Splicing factor PRP-19 regulates mitochondrial stress response

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Abstract

Animals respond to mitochondrial perturbation by activating the mitochondrial unfolded protein response (UPRmt) to induce the transcription of mitochondrial stress response genes. In Caenorhabditis elegans, activation of UPRmt allows the animals to maintain organismal homeostasis, activate the innate immune response, and promote lifespan extension. Here, we show that splicing factors such as Precursor RNA processing 19 (PRP-19) are required for the induction of UPRmt in C. elegans. PRP-19 also modulates mitochondrial perturbation-induced innate immune response and lifespan extension. Knockdown of PRP-19 in mammalian cells suppresses UPRmt activation and disrupts the mitochondrial network. These findings reveal an evolutionarily conserved mechanism that maintains mitochondrial homeostasis and controls innate immunity and lifespan through splicing factors.

Keywords: mitochondria; unfolded protein response; stress response; splicing; lifespan

Introduction

Living organisms actively face challenges from the ever-changing environment. The ability to sense and respond to environmental changes is critical for organismal survival. As essential cellular organelles, mitochondria are constantly challenged by intrinsically stimuli such as reactive oxygen species (ROS) and extrinsic pathogens or xenobiotics [1]. Failure to respond to mitochondrial stresses can result in multiple diseases including neurodegenerative disorders [2, 3].

In Caenorhabditis elegans, mitochondrial perturbation activates a surveillance program named the mitochondrial unfolded protein response (UPRmt), which initiates mitochondrion-to-nucleus communication to elevate the transcription of genes encoding mitochondrial chaperones and proteases, thereby buffering the mitochondrial folding environment [4, 5]. UPRmt also increases animal fitness by eliciting metabolic reprogramming [6], increasing innate immunity, and promoting longevity [1, 7–11]. In C. elegans, the transcriptional regulation of UPRmt genes is mainly regulated through two axes driven by ATFS-1 or DVE-1 [10]. ATFS-1 is a transcription factor that contains an N-terminal mitochondrial targeting sequence and a C-terminal nuclear localization sequence. Under normal conditions, ATFS-1 is imported into mitochondria and degraded by the mitochondrial protease LONP-1. Upon mitochondrial perturbation, the impaired mitochondrial import results in the nuclear accumulation of ATFS-1 and elevated transcription of UPRmt genes [12]. DVE-1 is a homeobox protein homologous to human SATB1/SATB2, which functions as a genome organizer. During mitochondrial stress, DVE-1 accumulates in the nucleus and coordinates with histone deacetylase HDA-1 to promote the transcription of UPRmt genes [9, 11, 13].

Spliceosome is a dynamic RNA–protein complex composed of five core small nuclear ribonucleoprotein particles (snRNPs) and around 100 cofactors. It removes the non-coding sequences (introns) and ligates the coding sequences (exons) in precursor messenger RNA (pre-mRNA) [14–16]. The Precursor RNA processing 19 (Prp19) complex, also known as NineTeen Complex (NTC), plays an important role in the catalytic activation of spliceosome [17]. Spliceosomes are highly conserved across species. Interestingly, recent studies have shown that the splicing machinery can regulate gene expression through a splicing-independent function by affecting transcription and chromatin remodeling [18–20]. For instance, splicing factors can interact with RNA Polymerase II C-terminal domain to promote transcription elongation [21, 22].

In this study, we explore the effect of splicing factors on UPRmt in a physiologically relevant setting. We show that upon mitochondrial perturbation, splicing factors such as PRP-19 are required for the induction of UPRmt in C. elegans. PRP-19 also plays an essential role in promoting innate immunity and lifespan extension under mitochondrial stress conditions. Moreover, PRPF19, the mammalian ortholog of PRP-19, regulates mitochondrial homeostasis in mammals. In summary, our results reveal an essential function of splicing factors in UPRmt signaling.

Results

Splicing factor PRP-19 is required for the activation of UPRmt

We previously completed a genome-wide RNAi screen to identify genes required for the induction of UPRmt in C. elegans [1].
Precursor RNA processing 19 (prp-19), a gene with an essential role in splicing in *C. elegans*, is one of the hits from our screen. Knockdown of prp-19 by RNAi suppressed the activation of UPR mt induced by Antimycin A, a mitochondrial electron transport chain (ETC) complex III inhibitor, or RNAi of the nucleus-encoded mitochondrial metalloprotease gene *spg-7* (Fig. 1a and b). In worms with mitochondrial perturbation induced by *spg-7* RNAi, RNAi of prp-19 also impaired the expression and induction of the endogenous mitochondrial stress response gene *hsp-6* (Fig. 1c). To exclude the possibility that prp-19 RNAi may generate off-target effects, we overexpressed a codon-optimized PRP-19, the expression of which could not be suppressed by prp-19 RNAi (Fig. 1d). Overexpression of PRP-19 in prp-19 RNAi worms restored the induction of UPR mt (Fig. 1e). PRP-19 is mainly expressed in the nuclei of intestinal cells (Fig. 1f). The subcellular localization and the expression level of PRP-19 were not affected by mitochondrial perturbation (Fig. 1f and g). Notably, prp-19 RNAi also affected the induction of the endoplasmic reticulum (ER) stress reporter *hsp-4p::gfp* when the worms were challenged with *hsp-4* RNAi or tunicamycin (Fig. 1h and i). Conversely, the heat shock response and the induction of *hsp-16.2p::gfp* reporter were not affected by knockdown of prp-19 (Fig. 1j).

