3-Ketoacyl thiolase delays aging of *Caenorhabditis elegans* and is required for lifespan extension mediated by *sir-2.1*

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Studies of long-lived *Caenorhabditis elegans* mutants have identified several genes that function to limit lifespan, i.e., loss-of-function mutations in these genes promote longevity. By contrast, little is known about genes that normally act to delay aging and that when mutated cause premature aging (progeria). To seek such genes, we performed a genetic screen for *C. elegans* mutants that age prematurely. We found that loss-of-function mutations of the ketoacyl thiolase gene *kat-1* result in an increased accumulation of the lipofuscin-like fluorescent aging pigment, shortened lifespan, early behavioral decline, and other abnormalities characteristic of premature aging. These findings suggest that *kat-1* acts to delay *C. elegans* aging. *kat-1* encodes a conserved metabolic enzyme that catalyzes the last step of fatty acid oxidation and was previously shown to regulate fat accumulation in worms. We observed that *kat-1* is required for the extension of lifespan and enhanced thermotolerance mediated by extra copies of the deacetylase gene *sir-2.1*. *kat-1* acts independently of other known pathways that affect longevity. Our findings suggest that defects in fatty acid oxidation can limit lifespan and accelerate aging in *C. elegans* and that *kat-1*-mediated fatty acid oxidation is crucial for overexpressed *sir-2.1* to delay aging.

progeria | lipofuscin | fatty acid oxidation | sirtuins | protein deacetylation

Over the past few decades, experiments using *Caenorhabditis elegans* and other organisms have resulted in progress toward an understanding of molecular mechanisms of aging (1–3). Loss-of-function mutations in single *C. elegans* genes can substantially increase lifespan (4–7), indicating that lifespan determination of nematodes has a major genetic component. Research in the aging field has largely focused on lifespan-extending mutations in genes that normally function to reduce longevity. This focus has been primarily a consequence of the difficulties in distinguishing accelerated aging from sickness of short-lived mutants.

Though longevity remains the most commonly used measure of the rate of nematode aging, additional biomarkers of *C. elegans* aging have been characterized. These biomarkers include behavioral decline and tissue aging (8–11). Aging of *C. elegans* is characterized by a progressive decline in locomotion, both in speed and coordination (9, 10, 12), and decreased defecation and pharyngeal pumping rate (8, 9, 13). Old nematodes, like old humans, show progressive tissue and organ deterioration: muscles, gonad, intestine, and connective tissues degenerate in aged worms (9–11).

The intestinal cells of aging *C. elegans* accumulate an autofluorescent aging pigment similar to the aging pigment lipofuscin present in postmitotic mammalian cells (13, 14). Lipofuscin accumulates in the secondary lysosomes (14–16) and consists primarily of lipid peroxidation products and oxidized proteins that resist proteolytic degradation (17). The amount of lipofuscin gradually increases throughout *C. elegans* adulthood, and autofluorescent granules can be detected in aging adult nematodes by fluorescence microscopy.

Accumulation of lipofuscin, tissue degeneration, and behavioral decline characterize aged worms and can be used to seek progeroid mutants. Such mutants might define a new class of aging genes.

Here we report the identification and characterization of progeroid mutants of *C. elegans* defective in the gene *kat-1* (3-ketoacyl thiolase). We show that *kat-1* functions to delay *C. elegans* aging and that *kat-1* is specifically required for the increased lifespan of worms bearing extra copies of the *sir-2.1* protein deacetylase gene.

Results

To identify genes that act to delay aging, we sought mutants that age prematurely, using accumulation of the autofluorescent pigment lipofuscin as our marker. We used a fluorescence-equipped dissecting microscope to screen for mutants that showed a premature increase of lipofuscin. Old wild-type adults and animals with a *daf-16* loss-of-function mutation, which age prematurely, accumulated high levels of lipofuscin (13) (Fig. 1 *A* and *B*). We mutagenized wild-type hermaphrodites and screened F$_2$ offspring for an increased accumulation of intestinal autofluorescence. Overall, we screened ≈20,000 mutagenized haploid genomes and obtained 11 mutant strains that reliably exhibited premature accumulation of intestinal autofluorescence (Fig. S1, and see SI Text for details).

