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Curcumin Extends Life Span, Improves Health Span, and Modulates the Expression of Age-Associated Aging Genes in *Drosophila melanogaster*

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Abstract

Background: Curcumin, an extract from the rhizome of the plant *Curcuma longa* (turmeric), has been widely used as a spice and herbal medicine in Asia. It has been suggested to have many biological activities, such as antioxidative, antiinflammatory, anticancer, chemopreventive, and antineurodegenerative properties. We evaluated the impact of curcumin on life span, fecundity, feeding rate, oxidative stress, locomotion, and gene expression in two different wild-type *Drosophila melanogaster* strains, Canton-S and Ives, under two different experimental conditions.

Results: We report that curcumin extended the life span of two different strains of *D. melanogaster*, an effect that was accompanied by protection against oxidative stress, improvement in locomotion, and chemopreventive effects. Life span extension was gender and genotype specific. Curcumin also modulated the expression of several aging-related genes, including *mth*, *thor*, *InR*, and JNK.

Conclusions: The observed positive effects of curcumin on life span and health span in two different *D. melanogaster* strains demonstrate a potential applicability of curcumin treatment in mammals. The ability of curcumin to mitigate the expression levels of age-associated genes in young flies suggests that the action of curcumin on these genes is a cause, rather than an effect, of its life span–extending effects.

Introduction

Over the last few decades, studies on aging have successfully identified genes and signaling pathways that regulate life span in model organisms.[1] These studies have significantly extended our understanding of the aging process at a molecular genetic level. Genes that can extend lifespan when mutated are often called longevity assurance genes (LAGs).[2] In *Drosophila melanogaster*, several LAGs have been identified, including *insulin receptor* (*InR*),[3] *methuselah* (*mth*),[4] EcR,[5] Indy,[6] superoxide dismutase (SOD) and Catalase,[7] *heat shock protein 70* (*hsp70*),[8] peptide methionine sulfoxide reductase (PMSR),[9] c-jun N-terminal kinase (JNK),[10] and sir2 and p53.[11,12] Although the manipulations of such genes may not be applied to humans due to ethical reasons and technical difficulties, the revealed genetic pathways give us clues for pharmacological interventions to postpone or slow the aging process. Several pharmacological interventions have been shown to increase the life span of *D. melanogaster* and *Caenorhabditis elegans*.[13–15] Inhibition of the target of rapamycin (TOR) signaling pathway by genetic or pharmacological intervention extends life span both in nematodes and flies.[16,17] Recently, Harrison et al. provided evidence that pharmacological intervention of TOR signaling in the aging process is reproducible in a mammalian model.[18] There are numerous published reports regarding antiaging compounds, but many of these reports lack the evaluation of secondary physiological confounds that could have resulted in artificial antiaging effects.[19]

Curcumin, a yellow pigment extracted from the rhizome of the plant *Curcuma longa* (turmeric), has been widely used as a spice, food additive, and herbal medicine in Asia. It is of

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particular interest because it has various biological activities, such as antioxidative, antiinflammatory, anticancer, and antineurodegenerative.[20,21] With its powerful antioxidant and antiinflammatory activities, which may counteract the main causes of aging, curcumin has been considered as a potential antiaging compound.[22] The only published report on the impact of curcumin on life span reported that mice fed a diet containing tetrahydrocurcumin (a more potent biometabolite of curcumin) had significantly longer average life spans than control mice. However, this study was confounded by a potential caloric restriction effect as measured by weight loss. Mice fed on a diet containing tetrahydrocurcumin significantly reduced their food consumption, which could have then led indirectly to an increase in life span as a "phenocopy" of caloric restriction.[23] To date, no study has systematically evaluated the effects of curcumin on the life span of model animals and controlled for a number of physiological confounds of aging.

In this study, using two different strains of *Drosophila*, Canton-S and Ives flies, we evaluated the impact of curcumin on life span and health span. Our study has a number of unique features, because reproducibility of life span studies is a major concern in aging research. We are the first to report that curcumin extended the life span of two different wild-type *D. melanogaster* strains under two different experimental conditions without compromising health span. In addition, we were able to show that curcumin modulated the expression of several LAGs, including *mth*, *InR*, and JNK.

Materials and Methods

Drosophila melanogaster populations and curcumin supplementation

Experiments were performed in two different countries under different experimental conditions using two different strains of flies, wild-type Canton-S flies in Korea, and the Ives flies ("IV") from Amherst, Massachusetts. Life span, stress resistance, and fecundity assays were performed using both strains of flies. In Korea, stock solutions of curcumin from Agros (Geel, Belgium) were prepared and added to the fly food. All feeding assays were performed in 500 mL cages under a 12-h light/dark cycle. In the United States, curcumin powder from Sigma-Aldrich was mixed with the yeast solution that was added on the top of 1 mL of food in each vial under constant illumination. In both countries, assays were performed in 50% humidity and 25°C.

In Korea, adult sucrose-yeast diets were prepared with 10 g sucrose, 10 g yeast, 1.1 mL of 20% tegosept (wt/vol in ethanol), and 0.79 g agar per 100 mL in water. Different concentrations of stock solutions of curcumin were made in ethanol. After boiling, medium was cooled to 60°C, and curcumin stock solution, or ethanol alone, was added by a volume of 19:1. Next, 5 mL of medium was distributed into 10-×75-mm shell vials, stored at 4°C, and used within 2–3 weeks. Larvae of the Canton-S strain were grown on standard CSY medium (5.2 g cornmeal, 11 g sucrose, 11 g nutritional yeast [MP Biomedicals, Solon, OH], 1.1 mL of 20% tegosept, 0.79 g agar per 100 mL in water) supplemented with several grains of live yeast.[24] In the United States, stocks were raised from larvae in controlled densities of 50–80 eggs per 8-dram vial with 5 mL of standard banana–molasses food and were kept at 25°C with constant illumination. Curcumin was supplemented to adults only and was mixed with yeast solution (50 mL DI H_2O, 2.0 g yeast, 2.0 mL 1% acetic acid) to obtain the desired concentration. The control groups contained only yeast solution.