PRP-19 is a core component of the large Prp19C/NTC complex mentioned above. This protein complex consists of 8 core proteins and more than 30 other proteins in mammals [23]. The best characterized function of Prp19C/NTC is its role in the catalytic activation of the spliceosome [17, 24]. Prp19C/NTC has also been shown to play a role in transcription elongation and the maintenance of genome stability [25–27]. To see if other splicing factors similarly modulate UPR mt, we individually knocked down a

**Figure 1** prp-19 is required for UPR mt activation. (a) Fluorescence images of *hsp-6p::gfp* worms at day 1 adulthood. Top: *hsp-6p::gfp* worms were fed on control RNAi and then treated or untreated with Antimycin A or *spg-7* RNAi. Bottom: *hsp-6p::gfp* worms were fed on prp-19 RNAi and then treated with or without Antimycin A or *spg-7* RNAi. Scale bar, 200 µm. (b) Immunoblotting of GFP protein level in *hsp-6p::gfp* worms. (c) Quantitative RT-PCR of endogenous *hsp-6* mRNA levels in *qpr-4* RNAi-fed worms on control or prp-19 RNAi and treated with or without *spg-7* RNAi. **P < .005.** (d) Quantitative RT-PCR of codon-optimized transgenic prp-19 mRNA levels in *hsp-6p::gfp*, *prp-19p::mcherry::prp-19* raised on control or prp-19 RNAi and treated with control or *atp-2* RNAi. n.s., no significance. (e) Fluorescence images of *hsp-6p::gfp* (top) or *hsp-6p::gfp*, *prp-19p::mcherry::prp-19* (bottom) animals grown on control or prp-19 RNAi. Worms were then untreated or treated with *atp-2* RNAi. Overexpressed prp-19 was codon optimized. Scale bar, 200 µm. (f) Fluorescence images of *prp-19p::gfp*, *prp-19p::gfp* worms grown on control or *cco-1* RNAi. Scale bar, 100 µm. (g) Quantitative RT-PCR of endogenous *prp-19* mRNA levels in N2 worms raised on control or *atp-2* RNAi. (h) Fluorescence images of *hsp-4p::gfp* worms at day 1 adulthood. (i) Fluorescence images of *hsp-4p::gfp* worms grown on control or prp-19 RNAi. (j) Fluorescence images of *hsp-16.2p::gfp* worms at day 1 adulthood. *hsp-16.2p::gfp* worms grown on control or prp-19 RNAi, followed by heat shock treatment. Scale bar, 200 µm.
broad spectrum of splicing factors in different spliceosome subunits, including many splicing factors functioning outside of the Prp19C/NTC complex (Fig. 2a). RNAi of most of the splicing factors strongly suppressed the induction of UPR^mt and UPR^ER, but not the heat shock response (Fig. 2b–d). These results suggest that PRP-19 regulates UPR^mt activation through its function in splicing.

Alternative splicing (AS) is a post-transcriptional process in eukaryotes that produces different isoforms of mRNAs and increases the diversity of gene expression. AS events are divided into five main patterns: use of alternative 3′ (acceptor) splice sites (A3SS), use of alternative 5′ (donor) splice sites (A5SS), mutually exclusive exon (MXE) usage, retention of introns (RI), and skipping of exons (SE) [28]. To test if any AS events are required for the production of factors that play a role in UPR^mt regulation, we performed RNA-seq experiments to detect global transcriptome alteration. Mitochondrial perturbation induced by atp-2 RNAi only caused 8 alternatively spliced transcripts in total, whereas knockdown of prp-19 alone or knockdown of prp-19 together with atp-2 resulted in 117 or 102 AS events, respectively (Supplementary Fig. S1a and b and Supplementary Table S1). We validated some of the AS events and confirmed the RNA-seq results (Supplementary Fig. S1c–f). However, UPR^mt genes were not alternatively spliced (Supplementary Table S1). We also found no evidence that alternatively spliced genes play a role in UPR^mt activation.

**PRP-19 functions downstream of ATFS-1 to modulate mitochondrial stress response**

To explore the molecular mechanism by which PRP-19 modulates UPR^mt, we tested if knockdown of prp-19 affected the functions of known components in the UPR^mt pathway. In C. elegans, the transcriptional induction of UPR^mt is mainly governed by two transcription factors, ATFS-1 and DVE-1 [10, 12, 13]. In addition, a histone deacetylase HDA-1 functions in concert with DVE-1 to activate the transcription of UPR^mt genes [11]. Unlike hda-1 RNAi,

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**Figure 2** Splicing factors are required for UPR^mt activation. (a) List of splicing factors tested that affect the activation of UPR^mt. (b) hsp-6p::gfp animals raised on the indicated RNAi in the presence or absence of Antimycin A or cco-1 RNAi. Scale bar, 200 µm. (c) hsp-6p::gfp animals raised on the indicated RNAi in the presence of hsp-4 RNAi. Scale bar, 200 µm. (d) Fluorescence images of hsp-16.2p::gfp animals raised on the indicated RNAi followed by heat shock at 37°C for 1 h. Scale bar, 200 µm.
which reduces the DVE-1 protein level [11], knockdown of prp-19 actually promoted the nuclear accumulation of DVE-1 and elevated the DVE-1 protein level (Supplementary Fig. S2a). Consistent with this, prp-19 RNAi also promoted the nuclear accumulation of HDA-1 and elevated the HDA-1 protein level (Supplementary Fig. S2b and c). Moreover, knockdown of prp-19 promoted the interaction between DVE-1 and HDA-1 (Supplementary Fig. S2d). Collectively, these results indicate that PRP-19 may not regulate UPRmt gene expression through DVE-1 and HDA-1.

We then tested if PRP-19 modulates UPRmt gene expression via ATFS-1. The transcription factor ATFS-1 harbors an amino-terminal mitochondrial targeting sequence and a carboxy-terminal nuclear localization sequence. Upon mitochondrial perturbation, the import efficiency of mitochondria is decreased, leading to the nuclear accumulation of ATFS-1 [12]. Deletion of the N-terminal amino acids 1–32 of ATFS-1 disrupts its mitochondrial targeting signal, resulting in constitutive nuclear accumulation of ATFS-1 and activation of UPRmt gene expression [12]. We employed a transgenic strain that allows the expression of ATFS-1Δ1–32.myc::GFP upon heat shock and noticed that expression of the UPRmt gene hsp-60 also requires prp-19 (Fig. 3a). Knockdown of prp-19 did not affect the expression and nuclear accumulation of ATFS-1Δ1–32.myc::GFP after heat shock (Fig. 3a), suggesting that PRP-19 acts downstream of ATFS-1 once it enters the nucleus.

