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Compensatory regulation among ER chaperones in *C. elegans*

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Abstract **We have identified a particularly clear case of compensatory transcriptional regulation among ER chaperones in *Caenorhabditis elegans* using a GFP reporter transgene that is under the control of the promoter of *hsp-4*, a *C. elegans* homolog of GRP-78/BiP. Knockdown by RNA interference of 9 known or predicted ER chaperones induced *hsp-4* upregulation via the *ire-1*/*xbp-1* signaling cascade employed in the unfolded protein response. We show that this compensatory regulation is specific for ER chaperones, not dependent on RNA interference, and required for maintaining viability in worms containing a deletion of the *hsp-3* gene.**

Keywords: Endoplasmic reticulum; Chaperone; RNAi; Transcriptional regulation; *Caenorhabditis elegans*

1. Introduction

The determination of gene function by classical genetic approaches is currently being extended by "reverse" genetic approaches such as construction of genome wide deletion libraries [1–3] or high throughput RNA interference screens [4–6]. All of these approaches can be confounded by genetic redundancy or compensatory regulation, which can obscure phenotypic changes resulting from loss of specific gene function. Similarly, many mouse knock-out models have had surprisingly minimal phenotypes [7,8], presumably due to compensatory gene expression changes. The molecular mechanisms underlying these predicted compensatory changes have often remained unidentified.

RNA interference (RNAi) has become a particularly powerful tool for studies in *Caenorhabditis elegans*, as the development of genome wide "feeding libraries" has allowed high throughput gene inactivation studies based on propagating *C. elegans* worms on *Escherichia coli* clones engineered to express dsRNA that corresponds to specific *C. elegans* genes [7,9,10]. We have previously reported the use of feeding RNAi to investigate the role of chaperone proteins in the toxicity of the human β amyloid peptide (Aβ) in a transgenic *C. elegans* Alzheimer's disease model [11]. In these studies, co-immunoprecipitation followed by mass spectrometry was used to demonstrate interaction between intracellular Aβ and a number of endogenous chaperone proteins, including HSP-1 (a cytoplasmic HSP70), HSP-16 (an αB-crystallin homolog), and HSP-3 (homologous to endoplasmic reticulum (ER) HSP70 GRP78/BiP). By analogy to other studies demonstrating a protective effect of HSP70 with respect to toxic protein aggregates [12], we anticipated RNAi knockdown of HSP-3 would exacerbate Aβ toxicity in this model. When we failed to observe the expected enhancement of Aβ toxicity, we investigated whether a change in the expression of *hsp-4* (the second GRP78/BiP homolog in *C. elegans*) might compensate for loss of *hsp-3* function. In addition to their sequence similarity, *hsp-3* and *hsp-4* are both upregulated in response to ER stress, and contain similar upstream regulatory elements [13]. Here, we show that compensatory upregulation of *hsp-4* does in fact occur in response to loss of function of either *hsp-3* or a number of other ER-resident chaperones, and this compensatory gene expression depends upon the unfolded protein response (UPR) regulatory system.

2. Materials and methods

2.1. Strains

The following *C. elegans* strains were used in this study: SJ4005 (*zcIs4*[*hsp-4*::GFP] V), SJ17 (*xbp-1*(*zc12*) III; *zcIs4* V), SJ30 (*ire-1*(*zc14*) II; *zcIs4* V), RB1104 (*hsp-3*(*ok1083*) X) and CL684 (*zcIs4*[*hsp-4*::GFP] V; hsp-3(ok1083) X). The *zcIs4* reporter transgene is inserted into chromosome V, and thus is unlinked to the endogenous *hsp-4* gene (on chromosome II).

