30. Subdecadal correlations are based on annual mean data after removing a 10-year running mean. The 10- to 50-year correlations are based on the difference between the 10- and 50-year running means, and greater than 10-year correlations are based on 10-year running means alone. The threshold for significance (at the $P = 0.05$ level) for the sub–10-year, 10 to 50-year, and greater than 10-year correlations are 0.15, 0.62, and 0.47, respectively.
31. Potential predictability is given by

$$\frac{\text{var(ens. mean)} - \text{var(control)}/4}{\text{var(ens. mean)} - \text{var(control)}/4 + \text{var(control)}}$$

where var(ens. mean) is the variance of the decadal mean temperatures in the ensemble mean and var(control) is the variance of the decadal mean temperatures in the control integration. The var(control)/4 terms account for finite ensemble size, giving a measure of potential skill in predicting a single observed series with a hypothetical infinite ensemble, assuming (as appears to be the case for all diagnostics considered) linear superposition of signals and noise, and normal distributions.
32. G. J. Boer, *Clim. Dyn.* **16**, 469 (2000).
33. M. Collins, M. R. Allen, *Hadley Centre Technical Note No. 21* (Hadley Centre for Climate Prediction and Research, Bracknell, UK, 2000).
34. We apply a standard optimal detection methodology, as used in previous studies (*3, 13, 35*) by projecting decadal mean temperature changes onto the main modes of internal variability. The procedure is optimal in giving more weight to less variable components of the patterns. We calculate the probability that the ensemble mean is consistent with the observations, where the details of the optimization procedure are given in Tett *et al.* (*10*) and we do not scale the ALL signal. We analyze the period 1 December 1899 to 30 November 1999 and restrict the analysis to the highest 40 spatiotemporal modes of variability, the maximum that is estimated to be adequately sampled by the control (*10*).
35. M. R. Allen, S. F. B. Tett, *Clim. Dyn.* **15**, 419 (1999).
36. P. A. Stott, S. F. B. Tett, G. S. Jones, M. R. Allen, J. F. B. Mitchell, G. J. Jenkins, data not shown.
37. D. T. Shindell, R. L. Miller, G. A. Schmidt, L. Pandolfo, *Nature* **399**, 452 (1999).
38. T. J. Osborn, K. R. Briffa, S. F. B. Tett, P. D. Jones, R. M. Trigo, *Clim. Dyn.* **15**, 685 (1999).
39. D. E. Parker, P. D. Jones, C. K. Folland, A. Bevan, *J. Geophys. Res.* **99**, 14373 (1994).
40. A. Jones, D. L. Roberts, M. J. Woodage, *Hadley Centre Technical Note No. 14* (Hadley Centre for Climate Prediction and Research, Bracknell, UK, 1999).
41. B. A. Albrecht, *Science* **245**, 1227 (1989).
42. M. R. Allen, P. A. Stott, J. F. B. Mitchell, R. Schnur, T. L. Delworth, *Nature* **407**, 617 (2000).
43. J. U. Beer *et al.*, in *The Sun as a Variable Star: Solar and Stellar Irradiance Variability*, Proceedings of IAU Colloquium 143, 20 to 25 June 1993, J. M. Pap, C. Frohlich, H. S. Hudson, S. K. Solanki, Eds. (Cambridge University Press, Cambridge, 1994), pp. 291–300.
44. M. Lockwood, R. Stamper, M. N. Wild, *Nature* **399**, 437 (1999).
45. D. V. Hoyt, K. H. Schatten, *J. Geophys. Res.* **98**, 18895 (1993).
46. P.A.S., S.F.B.T., G.S.J., and G.J.J. were funded by the UK Department of the Environment, Transport and the Regions under contract PECD 7/12/37. M.R.A. was supported by a Research Fellowship from the UK Natural Environmental Research Council. J.F.B.M. was supported by the UK Public Meteorological Service Research and Development Programme. Supplementary support was provided by European Commission contract ENV4-CT97-0501 (QUARCC). We thank the reviewers and N. Rayner for their comments on the manuscript, and the many colleagues who developed HadCM3.

