



Regulation of Life-Span by Germ-Line Stem Cells in *Caenorhabditis elegans*
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sequence of calcium oscillations in the cell cycle is to trigger centrosome duplication, and in particular we suggest that IP₃-dependent calcium oscillations regulate the initiation of centrosome duplication through CaMKII activation. Consistent with the requirement for CaMKII to trigger centrosome duplication, chelation of calcium in cycling *Xenopus* egg extracts blocks the cell cycle before S phase entry, when centrosome duplication starts (13). CaMKII is localized on centrosomes (15, 16) and phosphorylates several centrosomal proteins in vitro (16). These proteins may be candidate substrate(s) for initiation of centrosome duplication by CaMKII.

References and Notes

1. J. Marx, *Science* **292**, 426 (2001).
2. K. Fukasawa, T. Choi, R. Kuriyama, S. Rulong, G. F. Vande Woude, *Science* **271**, 1744 (1996).
3. E. H. Hinchcliffe, G. Sluder, *Genes Dev.* **15**, 1167 (2001).
4. E. H. Hinchcliffe, C. Li, E. A. Thompson, J. L. Maller, G. Sluder, *Science* **283**, 851 (1999).
5. K. R. Lacey, P. K. Jackson, T. Stearns, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2817 (1999).
6. Y. Matsumoto, K. Hayashi, E. Nishida, *Curr. Biol.* **9**, 429 (1999).
7. P. Meraldi, J. Lukas, A. M. Fry, J. Bartek, E. A. Nigg, *Nature Cell Biol.* **1**, 88 (1999).
8. M. Okuda *et al.*, *Cell* **103**, 127 (2000).
9. H. A. Fisk, M. Winey, *Cell* **106**, 95 (2001).
10. A. R. Means, *FEBS Lett.* **347**, 1 (1994).
11. H. Schulman, *Curr. Opin. Cell Biol.* **5**, 247 (1993).
12. C. A. Swanson, A. P. Arkin, J. Ross, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1194 (1997).
13. A. A. Tokmakov, K. I. Sato, Y. Fukami, *J. Cell. Biochem.* **82**, 89 (2001).
14. M. A. Sanders, J. L. Salisbury, *J. Cell Biol.* **124**, 795 (1994).
15. Y. Ohta, T. Ohba, E. Miyamoto, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5341 (1990).
16. S. F. Pietromonaco, G. A. Seluja, L. Elias, *Blood Cells Mol. Dis.* **21**, 34 (1995).
17. R. Y. Tsien, *Biochemistry* **19**, 2396 (1980).
18. Y. Matsumoto, J. L. Maller, unpublished data.
19. E. Schiebel, *Curr. Opin. Cell Biol.* **12**, 113 (2000).
20. The S phase-arrested extracts were prepared as described (4). Briefly, freshly made cycling *Xenopus* egg extracts were aliquoted into 25 μ l per tube. Aphidicolin (10 μ g/ml, CalBiochem) and 400 demembrated sperm nuclei per microliter of extract were added before S phase started and the extracts were incubated for 1 or 6 hours at 2°C. For analysis, the S phase-arrested extracts were treated with nocodazole (30 μ g/ml, CalBiochem) for 15 min at 20°C, and kept on ice for 15 min. Centrosomes were then centrifuged onto a cover slip as described (21) and fixed with 1:1 methanol/acetone for 10 min at -20°C. For centrosome duplication assays, γ -tubulin monoclonal antibody (mAb) (Sigma) was used. For double immunostaining of centrioles, we used a rabbit polyclonal antibody to γ -tubulin (Sigma), α -tubulin mAb (Sigma), and centrin mAb 20H5 (a gift of M. Winey) (14).
21. L. Evans, T. Mitchison, M. Kirschner, *J. Cell Biol.* **100**, 1185 (1985).
22. M. J. Berridge, R. F. Irvine, *Nature* **341**, 197 (1989).
23. T. K. Ghosh, P. S. Eis, J. M. Mullaney, C. L. Ebert, D. L. Gill, *J. Biol. Chem.* **263**, 11075 (1988).
24. T. Lorca *et al.*, *Nature* **366**, 270 (1993).
25. R. J. Colbran, Y. L. Fong, C. M. Schworer, T. R. Soderling, *J. Biol. Chem.* **263**, 18145 (1988).
26. CaMKII(281-309) (CalBiochem) was added to CSF extracts on ice. After addition of calcium to 0.6 mM, the extracts were incubated at room temperature for the indicated time. For cyclin B immunoblots, a sheep affinity-purified polyclonal antibody to cyclin B2 was