2.2. RNAi

All *E. coli* RNAi feeding clones were from the Ahringer genome-wide library purchased from MRC Geneservice. Lists of *C. elegans* candidate chaperone proteins were generated by identifying all genes in Wormbase (http://www.wormbase.org) encoding proteins with the following Pfam domains: PF00012 (HSP70 protein), PF00011 (Hsp20/alpha crystallin family), PF00226 (DnaJ domain), PF00183 (HSP90 protein), or PF00118 (TCP-1/cpn60 chaperonin family). Genes encoding candidate ER-resident proteins were selected by identifying all genes encoding proteins with INTERPRO domain IPR000886, which encodes predicted ER targeting signals, and additional chaperone-related genes (e.g., *bag-1*, prefoldin homolog C55B7.5) were identified based on Wormbase annotations. These lists were cross-referenced with the Ahringer RNAi clone list, yielding 87 RNAi clones encoding putative chaperone and/or ER-resident proteins. (The Ahringer library contains RNAi clones for ~86% of all identified *C. elegans* genes, so clones were not recovered for every candidate gene.) Nematode Growth Media plates were spotted with *E. coli* RNAi clones as described [14]. To assay the effect of RNAi clones on expression of the *hsp-4*::GFP reporter transgene, RNAi plates were seeded with first larval stage *zcIs4* worms and incubated at 20 °C for 3–4 days, and then scored at the fourth larval or young adult stage.

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Abbreviations: ER, endoplasmic reticulum; GFP, green fluorescent protein; UPR, unfolded protein response

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2.3. Microscopy

Induction of the *hsp-4*::GFP reporter transgene was initially assayed by examining RNAi plates with a Leica MZ12 epifluorescence dissecting microscope. Worms of interest were subsequently imaged using a Zeiss Axioskop microscope equipped with a digital image acquisition system (Intelligent Imaging Innovations).

2.4. Fluorescence quantitation

Overall GFP fluorescence of RNAi-treated populations was assayed using a Tecan GENios microplate reader. Fifty young adult animals were recovered by hand from RNAi plates and transferred in 50 μl of S buffer [15] to a well of a 96-well microtiter plate (clear, flat bottom wells), and total GFP fluorescence was measured using 485 nm excitation and 535 nm emission filters. Triplicate populations were used for each determination and background signals (determined by assaying populations of wild-type *C. elegans* animals in parallel) were subtracted prior to calculating average relative fluorescence.

2.5. Quantitative RT-PCR

Specific *C. elegans* transcript levels were determined as previously described [16], using an ABI Prism 7000 real-time PCR machine (Applied Biosystems). *hsp-3* and *hsp-4* transcript levels were normalized to the levels of *ama-1*, the sole RNA polymerase II in *C. elegans*, and the normalized levels for *zcIs4* animals exposed to *hsp-3* and vector-only RNAi were compared. The specific primer pairs used were: *hsp-3* – GGATAAGGGAACCGGAAACAA (f), TGATCATGCGCTCGATGTCT (r); *hsp-4* – GCAGATGATCAAGCCCAAAAAG (f), GCGATTTGAGTTTTCATCTGATAGG (r); *ama-1* – GCGGTCAGAAAGGCTATCGA (f), AGCAGTGCCAAATGTCGGTAAT (r).

3. Results

To examine *hsp-4* expression, we made use of a transgenic *C. elegans* strain (SJ4005) that is homozygous for a reporter transgene (*zcIs4*) integrated into chromosome V. The *zcIs4* transgene consists of a transcriptional fusion of the *hsp-4* promoter to green fluorescent protein (GFP) [17]. The *hsp-4* gene has previously been shown to be transcriptionally upregulated in response to treatments inducing ER stress [13], and as expected, the *zcIs4* reporter is also strongly upregulated in response to treatments that induce ER stress [17]. As shown in Fig. 1A, inhibition of *hsp-3* expression by exposure of the *zcIs4* strain to a feeding RNAi strain targeting *hsp-3* leads to a strong increase in GFP expression in comparison to control RNAi treatment, reflecting an upregulation of the *hsp-4* gene. Quantification of fluorescence levels indicates an approximately 10-fold increase in *hsp-4*::GFP reporter expression as the result of RNA interference of *hsp-3* (Fig. 1B). This increase in GFP is not due to effects on the stability of the GFP protein itself, as other unrelated GFP transgenes do not show increased expression (data not shown). Quantitative RT-PCR (using gene-specific probes) performed on *zcIs4* animals similarly exposed to *hsp-3* RNAi revealed an ~2-fold decrease in endogenous *hsp-3* mRNA (0.48-fold control RNAi treatment, range +0.13, −0.11) accompanied by a ~6-fold increase in endogenous *hsp-4* mRNA (6.19-fold control RNAi treatment, range +1.99, −1.51). These results demonstrate that *hsp-3* feeding RNAi does knockdown endogenous *hsp-3* mRNA, and the increase in reporter GFP does reflect endogenous *hsp-4* transcription.

