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Hormesis and aging in *Caenorhabditis elegans*

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Abstract

Hormesis has emerged as an important manipulation for the study of aging. Although hormesis is manifested in manifold combinations of stress and model organism, the mechanism of hormesis are only partly understood. The increased stress resistance and extended survival caused by hormesis can be manipulated to further our understanding of the roles of intrinsic and induced stress resistance aging. Genes of the dauer/insulin/insulin-like signaling (IIS) pathway have well-established roles in aging in *C. elegans*. Here we discuss the role of some of those genes in the induced stress resistance and induced life extension attributable to hormesis. Mutations in three genes (*daf-16*, *daf-18*, and *daf-12*) block hormetically-induced life extension. However, of these three, only *daf-18* appears to be required for a full induction of thermotolerance induced by hormesis, illustrating possible separation of the genetic requirements for stress resistance and life extension. Mutations in three other genes of this pathway (*daf-3*, *daf-5* and *age-1*) do not block induced life extension or induced thermotolerance; *daf-5* mutants may be unusually sensitive to hormetic conditions.

1. Introduction

The number of widely-accepted environmental interventions that slow aging is limited. Such interventions can be broadly categorized as caloric restriction, pharmacological interventions and hormetic treatments. When these interventions are completely understood, some overlap of mechanisms will likely be found. Caloric restriction is the most widely studied mechanism of life extension and has been studied extensively in numerous model organisms, especially rodents, but also *Caenorhabditis elegans* (Houthoofd et al., 2003; Klass, 1977; Lakowski and Hekimi, 1998). Another environmental intervention - drug treatments - has also been used to prolong survival in nematodes (Evason et al, 2005; Melov et al, 2000). A third type of intervention – hormesis – has become recognized more recently as a reliable mechanism for extending lifespan, and *C. elegans* is also a reliable model for further investigation of the mechanisms underlying this intervention, (Cypser and Johnson 2002, 2003; Lithgow et al., 1994; 1995).

Hormesis was reported as early as 1887 (for review, see Calabrese et al., 1999a). The term “hormesis was first coined by Southam and Ehrlich (1943), and can be defined as “stimulatory effects of low doses of substances known to be toxic at higher doses”. Hormesis typically stimulates the response 20–60% over the controls. These effects are typically found in response to doses of 20–25% of the minimum toxic dose, and the stimulatory dosages generally have a 10-fold range (Calabrese and Baldwin 1999b). It should be understood that hormesis is not a result of the culling of weak individuals from a population subjected to stress, followed by

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increased survival of those individuals who survived the stress because of intrinsic greater robustness. Experiments using large populations have demonstrated both increased stress resistance (Khazaeli et al., 1997) and increased life expectancy (Khazaeli et al., 1997; Michalski et al., 2001) without significant mortality

Resistance to the seemingly paradoxical effects of hormesis necessitated the extended and exhaustive documentation of the phenomenon, an effort led primarily by Edward Calabrese (Kaiser, 2003). Those efforts produced both practical and theoretical benefits. The practical benefit was an additional set of tools useful in studying survival, following the application of mild stress. The theoretical aspect was further support for the inverse correlation observed between stress resistance generally and improved survival. This review will focus on the effects of hormesis upon subsequent stress resistance and life span and also explore how the IIS pathway, known to modulate aging in the worm, also modulates hormesis.

2. The breadth and reality of hormesis

Hormetic effects have been documented in diverse combinations of stressors and recipient organism. Such sub-lethal stress pretreatments have been shown to increase germination growth in plants, cell proliferation in culture, protein turnover, subsequent stress resistance, and life expectancy. Stressors reported to increase subsequent stress resistance include heat, cold, hypergravity and pesticides. Increased life span has been reported after a similarly diverse number of hormetic treatments including heat, cold, hypergravity, ionizing radiation; exercise, electric shock, and wounding accompanied by regrowth (Martinez, 1996; Minois, 2000). Some of the earliest work in *C. elegans* by Lithgow et al (1994; 1995) demonstrated hormesis in response to sub-lethal heat treatment, affecting both subsequent heat resistance as well as longevity. We have expanded on that work to demonstrate the close relationship between the amount of exposure to a toxin and the level of induced stress resistance or induced life extension in *C. elegans* (Cypser and Johnson, 2002; Figure 1).

