Each species has a characteristic life-span, ranging from 10 days for the nematode Caenorhabditis elegans to 80 years for humans. Despite these vast differences in life-span, shared features of aging in diverse species support the existence of a common mechanism for life-span determination (7). Reductions in caloric intake, insulin/insulinlike growth factor–I (IGF-I) signaling, and free radical levels can lengthen the life-span of C. elegans daf-2 mutant (8–10), or the downstream age-1 phosphoinositide 3-kinase gene (11). Studies of age-1 (12, 13) showed that cin3 mRNA was confirmed to continue in aged animals lacking daf-2 activity from the entire AB cell lineage, which generates nearly all of the hypodermis and nervous system and half of the pharynx, have extended life-spans (14). However, extended life-spans (15). Moreover, animal lacking daf-2 activity from blastomere daughters of AB, which generate about half of the hypodermis, nervous system, and pharynx, did not show extended life-spans. These studies showed that daf-2 can act nonautonomously to regulate life-span but did not assign daf-2 longevity control to particular cell types. To define the cell type(s) from which the daf-2 insulinlike signaling pathway functions to control C. elegans life-span, metabolism, and development, we restored daf-2 pathway function to restricted cell types by using distinct promoters to express daf-2 or age-1 cDNAs in either neurons, intestine, or muscle cells of a daf-2 or age-1 mutant (16–22). Long life-span, metabolic changes, and dauer arrest were tested in these transgenic animals (Table 1). Because regulation of longevity may require gene activity over the entire life of the animal, the expression of green fluorescent protein (GFP) fusions to these promoters was confirmed to continue in aged animals (23).

The long life-span of daf-2 and age-1 mutants was rescued by neuronal expression of daf-2 or age-1, respectively, with the pan-neuronal unc-14 promoter (16, 24). Neurally restricted age-1 expression fully restored wild-type adult life-span to an age-1 (mk44) null mutant (Fig. 1). This rescue is comparable to the positive control, ubiquitous expression of age-1 from the dpy-30 promoter in the age-1 mutant (17, 25). Neurally restricted daf-2 expression from the unc-14 promoter also rescued the long life-span of daf-2(e1370) mutants, although not

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**Regulation of C. elegans Life-Span by Insulinlike Signaling in the Nervous System**

Catherine A. Wolkow,†* Koutarou D. Kimura,‡* Ming-Sum Lee,†* Gary Ruvkun††

An insulinlike signaling pathway controls Caenorhabditis elegans aging, metabolism, and development. Mutations in the daf-2 insulin receptor–like gene or the downstream age-1 phosphoinositide 3-kinase gene extend adult life-span by two- to threefold. To identify tissues where this pathway regulates aging and metabolism, we restored daf-2 pathway signaling to only neurons, muscle, or intestine. Insulinlike signaling in neurons alone was sufficient to specify wild-type life-span, but muscle or intestinal signaling was not. However, restoring daf-2 pathway signaling to muscle rescued metabolic defects, thus decoupling regulation of life-span and metabolism. These findings point to the nervous system as a central regulator of animal longevity.

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**References and Notes**

17. H. Vakis, unpublished results.
29. N. Larmarche et al., Cell 87, 519 (1996).
30. We thank T. Finkel, L. Feig, D. Toksoz, A. Hall, and J. Darnell for plasmids and adenoviruses and are also indebted to S. Takahashi for excellent technical assistance. Supported by KO8-HL-03547 (A.R.S.), NIH GM-54304 and GRASP Digestive Disease Center P30-DK43928 (B.H.C.), and NIH-CM-51586 (K.L.G.).

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as completely as the comparable age-1 rescued animals, but to the same extent as ubiquitous daf-2 expression from the dpy-30 promoter (Fig. 2). The long daf-2(e1370) lifespan is also rescued when daf-2 is expressed from the unc-119 promoter, another neuron-specific promoter (18,18).

Animals with age-1 expression restricted to a smaller set of neurons were also examined. The promoter for the mechanosensory neuron-specific beta-tubulin mec-7 was used to express age-1 in about 10 neurons, including the six touch neurons (19). age-1 activity in these neurons showed little or no rescue of the long lifespan phenotype (Fig. 1), indicating that this neural type or this small number of neurons does not contribute in a major way to longevity control.

In contrast to neuronal expression of daf-2 and age-1, restoration of daf-2 pathway activity to muscles from the promoter for muscle myosin, unc-54, was not sufficient to rescue the long lifespan of daf-2 or age-1 mutants (Figs. 1 and 2 and Table 1) (20). Similarly, expression of daf-2 or age-1 in the intestine, the major site of fat storage, from the ges-1 promoter does not rescue life-span as efficiently as neural expression of these genes (21). Intestinally restricted daf-2 expression showed weak rescue of the long lifespan of daf-2(e1370), whereas intestinal age-1 expression did not rescue the long lifespan of age-1(mg44) mutants. The lack of longevity rescue was observed in multiple transgenic lines for both daf-2 and age-1. In addition, the muscle or intestinal age-1 and intestinal daf-2 transgenes expressed sufficient gene activities to partially rescue dauer arrest phenotypes, showing that the fusion genes were functional.

