

PI3-kinase inhibition induces dauer formation, thermotolerance and longevity in *C. elegans*[☆]

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Abstract

The effects of 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), an inhibitor of mammalian phosphatidylinositol 3-OH kinase, was tested on an insulin signaling-like pathway in the nematode *Caenorhabditis elegans*. Populations of *C. elegans* were treated with LY294002 at different stages of the life cycle, and its effects on development, thermotolerance and longevity were assessed. At concentrations of 160 μ M and above, LY294002 significantly induced both dauer formation and thermotolerance. Treatment of adult worms also resulted in a small, but significant, increase in life span. The results presented are consistent with the view that a neuroendocrine signaling pathway functions in adult worms to determine stress resistance and longevity. © 1999 Elsevier Science Inc. All rights reserved.

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1. Introduction

Caenorhabditis elegans is a comprehensively studied, free-living nematode that has proved to be an excellent system for studies in neuroscience and aging [35]. This species' influence on the field of aging stems from the existence of a series of single-gene mutations that greatly extend its life span (the Age phenotype) [5,6,13,17,19,27,47] and reduce the acceleration of mortality rate normally associated with chronological age [10].

C. elegans has a 3-day life cycle with four larval stages (L1–L4) before its final molt into a reproducing adult. Adults normally live for ~20 days in the laboratory when cultured in the presence of a bacterial food source [11]. Populations of *C. elegans* exist primarily as self-fertilising hermaphrodites, with males occurring at a ratio of ~1:500.

Mutations that extend *C. elegans* life span (Age mutations) tend to be pleiotropic, effecting its development and early-life traits [22]. Many of the proposed mechanisms of

life span extension by Age mutations come from studies of these associated phenotypes. A major class of Age mutations are known also to regulate the formation of a specialized larval diapause stage called the dauer [13,19,27]. Dauer larvae develop from L1 worms during nutritional deprivation in response to a neuroendocrine signal [34]. The dauer larva is nonfeeding, nonreproducing, stress resistant, and lives four to eight times longer than the adult [16]. The environmental cues for dauer development are the absence of bacteria and the presence of a pheromone produced by adult and larval worms. Consequently, dauer formation is dependent on the nutritional status of the immediate environment and the density of the worm populations. Dauer formation depends on the integrity of a set of neurons in the chemosensory amphids on either side of the head. In good nutritional conditions, three neurons (ADF, ASG, ASI) act to prevent dauer development, and a further neuron (ASJ) signals the exit from dauer [34] when starved dauers are fed.

A network of over 30 genes control dauer formation (*daf* genes). These genes fall into partially redundant pathways, and mutations are of two types: those that confer dauer formation even in good nutritional conditions (Dauer formation-constitutive, or *Daf-c*) and those that result in the failure to form normal dauers (Dauer formation-defective, or *Daf-d*). Genetic analysis has indicated that a number of *Daf* genes also influence the life span of the reproducing

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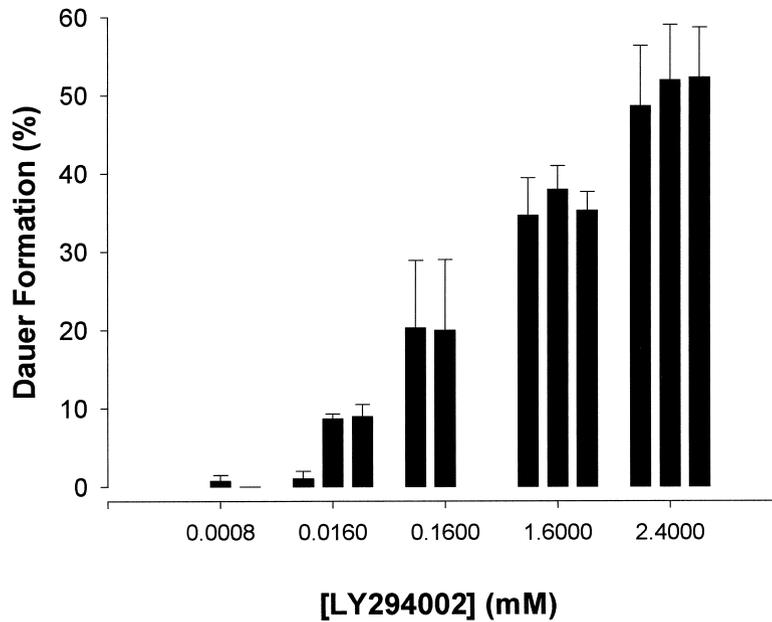