11 September 2000; accepted 7 November 2000

Extended Life-Span Conferred by Cotransporter Gene Mutations in *Drosophila*

Blanka Rogina, Robert A. Reenan, Steven P. Nilsen, Stephen L. Helfand*

Aging is genetically determined and environmentally modulated. In a study of longevity in the adult fruit fly, *Drosophila melanogaster*, we found that five independent P-element insertional mutations in a single gene resulted in a near doubling of the average adult life-span without a decline in fertility or physical activity. Sequence analysis revealed that the product of this gene, named *Indy* (for *I'm not dead yet*), is most closely related to a mammalian sodium dicarboxylate cotransporter—a membrane protein that transports Krebs cycle intermediates. *Indy* was most abundantly expressed in the fat body, midgut, and oenocytes: the principal sites of intermediary metabolism in the fly. Excision of the P element resulted in a reversion to normal life-span. These mutations may create a metabolic state that mimics caloric restriction, which has been shown to extend life-span.

Single gene mutations can greatly enhance our understanding of complex biological processes such as aging. Mutations in *Caenorhabditis elegans* and mice have highlighted the importance of hormone signal transduction, mitochondrial function, food intake, and the growth hormone–prolactin–thyroid-stimulating hormone system in life-span extension (*1–9*). To date, only one mutation that extends life-span in *Drosophila* has been reported. A partial loss-of-function mutation in the *methuselah* (*mth*) gene extends the average life-span of *Drosophila* by 35%, but neither the function of the *methuselah* gene product nor its tissue localization is known (*10*). In mammals, the only intervention that extends life-span is caloric restriction, and it has been postulated that the mechanism by which some of the mutations in *C. elegans* (for example, *daf*) extend life-span may be through a similar alteration in energy use (*5–7*).

In studies of *Drosophila* enhancer-trap lines (*11*), we noticed that male and female flies of two lines, 206 and 302, showed a doubling of mean life-span (from ~37 to ~70 days) and a 50% increase in maximal life-span. This occurred when only one copy of the enhancer-trap chromosome was present (in heterozygotes) (Fig. 1). Chromosomal in situ hybridization revealed that the P element in both 206 and 302 was inserted at the same cytological location (*12*). Genomic DNA flanking the site of insertion in the two enhancer-trap lines (206 and 302) was obtained by plasmid rescue (*13*) and sequenced. The insertion sites in the 206 and 302 enhancer-trap lines were 5753 base pairs (bp) from each other and were in the same gene, which we have named *Indy* (for *I'm not dead yet*).

Sequence analysis identified three expressed sequence tags (ESTs) from the *Drosophila* genome project (LD13803, LD16220, and HL01773). Genomic and cDNA sequences predicted a 572–amino acid protein with 34% identity and 50% similarity to human and rat renal sodium dicarboxylate cotransporters (*14–16*) (Fig. 2). Mammalian dicarboxylate cotransporters are membrane proteins responsible for the uptake or reuptake of di- and tricarboxylic acid Krebs cycle intermediates such as succinate, citrate, and alpha-ketoglutarate. They are found in a variety of tissues, including brush border cells of the small intestine, colon, and placenta; the basolateral membrane of perivenous cells in the liver; and epithelial cells of the renal proximal tubule and the brain (*14–16*).

Information on the chromosomal location of *Indy* was used to identify additional mutations in the *Indy* gene from other laboratories. We examined several candidate lines with P-element insertions in the same cytogenetic region as *Indy* and found a third enhancer-trap line with a P element inserted 734 bp from the site of the 206 insertion (Fig. 2A). As a heterozygote, this line, 159, showed the same extension in life-span (Fig. 1). Two further P-element insertions in *Indy* were obtained through site-selected mutagenesis of the *Indy* locus. In a polymerase chain reaction–based screen of 10,000 mutagenized third chromosomes, we identified two new insertions into the *Indy* locus (*12*) (Fig. 2A). Flies heterozygous for either of these new alleles of *Indy* also showed a large extension in life-span (*12*).

Department of Genetics and Developmental Biology, School of Medicine, University of Connecticut Health Center, 263 Farmington Avenue, Farmington CT 06030, USA.