After entering the nucleus, ATFS-1 binds to the promoter of UPRmt genes and activates their transcription. A group of ATFS-1-dependent UPRmt genes, including mitochondrial chaperone genes hsp-6 and hsp-60, mitochondrial import complex genes tom7-m and tim32, and innate immune response genes cyp14a4 and ugt61, also require the presence of PRP-19 for their induction during mitochondrial stress (Fig. 3b). We therefore sought to test if lack of PRP-19 affects the binding of ATFS-1 to the promoters of UPRmt genes. Again, we employed a transgenic strain which expresses ATFS-1Δ1–32.myc::GFP upon heat shock induction. Chromatin immunoprecipitation (ChIP) coupled with quantitative PCR (qPCR) was performed using anti-GFP antibody to pull-down ATFS-1Δ1–32.myc::GFP (Fig. 3c). A significant amount of ATFS-1Δ1–32.myc::GFP was associated with the promoters of hsp-6 and hsp-60 after heat shock induction. However, knockdown of prp-19 did not impair, but rather promoted the binding of ATFS-1 to the promoters of hsp-6 (Fig. 3d). Therefore, PRP-19 and spliceosome seem to function in a step after ATFS-1 associates with the promoter of mitochondrial stress response genes to modulate UPRmt activation.

Interestingly, a recent study has shown that spliceosomal repression directly affects gene transcription in mouse embryonic stem cells (ESCs), resulting in decreased expression of pluripotent genes, but not affecting the expression of totipotent genes [29]. This study proposed that the greater length and increased number of introns in pluripotent genes relative to totipotent genes may explain their transcriptional regulation by spliceosomal repression. However, we analyzed the number and length of introns in the UPRmt genes and noticed no difference compared with other genes not regulated by the mitochondrial stress response (data not shown). Therefore, at this stage, the detailed mechanism of how the transcription of UPRmt genes is specifically regulated by the splicing factors remains elusive.

**PRP-19 modulates mitochondrial stress response in mammals**

We further examined if PRP-19 plays a conserved role in modulating mitochondrial homeostasis in higher eukaryotes. Interestingly, the expression levels of PRPF19 (mammalian ortholog of PRP-19) strongly correlate with the mitochondrial proteases YME1L1 and LONP1, the mitochondrial import inner membrane translocase TIMM17A, the asparagine synthetase ASNS, and the mitochondrial chaperones HSPE1, HSDF1, and HSPA9 in various human tissues (Fig. 6a). To validate the function of PRPF19 in mediating mitochondrial stress response in mammals, we used shRNA to knock down PRPF19 in HEK293T cells and examined the expression levels of mitochondrial stress response genes. Deficiency of PRPF19 suppressed the induction of mitochondrial stress response genes in cells treated with FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone), a potent uncoupler of mitochondrial oxidative phosphorylation (Fig. 6b). Knockdown of PRPF19 impaired both basal and ATP-linked respiration in the presence or absence of mitochondrial perturbation (Supplementary Fig. S3). In the presence of Antimycin A, mitochondrial morphology was disrupted more extensively in PRPF19 knockdown cells than in wild-type cells (Fig. 6c and d). Taken together, these results reveal an evolutionarily conserved role of PRPF19 in mediating mitochondrial stress response.

**PRP-19 is essential for UPRmt-mediated innate immunity and lifespan extension**

Mitochondrial function is actively challenged by wild microbes in the natural habitats of *C. elegans* [1]. As a defense mechanism, UPRmt also initiates the innate immune response [1, 7] and promotes lifespan extension [8, 10, 11]. Therefore, we further tested the function of PRP-19 in innate immunity and lifespan regulation. *irg-1p::gfp* has been used as a reporter strain for the induction of innate immune response [1, 30]. We challenged the *irg-1p::gfp* transgenic worms with a *Pseudomonas aeruginosa* strain isolated from natural habitats of *C. elegans*. This pathogen has been shown to disrupt mitochondrial function and activate UPRmt [1]. Knockdown of prp-19 suppressed the induction of *irg-1p::gfp* upon pathogen infection (Fig. 4a). Deficiency of prp-19 also suppressed the endogenous induction of several other immune response genes and reduced the survival rate of *C. elegans* when they were exposed to *Pseudomonas aeruginosa* (Fig. 4b and c). Taken together, these results indicate that PRP-19 plays a critical role in mediating the innate immune response.

The physiological function of PRP-19 was assessed by examining lifespan regulation in the presence or absence of mitochondrial stress. Under normal conditions, prp-19 RNAi had a minor shortening effect on worm lifespan (Fig. 5a). However, knockdown of prp-19 greatly suppressed the lifespan extension in atp-2 RNAi-treated worms (Fig. 5a). To test the knockdown efficiency of double RNAi, we performed qPCR experiments and showed that the reduction in atp-2 expression achieved with a mix of atp-2 RNAi plus prp-19 RNAi is similar to that with atp-2 RNAi plus the control RNAi (Fig. 5b). In addition, knockdown of prp-19 suppressed the lifespan extension of worms carrying a mutation in the mitochondrial gene isp-1 (Fig. 5c). Age-related decline of protein homeostasis results in the toxic accumulation of protein aggregates, including aggregates of proteins containing polyglutamine (polyQ) repeats [2, 11]. Consistent with the role of PRP-19 in lifespan regulation, knockdown of prp-19 impaired the mobility of worms expressing polyQ repeats (Fig. 5d) and significantly increased the number of polyQ aggregates in *C. elegans* (Fig. 5e and f). Collectively, these results suggest that PRP-19 is essential for the UPRmt-mediated beneficial impact to alleviate age-related pathology.
Discussion

