Coenzyme Q and Aging in the fruit fly *Drosophila melanogaster*.

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ABSTRACT

Aging is the consequence of the gradual accumulation of molecular and cellular damage during life. Oxidative damage due to mitochondrial malfunction seems to be the main contributor to aging. Although, recently it has been proposed that reverse electron transport participate in signalling more than in damage the cell by ROS production. Other molecules has been described to take part in the aging process, as they are NAD, antioxidants and several microRNAs, as well new pathways that regulate the progression of aging. In addition, gene regulation due to epigenetic modification seems to be the responsible of providing a protective or permissive environment to age faster or slower. In this chapter, we review these things using the fruit fly as a model organism.

Keywords: aging, *D. melanogaster*, Coenzyme Q, NAD, longevity, Reactive oxygen species.

1. INTRODUCTION

It is generally accepted that aging is a consequence of the gradual accumulation of molecular and cellular damage during life. The 'Free Radical Theory of Ageing' (Harman 1956) updated to the "Mitochondrial Free Radical Theory of Ageing" (Harman 1972) (MFRTA) proposed that Reactive Oxygen Species (ROS) produced as by-products during normal metabolism cause the accumulation of oxidative damage. Accumulation of oxidative damage, such as oxidized DNA and proteins, or even DNA breaks, may cause the fall of biological systems. Thus, oxidative damage can lead to higher mitochondrial ROS production through a vicious circle mechanism. Although MFRTA is the most popular damage theory, there are many other theories proposing alternative mechanisms of damage e.g. telomere attrition, accumulation of cellular debris, epigenetics changes, etc. (Lopez-Otin et al. 2013). Recently, a new paradigm has been postulated where damage is not a cause but a consequence of a genetic program or "quasi-program" driven by continuous activation of pro-growth pathways such as nutrient sensing pathways controlled by Target of Rapamycin (Blagosklonny 2006).

Although, the proximal cause of aging remains unknown, it is clear that manipulation of metabolism alter the rate of aging. Three different metabolic pathways have been shown to affect the rate of aging: (1) the activity level of the electron transport chain in mitochondria, (2) the Insulin-like growth factor (IIS) signalling pathway, and (3) the Sirtuin pathway responsive to caloric restriction. Although these pathways affect aging separately, they have additive increases in lifespan (Taylor and Dillin 2011).

Aging is the greatest known risk factor for most non-communicable human diseases. Apart of the obvious ethical reasons, the use of humans in aging research is complicated due to our extreme long natural life span and to several other environmental and social factors that would make impossible to control lifespan studies properly. Cellular models of human disease provide important information about the underlying mechanisms; however, they have limitations that need to be overcome by *in vivo* models. Therefore, the use of animal models in aging research is instrumental to understand the proximal cause of aging and find new therapies to delay, prevent or reverse age-related diseases (Mitchell et al. 2015).

Here, we review some of the most recent findings on aging research using the fruit fly *Drosophila melanogaster* as animal model. In addition, we report the latest interventions that extend life span and health span in *Drosophila*.

2. AGING IN FLIES

Drosophila melanogaster has been widely used for biological research in studies of genetics, development and organogenesis, physiology, pathogenesis, pharmacology, degenerative diseases, cardiomyopathies and inflammatory diseases, cancer and aging (Fernandez-Ayala et al. 2014). *Drosophila* has also been used to model mitochondrial disease in order to understand the underlying genetic and physiological mechanism and to try new therapies including gene therapy, or even for mitochondrial gene therapy (Fernandez-Ayala et al. 2014). *Drosophila* is perfect for aging studies because it is easy to culture, has a short lifespan and life cycle, produces abundant progeny and has a powerful genetics that allows spatiotemporal manipulation of gene expression.

Here, we will review some of the most exciting research recently published using *Drosophila melanogaster* in the aging field. We will briefly describe those pathways that seem to regulate aging rate, how age-related pathologies are studied in flies and how epigenetic modifications affect gene regulation in old individuals. For the sake of space, we will focus on the role of mitochondria and ROS, how manipulations during development affect adult lifespan, and how supplements in the diet can extend healthy lifespan. For a full description of a gene, function or the biological process where is involved, the reader is advised to visit FlyBase (<http://flybase.org>). The annotation symbol ("CG") of a gene is shown next to the name of the gene.

2.1. The role of mitochondria in *Drosophila's* aging

Mitochondrial dysfunction is one of the few universal hallmarks of aging (Lopez-Otin et al. 2013). Aged individuals are characterized by reduced bioenergetics capacity, increased ROS production, more oxidative damage in mitochondrial DNA and RNA, and accumulation of mutations in mtDNA. Although, mutations in superoxide dismutase 1 and 2 increase superoxide levels and shorten lifespan (Phillips et al. 1989). The role of mitochondrial ROS in *Drosophila's* aging remains controversial (see below).

Reduction in ATP generation caused by reduced mitochondrial function can compromise cellular viability triggering cell death. Accordingly, interruption of mitochondrial protein translation in *tko25t* mutants, due to a point mutation in the nuclear gene that encodes the

mitoribosomal protein S12 (CG7925), causes a severe phenotype characterized by reduction in mitochondrial respiration, development delay, and a dramatic shortening in lifespan, reproducing many of the symptoms observed in human mitochondrial disorders (Toivonen et al. 2001). A subsequent transcriptomic analysis revealed systematic and compensatory changes in the expression of genes involved in metabolism, including up-regulation of lactate dehydrogenase and many other genes related to the catabolism of fat and proteins, the TCA cycle and various anaplerotic pathways, as well as several pathways involved in gut transport and absorption of lipids and proteins. Overall, these results indicate that OXPHOS dysfunction is perceived physiologically as a starvation for particular biomolecules, inducing mitochondria to trigger a general programmed response similar to those produced in response to caloric restriction (Fernandez-Ayala et al. 2010). However, caloric restriction or mild mitochondrial dysfunction (e.g. mild knock-down of components of the electron transport chain) extends lifespan (Partridge et al. 2005; Copeland et al. 2009) because their much more moderate effect on mitochondrial function.