The aging and metabolic outputs of daf-2 pathway signaling are separable. Restoring age-1 function ubiquitously to the nervous system or to muscle rescued the metabolic defects of age-1 mutants (Table 1) (26). Paradoxically, given that the intestine is the major fat storage depot, expression of age-1 in the intestine only weakly rescued the metabolic defects. Ubiquitous and neuronal, but not intestinal or muscle, daf-2 expression reduced the level of fat accumulation in daf-2 mutants. The rescue of the metabolic phenotype was highly correlated with the rescue of dauer arrest by these transgenes (see below), suggesting that the metabolic rescue may be a consequence of dauer arrest rescue, or vice versa.

An important finding is that rescue of metabolic defects in daf-2 pathway mutants is not correlated with rescue of long lifespan. Shifting metabolism away from fat accumulation, by restoring age-1 activity to muscle or intestine, is not sufficient to induce a short life-span. Because intestine and muscle are major sites of metabolic storage and activity, it is significant that they are not the major organs of longevity control. Rather, the lack of daf-2 pathway signaling in the nervous system of these chimeric animals may induce their long lifespan.

The dauer arrest phenotype of daf-2 pathway mutants was rescued most effectively by restoring signaling to neurons (Table 1) (27). Expression of age-1 in muscle, intestine, or the mec-7-expressing neurons also rescued dauer arrest, but less efficiently than pan-neuronal expression. However, expression of daf-2 in muscle, unlike of age-1, did not rescue dauer arrest.

The conclusion that it is the expression of age-1 or daf-2 within the nervous system that rescues aging depends on the unc-14 or unc-119 promoters driving expression only in neurons. One measure of specificity is that GFP fusions of these promoters show only expression in the expected tissues at all stages tested (23). However, weak expression below the detection limit of GFP in other cell types is possible. The phenotypes of age-1 and daf-2 allelic series show that the highest gene activities are needed for life-span regulation and less is needed for regulation of metabolism and dauer arrest (for example, maternally contributed age-1 activity can rescue both metabolism and dauer arrest, but not the longevity phenotype). Substantial age-1 and daf-2 gene activity is probably required to allow such potent longevity rescue in the nervous system, suggesting that weak promoter promiscuity is not a problem. Expression level differences between the neuronal, muscle, and intestinal promoters also do not appear to account for more potent life-span rescue by transgenes expressed from neuronal-specific promoters. We observed high levels of GFP expression from the muscle-specific unc-54 promoter, relative to the other promoters used (28). Consistent with this observation, unc-54 is more abundant in the 100,000 sequence C. elegans expressed sequence tag (EST) database, which contains 90 unc-54 ESTs compared with 14 unc-14 ESTs and 2 ges-1 ESTs. Thus, it is the activation of the daf-2 pathway in the nervous system in particular, rather than high expression levels in any tissue, that rescues the longevity extension of daf-2 pathway mutations.

Genetic mosaic analyses of daf-2 support the interpretation that daf-2 signaling from the nervous system controls longevity. Wild-

Table 1. Phenotypes of animals with cell-type-restricted daf-2 pathway signaling.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cell type with age-1 or daf-2</th>
<th>Life-span* (days ± SD)</th>
<th>Intestinal fat level (% of population)</th>
<th>Fertile adults % of pop.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>10.3 ± 1.9 (402)</td>
<td>83</td>
<td>15</td>
</tr>
<tr>
<td>age-1(−) background</td>
<td>None</td>
<td>19.5 ± 5.1 (362)</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>age-1(−) [m+n−]</td>
<td>Pdpy-30::age-1 All cells</td>
<td>11.6 ± 3.4 (198)</td>
<td>97</td>
<td>3</td>
</tr>
<tr>
<td>Punc-14::age-1 All neurons</td>
<td>10.5 ± 3.7 (198)</td>
<td>97</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Pmec-7::age-1 Ten neurons</td>
<td>Punc-54::age-1 Muscle</td>
<td>21.2 ± 6.7 (201)</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Pjes-1::age-1 Intestine</td>
<td>18.8 ± 5.8 (343)</td>
<td>20</td>
<td>80</td>
<td>66</td>
</tr>
<tr>
<td>daf-2(−) background</td>
<td>Pdpy-30::daf-2 All cells</td>
<td>28.8 ± 4.8 (101)</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>daf-2(−)</td>
<td>Punc-14::daf-2 All neurons</td>
<td>16.8 ± 3.9 (101)</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Punc-119::daf-2 All neurons</td>
<td>Punc-54::daf-2 Muscle</td>
<td>18.3 ± 9.8 (128)</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>Pjes-1::daf-2 Intestine</td>
<td>24.9 ± 9.3 (143)</td>
<td>10</td>
<td>90</td>
<td>35</td>
</tr>
</tbody>
</table>

