growth factor stimulation, and ROS is known to activate Src and Jak family kinases (*11*, *23*, *24*). Thus, Rac1 may both localize STAT3 to kinase complexes and contribute to the activation of the kinases themselves.

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

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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.

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 (*1*). Reductions in caloric intake, insulin/insulinlike growth factor–I (IGF-I) signaling, and free radical levels can lengthen the life-span of animals as divergent as nematodes, *Drosophila*, and mammals (*1*–*3*). Mutations that decrease *C. elegans daf-2* insulin/IGF-I–like receptor or *age-1* phosphoinositide 3-kinase signaling result in severalfold extension of adult life-span (*2*, *4*, *5*) and increased accumulation of fat (*2*, *6*–*8*). Null mutations in *daf-2* or *age-1* cause constitutive arrest at the dauer larval stage; dauer larvae have slowed metabolic rates, store large amounts of fat, express high levels of antioxidant enzymes such as catalase and superoxide dismutase (SOD), and live longer than reproductive adults (*9*). One reasonable hypothesis is that free radicals generated as by-products of metabolism damage cellular components (*10*). The lower level of free radicals in *daf-2* insulinlike signaling mutants is essential for life-span extension: The life-span extension in a *daf-2* mutant requires the activity of a cytosolic catalase *ctl-1* (*11*).

The cells where *daf-2* pathway signaling is required for signaling normal life-span are not known. Insulinlike signaling may regulate metabolism and free radical production directly in aging skin or muscle, or these pathways may act in key signaling centers that then coordinately control the senescence of the entire organism. In addition, it is not clear whether insulin/IGF-I regulation of life-span is simply coregulated with metabolism or whether the metabolic shifts are mechanistically connected to the life-span regulation. Several components of the *daf-2* pathway, such as *akt-1*, *pdk-1*, and *daf-16*, are widely expressed throughout development (*12*–*14*). Studies of *daf-2* genetic mosaic animals showed that 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 (*15*). However, mosaic animals 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*). Neuronally restricted *age-1* expression fully restored wild-type adult life-span to an *age-1(mg44)* 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*). Neuronally 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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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*).

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 life-span 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 life-span 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 life-span of *daf-2(e1370)*, whereas intestinal *age-1* expression did not rescue the long life-span 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 life-span. 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 life-span.

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.

Genotype	Cell type with *age-1* or *daf-2*	Life-span* (days ± SD) (*n*)	Intestinal fat level (% of population)			Fertile adults	
			Low	High	(*n*)	% of pop.	(*n*)
Wild type	Wild type	10.3 ± 1.9 (402)	83	17	(51)	100	(148)
age-1(−) background							
age-1(−) (m+z−)†	None	19.5 ± 5.1 (362)	75	25	(20)	100	(>100)
age-1(−) (m−z−)‡	None	–	5	95	(41)	0	(>100)
Pdpy-30::age-1	All cells	11.6 ± 3.4 (198)	94	6	(75)	100	(1185)
Punc-14::age-1	All neurons	10.5 ± 3.7 (198)	97	3	(35)	100	(749)
Pmec-7::age-1	Ten neurons	17.9 ± 6.8 (160)	100	0	(39)	65§	(897)
Punc-54::age-1	Muscle	21.2 ± 6.7 (201)	95	5	(38)	55§	(1400)
Pges-1::age-1	Intestine	18.8 ± 5.8 (343)	20	80	(66)	50§	(597)
daf-2(−) background							
daf-2(−)	None	28.8 ± 4.8 (101)	5	95	(20)‖	0	(175)
Pdpy-30::daf-2	All cells	15.0 ± 2.9 (21)	30	70	(18)	73	(161)
Punc-14::daf-2	All neurons	16.8 ± 3.9 (101)	25	75	(11)	95	(173)
Punc-119::daf-2	All neurons	18.3 ± 9.8 (128)	nt¶	nt¶	–	38	(513)
Punc-54::daf-2	Muscle	24.9 ± 9.3 (143)	0	100	(18)	0	(335)
Pges-1::daf-2	Intestine	19.2 ± 3.5 (197)	10	90	(35)	8	(911)