To determine the generality of this compensatory regulation, we tested the response of the *zcIs4* transgene to RNA inhibition of genes encoding other chaperone and ER resident proteins. We identified 87 known or predicted chaperone and/or ER-resident proteins represented in the Ahringer genome-wide RNAi library (see Supplementary table). All of these clones were tested for their ability to induce the *zcIs4* reporter transgene, and eight additional genes were identified whose RNAi knockdown reproducibly caused induction (see Table 1). Seven of these genes encode likely ER-resident proteins, based on the presence of ER retention motifs in their C-termini. Among these inducer RNAi clones was *hsp-4* itself, indicating an autoregulatory loop for *hsp-4* expression. Interestingly, no effect on *zcIs4* reporter induction was observed for any of the cytoplasmic HSP70, DNAJ, αB-crystallin, or chaperonin family RNAi clones tested.

The strong bias for ER-associated inducer genes suggested that the compensatory regulation of *hsp-4* might occur via the same regulatory mechanism underlying UPR. We therefore

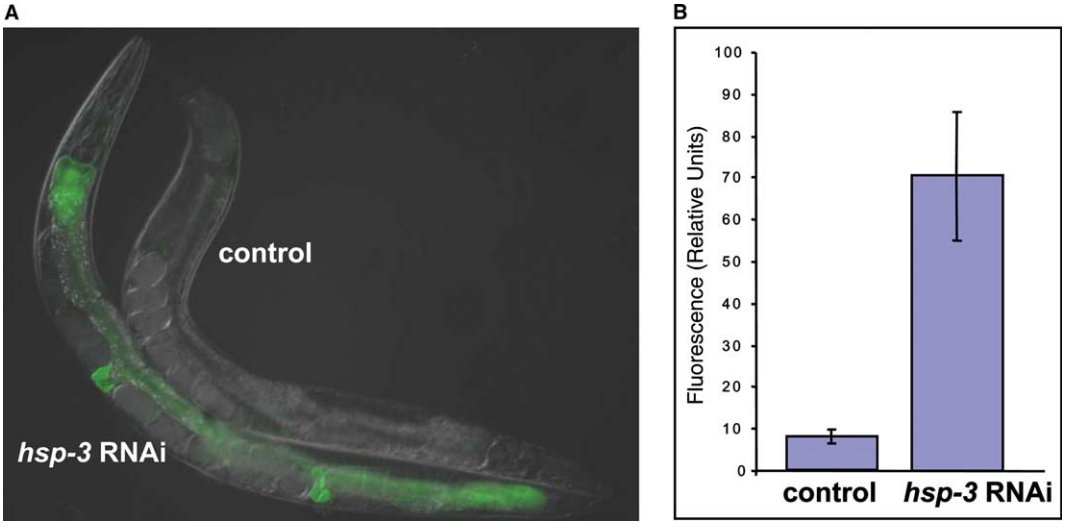

Fig. 1. *hsp-3* RNAi induces *hsp-4*::GFP reporter. (A) Adult *zcIs4* (*hsp-4*::GFP) animals propagated on control (L4440 vector only) or *hsp-3* RNAi. Note induction of GFP expression by *hsp-3* RNAi, particularly in the intestine and spermatheca (digitally fused DIC/epifluorescence image). (B) Microplate reader determination of overall fluorescence of *hsp-3* and control RNAi-treated *zcIs4* populations. Error bars represent S.E. of the mean.

