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DOI: 10.1126/science.1136610

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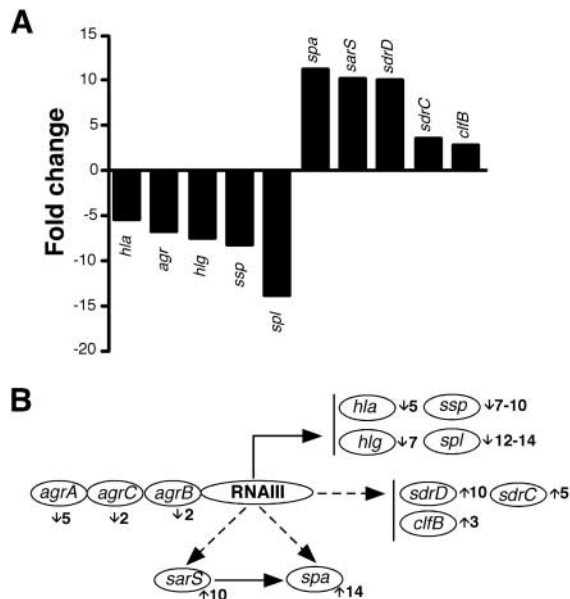
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**Fig. 4.** *S. aureus* PVL-positive strains show an altered transcriptional profile. (A) Fold increase or decrease levels of transcript from selected genes. Total RNA extracted from cultures grown to stationary phase. Genes were considered to be induced or repressed in the PVL phage if they were transcribed at least twice or half as much as those of  $\Delta$ PVL phage. The shown transcripts encode *agrA-C*, accessory gene regulator system; *sarS*, staphylococcal accessory regulator S; *spa*, staphylococcal protein A; *sdrD*, serine-aspartate repeat protein D; *sdrC*, serine-aspartate repeat protein C; *clfB*, clumping factor B; *hla*, alpha toxin; *ssp*, a representative of serine proteases *sspB* and *sspC*; *spl*, a representative of *splA-F* proteases. (B) A schematic overview of the interactions between regulators involved in cell wall-anchored and secreted protein genes (full and broken lines indicate positive and negative regulation, respectively) based on previously published data. Numbers next to the gene name indicate fold change based on microarray analysis (upward arrow indicates up-regulation, downward arrow indicates down-regulation). The down-regulation of RNAIII (the effector of the *agr* system) results in the down-regulation of secreted protein genes (*hla*, *hlgC*, *hlgB*, and proteases) and the up-regulation of *sarS* and cell wall-anchored proteins (*spa*, *sdrD*, *sdrC*, *clfB*). In addition, the up-regulation of *sarS* results in the up-regulation of *spa*.



13. I. Kullic, P. Giachino, T. Fuchs, *J. Bacteriol.* **180**, 4814 (1998).  
 14. M. J. Horsburgh *et al.*, *J. Bacteriol.* **184**, 5457 (2002).  
 15. B. Said-Salim *et al.*, *J. Clin. Microbiol.* **43**, 3373 (2005).  
 16. A. L. Cheung, K. Eberhardt, J. H. Heinrichs, *Infect. Immun.* **65**, 2243 (1997).  
 17. E. Huntzinger *et al.*, *EMBO J.* **24**, 824 (2005).  
 18. N. Vojtov, H. F. Ross, R. P. Novick, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10102 (2002).  
 19. P. Thomas, M. Riffelmann, B. Schweiger, S. Dominik, C. H. von Konig, *Pediatr. Infect. Dis. J.* **22**, 201 (2003).  
 20. L. O'Brien *et al.*, *Mol. Microbiol.* **44**, 1033 (2002).  
 21. We thank S. L. Mueller-Ortiz and S. M. Drouin for their invaluable help establishing the animal model; Z. Chronos for his input in the interpretation of histological samples; C. Badiou for technical assistance; G. Lina for scientific advice; and S. Foster, T. Foster, B. Fournier, R. Novick, and P. McNamara for providing strains. This work was supported by grants from the TAMUS HSC to M.G.B., from the French Ministry of Research to F.V. and NIH AIO20624 to M.H. Support from the Neva and Wesley West and the Hamill Foundations were awarded to M.H. The authors declare that they have no competing financial interests. Microarray data are deposited in the GEO database (<http://www.ncbi.nlm.nih.gov/projects/geo/>) under the accession number GPL4653.

**Supporting Online Material**

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3 November 2006; accepted 19 December 2006  
 Published online 18 January 2007;  
 10.1126/science.1137165  
 Include this information when citing this paper.

9. S. de Bentzmann *et al.*, *J. Infect. Dis.* **190**, 1506 (2004).  
 10. N. Palmqvist, T. Foster, A. Tarkowski, E. Josefsson, *Microb. Pathog.* **33**, 239 (2002).  
 11. M. I. Gomez *et al.*, *Nat. Med.* **10**, 842 (2004).  
 12. J. Oscarsson, C. Harlos, S. Arvidson, *Int. J. Med. Microbiol.* **295**, 253 (2005).

