18) and mediating the induction of *zip-3* mRNA transcription during mitochondrial dysfunction caused by *spg-7* (RNAi), *clk-1* mutation, and paraquat treatment (*SI Appendix*, Fig. S1 *C*–*E*) (4). *zip-3* was also transcriptionally induced in a strain expressing constitutively active ATFS-1, which harbors a mutation that impairs the MTS and promotes its nuclear accumulation (11, 19) (*SI Appendix*, Fig. S1*F*). Importantly, the bZIP domain of ZIP-3 was one of four *C. elegans* bZIP domains previously found to dimerize with the bZIP domain of ATFS-1 in vitro (20), suggesting heterodimer formation between ATFS-1 and ZIP-3 may affect ATFS-1 function.

Intriguingly, when raised on *E. coli*, *zip-3(gk3164)* loss-of-function mutants displayed modest UPR^{mt} activation (Fig. 2*A* and *SI Appendix*, Fig. S2*A*), suggesting that ZIP-3 is either required for mitochondrial function or functions as a negative regulator of activated ATFS-1. Impressively, the UPR^{mt} was strongly activated in *zip-3(gk3164)* worms raised on several pathogenic *P. aeruginosa* strains within 12 h (Fig. 2*A* and *SI Appendix*, Fig. S2*B*), unlike in wild-type worms (21). Moreover, while UPR^{mt} activation in *clk-1(qm30)* worms was repressed during *P. aeruginosa* infection (Fig. 1*D* and *SI Appendix*, Fig. S1*B*), *clk-1(qm30);zip-3(gk3164)* worms displayed robust activation of the UPR^{mt} following 48 h *P. aeruginosa* exposure (Fig. 2*B*). These data demonstrate that ZIP-3 is required for UPR^{mt} inhibition during *P. aeruginosa* infection.

We next sought to determine the physiological impact of UPR^{mt} inhibition by *P. aeruginosa* during infection. Impressively, *zip-3(gk3164)* worms survived significantly longer than wild-type worms during *P. aeruginosa* exposure (Fig. 2*C*), in a manner dependent on *atfs-1* (Fig. 2*C*). Furthermore, the prolonged survival of *zip-3(gk3164)* worms on *P. aeruginosa* was comparable to the prolonged survival conferred by the constitutively active allele *atfs-1(et15)* (*SI Appendix*, Fig. S2*C*). As with the *atfs-1(et15)* strain (12), the intestinal colonization of *P. aeruginosa* was reduced in *zip-3(gk3164)* relative to wild-type worms, which also required *atfs-1* (Fig. 2 *D* and *E*). Combined, these data indicate that *zip-3*–deletion worms are resistant to *P. aeruginosa* in a manner dependent on UPR^{mt} activation, suggesting that *zip-3* negatively regulates *atfs-1*.

ZIP-3 Stability Is Regulated by the Ubiquitin Ligase WWP-1 and Proteasomal Degradation. To better understand the mechanism by which ZIP-3 is regulated and impacts the UPR^{mt}, transgenic strains were generated in which GFP or a ZIP-3::GFP fusion protein was expressed via the *zip-3* promoter. GFP was expressed at high levels, indicating that the *zip-3* promoter was active (Fig. 3*A*). However, ZIP-3::GFP was difficult to detect, suggesting that the fusion protein has a relatively short half-life (Fig. 3*A*). Impressively, inhibition of a core proteasomal subunit via *pbs-1* (RNAi) caused ZIP-3::GFP accumulation within intestinal nuclei (Fig. 3*B*), indicating that in addition to transcriptional regulation, ZIP-3 is also regulated by protein stability.