Aging has been associated with a progressive decline of mitochondrial function accompanied with a reduction of physical activity in *Drosophila melanogaster* (Peleg et al. 2016). Accordingly, increasing mitochondrial turnover of damaged mitochondria by over-expressing the master regulator of mitophagy: *parkin* (CG10523) extends lifespan (Rana et al. 2013). Similarly, boosting mitochondrial function by over-expressing *spargel* (CG9809) in the intestine also increases longevity (Rera et al. 2011). However, aging can be much more complex than anticipated with unknown players participating in determining aging rate. For example, there is a growing interest in the role of metabolic intermediates (e.g. Krebs's cycle intermediates) in triggering cancer and other pathological processes (Yang et al. 2013). Interestingly, cellular metabolism during early aging showed an increment of both several mitochondrial metabolic substrates, like Acetyl-CoA, citrate and isocitrate, and their related-enzymatic activities, which are accompanied with an increment in oxygen consumption at middle age (Peleg et al. 2016). Acetyl-CoA is a key metabolite in the central metabolism, and also a cofactor for the acetylation of lysine residues. Protein-acetylation of specific aminoacids is a key posttranslational modification for many metabolic enzymes as well as nuclear regulators of gene expression, mainly histone proteins (Graff and Tsai 2013). Changes in acetyl-coA levels could trigger alterations in metabolic function that would explain some of the phenotypes observed during aging. However, it is still unclear if the aging process leads to metabolic alterations, or if changes in the metabolic state of an organism trigger the process of aging.

Recent research suggests that it is possible to reverse mitochondrial decay with dietary supplements that increase cellular levels of NAD (nicotinamide adenine dinucleotide). NAD is a central coenzyme in the Krebs's cycle, but also participates in other metabolic redox reactions, being a precursor for several cellular signalling molecules, and a substrate for posttranslational modifications to proteins. *CG9940* encodes the NAD⁺ synthase protein that catalyses the final step in de novo NAD⁺ biosynthesis, and transfers the amide from either ammonia or glutamine to nicotinic acid adenine di-nucleotide (NaAD). The over-expression of *CG9940* enhances cardiac output and reduces heart failure in aged flies, delays age-related mobility decline, and prolongs lifespan (Wen et al. 2016). Conversely, knock-down of *CG9940* by RNA-interference has negative effects on these parameters. Also, expression levels of the NAD⁺ synthase regulates the adaptation to exercise in aging (Wen et al. 2016). Over-expression of *CG9940* boosts the response to exercise increasing cardiac function, mobility, and lifespan in old flies, whereas a low expression of the gene reduces the capacity of flies to respond to the stress caused by exercise.

2.2. The role of ROS in longevity in *Drosophila*

The role of ROS in determining *Drosophila*'s lifespan is unclear with different studies reporting opposite results (Sanz 2016b). As predicted by MFRTA, depletion of both SOD1 and SOD2 either in muscle or in nervous system produces ROS accumulation, impairment of locomotive activity and shortens lifespan (Oka et al. 2015). However, other studies have shown results that do not support MFRTA. For example, although mitochondrial ROS production has been shown to negatively correlate with lifespan in wild type strains (Sanz et al. 2010a), experimental reduction of ROS levels by ectopic expression of an alternative oxidase from *Ciona intestinalis* (AOX) does not extend lifespan in flies (Sanz et al. 2010a). AOX expression is able to complement the semilethality of partial knockdown of both *cyclope* (*CG14028*, COXVIc) and the complex IV assembly factor *Surf1* (*CG9943*) (Fernandez-Ayala et al. 2009). Moreover, it also rescues the locomotor defect and the excess of mitochondrial ROS production caused by mutations in *dj-1beta*, a *Drosophila* homolog of the human Parkinson's disease gene DJ-1. These results indicate that ectopic expression of this ubiquinol oxidase can restore the electron flow in the mitochondrial respiratory chain when it is blocked, thereby decreasing the high level of ubiquinol and restoring the physiological properties of Coenzyme Q, and subsequently inhibiting the deleterious effects that mitochondrial defects produce on health and lifespan. However,

these modifications do not produce higher life span in wild type animals besides their effect decreasing mtROS (Sanz et al. 2010a). Furthermore, mutations in *dj-1 β* (CG1349) increase mitochondrial ROS levels and oxidative damage reducing fly activity, but do not short lifespan (Stefanatos et al. 2012). These results support that other mitochondrial-related factors apart of ROS are affecting longevity in *Drosophila* and/or that ROS act via signalling and not modulating oxidative stress.

ROS are essential cellular messengers (Reczek and Chandel 2014) and disruption of ROS signalling reduces cellular viability (Hamalainen et al. 2015). Interestingly, boosting ROS levels can extend lifespan by a mechanism that has been called mitohormesis (Ristow and Zarse 2010). Mitochondrial complex I (CI) has been proposed as a crucial regulator of animal longevity (Stefanatos and Sanz 2011). Mutations in CI are frequently associated with multiple pathologies in humans and flies, and compensation of CI function by an alternative NADH internal dehydrogenase (NDI1) from *Saccharomyces cerevisiae* extends lifespan (Sanz et al. 2010b). Paradoxically, mild knock-down of the complex I (CI) subunit *ND-75* (CG2286) increases ROS levels and extends lifespan in a ROS-dependent manner (Owusu-Ansah et al. 2013). Disruption of CI causes the activation of the mitochondrial unfolded protein response that represses IIS inducing lifespan-extension. Similarly, allotopic expression of NDI1 increases the generation of ROS via reverse electron transport at respiratory CI and extends lifespan (Scialo et al. 2016). In theory, mitohormesis extends lifespan by increasing endogenous antioxidant defences (Ristow and Zarse 2010). However, overexpression of antioxidant does not increase lifespan (Sanz 2016a), and extension in lifespan conferred by increasing ROS at CI is suppressed by antioxidants (Owusu-Ansah et al. 2013; Scialo et al. 2016). This supports that ROS act via signalling and not by reducing oxidative stress through boosting antioxidant defences.

