Systemic regulation of mitochondria by germline proteostasis prevents protein aggregation in the soma of C. elegans

Giuseppe Calculli¹, Hyun Ju Lee¹, Koning Shen², Uyen Pham¹, Marija Herholz¹, Aleksandra Trifunovic¹,³,⁴, Andrew Dillin², David Vilchez¹,³,⁵*

Protein aggregation causes intracellular changes in neurons, which elicit signals to modulate proteostasis in the periphery. Beyond the nervous system, a fundamental question is whether other organs also communicate their proteostasis status to distal tissues. Here, we examine whether proteostasis of the germ line influences somatic tissues. To this end, we induce aggregation of germline-specific PGL-1 protein in germline stem cells of Caenorhabditis elegans. Besides altering the intracellular mitochondrial network of germline cells, PGL-1 aggregation also reduces the mitochondrial content of somatic tissues through long-range Wnt signaling pathway. This process induces the unfolded protein response of the mitochondria in the soma, promoting somatic mitochondrial fragmentation and aggregation of proteins linked with neurodegenerative diseases such as Huntington’s and amyotrophic lateral sclerosis. Thus, the proteostasis status of germline stem cells coordinates mitochondrial networks and protein aggregation through the organism.

INTRODUCTION
Aggregation-prone mutations challenge the integrity of the cellular proteome, leading to protein aggregates that cause malfunction and death of postmitotic cells such as neurons (1, 2). This demise in protein homeostasis (proteostasis) contributes to the onset of distinct disorders, including Alzheimer’s disease, Huntington’s disease, and amyotrophic lateral sclerosis (ALS) (1, 2). Besides intracellular deficits in proteostasis, growing evidence indicates that the proteostasis status can be communicated between somatic tissues (3–7). To date, inter-organ communication of proteostasis has been essentially attributed to the nervous system, which coordinates proteostasis networks through the organism (3–7). For instance, Caenorhabditis elegans neurons that express aggregation-prone proteins elicit signals to regulate proteostasis in the intestine (3). Moreover, activation of the unfolded protein response of the endoplasmic reticulum in glial cells induces a similar stress pathway in the intestine (3).

Since the germ line can influence somatic function (8–12), we asked whether proteostasis deficits in germline cells impinge on distal tissues such as the nervous system. An intrinsic characteristic of germline cells is the assembly of germ granules, specific membraneless ribonucleoprotein organelles that are constitutively found in the germline cytoplasm of all animals (13, 14). The intracellular localization and structure of germ granules is conserved from invertebrates to mammals (14). In adult animals, germ granules exhibit a perinuclear distribution and reside close to mitochondria, forming complexes with germline-specific structures such as the mitochondrial cloud and intermitochondrial cement (14–16). The formation of germ granules relies on the self-association of RNA binding proteins (17, 18). However, these proteins are also prone to aggregation and can lead to aberrant granules (18–20). In C. elegans, germ granules are known as P granules, and their assembly depends on the self-interaction domains of the RNA binding protein PGL-1 or its redundant paralog PGL-3, which are specifically expressed in germline cells (14, 17, 21, 22).

Here, we find that aggregation of PGL-1 in germline stem cells (GSCs) not only impinges on the mitochondrial network of the germ line but also diminishes the mitochondrial content of somatic tissues via long-range Wnt signaling. The subsequent activation of the unfolded protein response of the mitochondria (UPRmt) leads to mitochondrial fragmentation and aggregation of disease-related proteins in somatic tissues such as the nervous system, muscle, and intestine. Together, our findings suggest a cell nonautonomous mechanism whereby proteostasis of germline-specific, nonmitochondrial factors determine mitochondrial function and protein aggregation across multiple tissues.

RESULTS
Loss of germline-specific CEYs triggers PGL-1 aggregation in the germ line
To assess whether proteostasis of germline cells triggers cell non-autonomous pathways, we targeted Y-box binding proteins (YBX), a typical component of ribonucleoprotein granules that prevents aberrant aggregation of self-interacting proteins (19, 23, 24). The C. elegans genome encodes four YBX proteins, i.e., CEY (C. elegans Y-box)-1 to CEY-4 (19). A comprehensive study combining analysis of transcript levels with protein expression reporters previously demonstrated that CEY-1 and CEY-4 are present in both the soma and the germ line (19), whereas CEY-2 and CEY-3 are only expressed in the germ line (19) similar to PGL-1 (Fig. 1A). Thus, we focused on CEY-2 and CEY-3 to circumvent direct effects of CEY proteins on somatic tissues. Notably, single knockdown of either cey-2 or cey-3 (Fig. 1B) was sufficient to trigger the accumulation of PGL-1 into aberrant foci in the germ line, particularly in the mitotic region where GSCs are located (Fig. 1C). Likewise, we observed a similar

¹Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany. ²Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA. ³Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany. ⁴Institute for Mitochondrial Diseases and Aging, Medical Faculty, University of Cologne, Cologne, Germany. ⁵Faculty of Medicine, University Hospital Cologne, Cologne, Germany.
*Corresponding author. Email: dvilchez@uni-koeln.de
Fig. 1. Knockdown of germline-specific CEYs induces PGL-1 aggregation in GSCs. (A) mRNA levels of ubiquitously expressed act-2 and germline-specific cey-2, cey-3, and pgl-1 (means ± SEM of the relative expression to wild-type worms, n = 6 independent experiments). (B) Knockdown levels in day 3 adults [relative to vector RNA interference (RNAi), means ± SEM, n = 4]. (C) Immunostaining of germ lines with antibody to PGL-1 and 4′,6-diamidino-2-phenylindole (DAPI; nuclei). Knockdown of CEYs triggers aggregation of endogenous PGL-1 in GSCs of the mitotic region (that is, the first 20 rows of cells in the most distal part of the germ line). Scale bar, 20 μm. Images are representative of three independent experiments. (D) Knockdown of CEYs induces aggregation of PGL-1::green fluorescent protein (GFP) (detected by anti-GFP antibody). Right: SDS–polyacrylamide gel electrophoresis (PAGE) with antibodies to GFP and α-tubulin loading control. Images are representative of three independent experiments. (E) 5-Bromodeoxyuridine (BrdU) staining of proliferating GSCs in day 3 adults. Scale bar, 20 μm. (F) Percentage of BrdU-positive cells per total nuclei (DAPI) within the mitotic region (means ± SEM, n = 16 germ lines scored per condition from two independent experiments). (G) Total number of germline cells within the mitotic region (means ± SEM, n = 16 germ lines per condition, two independent experiments). (H) Number of eggs laid per worm every 24 hours (means ± SEM, n = 10 worms per condition, three independent experiments). (I) Percentage of hatched eggs (means ± SEM, n = 10 worms per condition, three independent experiments). Statistical comparisons were made by two-tailed Student’s t test for unpaired samples: ****P < 0.0001; NS, not significant (P > 0.05). (K) Life-span analysis (log-rank test, n = 96 worms per condition). Data file S1 contains replicate life-span experiments.
phenotype in *cey-2* functional null mutant worms (fig. S1). By filter trap assay, we confirmed aggregation of PGL-1 upon knockdown of germline-specific CEY proteins (Fig. 1D).