We next generated a strain expressing GFP::ZIP-3 from the native locus via CRISPR-Cas9. Importantly, GFP::ZIP-3 prevented UPR^{mt} activation during exposure to *P. aeruginosa*, unlike *zip-3* deletion, indicating that the fusion protein was functional (*SI Appendix*, Fig. S3*A*). To identify potential ubiquitin ligases that

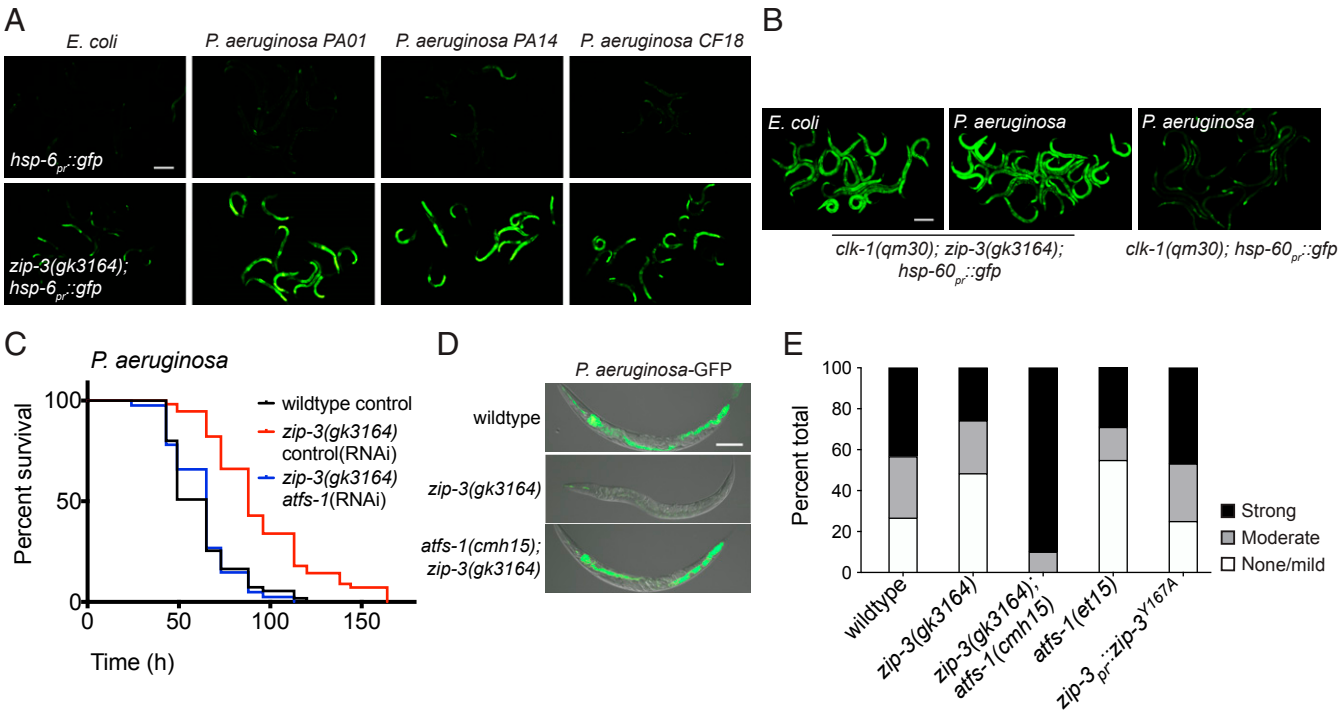

Fig. 2. The bZIP protein ZIP-3 is a negative regulator of ATFS-1 and the UPR^{mt}. (*A*) *hsp-6$_{pr}$::gfp* or *zip-3(gk3164);hsp-6$_{pr}$::gfp* worms on *E. coli* or pathogenic *P. aeruginosa* strains PA01, PA14, and CF18[21]. (Scale bar, 0.1 mm.) (*B*) *clk-1(qm30);zip-3(gk3164);hsp-60$_{pr}$::gfp* and *clk-1(qm30);hsp-60$_{pr}$::gfp* worms on *E. coli* or *P. aeruginosa*. (Scale bar, 0.1 mm.) (*C*) Survival of wild-type and *zip-3(gk3164)* worms on control or *atfs-1*(RNAi) exposed to *P. aeruginosa*. Statistics are in *SI Appendix*, Table S5. (*D*) Representative photomicrographs of wild-type, *zip-3(gk3164),* and *zip-3(gk3164);atfs-1(cmh15)* worms raised on *E. coli* and exposed to *P. aeruginosa*-GFP for 48 h. Images are overlays of differential interference contrast (DIC) and GFP. (Scale bar, 0.1 mm.) (*E*) Quantification of intestinal colonization of wild-type, *zip-3(gk3164), zip-3(gk3164);atfs-1(cmh15), atfs-1(et15),* and *zip-3$_{pr}$::zip-3^{Y167A}* worms. White, gray, and black bars denote no or mild infection, moderate infection, and strong infection, respectively. Thirty worms were analyzed per treatment.

