DR GEORGE L. SUTPHIN (Orcid ID : 0000-0002-3659-4678)

Article type : Short Take

ssssss Oxidative stress is a central pathomechanism in Alzheimer's disease (AD) and other diseases with tau pathology. The Nrf2 transcription factor induces detoxification enzymes and improves tau pathology and cognition. Its homologue in C. elegans is SKN-1. We previously showed that the worm tau homologue, PTL-1, regulates neuronal aging and lifespan. Here, we tested PTL-1's involvement in the stress response. ptl-1 mutant animals are hypersensitive to oxidative stress and are defective in stress-mediated nuclear accumulation of SKN-1. This defect can be rescued by PTL-1 re-expression under the control of the ptl-1 promoter. Given the close relationship between aging and stress tolerance, we tested lifespan and found that PTL-1 and SKN-1 regulate longevity via similar processes. Our data also suggest that PTL-1 functions via neurons to modulate SKN-1, clarifying the role of this protein in the stress response and longevity.

Key words: C. elegans; lifespan; neurons; oxidative stress; PTL-1; SKN-1.

Introduction, results and discussion

The most common form of dementia, AD, is characterised by Ab-containing plaques and neurofibrillary tangles composed of hyper-phosphorylated tau (Ittner et al., 2011).

Protein with tau-like repeats-1 (PTL-1) is the sole Caenorhabditis elegans homologue of the mammalian

tau/MAP2/MAP4 family (Goedert et al., 1996). PTL-1 has a predominantly neuronal expression pattern and functions in the nervous system to mediate kinesin-based transport (Tien et al., 2011).

Activation of Nrf2, a mediator of the oxidative stress response, reduces tau hyperphosphorylation and aggregation (Jo et al., 2014; Stack et al., 2014). Its C. elegans homologue, SKN-1, similarly regulates an oxidative stress response (An et al., 2003). SKN-1 exists in 3 isoforms (Bishop et al., 2007). Most studies have focused on isoforms b and c, and a SKN-1b/c::GFP transgenic line is available, facilitating expression studies (An et al., 2003). SKN-1b mediates dietary-restriction-mediated longevity (Bishop et al., 2007) and SKN-1b/c re-expression compensates for the loss of isoforms a/c in the oxidative stress response (An et al., 2005).

Loss of ptl-1 causes neuronal and organismal aging defects (Chew et al., 2013, 2014). As aging and stress pathways are intimately linked, we tested whether ptl-1 mutant animals are stress-sensitive. ptl-1 (ok621) and ptl-1(tm543) mutant worms showed decreased survival after exposure to H_2O_2 (Fig 1A). We next tested whether SKN-1 was affected by defective PTL-1. Wild-type animals carrying the ldIs7[SKN-1::GFP] transgene show reporter expression in the cytoplasm of intestinal cells that rapidly accumulates in the nucleus in response to stress. In contrast, in ASI neurons, SKN-1 is constitutively localised to nuclei (An et al., 2003)(Fig. 1Bi). In the following assays, we used azide stress as this was shown to effectively induce SKN-1 nuclear accumulation (An et al., 2003). In nonstress conditions, no SKN-1 nuclear accumulation was observed in any of the tested strains (data not shown). Both ptl-1 mutant strains displayed a defect in SKN-1 accumulation in intestinal nuclei in response to azide (Fig. 1Bii), which could be rescued by