The accumulation of PGL-1 aggregates did not diminish GSC proliferation and fecundity rates, indicating that these worms conserve a functional germ line (Fig. 1, E to J). Moreover, loss of germline-specific CEYs did not shorten life span, suggesting that PGL-1 aggregates do not induce organismal sickness or mortality (Fig. 1K and data file S1). Thus, single knockdown of either *cey-2* or *cey-3* did not impair features such as organismal viability, reproduction, and GSC self-renewal, providing a mean to examine whether the proteostasis status of GSCs modulates protein aggregation in the soma.

**Germline PGL-1 inclusions trigger aggregation of disease-related proteins in the soma**

To examine whether PGL-1 aggregation in the germ line determines somatic proteostasis, we used *C. elegans* that express expanded polyglutamine repeats (polyQ) throughout the nervous system, a bona fide model of neuronal protein aggregation (25–27). In these worms, neurotoxicity and protein aggregation correlate with increased length of the polyQ peptide, with a pathogenic threshold of 40 repeats (25). Notably, knockdown of germline-specific CEYs triggered a pronounced increase of polyQ67 aggregation in neurons, without affecting the total amounts of polyQ peptides (Fig. 2A). We further confirmed neuronal polyQ67 aggregation in single *cey-2* and double *cey-2*, *cey-3* functional null mutant worms (Fig. 2B). On the contrary, loss of CEY proteins did not induce aggregation of control polyQ19 peptides (Fig. 2C).

Neuronal expanded polyQ aggregation causes neurotoxicity and subsequently impairs coordinated movement, a disease-like phenotype (25). To assess the physiological consequences of neuronal polyQ aggregation induced by disrupted proteostasis in the germ line, we performed motility assays. We found that loss of germline-specific CEY proteins hasten the motility defects induced by polyQ67 expression (Fig. 2D). However, loss of *cey-2* or *cey-3* did not affect the motility of control polyQ19-expressing worms (Fig. 2D).

RNA interference (RNAi) against *cey-1*, a CEY protein expressed in both germline cells and neurons (19), also impaired proteostasis of PGL-1 in the germ line and triggered polyQ67 aggregation in neurons (Fig. 3, A to C). However, neuronal-specific knockdown of *cey-1* failed to impair not only proteostasis of PGL-1 in the germ line but also polyQ67 aggregation in neurons (Fig. 3, A, B, and D). In contrast, neuronal-specific down-regulation of the TRiC/CCT chaperonin complex, a direct regulator of expanded polyQ proteostasis (27, 28), was sufficient to promote polyQ67 aggregation in neurons (Fig. 3D and fig. S2). Likewise, loss of TRiC/CCT function induced polyQ67 aggregation in *glp-4* germline-lacking mutants, whereas
Notably, knockdown of aggregation-prone loss of PGL-1 alone does not impair the formation of germ granules. Given that germline-specific PGL proteins have redundant roles, RNAi against CEY factors does not induce polyQ67 aggregation in the neurons of germline-lacking worms (glp-4(bn2)). In contrast, knockdown of PGL-1 was sufficient to ameliorate neuronal polyQ67 dysregulation in cey-2 mutants (Fig. 3F), further supporting a role of cell nonautonomous signals triggered by defects in germline proteostasis.

The effects of PGL-1 aggregation on somatic proteostasis were not limited to the nervous system, since loss of germline-specific CEYs also triggered aggregation in C. elegans models that specifically express expanded polyQ peptides in the intestine or muscle (Fig. 4, B). This suggests that PGL-1 aggregation in the germ line modulates neuronal proteostasis. To assess this hypothesis, we performed rescue experiments using pgl-1 RNAi. Given that germline-specific PGL proteins have redundant roles, knockdown of aggregation-prone PGL-1:YFP under neuronal F25B3.3 promoter. Graph represents the relative expression to vector RNAi control (means ± SEM, n = 4). On the right, qPCR after neuronal-specific RNAi treatment in day 3 adult worms expressing Q67::YFP in neurons (means ± SEM, cey-1 (n = 4), cey-2 (n = 5), and cey-3 (n = 6)). RNAi rescued in the neurons alone of RNAi-deficient worms (sid-1(pk3321); unc-119p::sid-1, F25B3.3p::Q67::YFP). Immunostaining of germ lines in polyQ67-expressing worms with antibody to PGL-1 and DAPI (nuclei). On the left, knockdown of cey-1 induces aggregation of PGL-1 in the germ line. Scale bar, 20 μm. Images are representative of three independent experiments. (C) Knockdown of cey-1 increases polyQ67 aggregation in neurons (detected by anti-GFP antibody). The images are representative of four independent experiments. (D) Neuronal-specific knockdown of cey-1 does not induce polyQ67 aggregation in neurons. In contrast, neuronal-specific knockdown of cct-8, a subunit of the TRiC/CCT chaperonin complex, promotes polyQ67 aggregation in neurons. The images are representative of four independent experiments. (F) Knockdown of germine-specific pgl-1 rescues the increased polyQ67 aggregation phenotype in the neurons of cey-2 mutants. The images are representative of three independent experiments. All statistical comparisons were made by two-tailed Student’s t test for unpaired samples. P values: **P < 0.01, ****P < 0.0001, and NS, P > 0.05.

