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## The dynamin-related protein DRP-1 and the insulin signaling pathway cooperate to modulate *C. elegans* longevity

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### Summary

Here we report that inactivation of the *C. elegans* dynamin-related protein DRP-1, a key component responsible for mitochondrial fission and conserved from yeast to humans, dramatically enhanced the effect of reduced insulin signaling (IIS) to extend lifespan. This represents the first report of a beneficial impact of manipulating mitochondrial dynamics on animal lifespan and suggests that mitochondrial morphology and IIS cooperate to modulate aging.

### Keywords

mitochondria; fission protein DRP-1; insulin signaling; aging; *C. elegans*

Mitochondria are dynamic organelles able to undergo frequent morphological and numeral changes. A delicate balance between mitochondrial fusion and fission is critical for broad aspects of animal physiology, including apoptosis and control of mitochondrial inheritance and quality (Seo *et al.* 2010). From yeast to human, deregulations of mitochondrial network equilibrium as evident by disrupted mitochondrial morphology and accumulation of abnormally shaped mitochondria have been associated with senescence, aging and aging-related diseases (Sohal 1975; Yasuda *et al.* 2006; Lee *et al.* 2007). However, no direct evidence implicates mitochondrial dynamics in longevity determination in animals. The only evidence that mitochondrial plasticity positively impacts lifespan was shown in fungal models, in which reduced mitochondrial fission led to increased lifespan (Scheckhuber *et al.* 2007; Palermo *et al.* 2010). Mitochondrial dynamics are governed by molecular machineries that are highly conserved (Okamoto & Shaw 2005). The dynamin-related protein DRP-1 is the only profession protein identified in *C. elegans* and is demonstrated to control the scission of the mitochondrial outer membrane (Labrousse *et al.* 1999; Westermann 2010).

To explore how mitochondrial fission can impact animal lifespan, we monitored the lifespan of worms either treated with *drp-1* RNAi or bearing a putative null-mutation in the *drp-1* gene (Breckenridge *et al.* 2008). The mean lifespan of wild-type worms treated with *drp-1* RNAi or *drp-1* mutant worms was indistinguishable from that of control animals (Fig. 1A-B, Table S1), even though their mitochondrial morphology are greatly disrupted (Labrousse *et al.* 1999, Fig. 2B-C and S1), indicating that reduced mitochondrial fission does not affect *C. elegans* lifespan under normal culturing condition

The insulin/IGF-1 signaling (IIS) pathway is a key longevity pathway and *C. elegans* mutants with reduced IIS, such as the phosphatidylinositol 3-kinase *age-1* mutant

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(Friedman & Johnson 1988; Morris *et al.* 1996) and the tyrosine kinase insulin/IGF receptor *daf-2* mutant (Kenyon *et al.* 1993), are long-lived (Fig.1A,C-D). Given that mitochondrial fission plays a critical role in insulin secretion in mammals (Yoon *et al.* 2010), we next tested how inactivating *drp-1* might affect the longevity of IIS mutants. Strikingly, both *age-1* and *daf-2* mutants treated with *drp-1* RNAi showed substantial further increase in mean lifespan (>65% compared to *age-1* and *daf-2* single mutant on control RNAi; Fig.1A, Table S1). Similarly, *age-1;drp-1* and *daf-2;drp-1* mutants exhibited a >75% increase in mean lifespan and an extension by up to 30 days in maximum lifespan when compared to their single mutant counterparts (Fig.1C-D, Table S1). Importantly, the enhancement of both the mean and maximum lifespans of *age-1* mutants by the *drp-1* mutation could be rescued by a transgene expressing *drp-1* (Fig.S2). We also tested whether *drp-1* inactivation will impact the lifespan of other long-lived strains, including worms undergoing the bacteria deprivation paradigm of dietary restriction (Kaeberlein *et al.* 2006), and worms with mild impairment of the mitochondrial respiratory chain (Felkai *et al.* 1999; Feng *et al.* 2001). We detected no or marginal effects (Table S2). Altogether, we demonstrated that inactivating *drp-1* specifically and robustly synergizes with reduced IIS to prolong longevity.