regulate GFP::ZIP-3 degradation, we initially examined previous transcriptional profiling data of those mRNAs induced during mitochondrial dysfunction (4). Of the seven ubiquitin ligases examined (*SI Appendix*, Table S6), only *wwp-1*(RNAi) caused an accumulation of GFP::ZIP-3 within intestinal nuclei (Fig. 3*B*). WWP-1 is a well-conserved HECT domain ubiquitin ligase with over 20 known substrates in mammals (22). WW domain ubiquitin ligases bind specifically to PY motifs (-PPxY-) within their substrates. Interestingly, ZIP-3 harbors a single PY motif (-PPPY-) (Fig. 3*C*), suggesting that WWP-1 interacts directly with ZIP-3 to ubiquitinate and target it for degradation (23, 24). To examine the role of the PY motif, the -PPxY- motif in GFP::ZIP-3 was altered to either -PPxA- or -PPxF- via CRISPR. Furthermore, like *wwp-1* (RNAi), either amino acid substitution caused accumulation of GFP::ZIP-3 in the intestinal nuclei (*SI Appendix*, Fig. S3*B*). Combined, these data indicate that ZIP-3 is recognized and ubiquitinated by WWP-1 and degraded by proteasomes.

Importantly, ZIP-3 stabilization caused by either *wwp-1*(RNAi) or ZIP-3^{PPxA} was sufficient to repress *clk-1(qm30)*–induced UPR^{mt} activation (Fig. 3*D* and quantified in *SI Appendix*, Fig. S3*C*), consistent with ZIP-3 being a negative regulator of ATFS-1. We next examined the impact of ZIP-3 stabilization on UPR^{mt} activation caused by the constitutively active allele *atfs-1(et15)*. ATFS-1^{et15} harbors an impaired MTS, which causes nuclear accumulation of ATFS-1 and constitutive activation of the UPR^{mt} independent of mitochondrial stress (19). Impressively, ZIP-3^{PPxA} reduced UPR^{mt} activation in *atfs-1(et15)* worms (Fig. 3*E* and quantified in *SI Appendix*, Fig. S3*D*), indicating that ZIP-3 inhibits the activated form of ATFS-1, rather than perturbing mitochondrial function, consistent with ZIP-3 harboring an NLS and being localized in the nucleus (Fig. 3*B* and *SI Appendix*, Fig. S3*B*).

ZIP-3 Limits a Subset of ATFS-1–Dependent Transcripts That Confer Resistance to *P. aeruginosa*. Because the resistance of *zip-3*–deletion worms to *P. aeruginosa* required *atfs-1*, we identified the *atfs-1*–regulated transcripts increased in *zip-3(gk3164)* worms exposed to *P. aeruginosa* (25), as they potentially comprise genes that confer pathogen resistance. Following 18 h of *P. aeruginosa* exposure, 1,082 mRNAs were induced in *zip-3(gk3164)* worms relative to wild-type worms (*SI Appendix*, Fig. S4*A* and Table S1). Of those, the induction of 108 mRNAs required *atfs-1* during mitochondrial dysfunction caused by *spg-7*(RNAi) (26) (*SI Appendix*, Fig. S4 *B* and *C* and Tables S2 and S3), consistent with ZIP-3 inhibiting activated ATFS-1. These mRNAs include components involved in mitochondrial recovery (Fig. 3 *F* and *G*), innate immunity (Fig. 3 *H* and *I*), iron acquisition (Fig. 3*J*), and fat metabolism (Fig. 3*K*). Of note, many of the mRNAs altered in *zip-3(gk3164)* worms exposed to *P. aeruginosa* were not affected by *atfs-1* deletion during mitochondrial stress (*SI Appendix*, Fig. S4*C*), indicating ZIP-3 has roles independent of ATFS-1 and the UPR^{mt}. Furthermore, *zip-3(gk3164);atfs-1(cmh15)* worms developed slower than either the *zip-3(gk3164)* or *atfs-1(cmh15)* mutants alone, also consistent with *atfs-1*–independent roles for *zip-3* (*SI Appendix*, Fig. S5).

