MEDICINE

Longevity Regulation by *Drosophila* Rpd3 Deacetylase and Caloric Restriction

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Genetic studies of single gene mutations are revealing mechanisms and pathways that regulate longevity across distant species (*1*). One such mechanism is an alteration in histone deacetylase activity. Abolishing expression of the Rpd3 deacetylase (*2*) or increasing expression of the Sir2 deacetylase (*1*) increases life-span in yeast; Sir2 mediates increased nematode longevity as well (*1*). Caloric restriction is an intervention that increases life-span in mammals, insects, nematodes, and yeast (*1*, *3*). Although the molecular pathways underlying the response to caloric restriction are yielding to genetic analysis in yeast (*1*), there is little information on how this response is regulated in metazoans. We investigated the relationship between histone deacetylases, caloric restriction, and longevity in *Drosophila*.

Greatly reduced Rpd3 levels are lethal in *Drosophila* (*4*), but partial reduction of Rpd3 levels has not been evaluated for its effect on life-span. We found that males heterozygous for either a hypomorphic (partial loss-of-function) or null mutation of *rpd3* have life-span extension of 33% and 41 to 47%, respectively (Fig. 1A). Females heterozygous for the hypomorphic allele have a 52% increase in life-span, whereas females carrying the null mutation have only modest changes in life-span (maximum but not median life-spans are increased). The presence of large increases in life-span for males carrying both types of allele indicates that the effect is specific to the *rpd3* locus. The different results for females may indicate a greater sensitivity to the predicted lower levels of Rpd3 in individuals carrying the null mutation compared with individuals carrying the hypomorphic allele.

To further explore the parallels between life extension in yeast and *Drosophila*, we examined the effect of caloric restriction on normal-lived control and long-lived *rpd3* mutants. Longevity is increased to approximately the same extent in control flies fed a low-calorie diet and *rpd3* mutants fed a normal diet (Fig. 1B). In addition, caloric restriction of the *rpd3* mutants shows no further extension of life-span (Fig. 1B). The lack of an additive increase in longevity is not due to a physiological cap for life-span extension, because *rpd3* females that were kept as virgins did have a further extension of longevity [see (*14*) in supporting online material (SOM) (*5*)]. Furthermore, at least one other mutation in *Drosophila*, *Indy*, increases life-span to a greater extent (>90%) (*6*). It has previously been demonstrated that caloric restriction of flies leads to a moderate but significant down-regulation of Rpd3, analogous to the decreases obtained in heterozygotes carrying *rpd3* mutations (*7*, *8*). The data suggest that life-span extension by the *rpd3* mutation is within a pathway related to caloric restriction.

Given the evidence connecting histone deacetylases to life-span extension, we wanted to determine whether *Drosophila* longevity was generally responsive to changes in histone acetylation. Increased acetylation (*9*) was achieved by mutating an independent locus, *Su(var)2-1*^{01}. This had virtually no effect upon life-span (*5*). The effects of Rpd3 therefore appear to be specific, mediated by its targeting to particular genes and/or histone residues. The life-span extension obtained by feeding the drug phenylbutyrate to adult *Drosophila* may operate by a similar mechanism (*10*).

Studies in yeast have implicated Sir2 as an important element in the life-extending effects of caloric restriction (*1*). We found that under our two life-extending conditions, *rpd3* mutants fed normal food and wild-type flies fed low-calorie food, Sir2 expression was increased twofold (Fig. 1C). Our results highlight the conservation of longevity regulation across distant species boundaries and suggest a genetic pathway that begins with caloric restriction and proceeds to down-regulation of Rpd3, followed by Sir2-independent regulation of longevity effector genes (gene activation) and/or increased Sir2 levels and Sir2-dependent modulation of longevity effector genes (gene silencing).

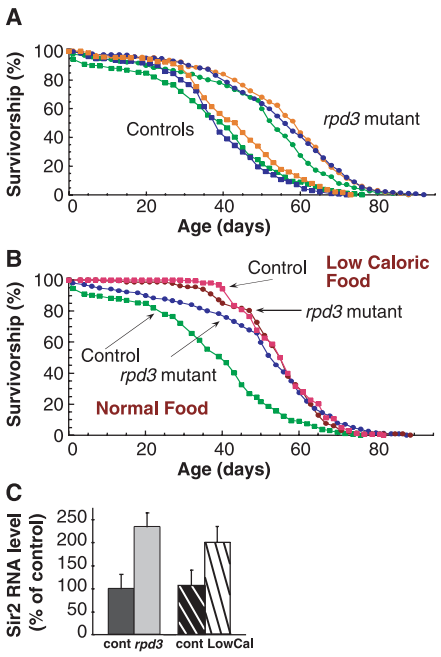

Fig. 1. (**A**) Life-span is extended when levels of the rpd3 histone deacetylase are reduced. Survival curves are for males heterozygous for either a hypomorphic (*rpd3*$^{P\text{-}UTR}$) (green circles) or null (*rpd3*^{def24}) *rpd3* mutation; the null allele was tested with two independent crossing schemes, paternal (blue circles) and maternal (orange circles). Controls are genetically matched (squares) [see (*11*) in SOM]. Median life-span increases are 47% for the paternally derived null allele, 41% for the maternally derived null allele, and 33% for the hypomorphic allele; mean increases are very similar. Flies were maintained at 25°C and scored for survival as in (*6*); number of flies per sex in each genotype was 300 to 400. (**B**) Caloric restriction does not further extend the life-span of long-lived *rpd3* mutants. Survival curves are for males heterozygous for either a hypomorphic *rpd3* allele (*rpd3*$^{P\text{-}UTR}$) (circles) or a control allele (*rpd3*$^{P\text{-}1.8}$) (squares) (*5*). Median 25°C life-spans for control *rpd3*$^{P\text{-}1.8}$/+ flies on normal (green) and low calories (red) [0.5 SY, sugar yeast, as in (*3*)] are 39 and 55 days, respectively (a 41% increase). Median life-spans for mutant *rpd3*$^{P\text{-}UTR}$/+ flies on normal (blue) and low calories (brown) are 52 and 55 days, respectively. Mean life-spans are similar. (**C**) Life-extending *rpd3* mutations and caloric restriction increase Sir2 expression. RNA levels were measured by reverse transcriptase polymerase chain reaction [see (*15*) in SOM] in whole male flies from control *rpd3*$^{P\text{-}1.8}$/+ (black) and mutant *rpd3*$^{P\text{-}UTR}$/+ (solid gray) flies fed normal food or from wild-type flies fed normal (dark stripes) or low-calorie food (light stripes; 0.5 SY). Sir2 increased 234% in the mutant *rpd3*$^{P\text{-}UTR}$/+ background (SE 31%, $N = 6$) and 192% in low calories (SE 33%, $N = 6$).

References and Notes

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Supporting Online Material
www.science.org/cgi/full/298/5599/1745/DC1
Materials and Methods
Table S1

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