The ability of the nervous system to sense cellular stress and coordinate protein homeostasis is essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPRER), become essential for organismal health.

During aging, there is an organism-wide loss of protein homeostasis, exacerbated by the inability to mount an effective unfolded protein response of the endoplasmic reticulum (UPRER), which likely contributes to tissue damage and increased susceptibility to disease (1–3). The age-dependent decline in the ability to induce the UPRER can be prevented by the selective overexpression of constitutively active xbp-1s in neurons. Neuronal XBP-1s leads to cell nonautonomous activation of the UPRER in distal cells and rendered animals more resistant to protein aggregation and chronic ER stress. Mutants deficient in neuropeptide processing and secretion suppressed glial cell nonautonomous induction of the UPRER and life-span extension. Thus, astrocyte-like glial cells play a role in regulating organismal ER stress resistance and longevity.

**Fig. 1. Glial xbp-1s extends life span and induces cell nonautonomous UPRER.** (A) Survival of animals expressing xbp-1s in most glia [ptr-10p::xbp-1s, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (B) Survival of animals expressing xbp-1s in four amphid and phasmid sheath glia [fig-1p::xbp-1s, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (C) Survival of animals expressing xbp-1s in four cephalic sheath glia [hlh-17p::xbp-1s, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (D and E) Fluorescent micrograph (D) and quantification (E) of UPRER reporter worms (hsp-4p::GFP) expressing hlh-17p::xbp-1s (left). hlh-17p::GFP reporter worms, pseudo-colored red (right), are shown. Data in (D) are representative of n > 10. Scale bars, 250 μm. Quantification of hsp-4p::GFP fluorescence using COPAS biosorter was normalized to time of flight (length) and extinction (thickness) of animals. Results are shown relative to hsp-4p::GFP alone (control) with error bars representing means ± SD. One-way analysis of variance (ANOVA) Tukey’s post hoc test, n = 2, ****P < 0.0001. Life spans are representative of n = 3. See table S1 for life-span statistics.
Fig. 2. Cell nonautonomous induction of the UPRER is dependent on \( \text{xbp-1} \), but not \( \text{atf-6} \) or \( \text{pek-1} \). (A) Fluorescent micrographs of day 1 \( \text{hsp-4p::GFP; hlh-17p::xbp-1s} \) animals grown on control empty vector (EV), \( \text{atf-6} \), \( \text{pek-1} \), or \( \text{xbp-1 RNAi} \) from hatch. Scale bar, 250 \( \mu \)m; \( n = 3 \). (B) Survival of control (N2) and \( \text{hlh-17p::xbp-1s} \) animals grown on EV control RNAi or RNAi-targeting \( \text{xbp-1} \). See table S1 for lifespan statistics; \( n = 2 \). (C) Volcano plot of whole-animal transcriptional profiling from \( \text{hlh-17p::xbp-1s} \) animals compared with wild type (N2). \( \text{xbp-1} \) is highlighted in red. Note that \( \text{aex-5} \) (gray) was detected as highly overexpressed because of a small \( \text{aex-5} \) promoter and exon fragment present in the 3' untranslated region in the backbone plasmid used for all constructs. All \( \text{aex-5} \) reads aligned to this short fragment. (D) The UPRER is activated in animals expressing \( \text{xbp-1s} \) in CEPsh glia compared with N2, shown by fold change of two gene groups: UPRER (GO: 0030968 and 1900103) or \( \text{xbp-1} \) targets (19). The line inside the box represents the median change of the gene group. *** \( P < 0.001 \). GO enrichment analysis for genes with a fold change \( P \) value < 0.05 for terms with a false discovery rate \( Q \) value < 0.05 can be found in table S3.

