Genes down-regulated in spaceflight are involved in the control of longevity in *Caenorhabditis elegans*

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How microgravitational space environments affect aging is not well understood. We observed that, in *Caenorhabditis elegans*, spaceflight suppressed the formation of transgenically expressed polyglutamine aggregates, which normally accumulate with increasing age. Moreover, the inactivation of each of seven genes that were down-regulated in space extended lifespan on the ground. These genes encode proteins that are likely related to neuronal or endocrine signaling: acetylcholine receptor, acetylcholine transporter, choline acetyltransferase, rhodopsin-like receptor, glutamate-gated chloride channel, shaker family of potassium channel, and insulin-like peptide. Most of them mediated lifespan control through the key longevity-regulating transcription factors DAF-16 or SKN-1 or through dietary-restriction signaling, singly or in combination. These results suggest that aging in *C. elegans* is slowed through neuronal and endocrine response to space environmental cues.

Lifespan and aging rate in metazoans are influenced by environmental factors, including temperature1, oxygen2, pheromone3, and food intake4. Accordingly, perturbation of sensory perception or signaling of mechanical, chemical, or osmotic stimuli changes the lifespan of *Caenorhabditis elegans*5. Microgravity has been shown to induce several physiological or pathological changes including disturbance of the sense of equilibrium and loss of muscle and bone mass, mainly from the observations in spaceflight6. However, how microgravity affects organismal aging and lifespan has not been well understood. This issue is not only intriguing from a basic scientific perspective but also important from a space medical aspect, because the duration of space missions will be increasing such as those for human exploration of other planets or colonization of the moon. To obtain some basic insights into aging in space, we examined an aging marker in space-flown *C. elegans* and explored the involvement of the genes whose expression was changed during spaceflight, in the control of lifespan.

**Results**

During the International *C. elegans* Experiment First (ICE 1st) project7, we investigated the effect of spaceflight on the formation of aggregates of a 35-glutamine repeat (Q35) in *C. elegans* transgenically expressing the (CAG)35-yellow fluorescent protein (YFP) gene in muscle, which normally increases with advancing age8 (Fig. 1). Q35 aggregate formation expressed as the number of aggregates per worm was found to be lower in worms flown in space from the L1 larva stage and L4/young adult stage than in matched ground control worms (Fig. 2A, B). This difference may be because of the possible changes in growth rate induced by spaceflight. However, growth of worms has been reported to be unaffected by spaceflight9,10. Moreover, the number of aggregates per body length was lower in space-flown worms than in ground control worms (Fig. 2D, E). This indicated that the spaceflight-induced suppression of Q35 aggregates was not ascribed to the spaceflight-induced changes in growth rate. Numbers of Q35 aggregates per total YFP fluorescence intensity in each worm, an indicator of Q35 expression, was also lowered by spaceflight (Fig. 2F, G). This showed that the spaceflight-induced suppression of Q35...
aggregates was not due to spaceflight-induced changes in Q35 expression. These results suggest that biomarkers of aging are expressed more slowly in space-flown C. elegans in than ground control worms.

Further, these findings led us to propose a working hypothesis that the space environment changes the expression of genes involved in the control of aging. To identify the possible longevity-control genes, we first used the data from a DNA microarray experiment conducted to examine changes in gene expression in response to spaceflight\(^9\) (The data set was deposited in the Gene Expression Omnibus (GEO) database (accession number: GSE36358) and WormBase (www.wormbase.org)), which showed that the expression of 48 genes increased by more than two-fold, and that of 199 genes decreased to less than half in the spaceflight conditions relative to the ground control\(^9\). Among these genes, we noticed that eleven genes likely related to neuronal or endocrine signaling were down-regulated in space-flown worms. We confirmed these observations and quantitatively evaluated the extent of decreased mRNA expression using real-time RT-PCR (Fig. 3). Second, we examined the effects of the inactivation of these eleven genes by loss- or reduction-of-function mutations and/or feeding RNA interference (RNAi) on the lifespan under ground laboratory conditions. We found that the inactivation of each of seven genes among these eleven genes by loss- or reduction-of-function mutations in all of these genes, except shk-1, cha-1, and cha-1, was not due to spaceflight-induced changes in Q35 expression. These results suggest that biomarkers of aging are expressed more slowly in space-flown C. elegans in than ground control worms.

