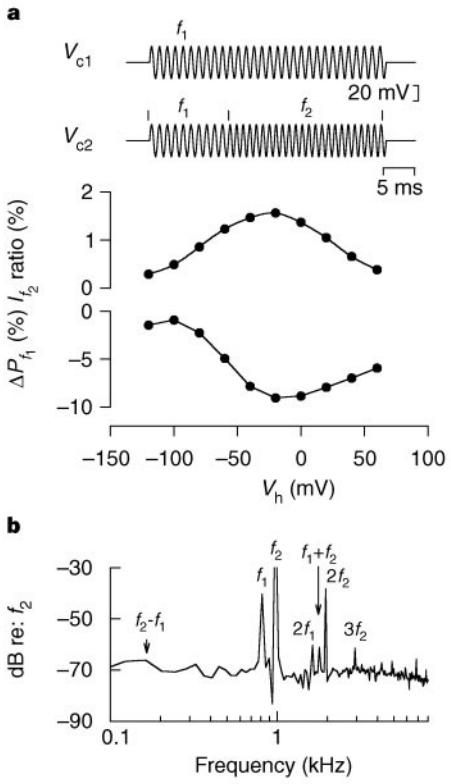

Figure 4 Both OHCs were voltage clamped to –40 mV and independently stimulated with pure sine-wave voltages. **a**, The upper two traces show the voltage stimuli delivered independently to each cell. During the initial part of the stimulus duration, each cell received a 20 mV peak sine wave at $f_1 = 813.8$ Hz; in the latter three-quarters of the stimulus duration, the OHC 2 frequency was switched to 976.5 Hz. Fast Fourier transform analysis was limited to the last half of stimulus duration to avoid transient responses. Comparisons were made before and after uncoupling by collapsing OHC 2. The upper plot shows the ratio of mechanically induced f_2 current (I_{f2}) in OHC 1 due to f_2 voltage stimulation in OHC 2. The ratio of the power of the f_1 component in the response of OHC 1 before and after OHC 2 was collapsed was also obtained. The change in this ratio (ΔP_{f1}) is shown in the lower plot. Negative percentage means that the f_1 response after collapse of OHC 2 was larger than that before; in other words, the mechanically induced f_2 component in OHC 1 suppressed the f_1 response in OHC 1. **b**, The spectrum of current response in OHC 2. The magnitude (in dB) is referenced to f_2 magnitude. Besides harmonic distortion products, the peak of sum frequency distortion ($f_1 + f_2$) is clearly visible.

lateral interactions may allow basilar membrane motion to be selectively enhanced at a particular location where passive vibration is maximal. In nonlinear systems, 10% feedback can have enormous consequences. The likely increase in OHC coupling with increasing frequency, supported by morphological evidence[17], may explain the finding that tuning is sharper at higher characteristic frequencies. In this model, distortion in the cochlea arises from the very process that promotes greater frequency resolution. □

Methods

Pieces of the organ of Corti, containing between 3 and more than 50 OHCs with associated Deiters' cells, were freshly isolated from the guinea-pig cochlea, and adjacent OHCs were separately whole-cell voltage clamped at room temperature using an Axon 200A and 200B amplifier. Membrane voltages were corrected for the effects of residual series resistance, which ranged from 3 to 7 MΩ. We used ionic blocking solutions to remove voltage-dependent ionic conductances so that capacitive currents could be analysed in isolation[29]. Gating currents were extracted using the P/-4 technique[30]. Pipette crosstalk artefacts at the onset of traces (first 50 μs) were subtracted from the data, using the artefacts obtained in the absence of mechanical coupling. All data collection and most analyses were performed with an in-house-developed, Windows-based whole-cell voltage-clamp program, jClamp (www.med.yale.edu/surgery/otolar/santos/jclamp.html), using a Digidata 1200 board (Axon).