# Regulation of *Drosophila* Life Span by Olfaction and Food-Derived Odors

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Smell is an ancient sensory system present in organisms from bacteria to humans. In the nematode *Caenorhabditis elegans*, gustatory and olfactory neurons regulate aging and longevity. Using the fruit fly, *Drosophila melanogaster*, we showed that exposure to nutrient-derived odorants can modulate life span and partially reverse the longevity-extending effects of dietary restriction. Furthermore, mutation of odorant receptor *Or83b* resulted in severe olfactory defects, altered adult metabolism, enhanced stress resistance, and extended life span. Our findings indicate that olfaction affects adult physiology and aging in *Drosophila*, possibly through the perceived availability of nutritional resources, and that olfactory regulation of life span is evolutionarily conserved.

**A**s in many species, reduced nutrient availability (dietary restriction) increases life span in the fruit fly, *Drosophila melanogaster*, and leads to alterations in age-dependent patterns of gene expression, physiology, and behavior (1–4). Acute nutrient manipulation causes sudden and rapid changes in age-specific mortality (5, 6). Whole-genome expression data, containing age-dependent patterns of gene expression in diet-restricted long-lived flies and fully fed control flies (7), revealed that expression

of genes encoding odorant-binding proteins was strongly affected by both age and nutrient availability (fig. S1).

To determine whether detection of food-related odors is sufficient to affect fly life span, we measured the life spans of flies in the presence and absence of odorants from live yeast. Yeast odorants were used because demographic and gene-expression data suggested that yeast availability is a major component of the longevity response to diet in *Drosophila* (7–9). Exposure to

yeast odorants reduced life span in long-lived flies from two laboratory fly strains (Canton-S and yw) that had been subjected to dietary restriction (Fig. 1, A and C). Life span was further reduced when flies were allowed to consume yeast paste. The magnitude of the odorant effect was variable and usually small, relative to that caused by the consumption of yeast paste; odorant-mediated life-span reductions ranged from 6 to 18% in Canton-S flies and from 7 to 8% in yw flies (Fig. 1C). Such variability is reminiscent of the dietary-restriction response in flies, which depends on genetic background (8). Odorants are therefore sufficient to modulate life span, and currently unidentified odors may alter longevity with greater potency.

We tested whether diet-restricted flies might exhibit altered feeding behavior or altered in-

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vestment in reproduction when exposed to nutrient-related odors, thereby accounting for the longevity effect. Increases in the intensity of either of these behaviors would reduce life span by compensating for our diet-restriction procedure or by augmenting the costs of reproduction, respectively. In our experiments, neither food consumption (as measured by the rate of dye ingestion, proboscis extension, and fecal output) nor fecundity was affected by yeast odorants (Fig. 1D and figs. S2 and S3). Be-

