

Supporting Online Material

RT-PCR analysis

Total RNA was extracted from approximately 20000 synchronized, sterile animals using trizol. To avoid mRNA contamination from eggs, which may not be susceptible to bacterial RNAi, we determined the efficacy of our RNAi treatments using a sterile strain, *fer-15(b26)*, *fem-1(hc17)*. Before harvest, animals were exposed to bacteria containing the RNAi vector or containing the *daf-2* RNAi construct from the L1 until the L4 larval stage or from day 8 until day 10 of adulthood. 4 ug of total RNA was used for one round of reverse transcription (RT) using oligo dT primers. Serial dilutions of the RT reaction (1:1 – 1:24) was used for PCR reaction using *daf-2* specific primers (5'-GGCACCGGTGCGGGAGCATTGAAACGAACAAAACACATC-3', 5'-TCCAGCACATTTTCATCACCTTATACC-3') to the 3' end of *daf-2*. RNAi was directed to a non-overlapping 5' end of *daf-2*. Serial dilutions of the RT reaction (1:1 – 1:20) was used for PCR reaction using *daf-16* specific primers (5'-ATCTATGATGATCTAGAATTCCCATCATGGG -3', 5'-CAAATCAAATGAATATGCTGCCCTCCAGC -3') to the 3' end of *daf-16*. RNAi was directed to a non-overlapping 5' end of *daf-16*. 4 µl of a 50 µl PCR reaction was analyzed on agarose gels using ethidium bromide.

Dauer assays

Wild-type hermaphrodites were allowed to lay eggs onto the control RNAi bacteria or *daf-2* RNAi bacteria at 20°C. The eggs were then shifted to 27°C and the presence of dauer larvae were scored 48 hours later when animals would normally be reproductive adults.

Lifespan, reproduction and stress assays

Lifespan, reproduction and stress assays were conducted at 20°C. We used the pre-fertile period of adulthood as the t=0 for lifespan analysis. Strains were grown at 20°C at for at least two generations before use in lifespan analysis. We used Statview 5.0.1 (SAS) software for statistical analysis and to determine means and percentiles. In all cases, *P* values were calculated using the logrank (Mantel-Cox) method.

The total number of progeny born to a single worm over time was measured in the following way. Briefly, worms hatched within a 1 hour period were collected and allowed to develop to the L4 stage. Once in the L4 stage, worms were individually placed onto separate plates. In all cases, at least 15 worms were used for each analysis. Worms were transferred to new plates every 12 hours and the resulting progeny were allowed to grow for two days until counted for progeny measurements. The % of total progeny was calculated for each time point by dividing the number of progeny produced on a time point by the total number of progeny produced over the course of the experiment.

For stress resistance assays, wild-type animals were transferred to bacteria expressing *daf-2* dsRNA at the indicated times. After reproduction had ceased, at day 5 of adulthood, worms were submerged in 50 µl of 0.4M paraquat dissolved in S-basal buffer

at 20°C. Death was determined on an hourly basis by the lack of movement after prodding with a platinum wire. At least 40 worms were used for each analysis.

RNAi inhibition using *dcr-1*

To lower *daf-2* activity during the larval stages only, wild-type animals were grown on bacteria expressing *daf-2* dsRNA and then shifted to bacteria expressing *dcr-1* dsRNA as day 1 adults. Control animals were grown during development on the RNAi bacteria containing the vector only and then shifted to *dcr-1* RNAi bacteria as day 1 adults. Animals were grown at 25°C.

Table 1. Effects of *daf-2* RNAi and *daf-16* RNAi on lifespan and brood size.

Treatment	Mean Lifespan ± s.e.m. (days)	<i>p</i> †	75 th Percentile* (days)	Average Brood Size ± SD ^Δ	(Total #Animals Died/Total) [§]
N2 shifted to <i>daf-2</i> RNAi as:					
Egg	35.5 ± 1.9	<0.0001 ‡	48	339 ± 41	61/81
L1	35.4 ± 2.6	<0.0001 ‡	52	354 ± 42	33/52
L2	36.2 ± 2.3	<0.0001 ‡	48	373 ± 43	41/48
L3	34.6 ± 1.9	<0.0001 ‡	48	380 ± 44	52/74
L4	35.3 ± 1.9	<0.0001 ‡	48	340 ± 47	56/71
Pre-Fertile Adult	29.1 ± 1.3	<0.0001 ‡	31	344 ± 46	56/71
Day 1 Adult	34.3 ± 2.0	<0.0001 ‡	45	386 ± 88	40/53
Day 2 Adult	29.9 ± 2.3	<0.0001 ‡	41	361 ± 53	36/37
Day 3 Adult	26.6 ± 1.8	0.0006 ‡	35	341 ± 50	42/52
Day 4 Adult	26.9 ± 1.8	0.0001 ‡	33	354 ± 53	33/39
Day 5 Adult	23.7 ± 1.0	0.0051 ‡	28	N.D.	27/35
Day 6 Adult	22.3 ± 1.4	0.0072 ‡	28	N.D.	35/44
Day 8 Adult	19.0 ± 0.8	0.5719 ‡	22	N.D.	49/53
Vector(control)	19.7 ± 0.8		22	371 ± 57	46/53
<i>daf-2(e1370)</i> shifted to <i>daf-16</i> RNAi as:					
Egg (α)	17.9 ± 0.6	<0.0001 [∅]	21	269 ± 39	35/39
Egg (β)	19.1 ± 0.8	<0.0001¥	25	N.D.	32/47
L1	17.8 ± 0.4	<0.0001 [∅] 0.8509 [#]	21	262 ± 37	74/86
L2	17.8 ± 0.4	<0.0001 [∅] 0.7903 [#]	21	295 ± 35	67/73
L2/L2d	17.1 ± 0.3	<0.0001 [∅]	20	312 ± 42	67/72