Upon mitochondrial perturbation, UPR$^\text{mt}$ is activated to initiate a mitochondrion-to-nucleus crosstalk to activate the transcription of mitochondrial stress response genes, as well as innate immune response genes. Here, we report that splicing factors such as PRP-19 are required for the activation of UPR$^\text{mt}$ in C. elegans. In addition, we show that PRPF19 (mammalian ortholog of PRP-19) also mediates mitochondrial homeostasis in mammals.

prp-19 is an essential gene. prp-19 RNAi causes sterility and embryonic lethality. Therefore, in several experiments (Figs 1c and 4c and Supplementary Fig. S1), we used the temperature-sensitive sterile glp-4 mutant strain to avoid collecting the embryos generated in the control RNAi group, which may affect the results. Knockdown of prp-19 suppresses the induction of both UPR$^\text{mt}$ and UPR$^\text{ER}$, but not the induction of the heat shock response (Fig. 1a–c, h–j), which reveals the specificity of PRP-19 in regulating stress.

Figure 3 PRP-19 functions downstream of ATFS-1. (a) Fluorescence images of hsp-16.2p::atfs-1$^{1-32$.myc};hsp-60p::gfp, atfs-1(tm4525) (top) or hsp-16.2p::atfs-1$^{1-32$.gfp (bottom) animals raised on control, prp-19 or atfs-1 RNAi, and followed by heat shock treatment. Scale bar, 200 µm. (b) Heat map of the expression levels of the prp-19-dependent genes whose promoters are bound by ATFS-1 during mitochondrial stress. $\log_2$(fold change) $> 1$, $P < 0.05$. (c) Diagram of the ATFS-1 ChIP assay. (d) Anti-GFP ChIP-QPCR of hsp-6 or hsp-60 promoters. hsp-16.2p::atfs-1$^{1-32$.myc::gfp worms were raised on control or prp-19 RNAi, followed by heat shock treatment to induce the expression of ATFS-1$^{1-32$.myc::GFP. $n = 2$, $P < 0.05$. n.s., no significance.
responses. Knockdown of prp-19 promotes the accumulation of polyQ35::YFP aggregates in muscles. We speculate that the effect of PRP-19 on age-associated polyQ accumulation is due to UPRmt but not UPRER based on the following two reasons: (i) it has been shown that the accumulation of polyQ35::YFP aggregates in muscles is unaffected by the expression of xbp-1s, which activates UPRER [31], and (ii) our previous studies showed that knockdown of the histone deacetylase HDA-1, which specifically regulates UPRmt but not UPR ER, promotes polyQ35::YFP aggregate accumulation in muscles [11].

It has been reported that PRP-19 has both pre-mRNA processing factor and E3 ubiquitin ligase activities [32]. The E3 ligase activity of PRP-19 is critical for the catalytic activation of the spliceosome [23]. On the other side, Prp19 has been reported to require components of the Prp19C/NTC complex for the E3 activity [33]. Taking these findings together, it is very difficult to separate the putative E3 activity of PRP-19 from the splicing activity. We knocked down several splicing factors that function outside the Prp19C/NTC complex (Fig. 2b–d), and found that many of those factors also affect UPRmt. These results suggest that PRP-19 may modulate UPRmt through its function in splicing.

Splicing has been shown to occur and function co-transcriptionally. Although PRP-19 and other splicing factors modulate the activation of UPRmt, we did not observe extensive AS events when C. elegans was challenged with mitochondrial perturbation (Supplementary Fig. S1a). Therefore, the splicing machinery might regulate transcription through a splicing-independent mechanism. At this stage, how the splicing machinery specifically modulates the transcription of mitochondrial stress response genes upon mitochondrial inhibition remains speculative. We speculate that PRP-19 functions downstream of ATFS-1 based on the following reasons: (i) knockdown of prp-19 impairs the induction of the UPRmt gene hsp-60 driven by the expression of ATFS-1Δ1-32, (ii) knockdown of prp-19 impairs the induction of ATFS-1-dependent UPRmt genes, (iii) lack of PRP-19 does not affect the binding of ATFS-1 to the promoters of UPRmt genes, and (iv) knockdown of other splicing factors besides PRP-19 also impairs the induction of UPRmt genes. Notably, ATFS-1-dependent transcription of hsp-6 is basally active throughout worm development, and it can be further activated by diverse mitochondrial perturbations. If PRP-19 acts downstream of ATFS-1 to modulate the transcription of UPRmt genes, knockdown of prp-19 by RNAi may suppress the basal expression level of hsp-6 under normal conditions (Fig. 1c). Currently, we do not fully understand how PRP-19 and other splicing factors regulate ATFS-1-dependent transcription. One possibility is that splicing factors may specifically interact with both ATFS-1 and the C-terminal domain of RNA Polymeerse II under mitochondrial stress conditions to promote the transcriptional elongation of UPRmt genes [21, 22]. Further analysis comparing ChIP-seq results using antibodies against RNA Polymeerse II and PRP-19 may provide evidence of how splicing factors regulate the transcription of UPRmt genes. Nucleosome positioning experiments may also shed light on the effect of PRP-19 on transcription. However, it is also possible that PRP-19 may function in parallel to ATFS-1.

Materials and methods
Materials
Reagent and resource information is listed in Table 1.