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Signals from the reproductive system regulate the lifespan of *C. elegans*

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Understanding how the ageing process is regulated is a fascinating and fundamental problem in biology. Here we demonstrate that signals from the reproductive system influence the lifespan of the nematode *Caenorhabditis elegans*. If the cells that give rise to the germ line are killed with a laser microbeam, the lifespan of the

animal is extended. Our findings suggest that germline signals act by modulating the activity of an insulin/IGF-1 (insulin-like growth factor) pathway that is known to regulate the ageing of this organism. Mutants with reduced activity of the insulin/IGF-1-receptor homologue DAF-2 have been shown to live twice as long as normal[1–3], and their longevity requires the activity of DAF-16, a member of the forkhead/winged-helix family of transcriptional regulators[1,2,4,5]. We find that, in order for germline ablation to extend lifespan, DAF-16 is required, as well as a putative nuclear hormone receptor, DAF-12 (refs 6, 7). In addition, our findings suggest that signals from the somatic gonad also influence ageing, and that this effect requires DAF-2 activity. Together, our findings imply that the *C. elegans* insulin/IGF-1 system integrates multiple signals to define the animal's rate of ageing. This study demonstrates an inherent relationship between the reproductive state of this animal and its lifespan, and may have implications for the co-evolution of reproductive capability and longevity.