Fig. 3. Expression of \( \text{xbp-1s} \) in glial cells protects animals against protein aggregation and chronic ER stress. (A) Fluorescent micrograph and quantification of age-dependent accumulation of polyQ44-YFP aggregates in control animals or animals expressing \( \text{hlh-17p::xbp-1s} \). Control animals average 3.5 puncta per animal, compared with 1.3 in \( \text{hlh-17p::xbp-1s} \) animals (\( P < 0.0001 \)). Scale bar, 250 \( \mu \)m; \( n = 2 \). (B) Survival of animals transferred to tunicamycin-containing plates at day 1 of adulthood. CEPsh glial ablation via \( \text{hlh-17p::recCasp} \) suppresses \( \text{hlh-17p::xbp-1s} \) ER stress resistance. \( n = 2 \). (C) Fluorescent micrograph of \( \text{hsp-4p::GFP} \) reporter worms expressing \( \text{hlh-17p::xbp-1s} \) and \( \text{hlh-17p::xbp-1s; hlh-17p::recCasp} \). GFP puncta in \( \text{hlh-17p::recCasp} \) strain represent co-injection (coinj.) marker for \( \text{hlh-17p::recCasp} \) transgene, which is expressed in coelomocytes. White bracket marks distal intestine, where induction of cell nonautonomous UPRER is reduced in animals expressing \( \text{hlh-17p::recCasp} \). Scale bar, 250 \( \mu \)m; \( n = 3 \).
showed induction of hsp-4p::GFP in the CEPsh glia and in the distal intestine and pharynx (Fig. 1, D and E). Notably, green fluorescent protein (GFP) expression was limited to CEPsh glial cells in hlh-17p::GFP reporter animals (fig. S1C) (9, 10, 13, 14, 16–18). These data suggest that xbp-1s expression in glial cells can induce cell nonautonomous UPRER in distal intestinal cells and that CEPsh glia have a unique role in regulating xbp-1s-mediated longevity.

To elucidate how CEPsh glia promote longevity via xbp-1s, we first tested whether life-span extension and cell nonautonomous activation of the UPRER from CEPsh glia was dependent on the known signaling components of the UPRER branches, PERK, ATF6, and XBP1, encoded by pek-1, atf-6, and xbp-1, respectively, in C. elegans. No difference was observed in hsp-4::GFP induction with pek-1 or atf-6 RNA interference (RNAi)–mediated knockdown in hlh-17p::xbp-1s animals (Fig. 2A and fig. S4).

However, knockdown of xbp-1 reduced GFP fluorescence of hlh-17p::xbp-1s; hsp-4p::GFP animals and abolished the life-span extension of hlh-17p::xbp-1s animals (Fig. 2, A and B, and fig. S4). Whole-worm RNA sequencing (RNA-seq) of hlh-17p::xbp-1s animals revealed 115 differentially expressed genes (adjusted P value <0.05), including a significant increase in xbp-1s–dependent transcripts (Fig. 2, C and D, and table S2) (19). Gene ontology analysis showed enrichment of genes involved in the immune response, stress response, and, as expected, response to ER stress (table S3).
would render these animals more resistant to age-dependent protein aggregation and chronic ER stress. Expression of xbp-1s in CEPsh glia notably reduced aggregation of yellow fluorescent protein (YFP)-tagged, Huntingdon-like polyglutamine protein in the intestine (with age) compared with controls (Fig. 3A). Furthermore, animals expressing xbp-1s in CEPsh glia exhibited an increase in survival when chronically exposed to tunicamycin, a chemical inducer of ER stress (Fig. 3B). Perturbing CEPsh glial development, using a partially penetrant reconstituted caspase (recCasp), abrogated the ER stress resistance of hh-17p::xbp-1s animals grown on tunicamycin-containing plates and decreased the median life span of hh-17p::xbp-1s animals grown on control plates (Fig. 3B and fig. S5, A and B). Moreover, distal UPRER was observed robust distal activation of the UPRER, neurons.

Next, we assessed whether overexpression of xbp-1s in CEPsh glia induces other stress responses known to affect protein homeostasis and longevity, such as the mitochondrial responses known to affect protein homeostasis and chronic ER stress. Expression of xbp-1s in CEPsh glia significantly extended life span of animals expressing xbp-1s and cell nonautonomous activation of the UPRER (Fig. 4, D and E) (2). Furthermore, we tested a loss-of-function mutation in the proprotein convertase, egl-3, which is deficient in neuropeptide processing, and found that induction of the cell nonautonomous UPRER by CEPsh glia was suppressed (Fig. 4, F and G, and fig. S8, A and B) (25). Blocking neuropeptide processing had no effect on cell autonomous xbp-4p::GFP induction in intestinal cells or cell nonautonomous activation of the UPRER in animals expressing neuronal xbp-1s (fig. S9, A to D, and Fig. 4, H and I). Thus, glial-mediated cell nonautonomous induction of the UPRER is dependent on neurons expressing xbp-1s.