*To whom correspondence should be addressed. E-mail: shelfand@neuron.uchc.edu

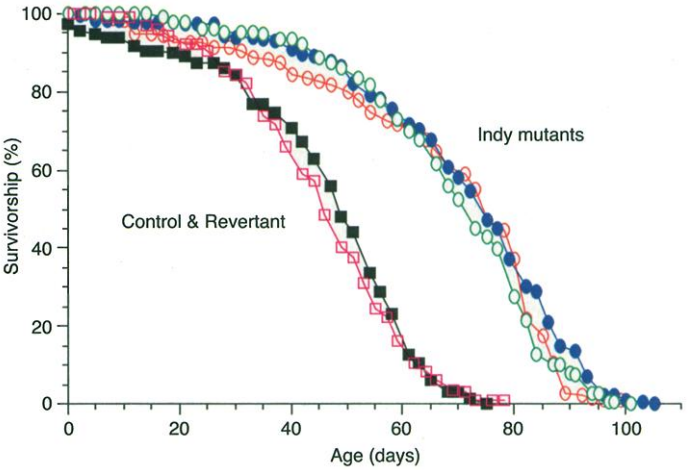
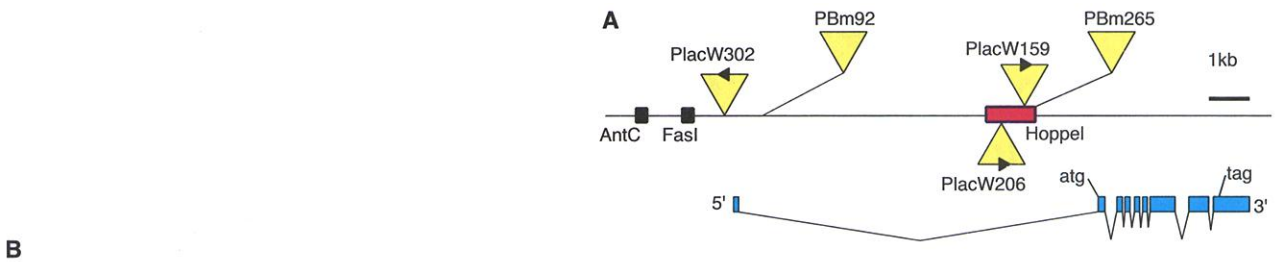


Fig. 1. Life-span extension in *Indy* mutants. Survival curves of males heterozygous for three different *Indy* mutations, a precise excision of the P -element from Indy 302 (revertant), and an enhancer-trap control are shown. All flies were tested as heterozygotes over a wild-type Canton-S strain. The *Indy* mutants are *Indy302* (open green circles), *Indy206* (solid blue circles), and *Indy159* (open orange circles) (see Fig. 2 for mutation map). The excision line (open red squares) is one of four exact excisions (sequence confirmed) of the P element obtained by mobilizing the P element from either the *Indy302* or *Indy206* line, using delta 2-3 transposase (*17*). The control (solid black squares) is one of four other enhancer-trap control lines from the same mutagenesis that generated *Indy302* and *Indy206* (*11*), tested as a heterozygote over Canton-S (*28*). A similar control survival curve was found for a control from the mutagenesis that gave rise to *Indy159* (*12*, *13*). The mean 25°C life-spans of controls were 37 days, whereas the mean life-spans for *Indy206*, *Indy302*, and *Indy159* were 71, 69, and 69 days, respectively. *Indy206*, *Indy302*, and *Indy159* extended mean life-span by 92, 87, and 87% respectively. Extension of 1% maximal life-span of these *Indy* mutants was greater than 45%. At 18°C, the increase in mean life-span conferred by *Indy* mutations approached 100%, whereas the increase in 1% maximum life-span approached 50% (*12*). Flies were maintained in a humidified, temperature-controlled environmental chamber at 25°C and were transferred to fresh food vials and scored for survival every 2 to 3 days as in (*20*). Each survivorship curve represents data from over 300 male flies. A total of 5430 male and female *Indy* heterozygote flies were tested.



