fewer cells (*5*). Expression of the constitutively active AtGPA1^{QL} increased etiolated hypocotyl length due to increased cell elongation, similar to the *Atrgs1*-null mutants (Fig. 4, C and G).

In light-grown seedlings, both null mutants of *AtRGS1* and lines overexpressing AtGPA1^{QL} produced longer primary roots compared with wild-type *Arabidopsis* or null mutants of *AtGPA1* (Fig. 4D). This increased root growth phenotype resulted from increased cell production in root meristems (Fig. 4G). These data suggest that increased activity of AtGPA1, either by expression of constitutively active AtGPA1^{QL} or through loss of AtRGS1 expression, results in increased cell proliferation in the apical root meristem. Inducible overexpression of *AtRGS1* (*7*) (fig. S4) produced a similar phenotype as loss of *AtGPA1* (Fig. 4F), further suggesting that AtRGS1 antagonizes the activation of AtGPA1. In addition, some loss-of-function *gpa1* phenotypes, such as paclobutrazol and sugar sensitivity, are the opposite in the *Atrgs1* mutants, indicating a role for activated GPA1 in other signaling pathways throughout development (figs. S5 and S6).

Heterologous expression of full-length AtRGS1 protein in Sf9 insect cells has not yet provided adequate expression levels for a biochemical test of GAP activity in vitro. Nevertheless, the evidence that null mutants of AtRGS1 phenocopy the constitutively active mutant form of AtGPA1 (AtGPA1^{QL}), that overexpression of AtRGS1 antagonizes the activation of AtGPA1 (Fig. 4), and that full-length AtRGS1 interacts with AtGPA1 in an AlF_4^--dependent manner (Fig. 3) suggests that AtRGS1 exerts GAP activity on AtGPA1 in vivo. Our results here support earlier findings that cell proliferation in plants is regulated by heterotrimeric G protein subunits and further extend those findings by showing that this regulation is cell type specific. It also reveals that cell proliferation control by the *Arabidopsis* G protein mechanistically involves either the unsequestered Gβγ subunit or the activated Gα subunit as the predominant regulatory element, depending on cell type.

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Supporting Online Material

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Figs. S1 to S6

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Demography of Dietary Restriction and Death in *Drosophila*

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Dietary restriction (DR) increases life-span in organisms from yeast to mammals, presumably by slowing the accumulation of aging-related damage. Here we show that in *Drosophila*, DR extends life-span entirely by reducing the short-term risk of death. Two days after the application of DR at any age for the first time, previously fully fed flies are no more likely to die than flies of the same age that have been subjected to long-term DR. DR of mammals may also reduce short-term risk of death, and hence DR instigated at any age could generate a full reversal of mortality.

Dietary restriction (DR) prolongs life-span and delays the onset of many age-related declines in function (*1–4*). In *Drosophila*, DR is applied by maintenance of adult flies on a food medium that contains roughly 35% less yeast and sugar than standard laboratory medium (*2*, *5*). Both mean and maximum life-span are increased under DR conditions (*5*). Age-specific mortality is a measure of the instantaneous hazard of death for an individual at a given age. Unlike survivorship analysis, which is a cumulative measure, age-specific mortality allows independent comparisons of vulnerability to death at different ages (*6*, *7*). In *Drosophila*, chronic DR results in a delay in the onset of a detectable aging-related increase in mortality (*5*). Once the mortality increase is detected, however, it proceeds at roughly the same rate in DR and control flies (*5*).

Interventions can lower adult mortality by slowing the accumulation of the irreversible damage that is characteristic of aging (aging-related damage), by reducing short-term vulnerability to death (risk), or by some combination of the two (*8*). We can distinguish these hypotheses experimentally for DR by examining the effect of past and current nutritional conditions on age-specific mortality. This type of approach has shown that, in *Drosophila*, increased reproductive activity in males (*8*) and yeast deprivation in females (*9*) result in a higher mortality that is entirely due to an increased risk of death. In contrast, Mediterranean fruit flies (*Ceratitis capitata*) switched from sugar only to sugar and yeast food were permanently affected by their previous diet (*10*). If DR acts solely by slowing the accumulation of aging-related damage, then the onset of DR would not lead to a drop in mortality rate, because the damage would not be reversed. However, DR would result in a slower subsequent accumulation of aging-related damage and, hence, a less rapid subsequent increase in

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mortality rate with age. If, instead, increased nutrient availability introduces a higher risk of death, then removal of this risk by DR would result in a sustained drop in the elevation of the mortality trajectory relative to that of permanently fully fed individuals. If DR increases life-span solely by reducing the short-term risk of death, then the mortality rates of previously fully fed individuals switched to DR would drop to the same levels as those seen in same-age individuals subjected to DR throughout adulthood. Hence, both hypotheses predict that the onset of DR at any age will increase life-span. Under the damage hypothesis, the mortality trajectory after the onset of DR has a lowered slope, whereas under the risk hypothesis the mortality rate shows a sustained drop in elevation. These hypotheses are not mutually exclusive.