2.3. Links between aging and development in *Drosophila*

Age-specific mortality rate reach a plateau in late life following the exponential increase in mortality rate that is characteristic of aging. However, depending on how a population develops, this is how aging is. Populations selected for fast development (9-10 day generation cycles) during several generations entered the late-life phase at an earlier age than flies selected for slow development (28 day generation cycles) (Shahrestani et al. 2016). Populations showed changes in age-specific rates, including differences in starvation resistance, desiccation resistance, time-in-motion and geotaxis. Age at which physiological

decline occurs matches with the age at which the transition from exponential mortality to late life occurs (Shahrestani et al. 2016). This result suggests that late-life is physiologically distinct from aging.

Some microRNAs are known to affect lifespan by post-transcriptionally silencing mRNAs that play critical, beneficial roles at early stages of the life cycle but are deleterious when expressed inappropriately at later stages (Chawla et al. 2016). In adult flies, miR-34 promotes longevity and maintains neuronal homeostasis by repressing *Eip74EF* (CG32180), a transcription factor required for progression through early development that regulates oogenesis and autophagic cell death (Liu et al. 2012). Similarly, other microRNAs such as let-7, miR-125 and mi-100 regulate the transcription factor *chinmo* (CG31666), which is involved in imaginal disc-derived wing morphogenesis, mushroom body development and dendrite morphogenesis (Chawla et al. 2016), during nervous system formation. The coordinate upregulation of the three former miRNAs in old flies induces brain degeneration and shortens life span, although it seems that they operate in two different stages (development and adulthood) instead of acting at the same time. For example, let-7 is predominantly responsible for regulating *chinmo* during nervous system formation, and miR-125 acts during adulthood. In contrast, loss of miR-125 induces ectopic *chinmo* expression only in adult brains (Chawla et al. 2016). Other microRNA that has been involved in aging is miR-1000, whose loss leads to shorten lifespan (Verma et al. 2015).

2.4. Pathways related to aging in *Drosophila*

Extensive research has identified several orthologous genes that modulate a few specific conserved genetic pathways that affect longevity, metabolism and development from yeast to humans. These pathways are conserved in *Drosophila* and have been extensively investigated in this model organism (Bitto et al. 2015).

mTOR is a serine/threonine protein kinase that promotes cellular growth and cell division in response to nutrients and growth factors. The mTOR protein acts in at least two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). While mTORC1 operates as a central regulator of longevity, by repressing autophagy and modulating mitochondrial metabolism, mTORC2 regulates the activity of several substrates involved in cytoskeleton reorganization and cell polarity (Bitto et al. 2015).

Interestingly, manipulation of TOR signalling via overexpression of TOR repressors extends *Drosophila*'s lifespan by interrupting nutrient sensing and mimicking dietary restriction (Kapahi et al. 2004). The intracellular signalling regulated by the protein kinase TOR modulates several biological processes inside the cell, such as ribosome biogenesis, translation, autophagy, cell proliferation and cytoskeletal changes required for cell growth. The primary tissues in which these pathways operate are the fat body and the nervous system. Additionally, both the expression of a dominant-negative version of TOR and the inhibition of TOR-complex 1 by rapamycin also extend lifespan (Kapahi et al. 2004). Moreover, TOR downregulation together with insulin signalling improves locomotion and cardiac function and confers neuroprotection against neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Hirth 2010; Partridge et al. 2011). Similarly, lifespan is extended in response to reduced levels of insulin-like peptides (Gronke et al. 2010) or by loss of function mutations in different components of the insulin signalling pathway, such as the insulin receptor (Tatar et al. 2001) or downstream substrates such as *chico* (CG5686) (Clancy et al. 2001; Slack et al. 2010).

There is a close connection between mitochondria and IIS. For example, IIS regulates mitochondrial respiration, while mitochondrial ROS fine-tune insulin receptor activation in neurons (Ramalingam and Kim 2015). The age-related reduction in mitochondrial function, including respiration and ATP production, can disrupt the insulin receptor activation in neurons leading to the development of cerebral insulin resistance in old age. Another important metabolic regulator is the AMP-activated protein kinase AMPK (CG3051). In *Drosophila*, activation of AMPK in neurons or in the gut increases life span and slows aging in a non-cell-autonomous manner, regulating both the uptake and oxidation of glucose, as well as the oxidation of fatty acids in response to changes in the relative abundance of AMP and ATP (Ulgherait et al. 2014). AMP:ATP ratio is strongly influenced by mitochondrial function. Paradoxically, metformin, a complex I inhibitor, increases the ratio AMP:ATP and activates AMPK, but does not extend lifespan in fruit flies (Slack et al. 2012) as it does in worms or mice (De Haes et al. 2014; Martin-Montalvo et al. 2013).

Repeated or chronic perturbations of circadian rhythms are strongly suspected to be detrimental to healthspan, affecting aging, locomotor capacity and longevity in both humans and flies. At the molecular level, circadian clocks are based on negative feedback loops (Allada and Chung 2010). The transcription factors Clock (*Clk*, CG7391) and Cycle (*Cyc*, CG8727) form a heterodimer that promotes the transcription of two negative elements, period (*per*, CG2647) and timeless (*tim*, CG3234), which form a heterodimer that inhibits

the activity of Clk/Cyc. The inhibition of Clk/Cyc results in *per/tim* degradation and releases of Clk/Cyc from repression, allowing the start of a new cycle. Mutations in *per* gene displays robust short 16-h rhythms, reduced longevity and decreased startle-induced locomotion in aged flies (Vaccaro et al. 2016). Interestingly, the adjustment of environmental light-dark cycles to the endogenous rhythms of 16 hours fully suppresses the acceleration of the age-related decline in *per* mutants, while accelerates it in wild-type flies.