B

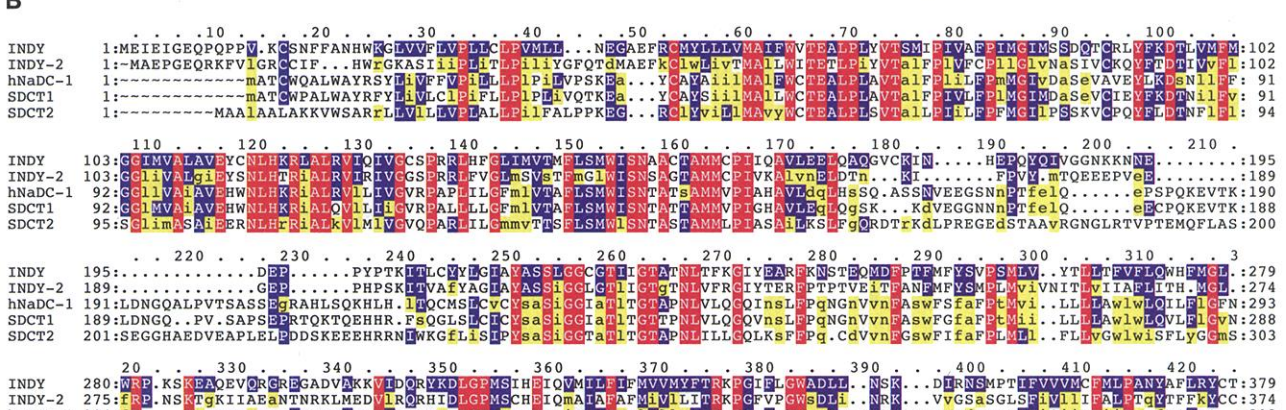


Fig. 2. (**A**) Genomic organization of the *Indy* locus, with insertion sites of all five P-element alleles. The organization of the *Indy* transcription unit is shown. Solid black boxes represent conserved regulatory sequences (AntC and FasI). The red rectangle represents the conserved Hoppel transposable element. *PlacW* insertion sites in the 206, 302, and 159 insertion lines are shown, as well as the orientation of the insertions. The positions of Birmingham-2 P-element insertions (PBm) in 92 and 265 insertion lines are also shown. The insertions in the 206, 159, and 265 lines are within a Hoppel element in the first intron of the *Indy* gene just upstream of the putative translational start site. The Hoppel element is present in the same position in wild-type animals, including P1 clones from the *Drosophila* Genome Project. The insertion in the 302 line is within 50 bp of the putative transcriptional start site. *PlacW* (10 kb) is not drawn to scale. (**B**) Sequence comparisons (*29*). The proteins most homologous to the Indy protein (GenBank accession no. AE003519) were identified by Blast. *Indy-2* is a highly homologous *Drosophila* gene (accession no. AE003728). hNaDC-1 (accession no. U26209) is a human dicarboxylate cotransporter, and SDCT1 (accession no. AF058714) and SDCT2 (accession no. AF081825) are rat sodium dicarboxylate cotransporters. Red boxes indicate identity across all proteins. Blue indicates identity to Indy. Yellow indicates amino acid similarity to Indy.

To confirm that the P-element insertion in *Indy* caused the life-span extension, we remobilized and excised the P element from the *Indy* gene in the 302 and 206 lines (*17*). Four independent lines of flies, shown by sequence analysis to carry exact excisions, reverted to normal life-span (Fig. 1). A nonexcision control line isolated at the same time, which passed through the same genetic background as the excision lines, remained long-lived (*12*).

The reporter protein β-galactosidase (β-Gal) had an identical pattern of expression in each of the three enhancer-trap lines (206, 302, and 159), despite the P-element insertions being almost 6.5 kb away from each other in the three lines. In adult flies, *Indy* was expressed in the fat body, midgut, and oenocytes (Fig. 3): organs that are thought to be the primary sites of intermediary metabolism, absorption, and metabolic storage in *Drosophila*. The fat body is involved in the metabolism and storage of fat, glycogen, and protein and is most often compared to the liver of vertebrates (*18*, *19*). *Indy* was also expressed at low levels in the halteres; portions of the alimentary canals, including the procardia and restricted regions of the esophagus and hindgut; and the base of the legs. These are regions that have been identified as storage depots for glycogen (*18*). Finally, *Indy* was expressed in a subset of cells in the third segment of the antennae (*20*).

To exclude the possibility that the extended life-span of the *Indy* mutants was due to the rescue of uncharacterized deleterious mutations accumulating in our wild-type Canton-S stock, we crossed the *Indy* mutation into several different genetic backgrounds that were distinct from our Canton-S stock. These included the *Hyperkinetic*, *Shaker*, and *drop dead* stocks, each of which was isolated from other wild-type stocks 25 to 30 years ago, as well as the long-lived laboratory-selected lines of Luckinbill (*21*, *22*). In all cases, there was an extension in life-span. For all genetic backgrounds, mean life-span was extended by 40 to 80%, except in the case of the long-lived lines of Luckinbill, in which life-span was additionally extended by only 15%. These data indicate that the mechanism by which *Indy* mutations extend life-span is a positive effect of the mutation on life-span and not simply of the rescue of deleterious mutations. The smaller increase in life-span seen with the laboratory-selected long-lived lines provides additional evidence that the mechanisms by which *Indy* acts to increase life-span may represent physiological systems already partially optimized by laboratory selection.

