Regulation of Lifespan in *Drosophila* by Modulation of Genes in the TOR Signaling Pathway

Pankaj Kapahi, Brian M. Zid, Tony Harper, Daniel Koslover, Viveca Sapin, and Seymour Benzer

Supplemental Experimental Procedures

Drosophila Strains

da-Gal4, *24B*-GAL4, *appl*-GAL4, and *gmr*-GAL4 strains were obtained from the *Drosophila* stock center (Bloomington, Indiana). *PO188*-GAL4 and *PO163*-GAL4 strains were obtained from Umea *Drosophila* stock center (Sweden). *DJ634*-GAL4 was isolated in our laboratory [S1]. All driver lines were outcrossed three to five times into the w^{1118} background. UAS-*dTsc1* and UAS-*dTsc2* strains were generously provided by Dr Duojia Pan [S2]. The UAS-$dTOR^{FRB}$, UAS-$dTOR^{TED}$, and UAS-$dTOR^{WT}$ transgenic lines were kindly provided by Dr. Thomas Neufeld [S3]. The UAS-$dS6k^{KQ}$ and UAS-$dS6k^{STDETE}$ strains were kindly provided by Dr. Mary Stewart [S4].

Lifespan Measurements

Crosses were set up between five to seven virgin females and three to six males of various strains. Progeny were collected 2–4 days after adult emergence by separating males and females under a brief (<2 min) carbon dioxide anesthesia. Flies were transferred every 3–4 days into fresh food vials, and the number of dead flies was recorded to obtain the survival curves. p values were calculated by using Graphpad prism software [S5].

Fly Food

Standard laboratory food was prepared as described by Lewis [S7]. The food consisted of 82% water, 0.45% agar, 5% dextrose, 2.5% sucrose, 8.3% cornmeal, 1.5% dried yeast, 0.06% phosphoric acid, and 0.4% propionic acid. The specially prepared food for testing how varying yeast-extract concentration modulates lifespan consisted of 82% water, 0.45% agar, 5% dextrose, 2.5% sucrose, 8.3% cornmeal, 0.06% phosphoric acid, 0.4% propionic acid, plus the specified concentration of yeast extract (Becton, Dickinson & Company, Cat. No. 212750). We have confirmed the observations that on high-yeast extract, flies show increases in fecundity, body weight, and a decrease in stress resistance (data not shown).

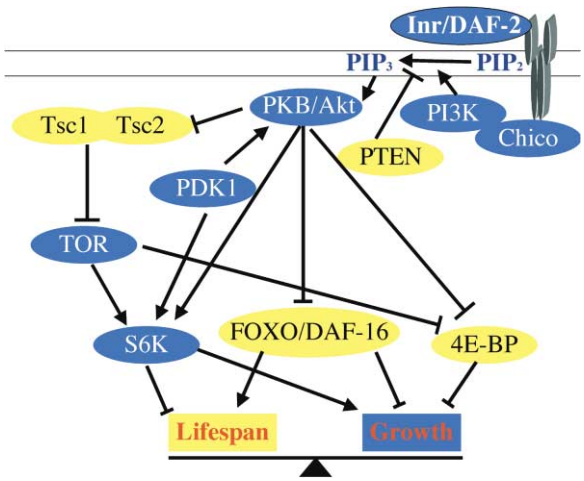

Figure S1. Signaling Pathways Balancing Growth and Lifespan

At left is the TSC-TOR signaling pathway, while on the right is the insulin/IGF-dependent PI3K-AKT pathway. Arrows represent molecular interactions based on studies in various model organisms [S6].

Weight Measurements

Flies were raised on standard laboratory food at 25°C, and weights of 3- to 5-day-old male flies were measured in four replicates of ten flies by using a Sartorius fine balance (Sartorius Genius Series, model ME).

Lipid-to-Protein Ratio

All lipid analyses were performed by using a commercially available kit (Stanbio laboratory). Protein measurements were made by using the Bradford assay (Biorad). Four replicates of ten male flies were used for analyzing protein-to-lipid ratios.

Starvation Test

Flies were raised on standard laboratory food at 25°C. Two to three vials of 30–40 males, 3–5 days old, were put into vials with 5 ml 1% agarose gel at 25°C. Starvation resistance was defined as the mean survival time under these conditions.

X-Gal Staining of Tissues

This was done as described [S1]. For each line, at least five 3- to 7-day-old adult males were cryosectioned. The sections were fixed for 20 min in 1% glutaraldehyde in PBS (pH 7.2), reacted with 0.1% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and incubated at 37°C. The incubation time ranged from a few to several hours, depending on the strain. The reaction was stopped by rinsing twice with PBS before mounting in 70% glycerol/PBS.

Supplemental References

S1. S1. Seroude, L., Brummel, T., Kapahi, P., and Benzer, S. (2002). Spatio-temporal analysis of gene expression during aging in Drosophila melanogaster. Aging Cell *1*, 47–56.

S2. Gao, X., and Pan, D. (2001). TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. Genes Dev. *15*, 1383–1392.

S3. Hennig, K.M., and Neufeld, T.P. (2002). Inhibition of cellular growth and proliferation by dTOR overexpression in Drosophila. Genesis *34*, 107–110.

S4. Barcelo, H., and Stewart, M.J. (2002). Altering Drosophila S6 kinase activity is consistent with a role for S6 kinase in growth. Genesis *34*, 83–85.

S5. Melov, S., Ravenscroft, J., Malik, S., Gill, M.S., Walker, D.W., Clayton, P.E., Wallace, D.C., Malfroy, B., Doctrow, S.R., and Lithgow, G.J. (2000). Extension of life-span with superoxide dismutase/catalase mimetics. Science *289*, 1567–1569.