*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*(−); *Pges-1::age-1* larvae develop into sterile adults. ‖*daf-2*(−) dauer larvae. ¶nt, 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 ectoderm 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 *ctl-1* cytosolic catalase reduce the life-span of *daf-2* mutants,

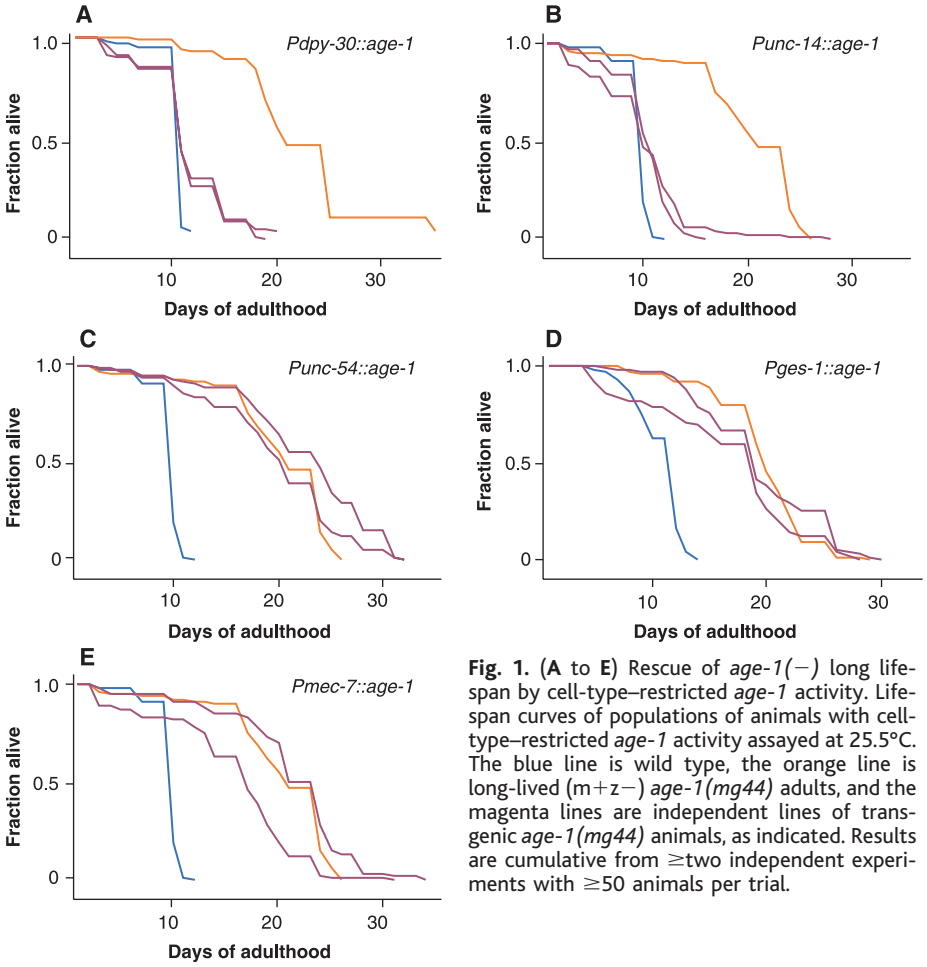

Fig. 1. (**A** to **E**) Rescue of *age-1(−)* long life-span by cell-type–restricted *age-1* activity. Life-span curves of populations of animals with cell-type–restricted *age-1* activity assayed at 25.5°C. The blue line is wild type, the orange line is long-lived (m+z−) *age-1(mg44)* adults, and the magenta lines are independent lines of transgenic *age-1(mg44)* animals, as indicated. Results are cumulative from ≥two independent experiments with ≥50 animals per trial.