- used. For immunoblots of phospho-Thr²⁸⁶ in CaMKII α , the membrane was incubated in 2% (v/v) goat serum (Sigma) containing rabbit anti-ACTIVE CaMKII polyclonal antibody (Promega). After washing in 0.5 M NaCl, 20 mM Tris-Cl (pH 7.5), and 0.05% (v/v) Tween-20, the immunoblot was developed with anti-ACTIVE qualified, horseradish peroxidase-conjugated donkey antibody to rabbit immunoglobulin G (Promega).
27. S. G. Miller, B. L. Patton, M. B. Kennedy, *Neuron* **1**, 593 (1988).
28. The activity of the holoenzyme of CaM kinase II purified from rat brain (a kind gift of M. D. Browning) was 2.14 μ mol min⁻¹ mg⁻¹ with synapsin I as substrate (29).
29. A. S. Nayak, C. I. Moore, M. D. Browning, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 15451 (1996).
30. J. Y. Su, R. E. Rempel, E. Erikson, J. L. Maller, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10187 (1995).
31. GST- Δ 34Xic1 (30) and GST-Cdc25A(C432A) (32) were prepared from recombinant baculovirus-infected Sf9 cells as described.
32. S. H. Kim, C. Li, J. L. Maller, *Dev. Biol.* **212**, 381 (1999).
33. Y. Matsumoto, J. L. Maller, unpublished data. Cdk2 phosphorylated on Tyr¹⁵ was detected as a band with increased electrophoretic mobility relative to Cdc2 in immunoblots of egg extracts using an antibody to phospho-Tyr¹⁵ of Cdc2 or Cdk2.
34. After incubation of the S phase-arrested extracts (4) for 6 hours, centrosomes and microtubules were centrifuged onto cover slips at room temperature

without depolymerization of microtubules. The cover slip was fixed with 99% methanol at -20°C for 5 min and then stained with a rabbit polyclonal antibody to γ -tubulin (Sigma) and α -tubulin mAb (Sigma).

35. Y. Matsumoto, J. L. Maller, unpublished data. Immunofluorescence staining was performed using mAbs (No-185 and No-63) to NO38, a *Xenopus* homolog of nucleophosmin (B23) (36). The antibodies were a kind gift of M. S. Schmidt-Zachmann. The extracts were fixed with methanol, methanol/acetone (1:1), or 10% methanol/10% formaldehyde, as described (8).
36. M. S. Schmidt-Zachmann, B. Hügler-Dorr, W. W. Franke, *EMBO J.* **6**, 1881 (1987).
37. We thank G. Sluder for providing adaptors for spinning down centrosomes from extracts and for help in setting up polarization microscopy in this laboratory; M. Winey for the antibody to centrin; M. D. Browning for the CaM kinase II purified from rat brain; M. S. Schmidt-Zachmann for the antibodies to NO38; members of this laboratory for helpful discussions and support, particularly F. Taieb for advice on the CaMKII experiments; and P. Eyers and E. Erikson for critical reading of the manuscript. Recombinant baculovirus-infected Sf9 cells were produced in the Tissue Culture/Monoclonal Antibody Core Facility at the University of Colorado Cancer Center (CA46934). Y.M. is an Associate and J.L.M. is an Investigator of the Howard Hughes Medical Institute.

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Regulation of Life-Span by Germ-Line Stem Cells in *Caenorhabditis elegans*

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The germ line of the nematode *Caenorhabditis elegans* influences life-span; when the germ-line precursor cells are removed, life-span is increased dramatically. We find that neither sperm, nor oocytes, nor meiotic precursor cells are responsible for this effect. Rather life-span is influenced by the proliferating germ-line stem cells. These cells, as well as a downstream transcriptional regulator, act in the adult to influence aging, indicating that the aging process remains plastic during adulthood. We propose that the germ-line stem cells affect life-span by influencing the production of, or the response to, a steroid hormone that promotes longevity.