To further explore the mechanism of lifespan extension by the inactivation of each of these genes, we investigated whether lifespan extensions are mediated through the DAF-16/FOXO transcription factor, which is a key factor in lifespan extension by reduction of insulin/IGF-1-like signaling (IIS)\(^13\), or through SKN-1, an Nrf-like xenobiotic-response factor, which is the other key factor in lifespan extension both by reduction of IIS\(^2\) and by dietary-restriction signaling\(^12\). Lifespan extensions caused by mutations either in ins-35, glc-4, unc-17, or shk-1 were totally abolished by daf-16 RNAi inactivation, whereas mutations either in gar-3, F57A8.4, or cha-1 still lived longer than wild-type worms under daf-16 RNAi (Fig. 4B, Supplementary Table S2 online). These results suggest that INS-35, GLC-4, UNC-17, and SHK-1 control lifespan through IIS/DAF-16 signaling. Further, the skn-1 RNAi completely abolished the lifespan extension induced by mutations in ins-35, glc-4, and shk-1, whereas mutants in unc-17, gar-3, F57A8.4, and cha-1 still lived longer than wild-type worms under skn-1 RNAi (Fig. 4C, Supplementary Table S2 online). These results suggest that INS-35, GLC-4, and SHK-1 control lifespan through SKN-1. To determine whether these lifespan extensions are mediated through dietary-restriction signaling, we examined the lifespan under the inactivation of eat-2, which induces feeding impairment-based dietary restriction\(^3\). The eat-2 RNAi further enhanced the extension of lifespan by mutations in ins-35, glc-4, unc-17, or gar-3, whereas the eat-2 RNAi shortened the lifespan of mutants of shk-1, cha-1, or F57A8.4 (Fig. 4D and Supplementary Table S2 online). These results suggest that SHK-1, CHA-1, and the F57A8.4 protein share a common lifespan control mechanism with dietary-restriction signaling.

In order to explore the involvement of these seven genes in the suppression of Q35 aggregate formation during spaceflight, we examined the effect of RNAi inactivation of some of these genes on Q35 aggregation. RNAi inactivation of gar-3, cha-1, and shk-1 reduced Q35 aggregation (Fig. 6), suggesting that GAR-3, CHA-1, and SHK-1 control Q35 aggregate formation.

We investigated whether these seven genes relate to the formation of dauer larvae, a long-lived growth arrest state under harsh environmental conditions. Mutation and RNAi of ins-35 and mutation of shk-1 were found to enhance pheromone-induced dauer formation, whereas mutations in glc-4, unc-17, or F57A8.4 suppressed it (Fig. 7A, B). These results suggest that INS-35 and SHK-1 may be related to dauer-associated life maintenance. Alternatively, some of these five genes may be involved in sensory perception or signaling related to dauer induction.