Age-dependent accumulation of fluorescence in the homozygous mutant isolates was comparable to that of animals bearing loss-of-function mutations in *daf-16* (example shown in Fig. 1 *A* and *B* is *n4430*, an allele of the *kat-1* gene identified below). We mapped *n4430*, *n4434*, *n4435*, and *n4501* to linkage group II (LGII), and *n4427*, *n4428*, *n4429*, *n4431*, *n4432*, *n4433*, and *n4436* to LGV. *n4434* and *n4435* failed to complement *n4430*, whereas *n4501* complemented *n4430* for increased autofluorescence accumulation. All LGV mutants were strongly semidominant and appeared to fail to complement each other. In short, the 11 isolates with high autofluorescence represent at least three complementation groups: two on LGII and one or more on LGV (Table 1), suggesting that at least three genes control the age-related accumulation of autofluorescence in *C. elegans*.

In their studies of aging *C. elegans*, Gerstbrein et al. (13) distinguished two distinct sources of autofluorescence: the aging pigment lipofuscin, which increases with age and has an emission range of 400–460 nm, and total protein fluorescence (mostly tryptophan fluorescence), which remains constant during adult-

Fig. 1. *kat-1* mutations cause increased fluorescence and short lifespan. (*A*) Intestinal fluorescence accumulation in young (1-d), middle-aged (5-d), and old (12-d) *kat-1(n4430)* adults compared with wild-type (N2) and progeroid *daf-16(mgDf50)* animals of the same ages. Intestinal autofluorescence was recorded using a DAPI filter (excitation 365–380 nm, emission 450 nm) with an exposure time of 1,000 ms. (Scale bar, 0.2 mm.) (*B*) Quantitation of the intestinal autofluorescence intensity in wild-type, *kat-1(lf)*, and *daf-16(lf)* animals using ImageJ software. Fluorescence intensity was calculated by subtracting background fluorescence (ranging from 90 to 110 arbitrary units) from total intestinal fluorescence. Five animals per genotype per time point were analyzed. *$P < 0.05$ compared with wild type of the same age. (*C*) *kat-1* mutants have shortened lifespans. Median lifespans in this experiment were: wild-type, 18 d, $n = 79$; *n4430*, 13 d, $n = 105$; *n4434*, 16 d, $n = 95$; *n4435*, 13 d, $n = 122$, *tm1037*, 13 d, $n = 89$. Lifespans af all four *kat-1* mutants were significantly different from that of the wild type ($P < 0.0001$).

hood and has an emission range of 330–380 nm. To elucidate which type of fluorescent pigment accumulated in our mutants, we extracted proteins from the mutant strains and tested fluorescence accumulation in worm lysates, comparing young to old animals of each strain as well as mutant to wild-type animals at different ages. Our lysis buffer was designed to extract proteins, so we were not expecting to detect lipofuscin components of lipid origin in lysates. Nonetheless, both wild-type animals and our mutants showed in whole-worm lysates an age-dependent increase in fluorescence with a spectrum similar to that of lipofuscin (420 nm emission peak), possibly because fluorescent moieties of nonprotein origin were also extracted during lysis. Both young and old adult LGV mutants showed levels of protein fluorescence substantially higher than those in the wild type, and LGII mutants also showed an age-dependent increase in protein fluorescence but had overall levels more similar to and possibly less than those of the wild type (Fig. S2). It is noteworthy that because wild-type animals showed an age-dependent increase in fluorescence accumulation by this method, and the LGII mutants did not show abnormally high levels in young adults, it is possible that not all features of aging are accelerated in the LGII mutants. To determine whether the isolated mutants show additional characteristics of premature aging, we performed tests to assess other aging parameters of the screen isolates, as described below.