Table 1
RNAi clones that reproducibly induce the *hsp-4*::GFP reporter transgene

Gene ID	Gene name	Description	C-terminal sequence
C15H9.6	*hsp-3*	GRP78/BiP (HSP70)	KDEL
F43E2.8	*hsp-4*	GRP78/BiP (HSP70)	HDEL
F54C9.2	*stc-1*	STCH (HSP70)	ISEL
T05E11.3		GRP94 (HSP90)	HSEL
K11D9.2	*sca-1*	SERCA Ca^{2+} channel	HNEL
Y38A10A.5	*crt-1*	Calreticulin	HDEL
C55B6.2	*dnj-7*	P58(IPK) (DNAJ)	FNWG
W10G11.19			RDEL
F58G1.4			HEEL

tested the response of the *zcIs4* transgene to the nine inducer RNAi clones in genetic backgrounds containing loss-of-function mutations in *ire-1* or *xbp-1*, two genes directly involved in the transcriptional response to tunicamycin-induced ER stress [13,17]. Both *ire-1*(*zc14*) and *xbp-1*(*zc12*) mutations suppressed the induction of *zcIs4* by all of the inducer clones, indicating that in all cases the compensatory upregulation of *hsp-4* occurred through the UPR pathway.

To exclude the possibility that the RNAi process itself might influence induction of the *hsp-4*::GFP reporter, we asked whether similar compensatory regulation would occur after genetic inactivation of an inducer gene. The deletion allele *hsp-3*(*ok1083*) was introduced into the *zcIs4* strain by classic genetic mating, and a strong induction of the GFP reporter was observed in the resulting strain homozygous for both the *hsp-3*(*ok1083*) deletion and the *zcIs4* reporter gene (see Fig. 2A). Quantification of this induction (Fig. 2B) indicated that deletion of the *hsp-3* gene resulted in an approximately 30-fold increase in *hsp-4*::GFP reporter expression. (The observation that genetic deletion of *hsp-3* induces the *hsp-4*::GFP reporter more strongly than *hsp-3* RNAi is consistent with the quantitative RT-PCR data showing this RNAi treatment does not completely abolish *hsp-3* function.) RNA interference of *ire-1* or *xbp-1* suppressed this induction of the *zcIs4* reporter, again illustrating the specific involvement of UPR regulatory cascade. This induction was not detectably suppressed by RNAi against other regulators of the UPR (ATF6, PERK), or against the sole known heat shock transcription factor in *C. elegans*, *hsf-1*.

Surprisingly, the *zcIs4*; *hsp-3*(*ok1083*) double homozygous strain also showed significant phenotypic sensitivity to *ire-1* or *xbp-1* RNAi treatments, with *ire-1* RNAi causing sterility and *xbp-1* RNAi resulting in larval arrest (Fig. 2A). In both our experiments and published studies [4,10,18,19] neither of these RNAi treatments caused obvious deleterious effects in wild-type animals. These synthetic deleterious interactions also occurred with the single *hsp-3*(*ok1083*) homozygous mutant (Fig. 3), but not with the *zcIs4* reporter strain. Similar severe developmental effects were observed in the converse experiment, when strains containing *ire-1*(*zc14*) or *xbp-1*(*zc12*) were treated with *hsp-3* RNAi. These results indicate that in the absence of *ire-1*/*xbp-1*-dependent compensatory regulation, *hsp-3* is effectively an essential gene.