**Discussion**

We identified seven genes, which were down-regulated in space and whose inactivation extended lifespan under ground conditions. These genes encode proteins that are conserved across animal phylogeny and that are likely related to neuronal or endocrine signaling. How do these genes play a role in longevity control? One possibility is that they are involved in longevity control through sensory perception or signaling of space environmental cues. Mutations that cause defects in sensory perception and signaling systems, related to mechanical, chemical, thermal, and osmotic stimuli and pheromones, have been reported to extend the lifespan of C. elegans\(^2\). SHK-1\(^16\) and GLC-4\(^40\) are expressed in sensory neurons and UNC-17 in interneurons in sensory signaling\(^42\). F57A8.4 encodes a homolog of rhodopsin that is known to sense light\(^43\), suggesting that it may be involved in sensing stimuli in space environments. Pheromone-induced dauer formation was inhibited by the inactivation of unc-17, glc-4, or F57A8.4 and enhanced by that of ins-35 or shk-1 (Fig. 7A, B), suggesting that some of these genes are involved in lifespan control through sensory perception or signaling processes overlapping with dauer-switching processes. Alternatively, it may be possible that...
the inactivation of \textit{ins-35} or \textit{shk-1} extends lifespan, as some abnormally efficient dauer formation mutants such as \textit{daf-2} display adult-lifespan extension, and it has been hypothesized that these mutants ectopically express a dauer-related efficient life maintenance capability in the adult stage\textsuperscript{20}. Meanwhile, \textit{ins-35} is likely involved in endocrine signaling in the control of dauer formation and lifespan, as some of the 40 insulin-like peptides participate in regulating dauer formation and lifespan in \textit{C. elegans} through a cell-nonautonomous mechanism\textsuperscript{21}. The inactivation of \textit{ins-35} as well as \textit{glc-4} and \textit{shk-1} extended lifespan through the DAF-16 transcription factor (Fig. 4B). Reduction in IIS is known to extend lifespan through DAF-16\textsuperscript{12} similar to some sensory signaling mutants\textsuperscript{5}. Together with the observation that the space environments and IIS reduction shared some common features in terms of their effects on gene expression\textsuperscript{22} and that a hypergravity environment induces DAF-16 localization to nuclei we suggest that DAF-16 also mediates responses to altered gravity levels (the hypergravity experiment used \textit{Escherichia coli} as food instead of CeMM\textsuperscript{23}). On the other hand, the lifespan extension by the inactivation of \textit{glc-4}, \textit{ins-35}, \textit{shk-1}, or \textit{cha-1} required SKN-1 (Fig. 4C). SKN-1 in sensory neurons was reported to mediate lifespan determination during dietary-restriction signaling\textsuperscript{14}, although SKN-1 in intestine also plays a role in lifespan determination through IIS\textsuperscript{13}. A second possibility is that the genes in the present study are involved in longevity control through motor neuron-muscle signaling. SHK-1 functions in muscle\textsuperscript{24} and is expressed in neurons\textsuperscript{16}. CHA-1 and UNC-17, which are expressed in motor neurons, and GAR-3, which is expressed in motor neurons and muscle\textsuperscript{26}, play roles in acetylcholine transmission in motor neuron-muscle signaling. \textit{cha-1} and \textit{unc-17} are encoded by a single polycistronic cluster. They share the first noncoding exon; therefore, a common promoter termed as “cholinergic promoter” regulates the expression of both genes\textsuperscript{27}. Increased acetylcholine signaling enhanced polyglutamine aggregation in post-synaptic muscle cells in \textit{C. elegans}, suggesting that excess neuronal stimuli disrupt a balance in protein homeostasis in the target muscle\textsuperscript{28}. Conversely, reduction in neuromuscular signaling may enhance protein structure stabilization, which has been reported to be associated with the enhancement of longevity\textsuperscript{29}. Consistent with these studies, several mutations and RNAi

**Figure 2 | Spaceflight reduced Q35 aggregate formation.** Q35-YFP transgenic worms were inoculated into CeMM with 40 \(\mu\)M FUdR at L4/young adult stage (A, D, F) or without FUdR at egg stage (B, E, G), and incubated on ground for 5 days at 12°C. Then they were space-flown or on ground for 2 days at 12°C and for next 9 days at 20°C. In B, E, and G, only adult worms in the samples containing L1, L2 and adult stage worms were measured as adult worms were considered as the worms originally inoculated (See Legend for Fig. 1). Numbers of Q35 aggregates were counted from the photographs of worms obtained by a fluorescence microscope (as in (C)) using ImageJ software (Analyze Particles). (A, B) Numbers of Q35 aggregates per worm are shown. * vs ** and # vs ##: \(p<0.001\) (Student’s t test). (C) Photographs of YFP fluorescence of the worms flown in space from L1 stage and matched ground control are shown. Bars show 100 \(\mu\)m. (D, E) Numbers of Q35 aggregate formation per body length are shown. The lengths of individual worms were measured using the ImageJ software. * vs ** and # vs ##: \(p<0.001\) (Student’s t test). (F, G) Ratio of Q35 aggregate formation of space-flown worms to those of ground control worms, per total body fluorescence intensity are shown. Whole-worm YFP fluorescence was quantified using the measurement and analysis software VH-HIA5 (Keyence). * vs **: \(p<0.05\) and # vs ##: \(p<0.001\) (Student’s t test). Numbers of worms examined were as follows, A, D and F: space-flown \((n=15)\) and ground control worms \((n=47)\); B, E and G: space-flown \((n=138)\) and ground control worms \((n=367)\). Data are expressed as the mean ± S.E.
treatments recently predicted to disrupt depolarization of the muscle membrane have been shown to alter protein homeostasis within muscle\(^\text{20}\). In fact, the inactivation of *gar-3*, *cha-1*, and *shk-1* suppressed Q35 aggregation (Fig. 6), suggesting that GAR-3, CHA-1, and SHK-1 play a role in protein structure stability in muscle during motor neuron-muscle signaling. In case of *cha-1*, it has been shown that loss-of-function mutants evoke muscle protein degradation, which is suppressed by inhibitors of the ubiquitin-proteasome activity\(^\text{17}\). Polyclonal aggregation was also reported to be regulated by genes related to the ubiquitin-proteasome system\(^\text{35}\). These neuromuscular signaling changes may affect the muscle protein metabolism observed during spaceflight\(^\text{18}\). Alternatively, the possibility remains that SHK-1, which is expressed in sensory neurons\(^\text{24}\), controls polyclonal aggregation through a mechanism such as thermosensory neuronal circuit nonautonomously modulates protein misfolding in muscle and intestine cells via Ca\(^2+\)-dependent dense core vesicle neurosecretion\(^\text{17}\). The Ca\(^2+\)-dependent dense core vesicle neurosecretion mechanism modulates the HSP-70 levels in muscle to affect polyclonal aggregation\(^\text{39}\), and HSP-70 levels are elevated in response to spaceflight\(^\text{22}\). In either case, the suppression of Q35 aggregation observed in space might be explained in part by reduced expression of aggregation\(^\text{33}\), and HSP-70 levels are elevated in response to spaceflight\(^\text{22}\). In either case, the suppression of Q35 aggregation observed in spaceflight experiment and ground control. The mRNA levels of each gene were adjusted to that of gpd-2 (glyceraldehyde-3-phosphate dehydrogenase) mRNA, which was used as the internal standard. Bar graphs are ratios of the mRNA levels in spaceflight to those on ground (%). Data are expressed as the mean ± S.E. (n=3). All of them were significantly different (p<0.05) compared with ground control (Student's t-test).