re-expression of PTL-1 under control of the ptl-1 promoter (Fig 1Bii). We next tested whether GCS-1, a detoxification enzyme that is induced by SKN-1, is affected by mutations in ptl-1. ldIs3[Pgcs-1::gfp] expression is low under normal conditions (Fig S1) but is induced in the intestine under stress conditions (Fig 1Ci) (An et al., 2003). Pgcs-1::gfp induction in response to stress was defective in ptl-1 mutants, and this defect was rescued by PTL-1 re-expression (Fig. 1Cii). We also found that the induction of two other SKN-1 targets, gst-4 (Park et al., 2009) and hsp-4 (Glover-Cutter et al., 2013), following azide treatment was compromised in ptl-1 mutants and could be rescued by PTL-1 re-expression (Fig S2). We previously showed that ptl-1 mutants are short-lived (Chew et al., 2013, 2014). Others reported that skn-1(zu67), which affects SKN-1a and c, also confers a short-lived phenotype (An et al., 2003). Interestingly, ptl-1;skn-1 double-mutant animals did not have a significantly different lifespan compared to skn-1 or ptl-1 single-mutant animals (Fig 1D, Table S1), suggesting that SKN-1 and PTL-1 regulate lifespan via the same pathway.

Using the pan-neuronal aex-3 promoter, we re-expressed PTL-1 to test whether neuronal PTL-1 regulates SKN-1. This was sufficient to rescue the defect in sensitivity to H

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(Fig S3), SKN-1 nuclear accumulation (Fig 2Ai), Pgcs-1::gfp induction (Fig. 2Aii) and induction of gst-4 and hsp-4 (Fig S2) in ptl-1 null mutants in response to stress. These data suggest a role for neuronal PTL-1 in regulating intestinal SKN-1. However, as aex-3 is also reported to function in the intestine (Mahoney et al., 2008), a contribution from non-neuronal tissues to the observed rescue of ptl-1 mutant phenotypes cannot be excluded. We therefore also performed RNAi knockdown of ptl-1 and found that SKN-

1 nuclear accumulation in response to stress is only compromised when the nervous system is sensitised to RNAi, supporting a role for neuronal PTL-1 in intestinal SKN-1 regulation (Fig S4). Given that SKN-1b is expressed in ASI neurons (An et al., 2003; Bishop et al., 2007), we tested whether re-expressing PTL-1 in ASI neurons alone, using a gpa-4

Genetic interaction with temperature is an important determinant of nematode longevity

Authors: Hillary Miller[1], Marissa Fletcher[2], Melissa Primitivo[2], Alison Leonard[2], George L. Sutphin[2], Nicholas Rintala[2], Matt Kaeberlein[2], and Scott F. Leiser[1,3,4*]

Affiliations:

[1]Cellular and Molecular Biology Department, University of Michigan, Ann Arbor, MI 48109, USA.

[2]Department of Pathology, University of Washington, Seattle, WA 98195, USA.

[3]Molecular & Integrative Physiology Department, University of Michigan, Ann Arbor, MI 48109, USA.

[4]Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA.

*Correspondence to: Scott F. Leiser, leiser@umich.edu

Key words: C. elegans; lifespan; temperature; aging; bacteria

Summary:

As in other poikilotherms, longevity in *C. elegans* varies inversely with temperature; worms are longer-lived at lower temperatures. While this observation may seem intuitive based on

thermodynamics, the molecular and genetic basis for this phenomenon is not well understood. Several recent reports have argued that lifespan changes across temperatures are genetically controlled by temperature-specific gene regulation. Here, we provide data that both corroborate those studies and suggest that temperature-specific longevity is more the rule than the exception. By measuring the lifespans of worms with single modifications reported to be important for longevity at 15, 20, or 25°C, we find that the effect of each modification on lifespan is highly dependent on temperature. Our results suggest that genetics play a major role in temperature-associated longevity and are consistent with the hypothesis that while aging in *C. elegans* is slowed by decreasing temperature, the major cause(s) of death may also be modified, leading to different genes and pathways becoming more or less important at different temperatures. These differential mechanisms of age-related death are not unlike what is observed in humans, where environmental conditions lead to development of different diseases of aging.

Introduction, results and discussion

The aging process has been described as stochastic – a probabilistic degeneration of cellular function that may be explained in sufficient detail by thermodynamic principles (Conti, 2008). Thermodynamics and the kinetics of chemical reactions provide the most rudimentary understanding of how physiological processes change as temperature changes. Described most simply, the rates of various chemical reactions increase as temperature increases, resulting in an increased rate of biochemical processes and, possibly, a corresponding increase in the rate of aging. Consistent with this model, lowering the ambient temperature of poikilotherms such as *C. elegans, D. melanogaster and C. bellottii,* and decreasing a mouse's body temperature can increase lifespan (Conti et al., 2006; Hosono et al., 1982; Lamb, 1968; Loeb and Northrop, 1916).