To determine the importance of these two mechanisms of life-span extension by DR in *Drosophila*, nutritional conditions were manipulated and age at death was assessed in 7492 individuals. Age-specific mortality trajectories for female flies subjected to DR from the onset of adulthood showed the characteristic delay in the onset of detectable aging-related mortality, compared to those maintained on full feeding (Fig. 1A). When fully fed flies were switched to DR on days 14 or 22 of adulthood, there was a rapid and complete reduction in age-specific mortality to the levels seen in permanent DR flies (Fig. 1A). Within 48 hours, the mortality of these switched cohorts had declined to the level of flies maintained on DR throughout adult life, and after this point the two mortality trajectories were indistinguishable. Males showed a similar response (Fig. 2A).

These results demonstrate that age-specific mortality of the DR flies depends only upon their age and their current nutritional status, with past nutrition having no detectable effect. DR therefore lowered mortality entirely as a consequence of a lower short-term risk of death, and the accumulation of aging-related damage remained unaffected. In reciprocal switches from DR to fully fed conditions, mortality levels showed a rapid (within 48 hours) increase (Fig. 1B). In females, subsequent mortality was reduced in the switched groups compared to mortality of the permanently fully fed flies, and the magnitude of this reduction was greater in the group that was switched later. Long-term DR therefore either impeded the females' ability to respond to full feeding or protected against its increased risk. Males showed no such effect, and subsequent mortality was slightly higher in individuals with a history of DR (Fig. 2B).

We performed a similar experiment examining the effect of current and past experimental temperature on mortality in *Drosophila*. In sharp contrast to the effects of DR, lowered temperature, which also increases life-span in ectotherms (*11*, *12*), reduced the accumulation of aging-related damage. Flies cultured at a lower temperature exhibited a reduction in the slope of the mortality trajectory, rather than a delay in the time when aging-related mortality could first be detected (Fig. 3A), as has been previously reported (*13*). When flies were switched from 27°C to 18°C environments (Fig. 3A), the increased mortality driven by life at a higher temperature persisted in the switched flies compared to the 18°C control flies. This effect of thermal history was greater the later the age at which the switch was made. After the switch, the subsequent rate of increase in mortality with age reflected the new temperature: It was lower in the switched flies currently at 18°C than in the flies permanently at 27°C. Flies switched from high to low temperature at various adult ages therefore showed slower demographic aging. The reciprocal switch, from 18°C to 27°C (Fig. 3B), produced similar findings: The lower mortality seen in flies at the lower temperature persisted in the switched flies, and to a greater extent the later the switch was made. After the switch, the rate of increase in mortality rate with age rose to become indistinguishable from that seen in flies kept permanently at the high temperature. These results demonstrate that higher temperature increases the rate of aging by inflicting permanent debilitation and that thermal history is a major determinant of mortality. This is in sharp contrast to the effect of DR on mortality, in which there is no memory of past feeding.

These findings support the hypothesis that DR in *Drosophila* extends life-span solely by reducing the short-term risk of death. DR and control flies accumulate irreversible, aging-related damage at the same rate, but the accumulated damage produces a detectable increase in the death rate at later ages in the DR flies. Death occurs when the combined effects of risk and damage are sufficiently great, and a lowering of risk by DR holds the flies below this death threshold for longer, in some support of the set-point model of life-span extension by