The longevity effect of reduced IIS is often associated with increased resistance to stress (Johnson *et al.* 2001). Surprisingly, wild-type and insulin mutant worms with *drp-1* deletion were more sensitive to the oxidizing agent paraquat and to heat stress than their counterparts with wild-type *drp-1* (Fig.2A, Table S3). The vulnerability of the *age-1;drp-1* mutant to heat stress is due to *drp-1* loss as it can be rescued by a transgene overexpressing *drp-1* (Fig.S2). Therefore, inactivating *drp-1* specifically prolongs longevity, but not stress resistance, of worms with reduced IIS, suggesting that manipulation of mitochondrial fission uncouples the function of IIS in aging and stress resistance. However, we cannot exclude that inactivating *drp-1* causes an early stress in young worms but will confer stress resistance later in life.

We next examined how inactivation of *drp-1* affects mitochondrial morphology by comparing the indices of circularity (a mathematical estimation of circular shapes) of individual mitochondrion (see Supplementary Materials and Methods). We found that mitochondrial morphology was similarly disrupted in single *drp-1* mutant and in *IIS;drp-1* mutants (Fig.2C), suggesting DRP-1 does not cooperate with IIS to regulate mitochondrial fission. Interestingly, our data also revealed that mitochondria of *daf-2* and *age-1* single mutants presented lower indices of circularity than those of wild-type animals (Fig.2B-C).

Signaling from DAF-2 and AGE-1 results in cytoplasmic retention of the FOXO transcription factor DAF-16, and DAF-16 translocation into the nucleus in *age-1* and *daf-2* mutants enables DAF-16-mediated regulation of target genes that contribute to diverse functional outcomes (Hekimi *et al.* 2001; Lee *et al.* 2001). Because the prolonged lifespan of *age-1* and *daf-2* mutants are fully dependent on *daf-16* (Dorman *et al.* 1995), we next tested whether the extraordinary longevity of the *IIS;drp-1* mutants also requires *daf-16*. We found that *daf-16* RNAi completely abolished the synergistic effect of *drp-1* and *daf-2/age-1* mutations (Fig.1C-D, Table S1). Interestingly, we did not detect any changes in DAF-16::GFP sub-cellular localization upon *drp-1* inactivation (Fig.2D). Thus, the mechanism allowing DRP-1 to cooperate with IIS in modulating lifespan fully depends on DAF-16 but is unlikely to be mediated *via* DAF-16's nuclear translocation.

In considering a mechanism that enables *drp-1* inactivation to specifically synergize with IIS mutants to extend lifespan, several possibilities come to mind. In mammals, mitochondrial fission is critical for insulin secretion (Zorzano *et al.* 2009; Yoon *et al.* 2010). Thus, *drp-1* inactivation in worms may similarly interfere with insulin secretion and further reduce IIS in *age-1/daf-2* mutants. This possibility is unlikely however as DAF-16 nuclear translocation

was not enhanced in *IIS;drp-1* double mutants. Although we found that *age-1* and *daf-2* single mutants show slight alterations in mitochondrial morphology, our data do not support the possibility that mitochondrial dynamics was further perturbed in *IIS;drp-1* double mutants, as mitochondrial morphology in those mutants were similar to *drp-1* single mutant. On the other hand, because the *drp-1* single mutant has a brood size defect (Breckenridge *et al.* 2008), it is possible that germline proliferation is inhibited when *drp-1* is inactivated, and defective germline proliferation is known to synergize with IIS mutant to increase lifespan (Hsin & Kenyon 1999; Spanier *et al.*). Alternatively, similar to what was shown with mitochondrial prohibitins (Artal-Sanz & Tavernarakis 2009), inactivation of *drp-1* may perturb lipid metabolism, a process highly dependent on mitochondrial activity, to synergize with IIS mutants to extend lifespan. Lastly, *C. elegans* DRP-1 is shown to regulate apoptosis under sensitized conditions (Breckenridge *et al.* 2008). Intriguingly, *daf-2* mutants exhibit enhanced germline apoptosis (Pinkston *et al.* 2006). It is possible that the apoptotic function of DRP-1 is induced in IIS mutants, and loss of *drp-1* may block apoptosis in IIS mutants and further promote longevity. Interestingly, in fungal models, mitochondrial fission modulates wild-type lifespan by interfering with apoptosis (Scheckhuber *et al.* 2007; Palermo *et al.* 2010). It is possible that fungal aging is more closely connected to apoptosis (Rockenfeller & Madeo 2008) than *C. elegans* aging, providing an explanation for why reduced mitochondrial fission affects wild-type lifespan in fungi but not in worms.