In *C. elegans*, animals that develop and age at 15°C ("low temperature") are long-lived compared to wild-type animals grown at 20°C (~ room temperature), whereas wild-type worms that develop and age at 25°C ("high temperature") are short-lived compared to wild-type worms grown at 15°C or 20°C (Fig 1). This "temperature law" has been described as widely accepted, but not tested beyond limited number of strains (Zhang et al., 2015).

While the “temperature law” is observed among wild-type organisms, the interplay between genetics and temperature is not well understood. Multiple recent reports suggest that the effects of temperature on longevity are genetically controlled, and that both heat and cold modify transcriptional pathways that effect lifespan (Chen et al., 2016; Ewald et al., 2015; Horikawa et al., 2015; Lee and Kenyon, 2009; Leiser et al., 2011; Xiao et al., 2013; Zhang et al., 2015). To better understand the interplay between temperature and longevity, we measured the lifespans of worms with genetic manipulations known to affect longevity at 15°C, 20°C, or 25°C. Figure 1 illustrates six examples of how longevity can be impacted across temperatures, representing conditions that:

- robustly increase lifespan at all temperatures (*daf-2* RNAi)
- robustly decrease lifespan at all temperatures (*rhy-1(ok1402)*)
- decrease lifespan at high but not low temperature (*daf-16(mu86)*)
- increase lifespan at high temperature but decrease lifespan at low temperature (*rsks-1(ok1255)*)
- increase lifespan at low temperature but not high temperature (*cep-1(gk138)*)
- do not alter lifespan at any temperature (*cah-4* RNAi)

Having established that relative longevity can vary across temperatures, we next asked whether this variability is common among conditions known to modify longevity. We tested nearly fifty genotypes and interventions previously reported to affect lifespan (Fig S1-3 and Table S1-2) and found that relative longevity was consistently inconsistent across temperatures. However, there are consistent trends within longevity pathways, where strains/conditions known to have opposing effects are also affected by temperature oppositely (Figure 2A-B, Fig S5A-D). We used Cox regression analysis to assess the interaction between each longevity intervention and temperature. The hazard ratios, which represent the cumulative risk of death throughout a worm’s lifespan, confirm the interaction between condition (genotype, RNAi, etc.) and temperature and clearly separate the conditions into three categories: approximately one third (15/43) of the interventions show an increased hazard ratio (significantly “better” at higher temperature), one third (14/43) show a decreased hazard ratio (significantly “better” at lower temperature), and one third (14/43) show no interaction between

genetic manipulation and temperature (Fig 2C,D). The changes in hazard ratio are frequently ~two-fold and are clearly not random, as evidenced by reciprocal results for genes that are known to have opposite effects within the same pathway (e.g. *daf-2(e1370)* vs. *daf-16(mu86)*, *vhl-1(ok161)* vs. *hif-1(ia4)*) (Fig S6). Heat-map analysis with hierarchical clustering segregate the tested conditions (Fig S7) into the groups described in Fig 1.

In summary, we find significant interaction between longevity interventions and environmental temperature in two-thirds (29/43) of the cases examined, indicating that a temperature-independent effect on longevity is more the exception than the rule (Fig 2C-D). This variation confirms that genetics play a substantive role in temperature-dependent longevity that cannot be explained solely by the rules of thermodynamics and chemical kinetics.