***C. elegans* Responds to *P. aeruginosa*–Produced Phenazines to Activate the UPR^{mt}.** Next, we took advantage of the robust UPR^{mt} activation that occurred in *zip-3(gk3164)* worms exposed to *P. aeruginosa* to identify the pathogen-produced molecules that perturb mitochondrial function and activate the UPR^{mt}. Interestingly, *P. aeruginosa* unable to produce phenazines (*P. aeruginosa-Δphz*) (27, 28) did not activate the UPR^{mt} in worms lacking *zip-3* (Fig. 4*A*). Moreover, phenazine treatment was sufficient to perturb mitochondrial function (Fig. 4*B*) and activate the UPR^{mt} (Fig. 4*C*) in

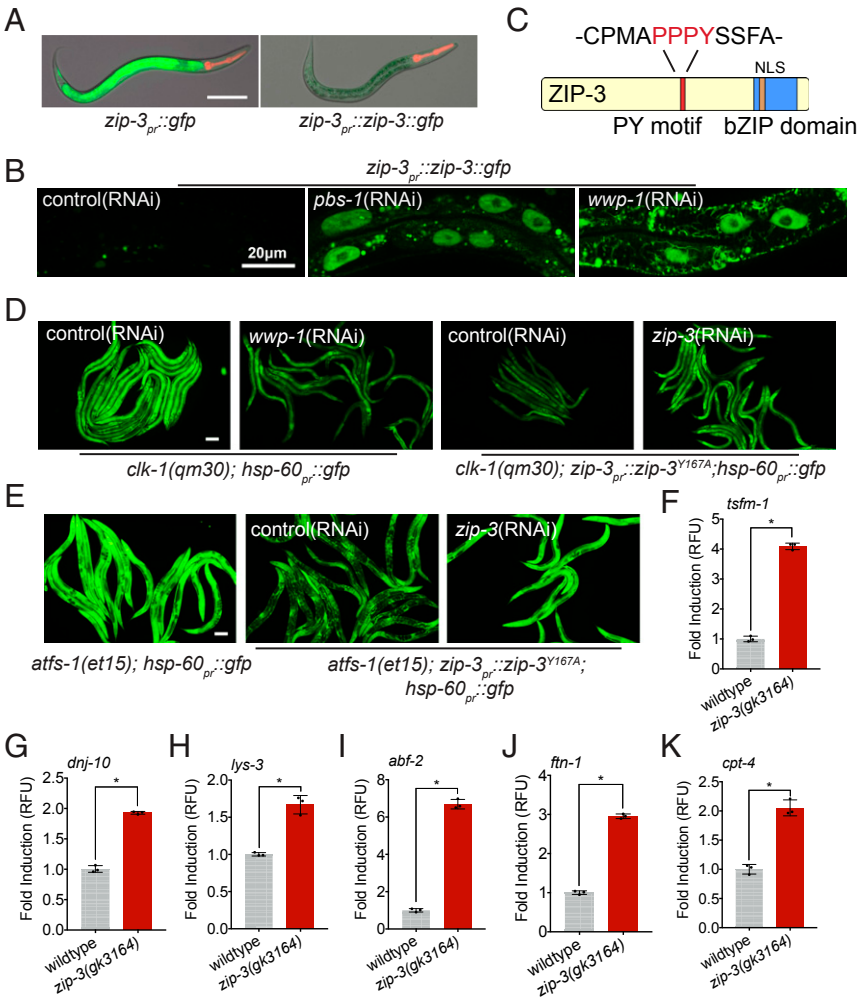

Fig. 3. ZIP-3 is regulated by proteasomal degradation and represses *atfs-1*–dependent transcription. (*A*) Representative photomicrographs of *zip-3*$_{pr}$*::gfp* and *zip-3*$_{pr}$*::zip-3::gfp* worms raised on *E. coli*. Images are overlays of differential interference contrast (DIC) and GFP. (Scale bar, 0.1 mm.) (*B*) Representative intestinal images of *zip-3*$_{pr}$*::zip-3::gfp* in worms on control, *pbs-1*(RNAi), or *wwp-1*(RNAi). (Scale bar, 0.02 mm.) (*C*) ZIP-3 protein. (*D*) *clk-1(qm30);hsp-60*$_{pr}$*::gfp* worms on control or *wwp-1*(RNAi) and *clk-1(qm30);zip-3*^{Y167A}*;hsp-60*$_{pr}$*::gfp* on control or *zip-3*(RNAi). (Scale bar, 0.1 mm.) Quantification is given in *SI Appendix*, Fig. S3*C*. (*E*) *atfs-1(et15);hsp-60*$_{pr}$*::gfp* or *atfs-1(et15);zip-3*^{Y167A}*;hsp-60*$_{pr}$*::gfp* on control or *zip-3*(RNAi). (Scale bar, 0.1 mm.) Quantification is given in *SI Appendix*, Fig. S3*D*. (*F*–*K*) *tsfm-1*, *dnj-10*, *lys-3*, *abf-2*, *ftn-1*, and *cpt-4* transcripts as determined by qRT-PCR in wild-type or *zip-3(gk3164)* worms on *P. aeruginosa* ($n = 3$, ±SD); $^*P < 0.05$ (Student's *t test*). RFU, relative fluorescence unit.

worms raised on nonpathogenic *E. coli*, consistent with phenazines being redox-active compounds that can perturb OXPHOS (3, 29). Importantly, worms expressing degradation-resistant ZIP-3^{Y167A} were unable to activate the UPR^{mt} upon phenazine exposure (Fig. 4*C*), consistent with ZIP-3 repressing UPR^{mt} activation during *P. aeruginosa* exposure. Of note, *P. aeruginosa* strains unable to produce cyanide or multiple siderophores still caused UPR^{mt} activation, suggesting that in this assay, the most potent mitochondrial toxins are phenazines (*SI Appendix*, Fig. S6).