Experimental model and subject details
Worms were maintained on Nematode Growth Medium (NGM) plates seeded with OP50 bacteria under normal conditions and grown on RNAi plates supplemented with 1.2 mg/ml Isopropyl β-D-thiogalactoside (IPTG) in NGM plates seeded with RNAi bacteria. HEK293T cells and HeLa cells were obtained from ATCC. Cells were cultured in DMEM high glucose medium supplemented with 10% (v/v) fetal bovine serum at 37°C.
Methods

RNA interference

All the RNAi bacteria were obtained from the Ahringer library. RNAi bacteria were grown in liquid lysogeny broth (LB) medium containing 50 μg/ml carbenicillin at 37°C overnight. IPTG (0.2 μg/ml) was added to the RNAi bacterial culture and incubated at 37°C for 4 h to induce the expression of double-strand RNA (dsRNA). Concentrated RNAi bacteria (25x) were seeded onto RNAi plates with 1.2 mg/ml IPTG. Synchronized L1 worms were raised on the RNAi plates at 20 or 25°C.

For shRNA knockdown in mammalian cells, 5 x 10⁵ HEK293T cells in six-well plates were transfected with the plasmid mixture containing 1.5 μg pLKO.1, 0.9 μg psPAX, and 0.6 μg pMD2.G. Medium was changed 12 h after transfection. Transfected cells were cultured for another 36 h to allow lentivirus production. Cells (5 x 10⁵) in 12-well plates were cultured in a virus-containing medium supplemented with 8 μg/ml polybrene for 24 h. Then, 1–2.5 μg/ml puromycin was added to the culturing medium for positive selection.

Induction of UPR<sup>mt</sup>

To induce UPR<sup>mt</sup> with Antimycin A, synchronized L1 worms were raised on 6-cm RNAi plates at 20°C for 48 h. Four hundred microliters of M9 buffer containing 10 μg Antimycin A were dropped to the surface of worm plates. Fluorescent images were taken after another 24 h.

To induce UPR<sup>mt</sup> with RNAi bacteria, synchronized L1 worms were raised on RNAi plates seeded with indicated RNAi bacteria at 20°C (N2) or 25°C (glp-4(bn2)) for 24 h. Secondary RNAi bacteria (cco-1, atp-2, or spg-7 RNAi) pre-induced with IPTG were then added to the plates. Worms were collected or imaged after 48 h.

To induce UPR<sup>mt</sup> in mammalian cells, cells were treated with 20 μM FCCP for 12 h.

Induction of UPR<sup>ER</sup> and heat shock response

To induce UPR<sup>ER</sup> with hsp-4 RNAi, synchronized L1 worms were grown on RNAi plates seeded with the indicated RNAi bacteria at 20°C for 24 h. hsp-4 RNAi bacteria pre-induced with IPTG were
then added to the plates. Fluorescence images were taken after another 48 h.

To induce UPR\textsuperscript{mt} with tunicamycin, synchronized L1 worms were grown on RNAi plates seeded with control RNAi bacteria, or prp-19 RNAi bacteria diluted with control RNAi for 72 h. Gravid adult animals were bleached and the eggs were collected. Synchronized L1 worms were then grown on RNAi plates seeded with the indicated RNAi bacteria in the presence or absence of tunicamycin (10 $\mu$g/ml NGM) at 20°C. Worms were collected after 48 h.

To induce heat shock response, synchronized L1 worms were raised on RNAi plates at 20°C for 24 h. The worms were then raised at 37°C for 1 h and transferred back to 20°C. Worms were imaged after 24 h.

**Microscopy**

Worms were placed on 2% agarose pads in 100 mM NaN\textsubscript{3} diluted with M9 buffer. The images were taken with a Zeiss Imager M2 microscope. HeLa cells stably expressing TXN2-GFP were treated with 10 $\mu$g/ml Antimycin A for 3 h to induce mitochondrial stress. Cells were washed with pre-cold phosphate-buffered saline (PBS) once and fixed with 4% paraformaldehyde fix solution for 10 min. Mitochondrial morphology images were taken with a Zeiss 880 Airyscan FAST microscope.

**Western blotting**

Worms cultured on 6-cm plates under the indicated conditions (~1000 worms) were harvested and washed several times with M9 buffer until the supernatant was clear. worms were centrifuged and worm pellets were suspended with 4 $\times$ NuPAGE LDS loading buffer. The samples were then boiled at 95°C for 10 min. Proteins were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were then blocked with 5% milk in PBST (1% Tween-20 in PBS) followed by incubation with primary and secondary antibodies.

