Drosophila melanogaster: A Prime Experimental Model System for Aging Studies

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Abstract Aging, the process of growing old is largely characterized by gradual deterioration of normal cellular functions, leading to progressive and steady decline in the biological, physical and psychological abilities. The phenomenon of aging is genetically determined and environmentally modulated. This is one of the most common yet mysterious aspects of biological studies, even after being a subject of interest to humans since the beginning of recorded history. Moreover, precise molecular basis of aging remains poorly understood, in part, because we lack a large number of molecular markers which could be used to measure the aging process in specific tissues. Moreover, limitations of human genetics and associated ethical issues further make it difficult to identify or analyze candidate gene(s) and pathways in greater details, and with the fact that the basic biological processes remain conserved throughout phylogeny; model organisms from bacteria to mammals have been utilized to resolve different aspects of aging. Classical model system such as *Drosophila melanogaster* has emerged as an excellent system to elucidate essential genetic/cellular pathways of human aging, due to its short generation time, availability of powerful genetic tools and functionally conserved physiology. Several key cellular events and signaling cascades have been deciphered by utilizing *Drosophila* as system of aging research and continues to add novel insights into this complex process. Present article attempts to introduce *Drosophila* as a model system for aging studies and also provides a brief overview of its decades of contribution in aging research.

Keywords *Drosophila* · Aging · Molecular chaperone · Oxidative stress · Insulin signalling · Neurodegeneration

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1 Introduction

Aging is not a passive activity, but an actively regulated complex process or collection of gradual senescence processes at both physiological and cellular levels. Some of the most prominent characteristics of aging include progressive decrease in physiological capacity, reduced ability to respond adaptively to environmental stimuli, increased vulnerability to infection and complex diseases and, increased mortality. Aging at large, is genetically determined and environmentally modulated. Aging activates some irreversible series of biological changes that inevitably result in death of the organism. Although, the causes of these changes may be entirely unrelated in different cases implying no common mechanism, yet they often imply a mutual element of descent. Therefore, aging is one of the most common yet mysterious aspects of biological studies, even after being a subject of interest to human race since the beginning of recorded history.

Decades of research on aging has found several genes and many biological processes those are associated with them; however, several fundamental questions continue to be intensely debated. Some of such unanswered questions are: (i) How many biological processes contribute to aging? What are they? (ii) Is it possible to reverse the phenomenon of aging? (iii) Can a single gene mutation recapitulate all the aging induced consequences? Also, the molecular basis of aging remains poorly understood, in part, because we lack a large number of molecular markers of aging which can be used to measure the aging process in specific tissues. Thus, unravelling the mysteries of aging is still on the frontier of biomedical research.

The last two decades have witnessed a tremendous upsurge in the genetic analyses of aging, with a greater emphasis towards the elucidation of the molecular mechanisms, pathways, and physiological processes implicated in longevity. Since the limitations associated with human genetic studies make it difficult to identify or analyze candidate gene(s) and pathway(s) in greater details, and with the fact that the basic biological processes remain conserved throughout phylogeny, model organisms from bacteria to mammals have been utilized to resolve different aspects of aging. However, classical model systems such as *Caenorhabditis elegans* and *Drosophila melanogaster* have emerged as excellent systems to elucidate essential genetic/cellular pathways of human aging. *Drosophila* particularly, holds tremendous promise for identifying genes and to decipher other mechanisms which influence age-related functional declines. Some of the major advantages associated with *Drosophila* have been discussed below:

2 *Drosophila melanogaster* as a Model Organism for Aging Research

D. melanogaster, commonly known as "fruit fly" is one of the most studied organisms in biology, particularly in genetics and developmental biology (Fig. 1a). Some of the major advantages of using *Drosophila* for aging related studies include

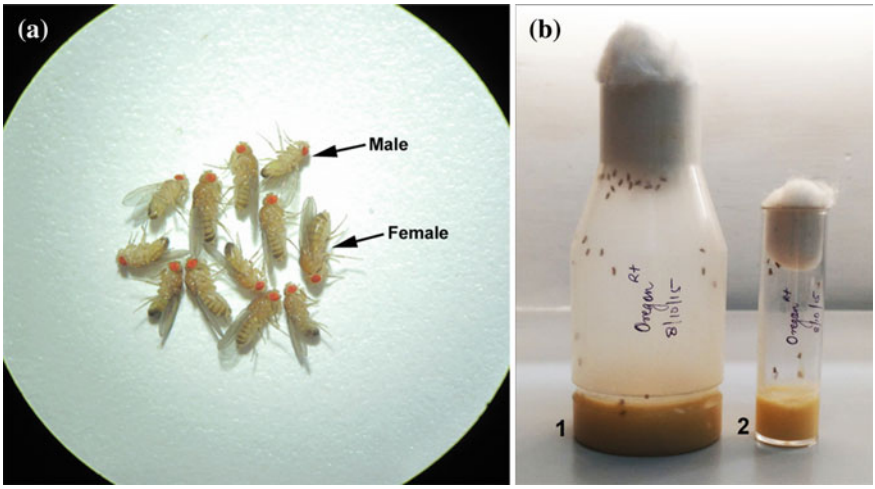

Fig. 1 **a** Mixed population (male and female) of wild type (*Oregon* R^+) *Drosophila melanogaster* as appears under stereozoom binocular microscope. **b** Wild type (*Oregon* R^+) *Drosophila melanogaster* raised on cornmeal in culture bottle (1) and vial (2)