2.1. The basis of hormesis

The biological mechanisms underlying hormesis are far from clear. The hormetic response may require transcription, translation, and/or post-translational protein modification, such as phosphorylation. Calabrese and Baldwin (2002) have suggested that hormesis is best defined by a description of dose and response, and that "…no single hormetic mechanism is expected, but a common evolutionary-based homeostasis maintenance regulatory strategy is evident."

The same authors point out that a given hormetic response may reflect one of two forms, either a direct stimulation or an overcompensation response. Minois (2000) has proposed an alternative description wherein hormesis is seen as a consequence of metabolic regulation coupled to the expression of stress response proteins. These two views are not mutually exclusive; however a full discussion of the molecular nature of hormesis is beyond the scope of this review.

2.2. Dietary restriction as hormesis

The best-documented and most powerful non-genetic intervention known to extend life span, in a broad variety of species, is caloric restriction (McCay 1935; Weindruch and Walford 1988; Yu 1994; for review, see Weindruch 1996). Johnson et al. (1996) and Masoro (1998, 2000) have suggested that caloric restriction may be a form of hormesis. Animals restricted to a balanced diet, containing as little as 60% of the calories they would consume *ad libitum*, have life expectancies up to 50% greater than *ad libitum* controls. This argument requires that caloric restriction be considered a stressor, and in fact caloric restriction does promote the expression of stress response factors, including corticosterone, glucocorticoids (Masoro 1995) and heat

shock proteins (Aly et al., 1994; Heydari et al., 1996; Moore et al., 1998). Caloric restriction can be induced in the worm in multiple ways. Klass (1977) demonstrated caloric restriction in response to reduced food concentration. The Eat mutations in *C. elegans* produce physiological defects which slow feeding, and also display increased lifespan (Lakowski and Hekimi 1998) and thermotolerance (Johnson et al., 2001). Houthoofd et al. (2003) have shown that axenic medium can extend life span about three-fold independently of *daf-16*. Indeed when this form of caloric restriction is used to treat a *daf-2* mutant, an eight-fold extension in life can be achieved (Houthoofd et al., 2004).

Complete caloric deprivation – that is, starvation - can also serve as a hormetic treatment to extend life span of *C. elegans*. Young adult worms deprived of all food for 1 – 3 days display an increase in mean life span of 30 – 40%. Interestingly, the relative size of this effect appears to be reduced in animals carrying a mutation in *age-1*. It may be that the *age-1* mutants have nearly maximized the effect, rendering sizable further increases in life span unattainable by the same mechanism (Figure 2). Additionally, a mutation in *daf-16* appears to reduce this effect (Tedesco and Johnson, unpublished).

However, Neafsey (1990) has concluded that mortality analysis of caloric restriction and other forms of hormesis indicate that they are different entities. In addition, the nematode Eat mutant which displays the greatest increase in life span [*eat-2(ad465)*] can have its intrinsic resistance to heat increased even further by a hormetic heat pretreatment (Figure 3). Although it is not clear that the effects of both caloric restriction and heat pre-treatment have been maximized, these results are consistent with the two effects being qualitatively different. Whether these (presumably) additive effects for thermotolerance translate into increased life expectancy has not been tested. Taken together, these results indicate that caloric restriction may be a hormetic phenomenon in the broad sense, but the contributions of caloric restriction and other sub-lethal stress pretreatments may contribute independently to increased stress resistance and life expectancy.