Life span assays

Newly eclosed Canton-S adult flies were collected over 48 h and were randomly assigned to 500-mL demography cages to the final density of 80 females and 80 males per cage. Food vials at 0, 50, 100, 500, and 1,000 μM concentrations were affixed to separate cages and changed every 2–3 days, at which time dead flies were removed and recorded. These doses were selected after performing a number of preliminary dose-finding studies. Three replicate cages were established for the each curcumin dose.

In the United States, to determine the optimal dosage to be used in the life span assay, the impact of curcumin on mortality was evaluated using four different concentrations of curcumin (0, 125, 250, 500 μM). The duration of the mortality assays were only 4 weeks because in Ives flies this period is considered aging phase,[25] and a major increase in mortality occurs at week 4. Compounds that decrease mortality during these 4 weeks appear to have a positive impact on life span.[15] Adults were collected upon 24 h of emergence and separated into vials. For each dose, a population size of 320 flies per sex was tested. Each vial contained 4 males and 4 females, with a total of 80 vials per dose. Flies were transferred to new vials containing 1 mL standard banana food and 75 μL yeast–drug solution every 2 days. The number and sex of dead flies were recorded during each transfer. The density of each vial was maintained at 6–10 flies with approximately even numbers of males and females as the population decreased. When comparing flies from different treatments, all preliminary rearing was carried out in parallel. Because curcumin was observed to have a beneficial effect on mortality at 250 μM for both sexes, the life span assay was conducted only with this dose. A concentration of 250 μM of curcumin was administered to 200 flies of each sex and compared to the control group with the same size population. Flies were collected from the stock and set up in vials no more than 24 h after emergence. Each vial contained 5 males and 5 females, with a total of 40 vials per dose. Food and drug–yeast solutions were prepared the same way as the mortality assay, mentioned above. Flies were transferred to new vials every 2 days until no survivors remained. The number and sex of dead flies were recorded at each transfer.

Fecundity assay

Within the first 24 h of emergence, adult flies were collected and each vial was set up with a density of 1 female and 2 males in Korea and 1 female and 1 male in the United States. Each female was allotted exactly 24 h to lay eggs. Flies were transferred to new vials daily, and fecundity (number of eggs laid by each female) was evaluated for 10 days.

In Korea, each vial contained sucrose–yeast media mixed either with ethanol (0 μM) or curcumin at 100 μM. A total of 10 vials were tested per treatment. In the United States, each vial contained sucrose-based charcoal food and 75 μL of 0.00, 125, 250, or 500 μM curcumin–yeast solution. A total of 80 vials were tested per dose.

Feeding assay

In Korea, newly eclosed adult flies were kept in the cages with food vials containing sucrose–yeast diets with curcumin and without curcumin. To perform a feeding assay, 30 female flies were transferred into the vials containing sucrose–yeast diets with bromophenol blue dye (0.05% wt/vol) or sucrose–yeast diets with the dye and curcumin. After 10 min of feeding, the fed flies were anesthetized, washed with phosphate-buffered saline (PBS), and homogenized in 1 mL of distilled water. The absorbance of the 100 times diluted homogenate was measured at 595 nm by a spectrophotometer.[26]

Stress resistance assay

The pretreatment feeding portion of the oxidative stress assay with Ives flies was performed similar to the life span assay mentioned above, but flies were only treated for 14 days. A population size of 800 flies of each sex were exposed to either yeast or curcumin–yeast solution. On day 14, 4 males and 4 females were placed in a new vial containing one 2.5-cm diameter paper towel circle with 300 μL of 5% sucrose solution containing one of the free radical generators: hydrogen peroxide, paraquat, and ferric nitrilotriacetate (Fe-NTA). The control and curcumin-pretreated groups were exposed to each of the three free radical generators. The number of deaths was recorded every 4 h until all flies had died, or 36 h had elapsed since exposure to free radical generators began.

Locomotion

We tested spontaneous locomotion and vertical climbing ability using the Drosophila Activity Monitor DAM2 (Trikinetics, MA). The flies were fed curcumin as described above for life span assays. Spontaneous locomotion was measured at weeks 1, 2, 3, 4, and 5. Vertical climbing ability was measured at week 1 and week 5. At each weekly interval, the flies were lightly anesthetized for the purpose of sex classification and to be transferred into 5-mm tubes needed for the DAM2 system. To avoid CO_2-dependent reductions in locomotor activity, all flies were give a 60-min acclimation period to recover and to acclimate to their new environment. Data were collected at 1-min intervals for 120 min, resulting in a total of 120 reading per fly. The paired *t*-test was used to determine significant differences between the control and curcumin-fed groups.

Chemoprevention assay

Within the first 24 h of emergence, adult Ives flies were collected and 4 males and 4 females were placed in each vial with standard banana food and 75 μL of 250 μM curcumin–yeast mixture. Another 80 vials were prepared the same way, but with only yeast. The flies were transferred to new vials every day for 4 days. After 4 days of pretreatment with curcumin or yeast only, each female was transferred to its own vial with one male, and supplemented with MTX at 0, 3, 6, or 12 mg/mL. Vials were changed daily, and fecundity was evaluated every day for 4 days. Finally, ovaries were dissected on day 4 prior to MTX exposure, and also on the last day of MTX treatment (day 8). Pictures were taken of the ovaries by microscopy to observe and evaluate the differences between curcumin pretreatment and no pretreatment.