*Results are sum of two independent lines for each construct, except for Punc-14::daf-2 and Pdpy-30::daf-2 (one line each). When data from multiple lines were summed, the independent data from each line were consistent. ¶ Maternally rescued age-1(−) progeny of age-1(−) hermaphrodites. † Progeny of maternally rescued age-1(−) hermaphrodites. § Partial rescue of dauer arrest, resulting in development into sterile adults, was observed in the following cases: 18% of age-1(−); Pmec-7::age-1 larvae, 42% of age-1(−); Punc-54::age-1 larvae, and 7% of age-1(−); Pjes-1::age-1 larvae develop into sterile adults. ¶¶ Maternally rescued daf-2(−) dauer larvae. ¶¶¶ Not tested.
type life-span required daf-2 pathway activity in the AB blastomere descendents, which include nearly all of the nervous system as well as much of the ecdoderm and half of the pharynx (15). Thus, although those studies could not map daf-2 pathway longevity control specifically to neurons, they are consistent with the results of the transgenic approach reported here. It may be important that the highest DAF-2 abundance revealed by antibodies to DAF-2 is in the nerve ring (29).

The more potent regulation of longevity by neuronal daf-2 pathway signaling could represent distinct outputs from some or all neurons or, simply, that neuronal promoters restore daf-2 pathway activity to more cells than muscle or intestinal promoters. The adult hermaphrodite nematode contains 302 neuronal cells, 95 body-wall muscle cells, and 20 intestinal cells. Although neurons constitute the largest number of cells, the total mass of neurons, which are smaller than nematode muscle or intestinal cells, is considerably less than the mass of muscle or intestinal cells. Further analysis of animals with daf-2 pathway signaling restored to restricted neuronal subtypes should elucidate whether C. elegans life-span is controlled by a specific set of neurons or, alternatively, by a quorum of neurons that can be of any neuronal subtype. Although mammalian insulin signaling in the nervous system has not yet been examined for longevity control, there is evidence that insulin signaling in neurons and neuroendocrine cells controls feeding and metabolism (30, 31).

Expression of daf-2 pathway genes in muscle, intestine, or the mec-7–expressing neurons can regulate dauer arrest and metabolism but not life-span. The daf-2 pathway-mediated regulation of dauer arrest and metabolism can be decoupled from life-span regulation, and these represent distinct outputs of the daf-2 insulin-like signaling pathway. daf-2 pathway signaling in neurons may result in the production of a senescence-inducing neuroendocrine output that is not produced in muscle or intestine. Intestinal and muscle cells may contribute dauer and metabolic regulatory signals. The somatic gonad has been shown to affect life-span through the daf-2 pathway (32). The life-span signals from the somatic gonad may act to regulate neuronal daf-2 pathway activity. C. elegans life-span is also extended 1.5-fold when daf-2 activity was lost from the EMS lineage, which contributes the intestine, some pharyngeal cells, the somatic gonad, and the sex muscles, suggesting that daf-2 signaling in one or several of these cell types is also necessary for normal aging (15). Our results also point to a minor role of intestinal daf-2 pathway signaling in aging.

How does daf-2 signaling from neurons control life-span? C. elegans dauer larvae express high levels of the free radical–scavenging enzymes, catalase and SOD (9). The expression of catalase and Mn-SOD is transcriptionally regulated by DAF-16, the major target of daf-2 pathway signaling (11, 12, 33). Furthermore, mutations in cdc-1 cytosolic catalase reduce the life-span of daf-2 mutants.
showing that ctl-1, and possibly other free radical–scavenging enzymes, are required for long life-span (11). Neurons may be particularly sensitive to free radical damage during aging. In fact, overexpression of Cu/Zn SOD in only motorneurons can extend Drosophila life-span by 48% (3).

We propose that neuronal DAF-2 activity maintains relatively low levels of free radical–scavenging enzymes, such as SOD-3 and CTL-1, by antagonizing the DAF-16 transcription factor. Loss of DAF-2 activity from neurons, relieving the negative regulation of DAF-16, induces higher expression levels of these free radical–scavenging enzymes, thereby protecting neurons from oxidative damage. By this model, neuronal daf-2 signaling might regulate an organism’s life-span by controlling the integrity of specific neurons that secrete neuroendocrine signals, some of which may regulate the life-span of target tissues in the organism. Our results, together with those from Drosophila, suggest that oxidative damage to neurons may be a primary determinant of life-span.