When *C. elegans* hatches, its gonad consists of four precursor

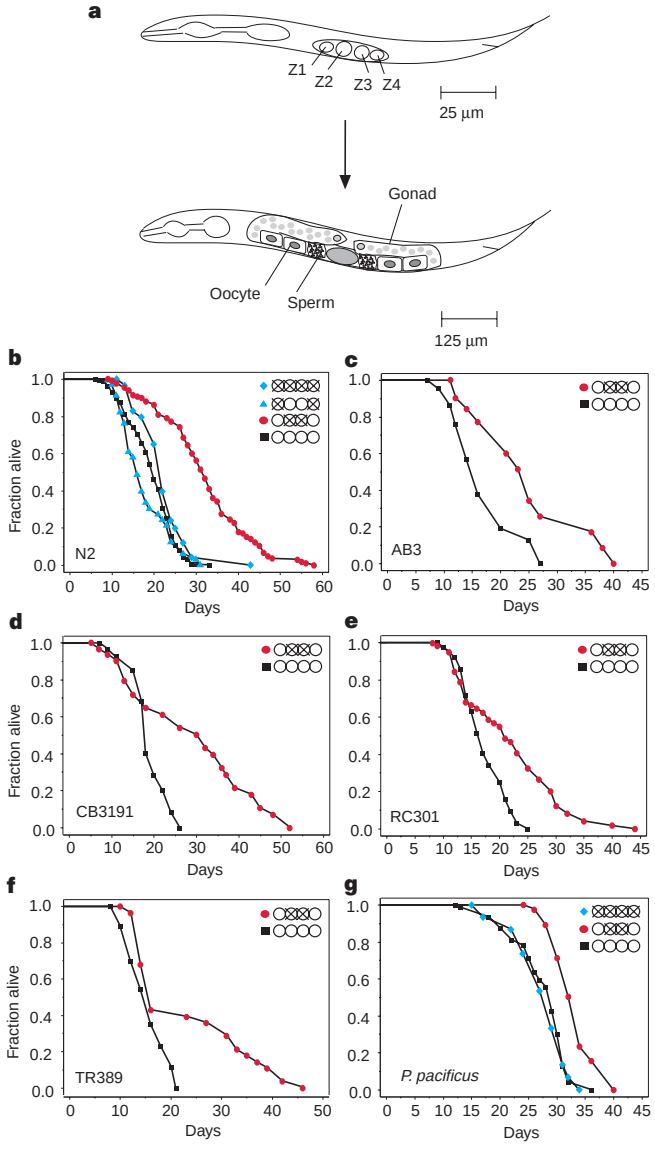

Figure 1 Response of wild-type nematodes to ablation of germline and somatic-gonad precursors. **a**, The *C. elegans* gonad. At hatching (top panel), the gonad consists of four cells. Z1 and Z4 give rise to the somatic gonad, and Z2 and Z3 give rise to the germ line of the adult (lower panel). Wild-type hermaphrodites have approximately 300 progeny; however, progeny production does not affect lifespan, since *fem* mutants (which lack sperm) have normal lifespans (also see text)[17]. The somatic gonad is needed for germline development[14], and in all of our experiments, ablating only the somatic gonad had the same effect as ablating both the somatic gonad and germ line. **b**–**g**, Survival curves. The curves shown represent the sum of all animals examined in one or more experiments (each experiment consisting of sets of ablated animals and control animals that were born at the same time). *n*, total number of animals observed (the number of independent experiments performed is given in parentheses); *m*, mean lifespan; –, ablated. *P* values were calculated between the experimental and control animals examined in a single experiment. **b**, Wild type (strain N2). Intact control, $n = 438(10)$, $m = 19.4 \pm 0.29$; Z2/3(–), $n = 146(7)$, $m = 31.8 \pm 0.97$, $P \leq 0.0001$ for each of 6 experiments (in the 7th, $P = 0.0023$); Z1/2/3/4(–), $n = 34(1)$, $m = 22.5 \pm 1.16$, $P = 0.33$ (similar results have been reported previously[1]); Z1/4(–), $n = 34(2)$, $m = 17.8 \pm 1.00$, $P = 0.87$ (experiment 1) and 0.35 (experiment 2). A second laboratory strain of N2, MRC N2, responded similarly to Z2/3 and Z1/4 ablation (data not shown). **c**–**f**, Survival of wild isolates of *C. elegans* following Z2/3 ablation. **c**, AB3 is an Australian strain. Intact control, $n = 35(1)$, $m = 17.1 \pm 1.33$; Z2/3(–), $n = 32(1)$, $m = 25.1 \pm 2.44$, $P = 0.0056$. **d**, CB3191 is an isolate from California. Intact control, $n = 33(1)$, $m = 19.0 \pm 0.80$; Z2/3(–), $n = 31(1)$, $m = 29.0 \pm 2.60$, $P = 0.0004$. **e**, RC301 is a German strain. Intact control, $n = 72(2)$, $m = 17.2 \pm 0.63$; Z2/3(–), $n = 69(2)$, $m = 21.8 \pm 1.15$, $P = 0.16$ and 0.0034. **f**, TR389 is from Wisconsin. Intact control, $n = 30(1)$, $m = 15.6 \pm 0.71$; Z2/3(–), $n = 31(1)$, $m = 23.4 \pm 2.09$, $P = 0.0012$. AB3 and RC301 exhibit clumping behaviour, the other strains do not[9]. **g**, *P. pacificus* (family *Diplogastridae*). $n = 83(2)$, $m = 27.5 \pm 0.82$; Z2/3(–), $n = 39(1)$, $m = 33.3 \pm 0.69$, $P < 0.0001$; Z1/2/3/4(–), $n = 29(1)$, $m = 27.6 \pm 1.14$, $P = 0.64$.

cells, called Z1, Z2, Z3 and Z4 (ref. 8). Z1 and Z4 give rise to the somatic gonad, and Z2 and Z3 give rise to the germ line (Fig. 1a). Previous studies have shown that if the entire gonad (Z1/2/3/4) is ablated with a laser microbeam at hatching, lifespan is unaffected; this suggests that the production of progeny *per se* does not influence the ageing process[1]. However, we found that if we ablated the germline precursor cells at hatching but left the somatic-gonad precursors intact, the animals remained active and healthy longer than normal and lived approximately 60% longer (Fig. 1b). Thus, in normal animals, a signal that depends on the germ line accelerates ageing.

To determine whether this phenomenon was confined to laboratory strains of *C. elegans*, we examined various wild *C. elegans* strains from around the world[9]. We found that all these strains lived longer after ablation of the germline precursors (Fig. 1c–f). We also ablated the germline precursors in *Pristionchus pacificus*, a nematode species thought to have diverged from *C. elegans* more than 100 million years ago[10], and found that its lifespan also increased (Fig. 1g). This phenomenon may therefore be conserved among different nematode families.

We next investigated which genes might be involved in this signalling system. *daf-16* activity is known to be required for the longevity of *daf-2* mutants. We found that *daf-16* is also required for the longevity of germline-ablated animals, because ablating the germline precursors in *daf-16* null mutants had no effect on lifespan (Fig. 2a). *daf-16* mutants do not have long lifespans, as would be expected if they were defective in the production or activity of the germline signal. Thus, we favour the interpretation that in normal animals, signals from the germ line inhibit DAF-16 activity. When the germline precursors are ablated, DAF-16 activity rises and lifespan is extended.

As both *daf-2* mutations and germline ablation increase lifespan, we investigated whether their effects might be additive or synergistic by ablating the germline precursor cells in four *daf-2* mutants, *e1370*, *m596*, *e1368* and *m41* (ref. 11). In each case, lifespan was extended dramatically (Fig. 3a–d); on average, the germline-ablated animals lived almost twice as long as intact *daf-2* controls, that is, almost four times as long as wild-type animals. Some of these animals lived as long as four months, remaining active much longer than intact *daf-2* animals.