Three Isolates Prematurely Display Multiple Attributes of Old Age. Progeroid mutants should have shorter lifespans than wild-type animals. We performed lifespan analyses of our mutants (Table 1). Six had 25–40% shorter lifespans than the wild type, and five had slightly shorter or normal lifespans (Table 1). Notably, all three allelic LGII mutants had short lifespans, with mean lifespans of 14–15 d, compared with 21 d for the wild type (Fig. 1*C* and Table 1). As will be described below, these three mutants carry alleles of the gene *kat-1*. The other LGII mutant, *n4501*, had only a slightly

Table 1. Genetic and phenotypic analyses of screen isolates

Genotype	Phenotype[a]	LG	Comp. group	Behavioral decline	Median lifespan 20 °C (25 °C)
Wild type	—	—	—	Normal	21 (12)
kat-1(n4430)	Weakly SD	II	1	Premature	14* (11*)
kat-1(n4434)	Weakly SD	II	1	Premature	14* (9*)
kat-1(n4435)	Weakly SD	II	1	Premature	14* (10*)
n4501	Recessive	II	2	Normal	18* (11*)
n4427	SD	V	3[b]	Normal	16* (11*)
n4428	SD	V	3[b]	Normal	19* (11*)
n4429	SD	V	3[b]	Normal	19* (11*)
n4431	SD	V	3[b]	Normal	19* (11*)
n4432	SD	V	3[b]	Normal	12* (10*)
n4433	SD	V	3[b]	Normal	19* (11*)
n4436	SD	V	3[b]	Normal	16* (11*)

[a]Refers to autofluorescence accumulation.
[b]These mutations might or might not be allelic; they cause a SD phenotype.
    Assays were performed as described in *Materials and Methods*. SD, semidominant; LG, linkage group; Comp., complementation. *$P < 0.05$.

(15%) shorter lifespan, and of the seven LGV mutants, three had short lifespans, and the other four had nearly wild-type lifespans.

We also characterized the age-dependent behavioral decline of our mutants. The most obvious behavioral abnormality associated with *C. elegans* aging is a progressive slowing of the rates of feeding (pharyngeal pumping) and locomotion, as well as uncoordinated locomotion (11, 13). *kat-1* mutants showed a premature onset of uncoordinated locomotion and paralysis, as did progeroid *daf-16 (lf)* mutants (Fig. 2). The *kat-1* mutants *n4430*, *n4434*, and *n4435*, but none of the other mutants, including those with short lifespans, showed a premature decline in both locomotion and pumping (Fig. 2 and Fig. S3).

We performed electron microscopic analyses to examine tissue integrity in aging wild-type and *kat-1(n4430)* mutant animals. Old wild-type animals show a striking decline in the integrity of many organs, including the gonad, intestine, and muscle. Aging *C. elegans* undergo marked gonad degeneration, and old animals lack a detectable gonad, though yolk and lipofuscin accumulate within their intestines (10, 11). The apparent size of the intestinal lumen increases as old animals become bloated with bacteria, and the intestinal cells of old worms show membrane swirls typical of necrosis (10). Old adults also accumulate electron-dense material in the intestine and other tissues (11). *kat-1(n4430)* animals showed early degeneration of the gonad compared with the wild type (Fig. 3 *A* and *C–E*). The intestines of *kat-1* animals showed multiple signs of premature decline: increased bloating of the intestinal lumens (Fig. 3 *B*, *C–E*, *H*, and *J*) and early appearance of membrane swirls (Fig. 3 *F*, *H*, and *J*). *kat-1(n4430)* animals also had greatly increased accumulation of electron-dense material (Fig. 3 *G*, *I*, and *J*) compared with the wild type.

We conclude that *kat-1* mutants have short lifespans and also display precocious onset and progression of many but not all features of *C. elegans* aging: we observed prematurely increased accumulation of lipofuscin-like pigment, early behavioral decline, and tissue degeneration. We failed to observe increased fluorescence in *kat-1* mutant lysates but did observe increased fluorescence in lysates of old wild-type animals.

n4430, n4434, and n4435 Are Alleles of kat-1, Which Encodes a Conserved Thiolase Involved in Fatty Acid Metabolism.