As an additional measure of the separation between neuronal and glial induction of peripheral UPRER, we removed CEPsh glial cells in animals expressing xbp-1s solely in neurons, and cell nonautonomous activation of the UPRER remained intact (fig. S10). Thus, neuronal activation of the peripheral UPRER via xbp-1s is independent of CEPsh glia. Next, we investigated whether combinatorial xbp-1s overexpression in both neurons and CEPsh glia would result in an additive increase in activation of the UPRER and life-span extension. Animals overexpressing xbp-1s in both neurons and CEPsh glia induced xbp-4p::GFP and extended life span to a greater degree than animals expressing xbp-1s only within CEPsh glia or neurons (fig. S11, A and B, and Fig. 4J).

To identify the cell type responsible for secreting the peptides mediating cell nonautonomous UPRER, we expressed wild-type unc-31(cDNA) in either neurons or glia in hh-17p::xbp-1s; unc-31(e928) animals. Neuronal unc-31(cDNA) did not restore activation of the UPRER in the intestine of hh-17p::xbp-1s; unc-31(e928) animals (fig. S12, A and B). In contrast, expression of unc-31(cDNA) in CEPsh glia oregl-3(cDNA) in CEPsh glia or all glia led to an increase in activation of the UPRER, albeit a modest increase (fig. S12, C and D). These data suggest that the neuropeptides required for glial-mediated cell nonautonomous activation of the UPRER do not originate from neurons but are secreted, in part, by glial cells themselves. Lastly, we sought to determine whether neuropeptide signaling was mediating longevity in hh-17p::xbp-1s animals. Loss-of-function egl-3 mutants are inherently long-lived because of reduced insulin and IGF-1 signaling (26). However, we did not observe an additive increase in survival of hh-17p::xbp-1s animals harboring the egl-3(ak979) mutation, suggesting that lifespan extension of hh-17p::xbp-1s animals requires neuropeptides (Fig. 4K).

Previously, cell nonautonomous stress signaling from the brain to the periphery has been ascribed only to neurons. However, our data identify a subtype of astrocyte-like glial cells that coordinate systemic protein homeostasis and aging via neuropeptide signaling—a distinct mechanism from that initiated by neuronal XBP-1s (fig. S13). This suggests there is regional and functional specificity of glial cells to control physiology and aging that evolved as early as the nematode. We speculate that, depending on the physiological cue received by the nervous system, either neurons or glia can signal via XBP-1s to peripheral tissues to coordinate organ systemic protein homeostasis.
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S.U.T. performed life spans, worm crosses, and COPAS biosorting
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repository, under accession number BioProject PRJNA589459. Further
information and requests for reagents may be directed to
dillinlabmaterials@berkeley.edu and will be fulfilled by A.D.

SUPPLEMENTARY MATERIALS
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Materials and Methods
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View/request a protocol for this paper from Bio-protocol.

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Four glial cells regulate ER stress resistance and longevity via neuropeptide signaling in *C. elegans*


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**Taking the stress out of life**

In the model organism *Caenorhabditis elegans*, a roundworm, it has been shown that neurons can communicate proteostasis to the periphery to affect aging. Frakes *et al.* have now identified astrocytike glial cells that also act as central regulators of systemic protein homeostasis and aging (see the Perspective by Miklas and Brunet). They found that the life span of *C. elegans* can be extended by expression of a constitutively active version of the transcription factor XBP-1s, which mediates the unfolded protein response of the endoplasmic reticulum (UPR ER), in a specific subset of glial cells. Glial XBP-1s initiates induction of the UPR ER in distal intestinal cells, which makes the worms more resistant to chronic ER stress. Neuropeptide signaling was required for glial-mediated longevity and induction of the peripheral UPR ER, suggesting a distinct mechanism from that initiated by neuronal XBP-1s. Thus, in this animal model of aging, a mere four cells can control organismal physiology and aging.

*Science*, this issue p. 436; see also p. 365

**REFERENCES**

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