Published in final edited form as:
Aging Cell. 2010 February ; 9(1): 100–103. doi:10.1111/j.1474-9726.2009.00537.x.

Perturbation of mitochondrial complex V alters the response to dietary restriction in *Drosophila*

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Abstract

Studies in a broad spectrum of model organisms have reported that dietary restriction (DR) is associated with an increase in mitochondrial electron transport chain (ETC) function. However, the question of whether ETC function is required for DR-mediated longevity remains controversial. Here, we report that genetic and pharmacological interventions that target mitochondrial complex V affect *Drosophila* lifespan in a nutrient-dependent manner. These findings support a requirement for mitochondrial complex V in DR-mediated longevity in flies.

Keywords

Drosophila; mitochondria; dietary restriction; electron transport chain

Numerous correlative studies have suggested that alterations in mitochondrial electron transport chain (ETC) function may play a role in mediating the pro-longevity effects of dietary restriction (DR) (Lin *et al.* 2002; Nisoli *et al.* 2005; Bishop & Guarente 2007; Guarente 2008). At the same time, genetic studies in both invertebrate (Ishii *et al.* 1998; Feng *et al.* 2001; Dillin *et al.* 2002; Lee *et al.* 2003; Walker *et al.* 2006; Copeland *et al.* 2009) and vertebrate (Dell'agnello *et al.* 2007; Lapointe & Hekimi 2008) model systems have shown that inactivation of genes important for ETC function can affect animal aging. However, an understanding of the causal relationship between ETC function and DR remains controversial. In yeast (Lin *et al.* 2002) and nematodes (Bishop & Guarente 2007), it has been reported that ETC function is required for DR-mediated longevity. However, these findings have been challenged by the observation that respiratory–deficient yeast cells display a robust DR response (Kaeberlein *et al.* 2005). In this study, we investigate the interaction between ETC function and DR in the fruit fly *Drosophila*.

Dilution of brewer's yeast has been proposed as an effective regimen for DR studies in *Drosophila* (Bass *et al.* 2007). We confirmed that this yeast DR paradigm produced longevity effects in our control flies (Figure 1A; Table 1). Reduction of brewer's yeast concentration in the media from 6% (rich media) to 1% (DR) increased lifespan in control flies. Moreover, a further reduction in yeast concentration to 0.2% yeast resulted in a shortened lifespan. Recently, we reported that knock-down of a complex V (*CG5389*) subunit resulted in increased longevity under our standard laboratory food conditions (3%

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Author Contributions: S.B, J.H, K.V and T.L designed and performed the experiments and analyzed the data. D.W.W designed experiments, supervised the work, analyzed the data and wrote the manuscript with helpful comments from S.B. and J.H.

yeast) (Copeland *et al.* 2009). Here, we examined the dependence of this complex V-mediated lifespan extension upon nutritional conditions. We used a ubiquitous expression GAL4 line, *daughterless (da)*-GAL4 to activate the *CG5389* RNAi transgene and compared survival of flies under rich, and DR media. Under rich media conditions, RNAi of *CG5389* conferred an 18% increase in mean survival compared to isogenic control flies (Figure 1B; Table 1). However, this longevity effect was abolished under DR conditions.

While using the *da-GAL4* driver activated the complex V RNAi transgene throughout development and adulthood, the DR regime was implemented exclusively during adulthood. To examine the impact of adult-specific RNAi of complex V on DR-mediated longevity, we used the mifepristone (RU486) inducible-GAL4 system (annotated P[Switch] or Gene-Switch (Osterwalder *et al.* 2001; Roman *et al.* 2001)). We used the ubiquitous tubulin (tub)-Gene-Switch (GS) driver line to examine the effect of activating RNAi of *CG5389* only during adulthood when the flies were first exposed to DR and rich media conditions. Uninduced control flies again displayed a robust increase in lifespan under DR conditions (Figure 1C; Table 1). In contrast, RNAi of complex V in adult flies greatly impaired DR-mediated life extension. Inducer had no impact on longevity in control flies (w^{1118}/*tub-GS*) under either nutritional regime (Table 1).