**RNA isolation and quantitative RT-PCR**

Worms raised on 6-cm plates under the indicated conditions (~1000 worms) were harvested and washed with M9 buffer for 20 min until the supernatant was clear. Worms were centrifuged and...
| Reagent or resource                                           | Source                          | Identifier                  |
|--------------------------------------------------------------|---------------------------------|-----------------------------|
| Antibodies                                                   |                                 |                             |
| Rabbit monoclonal anti-HA antibody                           | Cell Signaling Technology       | Cat# 3724; RRID: AB_1549585  |
| Rabbit polyclonal anti-GFP antibody                          | Abcam                           | Cat# ab290; RRID: AB_303395  |
| Rat monoclonal anti-alpha Tubulin antibody                   | Abcam                           | Cat# ab64332; RRID: AB_1140548 |
| Rabbit monoclonal anti-ACTB antibody                         | AbClonal                         | Cat# AC026 RRID: AB_2768234  |
| Bacterial and virus strains                                  |                                 |                             |
| Trans1-T1                                                    | Transgen                        | Cat# CD501-03                |
| TransStbl3                                                   | Transgen                        | Cat# CD521-02                |
| Pseudomonas aeruginosa                                        | Pellegrino et al. [7]           | PA14                        |
| Chemicals, peptides, and recombinant proteins                |                                 |                             |
| Sodium azide                                                 | Sigma                           | Cat# S8032                   |
| Isopropyl-β-D-thiogalactoside                                 | Amresco                         | Cat# 0487-100G               |
| Antimycin A                                                  | Sigma                           | Cat# A8674                   |
| Carbonyl cyanide 4 (trifluoromethoxy)phenylhydrazine         | MedChemExpress                  | Cat# HY-100410               |
| NuPAGE LDS loading buffer                                    | Thermo Fisher                   | Cat# NP0007                  |
| Pierce™ Anti-HA Magnetic Beads                               | Thermo Fisher                   | Cat# 88837                   |
| GFP-Trap Agarose Beads                                       | ChromoTek                       | Cat# gta-20                  |
| Critical commercial assays                                   |                                 |                             |
| One-Step gDNA Removal and cDNA Synthesis SuperMix           | Transgen                        | Cat# AE311-02                |
| SYBR Green PCR Master Mix                                    | Bio-Rad                         | Cat# 1725121                 |
| ChIP DNA Clean & Concentrator                                | Zymo                            | Cat# D5205                   |
| XF Cell Mito Stress Test Kit                                 | Agilent                         | Cat# 103015100              |
| Pierce™ Rapid Gold BCA                                       | Thermo Fisher                   | Cat# A53226                  |
| Deposited data                                               |                                 |                             |
| ATFS-1 CHIP-seq data                                         | Nargund et al. [6]              | GEO: GSE63803                |
| Raw and analyzed data                                        | This paper                      | GEO: GSE191294               |
| Human tissue RNA-seq data                                    | GTEx                            | https://www.gtexportal.org/home/datasets |
| Experimental models: cell lines                              |                                 |                             |
| Human: HEK293T cells                                         | ATCC                            | Cat# CRL-3216                |
| Human: Hela                                                  | ATCC                            | Cat# CRM-CCL-2               |
| Experimental models: *C. elegans* strains                    |                                 |                             |
| zcls13[hsp-6::gfp]                                            | CGC                             | SJ4100                       |
| zcls4[hsp-4p::gfp]                                            | CGC                             | S4005                        |
| dvls70[hsp-16.2::gfp]                                        | CGC                             | CL2070                       |
| zcls39[dve-1p::dve-1::gfp]                                    | CGC                             | SJ4197                       |
| agls17[irg-1p::gfp]                                          | CGC                             | AU133                        |
| rmils132[unc-54p::Q35::yfp]                                   | CGC                             | AM140                        |
| gfp[4-6m2]                                                   | CGC                             | SS104                        |
| N2                                                          | CGC                             | N2                           |
| isp-1(qm150)                                                 | CGC                             | MQ088                        |
| liuls1[hda-1p::hda-1::flag::ha; odr-1p::dsRed]                | YSL1                            | N/A                          |
| liuls2[hda-1p::hda-1::gfp; odr-1p::dsRed]                     | YSL2                            | N/A                          |
| liuls1[hda-1p::hda-1::flag::ha; odr-1p::dsRed]; zcls39[dve-1p::dve-1::gfp] | YSL3                            | N/A                          |
| gfp[4-6m2], rmils132[unc-54p::Q35::yfp]                       | YSL8                            | N/A                          |
| liuEx3[hsp-16.2p::atfs-1<32::gfp; odr-1p::dsRed]              | YSL8                            | N/A                          |
| Reagent or resource | Source | Identifier |
|---------------------|--------|------------|
| liuEx4[prp-19p: mcherry; prp-19; mec-7p:: yfp; zcls13[hs-6p:: gfp]] | liuEx4 | N/A |
| atfs1(tm4525); hsp16p: atfs1[1311:: yfp]; hsp-60p:: gfp; myo-3:: mCherry | Dr. Cole Haynes | N/A |
| hsp-16.2p: atfs-1[Δ132:: myc:: gfp] | Dr. Cole Haynes | N/A |

Oligonucleotides

| hsp-6 QPCR forward: 5’–3’ TCCAAAGTCTTCTCTACC | This paper | N/A |
| hsp-6 QPCR reverse: 5’–3’ CACGATCTCTGGCAACG | This paper | N/A |
| hsp-60 QPCR forward: 5’–3’ GACGGCAACTTGGTACCTG | This paper | N/A |
| hsp-60 QPCR reverse: 5’–3’ GCCCTCGAGAAGTCTCTACC | This paper | N/A |
| act-3 QPCR reverse: 5’–3’ TCCCTCGAGAAGTCTCTACC | This paper | N/A |
| prp-19 QPCR reverse: 5’–3’ TGGCTCTAGGGGATCG | This paper | N/A |
| prp-19 QPCR reverse: 5’–3’ CAGGTCACCAATGACGACG | This paper | N/A |
| mul-1 QPCR forward: 5’–3’ ATACACCTGTGGAACGCG | This paper | N/A |
| mul-1 QPCR reverse: 5’–3’ ATACACCTGTGGAACGCG | This paper | N/A |
| clec-4 QPCR forward: 5’–3’ GAGCGACACTGGTGACTGTG | This paper | N/A |
| clec-4 QPCR reverse: 5’–3’ CCATCCAGAATAGGTTGGCG | This paper | N/A |
| clec-265 QPCR forward: 5’–3’ CACCACACCCCTCACGTATG | This paper | N/A |
| clec-265 QPCR reverse: 5’–3’ GAGAATCTGGGCATGGCTGA | This paper | N/A |
| K08D8.5 QPCR forward: 5’–3’ TGTGCCGGAAATACGCTGAT | This paper | N/A |
| K08D8.5 QPCR reverse: 5’–3’ TAGCGTACGTTCCCTGAGGA | This paper | N/A |
| pals-23 QPCR forward: 5’–3’ AGGAAACGCTAAGCCAACCA | This paper | N/A |
| pals-23 QPCR reverse: 5’–3’ TCTGGTGGCATGGAATCC | This paper | N/A |
| rpl-32QPCR forward: 5’–3’ CTGGTTCCGGAACCAAGGTCA | This paper | N/A |
| rpl-32QPCR reverse: 5’–3’ GTCTGCGGACACGGTTATCA | This paper | N/A |
| hsp-4 QPCR forward: 5’–3’ ATTGAGTGGCTCGGAAGCAA | This paper | N/A |
| hsp-4 QPCR reverse: 5’–3’ GAACCCGATGGCAGCAAGTA | This paper | N/A |
| atp-2QPCR forward: 5’–3’ TCTCGAGGTAATGCAGGATT | This paper | N/A |
| atp-2QPCR reverse: 5’–3’ GACTTCTGGTCCGACTGGG | This paper | N/A |
| prp-19(codon-adapted) QPCR forward: 5’–3’ CCCTCTGCAAGGTCTCTGTC | This paper | N/A |
| prp-19(codon-adapted) QPCR reverse: 5’–3’ CTGGTTCCGGAACCAAGGTCA | This paper | N/A |
| hsp-6 ChIP-QPCR forward: 5’–3’ GCATCATTATTCTCCTAAAACTTG | This paper | N/A |
| hsp-6 ChIP-QPCR reverse: 5’–3’ GCATCATTATTCTCCTAAAACTTG | This paper | N/A |
| hsp-4 ChIP-QPCR forward: 5’–3’ ATGCTAATTTACATCAGAATAGACT | This paper | N/A |
| hsp-4 ChIP-QPCR reverse: 5’–3’ TGCCTCGAGAAGTCTCTACC | This paper | N/A |
| atp-2QPCR forward: 5’–3’ TCTCGAGGTAATGCAGGATT | This paper | N/A |
| atp-2QPCR reverse: 5’–3’ GACTTCTGGTCCGACTGGG | This paper | N/A |