3. The correlation between stress resistance and longevity

There is a strong correlation between increased stress resistance and life span extension among various mutants representing diverse species, including yeast, the nematode, the fly and the mouse (Finkel and Holbrook 2000). Numerous examples of the correlation between increased stress resistance and life span extension are known from *C. elegans* (Johnson *et al*., 2001). Furthermore, this correlation has been found in strains derived by selective breeding, in animals carrying single-gene mutations (Johnson et al, 2001), in transgenic animals, and in response to environmental interventions. However, while stress resistance appears to be necessary for life span extension, it is not sufficient; *C. elegans* carrying the *daf-4* or *daf-7* mutations are thermotolerant but not long-lived (Lithgow et al. 1995).

4. Genetics of induced thermotolerance in *C. elegans*

The IIS pathway mediates both life span and intrinsic stress resistance in *C. elegans*. Mutations in *age-1* or *daf-2* increase both life expectancy (Friedman and Johnson 1988a, b; Kenyon et al., 1993) and stress resistance (Barsyte et al., 2001; Larsen 1993; Lithgow et al., 1994, 1995; Murakami and Johnson, 1996; Vanfleteren 1993), and mutations in *daf-16* or *daf-18* suppress both the increased life span (Dorman et al., 1995; Kenyon et al., 1993; Larsen *et al*., 1995; Mihaylova et al., 1999) and the increased stress resistance (Barsyte et al., 2001; Murakami and Johnson 1996;). Wild-type animals display greater life span (Butov et al., 2001; Lithgow et al., 1995; Michalski et al., 2001; Yashin et al., 2001; 2002) and thermotolerance (Cypser and Johnson, 2002) in response to pretreatment with sub-lethal heat stress.

Given the well-established correlation between stress resistance and longevity and the importance of the IIS response pathway to *intrinsic* longevity, we wondered whether the same pathway might also modulate hormetic responses. We asked whether this pathway was required for heat-induced stress resistance, and heat-induced life extension attributable to hormesis.

4.1. Genetics of induced thermotolerance in *C. elegans*

We have found that several mutants of the IIS pathway display increased thermotolerance in response to pretreatment with sub-lethal doses of heat. Several dauer-defective mutants that interfere with signaling (*daf-3(e1376), daf-5(e1386), daf-12(m20), daf-16(m26), daf-16 (m27)* and *daf-16(mgDf50)*) were tested and these mutants all displayed induced thermotolerance similar to that of the wild-type (*daf-5* mutants displayed a significantly greater induced thermotolerance than did wild-type controls). In contrast, we found that although *daf-18(e1375) and daf-18(nr2037)* mutants do significantly increase their thermotolerance, the induced thermotolerance is still significantly less than that displayed by pretreated wild-type animals (Cypser 2002).

4.2. Genetics of induced life extension in *C. elegans*

We tested the effects of mutations in *daf-3*, *daf-5*, *daf-12*, *daf-16*, *daf-18*, and *age-1* on hormetic life extension caused by sub-lethal heat treatment. We found that three *Daf*-d mutants, *daf-12 (m20)*, *daf-16(m27)*, and *daf-18(nr2037)*, lack the ability to increase their life expectancy in response to this pretreatment (Cypser and Johnson, 2003). *daf-5* and *age-1* mutants (and *daf-3* mutants, although the evidence is not so robust) show induced life extension. *age-1* mutants overall display a significant increase in life span after heat pretreatment beyond that conferred by the *age-1* mutation. The *daf-5* mutant we tested was significantly shorter-lived than wild-type controls; however, it is striking that pretreatment with the conditions known to extend the life span of the wild type compensated for this defect.

5. Discussion

We conclude that genes of the IIS pathway are required for hormetic effects in *C. elegans* (Figure 4). *daf-18* is required for both hormetic life extension as well as hormetic thermotolerance. In spite of having no effect on induced thermotolerance, the *daf-12* and *daf-16* genes appear to be required for the hormetic life extension seen in response to the same conditions of heat pretreatment that induces thermotolerance. (In an interesting contrast, Fisher and Lithgow (2006) have demonstrated an allele-specific role for *daf-12* in *intrinsic* thermotolerance) Thus some separation of the genetic requirements for stress resistance and life extension is evident.