RNAi against CEY factors did not alter neuronal proteostasis in these worms (Fig. 3E). Thus, our results indicate that PGL-1 aggregation in the germ line modulates neuronal proteostasis. To assess this hypothesis, we performed rescue experiments using pgl-1 RNAi.
A and B). Similar to neurons, the intestinal and muscular polyQ aggregation phenotype was not accompanied by changes in the global levels of polyQ peptides in these tissues (Fig. 4, A and B). The accumulation of expanded polyQ aggregates in muscle cells has intracellular deleterious effects, leading to muscle dysfunction and loss of motility in C. elegans (29). The aggregation of muscle-expanded polyQ induced by loss of germ-line-specific CEYs also impaired coordinated movement (Fig. 4C).

Besides expanded polyQ peptides, we asked whether dysfunction of germline proteostasis also influences aggregation of other neurodegenerative disease–related proteins. To this end, we used established C. elegans models that express ALS-related mutant variants of FUS (Fused in Sarcoma) and TDP-43 (TAR DNA-binding protein 43) in the nervous system (30, 31). These worms replicate key features of ALS including protein aggregation and neurodegeneration, as reflected by loss of coordinated movement (30, 31). Notably, knockdown of germ-line-specific CEYs induced aggregation of distinct ALS-related mutant variants of FUS and TDP-43 in neurons (Fig. 5, A to C). Concomitantly, deficits in germline proteostasis accelerated the disease-like loss of motility in ALS models (Fig. 5, D and E). Together, our results indicate that the proteostasis status of the germ line determines aggregation of disease-related proteins in the soma, a process impaired by the accumulation of PGL-1 aggregates in germline cells upon loss of CEY proteins.

### PGL-1 aggregates impinge on the mitochondrial network of germline cells

To shed light on how proteostasis defects in the germ line influence somatic tissues, we first assessed the intracellular changes induced by PGL-1 aggregation in germline cells. For this purpose, we examined the proteome of isolated germ lines from C. elegans following cey-3 knockdown (data file S2). We found that PGL-1 aggregation results in up-regulated levels of 107 proteins in the germ line (data file S2). Among them, Gene Ontology Cellular Component (GOCC) analysis indicated enrichment for components of P granules [e.g., GLH-1 (Germ Line Helicase-1), MEX-5 (Muscle EXcess-5) and DDX-19 (DEAD boX helicase homolog-19)] and complexes involved in transcription [FACT (facilitates chromatin transcription) complex], splicing (e.g., spliceosomal tri–small nuclear ribonucleoprotein complex), and translation (e.g., 43S/48S preinitiation complexes and ribosomes) (fig. S3 and data file S3).

Besides CEY-3 levels, quantitative proteomics analysis revealed that another 72 proteins were significantly down-regulated in the germ line on cey-3 knockdown (data file S2). Among them, we found 24 mitochondrial proteins (data file S2). GOCC analysis of downregulated proteins indicated strong enrichment for components of distinct mitochondrial complexes required for mitochondrial organization and function (Fig. 6A and data files S2 and S3). For instance, we found factors involved in the mitochondrial electron transport chain (ETC) such as components of complex I/NADH (reduced form of NAD\(^+\))–ubiquinone oxidoreductase (NUO-5, NDUF-9, NDUF-2.2, C33A12.1, Y63D3A.7, Y94H6A.8, and Y54F10AM.5), complex III/ubiquinol-cytochrome c reductase (ISP-1 and UCR-1), complex IV/cytochrome c oxidase (COX-6A and COX-7C), and complex V/ubiquinol-cytochrome c oxidase (COX-6A and COX-7C), and complex V/proton-transporting adenosine triphosphatase (ATP) synthase complex (ATP-4, ASB-1, HPO-18, ASG-1, and Y69A2AR.18) (Fig. 6A and data files S2 and S3). In addition, we observed a decrease in components of complexes that regulate mitochondrial biogenesis and morphology, such as prohibitin (PHB-1), mitochondrial ribosomes (MRPL-44), or translocases of the outer (TOMM-20) and inner mitochondrial membrane (TIM-16) (Fig. 6A and data files S2 and S3).

The decrease in the protein amounts of several mitochondrial components induced by PGL-1 aggregation correlated with a reduction of the mRNA levels in the germ line (Fig. 6B). Besides downregulated expression of mitochondrial components, we also found changes in the morphology of mitochondrial networks that became more diffused through the cytoplasm of germline cells upon either knockdown or loss-of-function mutation of CEY factors (Fig. 6, C and D, and fig. S4). In addition, cey-2 functional null mutant worms exhibited a decrease of the mitochondrial DNA (mtDNA)/genomic DNA (gDNA) ratio in germline cells (Fig. 6E). Knockdown of pgl-1 rescued the low levels of mtDNA content in the germ line of cey-2 mutants (Fig. 6E), supporting that aggregation of PGL-1 impinges on the mitochondrial network of germline cells.
Germline PGL-1 aggregates influence mitochondrial proteostasis and morphology in the soma

Besides the intracellular alterations induced by PGL-1 aggregates in germline cells, we also found changes in the mitochondrial network of distal somatic tissues, suggesting a cell nonautonomous process mediated by the germ line. For instance, mitochondria formed more fragmented structures in muscle and intestinal cells upon knockdown of germline-specific CEY proteins (Fig. 7, A and B, and fig. S5). Similar to the intracellular effects caused by PGL-1 aggregation in the germ line, we also observed reduced expression of mitochondrial components and lower mtDNA content in somatic tissues (Fig. 7, C and D, and fig. S6). Knockdown of PGL-1 in the germ line was sufficient to rescue low mtDNA levels and alterations of mitochondrial morphology in the soma of cey-2 mutant worms (Fig. 7, D to F).