We molecularly identified the gene mutated in *n4430* animals using a combination of mapping with visible markers and polymorphisms, transgene rescue, and RNAi (see *SI Text* for details). We found that *n4430* and the allelic mutants *n4434* and *n4435* all carry mutations in the gene *T02G5.8* (Fig. S4).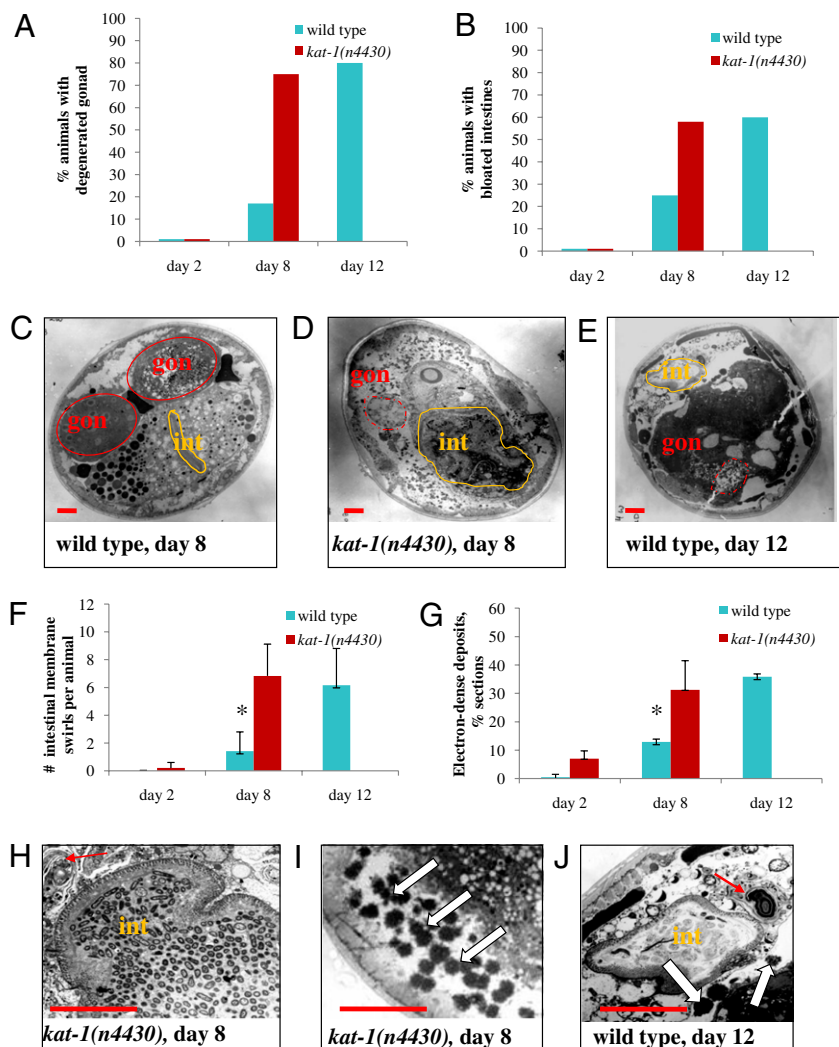 *T02G5.8* was previously identified in a RNAi-based screen for animals with abnormal fat metabolism and was named *kat-1* for ketoacyl thiolase-1 (18). *kat-1 T02G5.8* is highly conserved from worms to humans (Fig. S5) and is predicted to encode a protein with 52% identity and 70% similarity to the mammalian enzyme acetoacetyl CoA thiolase (EC 2.3.1.9), which is involved in the β-oxidation of fatty acids in mitochondria and peroxisomes (Kegg Pathway Database, http://www.genome.ad.jp/kegg/pathway.html). The three *kat-1* alleles identified in our screen are predicted to result in the substitutions of conserved amino acids (Fig. S5).

To analyze the null phenotype of *kat-1*, we obtained a deletion allele, *tm1037*, from the *C. elegans* Japanese knockout consortium (National Bioresource Project for the Experimental Animal Nematode *C. elegans*). *kat-1(tm1037Δ)* animals showed increased lipofuscin fluorescence, a shortened lifespan, and early behavioral decline comparable to those of the *kat-1* mutants *n4430*, *n4434*, and *n4435* (Figs. 1 and 2 and Fig. S6). Mortality rates of *kat-1(tm1037)* and *kat-1(n4430)* mutants were similar, and both increased prematurely compared with the wild-type animals, similarly to *daf-16 (lf)* mutants (Fig. S6A). Notably, the lifespan abnormalities of *kat-1 (tm1037)* and *kat-1(n4430)* mutants were also apparent when worms were grown without FUdR (Fig. S6B), a drug normally used in lifespan analyses and that we used in most of our studies; under some conditions, FUdR can mildly affect *C. elegans* lifespan (19). The injection of a PCR product containing multiple copies of the *kat-1* gene into *kat-1(n4430)* mutant animals rescued the lifespan defect of the *kat-1* mutants, resulting in normal (wild-type) lifespan (Fig. S6C). None of the transgenic lines overexpressing *kat-1* in the *kat-1(lf)* background had extended lifespan.

kat-1 Is Required for the Lifespan Extension Mediated by the Protein Deacetylase Gene sir-2.1.