This is the first report of a manipulation of mitochondrial dynamics that positively impacts lifespan in an animal. As components of the mitochondrial fission/fusion machinery and IIS are highly conserved, our observations are likely relevant to mitochondrial biology and longevity in mammals.

## Materials and Methods

All strains were obtained from the *Caenorhabditis* Genetic Center with the exception of the *drp-1 (tm1108)* strain provided by the National Bioresource Project for the Nematode. Lifespan assays were performed as in (Li *et al.* 2008) with slight modifications (see Supplementary Materials and Methods).

DAF-16 sub-cellular localization was evaluated using *daf-2(e1370);daf-16(mgDf47);xrls87* transgenic worms as in (Li *et al.* 2008) and (Padmanabhan *et al.* 2009) with slight modifications (see Supplementary Materials and Methods). The data shown in Fig.2D were obtained at Day 3 of adulthood.

Mitochondrial networks were visualized using tetramethylrhodamine (TMRE) staining or a *Pmyo-3::mito::GFP* construct. See Supplementary Materials and Methods for more detailed procedures.

Stress assays were performed on synchronized populations at the young adult stage or Day 3 of adulthood. For heat stress, synchronized worm populations were placed at 37°C for 8 hours and scored immediately after for survival. For paraquat-induced stress, synchronized worm populations were transferred onto plates seeded with a final concentration of 25mM paraquat. Similar results were obtained using 15 mM paraquat. The population was kept at 20°C and scored every day for survival. Each assay was repeated at least 3 times.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

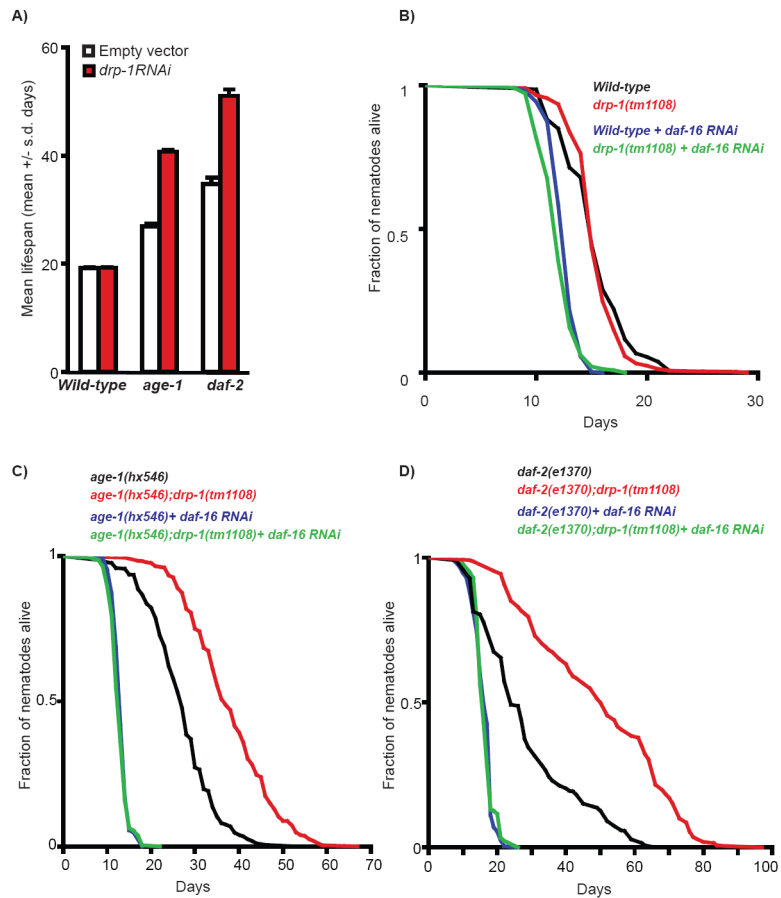
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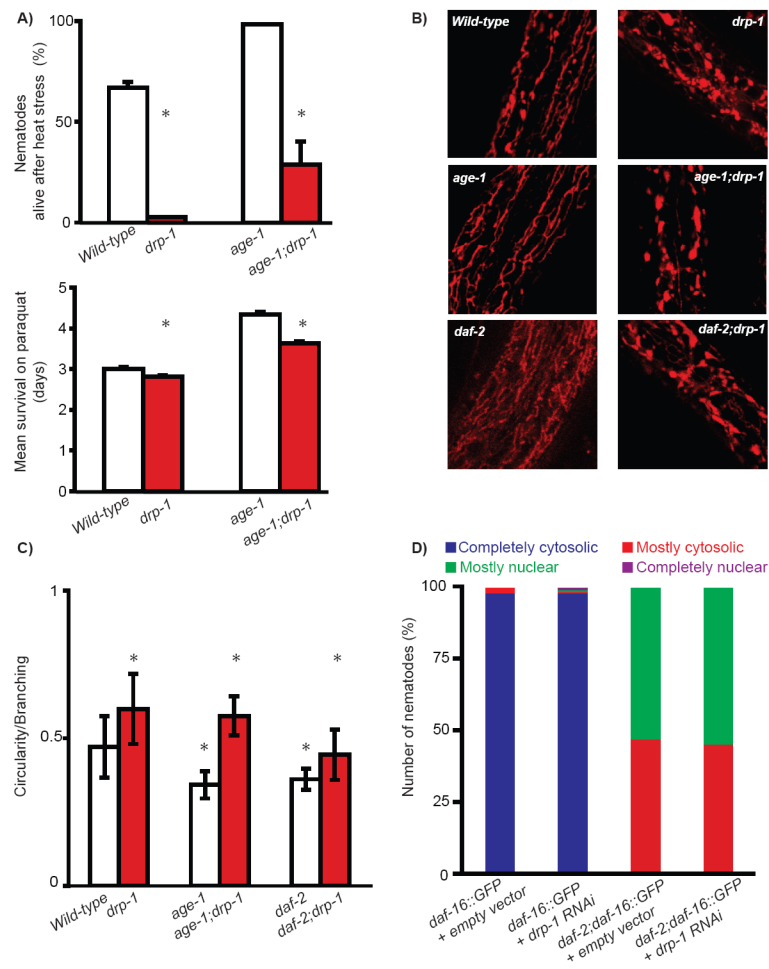
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**Figure 1. Loss of *drp-1* enhances the longevity phenotype of IIS mutant worms in a *daf-16*-dependent manner**