A decline in fertility (*23*, *24*) or a reduction in physical activity (*25*) can lead to an extension of life-span in flies. *Indy* long-lived heterozygote males and females were compared to controls and found to be normal or superior in fertility and fecundity (Table 1). Qualitative observations of flight, courtship, feeding behavior, and negative geotaxis revealed no significant differences between *Indy* long-lived males and females and control flies during early life. Differences occurred later in life when physical measures of behavior and locomotor function were maintained at high levels in the *Indy* long-lived animals but not in normal-lived controls. For instance, one physiological milestone of aging in flies is the onset of female infertility. *Indy* heterozygous long-lived females continued to produce viable adult offspring 40% longer on average than did control flies (23.2 versus 16.5 days). This was a true extension of the period of fertility and was not associated with a compensatory delay in fertility during early life, as is seen in laboratory-selected long-lived lines (*26*). *Indy* long-lived females showed the same early peak of egg laying and fertility as control females but sustained the ability to produce larger numbers of offspring for a longer period of time (Table 1). There was no alteration in the rate or timing of developmental events in *Indy* long-lived mutant animals, as in the *C. elegans clock* mutants (*3*). The time from egg to adult at 25°C was the same as that for normal-lived controls (9 to 10 days).

The expression of *Indy* in the fat body, gut, and oenocytes, and the amino acid sequence similarity of the Indy protein to dicarboxylate cotransporters, suggest that *Indy* may play a role in both the absorption of metabolites and in intermediary metabolism.

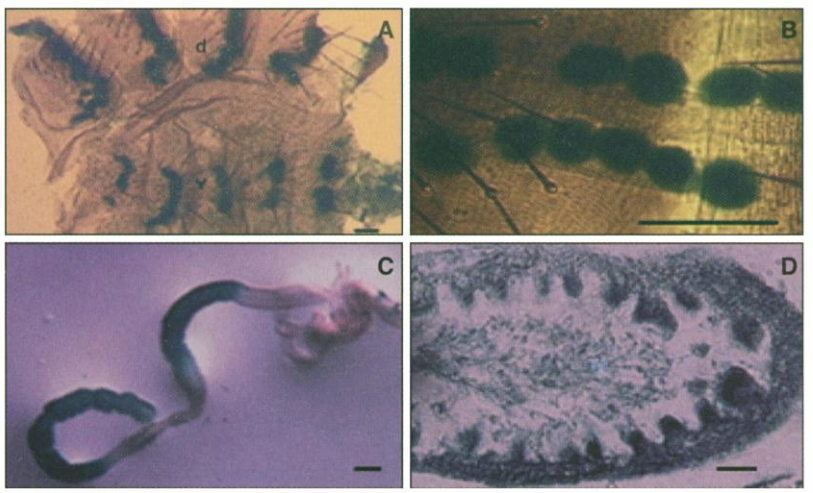


Fig. 3. Expression of *Indy* in adult flies. Whole-mount X-Gal staining showing nuclear localization of β-Gal in cells from lines carrying an enhancer-trap insert in the *Indy* gene: *Indy302*, *Indy206*, and *Indy159*. Expression is seen in oenocytes (**A** and **B**) and the gut (**C** and **D**). (A) Low-power view of oenocytes in the (v) ventral and (d) dorsal abdominal segments. (B) High-power view of dorsal midline oenocytes. (D) A 5-μm section showing X-Gal staining within the cells of the gut. Whole-mount staining was performed as in (*20*). After whole-mount X-gal staining, the tissue in (D) was postfixed in 6.25% glutaraldehyde, embedded in paraffin, and then sectioned. Scale bar in (A) through (C), 100 μm; in (D), 10 μm.

Table 1. Fertility and fecundity of long-lived *Indy* mutant animals. CS indicates the Canton-S control, 302/CS indicates heterozygotes of 302 and Canton-S, and 206/CS indicates heterozygotes of 206 and Canton-S. Single-pair mates were kept in individual vials. Each day throughout their entire life-span, they were passed to new vials, the number of eggs from the vial of the previous day was counted, and the vial was saved in order to determine the number of adult offspring.