Despite the systemic mitochondrial changes induced by loss of germline-specific CEY factors, the animals maintained similar levels of basal mitochondrial oxygen consumption when compared with control worms (fig. S7, A and B). Likewise, cey-2 mutant worms did not exhibit decreased levels of nicotinamide adenine dinucleotide (NAD⁺) and ATP compared with wild-type worms (fig. S7, C and D). Thus, these results suggest an up-regulation of the basal activity of mitochondria to compensate for the lower mitochondrial content, a process that could challenge the integrity of the mitochondrial proteome and activate proteostasis responses in the organelle. To assess this hypothesis, we used transgenic worms that express in hsp-6p::green fluorescent protein (GFP), a specific reporter of the activation of the UPR<sub>mt</sub> (32–34). Notably, knockdown of germline-specific cey-2 or cey-3 induced the expression hsp-6p::GFP.
CEY factors increased the somatic expression of hsp-60p::GFP (Fig. 8, F to H), a distinct specific reporter of the UPR mt (32–34). On the contrary, knockdown of germline-specific CEY factors did not induce the UPR of the endoplasmic reticulum (35) or the cytosolic...
heat shock response (fig. S8, A and B) (36). Thus, our data indicate that loss of germline proteostasis results in specific activation of the UPR\textsuperscript{mt}.

In C. elegans, the UPR\textsuperscript{mt} is induced when alterations in mitochondrial homeostasis promote the translocation of the transcription factor ATFS-1 from mitochondria to the nucleus (37). Knockdown of atfs-1 (activating transcription factor associated with stress) reduced UPR\textsuperscript{mt} induction in cey-2 mutant worms (Fig. 9, A and B). We found that loss of atfs-1 did not affect the levels of oxygen consumption (fig. S9), suggesting that UPR\textsuperscript{mt} induction is not required for the compensatory up-regulation of mitochondrial respiration in cey-2.

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mutants with altered mitochondria. Likewise, loss of atfs-1 did not further decrease mtDNA content in cey-2 mutants, indicating that this process precedes UPR<sup>mt</sup> induction (Fig. 9C). However, knockdown of atfs-1 was sufficient to rescue somatic alterations in the mitochondrial morphology of cey-2 mutant animals (Fig. 9D and E). Thus, UPR<sup>mt</sup> activation contributes to the fragmented mitochondrial phenotype induced by loss of germline proteostasis.

Since growing evidence links mitochondrial perturbations and UPR<sup>mt</sup> activation with the accumulation of protein aggregates in distinct neurodegenerative diseases (3, 38–42), we asked whether the systemic induction of UPR<sup>mt</sup> triggered by loss of germline proteostasis promotes aggregation of disease-related proteins in neurons. Loss of atfs-1 ameliorated the neuronal polyQ67 aggregation phenotype of cey-2 mutant worms (Fig. 9F). Concomitantly, atfs-1 RNAi also alleviated motility defects in these worms (Fig. 9G). Given that PGL-1 aggregation impinges on the mitochondrial network of germline cells, we asked whether disrupting mitochondrial function directly in the germ line is sufficient to trigger protein aggregation...
in the soma. To this end, we performed germline-specific knockdown of the mitochondrial ETC subunit cco-1 (cytochrome c oxidase-1). In contrast to PGL-1 aggregation (Figs. 2 and 3), disruption of germline mitochrondria through cco-1 RNAi was not sufficient to induce polyQ67 aggregation in neurons (fig. S10). However, knockdown of cco-1 at the organismal level including somatic tissues promoted neuronal polyQ67 aggregation (fig. S10). Together, our data suggest that aggregation of PGL-1 in the germ line is the key factor to regulate proteostasis in the soma rather than the intracellular effects induced by PGL-1 aggregation in the germline mitochondria. Hence, PGL-1 aggregation determines loss of mitochondrial content and subsequent UPRmit activation in somatic tissues, leading to mitochondrial fragmentation and the aggregation of disease-related proteins in the soma.
Germline proteostasis modulates somatic UPR\textsuperscript{mt} and protein aggregation through Wnt signaling

The Wnt signaling pathway acts as a signal to communicate information about proteostasis changes between tissues, activating the UPR\textsuperscript{mt} in distal organs (43). In C. elegans, EGL-20 is the only Wnt ligand that can induce long-range, cell nonautonomous events (43–46). While knockdown of egl-20 did not prevent the intracellular reduction of mitochondria content induced by PGL-1 aggregation in the germ line (Fig. 10A), it was sufficient to rescue the lower mitochondrial content in somatic tissues (Fig. 10B). Concomitantly, knockdown of

**Fig. 10. Proteostasis of germline cells coordinates mitochondrial function and protein aggregation in somatic tissues through Wnt signaling.** (A) Relative DNA (mtDNA)/gDNA ratio in isolated germ lines to wild-type + vector RNAi (means ± SEM, n = 6 biological replicates from three independent experiments). (B) Relative mtDNA/gDNA ratio in somatic tissues (isolated intestines + heads) to wild-type + vector RNAi (means ± SEM, n = 5 biological replicates from three independent experiments). (C) Knockdown of either mig-1 or egl-20 reduces UPR\textsuperscript{mt} induction in the soma of cey-2 mutant worms. Scale bar, 20 \(\mu\)m. Images are representative of two independent experiments. (D) Quantification of hsp-6p::GFP fluorescence relative to vector RNAi (means ± SEM, vector RNAi (n = 55 worms), mig-1 RNAi (n = 51 worms), and egl-20 RNAi (n = 55 worms) from two independent experiments). (E) Knockdown of either Wnt signal egl-20 or its receptor mig-1 ameliorates changes in the mitochondrial morphology of the muscle induced by loss of cey-2 function. Scale bar, 20 \(\mu\)m. Images are representative of three independent experiments. (F) Percentage of animals with normal, intermediate, or fragmented mitochondrial network morphology in the muscle (means ± SEM of three independent experiments; wild-type + vector RNAi = 40 worms, wild-type + mig-1 RNAi = 35 worms, wild-type + egl-20 RNAi = 41 worms, cey-2(ok902) + vector RNAi = 75 worms, cey-2(ok902) + mig-1 RNAi = 73 worms, and cey-2(ok902) + egl-20 RNAi = 89 worms). (G) Knockdown of either mig-1 or egl-20 ameliorates increased polyQ67 aggregation in the neurons of cey-2 mutants. The images are representative of three independent experiments. Statistical comparisons were made by two-tailed Student’s t test for unpaired samples. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, and NS, P > 0.05.

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either egl-20 Wnt ligand or its receptor mig-1 reduced the somatic induction of the UPR\textsuperscript{mt} triggered by PGL-1 aggregation in the germ line (Fig. 10, C and D), preventing mitochondrial fragmentation in the soma (Fig. 10, E and F). Likewise, loss-of-function mutations in egl-20 or mig-1 also prevented somatic mitochondrial fragmentation induced by loss of germline proteostasis (fig. S11, A and B).