endocrine signaling to induce some of “longevity-promoting” processes, including dietary-restriction signaling, stabilization of protein structure, or dauer-related efficient life maintenance during harsh conditions (Fig. 8). However, it should be noted that whether suppression of Q35 aggregation and the expression of the seven studied genes is due to microgravity or other environmental factors such as radiation is still uncertain, because the necessary experiments under the artificial gravitational field of the earth (1 G) in space environments have not been performed. Although further investigation regarding the effects of a decrease in the expression of the present genes comparable to the level in spaceflight and a simultaneous decrease in the expression of all these genes on lifespan is required, the present findings suggest that space-flown worms age more slowly compared with ground control worms and further predict that spaceflight extends worm lifespan. Male *Drosophila* lived shorter on the ground after spaceflight compared to controls that maintained on the ground through their lifetimes\(^\text{35}\). Further investigation is needed to compare aging and lifespan under microgravity and 1 G in space environments.

**Methods**

**Nematode strains and culture conditions.** Unless otherwise stated, the *C. elegans* strains were maintained at 20 °C on nematode growth medium (NGM) agar with *E. coli* OP50 as a food source, as previously described\(^\text{36}\). The N2 Bristol strain previously described\(^\text{16}\). The N2 Bristol strain was used as the wild-type *C. elegans*. The mutant strains used in this study were [D31; *gcl-4*](ok1212) II, [NL2099: *rfr-3(pk1426)* II, [BR1392: *shk-1(ok1581)* II, PR1152: *cha-1(n1152)* IV, TY1652: *cha-1(y226)* IV, CB933: *unc-17(n245)* IV, *F57A8.4(tm4341)* V, *VC670: gar-3*(gk337) V, TM290 ([x outcrossed]: ins-35([ok3297]) V, and Q35-VPF strain.

**Spaceflight experiment.** The spaceflight experiment was conducted as previously\(^\text{20}\). Briefly, eggs isolated after treatment of gravid adult worms with hypochlorite were incubated in a chemically defined liquid medium. Approximately 70 worms at the L4 larva/young adult stage were placed into a gas-permeable culture bag containing 2.5 mL CeMM with 40 μM 5-fluoro-2’-deoxyuridine (FUdR, Sigma Aldrich, St. Louis, MO, USA) which was used to prevent self-fertilization. Approximately 100 hatched L1 worms were also placed in a culture bag containing 2.5 mL CeMM without FUdR. Both of them were incubated for 5 days at 12 °C on the ground. After the launch, they were flown for 2 days at 12 °C on board the International Space Station for 9 days at 20 °C. When samples were returned to earth, they were flash frozen (freezing time <1 min) in liquid nitrogen within 2 h of landing\(^\text{21}\). Control worms underwent the same procedures at the same time on the ground.

**PolyQ aggregate measurement.** To measure polyQ aggregates, a *C. elegans* transgenic line expressing a chimeric fusion protein of a 35-glutamine repeat (Q35) and yellow fluorescent protein (YFP) in the body wall muscle, as described previously\(^\text{41}\), was used. The Q35 aggregates of worms were counted with a fluorescence microscope system (VB-G25/S200/L107010; Keyence CO., Osaka, Japan). Aggregates that were more than 1 μm long were defined as discrete structures.