		0.8995 [#]			
L2d/L3	17.2 ± 0.5	<0.0001 ^Ø 0.5973 [#]	20	315 ± 43	67/69
L4	18.6 ± 0.4	<0.0001 ^Ø 0.1954 [#]	22	318 ± 41	67/80
Pre-Fertile Adult	20.3 ± 0.4	<0.0001 ^Ø 0.0006 [#]	22	310 ± 29	71/73
Day 1 Adult	22.2 ± 0.5	<0.0001 ^Ø <0.0001 [#]	25	278 ± 26	62/67
Day 2 Adult	20.7 ± 0.5	<0.0001 ^Ø 0.0005 [#]	25	270 ± 23	73/76
Day 3 Adult	21.9 ± 0.5	<0.0001 ^Ø <0.0001 [#]	25	253 ± 20	75/85
Day 4 Adult	24.3 ± 0.6	<0.0001 ^Ø <0.0001 [#]	27	271 ± 20	76/83
Day 5 Adult	25.6 ± 1.0	<0.0001 ^Ø <0.0001 [#]	29	255 ± 20	47/63
Day 6 Adult	24.2 ± 0.7	<0.0001 ^Ø <0.0001 [#]	28	N.D.	69/73
Day 7 Adult	24.5 ± 0.7	<0.0001 ^Ø <0.0001 [#]	27	N.D.	67/85
Day 10 Adult	28.9 ± 1.0	<0.0001 [¥] <0.0001 [∞] <0.0001 ^Ω	33	N.D.	44/51
Day 15 Adult	33.9 ± 1.6	<0.0001 [¥] <0.0001 [∞] <0.0001 ^Ω	38	N.D.	40/47
Day 23 Adult	41.5 ± 2.3	0.0498 [¥] <0.0001 [∞] 0.0951 ^Ω	51	N.D.	39/55
Day 30 Adult	44.6 ± 2.2	0.2344 [¥] <0.0001 [∞] 0.4830 ^Ω	54	N.D.	45/56
Day 40 Adult	49.6 ± 2.7	0.4305 [¥] <0.0001 [∞] 0.3095 ^Ω	56	N.D.	43/53
Vector (α)	41.2 ± 1.7	<0.0001 [#]	50	263 ± 21	59/74
Vector (β)	47.3 ± 2.5	<0.0001 [∞]	59	N.D.	43/57
Repression of <i>daf-2</i> RNAi by <i>dcr-1</i> dsRNA:					
N2 grown on RNAi bacteria	12.5 ± 0.5	N.D.	14	N.D.	54/60

during development, then shifted to <i>dcr-1</i> RNAi bacteria					
N2 grown on <i>daf-2</i> RNAi bacteria during development, then shifted to <i>dcr-1</i> RNAi bacteria	13.7 ± 0.6	0.0417 [∞]	17	N.D.	45/50
N2 grown on <i>daf-2</i> RNAi bacteria during development and adulthood	28.8 ± 1.1	<0.0001 [∞]	33	N.D.	50/50

* The 75th percentile is the age when the fraction of animals alive reaches 0.25.

† *P* values were calculated for individual experiments, each consisting of control and experimental animals examined at the same time.

§ The total number of observations equals the number of animals that died plus the number censored. Animals that crawled off the plate, exploded or bagged were censored at the time of the event. Control and experimental animals were cultured in parallel and transferred to fresh plates at the same time. The logrank (Mantel-Cox) test was for statistical analysis.

Δ Average brood size was calculated from the total brood size of at least 15 animals cultured independently in each trial.

‡ Compared with N2 worms grown on HT115 bacteria harboring the RNAi plasmid vector at 20°C.

Compared to *daf-2(e1370)* mutant worms cultured continuously on HT115 bacteria harboring the *daf-16* RNAi plasmid Egg (α), at 20°C, which were analyzed at the same time.

∞ Compared to *daf-2(e1370)* mutant worms cultured continuously on HT115 bacteria harboring the *daf-16* RNAi plasmid, Egg (β), at 20°C, which were analyzed at the same time.

∅ Compared to *daf-2(e1370)* mutant worms cultured continuously on HT115 bacteria harboring the RNAi plasmid, Vector (α), at 20°C, which were analyzed at the same time.

¥ Animals containing vector only (β), at 20°C, which were analyzed at the same time, were compared to *daf-2(e1370)* mutant worms cultured continuously on HT115 bacteria harboring the RNAi plasmid.

Ω p value after resetting T_0 of lifespans to time at which RNAi treatment was initiated. For instance; T_0 was set to 10 for assays in which the experimental population was treated with RNAi at day 10. Animals containing vector only (β), at 20°C, were compared to *daf-2(e1370)* mutant worms cultured continuously on HT115 bacteria harboring the RNAi plasmid.

æ Compared with N2 worms grown on HT115 bacteria harboring the RNAi plasmid vector during development, then shifted to bacteria expressing *dcr-1* dsRNA at 25°C.