Prompted by these results, we asked whether the Wnt signaling triggered by loss of germline proteostasis underlies the aggregation of disease-related proteins in neurons. Loss-of-function mutations in egl-20 or mig-1 rescued the neuronal polyQ67 aggregation phenotype induced by cey-2 RNAi (fig. S11C). Similarly, knockdown of either egl-20 or mig-1 reduced polyQ67 aggregation in the neurons of cey-2 mutant worms (Fig. 10G). Moreover, RNAi against bar-1 (β-catenin) or pop-1 [TCF/LEF (T-cell factor/lymphoid enhancer factor) transcription factor], the canonical downstream effectors of Wnt pathway, also ameliorated neuronal aggregation and motility defects in polyQ67;cey-2 mutant worms (fig. S11, D and E). Collectively, our results indicate that the Wnt signaling pathway communicates proteostasis deficits from germline cells to somatic tissues.

**DISCUSSION**

Given the cumulative evidence that demonstrates a role of the germ line in the regulation of somatic function (8–10, 47, 48), here, we asked whether changes in the proteostasis status of germline cells can affect distal tissues. To identify long-range, cell nonautonomous mechanisms triggered by germline cells, we performed loss-of-function experiments of CEY proteins. Notably, we found that dysregulation of germline-specific, nonmitochondrial CEY proteins induces aggregation of the germ granule component PGL-1 followed by intracellular changes in the mitochondrial network of germline cells. Beyond intracellular effects, loss of germline proteostasis triggers a Wnt signaling pathway that influences the mitochondrial network of somatic tissues. Thus, we identify a cell nonautonomous mechanism that coordinates the proteostasis status of germline cells with systemic mitochondrial function, a capacity previously ascribed only to the nervous system. This cell nonautonomous mechanism originated in the germ line can trigger protein aggregation in somatic tissues depending on the proteostasis status of germline cells, establishing a regulatory link between the fitness of the reproductive system with systemic mitochondrial function and proteostasis. It is important to note that loss of germline-specific CEY factors induced systemic mitochondrial alterations and UPR\textsuperscript{mt} activation even in wild-type animals that do not express disease-related proteins in the soma (Fig. 8), further supporting that loss of germline proteostasis is sufficient to influence the UPR\textsuperscript{mt} in distal tissues. Whereas previous studies demonstrated that removal of the germ line can enhance proteostasis in somatic tissues by reallocation of metabolic resources (47), our results are particularly relevant because they indicate that dysregulation of proteostasis in the functional germline of reproductive organisms can hasten protein aggregation in the soma. Our results suggest that this interorgan communication of proteostasis is unidirectional since aggregation of polyQ67 peptides in neurons does not alter proteostasis of PGL-1 in the germ line (Fig. 3B).

Environmental and metabolic conditions such as stress or aging that impinge on proteostasis are tightly correlated with a decline of germline integrity in animals (49). Thus, we speculate that proteostasis deficits in germline cells ensued from stress conditions or aging could contribute to the dysregulation of mitochondrial function and aggregation of disease-related proteins that often appear in postmitotic tissues such as muscle or nervous system with age. In particular, our results indicate that the aggregation of germ granule proteins is sufficient to impinge on somatic proteostasis. Germ granules can convert into aberrant aggregates under stress conditions such as translational repression (20). In somatic tissues, the chronic stress and proteostasis failure ensued from aging can lead to persistent aggregates of membrane-less ribonucleoprotein organelles that may act as an intracellular nidus for the aggregation of disease-related proteins in neurons (50). However, the impact of aging in the proteostasis of germ granules and their self-associating protein components remains unknown. In these lines, it will be fascinating to examine whether germ granules components such as PGL-1 also aggregate during organismal aging and whether this process contributes to the age-associated aggregation of disease-related proteins in a cell nonautonomous manner.

Another important question raised by our findings is whether the germline-to-soma communication of proteostasis is evolutionary conserved. Similar to *C. elegans*, a recent study demonstrated that the neurons of mouse models can elicit cell nonautonomous pathways to regulate proteostasis in other somatic tissues (4). Besides invertebrates, germ granules are also constitutively present in the germ line of vertebrates with a similar intracellular localization close to mitochondria (13, 14). Moreover, their assembly and proteostasis rely on self-interacting RNA binding proteins similar to *C. elegans* (18). Thus, an intriguing possibility to be investigated is that aggregation of germ granules can also systemically influence the mitochondrial and proteostasis network of distal tissues in mammals. Together, our findings reveal a germline-to-soma communication whereby the proteostatic status of the germ line can have direct implications in somatic organismal health, a process that could shed light to understand the occurrence of aggregation events of disease-related proteins in the somatic tissues of adult organisms.

**MATERIALS AND METHODS**

*C. elegans* strains and maintenance

*C. elegans* strains were grown and maintained on standard nematode growth media seeded with *Escherichia coli* (OP50) bacteria. Wild-type (N2), SS104 ([glp-4(2b)]; M1994 [hsp-16.2p::GFP::hsp-16.2p::GFP::hsp-16.2p::GFP]), SS747 ([bnls1[pie-1::GFP::pgl-1 + unc-119(+)])], SJ4103 ([zcIs14[myo-3p::GFP(mit)]], AM141 ([rmls133[unc-54p::Q40:yellow fluorescent protein (YFP)]], SJ4100 ([zcs13[hsa-6p::GFP]]], SJ4058 ([zcs9[hsa-6p::GFP::lin-15(+)]]], SJ4005 ([zcs14[hsa-6p::GFP]]], CL2070 ([dvls170[hsa-16.2p::GFP + rol-6(su1006)]], MT1215 ([egl-20(n585)]], and CB3303 ([pig-1(c787)]]) were provided by the Caenorhabditis Genetics Center (CGC) (University of Minnesota), which is supported by the National Institutes of Health Office of Research Infrastructure Programs (P40 OD010440). RAFL291 (cey-2(ok902J]) was provided by R. Ciosk and was generated by outcrossing the RB988 strain (cey-2(ok902J]) four times to wild-type N2 (19). RB988 (cey-2(ok902J]) was made by the *C. elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation (Oklahoma, USA). RA4 (cey-3(rr11);cey-2(ok902J]) outcrossed twice to wild-type N2 was also a gift from R. Ciosk (19). AM23 ([rmls288[25B3.3p::Q19::CFP]] and AM716 ([rmls284[25B3.3p::Q27::YFP]]) strains were provided by R. I. Morimoto (25). MAH602 ([sqIs61[wha-6p::Q44::YFP + rol-6(su1006)]]) was provided by M. Hansen (51). ZM844