The observed variation in relative longevity with temperature is consistent with the hypothesis that distinct mechanisms determine nematode longevity at different temperatures (Fig 2E). As shown in the model, there are three distinct types of strains/conditions: those with similar slopes and hazard ratios to N2 (FMO-2 OE, *rhy-1(ok1402)*, etc.), "temperature dependent" strains/conditions that live comparatively longer at higher temperatures (e.g. DR, *rsks-1* (*ok1255)*, *daf-2(e1370)* or RNAi), and "temperature dependent" strains/conditions that live comparatively longer at colder temperatures (*vhl-1(ok161)*, *cep-1(gk138)*, SKN-1 OE). These three categories are further complicated by how they compare to wild-type overall, leading some strains to be consistently long-lived (e.g. *daf-2(e1370)* or RNAi) or short-lived (e.g. *rhy-1 (ok1402)*), whereas other strains vary in relative longevity depending on temperature (e.g. *cep-1(gk138)*). Together, these results suggest that testing strains/conditions at multiple temperature will not only define the robustness of an effect, but may provide clues as to the mechanism.

It has been suggested that protein quality control and the heat stress response are of primary importance for determining nematode longevity at 25℃ (Seo et al., 2013). Our data support this model; we find interventions that limit heat stress response (e.g., *daf-16(mu86)*) are detrimental at high, but not low, temperature, while interventions that improve protein homeostasis, such as dietary restriction or reduced expression of translation machinery (e.g.

rsks-1(ok1255), rpl-6 RNAi) show lifespan extension at high temperature. The relevant mechanisms affecting longevity at low temperature are less clear, particularly because relatively few aging studies are conducted at 15°C compared to 20°C or 25°C. It is possible a combination of a strain's ability to avoid age-associated vulval integrity defects (AVID), a healthspan phenotype primarily observed at colder temperatures (Leiser *et al.* 2016), and to better adapt to temperature-dependent changes to their bacterial food source (growth rate, metabolism, pathogenicity), leads to better outcomes in colder temperatures. We note that a subset of our data (*trpa-1(ok999), daf-16(mu86)* at 15°C) differ from other published works on whether strains are relatively short or long-lived at a given temperature (Horikawa et al., 2015; Xiao et al., 2013). While we did not directly test why these differences are observed, we expect that they are due to our lifespans using UV-killed bacteria for a food source and others using live bacteria. It is known that *daf-16* plays an important role in immunity (Singh and Aballay, 2009) in worms and both Xiao et al and Chen et al.'s reports describe a requirement for *daf-16* in their pathway. Our results agree with these reports on the slopes of the lifespans of these strains, and the differences we observe are consistent with immunity being more important at lower temperature. The difference between studies are similar to differences between live and UV-killed food experiments (which live longer) (Garigan et al., 2002), and are worth exploring in future studies as they may explain cold-dependent longevity mechanisms of insulin and *trpa-1(ok999)* signaling.

Our results demonstrate that the impact of temperature on relative lifespan is of greater importance than generally appreciated by the *C. elegans* aging field. The vast majority of published studies report the impact of different interventions on lifespan at a single temperature, usually either 20°C or 25°C. We suggest that studies reporting effects on lifespan should typically be performed at more than one temperature in order to understand the robustness of the effect and the interaction with temperature. As further mechanistic studies on the factors that control differences in the relative lifespan vs. temperature axis are completed, we expect that plausible links will be made between temperature-specific longevity in nematodes and specific diseases of aging in mammals.

Figure Legends.

Figure 1. Examples of different types of interactions between genotype, temperature, and lifespan. Panels A-F show survival curves and combined graphs plotting median lifespan vs temperature at 15°, 20°, and 25° for *daf-2* (RNAi), *rhy-1(ok1402), daf-16(mu86), rsks-1(ok1255)*, *cep-1(gk138),* and *cah-4* (RNAi) compared to wild-type (N2). Note that because they are developmentally delayed, *rhy-1* lifespans are shown from L4. All lifespans are available in the supplemental materials (Fig S1-3).