Fig. 1. Effect of LY294002 on dauer formation. The percentage of worms that formed dauer larvae (\pm SEM) is plotted for each of the concentrations of LY294002 ($n > 50$ for each experiment.). Each bar for each drug concentration represents a replicate experiment.

adult stage: loss-of-function mutations of either *daf-2*, which encodes a protein similar to vertebrate insulin-receptor [15], or *age-1*, which encodes a potential catalytic subunit of phosphatidylinositol-3-kinase (PI3K) [29], extending life span by up to 100% [6,13]. Loss-of-function mutations of *daf-16*, which encodes a forkhead transcription factor-like protein [21,32], suppresses both the Age phenotype and the Daf-c phenotypes of *age-1* and *daf-2* mutations [13,19,27]. Additionally, genes encoding homologues of mammalian Akt/PKB proteins act between *age-1* and *daf-16* [33]. In sum, an insulin signaling-like pathway is necessary for the normal development and life span of *C. elegans* [33]. At least one other tyrosine kinase receptor may also influence the life span through interactions with this pathway [31].

The cell and tissue specificity of the action of the insulin signaling-like pathway has been investigated by using a series of *daf-2* genetic mosaics [1]. It appears that *daf-2* may function in many cell types, but activity in neurons and support cells is sufficient to result in normal development and a normal life span.

Mutations that extend the life span, including mutations not in the insulin-signaling pathway, are known to also confer resistance to various environmental insults [7,18,25,26,30,44]. We have hypothesised that over expression of stress response genes, including molecular chaperone genes, would confer extended life span by slowing the accumulation of age-related macromolecular damage [23,26]. Here, in the current study, we investigated the use of a chemical inhibitor of PI3K activity to directly test the hypothesis that the insulin-signaling pathway is active, not just during development but in adult worms, as a repressor of stress

resistance and longevity. We have found that the inhibitor acts on first stage larvae to induce dauer formation. Further, we demonstrated that treatment of young adult worms results in thermotolerance and a small, but significant, life span extension.

2. Methods

2.1. Nematode strains, growth, and maintenance

The Bristol N2 (wild-type) strain and TJ1052[*age-1(hx546)II*] were obtained from the *Caenorhabditis* Genetic Center at the University of Minnesota. Worms were maintained on nematode growth medium (NGM) [42] agar plates

Table 1
Effect of LY294002 on intrinsic thermotolerance

Treatment	Mean survival \pm SD (min)	<i>p</i> -value ^a
Wild-type control	315 \pm 59	
Wild-type + 0.8 μ M	389 \pm 10	0.01
Wild-type + 16 μ M	377 \pm 11	0.01
<i>age-1(hx546)</i> control	466 \pm 92	0.0001
Wild-type control	261 \pm 65	
Wild-type + 163 μ M	303 \pm 89	0.001
<i>age-1(hx546)</i> control	400 \pm 93	0.0001
Wild-type control	268 \pm 71	
Wild-type + 1.6 mM	328 \pm 85	0.001
<i>age-1(hx546)</i> control	422 \pm 82	0.0001
Wild-type control	276 \pm 75	
Wild-type + 2.4 mM	326 \pm 94	0.001
<i>age-1(hx546)</i> control	425 \pm 89	0.0001

^a *p*-value based on Willcoxon (Gehan) statistic [20].

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