Killing the germ-line precursor cells, Z2 and Z3, extends the life-span of *C. elegans* by ~60% (1). This longevity is not a result of sterility, because removing the entire reproductive system (germ line and somatic gonad) has no effect on life-span. In order for germ line-ablated animals to live longer than normal, they require DAF-12, a nuclear hormone receptor, and DAF-16, a forkhead-family transcription factor. We found that this

effect could be reproduced genetically: *mes-1(bn7)* mutants, which lack germ cells, were long lived (Fig. 1A), as were *glp-1(q158)* mutants (Fig. 1B) (2, 3). *glp-1* encodes the receptor for a germ-line proliferation signal that is produced by the distal tip cells of the somatic gonad (4-7). In *glp-1(q158)* mutants, Z2 and Z3 generate only a few germ cells, which then enter meiosis and differentiate as sperm (7). In both mutants, life-span extension was suppressed by a *daf-16* null mutation and by ablation of the somatic gonad precursor cells (Fig. 1) (8, 9). Many other mutants with defective germ-line proliferation were also long lived [Web table 1, experiments A and B (10); (9)].

The germ-line precursors are stem cells that divide continuously during development.

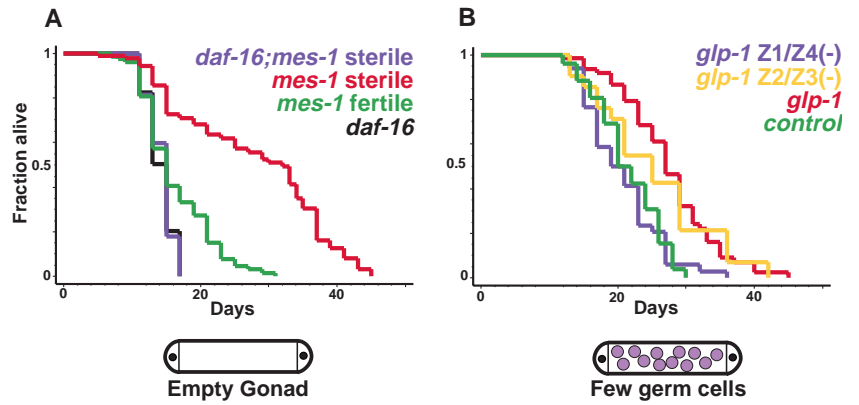
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Fig. 1. Life-spans of germ-line mutants. In each graph, experimental and control animals were grown in parallel. *n*, total number of animals observed in each experiment/total number of uncensored animals. *m*, mean adult life-span (days). (-), ablated. *P* values refer to experimental and control animals in a single experiment. **(A)** Life-spans of germ-line(-) mutants. At 20°C, *mes-1(bn7)* is 50% penetrant; half of the animals have normal germ lines (3). *mes-1(bn7)* fertile, *n* = 48/38, *m* = 15.8; sterile, *n* = 48/31, *m* = 29.8; Z1/Z4(-), *n* = 52/51, *m* = 17.2, *P* ≤ 0.0001. *daf-16(mu86)*, *n* = 54/26, *m* = 14.1; *daf-16(mu86); mes-1(bn7)* sterile, *n* = 62/22, *m* = 14.2, *P* = 0.90. Note that *daf-16* mutants are short-lived (25). **(B)** Life-spans of *glp-1(q158)* mutants. All of these strains contained the *dpy-19(e1259)* mutation. *dpy-19(e1259)* (control), *n* = 48/26, *m* = 21.5; *dpy-19(e1259) glp-1(q158)*, intact, *n* = 95/52, *m* = 27.4, *P* ≤ 0.0001; *dpy-19(e1259) glp-1(q158)*, Z2/Z3(-), *n* = 30/17, *m* = 25.3, *P* = 0.04; *dpy-19(e1259) glp-1(q158)*, Z1/Z4(-), *n* = 35/34, *m* = 20.7, *P* = 0.72.



As development progresses, germ cells located farthest from the distal tip cells enter meiosis and then differentiate into sperm (during the L4 stage) or oocytes (during adulthood) (6), but a pool of proliferating stem cells is maintained well into adulthood (Fig. 2).