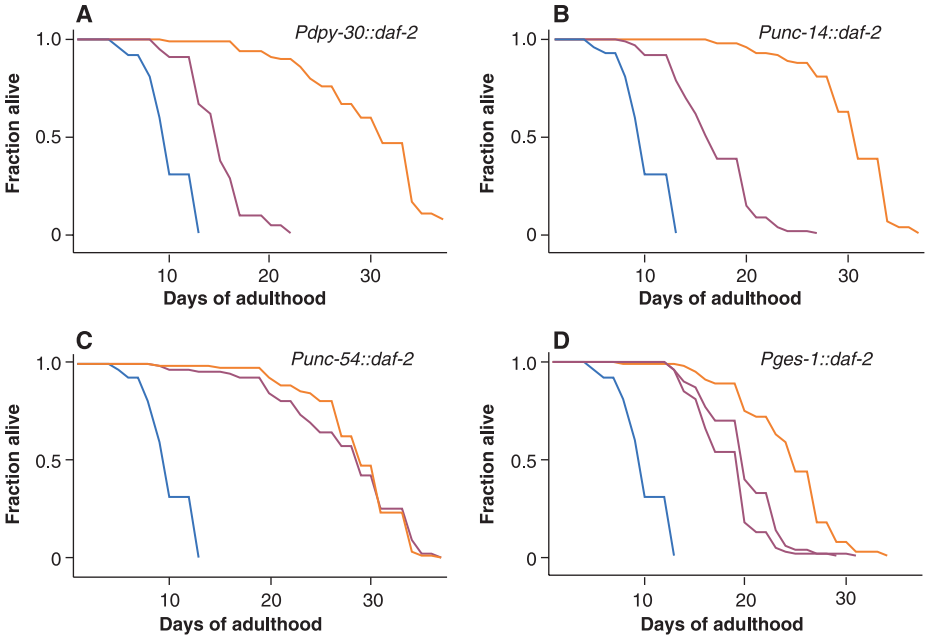

Fig. 2. (**A** to **D**) Rescue of *daf-2(−)* long life-span by cell-type–restricted *daf-2* activity. Life-span curves of populations with cell-type–restricted *daf-2* activity, as in Fig. 1.

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.

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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, $n = 37$ animals, one line). Animals with *Pges-1::GFP* showed a decrease in GFP intensity at adult day 8: 49% of animals showed intense/moderate GFP intensity, 27% showed low GFP intensity, and in 23% no GFP was detectable ($n = 114$ animals, two lines). In some animals, we observed *Punc-14::GFP* expression in the pharynx and/or intestine and *Pmec-7::GFP* expression in additional neurons and one body-wall muscle.
24. Aging assays were performed at 25.5°C with agar plates containing 5-fluorodeoxyuridine (FUDR; 0.1 mg/ml) to prevent growth of progeny. Animals were grown on nematode growth medium (NGM) plates until reaching the L4 or young adult stage at 25.5°C (*age-1* strains) or at 15°C (*daf-2* strains) and then transferred to FUDR-containing plates at 25.5°C. Animals were scored every 1 to 3 days subsequently and scored as dead when they no longer responded to gentle prodding 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.
25. 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+z−) animals. Thus, *age-1(mg44); Punc-14::age-1* and *age-1(mg44); Pdpy-30::age-1* animals are (m−z+) for *age-1* activity in the nervous system and ubiquitously, respectively. An (m−z−) *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+z− *age-1(mg44)*, but it is the only control available.
26. 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 hypodermis relative with positive [*age-1(mg44)* (m−z−) and *daf-2(e1370)* dauers] and negative (wild-type L4 larvae) controls.
27. 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 or 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.
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Structure of the Protease Domain of Memapsin 2 (β-Secretase) Complexed with Inhibitor

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Memapsin 2 (β-secretase) is a membrane-associated aspartic protease involved in the production of 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 subsite locations from S_4 to S_2' are well defined. A kink of the inhibitor chain at P_2' and the change of chain direction of P_3' and P_4' may be mimicked to provide inhibitor selectivity.

The accumulation of the 40- to 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 presenilin 1 (*2–4*). The β-secretase cleaves APP on the lumenal side of the membrane and its activity is the rate-limiting step of Aβ production in vivo (*5*). Both proteases are potential targets for inhibitor drugs against 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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