In agreement with our previous genetic results, flies exposed to oligomycin, a specific inhibitor of complex V, from the onset of adulthood did not show significant lifespan extension under DR conditions without shortening lifespan under rich media conditions (Figure 1D; Table 1). Taken together, our results indicate that ETC function plays an important role in mediating this DR paradigm (6% to 1% yeast). All three manipulations that target complex V (RNAi mediated by *da-GAL4*, RNAi mediated by *tub-GS* and oligomycin feeding) impaired the ability of DR to promote longevity. RNAi of complex V mediated by *da-GAL4* resulted in increased longevity under rich media and a shortening of lifespan upon DR. However, RNAi of complex V mediated by *tub-GS* and/or oligomycin feeding had no major impact on longevity under rich media and prevented an increase in longevity under DR. It is possible that variation in the degree of complex V inactivation in different tissues may be responsible for these phenotypic differences.

For a number of technical reasons, we conducted this study using male flies: 1) RNAi of ETC genes can decrease fecundity in female flies (Copeland *et al.* 2009). As reproductive status can dramatically affect feeding behavior in female flies (Carvalho *et al.* 2006) this may be a confounding factor in a study that manipulates nutrient availability. 2) The presence of eggs and subsequently larvae in the media affects the texture of the food, which may affect mortality and/or result in complicated effects on feeding behavior and hence drug (RU486 or oligomycin) dosage. 3) Using this DR (yeast restriction) paradigm, we observed robust effects on longevity in male flies which are not subject to the above variables.

Our study strongly suggest that DR results in changes in ETC function important for longevity. It will be interesting to determine the nature of these changes and whether they are evolutionarily conserved. One attractive hypothesis is that DR delays the onset of the age-related decline in ETC activity reported across the animal kingdom (Wallace 2005).

While this manuscript was under review, Zid *et al.* reported that RNAi of subunits from complex I and complex IV diminished the lifespan extension obtained upon DR in flies (Zid *et al.* 2009).

Methods

Fly stocks

Tubulin-Gene-Switch was a gift from S. Pletcher (University of Michigan, Ann Arbor, MI). The *CG5389* RNAi line was obtained from the Vienna *Drosophila* RNAi Center (Dietzl *et al.* 2007) and backcrossed into the *white*^{1118} background seven times. *da*^{G32}*-GAL4* was obtained from the Bloomington Stock Center. The age-related expression pattern of *da*^{G32}*-GAL4* was recently reported (Martin *et al.* 2009).

Fly food

One liter of stock medium consisted of 19 g of sucrose, 38 g of dextrose, 10 g of agar, 91 g of cornmeal, variable amount of Brewer's yeast (2, 10, 30, or 60 g), 11 mL of propionic acid, and 1.5 g of tegosept (1.5 g of tegosept dissolved in 15 mL of 95% ethanol).

Lifespan analysis

Lifespan studies were carried out at 25°C on a 12-hour light/dark cycle, with survivors transferred to fresh vials every 2-4 days. For Gene-Switch experiments, RU486 was administered by adding 200 μl of 0, 10, or 50 μg/ml RU486 dissolved in ethanol to the top of the fly food, as previously described. For adult-only induction, crosses were grown at 18°C during development. For oligomycin feeding, the drug was administered by adding 200 μl of 0, 5 or 25 μg/ml oligomycin dissolved in ethanol to the top of the fly food. Log-rank test was used to compare differences in survival curves.