Several pathways are known to control *C. elegans* aging, including insulin signaling, germ-line signaling, chemosensory signaling, mitochondrial function, dietary restriction (DR), and a pathway activated by the gene *sir-2.1* (20). To see whether *kat-1* acts to delay aging via one of these known longevity pathways, we crossed a *kat-1* mutation into backgrounds of long-lived *C. elegans* mutants and tested whether *kat-1* is required for the extended longevity of these mutants. We found that *kat-1* is not necessary for lifespan extension by low insulin signaling, chemosensory and ETC abnormalities, and DR (*SI Text* and Table S1). A *kat-1* mutation partially suppressed the long lifespan of *mes-1* mutants that lacked germ lines (see *SI Text* for details), suggesting that the effect of germ-line signaling on lifespan is partially *kat-1*-dependent.
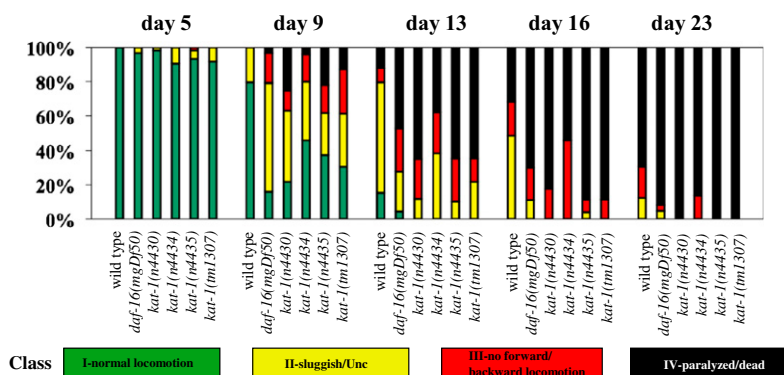
Fig. 2. Behavioral abnormalities of *kat-1* mutants. Aging animals were classified into four groups based on locomotion: I, animals with robust, coordinated sinusoidal locomotion (green bars); II, uncoordinated and/or sluggish animals (yellow bars); III, animals that did not move forward or backward but did move their heads or shudder in response to prodding (red bars); and IV, paralyzed/dead animals (black bars). Four *kat-1* mutants were tested: *n4430*, *n4434*, *n4435*, and *tm1037*. Wild-type animals (N2) and progeroid *daf-16(mgDf50)* mutants were used as controls. At least 60 animals per strain were tested for each time point. We used Student's *t* test to determine statistical significance of the differences between wild-type and mutant animals. For all mutants, differences in one or more classes were significant ($P < 0.05$).

By contrast, *kat-1* loss-of-function fully suppressed the lifespan extension caused by overexpression of the *sir-2.1* deacetylase. *sir-2.1* extends lifespan in response to stress by activating the fork-head transcription factor DAF-16 (21). Introducing a *kat-1* mu-tation into a strain in which *sir-2.1* is overexpressed eliminated the lifespan extension caused by *sir-2.1* and resulted in a short-ened lifespan similar to that of *kat-1* mutants (Table S1 and Fig. 4*A*). As mentioned previously, *kat-1* was not required for the extension of lifespan by a loss-of-function mutation in the insulin receptor gene *daf-2*, and *kat-1;daf-2* double mutants had long lifespans similar to these of the single *daf-2(lf)* mutants (Fig. 4*B*), indicating that *kat-1* is specifically required for the lifespan exten-sion mediated by *sir-2.1* overexpression.

sir-2.1-overexpressing animals accumulated less intestinal lip-ofuscin than did the wild type (Fig. 4*C*), consistent with the slower rate of aging and extended lifespan of *sir-2.1* overexpressors. Loss of *kat-1* function suppressed the reduced lipofuscin accumulation of *sir-2.1* overexpressors, as *sir-2.1*-overexpressing animals homo-zygous for a *kat-1* loss-of-function mutation accumulated more lipofuscin than did the wild type, just like *kat-1* mutants (Fig. 4*C*).