Last, we sought to gain insight into the relationship between virulence-mediated UPR^{mt} repression (Fig. 1) and the *P. aeruginosa*-produced phenazines that perturb mitochondrial function and activate the UPR^{mt}. One possibility is that UPR^{mt} repression increases phenazine potency by impairing a mitochondrial stress response. However, wild-type worm survival was similar upon exposure to wild type or *P. aeruginosa-Δphz* (Fig. 4*D*), suggesting the associated mitochondrial perturbation (Figs. 1*A* and 4*B*) did not decrease survival. Alternatively, UPR^{mt} repression may prevent the activation of an antimicrobial response initiated in response to phenazine-dependent mitochondrial perturbation (Fig. 4*C*).

To further examine these models, wild-type and *zip-3*–deletion worms were exposed to wild type or *P. aeruginosa-Δphz*. Surprisingly, *zip-3(gk3164)* worms were not resistant to *P. aeruginosa-Δphz* as they were to wild-type *P. aeruginosa*. In fact, the pathogenic strain unable to produce phenazines was considerably more toxic to worms lacking *zip-3* (Fig. 4*D*). We next examined the impact of *P. aeruginosa-Δphz* in *atfs-1(cmh15)* worms that cannot activate the UPR^{mt}. Consistent with the UPR^{mt} regulating an antibacterial response, *atfs-1(cmh15)* worms were more sensitive to *P. aeruginosa* than wild-type worms (Fig. 4*E*). However, survival of *atfs-1(cmh15)* worms was similar when exposed to *P. aeruginosa* or *P. aeruginosa-Δphz* (Fig. 4*E*). These findings suggest that the mitochondrial dysfunction caused by phenazines allows the host to initiate an antimicrobial response to prolong survival. However, in the absence of phenazines, the host is unable to engage the UPR^{mt}, resulting in decreased survival.

Discussion

The interactions between host cells and *P. aeruginosa* that facilitate pathogen detection and host response remain unclear.