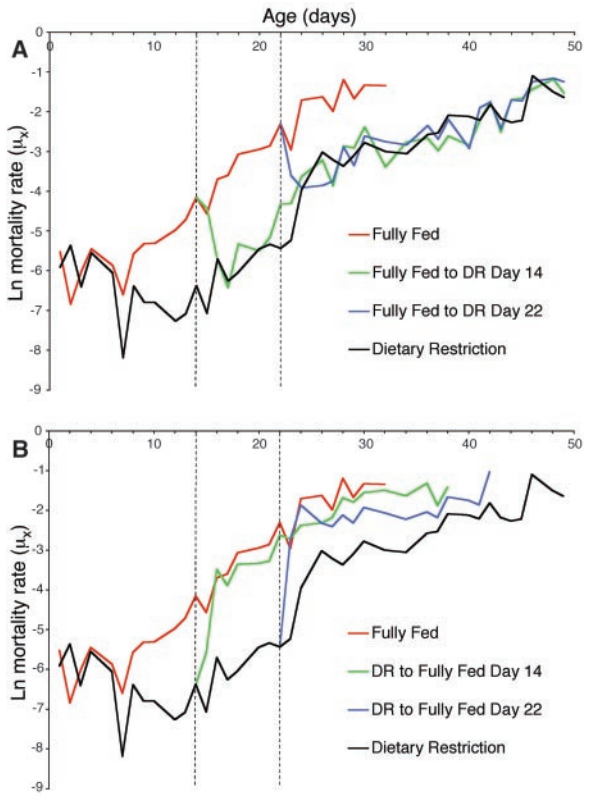

Fig. 1. Age-specific mortality rates of female *Drosophila* in response to the instigation or removal of a DR regime. Mortality rate (μx) is plotted on the natural log scale, because it increases exponentially with age (*21*). Dotted vertical lines represent days on which food regimes were switched (switch days). (**A**) Previously fully fed flies showed a rapid and complete reduction in mortality when switched to DR at 14 or 22 days of adulthood. Cox regression was used to avoid making assumptions about the shape of the trajectories post-switch. Within 48 hours of the switch, mortality trajectories of the switched cohorts were indistinguishable from those of same-age flies maintained on DR throughout adulthood (day 14 switch: $P = 0.79$, DR control $n = 2137$, switch $n = 1245$, risk ratio = 0.994; day 22 switch: $P = 0.94$, DR control $n = 912$, switch $n = 798$, risk ratio = 0.998). After both switch points, fully fed and DR flies differed significantly in mortality (from day 14 switch onwards: $P < 0.0001$, DR control $n = 2137$, fully fed control $n = 2198$, risk ratio = 0.419; from day 22 switch onwards: $P < 0.0001$, DR control $n = 798$, fully fed control $n = 501$, risk ratio = 0.491). (**B**) Reciprocal switches from restricted conditions to full feeding resulted in rapid and marked increases in log mortality rates within 48 hours. However, mortality remained significantly lower than that of fully fed controls ($P < 0.0001$). The protection offered by DR increased with time spent on the regime: 48 hours after the day 22 switch, the risk ratios of switched cohorts compared to those of fully fed controls were 0.831 for flies switched to full feeding on day 14, and 0.763 for flies switched to full feeding on day 22. There was a significant difference in mortality between the switched cohorts during this time ($P < 0.0001$).

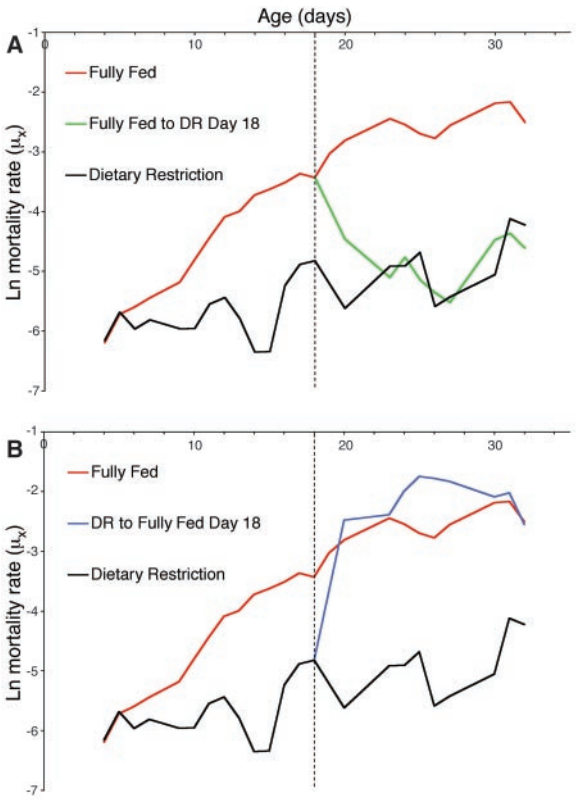

Fig. 2. Age-specific mortality rates of male *Drosophila* in response to the instigation or removal of a DR regime. Mortality rate (μx) is plotted on the natural log scale. The dotted vertical line represents the switch day. (**A**) Flies switched from full feeding to DR at day 18 showed a rapid and complete reduction in mortality. Cox regression was used to avoid making assumptions about the shape of the trajectories post-switch. Within 48 hours, subsequent mortality trajectories were indistinguishable from those of flies subjected to DR throughout adulthood: $P = 0.76$, DR control $n = 369$, switch $n = 297$, risk ratio = 1.04. After the switch, fully fed and DR flies differed significantly in mortality: $P < 0.0001$, DR control $n = 369$, fully fed control $n = 322$, risk ratio = 0.297. (**B**) In the reciprocal switch from DR to fully fed conditions at day 18, mortality rates rapidly increased. Subsequent mortality was higher in the switched flies than in the fully fed control flies: $P = 0.0007$, fully fed control $n = 322$, switch $n = 327$, risk ratio = 1.167. This experiment was terminated on day 32.