(A) Mean adult lifespan ( $\pm$  s.d) of wild-type, *age-1* and *daf-2* mutant worms treated with empty vector (empty bars) or *drp-1* RNAi (red bars). Note that quantitative PCR data showed that *drp-1* mRNA expression was reduced by  $\sim$ 50% in all strains upon *drp-1* RNAi treatment (data not shown). Adult lifespan of wild-type and *drp-1* mutant worms (B); *age-1* and *age-1*; *drp-1* mutant worms (C); *daf-2* and *daf-2*; *drp-1* mutant worms (D) treated or not with *daf-16* RNAi (as indicated). Quantitative data and statistical analysis are presented in Table S1.





**Figure 2. Loss of *drp-1* affects mitochondrial morphology and stress resistance without affecting DAF-16 sub-cellular localization**

(A) Percentage of worms alive ( $\pm$  s.d) after 8 hours at 37°C (upper graph) and mean survival ( $\pm$  s.d) upon treatment with 25 mM paraquat (bottom graph) in the indicated strains. \* indicates  $p < 0.001$  (Student's *t*-test for heat stress and log-rank test for paraquat). TMRE staining (B) and circularity index ( $\pm$  s.d) (C) of the mitochondrial network in the indicated strains. In (C), \* indicates  $p < 0.005$  when compared to single mutant counterpart and  $p < 0.005$  when compared to wild-type worms using a Student's *t*-test. In agreement with previous studies (Labrousse *et al.* 1999), *drp-1* mutant worms exhibited disrupted structure of the tubular mitochondrial network in which mitochondria tended to form large blebs as reflected by an increased circularity index. (D) DAF-16::GFP was categorized by sub-cellular localization in *daf-2* worms treated with empty vector or *drp-1* RNAi. The data obtained at Day 3 of adulthood are presented.