**Real-time RT-PCR.** To confirm the gene expression differences between ground control worms and space-flown worms, real-time PCR was performed. Total RNA of a mixed population of space-flight and ground control N2 worms was reverse-transcribed to cDNA using ExScript RT reagent kit (TaKaRa Bio Inc., Shiga, Japan) and iCycler thermal cycle (Bio-Rad Laboratories Inc., Hercules, CA, USA). The reaction mixture for measuring the cDNA quantity was prepared using Premix EX Taq (TaKaRa Bio) according to the manufacturer’s instructions. Reaction and fluorescence monitoring were done using a Thermal Cycler Dice Real Time System (Takara Bio). The following primers were used:

- **gpd-2 forward**, 5’-acgcgaagcatcctactcactac-3’; **gpd-2 reverse**, 5’-acgcgaagcagtaatacag-3’;
- **cha-1 forward**, 5’-tgacaccagctggcata-3’; **cha-1 reverse**, 5’-gcgaccttaaaaactgtgga-3’;
- **gagatga-3’**; **f57a8.4 reverse**, 5’-ctaggcacccgaac-3’;
- **gar-3 forward**, 5’-tgcacaccatgcagctaa-3’; **gar-3 reverse**, 5’-ggcacccgtaaaactgtgga-3’;
- **ins-35 forward**, 5’-actgcaccaatgcaataa-3’; **ins-35 reverse**, 5’-tgctgcaccaagc-3’;
- **ena-4 forward**, 5’-ctaatttctcagatttc-3’; **ena-4 reverse**, 5’-ctggacaccagactctg-3’;
- **unc-17 forward**, 5’-ccagccacatatattgcctgga-3’; **unc-17 reverse**, 5’-ctggacaccagcagtaga-3’;
- **shk-1 forward**, 5’-tgaccaaatgtaatgcttc-3’; **shk-1 reverse**, 5’-atgcgttcagctgctc-3’;
- **f57a8.4 forward**, 5’-ttgctgacacactgtaata-3’; **f57a8.4 reverse**, 5’-tgcgaatccacagcagtaga-3’;
- **cha-1 forward**, 5’-cgctggtgcgtacctgga-3’; **cha-1 reverse**, 5’-caatggcgcaccaatcctgga-3’;
- **lgc-54 forward**, 5’-ccaatgctaatggcccagc-3’; **lgc-54 reverse**, 5’-tgctgacagctgctg-3’;
- **f57a8.4 forward**, 5’-ctggacaccagcagtaga-3’; **f57a8.4 reverse**, 5’-tgcgaatccacagcagtaga-3’;
- **cha-1 forward**, 5’-ttgctggtgcgtacctgga-3’; **cha-1 reverse**, 5’-caatggcgcaccaatcctgga-3’;
- **gpd-2 forward**, 5’-acaaccaatcgcaatgctgtg-3’; **gpd-2 reverse**, 5’-aatgcgttcagctgctc-3’;
- **ins-35 forward**, 5’-actgcaccaatgcaataa-3’; **ins-35 reverse**, 5’-tgctgcaccaagc-3’;
- **ena-4 forward**, 5’-ctaatttctcagatttc-3’; **ena-4 reverse**, 5’-ctggacaccagactctg-3’;
- **unc-17 forward**, 5’-ccagccacatatattgcctgga-3’; **unc-17 reverse**, 5’-ctggacaccagcagtaga-3’;
- **shk-1 forward**, 5’-tgaccaaatgtaatgcttc-3’; **shk-1 reverse**, 5’-atgcgttcagctgctc-3’;
- **f57a8.4 forward**, 5’-ttgctgacacactgtaata-3’; **f57a8.4 reverse**, 5’-tgcgaatccacagcagtaga-3’;
- **cha-1 forward**, 5’-cgctggtgcgtacctgga-3’; **cha-1 reverse**, 5’-caatggcgcaccaatcctgga-3’;
- **lgc-54 forward**, 5’-ccaatgctaatggcccagc-3’; **lgc-54 reverse**, 5’-tgctgacagctgctg-3’;
Figure 4 | Lifespan of the mutants in the genes down-regulated by spaceflight. (A) The survival curves of N2 and the mutants in the genes that were down-regulated by spaceflight, on NGM with UV-killed OP50 are shown. The percentage of live worms is plotted against adult age. Lifespan of the mutants in the seven genes were longer than those of the wild type. Day 0 corresponds to the L4 molt. Data are one of two experiments, each of which gave similar results, detailed parameters of which are indicated in Supplementary Table S1. The survival curves of N2 and the mutants treated with daf-16 RNAi (B), skn-1 RNAi (C) and eat-2 RNAi (D) from the L1 stage until death. Data are one of two experiments detailed parameters of which are indicated in Supplementary Table S2 with the data of worms treated with mock-vector RNAi bacteria as control experiments.