Microarray analysis

Fifty male and 50 female Canton-S flies were collected from the control group and the 100 μM curcumin-fed group at two different ages—3 days old and 40 days old. Total RNA was extracted from each sample using TRIzol (Invitrogen) according to the manufacturer's protocol. Synthesis of cDNA and biotin-labeled cRNA, fragmentation, hybridization, washing, and scanning were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual (2005). Biotinylated cRNA probes were hybridized to the high-density *Drosophila* GeneChip 2.0 oligonucleotide microarrays (Affymetrix, Inc., Santa Clara, CA) and visualized with a streptavidin–phycoerythrin conjugate. Image processing and expression analysis were performed using Affymetrix GeneChip Operating System (GCOS). Statistical analysis was performed to evaluate global gene expression pattern using one-way analysis of variance (ANOVA). The differentially expressed genes were selected on the basis of statistical significance at a minimum two-fold change.

Gene ontology analysis

To interpret the microarray data, the DAVID program[27,28] was used. Groups of genes expressed differentially were annotated on the basis of the biological process by the GO database (DAVID bioinformatics resources, 2008; http://david.abcc.ncifcrf.gov/home.jsp).

Quantitative RT-PCR analysis

For quantitative RT-PCR analysis, ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster, CA) and SYBR Green PCR Core reagents (Applied Biosystems, Foster, CA) were used. Levels of mRNA were expressed as the relative fold change against the normalized *rp49* mRNA. The comparative cycle threshold (Ct) method (User Bulletin 2, Applied Biosystems, Foster, CA) was used to analyze the data. All experiments were repeated at least three times and the data was presented as the mean $\pm$ the standard error of the mean (SEM).

Statistical analyses

Data are presented as the mean $\pm$ SEM. Statistical analyses for demographic data were carried out using standard survival models in the JMP statistical package (ver 5.1) and Prism software (GraphPad, La Jolla, CA). The tests used and sample sizes for each experiment are indicated.

Results and Discussion

Extended life span and improved health span due to curcumin supplementation

Following dose-finding studies, we observed that life span extension by curcumin is sex and genetic background dependent. The optimal curcumin dose appears to vary with sex and genetic background. Curcumin at 100 μM extended the life span of Canton-S female flies by 19% (Fig. 1A, $p < 0.001$) and at 250 μM extended the life span of Ives male flies by 16% (Fig. 1D, $p = 0.004$). Phytochemical compounds