Aging mutants often show altered resistance to stress: *sir-2.1* and *daf-16* loss-of-function mutants are hypersensitive to heat stress, whereas *daf-2* loss-of-function mutants and *sir-2.1* over-expressors are stress resistant (6, 21) (Fig. 4 *D* and *E*). We tested the thermotolerance of *kat-1* mutants, alone and in *daf-2* loss-of-function and *sir-2.1* overexpression backgrounds. *kat-1* mutants

showed an increased sensitivity to heat, and *kat-1* was required for the stress resistance mediated by *sir-2.1* overexpression (Fig. 4*D*). By contrast, *kat-1* was not required for the increased ther-motolerance of *daf-2* mutants, as *kat-1;daf-2* double mutants had increased stress tolerance similar to that of *daf-2* single mutants (Fig. 4*E*). Our findings suggest that *kat-1* function is specifically required for the effect of *sir-2.1* overexpression on aging, stress resistance/thermotolerance, and lifespan.

Discussion

We isolated loss-of-function (lf) mutations in the *C. elegans* ketoacyl thiolase gene *kat-1* in a genetic screen for animals that age prematurely. The *kat-1* premature aging phenotype is remi-niscent of that of the well-characterized *C. elegans* progeroid *daf-16(lf)* mutant: both *kat-1* and *daf-16* mutants precociously accu-mulate high levels of autofluorescence associated with the aging pigment lipofuscin, show early behavioral decline typical of old age, have premature tissue aging, and are short-lived.

kat-1 is dispensable for the regulation of lifespan by most known longevity pathways, including the insulin-like pathway and that involving mitochondrial function, but is required for the lifespan extension caused by the overexpression of *sir-2.1*. We previously showed that SIR-2.1 can bind to and activate the transcription factor DAF-16 in a manner dependent on stress and the 14-3-3 gene *ftt-2* and acting in parallel to the insulin-like pathway (21). Like *ftt-2*, *kat-1* is not required for the longevity and heat stress
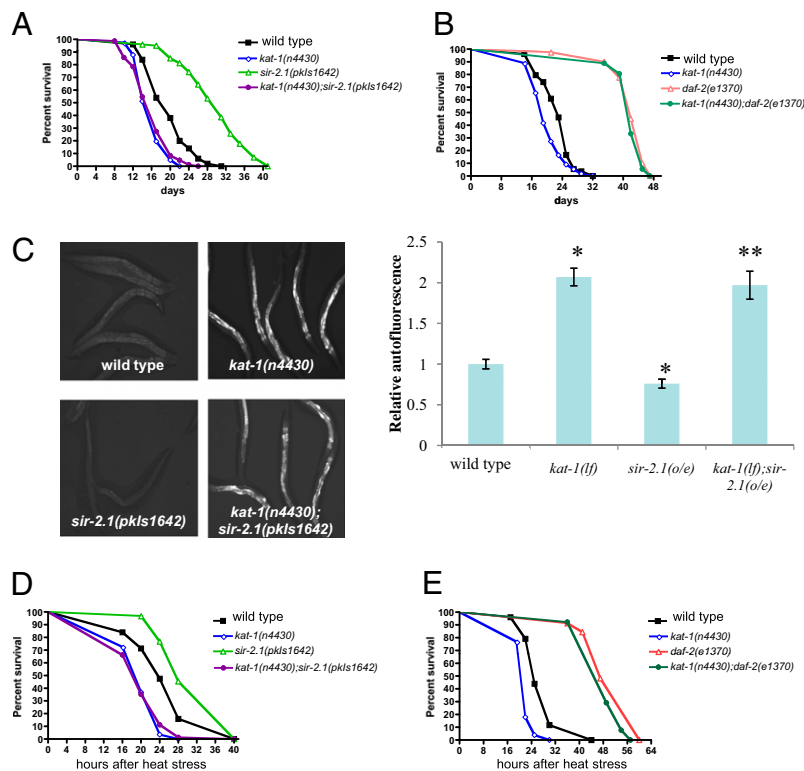
Fig. 4. *kat-1* is required for the increased longevity and thermotolerance caused by extra copies of *sir-2.1*. (*A*) The increased longevity of *sir-2.1*-over-expressing animals was suppressed by loss of *kat-1* function. Median lifespans were as follows: wild type (N2), 20 d, *n* = 50, *pkIs1642[sir-2.1]*, 31 d, *n* = 101, *kat-1(n4430)*, 17 d, *n* = 41, *kat-1(n4430);pkIs1642 [sir-2.1]*, 17 d, *n* = 84. (*B*) The increased longevity of *daf-2(lf)* mutants was not suppressed by the loss of *kat-1* function. Median lifespans were as follows: wild type (N2), 23 d, *n* = 76, *kat-1(n4430)*, 18 d, *n* = 67, *daf-2(e1370)*, 42 d, *n* = 92, *kat-1(n4430);daf-2(e1370)*, 41 d, *n* = 98. (*C*) Intestinal lipofuscin accumulation in young (2-d-old) wild-type animals (N2) compared with long-lived *sir-2.1*-overexpressing animals, with and without the *kat-1(n4430)* mutation. Note that *sir-2.1* animals accumulated less lipofuscin than the wild type, and the *sir-2.1*(o/e);*kat-1*(lf) animals accumulated more lipofuscin than did the wild type. Photographs were taken as described in Fig. 1*A*. *$P < 0.05$ vs. wild type, **$P < 0.05$ vs. *sir-2.1[pkIs1642]*. (*D*) *kat-1* is required for increased resistance to heat stress caused by *sir-2.1* overexpression. Median survival: wild type, 24 h, *n* = 63, *pkIs1642[sir-2.1]*, 28 h, *n* = 99, *kat-1(n4430)*, 20 h, *n* = 84, *kat-1(n4430);pkIs1642 [sir-2.1]*, 20 h, *n* = 71. (*E*) *kat-1* is dispensable for the increased resistance to heat stress of the insulin-like receptor mutant *daf-2(e1370)*. Median survival: wild type, 25 h, *n* = 77, *daf-2(e1370)*, 49 h, *n* = 65, *kat-1(n4430)*, 22 h, *n* = 78, *kat-1(n4430);daf-2(e1370)*, 47 h, *n* = 58.