Figure 2. Temperature vs. longevity across genotypes. Panels A-B plot median lifespan vs temperature at 15, 20, and 25°C for opposing genetic conditions in the longevity pathways of hypoxic signaling and antioxidant signaling normalized to wild-type (N2). Panels C-D show the Cox regression-calculated hazard ratios between each condition, separated into UV-killed and RNAi conditions, across temperatures. Panel E depicts a basic model. Significant ($p<0.01$) increased (*) and decreased (**) hazard ratios at 15°C compared to 25°C are denoted.

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Key words: C. elegans; lifespan; neurons; oxidative stress; PTL-1; SKN-1.

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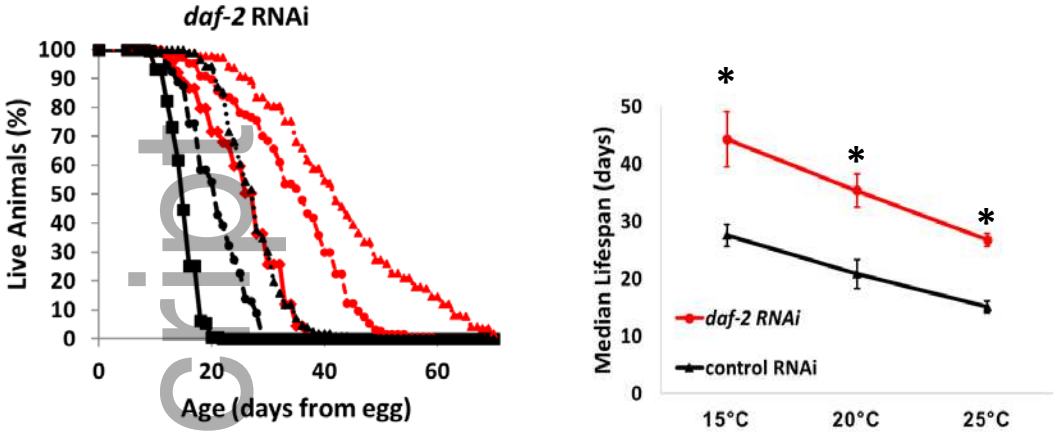
Figure 1.

A



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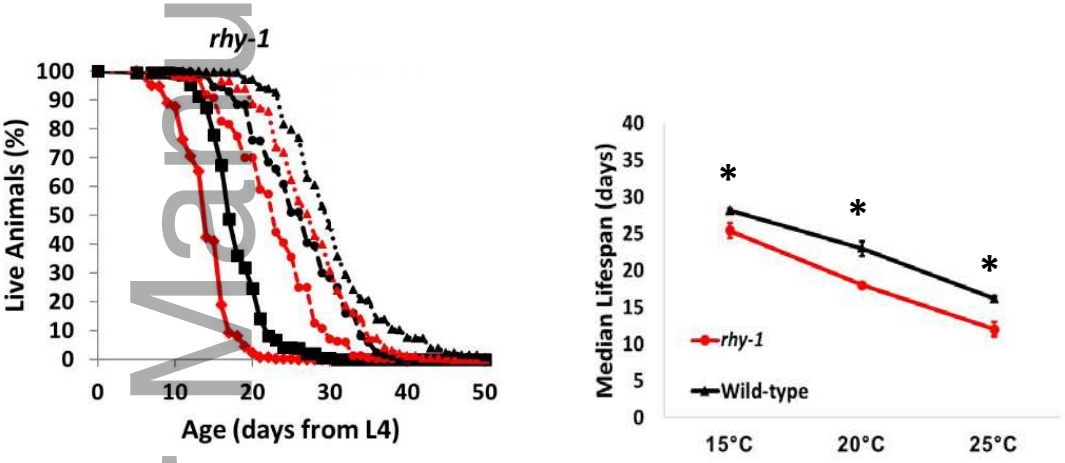