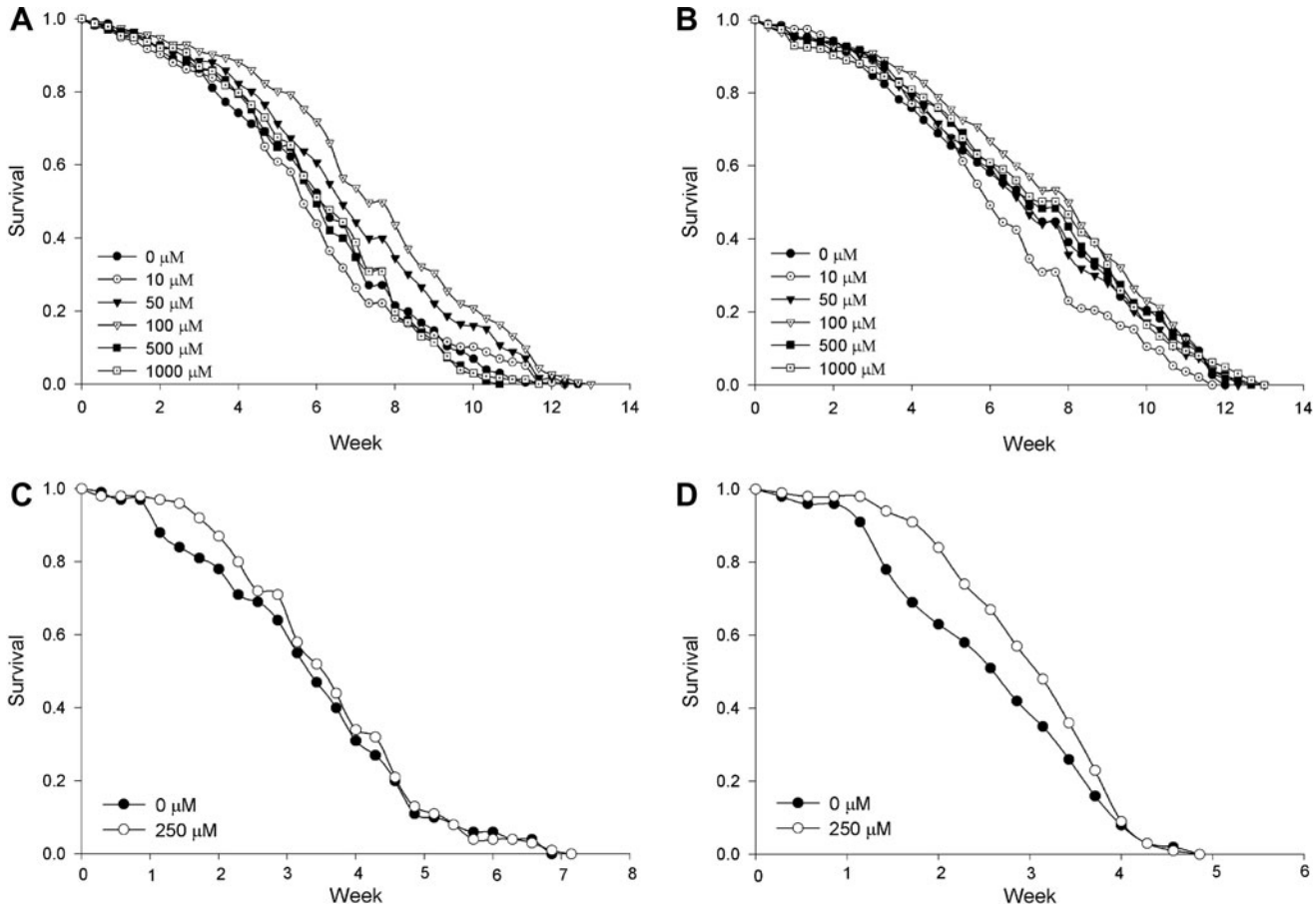

FIG. 1. The effect of curcumin on two strains of *D. melanogaster*. Curcumin extended life span in female Canton-S flies (**A**) at doses of 50 ($p < 0.001$) and 100 ($p < 0.001$) μM. However, no positive effect was detected in male Canton-S flies (**B**), and 10 μM curcumin appeared to shorten male Canton-S life span ($p = 0.0016$ with log rank test; $p = 0.0581$ with Wilcoxon test). The opposite result was found in Ives flies, where curcumin had no effect on females ($p = 0.41$) (**C**) but did extend life span in Ives male flies at 250 μM ($p = 0.0037$) (**D**).

tend to show toxicity when administrated in excess; however, curcumin did not show any toxic effect up to 1,000 μM in Canton-S flies and up to 1,500 μM in Ives flies. The difference in the magnitude of the optimal dose between the two strains of flies is consistent with previous reports, where only 5 μM of PBA was needed to increase the life span of Canton-S flies compared to 10 μ M needed to extend life span in W^{1118} flies.[14]

To determine if the observed life span extension was a direct effect of curcumin treatment or due to a curcumin-induced secondary physiological effect, we examined fecundity and caloric intake with curcumin supplementation. Reproductive output and life span are often negatively correlated both within and across species, and experimental repression of fertility is often sufficient to extend life span.[29] In both fly strains, Canton-S and Ives, curcumin did not reduce fecundity at the doses that life span extension was observed (data not shown). We also tested whether curcumin altered feeding behavior, because caloric restriction can increase life span. We observed that curcumin did not impact feeding behavior in Canton-S flies because there was no difference in feeding rate measured by intake of blue dye after curcumin feeding (data on file).

In addition to checking for the impact of curcumin on physiological confounds of aging such as fecundity and caloric restriction, we also tested the effect of curcumin on locomotor activity as a biomarker of health span. The association between locomotor activity and aging has been established in a variety of species, including *D. melanogaster*. In 1987, Le Bourg reported an association between locomotor activity and aging in *Drosophila*. He showed that spontaneous locomotor activity declined in females as they aged and increased in males up to week 5 and then began to decrease.[30] In another recent study, locomotion declined with aging in *Drosophila*, although males performed better than females at advanced age.[31] Spontaneous locomotion is an assessment of the flies walking behavior, thus giving a measurement of a nonstrenuous activity that can be interpreted as the basal activity level. Vertical climbing is an assessment of the animal's ability to complete a strenuous activity, because flies have to work against gravity to climb to the top of the vial, giving us insight into the animals' level of physical fitness.

Male Ives flies fed 250 μM curcumin showed a slight increase in spontaneous locomotion at days 7 and 14 as compared to controls, but there were no differences in

locomotion at days 21, 28, and 35. Comparatively, female flies fed 250 μM curcumin showed no difference in spontaneous locomotion at 28 days, but a slight increase in locomotion at day 35. Both male and female Ives flies fed curcumin had an enhancement in climbing ability: 42% and 15% in 7- and 35-day-old males, respectively, and a 30% enhancement in 35-day-old females (Fig. 2, $^{**}p < 0.01$, $^{***}p < 0.001$),

Because curcumin is proposed to have chemopreventive properties,[32–34] we evaluated its impact on the reproduction of the female Ives flies exposed to methotrexate (MTX), a commonly used chemotherapeutic drug with known adverse effects on female reproduction. Curcumin-pretreated flies had a significant increase in fecundity and preservation of normal ovarian morphology when exposed to MTX. After 3 days of exposure, MTX at 3 mg/mL and 6 mg/mL resulted in depression in fecundity and shorter, rounder ovarian follicles (ovarioles) in control flies. When flies were pretreated with curcumin, they had normal fecundity and normal and longer ovarioles. The positive impact of curcumin on locomotion and the chemopreventive effects on fly ovarian function indicate that curcumin life span extension properties are associated with improved health span and reproduction health.

Protection against oxidative stress by curcumin

Long-lived mutants often show elevated resistance to various environmental stresses,[35] including starvation and oxidative stress.[4,36] To assess whether curcumin protected flies against oxidative and environmental stresses, we subjected curcumin-fed flies to hydrogen peroxide (H_2O_2), paraquat, and starvation. A significant increase in the percent surviving was observed in Ives male and female flies that were pretreated with curcumin and exposed to H_2O_2 (Fig. 3C,D, $p < 0.0001$ and $p = 0.001$, respectively). In Ives flies, curcumin also protected the female flies against paraquat ($p = 0.008$), but did not exhibit a significant difference in