S6. Marygold, S.J., and Leevers, S.J. (2002). Growth signaling: TSC takes its place. Curr. Biol. *12*, R785–R787.

S7. Lewis, E.B. (1960). A standard new food medium. Drosoph. Inf. Serv. *34*, 117–118.

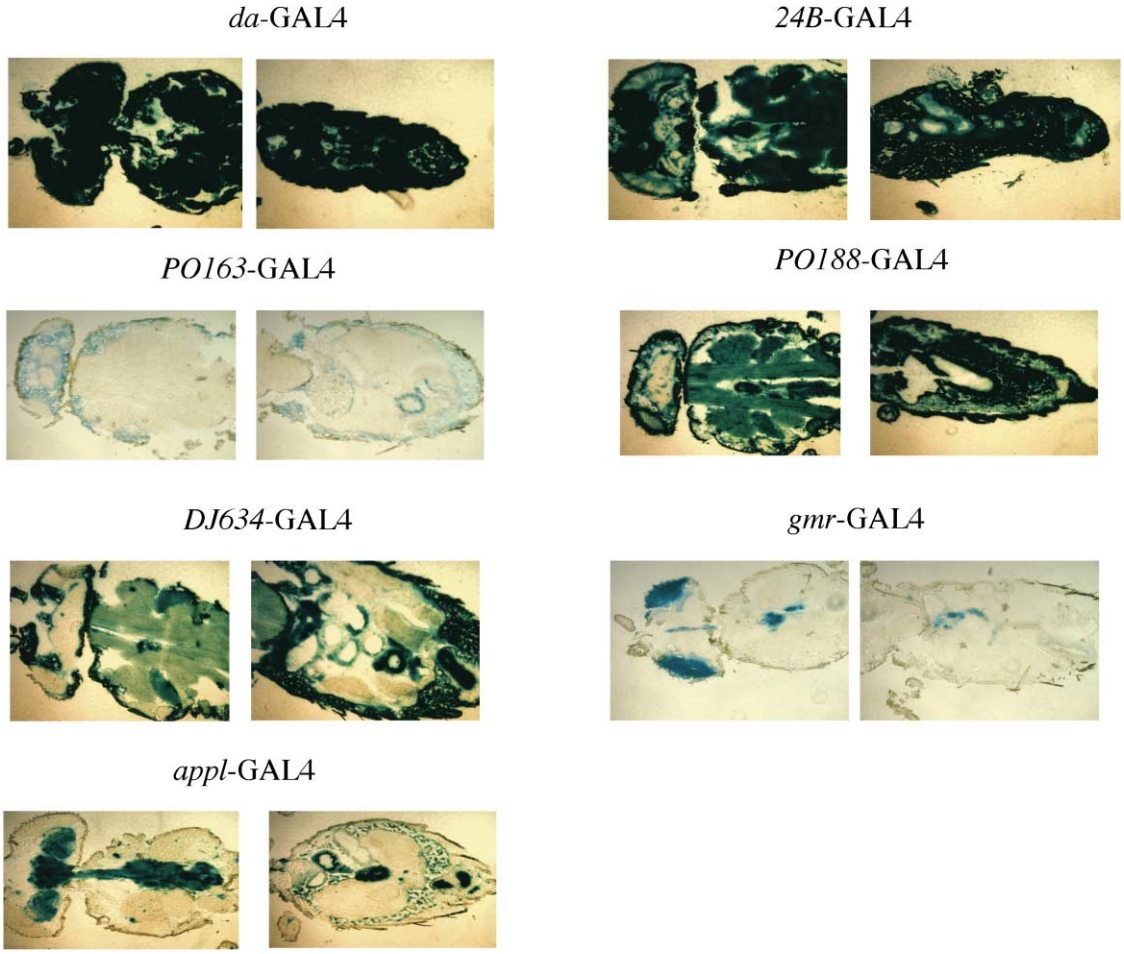

Figure S2. GAL-4 Expression Patterns of Various Drivers

X-gal staining of horizontal cryosections of 3- to 5-day-old adult males obtained by crossing various GAL-4 enhancer traps with a UAS-*lacZ* strain. The figures show qualitative differences in β-galactosidase expression. The sections were developed for various times, before stopping the enzymatic reaction, to best observe each distribution pattern, so they are not quantitative. The following expression patterns were observed for the various drivers: *gmr*-GAL4 in the eye, *appl*-GAL4 in the nervous system, and 24B-GAL4 and PO188-GAL4 in the muscle and fat tissues; DJ634-GAL4 and PO163-GAL4 were specific to fat tissue.

Table S1. Weight, Lipid-to-Protein Ratio, and Starvation Resistance of Male Flies of Different Genotypes in the TOR Signaling Pathway

Genotype	Weight/Fly (mg)	Lipid/Protein Ratio (Relative)	Starvation Resistance (hr)
+/*da*-GAL4	0.68 ± 0.01	1.00 ± 0.2	51 ± 12
da-GAL4/UAS-*dTsc2*	0.74 ± 0.02	1.07 ± 0.1	52 ± 10
+/UAS-*dTsc2*	0.68 ± 0.03	0.92 ± 0.05	51 ± 11
da-GAL4/UAS-*dTOR*^{FRB}	0.69 ± 0.02	0.89 ± 0.04	48 ± 11
+/UAS-*dTOR*^{FRB}	0.58 ± 0.01	0.95 ± 0.12	46 ± 12
da-GAL4/UAS-*dS6k*^{KQ}	0.58 ± 0.01	0.86 ± 0.02	51 ± 15
+/UAS-*dS6k*^{KQ}	0.63 ± 0.01	1.07 ± 0.13	41 ± 10

No statistically significant differences were observed among the strains.