It has been found that in the brain of old flies, sleep fragmentation induces ROS production, expression of insulin-like peptides and the insulin receptor, which are maintained even after 4 days of recovering normal sleep patterns (Williams et al. 2016). In this model, ROS production causes the expression of ARE genes in an attempt to reduce neuronal ROS levels, suggesting that the increment in ROS may damage mitochondria and induce the ER-UPR response, a cellular stress response related to the endoplasmic reticulum that is triggered by unfolded proteins (Williams et al. 2016). The ER-UPR response interrupts protein translation, induces the degradation of misfolded proteins, and activates the production of molecular chaperones involved in protein folding as an immediate response, however if stress is extended ER-UPR induces apoptosis. It has been proposed that higher ROS levels would improve insulin signalling in neurons, inhibiting cytochrome c release, and protecting neurons from ER-UPR induced apoptosis (Williams et al. 2016).

The ratio protein:carbohydrate in the fly diet is instrumental in determining the effect of dietary restriction on lifespan, with low protein diets extending lifespan (Mair et al. 2005; Partridge et al. 2005). Tryptophan hydroxylase (*Trh*, CG9122), the serotonin receptor 2A (5-HT2A, CG1056), and the solute carrier 7-family amino acid transporter (*Jhl-21*, CG12317) are required for the transient feeding preference for dietary protein after starvation through their role in establishing protein value (Ro et al. 2016). Interestingly, disruption of any of these genes increases lifespan independently of food intake, suggesting that the perceived value of dietary protein modulates lifespan via serotonin signalling. These results suggest that serotonin acts through an intracellular pathway mediated by the 5-HT2A receptor, in which the amino acid transporter, *Jhl-21*, is required for serotonergic evaluation of the dietary protein content, which subsequently affects lifespan (Ro et al. 2016).

2.5. Age-related pathologies that affect *Drosophila*

As we reported above, aging is characterized by an increase in ROS production, which has been proposed to cause neuronal insulin resistance, induces antioxidant systems that protect against oxidative damage and activates the expression of neuroprotective endoplasmic reticulum molecular chaperones in dopaminergic neurons of middle-aged flies to prevent the induction of apoptosis (Williams et al. 2016). Paradoxically, the elevated activity of antioxidants systems in Parkinson's and Alzheimer's disease may contribute to dysfunctional insulin receptor activation and central insulin resistance, which would lead to a decline in synapses and synaptic function (Freiherr et al. 2013). Related to that, inhibition of *dj-1alpha* (CG6646) gene, a homologue of the familial Parkinson's disease gene DJ-1, leads to oxidative stress, mitochondrial dysfunction, and dopaminergic neuron loss in *Drosophila* (Faust et al. 2009). Feeding flies with drugs that combine antioxidant and anti-inflammatory properties shows protective effects and improving many of the deleterious phenotypes associated with *dj1-alpha* mutation.

Progeria recapitulates many of the characteristics associated with aging such as neurodegeneration. It can be caused by defects in RNA metabolism and transport through the nuclear envelope. Lamin C (CG10119) is an intermediate filament with structural function that is responsible for nuclear pore distribution and mitotic nuclear envelope assembly. Mutations in *Lamin C* alter RNA export through the nuclear envelope and is used to model progeria in *Drosophila* (Li et al. 2016). The phenotype of Lamin C mutants includes progressive jumping and flight defects that are exacerbated with age. Interestingly, most of RNA affected by mutations in *Lamin C* are related with genes required for maintaining mitochondrial integrity and function, e.g. Marf (CG3869) single fly orthologue for mitofusins 1 and 2. These studies connect defects in RNA export through nuclear envelope to progressive loss of mitochondrial integrity and premature aging.

2.6. Gene regulation during aging

Considerable natural variation for lifespan exists within human and animal populations, including *Drosophila melanogaster*. Highfill and collaborators studied this genetic variation to elucidate the pathways and genes involved in aging (Highfill et al. 2016). The comparative genetic analysis of more than 800 lines, which grew under strict inbred conditions for several generations, mapped five quantitative trait loci that contribute to explain lifespan variation between the different inbred lines. RNA sequencing analysis comparing young *versus* old flies showed pathways affecting lifespan, and identified genes within the previous mapped

quantitative trait loci (Highfill et al. 2016). Old flies increased the expression of genes involved in antimicrobial defence, such as *Relish* (CG11992), and decreased the expression of those related to mitochondrial metabolism, including the electron transport chain (*UQCR-14*, CG3560) and the coenzyme Q biosynthesis (*coq2*, CG9613). This genetic variation could help to elucidate which pathways and genes are involved in aging, as well as genetic mechanisms underlying risk for age-related diseases.

It has been shown in several animal models that genetic differences and somatic mutations underlie longevity and that some environmental factors correlate with lifespan extension, such as a nutrient availability that can activate the stress response, reduce the basal metabolic rate or decrease fertility under dietary restriction situations (Sen et al. 2016). These observations support the idea of epigenetic mechanisms in modulating longevity pathways. Methylation of histones, particularly in histone 3, as for example the trimethylation in lysine 4 (H3K4me3) or in lysine 27 (H3K27me3) are epigenetic modifications that activate or repress transcription respectively, which had been directly linked to lifespan regulation in many organisms.