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For neuronal-specific RNAi experiments, we used the TU3041
strain (sid-1(pk3321); uIs69[pCF90(myo-2p::mCherry) + unc-
119p::sid-1]) provided by the CGC, in which RNAi treatment is only
effective in neurons (52). DVG196 (rmls284[F25B3.3p::Q67; YFP];
sid-1(pk3321); uIs69[pCF90(myo-2p::mCherry) + unc-119p::sid-1])
was generated by crossing AM716 to TU3041. Screening of sid-
1(pk3321) homozygote worms was done by polymerase chain reaction
(PCR) using the following primers: TATAGGATCCT-
and TTCGGGAAATGGCGCTTAAC. Screening of unc-119p::sid-1
was performed by using the following primers: TATAGGATCCT-
CAGAAAGGTGTCATGGTCTAGT. For germline-specific RNAi
experiments, we used the DCL569 strain (mksCi13[sun-1::rde-
1::sun-1-3 TR + unc-119(+)] II; rde-1(mk36)V) (53), a gift from
D. Chen (Nanjing University, China).

DVG195 strain (rmls284[F25B3.3p::Q67; YFP]; gpl-4(bn2])
was generated by crossing AM716 to SS104. For the generation of
DVG180 (rmls284[F25B3.3p::Q67; YFP]; cey-2(ok902)) and DVG183
(rmls284[F25B3.3p::Q67; YFP]; cey-3[rrr11]; cey-2(ok902)), AM716
was crossed to RAF291 and RAF4, respectively. To generate
DVG199 (zcs14[myo-3p::GFP(mit)]; cey-2(ok902)), we crossed SS4102
to RAF291. Screening of cey-2(ok902) and cey-3[rrr11]; cey-2(ok902)
homozygote worms was done by PCR using the following primers:
TGGGAAAGAAGGCAAGTTGCAAGTTCACATGGTGTCATGTGA
and ACCAAGGCGGACGACTCGTG.

DVG203 (zcs14[myo-3p::GFP(mit)]; mig-1(e1787)) and DVG201
strains (rmls284[F25B3.3p::Q67; YFP]; mig-1(e1787)) were generated
by crossing CB3303 to SJ4103 and AM716, respectively. Screening
of mig-1(e1787) homozygote worms was done by PCR using TGGA-
GGACAGCAAGGATCGAA, TTTTCACACTTCCAT
CACGACACAT, GATCTCTCCATCGGCAAGAAAAGA, and
ACCAAGGCGGACGACTCGTG.

RNAi constructs
RNAi-treated worms were fed E. coli (HT115) containing an empty
control vector (L4440) or expressing double-stranded RNAi. gpl-1, cct-8, atf-6, cco-1, egl-20, mig-1, and pop-1 RNAi constructs were
obtained from the Vidal RNAi library. cey-1, cey-2, cey-3, and bar-1
RNAi constructs were obtained from the Ahringer RNAi library. All
RNAi constructs were sequence-verified. Since concentrated pop-
1 and bar-1 RNAi resulted in organismal sickness, we used a 1:10
dilution in empty vector for our experiments to circumvent deleterious
effects induced by strong knockdown of these factors. All the other
RNAi constructs were not diluted in our experiments.

Life-span studies
Synchronized animals were fed from hatching on HT115 E. coli
carrying empty vector or RNAi constructs. Ninety-six adult animals
were used per condition and scored every day or every other day
(27). From the initial adult population, we censored the worms that
are lost or burrow into the media as well as those that undergo
bagging or exhibit “protruding vulva” phenotypes. n = total number
of uncensored animals/total number (uncensored + censored) of
animals in each independent experiment. We used Prism 6 software
to determine median life span and generate life-span graphs. To
determine mean life span, we used OASIS software (54). P values
were calculated using the log-rank (Mantel-Cox) method. The P values
refer to experimental and control animals in a single experiment.
Fig. 1K presents a representative experiment. See data file S1 for
statistical analysis and replicate data.

GSC proliferation assays
Worms were incubated with 33 mM solution of 5-bromodeoxyuridine
(BrdU) (Sigma-Aldrich) in S medium for 2 hours at 20°C. Worms
were washed with EBT buffer (1× egg buffer, 0.2% Tween 20, and
20 mM sodium azide). Then, worms were decapitated to extract the
germ line on a coverslip and covered with a poly-lysine–coated
microscope slide. The coverslip was removed, and the slide was
fast-frozen on dry ice. Then, the slide was fixed in methanol for
2 min at −20°C and washed with PBST. After blocking for
30 min in PBST containing 10% donkey serum, anti-BrdU antibody
(1:250; Abcam, ab6326) was added followed by overnight
incubation in a humid chamber. Anti-rat immunoglobulin G (IgG)
secondary antibody (1:500; Life Technologies 174474) was added
to 2 hours at room temperature. Last, slides were mounted with
Precision coverslip (Roth) using 4′,6-diamidino-2-phenylindole (DAPI)
fluoromount-G (SouthernBiotec 0100-20).

Egg counting and percentage of viable eggs
Synchronized L1 larvae were raised on HT115 E. coli carrying empty
vector, cey-2 RNAi, or cey-3 RNAi constructs. The number of eggs
during the self-reproductive period was measured by singly plating
late L4 worms and culturing them on the corresponding RNAi treat-
ment. Each adult worm was then transferred to a new plate every
24 hours, and the previous plate was kept for another 24 hours, when
the number of alive progeny (that is, visible as L1 larvae) was scored
to assess percentage of viable eggs. This procedure was repeated until
no live progeny were counted.