	Genotype of parents				
	CS male CS female	CS male 302/CS female	CS male 206/CS female	302/CS male CS female	206/CS male CS female
Pairs (*n*)	53	26	26	23	25
Eggs per pair (*n*)					
Up to day 14	788	961	942	792	781
Day 14 to 21	258	380	407	348	256
Total over life-span	1293	1747	1839	1490	1202
Offspring per pair (*n*)					
Up to day 14	344	735	779	412	390
Day 14 to 21	56	206	206	111	52
Total over life-span	403	980	1048	549	450

Thus, insertion of a P element in the *Indy* gene may alter normal metabolism. In mammals, a moderate caloric restriction increases life-span, whereas a more severe restriction leads to starvation and decreased life-span (*27*). We postulate that the level of *Indy* expression is critical for life-span extension and that P-element insertions in *Indy* reduce Indy expression. When the level of Indy is mildly reduced, as in flies heterozygous for *Indy* insertions, there is a large extension in life-span. The extension in life-span is less dramatic with a further reduction in Indy activity, such as that seen in *Indy* homozygous flies, in which a 10 to 20% increase in mean life-span was seen (*12*). This model predicts that a further reduction in Indy activity would shorten life-span, and indeed this was observed when *Indy* gene activity was further reduced by placing an *Indy* mutation over a chromosome deleted for the *Indy* region. Flies with only a single copy of a mutant *Indy* gene and no normal copy of *Indy* (*Indy* mutant over deletion for the *Indy* region) had a 10 to 20% shorter mean and maximum life-span than did homozygous *Indy* mutants (*12*).

The mechanism by which caloric restriction mediates life-span extension is not understood, but it is likely to involve alterations in energy utilization. In contrast to many of the previously identified genes associated with life-span extension in metazoans, which have indirect effects on metabolism (*5–9*), *Indy* appears to be directly involved in intermediary metabolism and thus may represent a new class of longevity genes. A genetically induced reduction in the amount or efficiency of a dicarboxylic acid cotransporter in the *Indy* mutants may be creating a metabolic state similar to caloric restriction. Further characterization of the *Indy* mutants may provide direct genetic insight into the role of energy balance and aging, and a point of access for genetic and pharmacological interventions for extending life-span.

References and Notes

1. T. E. Johnson, *Science* **249**, 908 (1990).
2. C. J. Kenyon, E. Chang, A. Rudner, R. Tabtiang, *Nature* **366**, 461 (1993).
3. B. Lakowski, S. Hekimi, *Science* **272**, 1010 (1996).
4. K. D. Kimura, H. A. Tissenbaum, Y. Liu, G. Ruvkun, *Science* **277**, 942 (1997).
5. J. Z. Morris, H. A. Tissenbaum, G. Ruvkun, *Nature* **382**, 536 (1996).
6. S. Ogg *et al.*, *Nature* **389**, 994 (1997).
7. S. Hekimi, in *The Molecular Genetics of Aging, Results and Problems in Cell Differentiation*, S. Hekimi, Ed. (Springer-Verlag, New York, 2000), vol. 29, pp. 81–112.
8. H. M. Brown-Borg, K. E. Borg, C. J. Meliska, A. Bartke, *Nature* **384**, 33 (1996).
9. A. Bartke, in *The Molecular Genetics of Aging, Results and Problems in Cell Differentiation*, S. Hekimi, Ed. (Springer-Verlag, New York, 2000), vol. 29, pp. 181–202.
10. Y. J. Lin, L. Seroude, S. Benzer, *Science* **282**, 943 (1998).
11. S. Boynton, T. Tully, *Genetics* **131**, 655 (1992).
12. B. Rogina, R. A. Reenan, S. P. Nilsen, S. L. Helfand, data not shown.
13. E. Bier *et al.*, *Genes Dev.* **3**, 1273 (1989).
14. X. Z. Chen, C. Shayakul, U. V. Berger, W. Tian, M. A. Hediger, *J. Biol. Chem.* **273**, 20972 (1998).
15. A. M. Pajor, *Am. J. Phys.* **270**, F642 (1996).
16. ———, *Annu. Rev. Physiol.* **61**, 663 (1999).
17. M. J. Palladino, L. P. Keegan, M. A. O'Connell, R. A. Reenan, *Cell* **102**, 437 (2000).
18. V. B. Wigglesworth, *The Principles of Insect Physiology* (Chapman and Hill, London, ed. 7, 1972).
19. L. Sondergaard, *Trends Genet.* **9**, 193 (1993).
20. S. L. Helfand *et al.*, *Genetics* **140**, 549 (1995).
21. The FlyBase database of the *Drosophila* genome projects and community literature, *Nucleic Acids Res.* **27**, 85 (1999) (http://flybase.bio.indiana.edu/).
22. L. Luckinbill, R. Arking, M. J. Clare, W. C. Cirocco, S. Buck, *Evolution* **38**, 996 (1984).
23. L. Partridge, A. Green, K. Fowler, *J. Insect Physiol.* **33**, 745 (1987).
24. K. Fowler, L. Partridge, *Nature* **338**, 760 (1989).
25. R. S. Sohal, P. B. Buchan, *Exp. Gerontol.* **16**, 157 (1981).
26. M. R. Rose, *Evolutionary Biology of Aging* (Oxford Univ. Press, New York, 1991).
27. R. S. Sohal, R. Weindruch, *Science* **273**, 59 (1996).
28. Because genetic background has such a powerful effect on life-span, the genetic backgrounds of the lines being compared must be as similar as possible. The *Indy302* and *Indy206* mutants are both from the mutagenesis reported in (*11*), where an effort was made to use "Cantonized" stocks. In addition, each enhancer-trap line was further backcrossed to a Canton-S stock with the w^{1118} mutation multiple times (*11*). Our controls included four other enhancer-trap lines from the original mutagenesis (*11*), compared as heterozygotes with the same Canton-S stock as the *Indy302* and *Indy206* lines. The control for *Indy159* was similar; we compared another enhancer-trap line insertion from the same mutagenesis (*13*). The control line had an insert in the same cytological region as *Indy*, but not inserted into the *Indy* locus.
29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