The relationship between stress resistance and life expectancy provides an important frame of context for the study of aging, and hormesis provides an important tool to disentangle improved survival from secondary phenotypes. The worm is an excellent model of induced stress resistance and induced life extension, because hormesis has been well-characterized in *C. elegans*. Indeed, Rea et al (2005) have shown that level of expression of *hsp-16* predicts both thermotolerance and survival of individual worms subsequent to hormetic conditions. Further investigations of hormetic phenotypes at the molecular and physiological level should contribute to our understanding of aging and the life-history tradeoffs that affect aging.

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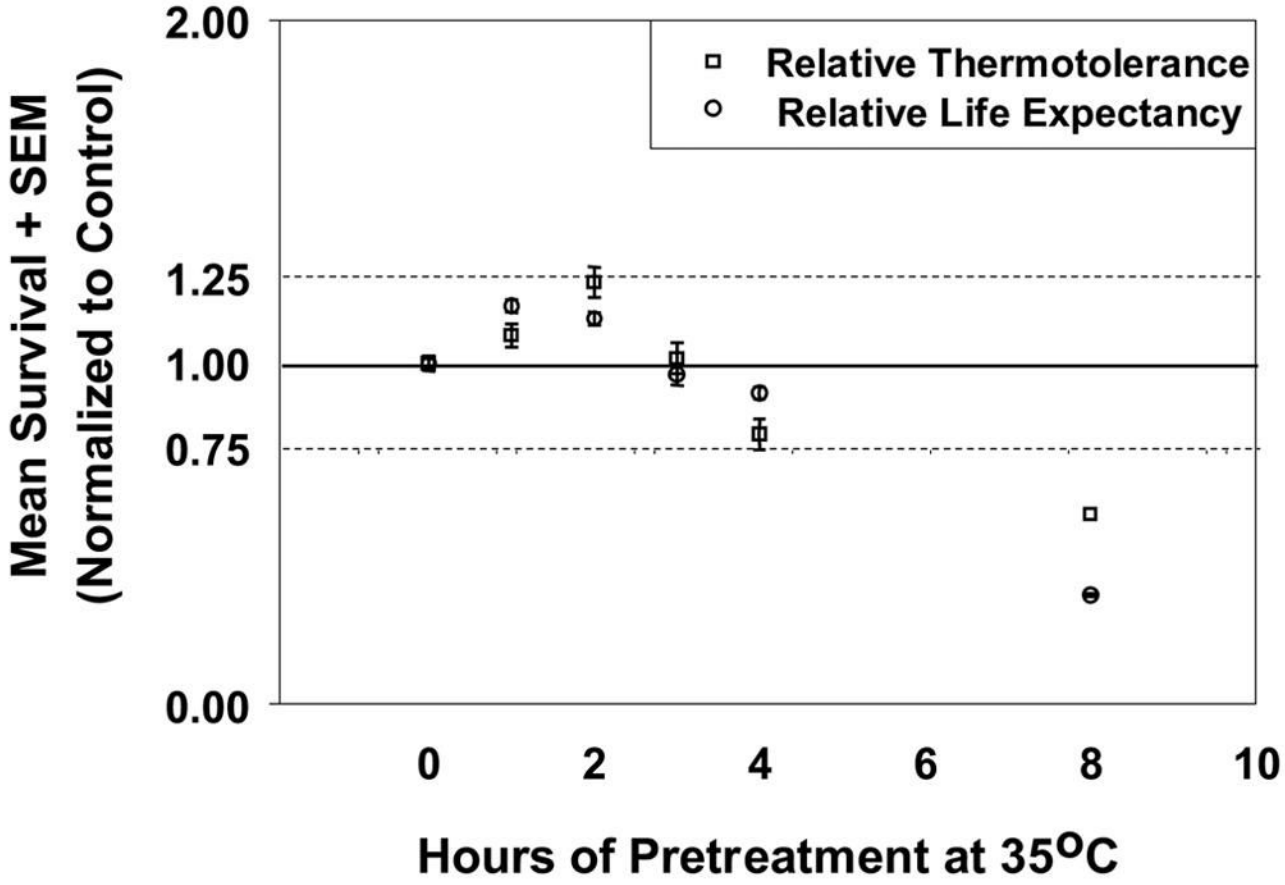