Because *daf-2* mutations and germline ablation had synergistic effects on lifespan, germline signals and DAF-2 activity may act in parallel to shorten lifespan by downregulating DAF-16 activity. Thus, when either DAF-2 activity or germline signalling is decreased, DAF-16 activity rises, and when both are decreased, DAF-16 activity rises even further. As these *daf-2* mutations are not null alleles[11], it is also possible that ablating the germline precursors of *daf-2* mutants increases lifespan by downregulating residual DAF-2 activity. We consider this interpretation less likely, however, because double mutants carrying mutations in *daf-2* and another gene thought to function in the *daf-2* pathway, the phosphatidylinositol-3-OH kinase *age-1* (ref. 12), have lifespans similar to those of *daf-2* single mutants[2,13].

In the wild type, the somatic gonad must be present for germline ablation to extend lifespan[1] (Fig. 1b). This is consistent with two models. First, germline cells could produce a signal that shortens lifespan while the somatic gonad produces a different signal that

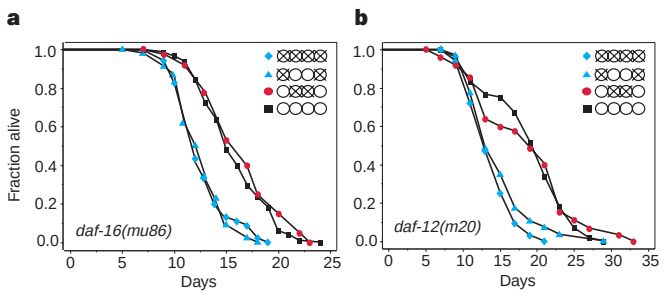

Figure 2 Responses of *daf-16* and *daf-12* mutants to ablation of germline and somatic-gonad precursors. **a**, Survival curves of the *daf-16* null mutant *mu86* (ref. 4). Intact controls, $n = 148(4)$, $m = 15.8 \pm 0.30$; Z2/3(–), $n = 42(1)$, $m = 16.6 \pm 0.68$, $P = 0.78$; Z1/2/3/4(–), $n = 53(2)$, $m = 12.9 \pm 0.34$, $P = 0.0018$ and 0.0095 in each of two experiments; Z1/4(–), $n = 45(2)$, $m = 12.6 \pm 0.36$, $P = 0.014$ and <0.0001. Ablating Z1 and Z4 also shortened the lifespans of two other *daf-16* alleles, *m26* and *m27*; ablating Z2 and Z3 in these animals did not significantly affect lifespan (data not shown). **b**, A *daf-12* putative null allele, *m20* (ref. 11). Intact control, $n = 105(3)$, $m = 19.2 \pm 0.61$; Z2/3(–), $n = 51(3)$, $m = 18.7 \pm 0.97$, $P = 0.70$, 0.89 and 0.46; Z1/2/3/4(–), $n = 32(1)$, $m = 14.0 \pm 0.52$, $P < 0.0001$; Z1/4(–), $n = 34(1)$, $m = 15.0 \pm 0.77$, $P = 0.0006$.

