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PEK-1 is crucial for hormesis induced by inhibition of the IRE-1/XBP-1 pathway in the *Caenorhabditis elegans mev-1* mutant

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ABSTRACT

The accumulation of unfolded proteins in the endoplasmic reticulum (ER) causes an imbalance of proteostasis and is related to many pathological conditions. In answer to this ER stress cells activate a network of three integrated signaling pathways consolidated as the unfolded protein response of the ER (UPR^{ER}), which is also present in the stress-sensitive *Caenorhabditis elegans* mutant *mev-1*. Whereas inhibition of one of those pathways by RNA-interference (RNAi) versus *xbp-1* results in reduced survival of *mev-1* nematodes under heat stress, additional knockdown of the *xbp-1* splicing activator *ire-1* results in a PEK-1-dependent hormetic response. In contrast, increased survival under *ire-1*/*xbp-1* double RNAi was found to be independent of the presence of HSP-4, an UPR^{ER}-specific chaperone, as evidenced under *ire-1*/*xbp-1*/*hsp-4* triple knockdown conditions. Moreover, *ire-1*/*xbp-1* double-RNAi significantly increased chymotrypsin-like proteasomal activity, which was completely blocked under additional RNAi versus *pek-1*.

In conclusion, we identified PEK-1 as a mediator of hormesis in the *mev-1* mutant of *C. elegans* which is induced by simultaneous inhibition of XBP-1 and its splicing activator IRE-1 and mediated through activation of the proteasome.

1. Introduction

Proteostasis is a complex network of protein quality control processes that ensure the maintenance and function of the proteome and thus provides an indispensable prerequisite for health and viability of the cell [1]. Endoplasmic reticulum (ER) stress, which results in the accumulation of misfolded proteins in the ER, is a common example of disturbed proteostasis. In answer to ER stress an adaptive response, the unfolded protein response of the ER (UPR^{ER}), is activated in order to restore the proteostatic equilibrium [2]. The UPR^{ER} is mediated by three parallel and partially complementary signaling pathways that regulate translation, chaperone expression and ER-associated degradation (ERAD) [3]. Each of these branches is controlled by an ER luminal sensor, i.e. PERK/PEK-1, IRE-1, and ATF-6. While PEK-1 is mainly responsible for global translation attenuation [4], ATF-6 is a transcription factor that activates the transcription of UPR genes [5]. IRE-1 is an ER transmembrane protein that catalyzes the splicing of *xbp-1* mRNA to initiate the generation of the active form of XBP-1 transcription factor [6]. The IRE-1/XBP-1 pathway in *Caenorhabditis elegans* is especially required for the metabolism of secreted proteins even during unstressed growth conditions [7] and for the proper localization of neuronal proteins [8]. Knockdown of *xbp-1* by RNA-interference (RNAi) leads to a reduced stress resistance in the nematode [9,10] and expression of a constitutively active form of XBP-1 was able to reverse age-onset loss of ER proteostasis [11]. Irrespective of the fact that *xbp-1* mRNA is a direct target of IRE-1 endonucleolytic activity and that processing is a precondition for the accumulation of activated *xbp-1* mRNA during the UPR [12], effects of IRE-1 on determination of germ cell fate, which are independent of its canonical downstream target *xbp-1*, have been described as well [13].

In the present study we investigated in how far the functional loss of IRE-1 affects the reduction of stress resistance observed under *xbp-1* RNAi. In order to identify downstream targets

Abbreviations: ABU, activated in blocked unfolded protein response; ANOVA, analysis of variance; ATF-6, activating transcription factor 6; BiP, immunoglobulin binding protein; *C. elegans*, *Caenorhabditis elegans*; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER associated degradation; IRE-1, inositol requiring enzyme 1; PERK/PEK-1, protein kinase RNA-like ER kinase; RNAi, RNA interference; ROS, reactive oxygen species; Suc-LLVY-AMC, succinyl-leucyl-leucyl-valine-aminomethyl-coumarine; UPR, unfolded protein response; UPR^{ER}, ER-specific UPR; UPS, ubiquitin-proteasome system; XBP-1, X-box binding protein 1.

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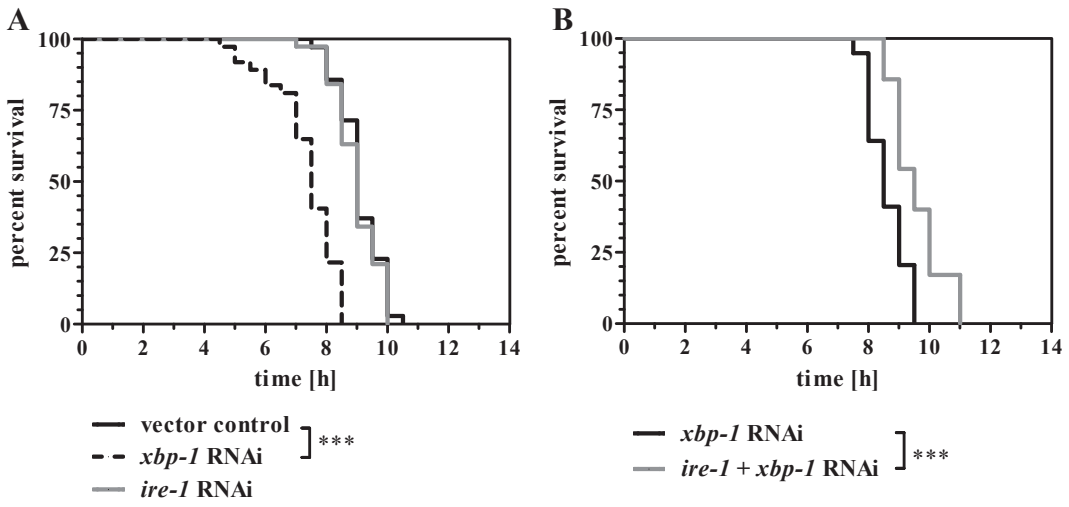