Western blot
Worms were lysed in protein lysis buffer [50 mM tris-HCl (pH 7.8),
150 mM NaCl, 1% sodium deoxycholate, 1 mM EDTA, 0.1% SDS,
1% Triton X-100, and protease inhibitor (Roche)] using a Precellys
homogenizer. Worm lysates were centrifuged at 10,000 rpm for
10 min at 4°C, and the supernatant was collected. Protein concen-
trations were determined with standard BCA (bicinchoninic acid
assay) protein assay (Thermo Fisher Scientific). Twenty micrograms
of total protein was separated by SDS–polyacrylamide gel electro-
phoresis (PAGE), transferred to nitrocellulose membranes (Millipore),
and subjected to immunoblotting. Western blot analysis was per-
formed with anti-GFP antibody (1:5000; AMSBIO, TP401), FUS
(1:1000; Abcam, ab154141), TDP43 (1:1000; Abcam, ab225710), and
α-tubulin (1:20,000; Sigma-Aldrich, T6199).

C. elegans germline and gut immunostaining
Day 3 adult worms were washed using EBT buffer (1× egg buffer,
20 mM sodium azide, and 0.2% Tween 20), decapsulated to ex-
tract the germ line and intestine on a coverslip, and covered with a

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poly-lysine–treated slide. The microscopy slide was then fast-frozen on dry ice, fixed in methanol for 2 min at −20°C, and washed with PBST followed by blocking with 10% donkey serum in PBST for 30 min. Samples were incubated overnight with anti–PGL-1 antibody (1:33; DSHB, K76) or anti–ATP5A (1:100; Abcam, ab14748) at room temperature. Alexa Fluor 546 goat anti-mouse IgM (1:500; Life Technologies, 2105681) and Alexa Fluor 546 goat anti-mouse IgG (1:500; Life Technologies, 1904466) were added for 2 hours. Slides were mounted with DAPI fluoromount-G (SouthernBiotech 0100-20).

**Imaging of myo-3p::GFP(mit) reporter**

For imaging of myo-3p::GFP(mit) reporter strain, day 3 adult worms were immobilized using 0.1% Azide in M9 buffer and covered with a coverslip. Images of worms were acquired with a Zeiss Axios Imager Z.1 fluorescence microscope. Qualitative assessment of mitochondrial morphology was made by scoring animals based on three categories: normal (interconnected mitochondrial network), intermediate (combination of interconnected mitochondrial network and isolated smaller mitochondria), or fragmented (mostly fragmented mitochondria).

**hsp-6, hsp-60, hsp-4, hsp-16.2 transcriptional reporter experiments and imaging**

SJ4100 (zcsIs13[hsp-6p::GFP]), SJ4058 (zcsIs9[hsp-60p::GFP + lin-15(+)]), SJ4005 (zcsIs4[hsp-4p::GFP]), and CL2070 (dvlIs70[hsp-16.2p::GFP + rol-6(sa1006)]) worms were cultured until day 3 of adulthood. For imaging, adult worms were immobilized using 0.1% Azide in M9 buffer and covered with a coverslip. Images of whole worms were acquired with a Zeiss Axio Zoom.V16 fluorescence microscope. To quantify GFP fluorescence, worms were outlined and quantified using ImageJ software.

**NAD⁺ and ATP detection**

Two hundred age-synchronized day 3 adult worms were collected and washed with M9 buffer. For NAD⁺ detection, we used a commercial colorimetric kit (NAD/NADH Assay Kit, #ab65348) following the manufacturer’s instructions. Briefly, the animals were homogenized in NAD⁺/NADH extraction buffer. Enzymes that may consume NADH were removed by filtering the samples through a 10 kD spin Column (ab93349) before performing the assay. Decomposition of NAD⁺ for NADH detection in samples was done by heating the samples to 60°C for 30 min. Quantification of NAD total and NADH was performed by colorimetric method (optical density 450 nm) using standard curve derived from known NADH concentrations. For ATP quantification, we used a colorimetric/fluorometric ATP assay kit (Abcam, ab83355) following the manufacturer’s instructions. First, worms were homogenized in ATP assay buffer followed by deproteinization using 4 M perchloric acid (Sigma-Aldrich, 244252) and 2 M KOH (Sigma-Aldrich, P-1767) method. Then, pH 1 was adjusted to 6.8 to 8. Samples were centrifuged at 13,000 g for 15 min at 4°C, and the supernatant was collected. Quantification of ATP content was measured by fluorometric [Ex/Em (Excitation/Emission) = 535/587 nm] method. ATP concentrations were determined using standard curve derived from fluorescence of known ATP concentrations. Levels of fluorescence were measured using multimode plate reader EnSpire (PerkinElmer). For both NAD⁺ and ATP detection, protein levels were normalized by a BCA protein assay kit (Pierce, Thermo Fisher Scientific).

**Oxygen consumption rates**

Oxygen consumption rates were measured and analyzed using the Seahorse XFe96 Analyzer (Agilent) using the protocol described in (55). Briefly, synchronized worms at day 3 of adulthood were washed in M9 buffer three times to remove residual bacteria and then transferred into the Seahorse plate wells at around 20 worms per well with at least five technical replicates per condition. Oxygen consumption was measured at room temperature of 20°C. Sodium azide treatment (Sigma-Aldrich) was used at 40 mM.

**Filter trap assay**

*C. elegans* strains were grown from hatching on HT115 *E. coli* carrying either empty vector or RNAi clones. At day 3 of adulthood, worms were collected with M9 buffer, and worm pellets were frozen with liquid N2. Frozen worm pellets were thawed on ice, and worm extracts were generated by glass bead disruption on ice in non-denaturing lysis buffer [50 mM Heps (pH 7.4), 1 mM EDTA, 150 mM NaCl, and 1% Triton X-100] supplemented with EDTA-free protease inhibitor cocktail (Roche). Worm debris was removed with 8000 g spin for 5 min. One hundred micrograms of protein extract was supplemented with SDS at a final concentration of 0.5% and loaded onto a cellulose acetate membrane assembled in a slot blot apparatus (Bio-Rad). Then, the membrane was washed with 0.2% SDS and SDS-resistant protein aggregates were assessed by immunoblotting using antibodies against GFP (1:5000; AMSBIO, TP401), FUS (1:1000; Abcam, ab154141), and TDP43 (1:1000; Abcam, ab225710). Extracts were also analyzed by SDS-PAGE/Western blot with anti-GFP, anti-FUS, anti-TDP43, and anti–α-tubulin (1:20,000; Sigma-Aldrich, T6199).