In flies, mutations in any of the subunits of Polycomb Repressive Complex 2 (PRC2), one of the H3K27 methyltransferases, reduce global levels of H3K27me3 and extend lifespan of male flies by derepressing target genes *Abd-B* (CG11648) and *Odc1* (CG8721). *Abd-B* belongs to the Hox gene family and encodes a homeodomain transcription factor that controls cell identity and contributes to the differentiation of posterior thorax and abdominal segment of the fly; *Odb1* is an Ornithine Decarboxylase that also plays a role in regulation of cell cycle. On the other hand, TRX antagonizes silencing mediated by PRC2 promoting the acetylation of H3K27. It had been described that mutations in *trx* (CG8651) increase H3K27me3 levels and suppress mutations of PRC2 subunits and their longevity phenotype. Since acetylation and methylation at the same site are mutually exclusive, increased acetylation results in both decreased methylation and loss of silencing at PRC2 target sites (Siebold et al. 2010). In addition, overexpression of *Lid* (CG9088), the histone demethylase homolog to the worm *rbr-2*, reduces the level of H3K4me3 and extends lifespan in *Drosophila*, while its knockdown shortens lifespan (Li et al. 2010).

Similarly, there is evidence that histone modifications affect lifespan of flies, altering the expression of specific target genes involved in stress tolerance or modulation of nutrient sensing pathways (Sen et al. 2016; Wood et al. 2016). Old flies showed a loss of activating

epigenetic marks like H3K4me3 and H3K36me3 and a gain of repressive epigenetic marks like H3K9me3 compared to young animals (Wood et al. 2010).

Acetylation of histones, another epigenetic modification that modifies gene activation, can affect fly longevity. The protein deacetylase Sirt1 (*CG5216*) maintains gene silencing in heterochromatin regions by epigenetic regulation of histone deacetylation. Overexpression of Sirt1 in *Drosophila* repressed the normal age-related loss of gene silencing in heterochromatin regions, as well as the loss of gene silencing undergoing genotoxic stress (Wood et al. 2016). Furthermore, it has been shown that spermidine inhibits the enzyme histone-acetyltransferase and generates chromatin states with a low acetylation grade. Because spermidine levels decline with age in several organisms, including *Drosophila*, the supplementation with spermidine increases lifespan (Eisenberg et al. 2009). The phenotype shown by these animals include upregulation of autophagy and improved stress response by gene activation due to histone acetylation. Alterations in the histone acetylation pattern, which alter the transcriptome, are shown in middle aged flies (Peleg et al. 2016). This can cause a general deterioration of the chromatin organization that underlies control of transcription during aging causing an increment in transcriptional noise and aberrant maturation of RNAs. These age-dependent changes in gene expression can be attenuated by environmental conditions such as dietary restriction, by the overexpression of heterochromatin components or by mutations in epigenetic regulators, such as histone modifying enzymes (Siebold et al. 2010).

In addition to histone modifications, epigenetic changes include chemical modifications of DNA, such as DNA methylation. DNA methylation is correlated with transcriptional repression and is important in genome imprinting, X-chromosome silencing, and repression of both centromeric and repetitive sequences (Sen et al. 2016). Although *Drosophila* has limited DNA methylation, overexpression of *Mt2* (*FlyBase* *CG10692*), the *Drosophila* C-5 cytosine methyltransferase, increases lifespan, whereas *Mt2* mutants are short lived (Lin et al. 2005). Because both 5mC (5-methyl-cytosine) and 6mA (6-methyl-adenine) have been localized to transposons, DNA methylation in flies could function to mark and repress transposition (Zhang et al. 2015). Furthermore, it has been described that transposons are de-repressed in both senescent human cells (Sen et al. 2016) and *Drosophila*'s tissues (Chen et al. 2016). Overexpression of transposable elements is responsible for cytotoxic phenotypes (Li et al. 2013), and inhibition of transposition prevents cytotoxicity and senescence (Wood et al. 2016). Therefore, the deregulation of DNA methyltransferases may

activate specific transcriptional programs in parallel with histone modification changes, being all together responsible for specific changes in gene expression found in aged flies.

Finally, dietary restriction represses expression of transposable elements as well as activation of genes located in heterochromatin during aging through changes in epigenetic factors (Wood et al. 2016). In *Drosophila*, the proteins Dicer-2 (CG6493) and Argonaute 2 (AGO2, CG7439), recruits the histone H3K9 methyltransferase Su(var)3–9 (CG43664) to catalyse formation of repressive heterochromatin at transposable elements sites. In order to do this, they use the small interfering RNA (siRNA) pathway. Accordingly, overexpression of Sir2, Su(var)3–9, and Dicer-2 mitigated the expression of transposable elements during aging and led to an increase in lifespan (Wood et al. 2016). The same effect was observed by decreasing the expression of the RNA-editing enzyme ADAR (adenosine deaminase acting on RNA, CG12598), which controls the efficiency of siRNA-directed silencing of transposable elements (Wood et al. 2016). These data support the retrotransposon theory of aging, which is based on the deleterious activation of epigenetically silenced transposons. According to this theory, maintenance of repressive heterochromatin preserves transposable elements silencing, prevents the damage caused by its activation and extends healthy life span.

3. COENZYME Q SUPPLEMENTATION AND AGING IN *DROSOPHILA*.

Little is known about the effect of Coenzyme Q supplementation on *Drosophila*'s aging. One of the main problems with Coenzyme Q supplementation is verified that is incorporated into the tissues and is able to participate in physiological processes such as electron transport. For example, coenzyme Q supplementation does not rescue phenotypes associated with mutation in *dj-1alpha* (Faust et al. 2009), whose human homologue DJ-1 causes Parkinson's disease in humans, although other antioxidants and anti-inflammatory compounds rescues most of the phenotypes in *dj-1alpha* mutants. Interestingly, antioxidant supplementation do not extend *Drosophila*'s lifespan (Sanz 2016a), whereas the role of anti-inflammatory compounds is not clear yet, although there is some evidence that they can have positive effects (He et al. 2014).

