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The Role of Mitochondria in *Drosophila* Aging

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Abstract

Understanding how alterations in mitochondrial function in different cells and tissues impacts the aging process remains one of the greatest challenges facing biogerontologists. Here, we discuss the recent upsurge in research in this area using the fruit fly *Drosophila melanogaster* as a model system. Topics that are discussed include age-related changes in mitochondrial function, mitochondrial oxidative stress and lifespan, life extension mediated by moderate knock-down of genes important for mitochondrial electron transport chain (ETC) function, and the relationship between dietary restriction and ETC activity. Finally, we review recent approaches to supplement the endogenous fly ETC with a single-subunit mitochondrial respiratory enzyme from yeast.

Keywords

Fly; Lifespan; longevity; *Ndi1*; respiratory chain

Cells depend upon healthy and efficient mitochondria to fuel vital metabolic processes. Therefore, it is not surprising that mitochondria play central roles in making life and death decisions for the cell. It also seems intuitive that effective mitochondrial function would be important for maintaining health at the organismal level. Indeed, there is a large amount of correlative data supporting the idea that as cells and, tissues age, there is a gradual decline in mitochondrial respiratory chain function (Wallace, 2005). Causal relationships can now be addressed by the application of genetic analysis. For example, if alterations in mitochondrial function are a cause of age-related morbidity and mortality then manipulating genes important for mitochondrial function should affect the aging process.

The fruit fly *Drosophila melanogaster* is the premier model system for studying how gene function(s) in spatially and temporally defined conditions modulate complex phenotypes. In the last few years, there have been a number of exciting and thought-provoking studies in flies that have shed light on the role of mitochondria in aging. In this review, we discuss these recent findings in the context of earlier research on mitochondria and aging.

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Age-related changes in mitochondrial structure and function

Almost exactly 40 years ago, in this journal, a study describing age-related changes in the heart of *Drosophila repleta* (Sohal, 1970) reported that mitochondria in old fly hearts display dramatic alterations in morphology--they are enlarged and contain intramitochondrial glycogen particles. Since then, other studies have reported specific changes in mitochondrial ultrasture in aged insects compared to that of young insects. For example, in a study examining the consequences of aging on *Drosophila* flight muscle, a very specific rearrangement of the cristae was reported (Walker and Benzer, 2004). Within individual mitochondria of aged flies, the cristae become locally rearranged in a pattern resembling a “swirl.” Exposure to acute oxidative stress resulted in the rapid and widespread accumulation of the same pathology even in young flies. The cristae involved in a swirl were shown to be deficient in respiratory enzyme cytochrome c oxidase (COX) activity, within an otherwise COX-positive mitochondrion. Moreover, the presence of the “swirls” is associated with a conformational change in cytochrome c and widespread apoptotic cell death in the fly flight muscle tissue.

The idea that alterations in mitochondrial functions play a key role in the decline of physiological vigor of animals during the aging process is not new (Harman, 1972). However, despite decades of research, the relationship between a decline in electron transport chain (ETC) function and aging remains somewhat controversial. The major criticism of published work reporting a decline in ETC activity with age is that many studies did not use reference markers for the mitochondrial content of their preparations. Therefore, it is possible that the observed declines in activity were due to higher levels of impurity (and a consequent lower level of mitochondria) in preparations from older tissue. More recently, a comprehensive study of ETC function in aging *Drosophila* that incorporated the activity of citrate synthase as a measure of mitochondrial purity and yield reported a selective reduction in certain aspects of respiration and electron transport (Ferguson et al., 2005). Specifically, the authors observed a significant age-related decline in COX activity (complex IV), but not of the other mitochondrial oxidoreductases examined. Moreover, pharmacological inactivation of complex IV in mitochondria isolated from young flies led to increased production of reactive oxygen species (ROS). These observations have lead to the concept of the “vicious cycle” in which an initial ROS-induced impairment of mitochondria leads to increased oxidant production that, in turn, leads to further mitochondrial damage. Another possible explanation behind the age-related decline in ETC function is the decline in expression of genes that are important for ETC activity (McCarroll et al., 2004).

The relationship between mitochondrial oxidative stress and longevity

The central idea of the oxidative stress theory of aging is that the accumulation of molecular damage caused by ROS contributes to the functional decline and increase in mortality observed in late life (Harman, 1956). Mitochondria are the primary source of cellular ROS and are therefore central to any discussion of the oxidative stress theory of aging. Studies in other species, such as *C. elegans* and mice, have challenged the importance of ROS in determining lifespan (Gems and Doonan, 2009). However, in *Drosophila*, there have been a number of studies that support an important role for mitochondrial ROS in modulating lifespan.