References and Notes
22. Supplementary material is available at Science Online at www.sciencemag.org/feature/data/1054300.shl.
23. GFP intensity was scored in wild-type animals after 8 days of adulthood at 25.5°C. Animals with Punc-14::GFP or Punc-54::GFP showed intense GFP fluorescence that was similar to that observed at larval stages (Punc-14::GFP, 92% of 8-day-old adults showed high GFP intensity, n = 24 animals, three lines; Punc-54::GFP, 92% of 8-day-old adults had high levels of GFP with a platinum wire. Life-span is defined as the day animals were at the L4 larval stage (time t = 0) until the day they were scored as dead. The results in Table 1 are the sum of at least two independent lines, except for Pdpy-30::daf-2 and Punc-14::daf-2, which are from one line each.
24. Neither Pdpy-30::age-1 or Punc-14::age-1 can provide maternal age-1 activity, as shown by the segregation of nontransgenic dauer-arrested animals, in contrast to the potent maternal rescue of dauer arrest in age-1(m+–) animals. Thus, age-1(mg44); Punc-14::age-1 and age-1(mg44); Pdpy-30::age-1 animals are (m+–) for age-1 activity in the nervous system and ubiquitously. An (m+–) age-1(mg44) animal develops into a long-lived dauer but cannot grow to reproductive adulthood, and the life-span cannot be directly compared. Thus, the rescuing activity of the strains bearing the Punc-14::age-1 and Pdpy-30::age-1 transgenes is underestimated by comparison with the m+– age-1 (mg44), but it is the only control available.
25. L4 animals grown at 20°C were fixed in 1% paraformaldehyde and subjected to three freeze-thaw cycles and then incubated on ice for 10 minutes. Fixed animals were washed and dehydrated through an ethanol series before staining with Sudan Black B solution. The level of fat accumulation was scored by comparing the relative size and number and density of fat droplets in the intestine and homogenates relative to positive age-1(mg44) (m+–) and daf-2(e1370) dauers and negative (wild-type L4 larvae) controls.
26. Eggs were laid by gravid adults overnight at 25.5°C (age-1 strains) or at 15°C (daf-2 strains) and then shifted to 25.5°C. Dauer and L4 larvae of young adults were scored 3 days after egg lay. At least two independent experiments were performed for each strain, and the results from each were summed.
27. We thank A. Fire, O. Hobert, J. McGehee, B. Meyer, Y. Ohshima, D. Pilgirm, and J. Szé for providing promot- er plasmids; P. Delerme and Y. Liu for technical assistance; and I. Mori and members of the Ruvkun lab for helpful discussions. This work was supported in part by NIH grant AG14161. C.A.W. was supported by a postdoctoral fellowship from the Leukemia and Lymphoma Society. K.D.K. was supported by the Jap- anese Society for the Promotion of Science and CREST of Japan Science and Technology Corporation.
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Structure of the Protease Domain of Memapsin 2 (β-Secretase) Complexed with Inhibitor
Lin Hong,1 Gerald Koelsch,1 Xinli Lin,1 Shili Wu,1 Simon Terzyan,2 Arun K. Ghosh,3 Xuenjun C. Zhang,2 Jordan Tang1,4†

Memapsin 2 (β-secretase) is a membrane-associated aspartic protease involved in the production of the β-amyloid peptide in Alzheimer’s disease and is a major target for drug design. We determined the crystal structure of the protease domain of human memapsin 2 complexed to an eight-residue inhibitor at 1.9 angstrom resolution. The active site of memapsin 2 is more open and less hydrophobic than that of other human aspartic proteases. The substrate locations from S0 to S4 are well defined. A kink of the inhibitor chain at P3 may be mimicked to provide inhibitor selectivity. The accumulation of the 40–42 residue β-amyloid peptide (Aβ) in the brain is a key event in the pathogenesis of Alzheimer’s disease (AD) (1). Aβ is generated in vivo through proteolytic cleavage of the membrane-anchored β-amyloid precursor protein (APP) by β- and γ-secretases. The γ-secretase activity, which cleaves APP within its transmembrane domain, is likely mediated by the transmembrane protein presenlin 1 (2–4). The β-secretase cleaves APP on the luminal side of the membrane and its activity is the rate-limiting step of Aβ production in vivo (5). Both proteases are potential targets for Alzheimer’s disease (AD). Our group (6) and others (7) recently cloned a human brain aspartic protease, memapsin 2 or BACE, and demonstrated it to be β-secretase. Memapsin

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