On the other hand, it has been shown that elimination of coenzyme Q from the diet shortens life span and accelerates the aging process (Palmer and Sackton 2003). Since *Drosophila*'s laboratory diet usually includes yeast, flies fed Q-less yeast live shorter. However, feeding

flies with a yeast deficient in the mitochondrial respiratory complex III showed similar results, suggesting that is mitochondrial functionality of the yeast, and not the absence of coenzyme Q the responsible for lifespan shortening (Palmer and Sackton 2003).

We finally conclude that new analyses should be done to clarify the role of coenzyme in *Drosophila* aging.

REFERENCES

- Allada R, Chung BY (2010) Circadian organization of behavior and physiology in *Drosophila*. Annual review of physiology 72:605-624. doi:10.1146/annurev-physiol-021909-135815
- Bitto A, Wang AM, Bennett CF, Kaeberlein M (2015) Biochemical Genetic Pathways that Modulate Aging in Multiple Species. Cold Spring Harbor Perspectives in Medicine 5 (11). doi:10.1101/cshperspect.a025114
- Blagosklonny MV (2006) Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. Cell cycle 5 (18):2087-2102
- Chawla G, Deosthale P, Childress S, Wu YC, Sokol NS (2016) A let-7-to-miR-125 MicroRNA Switch Regulates Neuronal Integrity and Lifespan in *Drosophila*. PLoS genetics 12 (8):e1006247. doi:10.1371/journal.pgen.1006247
- Chen H, Zheng X, Xiao D, Zheng Y (2016) Age-associated de-repression of retrotransposons in the *Drosophila* fat body, its potential cause and consequence. Aging cell 15 (3):542-552. doi:10.1111/ace.12465
- Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leivers SJ, Partridge L (2001) Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science 292 (5514):104-106. doi:10.1126/science.1057991
- Copeland JM, Cho J, Lo T, Jr., Hur JH, Bahadorani S, Arabyan T, Rabie J, Soh J, Walker DW (2009) Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. Curr Biol 19 (19):1591-1598. doi:10.1016/j.cub.2009.08.016
- De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, Braeckman BP, Schoofs L, Temmerman L (2014) Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proceedings of the National Academy of Sciences of the United States of America 111 (24):E2501-2509. doi:10.1073/pnas.1321776111
- Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstien B, Herker E, Fahrenkrog B, Frohlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, Madeo F (2009) Induction of autophagy by spermidine promotes longevity. Nature cell biology 11 (11):1305-1314. doi:10.1038/ncb1975
- Faust K, Gehrke S, Yang Y, Yang L, Beal MF, Lu B (2009) Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a *Drosophila* model of Parkinson's disease. BMC neuroscience 10:109. doi:10.1186/1471-2202-10-109
- Fernandez-Ayala DJ, Chen S, Kemppainen E, O'Dell KM, Jacobs HT (2010) Gene expression in a *Drosophila* model of mitochondrial disease. PloS one 5 (1):e8549. doi:10.1371/journal.pone.0008549
- Fernandez-Ayala DJ, Jimenez-Gancedo S, Guerra I, Navas P (2014) Invertebrate models for coenzyme q10 deficiency. Molecular syndromology 5 (3-4):170-179. doi:10.1159/000362751
- Fernandez-Ayala DJ, Sanz A, Vartiainen S, Kemppainen KK, Babusiak M, Mustalahti E, Costa R, Tuomela T, Zeviani M, Chung J, O'Dell KM, Rustin P, Jacobs HT (2009) Expression of the *Ciona intestinalis* alternative oxidase (AOX) in *Drosophila* complements defects in mitochondrial oxidative phosphorylation. Cell Metab 9 (5):449-460. doi:10.1016/j.cmet.2009.03.004

- Freiherr J, Hallschmid M, Frey WH, 2nd, Brunner YF, Chapman CD, Holscher C, Craft S, De Felice FG, Benedict C (2013) Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. *CNS drugs* 27 (7):505-514. doi:10.1007/s40263-013-0076-8
- Graff J, Tsai LH (2013) Histone acetylation: molecular mnemonics on the chromatin. *Nature reviews Neuroscience* 14 (2):97-111. doi:10.1038/nrn3427
- Gronke S, Clarke DF, Broughton S, Andrews TD, Partridge L (2010) Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. *PLoS genetics* 6 (2):e1000857. doi:10.1371/journal.pgen.1000857
- Hamalainen RH, Ahlqvist KJ, Ellonen P, Lepisto M, Logan A, Otonkoski T, Murphy MP, Suomalainen A (2015) mtDNA Mutagenesis Disrupts Pluripotent Stem Cell Function by Altering Redox Signaling. *Cell Rep* 11 (10):1614-1624. doi:10.1016/j.celrep.2015.05.009
- Harman D (1956) Aging: a theory based on free radical and radiation chemistry. *Journal of gerontology* 11 (3):298-300
- Harman D (1972) The biologic clock: the mitochondria? *Journal of the American Geriatrics Society* 20 (4):145-147
- He C, Tsuchiyama SK, Nguyen QT, Plyusnina EN, Terrill SR, Sahibzada S, Patel B, Faulkner AR, Shaposhnikov MV, Tian R, Tsuchiya M, Kaerberlein M, Moskalev AA, Kennedy BK, Polymenis M (2014) Enhanced longevity by ibuprofen, conserved in multiple species, occurs in yeast through inhibition of tryptophan import. *PLoS genetics* 10 (12):e1004860. doi:10.1371/journal.pgen.1004860
- Highfill CA, Reeves GA, Macdonald SJ (2016) Genetic analysis of variation in lifespan using a multiparental advanced intercross *Drosophila* mapping population. *BMC genetics* 17:113. doi:10.1186/s12863-016-0419-9
- Hirth F (2010) *Drosophila melanogaster* in the study of human neurodegeneration. *CNS & neurological disorders drug targets* 9 (4):504-523
- Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S (2004) Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol* 14 (10):885-890. doi:10.1016/j.cub.2004.03.059
- Li L, Greer C, Eisenman RN, Secombe J (2010) Essential functions of the histone demethylase lid. *PLoS genetics* 6 (11):e1001221. doi:10.1371/journal.pgen.1001221
- Li W, Prazak L, Chatterjee N, Gruninger S, Krug L, Theodorou D, Dubnau J (2013) Activation of transposable elements during aging and neuronal decline in *Drosophila*. *Nature neuroscience* 16 (5):529-531. doi:10.1038/nn.3368
- Lin MJ, Tang LY, Reddy MN, Shen CK (2005) DNA methyltransferase gene dDnmt2 and longevity of *Drosophila*. *The Journal of biological chemistry* 280 (2):861-864. doi:10.1074/jbc.C400477200
- Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, Zhu Y, Wang LS, Bonini NM (2012) The microRNA miR-34 modulates ageing and neurodegeneration in *Drosophila*. *Nature* 482 (7386):519-523. doi:10.1038/nature10810
- Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. *Cell* 153 (6):1194-1217. doi:10.1016/j.cell.2013.05.039