A direct way to test the mitochondrial oxidative stress theory of aging is to engineer flies with increased oxidative stress defenses. In pioneering work, using an inducible gene expression system that eliminates the confounding effects of genetic background that have plagued the field, it was shown that adult-specific overexpression of the mitochondrial Mn-Superoxide Dismutase (MnSOD) can extend lifespan in the fly (Sun et al., 2002). Similar effects were observed with the Cu/Zn-SOD (Sun and Tower, 1999). A complementary

approach to reduce oxidative stress would be to reduce the rate of ROS production within the mitochondrion. Towards this goal, investigators have expressed uncoupling proteins in different tissues of aging flies and examined the consequences on longevity. In one study, expression of the human uncoupling protein 2 (hUCP2) in adult neurons lead to a decrease in ROS production and an extension in lifespan (Fridell et al., 2005).

The studies described above demonstrate that reducing mitochondrial ROS can prolong lifespan in *Drosophila*. However, caution must be exercised in interpreting these findings. At present, it is not clear that these studies support the oxidative *damage* theory of aging--a reduction in ROS-induced macromolecular damage and a consequential increase in longevity. Another possibility is that the reduction in mitochondrial ROS may cause changes in cell signaling and/or gene expression and that these changes promote longevity independent of any effects of ROS-induced macromolecular damage. Although it will be difficult to separate these non-mutually exclusive mechanisms of life extension, transcriptional profiling of long-lived MnSOD-expressing flies has provided some direction (Curtis et al., 2007). Remarkably, it was found that the pattern of gene expression caused by overexpression of MnSOD was similar to that observed in long-lived *Caenorhabditis elegans* insulin-like signaling mutants and to the xenobiotic stress response. At present, however, it is not known whether these changes in gene expression are a cause or consequence of MnSOD-mediated life extension.

Moderate knock-down of electron transport chain (ETC) genes can extend *Drosophila* lifespan

Studies in *C. elegans* have shown that knock-down of certain ETC genes can prolong lifespan (Dillin et al., 2002; Lee et al., 2003). At face value, this appears paradoxical. It is important to keep in mind, however, that there exists a threshold of mitochondrial dysfunction, whereby severe mitochondrial dysfunction is detrimental for worm lifespan while moderate knock-down of ETC genes can promote longevity (Rea et al., 2007). A number of studies have reported that ETC dysfunction can also promote longevity in mice. Reduced activity of MCLK1, a mitochondrial enzyme necessary for ubiquinone biosynthesis, leads to a severe reduction of ETC function and a substantial increase in lifespan with no trade-off in growth or fertility (Lapointe and Hekimi, 2008; Liu et al., 2005). In addition, mice carrying a disruption in SURF1, a putative complex IV assembly factor, display a complex IV biochemical defect, markedly prolonged longevity and complete protection from kainic acid-induced neurotoxicity (Dell'agnello et al., 2007). Until recently, however, it was not clear whether knock-down of ETC genes could also extend life span in the fly.

Our group set out to address this question using transgenic RNAi lines from the Vienna *Drosophila* RNAi center (Dietzl et al., 2007). We used a ubiquitous GAL4 expression line, *daughterless* (*da*-GAL4), to systematically down-regulate nuclear-encoded components of the fly ETC (31 complex I subunits, three complex II subunits, five complex III subunits, six complex IV subunits, and eight complex V subunits) and studied the impact on viability and adult longevity (Copeland et al., 2009). Not surprisingly, most of the RNAi inductions caused lethality or shortened lifespan. However, a number of RNAi knock-downs were associated with increased life span (relative to the other RNAi lines and the control strain w^{1118}).

To avoid the potentially confounding issue of hybrid vigor, we used the mifepristone (RU486) inducible-GAL4 system (annotated P[Switch] or Gene-Switch (Osterwalder et al., 2001; Roman et al., 2001) to validate RNAi knock-downs isolated in the initial screen. We used the ubiquitous tubulin-Gene-Switch (*tub*-GS) driver line to manipulate three candidate longevity genes isolated in the pilot screen and two additional genes that resulted in larval

lethality in the *da*-GAL4 screen. Moderate RNAi knock-down of each of these five genes resulted in prolonged life span in female flies and variable effects on male lifespan.

Surprisingly, the long-lived flies with reduced expression of ETC genes did not show consistent defects in the assembly of the relevant respiratory enzymes or overall ATP levels. This may explain our observation that ETC-mediated longevity in the fly does not necessarily result in physiological trade-offs involving reduced growth and/or reproduction. Moreover, by taking advantage of tissue specific expression tools available in the fly, we observed that knock-down of certain complex I or complex IV genes in adult neurons was sufficient to extend lifespan.