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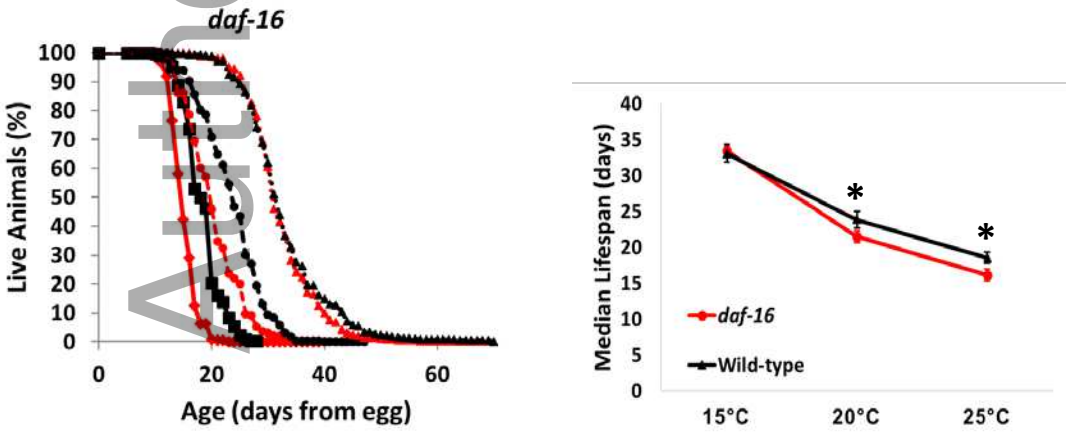
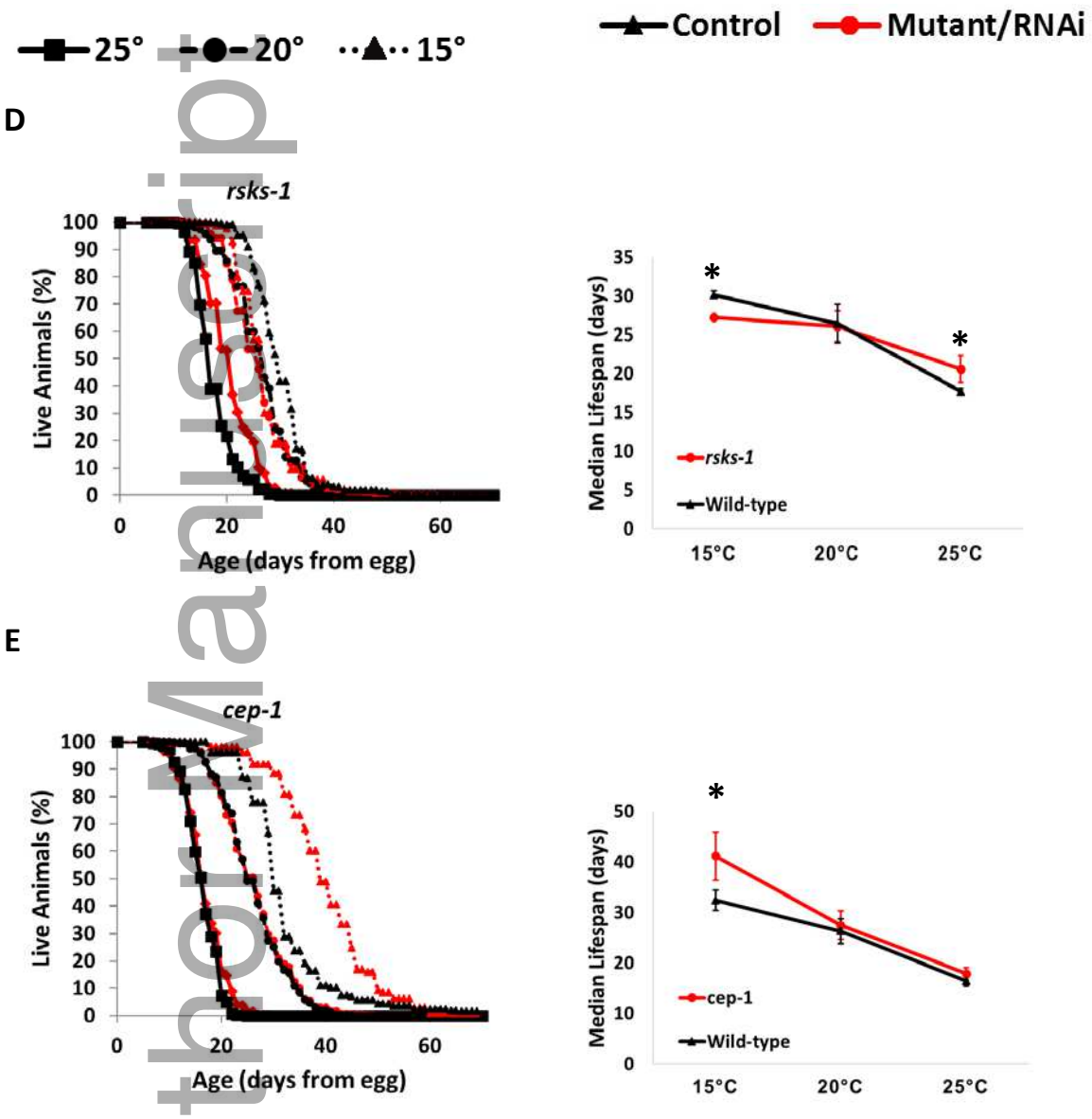