We found that neither sperm nor oocytes are required for the germ line to shorten life-span. Previously, *fem-3(e1996)* mutants, which do not produce sperm and develop as females, were found to have normal life-spans (11). We found that three additional female mutants, *fog-1(q180)*, *fog-2(q71)*, and *fog-3(q470)* (12–14), also had normal life-spans [Web table 1, experiments C and D (10)]. In *daz-1(tj3)* mutants, oocyte precursor cells arrest development at meiotic prophase and subsequently undergo apoptosis (15). We found that the life-spans of *daz-1* mutants were similar to those of the wild type (Table 1, experiment A). In addition, germ-line ablation extended the life-span of males, which do not produce oocytes, to the same extent as for control hermaphrodites (Table 1, experiment B).

To determine whether germ-line stem cells might influence life-span, we forced these cells to exit mitosis and enter meiosis at different times using a temperature-sensitive allele of *glp-1*, *e2141* (16). Surprisingly, we found that inducing this switch by shifting *glp-1(ts)* mutants to the nonpermissive temperature either during development or in early adulthood (when the animals were already producing progeny) extended life-span (Fig. 2). This demonstrates that germ-line stem cells do influence life-span, and that they can exert their influence in the adult. The life-span increase we observed after shifting L4 larvae or young adults was smaller than we observed after shifting younger animals. Thus, germ-line proliferation in the larva may also influence life-span.

We also asked whether excessive germ-line proliferation might shorten life-span. We tested two mutants in which germ-line stem cells fail