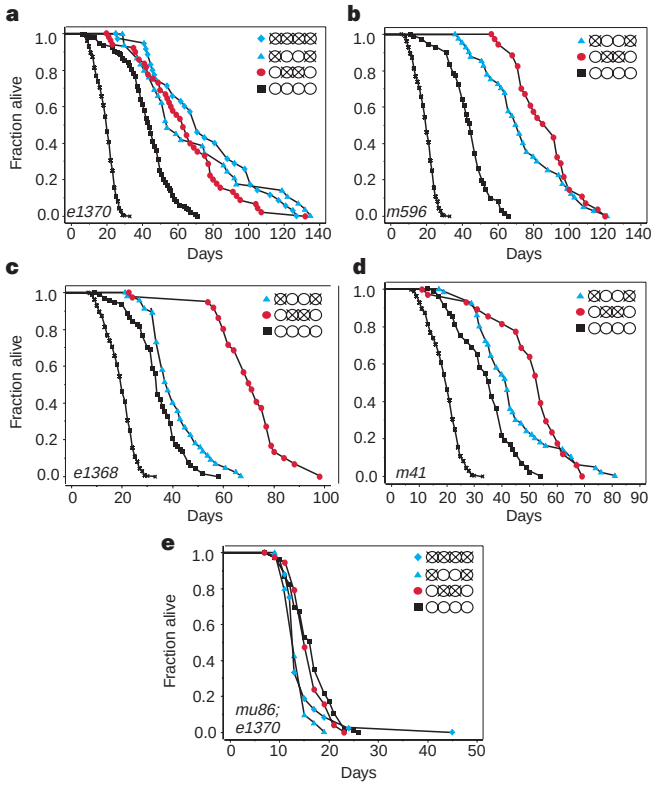

Figure 3 Responses of *daf-2* mutants to ablation of the germline and somatic-gonad precursors. **a–d**, Survival curves of intact N2 animals (left) are shown for comparison. **a**, Survival curve of *daf-2(e1370)*. Intact control, $n = 245(5)$, $m = 43.2 \pm 0.93$; Z2/3(–), $n = 59(2)$, $m = 64.5 \pm 3.47$, $P = 0.092$ (in an experiment in which $n = 13$) and <0.0001. Z1/2/3/4(–), $n = 40(1)$, $m = 75.7 \pm 4.68$, $P = 0.079$ vs the germline-ablated group, $P < 0.0001$ vs the control group. Z1/4(–), $n = 31(2)$, $m = 69.5 \pm 6.02$, $P = 0.33$ vs germline-ablated animals, $P = 0.019$ and <0.0001 vs control. **b**, *daf-2(m596)*. Intact control, $n = 89(2)$, $m = 43.9 \pm 1.34$; Z2/3(–), $n = 40(1)$, $m = 86.9 \pm 2.82$, $P < 0.0001$. Z1/4(–), $n = 43(1)$, $m = 73.9 \pm 3.43$, $P = 0.077$ vs germline-ablated, $P < 0.0001$ vs control. **c**, *daf-2(e1368)*. Intact control, $n = 110(3)$, $m = 34.3 \pm 1.01$; Z2/3(–), $n = 45(1)$, $m = 71.0 \pm 2.27$, $P < 0.0001$. Z1/4(–), $n = 45(2)$, $m = 41.1 \pm 1.47$, $P < 0.0001$ vs germline-ablated (for both experiments); $P < 0.0001$ (experiment 1, $n = 17$, $m = 49.1 \pm 2.25$) and $P = 0.43$ (experiment 2, $n = 28$, $m = 36.2 \pm 1.22$) vs control. **d**, *daf-2(m41)*. Intact control, $n = 90(2)$, $m = 34.3 \pm 1.20$; Z2/3(–), $n = 36(1)$, $m = 51.1 \pm 2.48$, $P < 0.0001$. Z1/4(–), $n = 51(2)$, $m = 43.9 \pm 1.91$, $P = 0.06$ vs germline-ablated, $P = 0.01$ and 0.02 vs control. The DNA sequences of the *m596* and *m41* mutations are not known. **e**, Survival curves of *daf-16(mu86); daf-2(e1370)* double mutants. Intact animals, $n = 155(4)$, $m = 16.4 \pm 0.38$; Z2/3(–), $n = 40(1)$, $m = 16.2 \pm 0.60$, $P = 0.03$ (this may not be significant since $P = 0.52$ if 91 additional control animals cultured but not born in parallel are included in the data set); Z1/2/3/4(–), $n = 50(2)$, $m = 14.6 \pm 0.77$, $P = 0.011$ and 0.24; Z1/4(–), $n = 46(1)$, $m = 13.7 \pm 0.31$, $P = 0.0081$.

lengthens lifespan. Second, germline ablation could cause the somatic gonad, which is normally silent, to emit a signal that extends lifespan. In principle, these two models could be distinguished by ablating the somatic-gonad precursors but not the germline precursors. Model 1 predicts that the animals would have short lifespans and model 2 that they would have normal lifespans. Unfortunately, this experiment is not possible because the germ line depends on the somatic gonad for its development[14]. However, we found that ablating the whole gonads of *daf-16* mutants shortened their lifespans relative to intact *daf-16* controls. This finding leads us to favour model 1, in which the germ cells and somatic gonad have opposite effects on lifespan. If *daf-16* mutants cannot respond to germline ablation but can respond to somatic-gonad ablation, killing the whole gonad would shorten lifespan (Fig. 2a).

We next investigated what gene activities might cause somatic-gonad ablation to shorten the lifespans of germline-ablated animals. One gene known to shorten lifespan is wild-type *daf-2*. Therefore, we asked whether the lifespan extension produced in *daf-2* mutants by ablating the germline precursors could be suppressed by ablating the somatic-gonad precursors. We found that, unlike in wild type, in two *daf-2* mutants, *e1370* and *m596*, it could not, since ablating either the germline precursors alone or both the germline and somatic-gonad precursors extended their lifespans (Fig. 3a, b). This suggests that the response to somatic-gonad ablation requires *daf-2(+)* activity. A simple model for DAF-2's role is that, in normal animals, signals from the somatic gonad promote longevity by inhibiting DAF-2 activity. When the somatic gonad is ablated, DAF-2 activity rises and lifespan is shortened.