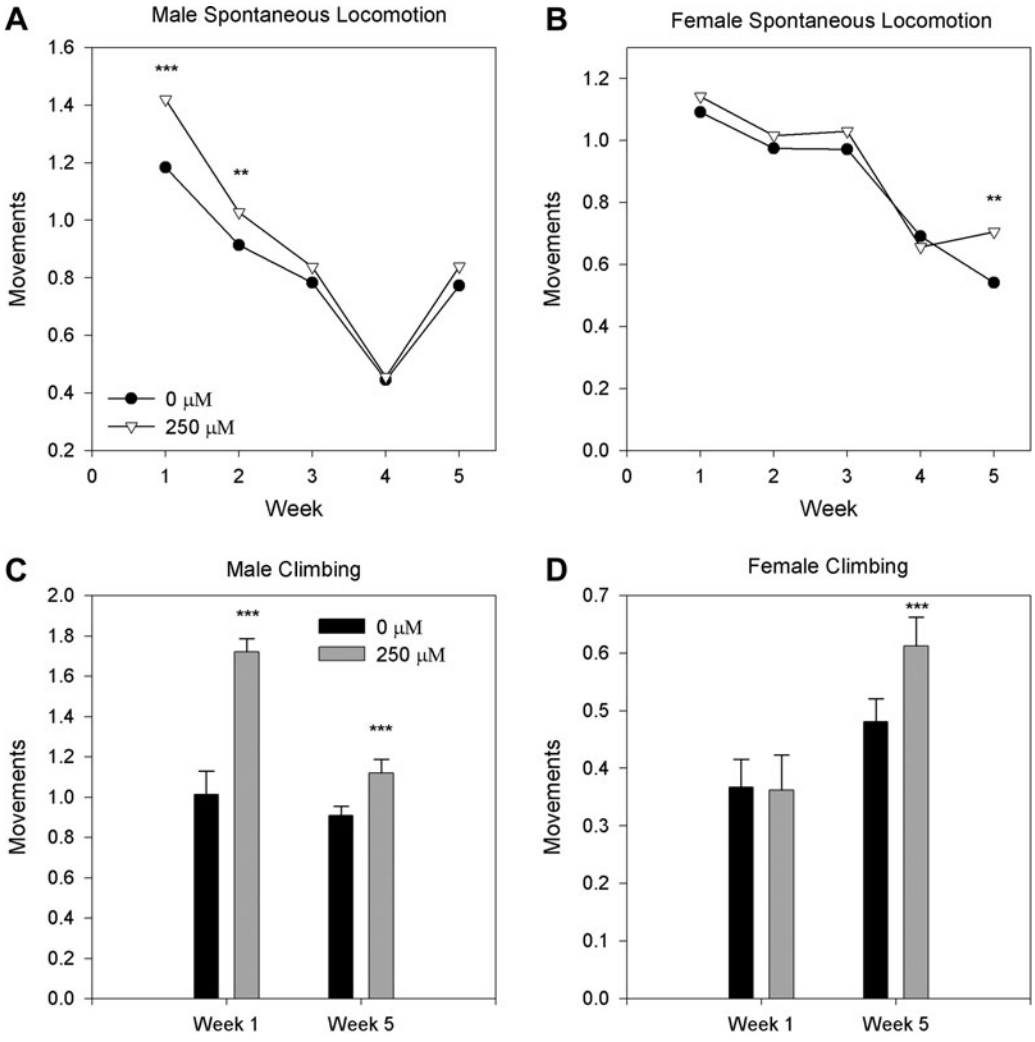

FIG. 2. The spontaneous locomotion and climbing ability of Ives flies administered 250 μM curcumin compared to control. Spontaneous locomotion of 1- and 2-week old males fed curcumin (**A**) and 5-week-old females fed curcumin (**B**) was slightly increased. Climbing ability of 1-week and 5-week-old males (**C**) and 5-week-old females (**D**) was significantly enhanced in flies administered curcumin. (**) $p < 0.01$, (***) $p < 0.001$.