Fig. 1. RNAi of *ire-1* inhibits survival reduction in response to *xbp-1* knockdown. Shown are Kaplan–Meier curves for survival of *mev-1* nematodes at 37 °C as a response to *xbp-1* or *ire-1* RNAi (A) or the combination of both RNAi versus *xbp-1* RNAi (B).

responsible for the observed effects, *pek-1* and *hsp-4* were knocked down. Using a fluorogenic substrate proteasome activity was assessed, in order to correlate effects on stress resistance with activities of the ubiquitin-proteasome-system (UPS).

2. Methods and materials

2.1. Reagents

SYTOX green nucleic acid stain was obtained from Life Technologies (Karlsruhe, Germany). The Brilliant II SYBR Green QRT-PCR Master Mix Kit was purchased from Agilent Technologies (Santa Clara, USA) and the Roti prep RNA MINI Kit was from Carl Roth (Karlsruhe, Germany). The Bio-Rad Protein Assay was purchased from Bio-Rad (Munich, Germany) and the proteasome inhibitor MG-132 was from Calbiochem/Merck (Darmstadt, Germany). Suc-LLVY-AMC and all other materials used were obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Strains and maintenance

C. elegans TK22 *mev-1(kn1)* and SJ4005 *zcIs4 [hsp-4p::GFP]* as well as *E. coli* OP50 were obtained from *C. elegans* Genetics Center (CGC, University of Minnesota, USA). Transgenic *C. elegans* nematodes expressing the *hsp-4p::gfp* transgene *zcIs4* in the TK22 *mev-1* mutant were generated by crossing, as previously described [10]. Nematodes were maintained on liquid nematode growth medium as previously described [14].

E. coli HT115 RNAi clones from the *C. elegans* ORF-RNAi library [15] or Ahringer library [16] were obtained from Source Bioscience (Cambridge, UK) and included a negative control (L4440), *xbp-1* (R74.3), *ire-1* (C41C4.4), *pek-1* (F46C3.1) and *hsp-4* (F43E2.8).

2.3. RNAi experiments

In order to achieve a gene-specific knockdown, RNAi was performed by feeding *E. coli* RNAi clones expressing gene-specific dsRNA, as described before [14]. Knockdown of the corresponding genes was verified by qPCR. Relative mRNA levels in nematodes cultured on RNAi bacteria were significantly lower ($p < 0.01$) than mRNA levels of the vector control (L4440) and were below 0.32 for all genes and gene combinations tested.

2.4. Quantitative real-time PCR

Total RNA was extracted from 2500 worms using the Roti®-Prep RNA MINI Kit. One-step-real-time PCR reactions were performed in triplicate using 1 µl of RNA template (10 ng/µl), the Brilliant II SYBR Green QRT-PCR Mastermix Kit and adequate primers in a Qiagen Rotor Gene-Q Real-time PCR Cycler (Hilden, Germany). Cycling conditions were 1 × 30 min 50 °C, 1 × 10 min 95 °C, 40 × [30 s 95 °C, 1 min 53 °C, 30 s 72 °C], 1 × 1 min 95 °C, 1 × [30 s 55 °C–95 °C]. Changes in target gene expression were calculated according to Pfaffl [18] using equation $(2)^{-\Delta\Delta CT}$. The fold change in the target gene was normalized to 18S rRNA and relative to the control expression for each sample. Primers used for qPCR were as follows: 18S-rRNA fw-5′-ATG GTT GCA AAG CTG AAA CT-3′, 18S-rRNA rev-5′-TCC CGT GTT GAG TCA AAT TA-3′, hsp-4-fw 5′-CGT TGA TTT TGA TTG CCT TC-3′, hsp-4-rv 5′-TGA TTC TGT TTC CTT GAT CG-3′, ire-1-fw 5′-GCT CTT CTC CTC TGT AAT TT-3′, ire-1-rv 5′-ATC GTA AAA CAG GCT CTT C-3′, xbp-1-fw 5′-ACG TAT TTA TGT GCT CCC AG-3′, xbp-1-rev 5′-TAT CAT CGC CAA GAA GTT GT-3′, pek-1-fw 5′-ATC CAT AAC AGA GCG ATG AT-3′, pek-1-rv 5′-ATC CAA TCT TCA AGG GTC TT-3′.

2.5. Survival analysis at 37 °C

Survival analysis under heat stress conditions was performed using a thermotolerance assay as previously described [14].

2.6. Fluorescence microscopy

Quantification of GFP-fluorescence was performed using the AMG EVOS fl digital fluorescence microscope (Bothell, USA). For epifluorescence microscopy nematodes were washed twice with M9/Tween20 buffer and subsequently anaesthetized by addition of 2 mM levamisole. GFP fluorescence was analyzed using an EVOS GFP Light Cube with an excitation at 470/22 nm and an emission at 510/42 nm. Images were taken at tenfold magnification and the quantification of fluorescence intensity was done using ImageJ (National Institute of Health, NIH).

2.7. Chymotrypsin-like proteasomal activity

The chymotrypsin-like proteasomal activity was quantified with