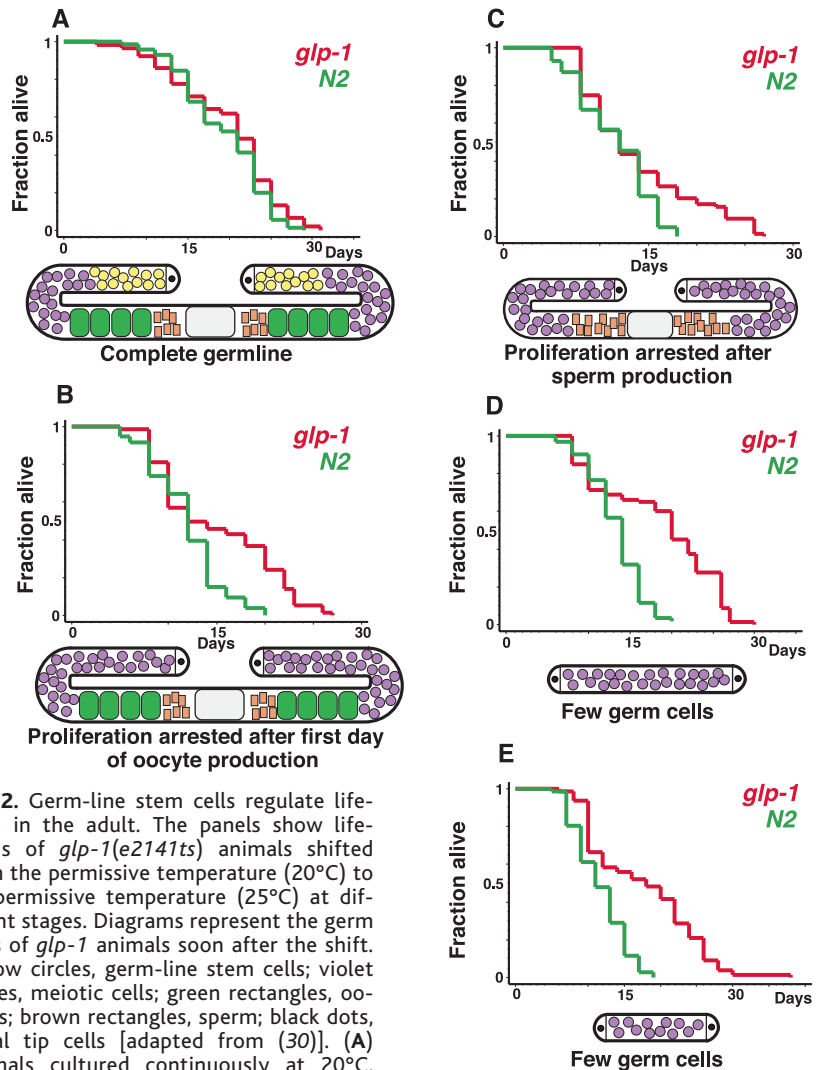


Fig. 2. Germ-line stem cells regulate life-span in the adult. The panels show life-spans of *glp-1(e2141ts)* animals shifted from the permissive temperature (20°C) to nonpermissive temperature (25°C) at different stages. Diagrams represent the germ lines of *glp-1* animals soon after the shift. Yellow circles, germ-line stem cells; violet circles, meiotic cells; green rectangles, oocytes; brown rectangles, sperm; black dots, distal tip cells [adapted from (30)]. **(A)** Animals cultured continuously at 20°C. Wild type (N2), *n* = 80/71, *m* = 19.4; *glp-1(e2141ts)*, *n* = 60/46, *m* = 19.9, *P* = 0.24. **(B)** Animals cultured at 20°C until day 1 of adulthood, and then shifted to 25°C. N2, *n* = 80/57, *m* = 11.9; *glp-1(e2141ts)*, *n* = 80/79, *m* = 15.0, *P* ≤ 0.0001. **(C)** Animals cultured at 20°C until L4 and then shifted to 25°C. N2, *n* = 80/64, *m* = 11.6; *glp-1(e2141ts)*, *n* = 65/64, *m* = 13.9, *P* = 0.008. **(D)** Animals shifted from 20°C to 25°C at L2. N2, *n* = 78/59, *m* = 13.3; *glp-1(e2141ts)*, *n* = 80/80, *m* = 18.6, *P* ≤ 0.0001. **(E)** Animals cultured continuously at 25°C. N2, *n* = 80/70, *m* = 11.6; *glp-1(e2141ts)*, *n* = 80/77, *m* = 17.7, *P* ≤ 0.0001. This experiment was repeated twice, yielding similar results (9).

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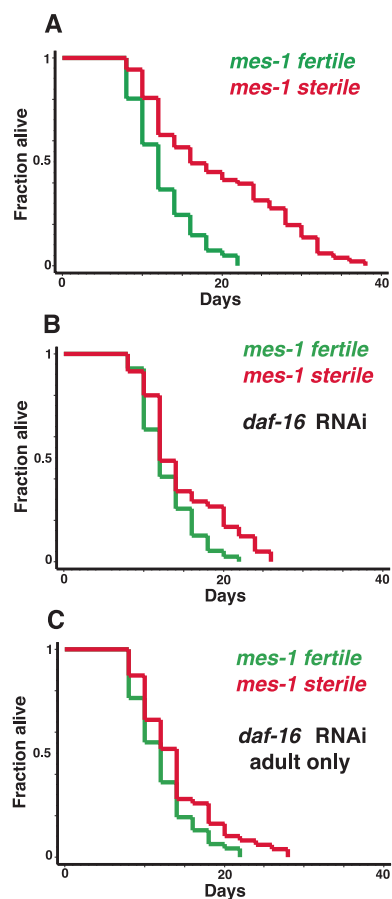


Fig. 3. Time of action of DAF-16 in germ-line regulation of life-span. Panels show the response of *mes-1(bn7)* fertile and sterile animals to a shift from control bacteria (vector only) onto bacteria expressing *daf-16* dsRNA. *daf-16* RNAi bacteria were grown on plates containing ampicillin, and carried an ampicillin-resistance gene. Bacteria carrying the plasmid vector alone were not ampicillin resistant. The sterile *Mes-1* phenotype was assayed using a dissecting microscope. (A) *mes-1(bn7)* mutants grown on bacteria carrying the plasmid vector. Fertile animals, $n = 70/41$, $m = 12.5$; sterile animals, $n = 66/51$, $m = 19.5$, $P \leq 0.0001$. (B) *mes-1(bn7)* grown on *daf-16* RNAi bacteria. Fertile animals, $n = 67/40$, $m = 12.9$; sterile animals, $n = 66/42$, $m = 14.9$, $P = 0.022$. (C) *mes-1(bn7)* shifted to *daf-16* RNAi bacteria at the onset of adulthood. Fertile animals, $n = 69/47$, $m = 12.2$; sterile animals, $n = 79/51$, $m = 14.1$, $P = 0.036$.

to differentiate and instead overproliferate, *glp-1(oz112gf)* and *gld-1(q485)* (17, 18). Both had short life-spans [Web table 1, experiments E and F (10)], although, in principle, this could also be caused by an independent, deleterious effect of germ-line overproliferation.