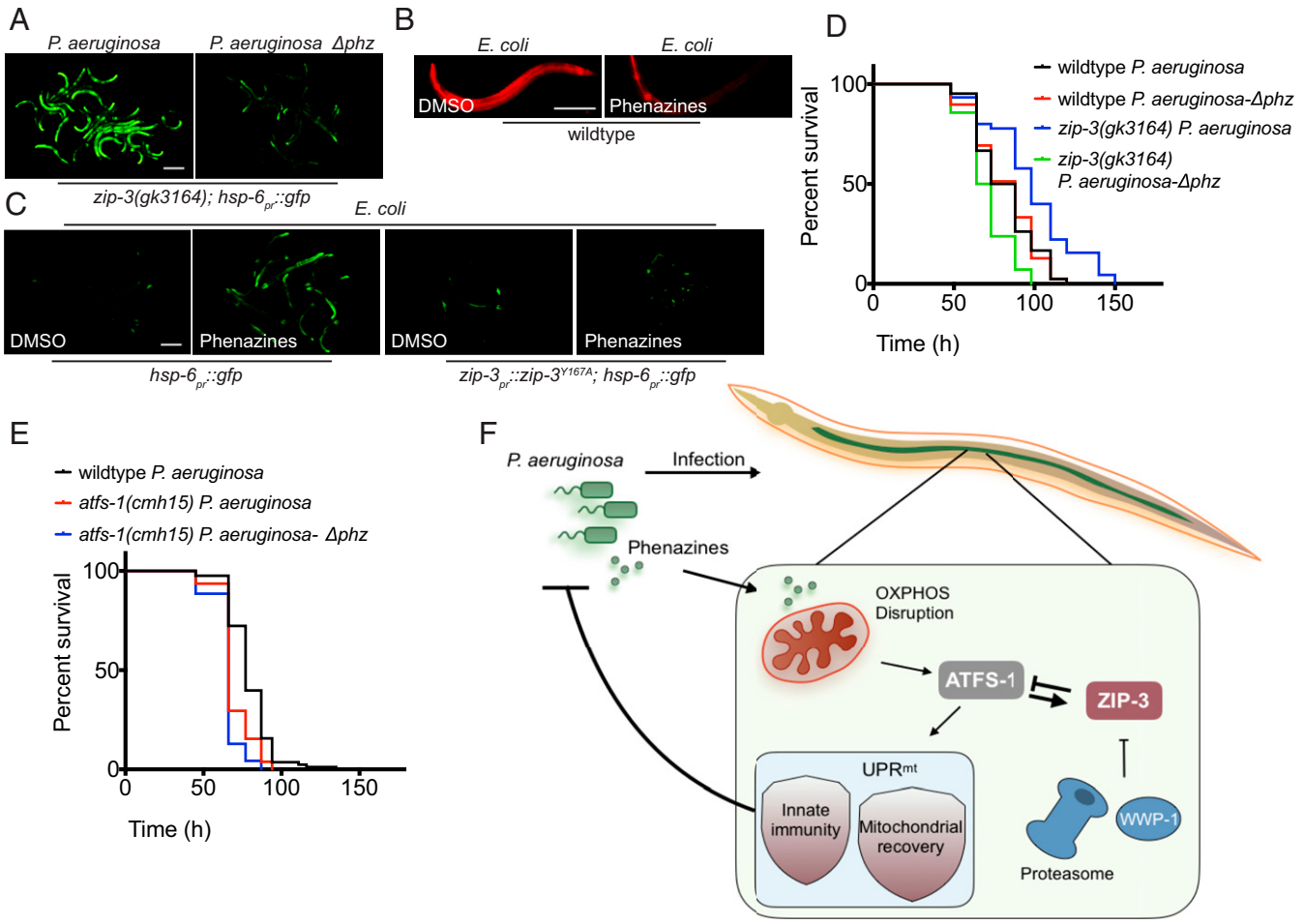

Fig. 4. *P. aeruginosa*-secreted phenazines allow worms to detect the pathogen and initiate a protective antibacterial response. (*A*) *zip-3(gk3164);hsp-6*$_{pr}$*::gfp* worms on wild-type *P. aeruginosa* or *P. aeruginosa-Δphz*. (Scale bar, 0.1 mm.) (*B*) Representative images of TMRE-stained wild-type worms on *E. coli* treated with DMSO or phenazines. (Scale bar, 0.1 mm.) (*C*) *hsp-6*$_{pr}$*::gfp* or *zip-3*^{Y167A}*;hsp-6*$_{pr}$*::gfp* worms raised on *E. coli* treated with DMSO or phenazines. (Scale bar, 0.1 mm.) (*D*) Survival of wild-type and *zip-3(gk3164)* worms exposed to *P. aeruginosa* or *P. aeruginosa-Δphz*. Statistics are given in *SI Appendix*, Table S5. (*E*) Survival of wild-type and *atfs-1(cmh15)* worms exposed to *P. aeruginosa* or *P. aeruginosa-Δphz*. Statistics are given in *SI Appendix*, Table S5. (*F*) Schematics of the interactions between *P. aeruginosa*, mitochondrial perturbation, and UPR^{mt} repression via ZIP-3.