resistance mediated by low insulin-like signaling but is required for the longevity mediated by *sir-2.1* overexpression. Recently, Gerhart–Hines et al. (22) demonstrated that mammalian SIRT1, the closest homolog of worm SIR-2.1, acts to increase fatty acid oxidation in mouse myocytes. Under conditions of nutrient deprivation, metabolism switches from glucose to fatty acid oxidation (23). It is plausible that *sir-2.1*, which is activated by a high NAD/NADH ratio (24), acts to induce and maintain fatty acid oxidation in response to limited food. For example, *sir-2.1* might specifically activate a subset of *daf-16* target genes that induce fatty acid oxidation. In this case, blocking fatty acid oxidation by reducing *kat-1* function might preclude this metabolic switch, render the animals less resistant to metabolic stress, and accelerate aging. Alternative models are also possible. For example, *kat-1* might function to promote *sir-2.1* activity. Either way, our findings suggest that the fatty acid oxidation enzyme KAT-1 acts in the SIR-2.1 pathway to delay aging and provide resistance to stress.

Efficient Fatty Acid β-Oxidation Can Slow Aging. Loss of function of the fatty acid oxidation thiolase gene *kat-1* causes accelerated aging in *C. elegans*. This effect of *kat-1* might be a consequence of an effect on fatty acid oxidation or might result from a *kat-1* function distinct from fatty acid oxidation, such as a direct role in lipofuscin accumulation.