Acknowledgments

The authors would like to thank Jeff Copeland and Margaret Wang for help with fly work and S. Pletcher, the Vienna *Drosophila* RNAi Center (Dietzl *et al.* 2007) and the *Drosophila* Stock Center (Bloomington) for fly stocks. D.W.W received support from the UCLA Older Americans Independence Center, NIH/NIA Grant P30-AG028748, and the content does not necessarily represent the official views of the National Institute on Aging or the National Institutes of Health. D.W.W also received support from the Ellison Medical Foundation, the American Federation for Aging Research, the UCLA Center for gene environment in Parkinson's Disease, and the Muscular Dystrophy Association. D.W.W is an Ellison Medical Foundation New Scholar in Aging.

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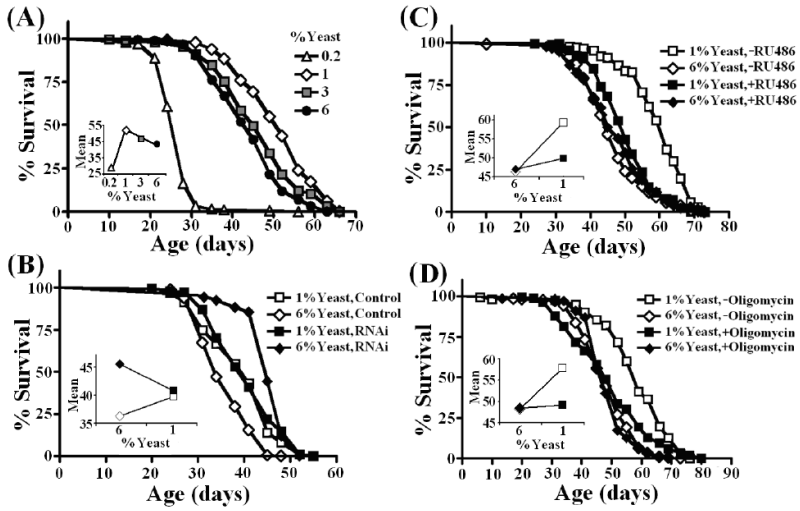

Figure 1. Genetic and pharmacological manipulations that target mitochondrial complex V affect fly lifespan in a nutrient-dependent manner

(A) Dietary restriction (1% yeast medium) extends *Drosophila* lifespan whereas malnutrition (0.2% yeast medium) shortens lifespan. **(B, C, D)** Perturbation of mitochondrial complex V affects DR-mediated extension of adult lifespan. Genotypes were as follows: **(A)** *Tub-Gene-Switch/w*^{1118}; **(B)** *da*^{G32}*-GAL4/w*^{1118} (Control) and *da*^{G32}*-GAL4/UAS-complexV-RNAi* (RNAi); **(C)** *Tub-Gene-Switch/UAS-complexV-RNAi* fed with or without RU486 (50 μg/ml during adulthood); **(D)** *Tub-Gene-Switch/w*^{1118} fed with or without oligomycin (25 μg/ml during adulthood). Complete survival data including statistical analysis, replicate *da*^{G32}*-GAL4* experiments and developmental feeding of oligomycin and RU486 are given in Table 1.

Table 1

The effects of yeast restriction on *Drosophila* longevity in the presence or absence of mitochondrial complex V inhibitors. UAS-RNAi targets the complex V subunit *CG5389*. Survival curves under yeast restriction (1% yeast) were compared to survival under rich media (6% yeast). The significance of the difference between survival curves was analyzed using log-rank test.