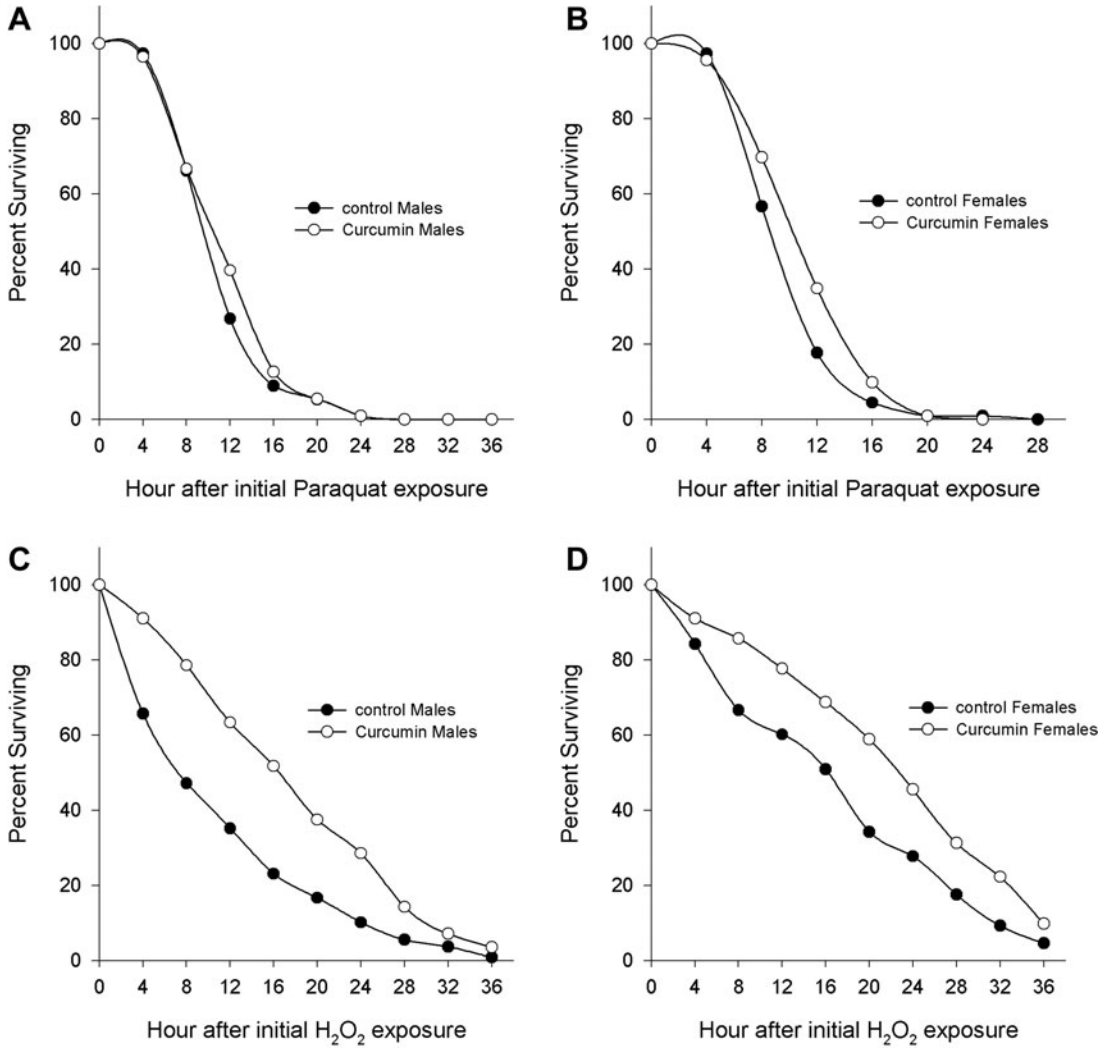

FIG. 3. Percent surviving of Ives males (**A**) and females (**B**) exposed to paraquat or males (**C**) and females (**D**) exposed to hydrogen peroxide (H_2O_2) after 14 days of supplementation with 0 or 250 μM curcumin. There was a statistically significant increase of percent surviving in females (**B**) fed curcumin compared to controls ($p = 0.008$). No significant difference was observed in males exposed to paraquat (**A**). There was a statistically significant increase of percent surviving in both males (**C**) and females (**D**) exposed to H_2O_2 with curcumin pretreatment compared to controls ($p < 0.05$).

percent surviving when being exposed to paraquat in males (Fig. 3A, $p = 0.27$). Of note, in Canton-S male and female flies, curcumin did not significantly increase resistances to paraquat or starvation (unpublished data; starvation $\chi^2 = 0.181$, $p = 0.893$, oxidative stress $\chi^2 = 1.275$, $p = 0.2588$). Our data are consistent with reported antioxidant properties of curcumin.[20,21,37,38]

Changes in the expression levels of genes affected by aging

To evaluate the impact of curcumin and aging on gene expression, we first determined which genes were affected by aging alone in Canton-S flies. The experiments were performed using mixture of males and females. Age-related

Table 1. Biological Processes Ontology of Probes with Significant Transcriptional Changes

Dataset	*Number of changed genes*	*Number of GO term in BP_level 5*	*Regulation*	*Number of changed genes*	*Number of GO term in BP_level 5*
1 day vs. 40 days	1383	71	Up	530	49
			Down	853	22
1 day 0 μM vs. 100 μM	420	33	Up	42	6
			Down	378	27
40 days 0 μM vs. 100 μM	879	39	Up	536	20
			Down	340	20

GO, Gene ontology.