Because the germ line acts in the adult to influence life-span, we also investigated the timing of DAF-16 transcription factor function. To do this, we reduced *daf-16* activity at specific times using RNA interference (RNAi) (19, 20). We cultured *mes-1* mutants on bacteria expressing *daf-16* double-stranded RNA either throughout life, or only during

Table 1. Life-spans of *daz-1(tj3)* mutants and germ line-ablated males. The animals described in each experiment (A and B) were grown in parallel (28, 29). P values are relative to the control animals grown in parallel. Life-spans were measured at 20°C.

Strain	Mean life-span (75th percentile) in days	No. of animals observed/no. of uncensored animals	P Value
<i>Experiment A</i>			
Wild type	19.3 (23)	80/71	—
<i>daz-1(tj3)</i>	21.0 (26)	65/41	0.080
<i>Experiment B</i>			
<i>unc-50(e306); him-5(e1490)</i> males	16.4 (23)	52/20	—
<i>unc-50(e306); him-5(e1490)</i> males Z2/Z3 (—)	23.9 (33)	42/27	0.008
<i>unc-50(e306); him-5(e1490)</i> hermaphrodites	15.9 (21)	58/39	—
<i>unc-50(e306); him-5(e1490)</i> hermaphrodites Z2/Z3 (—)	24.0 (30)	65/43	0.0001

adulthood, and found that both treatments completely suppressed their longevity (Fig. 3, B and C). Thus, DAF-16 appears to be required specifically in the adult to effect germ-line modulation of life-span.

Many long-lived mutants are resistant to heat and oxidative stress (21–24). We found that this was also true of germ line-ablated animals [Web table 2 (10)]. It is possible that resistance to oxidative damage causes longevity; alternatively, the germ line could influence both stress resistance and longevity independently of one another.

In summary, we have found that the aging process of *C. elegans* is modulated, during adulthood, by the activity of germ-line stem cells. How might these cells affect aging? One possibility is that germ-line proliferation shortens life-span by increasing energy expenditure, channeling resources that could otherwise be used for maintaining cellular integrity toward growth and reproduction. However, there does not appear to be a simple trade-off between reproduction (or energy expenditure) and aging in this system; for example, animals that lack the entire reproductive system are not long-lived. Another possibility is that the role of germ-line stem cells is simply to produce more germ-line tissue, which then influences life-span regardless of its state of differentiation. However, the gonads of *daz-1* mutants have a much smaller mass than those of *glp-1* mutants shifted to high temperature as adults, because oocyte precursors die instead of becoming large, mature oocytes (15). Yet *daz-1* animals are not long-lived. Therefore the germ-line stem cells may be uniquely capable of influencing life-span. We propose that stem-cell proliferation influences life-span by affecting either the production of, or the response to, a steroid hormone ligand for DAF-12, which, in turn, promotes longevity. In addition, a signal dependent on stem-cell proliferation must regulate the nuclear localization of DAF-16 in somatic nongonadal tissues (25).

Surprisingly, we found that the aging pro-

cess is subject to modulation by the germ line in the adult. It was particularly striking that DAF-16 acts exclusively in the adult to mediate germ-line modulation of aging. Consistent with this, ablating the germ line at hatching causes DAF-16 to accumulate in nuclei only during adulthood (25). In contrast, when life-span is extended by mutations in the *daf-16*-dependent insulin/IGF-1 system, DAF-16 accumulates in nuclei throughout development and into adulthood (25).

In conclusion, our findings demonstrate that germ-line stem cells preside over two fundamental processes in the life cycle of *C. elegans*: reproduction and aging. These cells initiate the cascade of germ line development, thereby generating the pool of mature gametes, and they also regulate a steroid-dependent system that accelerates aging. By governing both processes, germ-line stem cells may help to coordinate the rate of aging with reproduction. Because killing germ-line precursor cells in *Drosophila melanogaster* also extends life-span (26), it is possible that this system is evolutionarily conserved.

Note added in proof: While this paper was in press, Gerisch *et al.* (31) reported that the germ line may regulate the activity of *daf-9*, which encodes a cytochrome p450 and may produce a steroid ligand for *daf-12*.