4. Discussion

We have found that RNA interference of at least 9 genes can lead to the upregulation of an *hsp-4*/GFP reporter transgene. For all of these gene inactivations, the induction of the *hsp-4* GFP reporter depends upon the *ire-1*/*xbp-1* signaling pathway involved in the regulation of the UPR. The induction of *hsp-4* by *hsp-3* RNAi is probably the clearest example of a direct compensatory regulation, given that *hsp-3* and *hsp-4* encode GRP78/BiP family chaperones with presumably

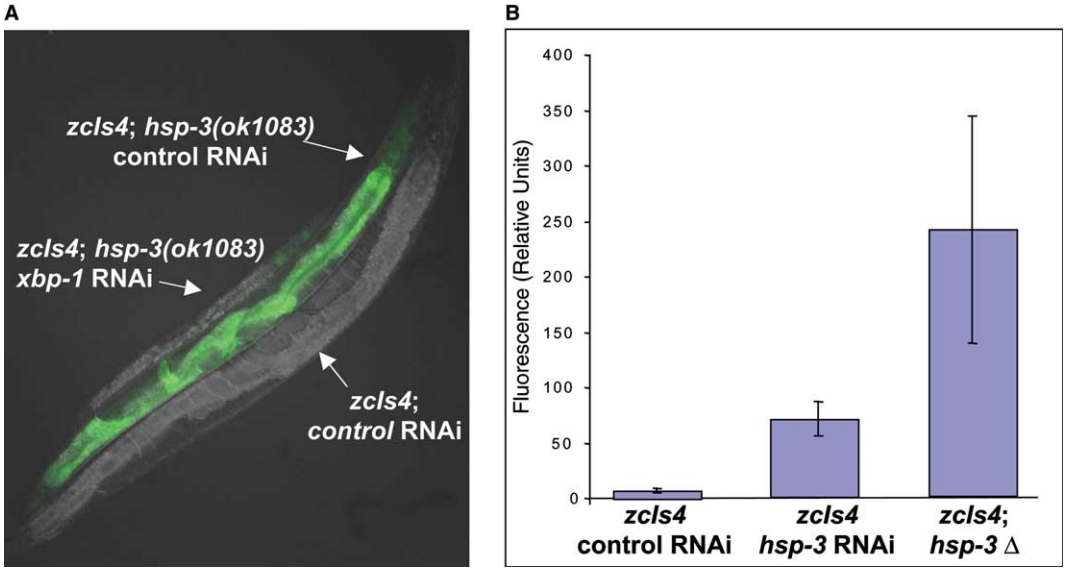

Fig. 2. Induction of *hsp-4*::GFP by *hsp-3* loss-of-function mutation requires *xbp-1* activity. (A) Introduction of a *hsp-3* deletion allele into the *zcIs4* reporter background leads to strong GFP induction (compare central worm to one on right). Exposure of *zcIs4*; *hsp-3*(*ok1083*) to *xbp-1* RNAi dramatically reduces fluorescence (compare left worm to central one). Note retarded development of the *xbp-1* RNAi treated *zcIs4*; *hsp-3*(*ok1083*) animal. (B) Microplate reader determination of overall fluorescence resulting from *hsp-3* RNAi or *hsp-3* genetic deletion. Error bars represent standard error of the mean.