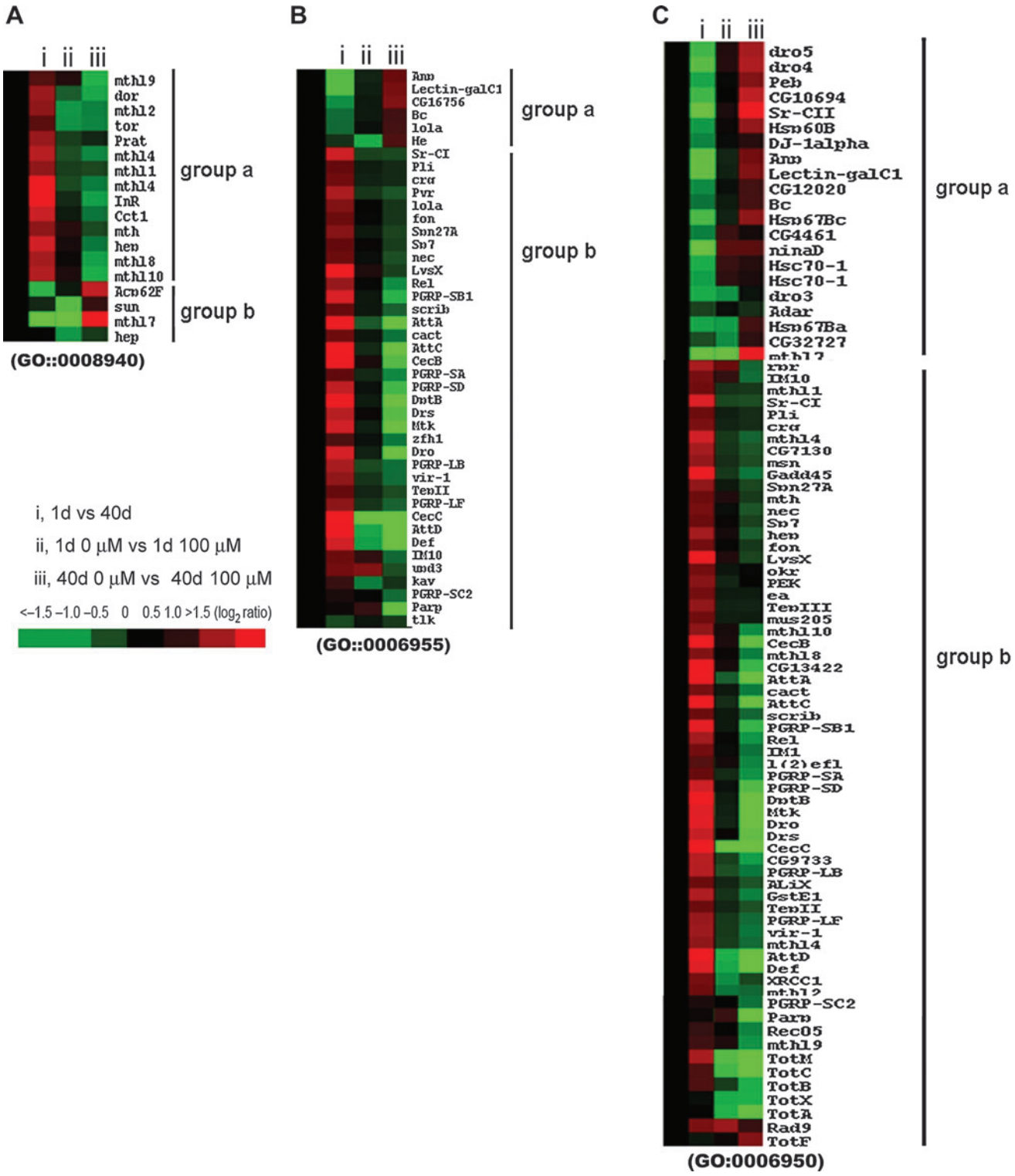

FIG. 4. Cluster analysis of expression profiles significantly associated with age-dependent and curcumin-dependent gene expression changes in Canton-S flies. Gene expression changes in the gene ontology (GO) categories most significantly associated with aging (**A**), immune response (**B**), and stress response (**C**) are represented by color-coding in bottom left. Green indicates downregulation of gene expression with aging; red indicates upregulation. The order of samples from left to right is day 1 versus day 40 flies (i), day 1 flies fed with 0 μM versus 100 μM curcumin (ii), and day 40 flies fed 0 μM versus 100 μM curcumin (iii).

changes in gene expression were defined as changes in expression levels that occurred between 3 and 40 days of age (median life span). Among the 18,880 probe sets in the Affymetrix GeneChip® *Drosophila* Genome 2.0 Array, 1,383 genes (unpublished data; 7.3%, $p < 0.05$) had statistically significant changes in expression levels during this time frame. A total of 530 genes were upregulated and 853 genes were downregulated in aged flies relative to young flies (unpublished data). We conducted a functional analysis for each cluster of age-related genes based on gene ontology (GO)[39] and found that about 70 biological processes were altered by aging in Canton-S flies ($p < 0.05$) (Table 1).

Data on Gene Ontology ID of Biological Processes for each experimental group are presented in supplementary tables (*see* www.liebertonline.rej).

Changes in the expression levels of genes affected by curcumin

We next determined the effect of curcumin on gene expression levels in young and aged flies. In newly eclosed flies fed control medium or 100 μM curcumin for 3 days, we identified upregulated 42 genes and 378 downregulated genes due to curcumin feeding. These were grouped into 33 biological processes ($p < 0.05$) (Table 1). By 40 days of age, 536 genes were upregulated and 340 genes were downregulated due to curcumin feeding. The latter genes were grouped into 39 biological processes ($p < 0.05$) (Table 1).

The effect of curcumin on age-related genes

To investigate whether the clustering of age-related genes correlates with functional groupings, we performed GO functional enrichment analysis on all of the significantly changed genes. Among the three independent GO categories (biological process [BP], molecular function, and cellular component), we focused on BP category. The BP category reflects gene induced biological outcomes such as "aging," "cell aging," or "multicellular organism aging" and more specific processes defined as "determination of adult life span" or "age-dependent telomere shortening."

For the evaluation of the effect of curcumin on age-related changes in gene expression, genes were grouped into three GO categories: Determination of adult life span (aging; GO:0008340), immune response (GO:0006955), and stress response (GO:0006950) (Fig. 4). The determination of adult life span category (aging; GO:0008340) includes the JNK pathway, the insulin-signaling pathway, target of rapamycin (*dTOR*), the insulin receptor (*dInR*), hemipterus (hep, the *Drosophila* homolog of JNKK), stunted, methuselah, and methuselah-like proteins. The immune response category (GO:0006955) includes antimicrobial peptides (AMPs), peptidoglycan recognition pattern receptors (PGRP-R), and Toll/NF-κB signaling pathway molecules. The expression levels of the majority of the genes in these two categories increased in response to aging in control diet-fed flies. However, their expression levels appeared to be either unaffected or modestly decreased by curcumin in young flies, but were more consistently decreased in curcumin-fed aged flies relative to young controls (Fig. 4A,B, group b). The third group, stress response (GO:0006950), includes the heat shock proteins (HSPs), various chaperones, as well as aging-related and immune response genes. Interestingly, gene expression levels for heat shock proteins decreased during aging in control diet-fed flies, but increased during aging in curcumin-fed flies (Fig. 4C, group a). However, other stress response genes appear to be downregulated by aging and upregulated by curcumin (Fig. 4C, group b).