References and Notes

- H. Hsin, C. Kenyon, *Nature* **399**, 362 (1999).
- Life-spans were determined at 20°C, or at 25°C (when noted), as described (1, 11). Animals that crawled off the plates, exploded, or, less frequently, died as a "bag of worms" were censored at the last day they were seen alive and were incorporated in the data set until that date, as described (27). Survival curves, mean life-spans and P values were calculated nonparametrically; log-rank tests were used to assess the similarity between each two groups using Statview 4.5 software (Abacus).
- S. Strome, P. Martin, E. Schierenberg, J. Paulsen, *Development* **121**, 2961 (1995).
- F. E. Tax, J. J. Yeagers, J. H. Thomas, *Nature* **368**, 150 (1994).
- S. T. Henderson, D. Gao, E. J. Lambie, J. Kimble, *Development* **120**, 2913 (1994).
- J. E. Kimble, J. G. White, *Dev. Biol.* **81**, 208 (1981).
- J. Austin, J. Kimble, *Cell* **51**, 589 (1987).
- Laser ablations were performed as described (1). Successful ablation was confirmed by examining

the adult reproductive systems with a dissecting microscope.

9. N. Arantes-Oliveira, J. Apfeld, C. Kenyon, data not shown.
10. Supplemental material is available at *Science Online* at www.sciencemag.org/cgi/content/full/295/5554/502/DC1.
11. C. Kenyon, J. Chang, E. Gensch, A. Rudner, R. Tabtiang, *Nature* **366**, 461 (1993).
12. M. K. Barton, J. Kimble, *Genetics* **125**, 29 (1990).
13. T. Schedl, J. Kimble, *Genetics* **119**, 43 (1988).
14. R. E. Ellis, J. Kimble, *Genetics* **139**, 561 (1995).
15. T. Karashima, A. Sugimoto, M. Yamamoto, *Development* **127**, 1069 (2000).
16. J. R. Priess, H. Schnabel, R. Schnabel, *Cell* **51**, 601 (1987).
17. L. W. Berry, B. Westlund, T. Schedl, *Development* **124**, 925 (1997).
18. R. Francis, M. K. Barton, J. Kimble, T. Schedl, *Genetics* **139**, 579 (1995).
19. Animals were fed bacteria (BL21-DE3) harboring either a plasmid with T7 promoters opposing each other to express dsRNA of *daf-16* (pAD43), or an identical plasmid with no insert (control vector)

- (pAD12). dsRNA production was induced by administering 100 μ l of 0.1 M IPTG to the bacterial lawn 2 to 3 hours before adding the worms. The animals were moved to fresh bacterial lawns every 4 to 7 days.
20. L. Timmons, D. L. Court, A. Fire, *Gene* **263**, 103 (2001).
21. G. J. Lithgow, T. M. White, S. Melov, T. E. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7540 (1995).
22. P. L. Larsen, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8905 (1993).
23. J. R. Vanfleteren, *Biochem. J.* **292**, 605 (1993).
24. L. Guarente, C. Kenyon, *Nature* **408**, 255 (2000).
25. K. Lin, H. Hsin, N. Libina, C. Kenyon, *Nature Genet.* **28**, 139 (2001).
26. C. M. Sgro, L. Partridge, *Science* **286**, 2521 (1999).
27. J. Apfeld, C. Kenyon, *Cell* **95**, 199 (1998).
28. A *daz-1/+* heterozygote, *daz-1(tj3)/mIn1[dpy-10(e128)mIs14]*, lived slightly longer than the wild type. This effect was present in gonad-ablated animals (9), indicating that this life-span extension was independent of the gonad.
29. We also ablated the germ line of wild-type males and found that the ablation increased life-span significantly (36% mean increase, $P \leq 0.0001$). In this experiment, a large number of animals (23/73 intact, and 40/69 ablated) crawled off the plates. Many of the ablated animals crawled off the plates at old ages, suggesting that the animals that would have lived the longest were lost.
30. T. Schedl, in C. *elegans II*, D. L. Riddle, T. Blumenthal, B. J. Meyer, J. R. Priess, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997), pp. 241–269.
31. B. Gerisch et al., *Dev. Cell* **1**, 841 (2001).
32. We thank the Kenyon lab members for very helpful discussions, A. Sugimoto for *daz-1*, and the *Caenorhabditis* Genetics Center for other strains. Supported by a predoctoral fellowship from the Portuguese Foundation for Science and Technology and by the Gulbenkian Foundation (N.A.-O.); by a Howard Hughes Medical Institute predoctoral fellowship (J.A.); and by a postdoctoral fellowship from the Damon Runyon–Walter Winchell Foundation (A.D.). This work was supported by a grant from the NIH to C.K.