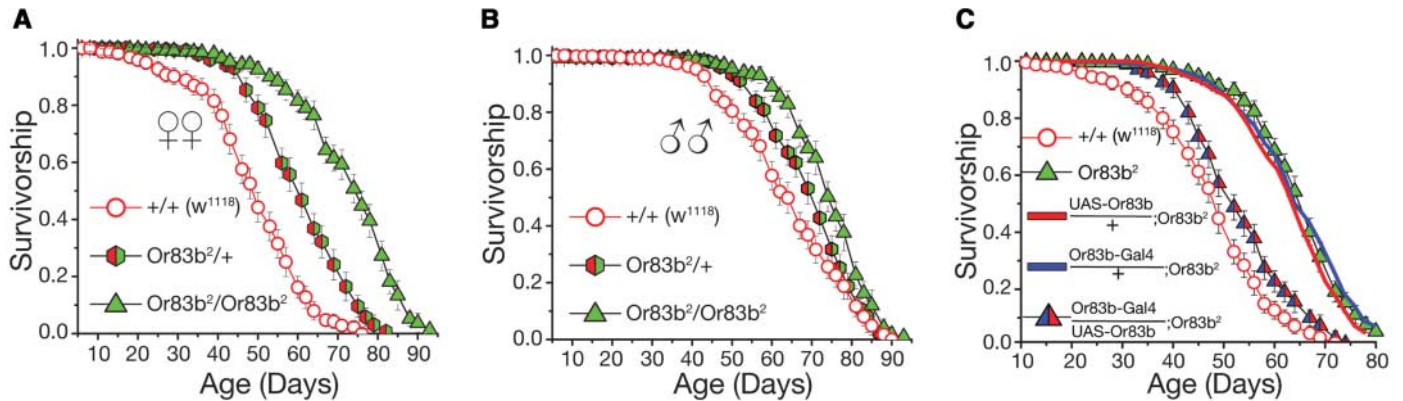
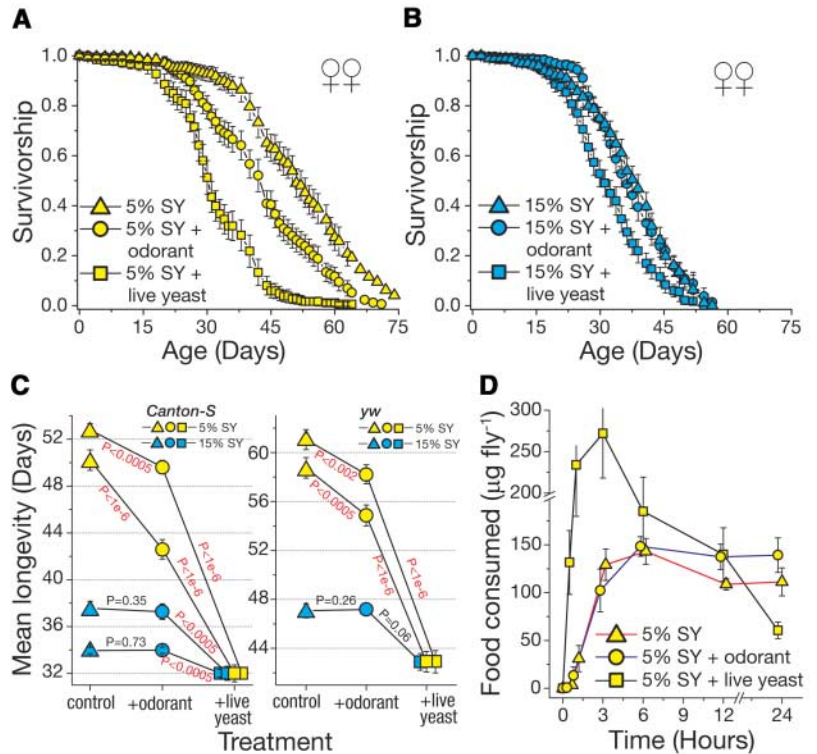
havioral alterations leading to increased nutrient intake or reproductive effort are therefore not responsible for reduced longevity upon exposure to yeast odorants.

The effects of yeast odorants on fly life span depend on nutrient availability. Longevity was not affected by yeast odorants when flies were fully fed (Fig. 1, B and C). Thus, the odor effect is a regulated biological response, and yeast odorants are not a generalized toxin that shortens life span. Our data support the hypotheses that

diet- and odorant-mediated regulation of aging act at least partly through the same molecular pathway and that nutrient-related odors can rescue, albeit incompletely, the extension of longevity through dietary restriction. Consequently, the beneficial effects of dietary restriction may be due in part to the decreased perception of nutrient availability.

We next asked whether the loss of olfactory function is sufficient to increase life span. In vertebrates and insects, each olfactory neuron ex-

**Fig. 1.** Exposure to yeast odorants alters life span in *Drosophila*. **(A)** Canton-S female flies were maintained under dietary restriction (5% SY medium) (triangles), with the addition of odorants from live yeast (circles) or yeast for consumption (squares). Sample sizes: control,  $n = 206$ ; + odorant,  $n = 247$ ; + live yeast,  $n = 193$ . **(B)** Canton-S female flies were unaffected by yeast odorants when fully fed (15% SY medium). Sample sizes: control,  $n = 269$ ; + odorant,  $n = 267$ ; + live yeast,  $n = 266$ . Error bars in (A) and (B) represent 95% confidence intervals on observed survivorship. **(C)** The effect of yeast odorants on longevity is repeatable and depends on diet. Replicate experiments showing mean life span (and SEM) for female *Drosophila* maintained under dietary restriction (5% SY, yellow symbols) and fully fed conditions (15% SY, blue symbols). Yeast odorants had no effect when flies were maintained in fully fed conditions ( $P > 0.05$  in all cases). Each point represents life spans of 5 to 10 cohorts and a minimum of 206 (maximum of 403) animals. **(D)** Food consumption is unaffected by yeast odorants. Mean food consumption (and SEM) as measured by dye uptake (see the supporting online material) is shown. Multivariate profile analysis reveals no significant treatment effects ( $P > 0.05$ ). The decline in dye uptake at later time points is a consistent finding that may reflect diurnal patterns of feeding and the dynamics of dye uptake and excretion. For survival data, block effects were adjusted for by analysis of variance (based on yeast paste treatments) to allow for comparisons across multiple experiments.  $P$  values were determined by means of a log-rank test.