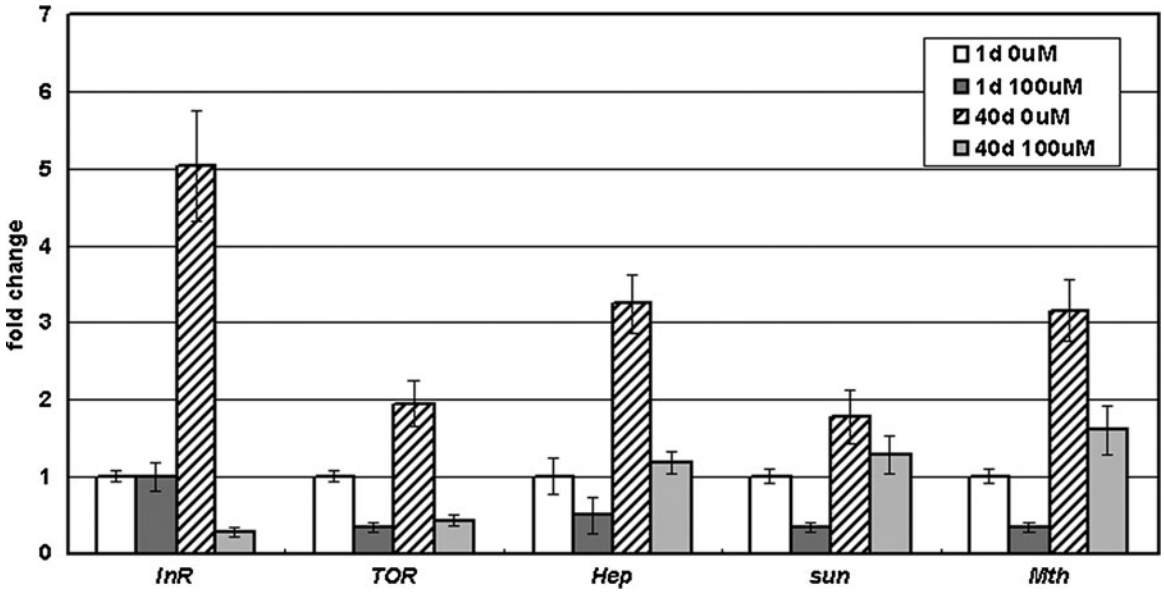

FIG. 5. Quantitative real-time PCR analysis of longevity assurance genes (LAGs) was used to validate the differential transcription data acquired from microarray analysis after 40-day aging in the diet of 100 μM curcumin compared with Canton-S flies reared on control diet. Data were shown as means ± standard error of the mean (SEM) for three biological replicates in real-time PCR experiments. *InR*, *Insulin receptor*; *TOR*, *target of rapamycin*; *Hep*, *hempiterus*; *sun*, *stunted*; *mth*, *methuselah*.

Our microarray data and primary analyses have been deposited in the Gene Expression Omnibus (GEO) database (accession numbers: GSE21182).

Validation of microarray results by quantitative RT-PCR

We validated our microarray studies for genes in the determination of adult life span GO category (aging; GO:0008340) by quantitative RT-PCR. Real-time PCR amplification from independent samples verified the increased expression of almost all tested genes that were upregulated in aged flies according to microarray analysis. In contrast, all of these genes were downregulated by curcumin in aged flies in agreement with our microarray data (Fig. 5). In aged flies, the expression of LAGs, such as *insulin receptor (InR), target of rapamycin (TOR)*, *hemipterus* (*hep*), *stunted (sun)*, and *methuselah (mth)*, were significantly increased but were decreased by curcumin feeding (Fig. 5). There are 14 *methuselah-like protein* (*mthl*) genes in *Drosophila*. Among them, the expression of six *mthl* genes showed similar expression pattern of *mth* gene (see Supplementary Figure S1; www.liebertonline.com/rej).

Insulin/insulin-like peptide signaling (IIS) is an evolutionary conserved pathway for regulating life span from yeast to human.[1] Independent mutation of the *InR* or the InR substrate *chico* leads to reduced insulin signaling and extended life span.[3,40] The TOR pathway interacts with IIS and also regulates life span. Inhibition of TOR signaling by overexpression of *Tsc1* or *Tsc2* extends life span in *Drosophila*.[17] In *Drosophila*, JNK signaling confers tolerance to oxidative stress and extends life span by inducing a protective gene expression program. Mutation in *hep*, a *Drosophila* homolog of *JNKK*, was more sensitive to oxidative stress and resulted in reduced shortened life.[10] Mth is a G protein–coupled receptor (GPCR) associated with longevity. *Drosophila mth* mutants have an extension of life span and increased resistance to stress.[4] Two peptides, SunA and SunB, which are products of the *stunted* gene, had previously been identified as the endogenous ligand for Mth. *sun* mutant flies, similar to *mth* mutants, have an increased life span and increased resistance to stress.[41]

Global transcription change provides an analytical tool to measure biological age and to evaluate at the molecular level the efficacy of pharmacological interventions designed to retard the aging process. Aging resulted in a differential gene expression pattern indicative of a higher expression of LAGs and lower expression of defense to stress response genes. Transcriptional patterns from curcumin fed-animals suggest that curcumin retards the aging process by causing a suppression of LAGs and enhancing the defense to stress response. These results reveal that curcumin may modulate the expression of age-related genes that may result in slowing the aging processes in *Drosophila*.

Conclusion

In this paper, we have presented data showing that curcumin increases life span in two different strains of flies, Canton-S and Ives flies, under two different feeding and experimental conditions. The life span extension was dependent on sex and genetic background and was not associated with compromised health span, as measured by its impact on locomotion. Age-associated genes were dramatically changed during aging process, which may be considered the phenotypic signature of the aging process. Curcumin delayed the onset of age-associated gene expression. Specifically, curcumin reduced expression of proteins in the insulin, JNK, and methuselah signaling pathways. Curcumin also modulated biological processes involved in the stress response. Given curcumin's therapeutic benefits in humans, it will be of interest to determine whether curcumin can affect the lifespan of mammalian models.

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