Figure 1.
Hormetic heat treatments induce both increased thermotolerance and improved survival. The similarity of the two dose-response curves is consistent with a relationship between stress resistance and aging. (Modified from Cypser and Johnson, 2002).

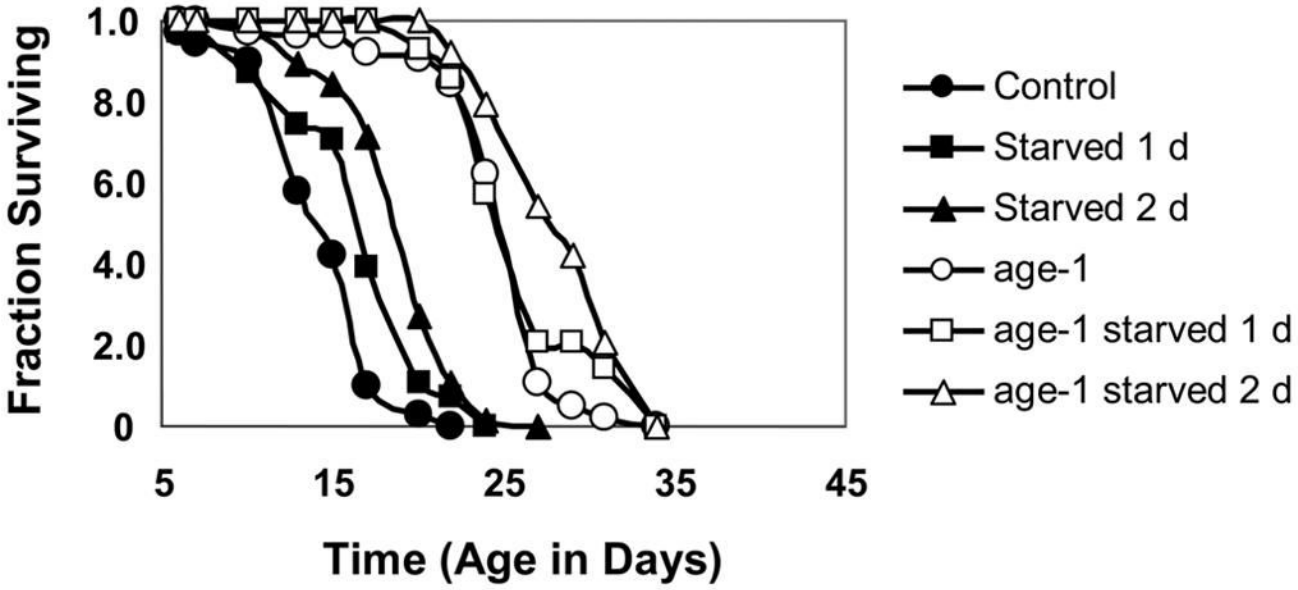

Figure 2.

Short-term starvation extends lifespan in *C. elegans*. *age-1* mutants display a relatively smaller effect size. Animals in experimental groups were transferred to liquid medium (S Basal) containing no food for 1 day or two days, then returned to survival medium (Johnson and Wood, 1982) for the duration of survival. Mean ± SEM (days) and p-value (log-rank) compared to unstarved control: For N2 (unstarved), 14.8 ± 0.3, NA; N2 (starved 1 day), 16.9 ± 0.4, $p < 0.001$; N2 (starved 2 days): 19.3 ± 0.3, $p < 0.0001$. For *age-1* (unstarved), 25.3 ± 0.4, NA; *age-1* (starved 1 day), 26.5 ± 0.4, p not significant; *age-1* (starved 2 days), 28.8 ± 0.4, $p < 0.0001$.