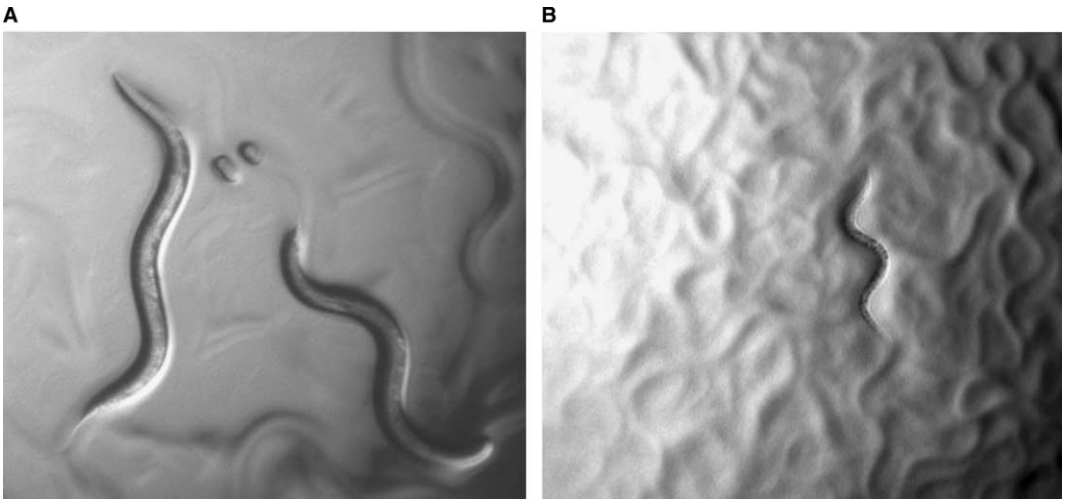

Fig. 3. *hsp-3* is essential for normal development in the absence of *xbp-1*-dependent compensatory regulation. *hsp-3*(*ok1083*) worms propagated from eggs for 4 days on control (panel A) or *xbp-1* (panel B) RNAi.

very similar functions. Our demonstration that an *hsp-3* deletion mutant is inviable in the absence of *ire-1*/*xbp-1* signaling illustrates the importance of this compensatory regulation in maintaining homeostasis of ER chaperone capacity, even in the absence of an exogenous ER stress. A similar observation has been made in yeast, where the viability of *LHS1* (an ER-localized HSP70-related protein) null mutants depends on IRE1 function [20]. Our results also demonstrate the compartment specificity of this regulation, as *hsp-4* was not upregulated in response to RNA interference of any cytoplasmic chaperone protein tested. Mitochondrial chaperones *hsp-6* and *hsp-60* have similarly been shown to be upregulated specifically in response to mitochondrial insults [21].

The *hsp-4*/GFP transgene is also induced in response to RNAi knockdown of two other putative ER chaperones, T05E11.3 (likely ortholog of GRP94/endoplasmin) and *stc-1* (homologous to mammalian STCH). Two inducer RNAi clones, W10G11.19 and F58G1.4, encode novel proteins containing predicted ER retention signals. Although these proteins lack informative homologies, our results suggest these proteins may have chaperone functions in the ER. RNAi of all these genes can also cause larval arrest or growth deficits [4,9,22], indicating that these genes have important functions not effectively compensated by the UPR.

Only one inducer RNAi clone, encoding the DnaJ domain-containing protein DNJ-7, lacked an apparent C-terminal ER retention signal (see Table 1). However, DNJ-7 does contain a predicted signal peptide and N-terminal transmembrane domain, suggesting that this is a type I ER protein with a cytoplasmic C terminus. The *dnj-7* gene encodes the likely ortholog of mammalian $p58^{IPK,}$ a protein originally identified as an inhibitor of the double stranded RNA-activated protein kinase PKR [23], and subsequently shown to function as a negative regulator of the PKR-like ER kinase (PERK) [24]. Although $p58^{IPK}$ silencing by $p58^{IPK}$ siRNA in HEK-293 cells has been reported to have no effect on GRP78/BiP mRNA levels [25], our results support a role for $p58^{IPK}$ in the attenuation of both the translational and transcriptional components of the UPR.

RNA interference of *crt-1* [26] and *sca-1* [27], the *C. elegans* orthologs of calreticulin and the SERCA Ca^{2+} pump, respectively, also result in *hsp-4* upregulation. Loss of function of these ER resident proteins would be expected to perturb ER calcium homeostasis. The induction of *hsp-4* by *crt-1* or *sca-1* RNAi is not surprising, given that thapsigargin, a known inducer of UPR, acts by perturbing ER calcium stores [28]. Loss of function mutations of *crt-1* have been shown to suppress neurodegeneration induced by dominant channel-activating mutations in members of the *C. elegans* degenerin family, such as *mec-4*(*d*) [29]. These studies demonstrated that *crt-1* mutations also reduce the expression of a *mec-4*::GFP translational fusion reporter construct, implying that these mutations suppress degeneration at least partially by reducing the expression of the toxic channel proteins. Our results suggest that this reduction in expression might result from a compensatory upregulation of *hsp-4* or other ER chaperones, leading to increased turnover of mutant channel proteins via ER-associated protein degradation (ERAD).