Genotype	% Yeast	RU486 dosage (Dev/Adult)	Oligomycin dosage (Dev/Adult)	Mean LS (days)	Mean LS extension (versus 6% yeast)	Sample size (n =)	p Value (log-rank)
TubGS/w^{1118}	0.2	0 μg/ml	0 μg/ml	26.7	-39.4 %	196	< 0.0001
TubGS/w^{1118}	1	0 μg/ml	0 μg/ml	51.28	16.4 %	180	< 0.0001
TubGS/w^{1118}	3	0 μg/ml	0 μg/ml	46.3	5.1 %	190	0.0026
TubGS/w^{1118}	6	0 μg/ml	0 μg/ml	44.05	0 %	185	-
TubGS/w^{1118}	1	0 μg/ml	0 μg/ml	58.18	20.5 %	173	< 0.0001
TubGS/w^{1118}	6	0 μg/ml	0 μg/ml	48.29	0 %	173	-
TubGS/w^{1118}	1	0 μg/ml	0/25 μg/ml	49.18	1.4 %	172	0.065
TubGS/w^{1118}	6	0 μg/ml	0/25 μg/ml	48.51	0 %	175	-
TubGS/w^{1118}	1	0 μg/ml	0 μg/ml	49.4	19.8 %	176	< 0.0001
TubGS/w^{1118}	6	0 μg/ml	0 μg/ml	41.23	0 %	181	-
TubGS/w^{1118}	1	0 μg/ml	5/25 μg/ml	40.98	0.1 %	183	0.0636
TubGS/w^{1118}	6	0 μg/ml	5/25 μg/ml	40.94	0 %	188	-
TubGS/w^{1118}	1	0 μg/ml	0 μg/ml	55.59	19.7 %	182	< 0.0001
TubGS/w^{1118}	6	0 μg/ml	0 μg/ml	46.44	0 %	183	-
TubGS/w^{1118}	1	0/50 μg/ml	0 μg/ml	54.04	15.5 %	186	< 0.0001
TubGS/w^{1118}	6	0/50 μg/ml	0 μg/ml	46.8	0 %	181	-
TubGS/UAS-RNAi	1	0 μg/ml	0 μg/ml	59.52	28 %	174	< 0.0001
TubGS/UAS-RNAi	6	0 μg/ml	0 μg/ml	46.5	0 %	183	-
TubGS/UAS-RNAi	1	0/50 μg/ml	0 μg/ml	50	6 %	180	0.0641
TubGS/UAS-RNAi	6	0/50 μg/ml	0 μg/ml	47.19	0 %	180	-
TubGS/UAS-RNAi	1	0 μg/ml	0 μg/ml	60.1	13.3 %	169	< 0.0001
TubGS/UAS-RNAi	6	0 μg/ml	0 μg/ml	53.06	0 %	180	-
TubGS/UAS-RNAi	1	10/50 μg/ml	0 μg/ml	56.57	3.9 %	139	< 0.0001
TubGS/UAS-RNAi	6	10/50 μg/ml	0 μg/ml	54.45	0 %	190	-

Genotype	% Yeast	RU486 dosage (Dev/Adult)	Oligomycin dosage (Dev/Adult)	Mean LS (days)	Mean LS extension (versus 6% yeast)	Sample size (n =)	p Value (log-rank)
da^{G32}-GAL4/w^{1118}	1	0 μg/ml	0 μg/ml	39.61	9.5 %	186	< 0.0001
da^{G32}-GAL4/w^{1118}	6	0 μg/ml	0 μg/ml	36.16	0 %	187	-
da^{G32}-GAL4/UAS-RNAi	1	0 μg/ml	0 μg/ml	40.7	-10.4 %	184	< 0.0001
da^{G32}-GAL4/UAS-RNAi	6	0 μg/ml	0 μg/ml	45.43	0 %	187	-
da^{G32}-GAL4/w^{1118}	1	0 μg/ml	0 μg/ml	43.42	13 %	217	< 0.0001
da^{G32}-GAL4/w^{1118}	6	0 μg/ml	0 μg/ml	38.44	0 %	210	-
da^{G32}-GAL4/UAS-RNAi	1	0 μg/ml	0 μg/ml	39.21	-13.2 %	217	< 0.0001
da^{G32}-GAL4/UAS-RNAi	6	0 μg/ml	0 μg/ml	45.19	0 %	211	-
UAS-RNAi/w^{1118}	1	0 μg/ml	0 μg/ml	45.8	9.6 %	169	< 0.0001
UAS-RNAi/w^{1118}	6	0 μg/ml	0 μg/ml	41.8	0 %	188	-