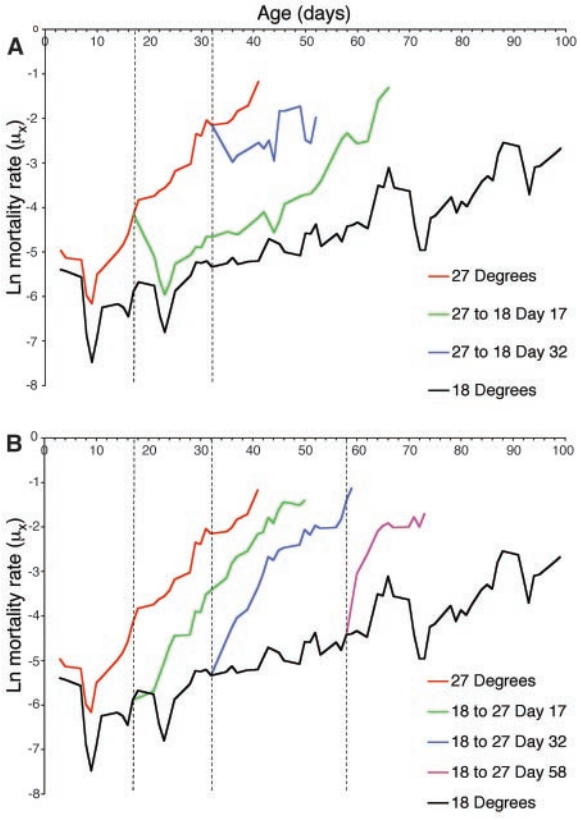

Fig. 3. Age-specific mortality rates of male *Drosophila* in response to switches in ambient temperature. The dotted vertical lines represent switch days. Cox regression was not used, because changing thermal treatment altered the slope of the mortality trajectories. Data were fitted to the Gompertz model ($\mu x = ae^{bx}$) where μx represents mortality rate at age x, a is the baseline mortality, and b is the change in mortality rate with age (the slope of the mortality trajectory) (*22*). Gompertz parameters are shown followed by the upper and lower 95% confidence limits in parentheses. (**A**) A lower experimental temperature reduced the slope of the mortality trajectory: 18°C control, $a = 0.0015$ (0.0012, 0.0017), $b = 0.038$ (0.035, 0.041); 27°C control, $a = 0.0021$ (0.0018, 0.0024), $b = 0.12$ (0.11, 0.12). Mortality of flies switched from 27°C to 18°C remained higher than that of flies maintained at 18°C throughout adulthood and the slope of the mortality trajectory dropped to become much more similar to that of the 18°C controls than the 27°C controls: day 17 switch, $a = 0.0037$ (0.0031, 0.0045), $b = 0.064$ (0.059, 0.069); day 32 switch, $a = 0.039$ (0.032, 0.048), $b = 0.060$ (0.059, 0.069). (**B**) In the reciprocal switch from 18°C to 27°C, the mortality trajectories of flies with a history of low temperature remained lower than that of 27°C controls. The slope of the mortality trajectories of switched cohorts increased to the levels of the 27°C controls: day 17 switch, $a = 0.0042$ (0.0035, 0.0052), $b = 0.13$ (0.12, 0.14); day 32 switch, $a = 0.013$ (0.011, 0.015), $b = 0.11$ (0.10, 0.12); day 58 switch, $a = 0.047$ (0.038, 0.058), $b = 0.093$ (0.077, 0.11).

DR (*14*). The crucial criterion for determining the roles of reduced risk and damage in the extension of life-span is the response of the mortality trajectory to switches between high- and low-mortality regimes. Although other interventions such as mutations in the insulin and insulin-like growth factor signaling pathway have been shown to extend life-span in *C. elegans*, *Drosophila*, and mice (*15*–*18*), it is not clear if these reduce risk, the rate of accumulation of aging-related damage, or both. DR initiated during middle age in mammals increases subsequent life-span (*19*, *20*), but this result is consistent with either the damage or risk hypothesis. The critical experiments in mammals have yet to be done.

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Supporting Online Material

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