**Fig. 2.** Mutation of *Or83b* increases life span. **(A and B)** Both female and male flies carrying the *Or83b*<sup>2</sup> mutation are long-lived. Sample sizes (females): +/+,  $n = 326$ ; +/-,  $n = 314$ ; -/-,  $n = 320$ . Sample sizes (males): +/+,  $n = 317$ ; +/-,  $n = 346$ ; -/-,  $n = 305$ . Error bars represent 95% confidence intervals, and statistical comparisons by means of a log-rank test and Cox regression yielded  $P < 1 \times 10^{-6}$  for all paired comparisons except (B) (males) where  $P = 0.001$  for +/+ versus +/- . **(C)** *Or83b*<sup>2</sup> homozygous mutant flies expressing *UAS-GFP:Or83b* under control of the *Or83b*-

Gal4 driver (bicolor triangles) have comparable life spans to those of background control animals ( $w^{1118}$ , open circles). Controls for the transgenic constructs had no effect on life span (compare solid red and blue lines to green triangles). Data are presented for females and are qualitatively similar for males (fig. S7). Experiments were carried out with the use of 15% SY media and were repeated by means of independent transgenic insertions with identical results. Error bars represent 95% confidence intervals on observed survivorship.

presses a small number of odorant receptors that impart response characteristics of the neuron to specific odors (10–13). Of the 62 putative odorant receptors in *Drosophila*, *Or83b* is atypical in that it is broadly expressed throughout olfactory tissues (14, 15). *Or83b* interacts with conventional odorant receptors and is required

for their localization to the neuronal dendrite (14–16). Loss-of-function mutations in *Or83b* limit spontaneous activity in many odorant-receptor neurons and severely reduce physiological and behavioral responses to a wide range of odorants (14, 16), including nutrient-related odors (fig. S4).

**Table 1.** Life-span data for female *Or83b* mutant *Drosophila*. Mean life spans of *Or83b*<sup>2</sup> homozygous mutant and wild-type control females. The *Or83b*<sup>2</sup> allele was backcrossed into each of three genetic backgrounds. For all comparisons, longevity extension in the mutant was calculated with respect to the wild-type background to which it had been backcrossed. All increases are statistically significant ( $P < 1 \times 10^{-6}$ ), determined by means of a log-rank test and Cox regression. \*YP represents live yeast paste added to the vials.

Genetic background	Nutrient level (% SY)	Control longevity			<i>Or83b</i> longevity			Absolute change	% Increase
		<i>n</i>	Mean	(SE)	<i>n</i>	Mean	(SE)		
Canton-S	3%	319	50.1	(0.77)	311	61.6	(0.86)	11.5	23.0%
	5%	319	50.1	(0.81)	304	62.5	(0.78)	12.3	24.6%
	7.5%	324	43.9	(0.80)	315	58.9	(0.83)	15.0	34.1%
	10%	313	44.8	(0.82)	318	58.6	(0.92)	13.8	30.7%
	15%	314	41.6	(0.69)	329	55.6	(0.76)	14.0	33.7%
yw	3%	249	58.0	(0.85)	234	76.1	(0.63)	18.1	31.2%
	5%	244	60.0	(0.83)	239	76.5	(0.60)	16.5	27.6%
	7.5%	254	62.0	(0.74)	236	76.0	(0.76)	14.0	22.6%
	10%	241	61.9	(0.74)	238	75.6	(0.81)	13.7	22.1%
	15%	242	50.5	(0.57)	246	69.4	(0.60)	18.9	37.4%
w <sup>1118</sup>	YP*	237	42.9	(0.84)	243	65.7	(0.73)	22.8	53.1%
	3%	315	51.9	(0.89)	318	75.6	(0.86)	23.7	45.6%
	5%	326	53.2	(0.90)	320	76.0	(0.81)	22.8	43.0%
	7.5%	333	49.6	(0.83)	319	78.0	(0.77)	28.4	57.3%
	10%	316	51.1	(0.79)	317	77.6	(0.84)	26.5	51.9%
	15%	314	49.3	(0.79)	319	72.7	(0.78)	23.4	47.3%

**Table 2.** Life-span data for male *Or83b* mutant *Drosophila*. Mean life spans of *Or83b*<sup>2</sup> homozygous mutant and wild-type control males. The *Or83b*<sup>2</sup> allele was backcrossed into each of three genetic backgrounds. For all comparisons, longevity extension in the mutant was calculated with respect to the wild-type background to which it had been backcrossed. All increases are statistically significant ( $P < 1 \times 10^{-6}$ ), determined by means of a log-rank test and Cox regression.