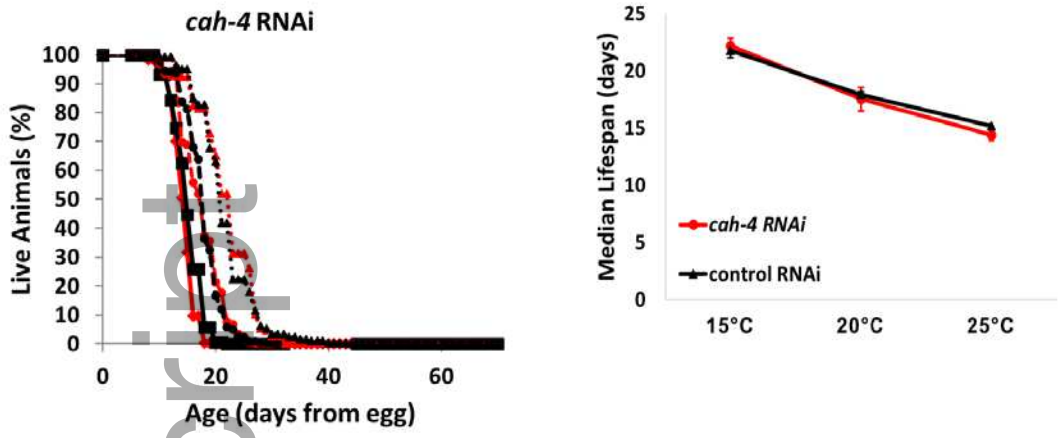
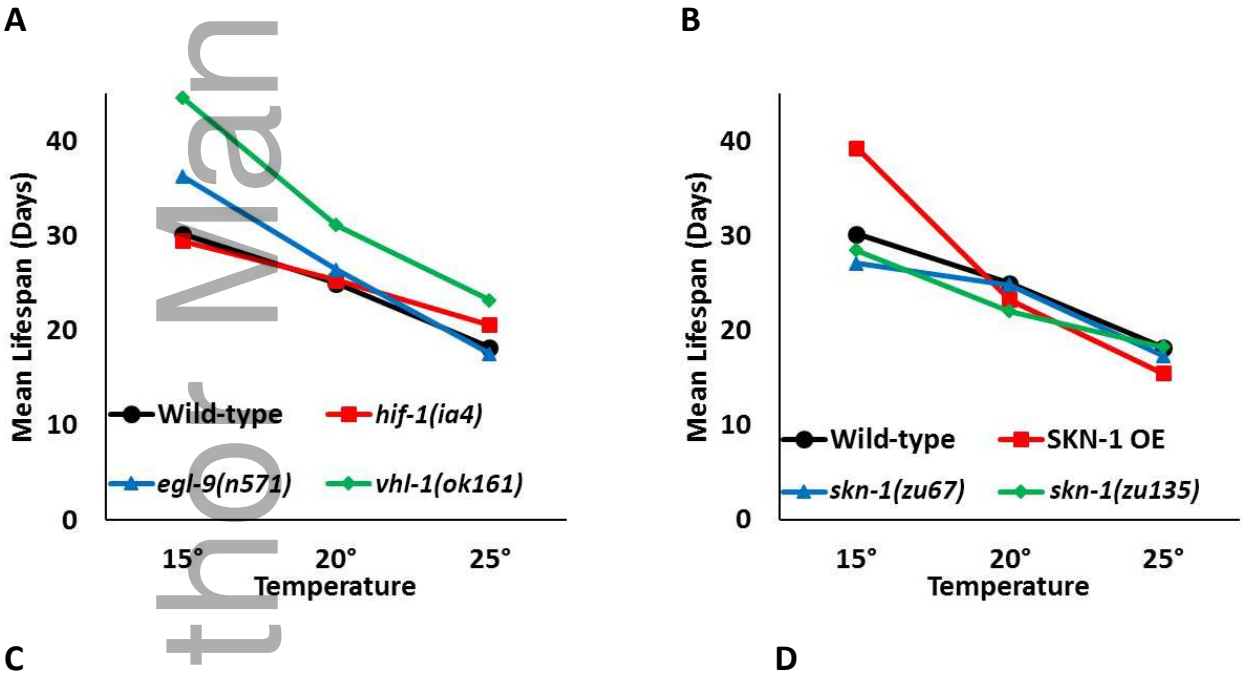