Table 1. Continued
Table 1. Continued

| Reagent or resource | Source | Identifier |
|---------------------|--------|------------|
| ShPRPF19 1# forward: 5’–3’ CCGGCGCTCAAGTCTCTAGCGCTCTGAGTACGCTGAGCTGTAAGCCTCGTGGTTTTT | This paper | N/A |
| ShPRPF19 2# forward: 5’–3’ CCGGCGCTCAAGTCTCTAGCGCTCTGAGTACGCTGAGCTGTAAGCCTCGTGGTTTTT | This paper | N/A |
| LONP1 QPCR forward: 5’–3’ CAACGACCAACCACCGAATG | This paper | N/A |
| LONP1 QPCR reverse: 5’–3’ CAGCTCTCTGGTGCAAAACT | This paper | N/A |
| YME1L1 QPCR forward: 5’–3’ CCCAGGGACTGGAAAGACAC | This paper | N/A |
| YME1L1 QPCR reverse: 5’–3’ GAGCATTCGGCTTTGGAAT | This paper | N/A |
| HSPA9 QPCR forward: 5’–3’ TGGTAGCGACTGGTGGGAAT | This paper | N/A |
| HSPA9 QPCR reverse: 5’–3’ ATTGGAAGCAGGACAATT | This paper | N/A |

Recombinant DNA
pBOBI vector
PLKO.1 vector
Software and algorithms
GraphPad Prism
rMATS 3.2.5
R 4.0.3

Resource availability: Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ying Liu (ying.liu@pku.edu.cn).

N/A, Not Applicable.

worn pellets were suspended with 1 ml TRizol reagent. Repeated freeze-thawing of worm samples was performed with liquid nitrogen five to six times before RNA isolation. HEK293T cells in 12-well plates were collected with PBS and suspended with 1 ml TRizol reagent. RNA was isolated using chloroform extraction, precipitated with isopropanol, and washed with 75% ethanol and 100% ethanol. cDNA was synthesized with One-Step gDNA Removal and cDNA Synthesis SuperMix. qPCR was carried out using SYBR Green PCR Master Mix. For quantification, transcript levels were normalized to act-3 or rpl-32 for worms and ACTB for mammals.

Immunoprecipitation
Approximately, 50 000 synchronized L1 worms were raised at 20°C on 15-cm RNAi plates seeded with pre-induced control RNAi or prp-19 RNAi bacteria. Late L4 worms were collected with PBS and suspended with 1 ml TRizol reagent. RNA was isolated using chloroform extraction, precipitated with isopropanol, and washed with 75% ethanol and 100% ethanol. cDNA was synthesized with One-Step gDNA Removal and cDNA Synthesis SuperMix. qPCR was carried out using SYBR Green PCR Master Mix. For quantification, transcript levels were normalized to act-3 or rpl-32 for worms and ACTB for mammals.

PA14 slow-killing assay
Pseudomonas aeruginosa (PA14) was cultured in 5 ml liquid LB containing 50 μg/ml ampicillin at 37°C overnight. Eighty microliters of the bacterial solution were dropped in the center of 3.5-cm slow-killing plates and incubated at 37°C for 24 h. The plates were then transferred to 25°C and incubated for another 24 h. Synchronized L1 glp-4 (bn2) worms were grown at 25°C on RNAi plates seeded with control RNAi or prp-19 RNAi. L4 worms were picked and transferred to the slow-killing plates at 25°C. The number of dead nematodes in the plates was counted every 12 h. Three biological replicates of the PA14 slow-killing assay were performed per condition.

Induction of nematode immune response
Pseudomonas aeruginosa (PA14) was cultured in 5 ml liquid LB containing 50 μg/ml ampicillin at 37°C overnight. Four hundred microliters of the bacterial solution were dropped into 6-cm slow-killing plates and incubated at 37°C for 24 h. The plates were then transferred to 25°C for another 24 h. L4 worms (N2) were washed off the plates after being treated with the indicated RNAi from L1, transferred to the slow-killing plates, and placed at 25°C for 2 h. Worms were harvested and washed with M9 buffer until the supernatant was clear. Worm pellets were then suspended in 1 ml TRizol reagent and prepared for RNA extraction.
Mobility assay
unc-54p::Q35::yfp animals were touched twice lightly with platinum wire on the tail. The total number of animals and those who could not move or change their positions after touching were counted on days 1, 7, and 11 of adulthood. Worms were randomly selected. Three biological replicates of the mobility assay were performed under each condition.