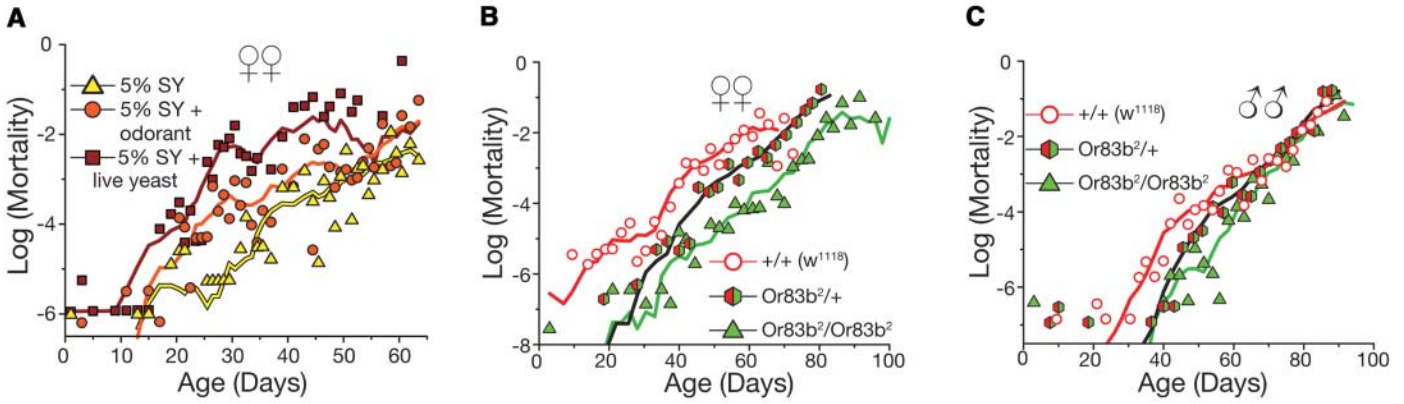
Genetic background	Nutrient level (% SY)	Control longevity			<i>Or83b</i> longevity			Absolute change	% Increase
		<i>n</i>	Mean	(SE)	<i>n</i>	Mean	(SE)		
Canton-S	3%	322	52.1	(0.83)	317	66.8	(0.75)	14.7	28.2%
	5%	315	53.2	(0.67)	282	66.3	(0.80)	13.1	24.6%
	7.5%	331	52.3	(0.71)	313	68.7	(0.77)	16.4	31.3%
	10%	330	52.0	(0.76)	296	67.5	(0.82)	15.6	30.0%
	15%	325	47.2	(0.74)	301	67.1	(0.85)	19.9	42.1%
yw	3%	257	63.6	(0.81)	238	73.8	(0.57)	10.3	16.2%
	5%	242	63.9	(0.89)	243	75.2	(0.57)	11.4	17.8%
	7.5%	251	64.4	(0.75)	240	74.3	(0.72)	9.9	15.4%
	10%	246	65.3	(0.92)	246	75.8	(0.67)	10.5	16.0%
	15%	244	60.1	(0.73)	222	70.3	(0.69)	10.1	16.8%
w <sup>1118</sup>	3%	317	63.2	(0.80)	328	69.1	(0.72)	6.0	9.5%
	5%	314	63.9	(0.77)	322	69.5	(0.77)	5.6	8.7%
	7.5%	322	63.6	(0.80)	324	73.6	(0.74)	10.0	15.7%
	10%	317	65.2	(0.77)	310	74.6	(0.77)	9.4	14.5%
	15%	317	64.4	(0.80)	305	73.8	(0.71)	9.4	14.5%

We measured the life span of flies carrying the *Or83b*<sup>2</sup> allele, in which the first five of seven transmembrane domains were replaced with the w<sup>+</sup> marker (14) (fig. S6). *Or83b*<sup>2</sup> homozygous flies lack detectable levels of *Or83b* mRNA and protein, which suggests that it is a null allele (14). Fully fed female *Or83b*<sup>2</sup> mutant flies exhibited a 56% increase in median life span when compared to appropriate wild-type animals (Fig. 2A). Males were also significantly long-lived, but the magnitude of the extension was generally smaller (Fig. 2B). Heterozygous flies exhibited intermediate longevity in both sexes, and heterozygous adult females showed a similar deficiency in attraction to live yeast paste (fig. S4, B and C). We found no evidence of such impairment in heterozygous mutant males (fig. S4D). It may be that odor-evoked behaviors are less affected or that different classes of odorants are critical for male longevity. Longevity was also extended when olfactory signaling was suppressed in *Or83b*-expressing neurons through the disruption of guanine nucleotide-binding protein (G protein) signaling (fig. S5). Because the *Or83b* mutant also disrupts G protein activity, it is possible that G protein function in olfactory neurons, rather than perception per se, influences life span.

To verify that the extended life span of *Or83b*<sup>2</sup> flies was not due to heterosis or to comparison against a relatively weak control stock, we backcrossed the *Or83b*<sup>2</sup> allele into two additional laboratory stocks (Canton-S and yw). In all cases, the longevity of mutant flies was considerably greater than that of their wild-type controls (Tables 1 and 2). The degree of life-span extension was independent of the longevity of the corresponding wild-type stock, establishing that loss of *Or83b* function extends life span in healthy animals (17).