- Mair W, Piper MD, Partridge L (2005) Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS biology* 3 (7):e223. doi:10.1371/journal.pbio.0030223
- Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R (2013) Metformin improves healthspan and lifespan in mice. *Nat Commun* 4:2192. doi:10.1038/ncomms3192
- Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R (2015) Animal models of aging research: implications for human aging and age-related diseases. *Annual review of animal biosciences* 3:283-303. doi:10.1146/annurev-animal-022114-110829
- Oka S, Hirai J, Yasukawa T, Nakahara Y, Inoue YH (2015) A correlation of reactive oxygen species accumulation by depletion of superoxide dismutases with age-dependent impairment in the nervous system and muscles of *Drosophila* adults. *Biogerontology* 16 (4):485-501. doi:10.1007/s10522-015-9570-3
- Owusu-Ansah E, Song W, Perrimon N (2013) Muscle mitohormesis promotes longevity via systemic repression of insulin signaling. *Cell* 155 (3):699-712. doi:10.1016/j.cell.2013.09.021
- Palmer MR, Sackton TB (2003) The effects of dietary coenzyme Q on *Drosophila* life span. *Aging cell* 2 (6):335-339
- Partridge L, Alic N, Bjedov I, Piper MD (2011) Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Experimental gerontology* 46 (5):376-381. doi:10.1016/j.exger.2010.09.003
- Partridge L, Piper MD, Mair W (2005) Dietary restriction in *Drosophila*. *Mechanisms of ageing and development* 126 (9):938-950. doi:10.1016/j.mad.2005.03.023
- Peleg S, Feller C, Forne I, Schiller E, Sevin DC, Schauer T, Regnard C, Straub T, Prestel M, Klima C, Schmitt Nogueira M, Becker L, Klopstock T, Sauer U, Becker PB, Imhof A, Ladurner AG (2016) Life span extension by targeting a link between metabolism and histone acetylation in *Drosophila*. *EMBO reports* 17 (3):455-469. doi:10.15252/embr.201541132
- Phillips JP, Campbell SD, Michaud D, Charbonneau M, Hilliker AJ (1989) Null mutation of copper/zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. *Proceedings of the National Academy of Sciences of the United States of America* 86 (8):2761-2765
- Ramalingam M, Kim SJ (2015) Insulin exerts neuroprotective effects via Akt/Bcl-2 signaling pathways in differentiated SH-SY5Y cells. *Journal of receptor and signal transduction research* 35 (1):1-7. doi:10.3109/10799893.2014.922576
- Rana A, Rera M, Walker DW (2013) Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. *Proceedings of the National Academy of Sciences of the United States of America* 110 (21):8638-8643. doi:10.1073/pnas.1216197110
- Reczek CR, Chandel NS (2014) ROS-dependent signal transduction. *Current opinion in cell biology* 33C:8-13. doi:10.1016/j.ceb.2014.09.010
- Rera M, Bahadorani S, Cho J, Koehler CL, Ulgherait M, Hur JH, Ansari WS, Lo T, Jr., Jones DL, Walker DW (2011) Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog. *Cell Metab* 14 (5):623-634. doi:S1550-4131(11)00388-3 [pii]
- 10.1016/j.cmet.2011.09.013