The nine genes identified in this study represent a minimal set of genes whose inactivation can lead to *hsp-4* upregulation. Although our RNAi treatments typically reproduced previously reported phenotypes, we do not know the effectiveness of each individual RNAi clone, and thus some *hsp-4*-inducing genes might have been missed. In addition, some chaperone genes (e.g., *daf-21*, an HSP90 homolog) are not represented in the Ahringer library, and inactivation of other classes of genes not tested might also lead to *hsp-4* upregulation. Urano and colleagues [30] have shown that RNA interference of *abu-1* also results in induction of the *zcIs4* reporter. The *abu-1* gene a member of a family of small, glutamine and asparagine-rich genes of unknown function that are upregulated in response to ER stress in *xbp-1* mutant animals. Thus, while we have not exhaustively determined all gene inactivations that lead to *hsp-4* induction, we have shown that RNAi knockdown of a specific set of ER-resident genes clearly leads to *hsp-4* transcriptional upregulation. We have not been able to address possible additional translational regulation of ER chaperones

due the unavailability of specific antibodies against these proteins.

Although it is widely recognized that interpretation of the results of large-scale gene inactivation studies may be complicated by genetic redundancy and compensatory regulation, specific phenotypes are routinely interpreted as resulting directly from loss of function of the targeted gene. We have identified a specific instance of compensatory regulation in *C. elegans* and illustrated how this might lead to misleading interpretation of an RNAi phenotype. Although we do not know how readily our observations with chaperone-related proteins can be extrapolated to RNAi screens in general, we note that some unexpected results from *C. elegans* RNAi screens could be explained by some forms of compensatory regulation. For example, high throughput RNAi screens have identified a large number of mitochondrial proteins whose inhibition results in increased longevity [31,32]. It is possible that these observed lifespan increases are not due to reduced mitochondrial function per se, but instead result from gene expression changes compensating for reduced mitochondrial function. In another instance, genes involved in both protein degradation (e.g., proteasomal subunits) and protein synthesis (e.g., ribosomal subunits) were identified in a genome-wide screen for RNAi treatments that enhanced aggregation of a polyglutamine repeat-GFP fusion protein [33]. This seemingly paradoxical result suggests that in the case of RNA interference of genes involved in protein synthesis, it may be the subsequent compensatory physiological changes that actually lead to increased aggregation of the reporter protein.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2005.04.062.