Expression of a rescuing *Or83b* transgene (15) under the control of an *Or83b*-Gal driver (14) restored normal life span to the *Or83b*<sup>2</sup> mutant flies (Fig. 2C). The effectiveness of this construct was verified by genomic polymerase chain reaction and visually (fig. S6), and it rescues most (but not all) olfactory phenotypes (14, 15). The *Or83b*-Gal4 driver and the upstream activating sequence–green fluorescent protein *Or83b* transgene (*UAS-GFP:Or83b*) were inserted into different genomic positions and none affected life span on their own (Fig. 2C and fig. S7). These rescue data and the persistence of the longevity phenotype through extensive backcrossing to three different genetic backgrounds provide compelling evidence that loss of function in *Or83b* is the cause of increased life span in these animals.

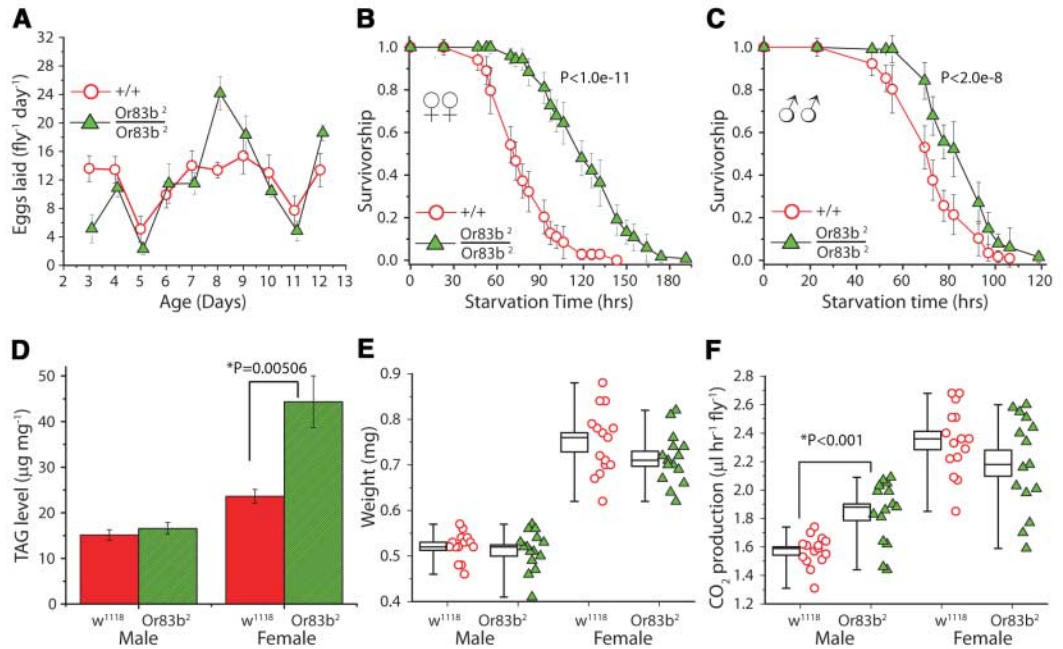
Olfactory signaling modulates life span primarily by altering the onset of demographic senescence (Fig. 3). Mortality analysis suggests that olfaction shifts the mortality curve to earlier (in the case of yeast odorants) or later (in



**Fig. 3.** Olfaction modulates the onset of demographic senescence. **(A)** Age-specific mortality rates for flies exposed to yeast odors expressed on the natural log scale. Corresponding survival data are presented in Fig. 1A. **(B and C)** Age-specific mortality rates for *Or83b* mutant **(B)** females and **(C)**

males and their controls (see also fig. S8). Corresponding survival data are presented in Fig. 2, A and B. Points represent observed mortality rates. Lines are obtained by smoothing hazard rates (3-day window) by means of a kernel smoother prior to log transformation.

**Fig. 4.** *Or83b*<sup>2</sup> flies exhibited altered physiology and enhanced stress resistance. **(A)** Total reproductive output through day 14 is not different between *Or83b* mutant and control females. Data represent average daily egg production per female (*n* = 60 females per genotype). Error bars represent 95% confidence intervals on the mean. *Or83b*<sup>2</sup> mutants had significantly lower egg production over the first observation period (two-tailed Student's *t* test, *P* = 0.0005) and higher levels of egg production at age 8 and 12 days (two-tailed Student's *t* test, *P* = 5.7 × 10<sup>-5</sup> and *P* = 0.009, respectively). **(B and C)** *Or83b*<sup>2</sup> homozygous mutant flies are starvation resistant. Sample sizes (females): +/+, *n* = 79; -/-, *n* = 79. Sample sizes (males): +/+, *n* = 100; +/-, *n* = 94. Statistical comparisons and *P* values were determined by means of a log-rank test. Error bars represent 95% confidence intervals on observed survivorship. **(D)** *Or83b*<sup>2</sup> homozygous mutant flies have increased triglyceride levels. Triglyceride levels are presented after normalization to total weight. Error bars represent 95% confidence intervals. TAG, triacylglycerol. **(E and F)** Weight and CO<sub>2</sub> production for



the case of *Or83b* mutation) ages. In females, the rate of increase in mortality with age was largely unaffected (Fig. 3, A and B, and fig. S8), an effect that is similar to diet restriction (1, 5). In males, the impact of olfaction on mortality rate was reduced later in life; mortality trajectories converge at the oldest ages (Fig. 3C).