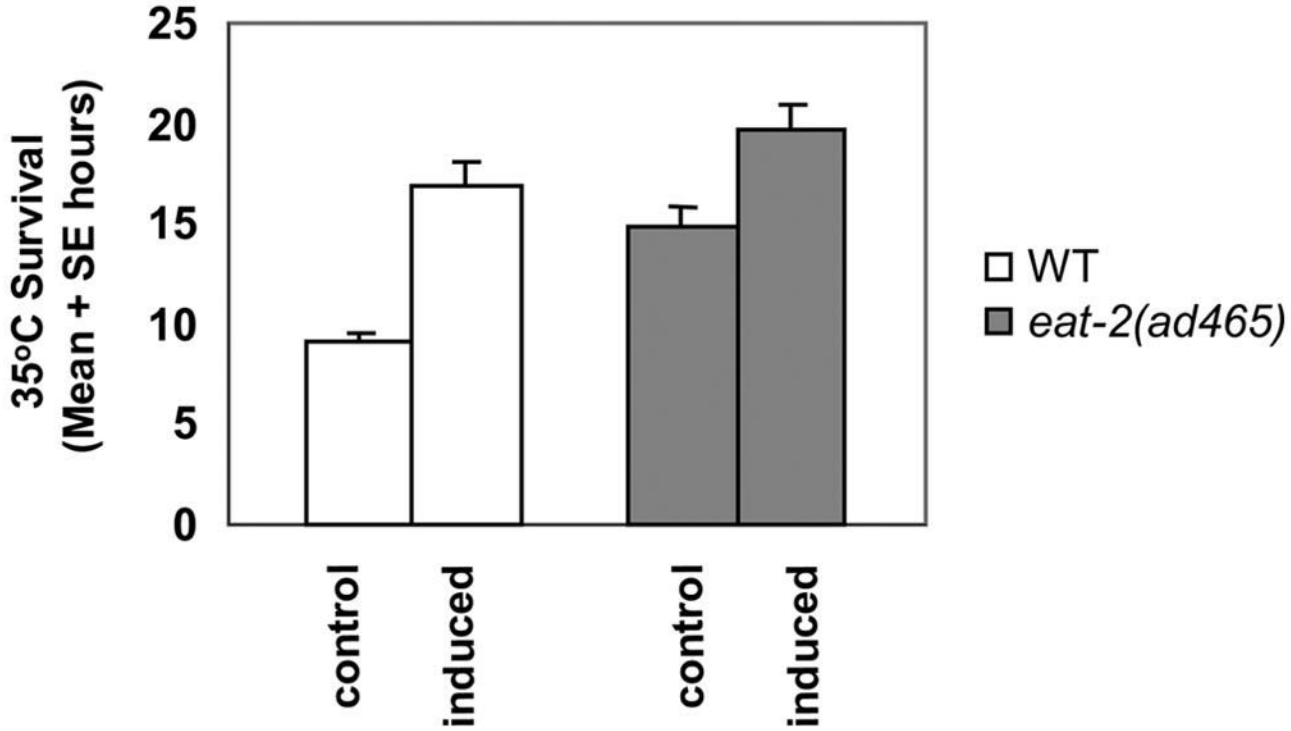

Figure 3.
Relative benefits derived from hormesis. Mean ± SEM (hours) and p-value by t-test from comparison of naïve and heat-pretreated animals (for N2): naive, 9.1 ± 0.4; pretreated, 16.9 ± 1.1, $p < 0.0001$, (for *eat-2[ad465]*): naive, 14.8 ± 0.8, pretreated, 19.8 ± 1.1, $p < 0.001$.