The fact that the same manipulation extends longevity in two different species does not necessarily mean that the underlying mechanisms are the same. In the case of mild knock-down of ETC genes, significant differences exist between the findings in the fly and worm. For example, the temporal requirements of ETC-mediated longevity appear to be different. In contrast to studies in the worm, where knock-down of ETC genes during development is sufficient for life span extension (Dillin et al., 2002), (Rera et al., 2010) found no evidence to suggest that knock-down of ETC genes during developmental alone can prolong fly lifespan. At the same time, we observed that adult-only knock-down of complex I genes could extend lifespan in *Drosophila* (Copeland et al., 2009). Similar findings (adult-only mediated life extension) have been observed in the fly for a complex IV subunit (Michael Rera & Herve Trichoire, personal communication).

An interesting and unsolved problem in this area is the discrepancy (with respect to longevity) between complex II deficiency and impairment of the other respiratory complexes. Unlike complex I, III, IV, and V, a lifespan extension from knock-down of complex II subunits has not been reported. The biochemical and phenotypic consequences of complex II deficiency in flies are very similar to complex II (*mev-1*) deficiency in worms (Ishii et al., 1998). Like worms, flies with compromised complex II function suffer from elevated oxidative stress and display hallmarks of rapid aging (Walker et al., 2006). An important difference is that unlike the other respiratory complexes, complex II is active in both the tricarboxylic acid (TCA) cycle and the ETC. However, it is not clear whether this is important in the phenotypic consequences of complex II deficiency.

Diet, mitochondrial respiratory chain activity and longevity

Dietary restriction (DR), reducing the food intake to ~60% of what an animal eats under *ad libidum* conditions, can extend lifespan in a wide range of species. Recent studies, in yeast, worms and mice, have suggested that alterations in ETC function may play a role in mediating the pro-longevity effects of DR (Guarente, 2008). In the last year, a number of studies have suggested that alterations in mitochondrial respiratory chain activity may play an important role in DR-mediated longevity in *Drosophila* as well.

To better understand the molecular mechanisms of DR-mediated longevity in the fly, genome-wide changes in mRNA translation were assayed upon DR (Zid et al., 2009). Remarkably, under DR translation of various nuclear encoded ETC components (complexes I and IV) and mitochondrial ribosomal proteins was enhanced, suggesting an overall increase in mitochondrial proteins. Moreover, flies under DR displayed an increase in both complex I and complex IV activity. Accordingly, RNAi-mediated knock-down of either complex I or IV subunits diminished the lifespan extension obtained upon DR. In our own lab, we observed similar findings regarding the role of mitochondrial complex V in DR-mediated longevity (Bahadorani et al., 2010a). Using both genetic and pharmacological treatments to perturb complex V exclusively in the adult stage, we observed that full complex V activity was required for DR-mediated longevity. At the same time, adult-only

complex V knock-down had no major impact on lifespan under rich media conditions (Bahadorani et al., 2010a; Copeland et al., 2009). Interestingly, RNAi knock-down of complex V during both development and adulthood leads to lifespan increases under rich media conditions (Bahadorani et al., 2010a; Copeland et al., 2009) but not under DR.

How do we interpret these findings? If DR leads to an increase in ETC activity, then it would make sense that genetic and/or pharmacological impairment of the ETC would reduce DR-mediated longevity, and these studies both demonstrate that flies with impaired respiration do not respond normally to DR (Bahadorani et al., 2010a; Zid et al., 2009). On the other hand then, how does knock-down of ETC genes result in life extension under normal nutritional conditions? In other words, why do flies with impaired respiratory chain activity live longer than those with an intact ETC?

Long-lived flies with reduced expression of ETC genes do not consistently display reduced physiological vigor or even reduced ATP levels (Copeland et al., 2009). Therefore, it seems unlikely that increased longevity results from a decreased metabolic rate. Similarly, in *C. elegans*, this mode of life extension appears not to result from a slower 'rate of living'. Instead, long-lived worms with defects affecting the ETC (e.g., *clk-1*) display alterations in gene expression reminiscent of the "retrograde response" in yeast and mammalian cell culture (Cristina et al., 2009). This conserved, and presumably adaptive response results in the up-regulation of genes predicted to metabolically remodel and protect the animal. At present, it is not known whether mitochondrial dysfunction in the fly results in similar changes in gene expression and/or if these changes are important in life extension via ETC gene knock-down.