References

[1] Winzeler, E.A., et al. (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285, 901–906.
[2] Giaever, G., et al. (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418, 387–391.
[3] Parks, A.L., et al. (2004) Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. Nat. Genet. 36, 288–292.
[4] Kamath, R.S., et al. (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421, 231–237.
[5] Boutros, M., et al. (2004). Genome-wide RNAi analysis of growth and viability in Drosophila cells. Science 303, 832–835.
[6] Zheng, L., Liu, J., Batalov, S., Zhou, D., Orth, A., Ding, S. and Schultz, P.G. (2004) An approach to genomewide screens of expressed small interfering RNAs in mammalian cells. Proc. Natl. Acad. Sci. USA 101, 135–140.
[7] Li, E., Sucov, H.M., Lee, K.F., Evans, R.M. and Jaenisch, R. (1993) Normal development and growth of mice carrying a targeted disruption of the alpha 1 retinoic acid receptor gene. Proc. Natl. Acad. Sci. USA 90, 1590–1594.
[8] Harada, A., et al. (1994) Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature 369, 488–491.
[9] Simmer, F., et al. (2003) Genome-wide RNAi of *C. elegans* using the hypersensitive rrf-3 strain reveals novel gene functions. PLoS Biol. 1, E12.
[10] Rual, J.F., et al. (2004) Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. Genome Res. 14, 2162–2168.
[11] Fonte, V., Kapulkin, V., Taft, A., Fluet, A., Friedman, D. and Link, C.D. (2002) Interaction of intracellular beta amyloid peptide with chaperone proteins. Proc. Natl. Acad. Sci. USA 99, 9439–9444.
[12] Lee, A.H., Iwakoshi, N.N. and Glimcher, L.H. (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol. Cell Biol. 23, 7448–7459.
[13] Shen, X., Ellis, R.E., Lee, K., Liu, C.Y., Yang, K., Solomon, A., Yoshida, H., Morimoto, R., Kurnit, D.M., Mori, K. and Kaufman, R.J. (2001) Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development. Cell 107, 893–903.
[14] Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G. and Ahringer, J. (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. Genome Biol., 2, RESEARCH0002.
[15] Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.
[16] Link, C.D., Taft, A., Kapulkin, V., Duke, K., Kim, S., Fei, Q., Wood, D.E. and Sahagan, B.G. (2003) Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. Neurobiol. Aging 24, 397–413.
[17] Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G. and Ron, D. (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 415, 92–96.
[18] Gonczy, P., et al. (2000) Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. Nature 408, 331–336.
[19] Piano, F., Schetter, A.J., Mangone, M., Stein, L. and Kemphues, K.J. (2000) RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. Curr. Biol. 10, 1619–1622.
[20] Tyson, J.R. and Stirling, C.J. (2000) LHS1 and SIL1 provide a lumenal function that is essential for protein translocation into the endoplasmic reticulum. EMBO J. 19, 6440–6452.
[21] Yoneda, T., Benedetti, C., Urano, F., Clark, S.G., Harding, H.P. and Ron, D. (2004) Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J. Cell. Sci. 117, 4055–4066.
[22] Maeda, I., Kohara, Y., Yamamoto, M. and Sugimoto, A. (2001) Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr. Biol. 11, 171–176.
[23] Melville, M.W., Katze, M.G. and Tan, S.L. (2000) P58IPK, a novel cochaperone containing tetratricopeptide repeats and a J-domain with oncogenic potential. Cell Mol. Life Sci. 57, 311–322, [20].
[24] Yan, W., Frank, C.L., Korth, M.J., Sopher, B.L., Novoa, I., Ron, D. and Katze, M.G. (2002) Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc. Natl. Acad. Sci. USA 99, 15920–15925.
[25] van Huizen, R., Martindale, J.L., Gorospe, M. and Holbrook, N.J. (2003) P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. J. Biol. Chem. 278, 15558–15564.
[26] Smith, M.J. (1992) A *C. elegans* gene encodes a protein homologous to mammalian calreticulin. DNA Seq. 2, 235.h–240.h.

[27] Zwaal, R.R., et al. (2001) The sarco-endoplasmic reticulum Ca^{2+} ATPase is required for development and muscle function in *Caenorhabditis elegans*. J. Biol. Chem. 276, 43557–43563.
[28] Jackson, T.R., Patterson, S.I., Thastrup, O. and Hanley, M.R. (1988) A novel tumour promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells. Biochem. J. 253, 81–86.
[29] Xu, K., Tavernarakis, N. and Driscoll, M. (2001) Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. Neuron 31, 957–971.
[30] Urano, F., Calfon, M., Yoneda, T., Yun, C., Kiraly, M., Clark, S.G. and Ron, D. (2002) A survival pathway for *Caenorhabditis elegans* with a blocked unfolded protein response. J. Cell Biol. 158, 639–646.
[31] Dillin, A., et al. (2002) Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.
[32] Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J. and Ruvkun, G. (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat. Genet. 33, 40–48.
[33] Nollen, E.A., Garcia, S.M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R.I. and Plasterk, R.H. (2004) Proc. Natl. Acad. Sci. USA 101, 6403–6408.