Figure 5 | The survival curves of N2 and the mutants in the genes that were down-regulated by spaceflight, in liquid CeMM. The percentage of live worms is plotted against adult age. Day 0 corresponds to the L4 molt. Mean adult lifespan ± S.E. (day), number of assayed worms and statistical significance with N2 wild type are: N2: 49.5 ± 0.7, n = 364; gar-3(gk337): 62.3 ± 1.1, n = 205, p < 0.001; ins-35(ok3297): 53.5 ± 1.3, n = 159, p < 0.01; glc-4(ok212): 59.0 ± 0.9, n = 286, p < 0.001; shk-1(ok1581): 55.2 ± 0.8, n = 204, p < 0.001; F57A8.4 (tm4341): 68.1 ± 1.2, n = 179, p < 0.001 and cha-1(p1532): 70.5 ± 1.1, n = 155, p < 0.001.

Figure 6 | The effect of RNAi of the genes on Q35 aggregate formation. The feeding RNAi of the designated genes against Q35-YFP transgenic worms was conducted as described in Methods for RNAi. L3 larval worms were placed on RNAi-expressing or mock-vector control bacteria. Worms were incubated for 2 days at 20 °C until they became gravid adults. The gravid adults were transferred to fresh RNAi-expressing bacteria lawns and allowed to lay eggs for 2 hours and removed and eggs were allowed to grow for subsequent assays. Aggregates of Q35-YFP were counted at 7 days after the treatment was started. Numbers of Q35 aggregates per worm are shown. Data are expressed as the mean ± S.E. * vs **: p < 0.001 (Student’s t test).
RNAi (66.1 conditions. The exchanges, some of which activate DAF-16 and/or SKN-1 transcription factors, induce “longevity-promoting” stimuli including microgravity, sensory perception, neuronal and endocrine signaling, and motorneuron-muscle signaling work through changes beyond the L2 stage under this condition.

Figure 7 | Pheromone-induced dauer formation in the mutants. Dauer formation of N2 and mutant worms (A) and RNAi fed rrf-3 worms (B) was scored after 3 days incubation with 1% crude pheromone extract at 25°C from the egg stage. The relative percentages of dauer arrest in the mutants compared with N2 (A) and in the RNAi fed rrf-3 worms compared with vector RNAi fed rrf-3 worms (B) are shown. Data are expressed as mean ± S.E. * vs ** and # vs ##; p<0.001 (Student’s t test). Percentages of dauer larvae (mean ± S.E. n=number of worms examined): N2 (19.9 ± 1.6%, n=367), gar-3(gk337) (16.9 ± 2.6%, n=418), ins-35(ok3297) (71.5 ± 3.9%, n=216), glc-4(ok212) (8.7 ± 2.0%, n=102), unc-17(e245) (1.3 ± 1.1%, n=171), shk-1(ok1581) (36.0 ± 3.1%, n=306), F57A8.4(tm341) (2.8 ± 1.4%, n=170), vector RNAi (16.2 ± 2.3%, n=215), and ins-35 RNAi (66.1 ± 3.1%, n=176). These data are from one of two or three trials, each of which gave similar results. cha-1(pr1152) worms did not grow beyond the L2 stage under this condition.

Figure 8 | Model for the action of longevity-control genes whose expressions are suppressed by spaceflight. In response to space-environmental stimuli including microgravity, sensory perception, neuronal and endocrine signaling, and motorneuron-muscle signaling work through changes in these gene activities. These changes, some of which activate DAF-16 and/or SKN-1 transcription factors, induce “longevity-promoting” processes including dietary-restriction signaling, stabilization of protein structure or dauer-related efficient life maintenance during harsh conditions.
dauer arrest. The dauer stage was confirmed by visual inspection of the animals as the presence of dark pigment granules and constriction of the body and pharynx and the loss of pharyngeal pumping.

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Author contributions

YH and SH conceived and designed the experiments. YH, SH, A. Higashibata, YM, YY, TK, A. Higashitani, and NI carried out the experiments and analysed the data. KK and TS prepared spaceflight experiments. NJS contributed materials. MT and NJS discussed results.

Additional information

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