The *Indy* gene represents another potential link between DR, mitochondria and lifespan determination. The INDY protein is a transmembrane transporter of Krebs cycle intermediates and is predominantly found at the plasma membrane of cells in the midgut, fat body, and oenocytes--all tissues important for the uptake, utilization, and storage of nutrients and the principal sites of intermediary metabolism in the fly (Inoue et al., 2002; Knauf et al., 2002). Almost a decade ago, it was reported that flies carrying P-element insertion mutations in *Indy* were long-lived (Rogina et al., 2000). Since then, however, the importance of *Indy* gene activity in modulating *Drosophila* lifespan has been questioned (Toivonen et al., 2007). Indeed, it appears that mutations in *Indy* do not extend lifespan in all genetic backgrounds (Toivonen et al., 2007; Wang et al., 2009). A recent study examining the relationship between *Indy* and DR reported that low nutrition conditions inhibit *Indy* expression and that *Indy*-mediated longevity is dependent on rich nutritional conditions (Wang et al., 2009). As different laboratories use different food recipes, this may explain the discrepancies reported by different laboratories regarding the role of *Indy* in regulating fly lifespan. This and other controversies regarding DR regimes in the fly highlights the pressing need for those of us using the fly as a model to study the aging process to reach a consensus regarding nutritional paradigms relevant for fly lifespan studies.

Trans-kingdom supplementation of the mitochondrial respiratory chain extends *Drosophila* lifespan

There is a growing body of evidence to suggest that DR is associated with an increase in mitochondrial respiratory chain activity (Guarente, 2008). As discussed above, this appears to be the case in *Drosophila* as well (Zid et al., 2009) and some studies have reported that normal respiration is required for DR-mediated longevity (Bahadorani et al., 2010a; Guarente, 2008; Zid et al., 2009). However, until recently the question of whether increased respiratory chain activity alone is sufficient to delay animal aging had not been addressed.

The most direct method for studying the effects of increased ETC activity with respect to lifespan would be to engineer animals to overexpress entire respiratory complexes. However, there exist significant technical barriers to this approach. For example, mitochondrial complex I (NADH–ubiquinone oxidoreductase), the major entry point for electrons into the respiratory chain, is comprised of at least 45 subunits encoded by both the nuclear and mitochondrial genomes. In contrast, the alternative NADH-ubiquinone oxidoreductase (*Ndi1*) of *Saccharomyces cerevisiae* mitochondria is composed of a single nuclear-encoded polypeptide (Luttik et al., 1998). Recently, we (Bahadorani et al., 2010a) and others (Sanz et al., 2010) have reported the generation and characterization of *Ndi1*-expressing flies. In our study, we observed that expression of NDI1 in *Drosophila* mitochondria is sufficient to increase complex I (NADH–ubiquinone oxidoreductase) activity. To determine whether *Ndi1* could promote an increase in the overall respiratory rate *in vivo*, we assayed CO_2 production in living flies and observed a significant increase in respiration in *Ndi1*-expressing flies (Bahadorani et al., 2010a). Therefore, exogenous expression of *Ndi1* in *Drosophila* represents an effective approach to investigate the impact of increased respiration on animal aging. Towards this goal, we used the Gene-Switch system to express NDI1 in different tissues and examine the impact on longevity. Expression of NDI1 in adipose tissue had a negative impact on longevity, while neuronal expression of NDI1 resulted in life extension. These contradictory effects of NDI1 expression in different tissues may explain our observation that ubiquitous expression of NDI1 had no major impact on longevity (Bahadorani et al., 2010a). However, Sanz and co-workers reported that ubuiquitous expression of NDI1 mediated by the constitutive *da*-GAL4 driver resulted in a significant increase in longevity (Sanz et al., 2010). At present, it is not clear whether this discrepancy between the two studies arose because of differences in genetic background, NDI1 expression level or nutritional conditions.

Taken together, both studies support the idea that *Ndi1* expression in a metazoan can retard the aging process. This represents a major breakthrough in the field and may open the door to therapeutic treatments for the diseases of aging. In future work, we hope to better understand the effects of *Ndi1* expression in different cells and tissues and in doing so understand the mechanisms underlying life extension.

CONCLUSIONS

The relationship between the rate of mitochondrial respiration and the rate of aging is a fundamental and fascinating question in biology. In the last year or so, significant advances have been made in this area by studies using the fruit fly as a model system. These studies, outlined above, represent the dawn of a new era of *Drosophila* research. Future challenges include understanding how age-related changes in mitochondrial activity impact tissue function and, ultimately, behavior. Like so many other important questions in biology, ‘the fly’ will surely provide many of the answers.

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