Mutations in *kat-1* were previously identified in a screen for genes that regulate fat accumulation (18): whereas *tub-1* mutants display a modest increase in fat storage, *kat-1 tub-1* double mutants have

significantly elevated fat accumulation. *tub-1* encodes the *C. elegans* homolog of mammalian Tubby, which is involved in glucose and fat metabolism. Mice homozygous for a Tubby loss-of-function mutation fail to activate carbohydrate metabolism and instead rely on increased fat metabolism for their energy needs (25). It is possible that *C. elegans tub-1* mutants have an increased rate of fat metabolism, which causes *kat-1;tub-1* double mutants to have higher levels of incompletely-processed fatty acids than do *kat-1* single mutants. That a loss of *tub-1* function results in an extension of lifespan (26) is consistent with the hypothesis that increased fat oxidation delays *C. elegans* aging. Interestingly, a recent study by Wang et al. (27) shows that in *C. elegans* fat hydrolysis modulated by a specific fat lipase is important for the lifespan extension mediated by a lack of germ-line signaling. A *kat-1* loss-of-function mutation partially suppressed the lifespan extension induced by a lack of the germ line in *mes-1* mutants. These findings further support the hypothesis that efficient fat metabolism can delay *C. elegans* aging.

Numerous studies over the past 30 y have revealed an association between aging and decreased fatty acid oxidation. Triglycerides and various products of incomplete lipid oxidation accumulate during old age, leading to so-called lipotoxicity, and this accumulation is associated with age-related disorders, such as type 2 diabetes, metabolic syndrome, and Alzheimer's disease (28–30). That loss of function of KAT-1, an enzyme involved in fatty acid breakdown, leads to accelerated aging suggests a causal link between the decline in fatty acid β-oxidation and aging.

Materials and Methods

Strains. We provide a thorough description of the *C. elegans* strains used in the *SI Text*.

Isolation of Mutants with Increased Lipofuscin Autofluorescence. We mutagenized N2 hermaphrodites with ethyl methanesulphonate (EMS) as described by Brenner (31). We screened F$_2$ animals equivalent to ≈20,000 mutagenized haploid genomes for high intestinal autofluorescence at day 2 or 3 of adulthood, using a dissecting microscope equipped with fluorescence optics; the filter set (Leica) was designed to detect low levels of lipofuscin autofluorescence: the excitation filter wavelength was 350–365 nm, and the emission filter range was 440–460 nm. For further details, see *SI Text*.

Mapping and Cloning. We cloned the gene mutated in *n4430* animals using a combination of visual mapping, polymorphism mapping, and RNAi. We injected a PCR product containing the entire *T02G5.8* ORF and 2 kb of the upstream promoter region into *n4430* animals and observed that transgenes containing the single gene *T02G5.8* rescued the phenotype of fluorescence accumulation of *n4430* mutants. For a detailed description of mapping and cloning, see *SI Text*.

Analyses of Lifespan, Stress Resistance, and RNAi. Unless stated otherwise, lifespan assays were performed by standard methods at 20 °C using plates containing 10 μM FUdR. We used Prism 4 GraphPad software, which uses nonparametric log-rank test that compares strain distributions, to perform statistical analyses of survival curves. RNAi lifespan assays were performed according to the standard RNAi feeding protocol (32). See *SI Text* for details.

Heat-shock assays were performed at 32 °C using 1-d adults. For each genotype, 50–70 animals were transferred to 60-mm Petri plates seeded with OP50 bacteria, and viability was scored every 2–8 h after the shift to 32 °C. Statistical analysis was performed using Prism 4 software as for the lifespan assays.

Behavioral Assays. Classification of aging animals into behavioral classes was done as described by Herndon et al. (11). At least 80 animals per genotype were assayed. Student's *t* test was applied to determine statistical significance of the differences.

Electron Microscopy. Adult hermaphrodites of various ages were fixed in a mixture of 0.8% gluteraldehyde and 0.8% OsO$_4$ in 0.1 M cacodylate buffer (pH 7.2) for 1 h at 4 °C in the dark. Worms were cut in this fixative using a razor blade; head regions were discarded, and animals were postfixed in 2% OsO$_4$ in 0.1 M cacodylate buffer overnight at 4 °C. Four posterior regions were then aligned in each agar block, and the agar blocks were dehydrated and infiltrated into an Epon-Araldite mixture. Sections of the midbody region about 30-nM thick were collected on Pioloform-coated slotted grids. Sections were poststained with 1% uranyl acetate and Reynolds lead citrate. Sections were viewed using a JEOL 1200EX electron microscope at 80 kV.

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Berdichevsky et al.