Surprisingly, in a different *daf-2* mutant, *e1368*, ablation of the somatic gonad almost completely suppressed the effect of ablating the germ line, as occurs in wild-type animals (Fig. 3c). Thus, this mutation seemed to uncouple two lifespan-shortening activities of DAF-2. Like other *daf-2* mutations, the *e1368* mutation increases

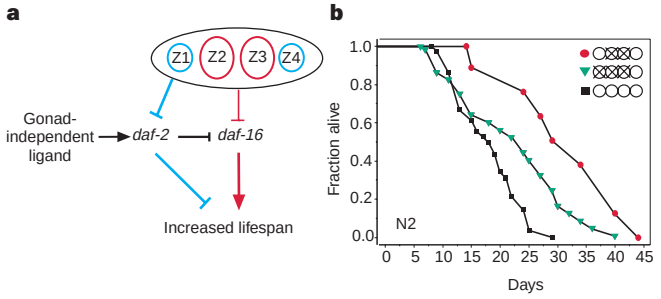

Figure 4 A model for the effects of reproductive signals on lifespan. **a**, In this model, two types of gonad-dependent signals influence lifespan. A signal from the germ cells decreases lifespan by downregulating the activity of *daf-16* (red lines) and *daf-12* (not shown). A counterbalancing signal from the somatic gonad increases lifespan by downregulating *daf-2* activity (blue lines). The somatic-gonad signal may be, or may control, a second insulin-like ligand for *daf-2*. In addition to signals from the reproductive system, a gonad-independent signal shortens lifespan by activating *daf-2*, which in turn downregulates *daf-16* activity. The site of integration of the three signals is not known. In principle, all these signals could act on the same cells, or different signals could act on different cells. Finally, we note that although both *daf-16* and *daf-12* function in the germline signalling pathway, only *daf-16* is absolutely required for the gonad-independent signalling pathway. In addition, *daf-12* may possibly have a role in somatic-gonad signalling that is influenced by *daf-2* (not shown, see text). **b**, Ablation of only one of the two somatic-gonad precursor cells does not fully suppress the effect of ablating the germline precursors ($P = 0.014$ between Z1/2/3(–) [$n = 38$, $m = 22.0 \pm 1.75$] and Z2/3(–) [$n = 10$, $m = 31.0 \pm 3.30$]; $P = 0.013$ between Z1/2/3(–) and intact control [$n = 40$, $m = 18.0 \pm 0.90$]). This suggests that the system is sensitive to the quantity of somatic-gonad signalling. Because the germline lineage is variable[8,14], it is not clear what effect ablating only one of the two germline precursors would have on germline development and final germ-cell number.

the lifespans of animals with intact gonads; therefore, it interferes with a gonad-independent activity of DAF-2 that reduces lifespan. However, unlike *daf-2(e1370)*, the *e1368* mutation did not abolish the response to somatic-gonad ablation. We saw a similar but less striking effect with the *m41 daf-2* mutant, which had a response intermediate between those of *e1370* and *m596* and that of *e1368* (Fig. 3d).

One possible reason that *e1368* behaves differently from other *daf-2* alleles is suggested by the molecular nature of these mutations. The *daf-2(e1370)* mutation, which blocks both *daf-2* activities, affects the putative tyrosine kinase domain of DAF-2 (ref. 3), whereas *e1368*, which affects only the gonad-independent activity, affects the putative ligand-binding domain[3]. Because intact *e1368* animals are long-lived, this lesion seems likely to prevent the DAF-2 protein from responding to an insulin-like ligand that is produced independently of the gonad. However, the fact that *e1368* can still respond to somatic-gonad ablation raises the possibility that this mutant DAF-2 protein retains the ability to respond to a second insulin-like ligand whose production depends on the somatic gonad. At present, no DAF-2 ligands have been identified, but the *C. elegans* genome contains a number of insulin/IGF-1-like sequences[15].