ChIP immunoprecipitation
ChIP assay was performed using the hsp-16.2p::atfs-1::32-myc::gfp strain. Synchronized L1 worms were raised in 15-cm RNAi plates containing control RNAi or prp-19 RNAi at 16°C until the worms reached the L4 stage. Animals were cultured at 37°C for 1.5 h and returned to 16°C for an additional 2 h. Approximately, 450,000 worms in each condition were collected with M9 buffer containing 0.01% Triton-X100. Worms were then washed with M9 buffer and pre-cooled PBS until the supernatant was clear.

After centrifugation at 2000 rpm for 1 min, worm pellets were suspended with 1 ml formaldehyde cross-linking solution (PBS containing 2% formaldehyde) and fixed at room temperature for 15 min. Glycine solution was added to the mixture to reach a final concentration of 125 mM and incubated at room temperature for 5 min. The mixture was centrifuged at 2000 rpm for 4 min and the pellets were washed with chilled PBS containing proteinase inhibitors three times. Two milliliters of SDS lysis buffer (1% SDS, 0.01 M EDTA pH 8.0, protease inhibitor cocktail) were added to the worm pellets and the mixture was placed on ice for 10 min. The animals were then fully ground with a homogenizer until no intact worms could be observed under a stereomicroscope. The worm lysates were fully sonicated (30% sonication power, cycled in a program of 10 s sonication and 10 s pause, 20 cycles). Lysates were centrifuged at 20,000 × g for 15 min at 4°C and the supernatants were collected. The protein lysates were quantified using the Pierce BSA kit. A small amount of each supernatant was taken as the input sample. Eight milliliters of ChIP dilution buffer (1% SDS, 11% Triton-X100, 1.2 mM EDTA pH 8.0, 16.7 mM Tris-HCl, 16.7 mM NaCl, proteinase inhibitor cocktail) were added to the supernatant. Half the volume of each worm lysate was incubated with GFP-Trap agarose (pre-coated with 5% BSA and 400 μg/ml Siniperca chuatsi sperm DNA) and the other half volume was incubated with control agarose beads. The mixture was rotated at 4°C for 4 h.

After incubation, beads were washed once with low salt washing buffer (1% SDS, 1% Triton-X100, 2 mM EDTA pH 8.0, 20 mM Tris-HCl pH 8.0, 150 mM NaCl), once with high salt washing buffer (1% SDS, 1% Triton-X100, 2 mM EDTA pH 8.0, 20 mM Tris-HCl pH 8.0, 500 mM NaCl), once with LiCl washing buffer (0.25 mM LiCl, 1% IGEPAL-CA630, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris pH 8.1) and finally twice with TE solution. After centrifugation, the supernatant was clear.

Mitochondrial respiration analysis
Mitochondrial respiration was measured using an Agilent Seahorse XF24 analyzer and Seahorse XF Cell Mito Stress Test Kit (Agilent). Briefly, 4 × 10^4 cells were plated per well with three replicates in Seahorse XF24 cell culture microplates (Agilent). Cells were grown overnight and treated with 10 nM Antimycin A for 30 min. The subsequent assay was performed according to the Agilent Seahorse manufacturer’s instructions. The final reports of OCR signals were generated using GraphPad software.

RNA-seq sample preparation and RNA-seq data analysis
Synchronized L1(p::-4) (bn2) worms were raised on RNAi plates seeded with control RNAi or prp-19 RNAi bacteria at 25°C for 24 h. atp-2 RNAi bacteria pre-induced with IPTG were then added. Worms were collected after an additional 48 h.

Differential AS events were analyzed with the rMATS tool (version: 3.2.5). Parameters: -t paired -len 101 -a 8 -c 0.05 -analysis U based on the RNA-seq data from the indicated conditions. Expression correlation analyses
Raw sequencing data were aligned to the ce11 reference genome using HISAT2 (version 2.1.0). Gene expression levels were quantified by feature Count (version 1.6.3) with the genomic annotation from UCSC (http://genome.ucsc.edu/cgi-bin/hgTables). Downstream analysis was performed using R (version 4.0.5). Differential expression analysis was performed by DESeq2. Genes with log2(fold change) >1 and adjusted P-value <0.05 were considered as differentially expressed genes. For clustering and heat map plots, we used the Pheatmap R package. Worm ATFS-1 ChIP-seq processed data [6] were obtained under accession GEO: GSE63803. The ATFS-1 binding genes were defined as the nearest genes to ATFS-1 ChIP-seq peaks.

Expression correlation analyses
Expression correlation analysis was performed using the expression data of PRPF19 and UPR^mch-related genes in various human tissue samples from the GTEx database (https://www.gtexportal.org/home/datasets). Gene expression levels in the above samples were compared by calculating Pearson’s correlation. Heat map presentation was performed by the ggplot2 function package in R language.

Quantification and statistical analysis
The experiments in this paper were all repeated at least three times unless otherwise indicated. Statistical analysis was performed using GraphPad software and Student’s t-test (two-tailed, unequal) to calculate the P values. IGV software was used to visualize the RNA-seq sequencing results.

Supplementary data
Supplementary data are available at Life Metabolism online.

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Conflict of interest statement
Ying Liu holds the position of Editorial Board Member for Life Metabolism, and is blinded from reviewing or making decisions for the manuscript.

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Author contributions
P.X. and Y.L. conceived the study and designed the experiments. P.X. and L.Z. performed all the experiments. W.D. and J.G. performed bioinformatic analysis. P.X. and Y.L. analyzed the data and wrote the manuscript.

Data availability
The accession number for the sequencing data reported in this paper is GEO:GSE191294.

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