**Motility assay**

Animals were grown on HT115 *E. coli* containing empty control vector or the indicated RNAi. At day 3 of adulthood, worms were transferred to a drop of M9 buffer. After 30 s of adaptation, the number of body bends was counted for 30 s. A body bend was defined as change in direction of the bend at the mid-body (25).

**Sample preparation for label-free quantitative proteomics and analysis**

Extruded germ lines of synchronized worms at day 3 of adulthood were lysed in urea buffer [8 M urea, 2 M thiourea, and 10 mM Hepes (pH 7.6)] by sonication and cleared using centrifugation (13,000 rpm, 10 min). Supernatants were reduced (1 mM dithiothreitol, 30 min), alkylated (5 mM iodoacetamide, 45 min), and digested with trypsin at a 1:100 (w/w) ratio after diluting urea concentration to 2 M. One day later, samples were cleared (16,000g, 20 min), and the supernatant was acidified. Peptides were cleaned up using stage tip extraction. The liquid chromatography–tandem mass spectrometry (LC-MS/MS) equipment consisted of an EASY-nLC 1000 coupled to the quadrupole-based QExactive instrument (Thermo Fisher Scientific) via a nanospray electroionization source. Peptides were separated on an in-house packed 50-cm column (1.9-μm C18 beads, Dr. Maisch) using a binary buffer system: (i) 0.1% formic acid and (ii) 0.1% formic acid in acetonitrile. The content of buffer B was raised from 7 to 23% within 120 min and followed by an increase to 45% within 10 min. Then, within 5 min, buffer B fraction was raised to 80% and held for further 5 min after which it was decreased to 5% within 2 min and held there for further 3 min before the next sample was loaded on the column. Eluting peptides were ionized by an
applied voltage of 2.2 kV. The capillary temperature was 275°C and the S-lens RF level was set to 60. MS1 spectra were acquired using a resolution of 70,000 [at 200 mass/charge ratio (m/z)], an Automatic Gain Control (AGC) target of 3 × 10³ and a maximum injection time of 20 ms in a scan range of 300 to 1750 Th. In a data-dependent mode, the 10 most intense peaks were selected for isolation and fragmentation in the HCD cell using a normalized collision energy of 25 at an isolation window of 2.1 Th. Dynamic exclusion was enabled and set to 20 s. The MS/MS scan properties were 17,500 resolution at 200 m/z, an AGC target of 5 × 10⁵ and a maximum injection time of 60 ms. All label-free proteomics datasets were analyzed with the MaxQuant software (version 1.5.3.8) (56). We used the label-free quantitative (LFQ) mode and used MaxQuant default settings for protein identification and LFQ quantification. All downstream analyses were carried out on LFQ values with Perseus (version 1.5.2.4) (57). Statistically significant differences were determined with Perseus software after correction for multiple testing following the Benjamini-Hochberg procedure to calculate FDR-adjusted P values.

Quantitative real-time PCR
Total RNA of whole animals was isolated from approximately 200 synchronized day 3 adult worms using RNAbeee (Tel-Test Inc.). For analysis of mitochondrial components in germ line, RNA of isolated germ lines from 50 synchronized day 3 adult worms was extracted using RNAbeee. In parallel, isolated intestines and heads were combined to extract RNA and examine expression of mitochondrial components in somatic tissues. CDNA was generated using a qScript Flex cDNA synthesis kit (Quintantio). SYBR green real-time quantitative PCR (qPCR) experiments were performed with a 1:20 dilution of cDNA using a CFC384 Real-Time System (Bio-Rad). Data were analyzed with the comparative ΔΔCt method using the geometric mean of cdc-42 and Y45F10D.4 as housekeeping genes (58). See data file S4 for details about the primers used for this assay.

mtDNA/gDNA ratio
Isolated germ lines or somatic tissues (intestines + heads) from 100 synchronized day 3 adult worms were incubated in lysis buffer [30 mM tris-HCl (pH 8), 8 mM EDTA, 100 mM NaCl, 0.7% NP-40, 0.7% Tween 20, and proteinase K (100 mg/ml)] for 1 hour at 65°C (59). Then, proteinase K was inactivated at 95°C for 15 min. The product was diluted 1:500, and 4 μl was loaded to quantify mtDNA by SYBR green real-time qPCR assay using the following primers for NADH dehydrogenase subunit 1 (nd-1): 5’-AGGCGTACATTATTGG-GGAAGAAGAC-3’ and 5’-AAGCTTGTGCTAATCCCATAAAT-GT-3’. The results were normalized to gDNA using the following primers for cox-4: 5’-GCGGAGATCCTGCTCAACTCCATGTA-3’ and 5’-GCGGAGATCCTGCTCAACTCCATGTA-3’. qPCR experiments were performed using a CFC384 Real-Time System (Bio-Rad), and data were analyzed with the comparative ΔΔCt method.

Statistical analysis
Sample sizes are indicated in the corresponding figure legends and the Supplementary Materials. Statistics were derived from at least n = 3 independent experiments. No data were excluded from the analyses. Statistical comparisons were performed with two-tailed Student’s t test for unpaired samples using GraphPad Prism 6.0. Data file S5 contains the source data underlying the graphical representations and corresponding statistical analysis used in the figures. For life-span experiments, statistical analysis was performed using the log-rank (Mantel-Cox) method (data file S1). Statistically significant differences from proteomics data were determined with Perseus (v. 1.5.2.4) (57) after correction for multiple testing following the Benjamini-Hochberg procedure to calculate FDR-adjusted P values. Data file S2 contains LFQ values and statistical analysis of all the identified proteins in the proteomics assay. Analysis of enriched GOCC from the proteomics data were performed using PANTHER (60). Data file S3 provides the number of significantly changed proteins and statistical analysis for all the enriched GOCC terms.

Supplementary materials

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/26/eabg3012/DC1

View/request a protocol for this paper from Bio-protocol.

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Systemic regulation of mitochondria by germline proteostasis prevents protein aggregation in the soma of *C. elegans*

Giuseppe Calcutti, Hyun Ju Lee, Koning Shen, Uyen Pham, Marija Herholz, Aleksandra Trifunovic, Andrew Dillin and David Vilchez

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