We next investigated whether olfactory function was required for longevity extension through diet manipulation. We measured male and female life span in different nutritional regimes ranging from severe nutrient

restriction [3% sugar/yeast (SY) media] to nutrient-replete conditions (15% SY media). Flies homozygous for the *Or83b*<sup>2</sup> mutation were consistently longer-lived than those of appropriate control stocks (Tables 1 and 2). Despite their exceptional longevity, life span was further increased in the *Or83b*<sup>2</sup> mutants by dietary restriction. *Or83b* is therefore not required for diet-mediated longevity. Consistent with the yeast odorant results, however, we do find evidence for interaction between the olfactory and diet pathways. The relative increase in median and mean longevity in

*Or83b*<sup>2</sup> homozygous mutant and wild-type flies. Each point represents one fly (*n* = 15 for each sex and genotype). Box plots represent the median (horizontal line), 95% confidence intervals (box), and range (whiskers) of the data.

*Or83b*<sup>2</sup> flies was significantly greater when flies were maintained in well-fed conditions (Tables 1 and 2), and mutant animals were partially resistant to changes in diet (fig. S9). Thus, the *Or83b* mutation extends longevity largely, but not exclusively, through a diet-independent pathway.

Reduced early reproductive output is not required for extended longevity in *Or83b*<sup>2</sup> mutants. We observed the largest life-span extension in very high nutrient conditions where flies were provided access to live yeast paste (see Table 1), and, under these conditions, homo-

zygous *Or83b*<sup>2</sup> mutant females had equal or greater reproductive output than control females (Fig. 4A). We consistently observed that *Or83b*<sup>2</sup> mutants showed moderately reduced egg production from 24 to 48 hours post-eclosion, but the reason for this is unclear. Feeding rates are not reduced in mature flies (fig. S10), but reduced reproduction may be due to a delay in the onset of adult feeding, because the chemotaxis ability of mutant flies is compromised.

*Or83b*<sup>2</sup> flies exhibited a range of phenotypes indicative of altered physiology and enhanced stress resistance. Females were more resistant to hyperoxia (mean longevity = 110 ± 2.04 hours and 123 ± 1.0 hours for control and *Or83b*<sup>2</sup> mutant females, respectively;  $P < 1 \times 10^{-6}$ , determined by means of a log-rank test). Mutants are resistant to starvation (Fig. 4, B and C), and females have significantly elevated levels of triglyceride, the primary lipid-storage molecule in *Drosophila* (Fig. 4D), despite their similar overall size and weight (Fig. 4E). Mutant males have higher but statistically indistinguishable levels of triglyceride, which suggests that life span and fat content are separable in this sex. The observed increases in longevity and stress resistance do not result from decreased metabolic rate (Fig. 4F).

Aging and longevity in *Caenorhabditis elegans* are regulated by sensory function through antagonistic effects of specific gustatory and olfactory neurons (18, 19). Although the specific environmental cues that regulate longevity in *C. elegans* are unknown, sensory regulation of aging largely involves insulin/IGF (insulin-like growth factor) signaling (18). Modulation of aging by gustatory neurons is entirely insulin signaling (i.e., *daf-16*)–dependent, whereas longevity extension by the ablation of olfactory neurons has a large *daf-16*–independent component (18).

Sensory systems and insulin-mediated longevity regulation are evolutionarily conserved (20, 21). Thus, as in *C. elegans*, olfaction may affect aging in *Drosophila* through altered insulin signaling and subsequent modulation of transcription factor dFOXO (the fly ortholog to *daf-16*). However, expression levels of *Drosophila* insulin-like peptides show no consistent differences in *Or83b*<sup>2</sup> mutant flies (fig. S11). Consistent with normal levels of insulin signaling, we found that expression of *Thor*—the *Drosophila* homolog of mammalian *4E-BP* and a primary target of dFOXO (22)—is not elevated in the body of mutant animals (fig. S11). Olfactory regulation of aging in *Drosophila* may therefore contain a substantial component that is independent of insulin signaling.

We have identified a nutrient-related olfactory cue (odorants from live yeast) and a gene involved in olfaction (*Or83b*) that limit fly life span. Olfactory-receptor function constrains the beneficial effects of dietary restriction, indicating that consumption is not the

only way that nutrient availability modulates longevity (4). Genetic dissection of the roles of conventional odorant receptors in the life span of *Drosophila* may reveal additional candidate odors and neural circuits for longevity regulation.