Another gene that can affect *C. elegans* lifespan is *daf-12*, which encodes a putative nuclear hormone receptor[6,7]. Mutations in *daf-12* slightly shorten the lifespans of otherwise wild-type animals[2,11] and also affect the lifespans of *daf-2* mutants (see below). We ablated the germline precursors in animals carrying *daf-12(m20)*, a putative null allele[11], and found that this did not affect lifespan (Fig. 2b). Thus, *daf-12* activity is required for germline ablation to extend lifespan. We also ablated the whole gonads of *daf-12* mutants and found that their lifespan was shortened (Fig. 2b). This finding is consistent with the model that DAF-12, like DAF-16, functions in the germline but not the somatic-gonad signalling pathway.

The *daf-12(m20)* mutation is known to lengthen the lifespans of *daf-2(e1370)* mutants but not those of *daf-2(m41)* mutants[2,11]. Similarly, loss of the whole gonad lengthens the lifespans of *daf-2(e1370)* mutants, but has a milder effect on those of *daf-2(m41)* mutants (Fig. 3a, d). The similar effects of whole-gonad ablation and *daf-12* mutations suggest that in *daf-2* mutants, *daf-12* activity could be required for signalling from both the somatic gonad and the germ line. However, it is difficult to reconcile this hypothesis with the finding that *daf-12* is not required for somatic-gonad signalling in an otherwise wild-type background. Perhaps DAF-12 activity is influenced by DAF-2.

Together, our findings can be explained by several models, but because of its relative simplicity we favour the model shown in Fig. 4a. In normal animals an insulin-like, gonad-independent ligand activates DAF-2, which in turn downregulates DAF-16 and shortens lifespan. Signals from the germ line also shorten lifespan by downregulating a pathway involving both DAF-16 and DAF-12. If germline signalling is inhibited, the activity of this DAF-16/DAF-12-dependent pathway rises and lifespan is increased. In addition, the somatic gonad produces a signal that lengthens lifespan by inhibiting DAF-2 activity. This somatic-gonad signal may be, or may control, a second insulin-like DAF-2 ligand. If somatic-gonad signalling is inhibited, changes in the level of this ligand lead to increased DAF-2 activity and shortened lifespan. Thus, in this model the reciprocal activities of germline and somatic-gonad signals arise because one signal acts on DAF-16, which lengthens lifespan, and the other acts on DAF-2, which shortens lifespan.

This model predicts that ablating germline or somatic-gonad precursors in *daf-16; daf-2* double mutants would have no effect. We found that ablating the germline precursors had no apparent effect, but that ablating the whole gonad shortened lifespan in a small but statistically significant way (Fig. 3e). This suggests that other factors may also contribute to this pathway.

We also investigated whether the lifespan of *C. elegans* was sensitive to quantitative differences in the level of somatic-gonad

signalling. We found that ablating one somatic-gonad precursor did not suppress the lifespan extension of germline-ablated animals to the same extent as ablating both precursors (Fig. 4b). Thus it seems likely that the animal can adjust its rate of ageing to the level of gonad signalling.

In addition to its role in ageing, the DAF-2/DAF-16 pathway controls the development of *C. elegans*[7]. When food is limiting, newly hatched animals do not progress to adulthood, but instead arrest development as reproductively immature dauer larvae until food is restored[7]. *daf-2* activity is required for animals to progress to adulthood (strong *daf-2* mutants form dauers constitutively), and *daf-16* activity is required for dauer formation (*daf-16* mutants cannot form dauers)[7]. Because of its role in dauer formation and because it encodes an insulin-receptor homologue, *daf-2* has been hypothesized to regulate the animal's development and rate of ageing in response to food signals, although this hypothesis has not been tested directly. In the present study, we have found that *daf-2* is also involved in a signalling pathway with a very different origin, the reproductive system. Thus, we speculate that the DAF-2/DAF-16 system may act globally to regulate the development and ageing of the animal in response to environmental signals such as food as well as internal signals reflecting the state of its reproductive system.

The signalling system described here has the interesting effect of placing the ageing process partially under the control of the germ line. This coupling could potentially have a beneficial effect on progeny production if the animal can monitor the state of its reproductive system and adjust its rate of ageing accordingly. In addition, coupling germline signals to lifespan could allow the processes of reproduction and ageing to co-evolve. For example, a mutation that slowed the growth of the germ line might simultaneously slow the animal's rate of ageing, enabling it to remain healthy and youthful long enough to produce progeny. □

Methods

Laser ablations. Ablations were performed on animals born within an hour of each other as described previously[16]. Survival of cells surrounding the gonad was monitored during the surgery, and successful ablation was confirmed by examining the germ cells and vulva under a dissecting microscope at the adult stage. The controls were grown in parallel with experimental animals and included some that were anaesthetized (1.0–1.5 M NaN_3) in parallel with ablated animals and some that were not. Anaesthesia had no effect on lifespan.