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Abnormal Vascular Function and Hypertension in Mice Deficient in Estrogen Receptor β

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Blood vessels express estrogen receptors, but their role in cardiovascular physiology is not well understood. We show that vascular smooth muscle cells and blood vessels from estrogen receptor β (ER β)-deficient mice exhibit multiple functional abnormalities. In wild-type mouse blood vessels, estrogen attenuates vasoconstriction by an ER β -mediated increase in inducible nitric oxide synthase expression. In contrast, estrogen augments vasoconstriction in blood vessels from ER β -deficient mice. Vascular smooth muscle cells isolated from ER β -deficient mice show multiple abnormalities of ion channel function. Furthermore, ER β -deficient mice develop sustained systolic and diastolic hypertension as they age. These data support an essential role for ER β in the regulation of vascular function and blood pressure.

Steroid hormones regulate a wide range of cellular events by activating a receptor family of transcription factors (1). Estrogens influence gene expression, growth, and cellular differentiation in target tissues by activating one or both of two estrogen receptors, ER α and ER β (2, 3). Estrogen receptors have been studied intensely in female reproductive physiology, but functional estrogen receptors

are also present and physiologically important in other tissues of both sexes, including the liver, brain, bone, and the cardiovascular system (4).

ER α and ER β both are expressed in vascular endothelial and smooth muscle cells, and in myocardial cells (5). ER β expression is induced in vascular cells following injury (6). Both estrogen receptors are necessary and sufficient for estrogen-mediated protection against measures of vascular injury in mice (7, 8). Estrogen receptors also regulate the expression of a number of vasodilator and vasoconstrictor proteins, including multiple components of the renin-angiotensin system (5). An association between an ER β gene polymorphism and systemic blood pressure in postmenopausal Japanese women (9) is the only genetic data to implicate estrogen receptors in blood pressure regulation.

Vascular tone is regulated by a complex set of variables that determine the contractile

state of vascular smooth muscle (10, 11). Inherited forms of hypertension have been shown to involve mutations in genes regulating renal salt reabsorption (12), but the pathogenesis in most individuals with hypertension remains unknown. Both acute and longer term changes in vascular tone due to estrogen are mediated principally by nitric oxide (5, 13–15). Endothelial-dependent vascular relaxation is mediated by production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) (16). Estrogen enhances production of NO by endothelial cells by increasing eNOS activity or expression of the eNOS gene, or both (17, 18). In vivo, estrogen enhances vasodilatation in both primates and humans with normal and abnormal endothelial function (5, 19, 20). Endothelial cell-independent vascular contraction is mediated by the direct actions of contractile agonists on vessel wall smooth muscle cells (11). Estrogen reduces vasoconstriction in vessels from which the endothelium has been removed from both humans and wild-type (WT) animals (21, 22), an effect that is blocked by pharmacologic inhibition of inducible nitric oxide synthase (iNOS) (22). To examine the role of estrogen receptors in vascular physiology, the existence of an estrogen effect on vasoconstriction was explored in vessels from WT mice.

We studied endothelial cell-independent vascular contraction (11, 22) in endothelium-denuded vascular rings from WT mice. Treatment of endothelial-denuded aortic rings from WT mice with 17 β -estradiol (E2) for 18 to 20 hours attenuated constriction to the alpha (1B)-adrenergic receptor agonist phenylephrine (PE) [decrease of 45% and 69%, respectively, $P < 0.05$ for both, (Fig. 1, A and B) (5, 21, 22)]. The attenuating effect of E2 on PE-induced constriction was partially reversed by treatment with the general inhibitor of nitric oxide synthases, N(G)-nitro-L-arginine (L-NNA) (Fig. 1A), and with the

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