Our findings indicate that *C. elegans* detects *P. aeruginosa* through phenazine-mediated disruption of OXPHOS and responds by initiating the UPR^{mt} via the transcription factor ATFS-1. However, the *P. aeruginosa* virulence response engages a host negative regulatory mechanism. ZIP-3 impairs active ATFS-1 and the associated mitochondrial-protective and antibacterial response, limiting a pathway that impairs intestinal colonization and prolongs host survival.

Studies in multiple organisms have suggested that phenazines are virulence factors that impair electron transport and mitochondrial function (30, 31). However, phenazines serve multiple functions for *Pseudomonas* species independent of infection. Perhaps most intriguing, phenazines are required to maintain redox balance in *Pseudomonas* biofilms where the internal bacteria are hypoxic (32, 33). Perhaps similarly, the *P. aeruginosa* lawn used in the *C. elegans* slow-killing assay is hypoxic as well (34). In both scenarios, phenazines serve as electron shuttles to maintain redox balance throughout the biofilm by facilitating the transfer of electrons to available oxygen. As electron shuttles, phenazines can also impair the eukaryotic electron transport chain.

Importantly, *P. aeruginosa* lacking phenazines remains pathogenic toward *C. elegans* and in the absence of ZIP-3 is considerably more toxic. We propose that the mitochondrial perturbation caused by phenazines is detected by ATFS-1 via mitochondrial surveillance, which in turn activates a response to promote mitochondrial function and eliminate the bacteria. However, *P. aeruginosa* exploits the host negative regulator ZIP-3 to repress UPR^{mt} activation. Consistent with this model, UPR^{mt} repression is dependent on the pathogenicity of *P. aeruginosa* (Fig. 1 *D* and *E*), while the phenazines that perturb mitochondrial function and activate the UPR^{mt} are secreted independent of the virulence response (Fig. 4*E*) (3, 27, 29, 31).

We have shown that ZIP-3 is a labile negative regulator of ATFS-1 that is degraded by proteasomes in a manner dependent on the ubiquitin ligase WWP-1. ZIP-3 stabilization is sufficient to inhibit the UPR^{mt} during mitochondrial dysfunction by impairing nuclear ATFS-1, likely by forming a heterodimer (20). However, it will be interesting to elucidate the inputs that determine ZIP-3 protein stability during mitochondrial stress. WWP-1 or ZIP-3 may receive additional inputs that either stimulate or impair ZIP-3 degradation. PY domain phosphorylation can influence interactions with WW domain ubiquitin ligases (35). Our data suggest that phosphorylation of the PY domain is required for ZIP-3 degradation as altering the PY domain from -PPPY- to -PPPF- within ZIP-3 prevented degradation (*SI Appendix*, Fig. S3*B*). However, the stimuli or potential tyrosine kinase or phosphatase remain to be identified. It will

also be exciting to determine the function of ZIP-3–mediated negative regulation in the absence of pathogens as multiple consequences of prolonged UPR^{mt} activation have been observed, including impaired development (19), loss of dopamine neurons (36), and the propagation of deleterious mitochondrial genomes (11, 37), indicating that UPR^{mt} activation must be strictly regulated by both positive and negative regulators.

Materials and Methods

The full details of worm strains and bacteria strains are described in *SI Appendix*. The procedures for tetramethylrhodamine, ethyl ester (TMRE) staining, the oxygen consumption assay, qPCR, RNA sequencing combined with phenazine treatment, *P. aeruginosa* slow-killing assays, and *P. aeruginosa* intestinal accumulation assays are described in *SI Appendix*. Image and statistical analysis is also described in *SI Appendix*.

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