- Ristow M, Zarse K (2010) How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Experimental gerontology* 45 (6):410-418. doi:10.1016/j.exger.2010.03.014
- Ro J, Pak G, Malec PA, Lyu Y, Allison DB, Kennedy RT, Pletcher SD (2016) Serotonin signaling mediates protein valuation and aging. *eLife* 5. doi:10.7554/eLife.16843
- Sanz A (2016a) Mitochondrial reactive oxygen species: Do they extend or shorten animal lifespan? *Biochim Biophys Acta*. doi:10.1016/j.bbabi.2016.03.018
- Sanz A (2016b) Mitochondrial reactive oxygen species: Do they extend or shorten animal lifespan? *Biochimica et biophysica acta* 1857 (8):1116-1126. doi:10.1016/j.bbabi.2016.03.018
- Sanz A, Fernandez-Ayala DJ, Stefanatos RK, Jacobs HT (2010a) Mitochondrial ROS production correlates with, but does not directly regulate lifespan in *Drosophila*. *Aging* 2 (4):200-223. doi:10.18632/aging.100137
- Sanz A, Soikkeli M, Portero-Otin M, Wilson A, Kempainen E, McIlroy G, Ellila S, Kempainen KK, Tuomela T, Lakanmaa M, Kiviranta E, Stefanatos R, Dufour E, Hutz B, Naudi A, Jove M, Zeb A, Vartiainen S, Matsuno-Yagi A, Yagi T, Rustin P, Pamplona R, Jacobs HT (2010b) Expression of the yeast NADH dehydrogenase Ndi1 in *Drosophila* confers increased lifespan independently of dietary restriction. *Proceedings of the National Academy of Sciences of the United States of America* 107 (20):9105-9110. doi:10.1073/pnas.0911539107
- Scialo F, Sriram A, Fernandez-Ayala D, Gubina N, Lohmus M, Nelson G, Logan A, Cooper HM, Navas P, Enriquez JA, Murphy MP, Sanz A (2016) Mitochondrial ROS Produced via Reverse Electron Transport Extend Animal Lifespan. *Cell Metab* 23 (4):725-734. doi:10.1016/j.cmet.2016.03.009
- Sen P, Shah PP, Nativio R, Berger SL (2016) Epigenetic Mechanisms of Longevity and Aging. *Cell* 166 (4):822-839. doi:10.1016/j.cell.2016.07.050
- Shahrestani P, Wilson JB, Mueller LD, Rose MR (2016) Patterns of physiological decline due to age and selection in *Drosophila melanogaster*. *Evolution; international journal of organic evolution*. doi:10.1111/evo.13065
- Siebold AP, Banerjee R, Tie F, Kiss DL, Moskowitz J, Harte PJ (2010) Polycomb Repressive Complex 2 and Trithorax modulate *Drosophila* longevity and stress resistance. *Proceedings of the National Academy of Sciences of the United States of America* 107 (1):169-174. doi:10.1073/pnas.0907739107
- Slack C, Foley A, Partridge L (2012) Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PLoS one* 7 (10):e47699. doi:10.1371/journal.pone.0047699
- Slack C, Werz C, Wieser D, Alic N, Foley A, Stocker H, Withers DJ, Thornton JM, Hafen E, Partridge L (2010) Regulation of lifespan, metabolism, and stress responses by the *Drosophila* SH2B protein, Lnk. *PLoS genetics* 6 (3):e1000881. doi:10.1371/journal.pgen.1000881
- Stefanatos R, Sanz A (2011) Mitochondrial complex I: a central regulator of the aging process. *Cell cycle* 10 (10):1528-1532. doi:15496 [pii]
- Stefanatos R, Sriram A, Kiviranta E, Mohan A, Ayala V, Jacobs HT, Pamplona R, Sanz A (2012) dj-1 β regulates oxidative stress, insulin-like signaling and development in *Drosophila melanogaster*. *Cell cycle* 11 (20):3876-3886. doi:10.4161/cc.22073

- Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS (2001) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292 (5514):107-110. doi:10.1126/science.1057987
- Taylor RC, Dillin A (2011) Aging as an event of proteostasis collapse. *Cold Spring Harbor perspectives in biology* 3 (5). doi:10.1101/cshperspect.a004440
- Toivonen JM, O'Dell KM, Petit N, Irvine SC, Knight GK, Lehtonen M, Longmuir M, Luoto K, Touraille S, Wang Z, Alziari S, Shah ZH, Jacobs HT (2001) Technical knockout, a *Drosophila* model of mitochondrial deafness. *Genetics* 159 (1):241-254
- Ulgherait M, Rana A, Rera M, Graniel J, Walker DW (2014) AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. *Cell Rep* 8 (6):1767-1780. doi:10.1016/j.celrep.2014.08.006
- Vaccaro A, Birman S, Klarsfeld A (2016) Chronic jet lag impairs startle-induced locomotion in *Drosophila*. *Experimental gerontology* 85:24-27. doi:10.1016/j.exger.2016.09.012
- Verma P, Augustine GJ, Ammar MR, Tashiro A, Cohen SM (2015) A neuroprotective role for microRNA miR-1000 mediated by limiting glutamate excitotoxicity. *Nature neuroscience* 18 (3):379-385. doi:10.1038/nn.3935
- Wen DT, Zheng L, Ni L, Wang H, Feng Y, Zhang M (2016) The expression of CG9940 affects the adaptation of cardiac function, mobility, and lifespan to exercise in aging *Drosophila*. *Experimental gerontology* 83:6-14. doi:10.1016/j.exger.2016.07.006
- Williams MJ, Perland E, Eriksson MM, Carlsson J, Erlandsson D, Laan L, Mahebal T, Potter E, Fredriksson R, Benedict C, Schioth HB (2016) Recurrent Sleep Fragmentation Induces Insulin and Neuroprotective Mechanisms in Middle-Aged Flies. *Frontiers in aging neuroscience* 8:180. doi:10.3389/fnagi.2016.00180
- Wood JG, Hillenmeyer S, Lawrence C, Chang C, Hosier S, Lightfoot W, Mukherjee E, Jiang N, Schorl C, Brodsky AS, Neretti N, Helfand SL (2010) Chromatin remodeling in the aging genome of *Drosophila*. *Aging cell* 9 (6):971-978. doi:10.1111/j.1474-9726.2010.00624.x
- Wood JG, Jones BC, Jiang N, Chang C, Hosier S, Wickremesinghe P, Garcia M, Hartnett DA, Burhenn L, Neretti N, Helfand SL (2016) Chromatin-modifying genetic interventions suppress age-associated transposable element activation and extend life span in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 113 (40):11277-11282. doi:10.1073/pnas.1604621113
- Yang M, Soga T, Pollard PJ (2013) Oncometabolites: linking altered metabolism with cancer. *The Journal of clinical investigation* 123 (9):3652-3658. doi:10.1172/JCI67228
- Zhang G, Huang H, Liu D, Cheng Y, Liu X, Zhang W, Yin R, Zhang D, Zhang P, Liu J, Li C, Liu B, Luo Y, Zhu Y, Zhang N, He S, He C, Wang H, Chen D (2015) N6-methyladenine DNA modification in *Drosophila*. *Cell* 161 (4):893-906. doi:10.1016/j.cell.2015.04.018