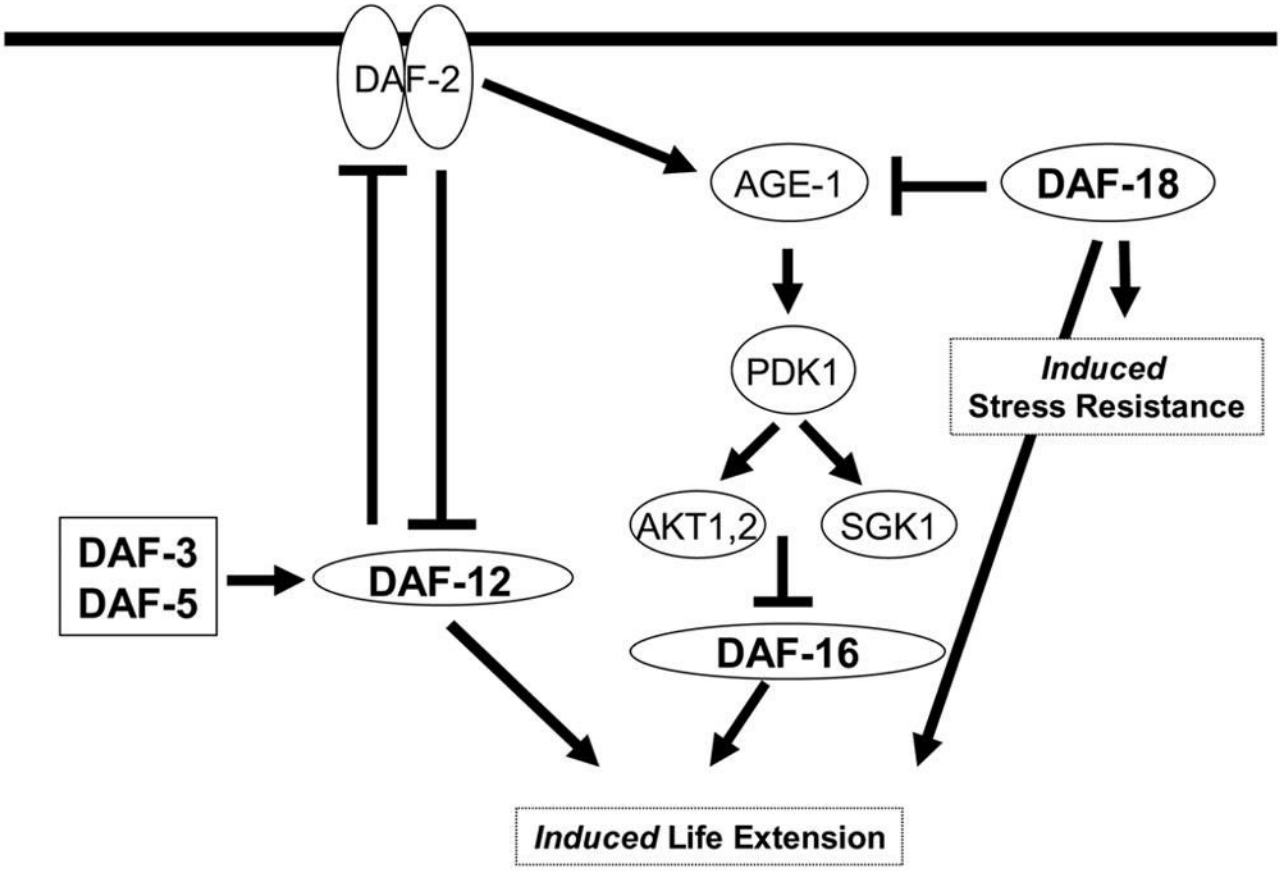

Figure 4.
Genes of the insulin/IGF-2 signaling pathway are required for induced stress resistance and induced life extension. *daf-18* appears to be required for heat-induced thermotolerance independently of *daf-16*. However, the *daf-12*, *daf-16* and *daf-18* mutants tested were all found to be defective for life extension induced by heat pretreatment. (Modified from Cypser and Johnson, 2003).