30. We thank J. Jack, B. Graveley, and M. Tanzer for helpful discussions. Supported by grants from the NIH-supported Claude Pepper Older Americans Independence Center at the University of Connecticut Center on Aging and the American Federation for Aging Research (B.R.); by NSF grant 9728737 and a grant from The Patrick and Catherine Weldon Donaghue Medical Research Foundation (R.A.R); and by the National Institute on Aging (grants AG14532 and AG16667), a Burroughs Wellcome Travel Grant, and a Donaghue Investigator Award from The Patrick and Catherine Weldon Donaghue Medical Research Foundation (S.L.H).

17 October 2000; accepted 14 November 2000

Docosahexaenoic Acid, a Ligand for the Retinoid X Receptor in Mouse Brain

Alexander Mata de Urquiza,[1] Suya Liu,[2]* Maria Sjöberg,[3]* Rolf H. Zetterström,[1] William Griffiths,[2] Jan Sjövall,[2] Thomas Perlmann[1]†

The retinoid X receptor (RXR) is a nuclear receptor that functions as a ligand-activated transcription factor. Little is known about the ligands that activate RXR in vivo. Here, we identified a factor in brain tissue from adult mice that activates RXR in cell-based assays. Purification and analysis of the factor by mass spectrometry revealed that it is docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid that is highly enriched in the adult mammalian brain. Previous work has shown that DHA is essential for brain maturation, and deficiency of DHA in both rodents and humans leads to impaired spatial learning and other abnormalities. These data suggest that DHA may influence neural function through activation of an RXR signaling pathway.

The nuclear hormone receptors are ligand-activated transcription factors [reviewed in (*1*)]. Included in this family are the so-called "orphan receptors," whose ligands have not been identified [reviewed in (*1, 2*)]. By screening chemical libraries, progress has been made in identifying ligands for these receptors [for examples, see (*3–8*)]; however, in most cases endogenous ligands have remained elusive.

[1]Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-171 77 Stockholm, Sweden. [2]Department of Medical Chemistry and [3]Department of Cell and Molecular Biology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: thomas.perlmann@licr.ki.se

RXR is an obligatory component of a large number of nuclear receptor heterodimers and is activated in vitro by the vitamin A metabolite 9-*cis* retinoic acid (9-*cis* RA), which binds with high affinity to the RXR ligand binding domain (*9, 10*); however, 9-*cis* RA acid has been difficult to detect in vivo (*11*). Nonetheless, because numerous studies have demonstrated striking synergism in biological responses in vitro and in vivo when both partners of the RXR-RAR heterodimer are activated by their respective ligand [for examples, see (*12–14*)], it seems likely that ligand-induced activation of RXR does occur in vivo. This conclusion has been corroborated by experiments with transgenic mice (*15, 16*).

To search for endogenous ligands that acti-