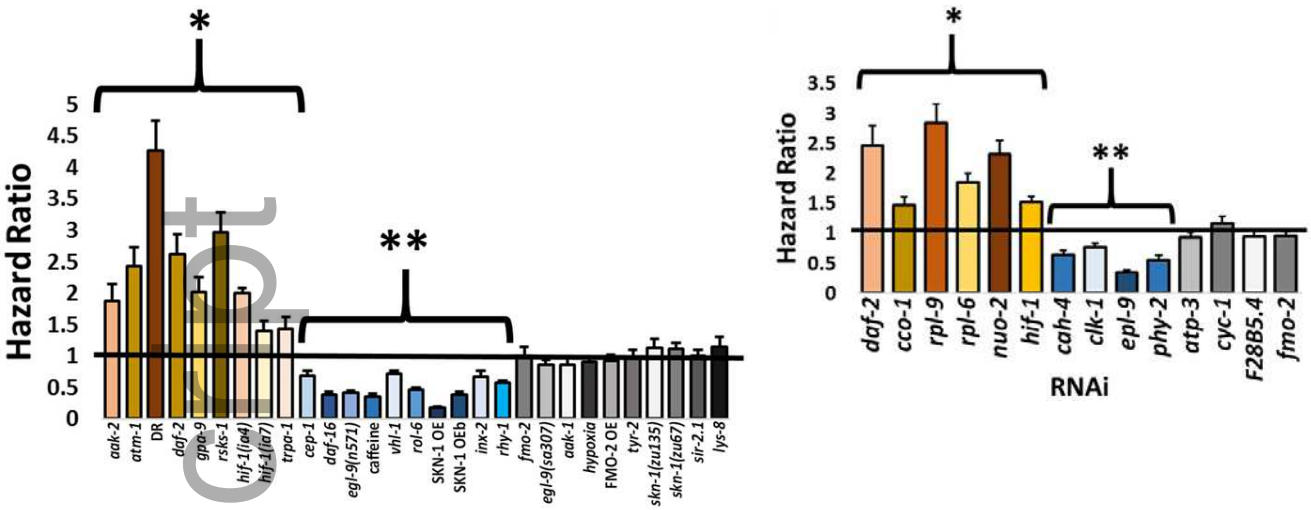


Figure 2.





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