Lifespan analysis. Lifespans were determined at 20 °C as described previously[1]. The animals were cultured on standard agar plates (about five animals per plate). Day of birth was used as the first time point. All strains reached adulthood by 3 days after hatching, except for *daf-2(m41)* animals, which reached adulthood 2 days later (see ref. 11). Animals were considered dead when they ceased moving and responding to prodding. If they crawled off the plate, 'exploded' or died as 'bags of worms', they were censored at the last day of life. This step incorporated those worms into the data set until their censor date. The following are censored proportions in all groups examined, where a minus sign signifies ablated: 117/438 (N2 control), 7/34 [N2 Z1/2/3/4(−)], 11/38 [N2 Z1/2/3(−)], 1/34 [N2 Z1/4(−)], 38/146[N2 Z2/3(−)], 18/35 (AB3 control), 19/32 [AB3 Z2/3(−)], 8/33 (CB3191 control), 3/31 [CB3191 Z2/3(−)], 38/72 (RC301 control), 17/69 [RC301 Z2/3(−)], 4/30 (TR389 control), 3/31 [TR389 Z2/3(−)], 56/83 (*P. pacificus* control), 14/29 [*P. pacificus* Z1/2/3/4(−)], 13/39 [*P. pacificus* Z2/3(−)], 38/148 [*daf-16(mu86)* control], 3/53 [*daf-16(mu86)* Z1/2/3/4(−)], 1/45 [*daf-16(mu86)* Z1/4(−)], 18/42 [*daf-16(mu86)* Z2/3(−)], 67/245 [*daf-2(e1370)* control], 5/40 [*daf-2(e1370)* Z1/2/3/4(−)], 2/31 [*daf-2(e1370)* Z1/4(−)], 13/59 [*daf-2(e1370)* Z2/3(−)], 27/89 [*daf-2(m596)* control], 3/43 [*daf-2(m596)* Z1/4(−)], 10/40 [*daf-2(m596)* Z2/3(−)], 34/110 [*daf-2(e1368)* control], 0/45 [*daf-2(e1368)* Z1/4(−)], 13/45 [*daf-2(e1368)* Z2/3(−)], 36/90 [*daf-2(m41)* control], 1/51 [*daf-2(m41)* Z1/4(−)], 15/36 [*daf-2(m41)* Z2/3(−)], 40/105 [*daf-12(m20)* control], 0/32 [*daf-12(m20)* Z1/2/3/4(−)], 4/34 [*daf-12(m20)* Z1/4(−)], 6/51 [*daf-12(m20)* Z2/3(−)], 49/155 [*daf-16(mu86); daf-2(e1370)* control], 2/50 [*daf-16(mu86); daf-2(e1370)* Z1/2/3/4(−)], 3/46 [*daf-16(mu86); daf-2(e1370)* Z1/4(−)], 14/40 [*daf-16(mu86); daf-2(e1370)* Z2/3(−)]. Survival curves, mean lifespans and *P* values were determined non-parametrically; log-rank tests were used to assess the similarity between two groups. Statview 4.5 software (Abacus) was used for all analyses.

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The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives

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The sympathetic, parasympathetic and enteric ganglia are the main components of the peripheral autonomic nervous system[1], and are all derived from the neural crest[2]. The factors needed for these structures to develop include the transcription factor Mash1 (refs 3–5), the glial-derived neurotrophic factor GNDF (refs 6–8) and its receptor subunits[9–12], and the neuregulin signalling system[13], each of which is essential for the differentiation and survival of subsets of autonomic neurons. Here we show that all autonomic ganglia fail to form properly and degenerate in mice lacking the homeodomain transcription factor Phox2b, as do the three cranial sensory ganglia that are part of the autonomic reflex circuits. In the anlagen of the enteric nervous system and the sympathetic ganglia, Phox2b is needed for the expression of the GDNF-receptor subunit Ret and for maintaining Mash1 expression. Mutant ganglionic anlagen also fail to switch on the genes that encode two enzymes needed for the biosynthesis of the neurotransmitter noradrenaline, dopamine-β-hydroxylase and