#### References and Notes

1. S. D. Pletcher *et al.*, *Curr. Biol.* **12**, 712 (2002).
2. J. Zheng, R. Mutcherson II, S. L. Helfand, *Aging Cell* **4**, 209 (2005).
3. T. G. Bross, B. Rogina, S. L. Helfand, *Aging Cell* **4**, 309 (2005).
4. G. B. Carvalho, P. Kapahi, S. Benzer, *Nat. Methods* **2**, 813 (2005).
5. W. Mair, P. Goymier, S. D. Pletcher, L. Partridge, *Science* **301**, 1731 (2003).
6. T. P. Good, M. Tatar, *J. Insect Physiol.* **47**, 1467 (2001).
7. W. Mair, M. D. W. Piper, L. Partridge, *PLoS Biol.* **3**, e223 (2005).
8. S. D. Pletcher, S. Libert, D. Skorupa, *Aging Res. Rev.* **4**, 451 (2005).
9. A. K. Chippindale, A. Leroi, S. B. Kim, M. R. Rose, *J. Evol. Biol.* **6**, 171 (1993).
10. L. B. Vosshall, A. M. Wong, R. Axel, *Cell* **102**, 147 (2000).
11. T. Elmore, R. Ignell, J. R. Carlson, D. P. Smith, *J. Neurosci.* **23**, 9906 (2003).
12. E. A. Hallem, M. G. Ho, J. R. Carlson, *Cell* **117**, 965 (2004).
13. S. A. Kreher, J. Y. Kwon, J. R. Carlson, *Neuron* **46**, 445 (2005).

14. M. C. Larsson *et al.*, *Neuron* **43**, 703 (2004).
15. R. Benton, S. Sachse, S. W. Michnick, L. B. Vosshall, *PLoS Biol.* **4**, e20 (2006).
16. E. M. Neuhaus *et al.*, *Nat. Neurosci.* **8**, 15 (2005).
17. W. C. Orr, R. S. Sohal, *Exp. Gerontol.* **38**, 227 (2003).
18. J. Alcedo, C. Kenyon, *Neuron* **41**, 45 (2004).
19. J. Apfeld, C. Kenyon, *Nature* **402**, 804 (1999).
20. A. Dahanukar, E. A. Hallem, J. R. Carlson, *Curr. Opin. Neurobiol.* **15**, 423 (2005).
21. M. Tatar, A. Bartke, A. Antebi, *Science* **299**, 1346 (2003).
22. M. Miron *et al.*, *Nat. Cell Biol.* **3**, 596 (2001).
23. We thank S. Lou, D. Skorupa, M. Tierney, and the Pletcher laboratory for help with *Drosophila* husbandry and metabolic assays; and J. Ferris for his technical assistance in the behavioral assays. C. Gendron, M. Tatar, three anonymous reviewers, and members of the Antebi laboratory provided comments on the manuscript. The research is supported by NIH (R01AG023166), Glenn Foundation, the Ellison Medical Foundation to S.D.P., and the American Federation for Aging Research to S.L. and S.D.P.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/1136610/DC1

Materials and Methods

Figs. S1 to S11

References

23 October 2006; accepted 23 January 2007

Published online 1 February 2007;

10.1126/science.1136610

Include this information when citing this paper.

## Redirection of Silencing Targets by Adenosine-to-Inosine Editing of miRNAs

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Primary transcripts of certain microRNA (miRNA) genes are subject to RNA editing that converts adenosine to inosine. However, the importance of miRNA editing remains largely undetermined. Here we report that tissue-specific adenosine-to-inosine editing of miR-376 cluster transcripts leads to predominant expression of edited miR-376 isoform RNAs. One highly edited site is positioned in the middle of the 5'-proximal half "seed" region critical for the hybridization of miRNAs to targets. We provide evidence that the edited miR-376 RNA silences specifically a different set of genes. Repression of phosphoribosyl pyrophosphate synthetase 1, a target of the edited miR-376 RNA and an enzyme involved in the uric-acid synthesis pathway, contributes to tight and tissue-specific regulation of uric-acid levels, revealing a previously unknown role for RNA editing in miRNA-mediated gene silencing.

Many developmental and cellular processes are regulated by microRNA (miRNA)–mediated RNA interference (RNAi) (1–4). After incorporation into the RNA-induced silencing complex, miRNAs guide the RNAi machinery to their target genes by forming RNA duplexes, resulting in sequence-specific mRNA degradation or translational repression (1, 2, 4). The generation of mature miRNAs requires the processing of primary transcripts (pri-miRNAs) (5), and A → I RNA editing occurs to certain pri-miRNAs (6–8).

Human chromosome 14 and syntenic regions of the distal end of mouse chromosome 12 harbor the miR-376 cluster of miRNA genes (9).

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