its short life span of 50–70 days, high fecundity (female lay up to 100 eggs per day), availability of powerful genetic tools, accessibility of stocks with many different alterations, knowledge of the complete genomic sequence and large homogeneous populations. In addition, ease of culturing and affordability of maintaining large populations within the confines of a laboratory further makes flies a remarkable model organism (Fig. 1b). Besides, absence of meiotic recombination in males and presence of balancer chromosomes allow populations of flies carrying heterozygous mutations to be maintained without undergoing any constant screening for the mutations. Moreover, completely sequenced and annotated genome distributed on four chromosomes makes *Drosophila* a well acceptable system to perform large-scale genetic screens for identification of potential modifiers of aging and disease related phenotype(s). One of the striking features of *Drosophila* is the existence of morphologically distinct developmental stages which includes embryonic, larval, pupal and adult phase (Table 1); thus, the sexually matured "aging" adults phase could be easily distinguished in the developing population.

Table 1 Different developmental stages in life cycle of *Drosophila melanogaster* at 25 °C

Stages	Time (in h)	Duration (in h)
Egg	0	24
First instar larvae	24	24
Second instar larvae	48	24
Third instar larvae	72	48
Pupa	120	72
Adult fly	192	–

Table 2 Effect of varying temperature on generation times of *Drosophila melanogaster*

Temperature (°C)	Generation time (days)
10	Viability decrease
20	13–15 days
25	10–12 days
35	Viability decrease

In several model organisms, it is not so conventional to visually distinguish the mature aging adults form immature or juvenile stage. Depending upon the temperature, *Drosophila* life cycle varies. Details of the different generation time corresponding to different temperature have been provided in the Table 2. Since anatomy and developmental process of *Drosophila* have been well worked out and therefore, creating environmental and genetic manipulations which alter aging dynamics and life span could be easily performed and scored. Besides, availability of the large number of mutants and transgenic lines at several *Drosophila* stock centers further makes it a popular model organism (Dietzl et al. 2007; Ryder et al. 2007).

Similarity of different genes and families which are structurally and functionally related in both *Drosophila* and mammals, makes flies a good model in human based research. It is increasingly clear now that *Drosophila* genome has approximately 75 % of known human disease genes and ~50 % of proteins have mammalian homologs (Reiter et al. 2001). Moreover, the adult fly harbors a well-coordinated sophisticated brain and nervous system, which makes it capable of exhibiting complex behaviors such as learning and memory, much like the human brain (Jones and Grotewiel 2011). Disruption of such well-coordinated motor behaviors leads to neuronal death and dysfunction. Mammalian aging related phenotypes such as locomotory and sensory impairments, learning disabilities, sleep like behavior etc. are well manifested in *Drosophila* (Jones and Grotewiel 2011). *Drosophila* lack a functional blood brain barrier which could otherwise prevent access of drugs to the tissues of central nervous system; as a result flies become extremely useful for pharmacological screening for identification of novel therapeutic drug targets (Jones and Grotewiel 2011). Interestingly, the response towards many drugs that has shown effects within the *Drosophila* CNS is quite similar as observed in mammalian systems (Wolf and Heberlein 2003; Pandey and Nichols 2011).

Drosophila provides powerful genetic tools which can easily manipulate gene expression in a tissue specific manner during various stages of life cycle. UAS-Gal4 system is a commonly used genetic tool to achieve ectopic expression of desired genes or to suppress the expression of a target gene by UAS-RNAi transgene (Brand and Perrimon 1993). Additionally, FLP-FRT system, a site- directed recombination technology, has been progressively used to manipulate the fly genome in vivo, under controlled condition (Theodosiou and Xu 1998). Utilizing this technology loss-of-function of any lethal gene can be easily studied in a given target organ in a spatially controlled manner, in the cases where model organism would not survive as a result of loss of this gene in other organs. The effect of

altered gene can also be studied over time, by using an inducible promoter to trigger the recombination activity late in development. This prevents the genetic alteration from affecting overall development of the organ, and also allows single cell comparison of the one lacking the gene to normal neighboring cells in the same environment.

In comparison to other model systems, a few additional advantages offered by *Drosophila* for aging studies include presence of almost fully differentiated post-mitotic cells throughout the adult fly, representing synchronized aging (Arking 1991). Enlightening the first aspect, the instigation of adulthood in *Drosophila* is said to occur only after the fly ecloses out of the pupal case. During this stage of its life, it becomes sexually mature and competent to reproduce and thus, aging is thought to be initiated (Shaw et al. 2008). This is in great disparity with other model systems where it is often difficult to find out when the organism has attained maturity (Helfand and Rogina 2003a). The second aspect has been focused on the rarely dividing neurons of the brain which makes the *Drosophila* brain an excellent model for the cytological studies and to relate with human aging (Herman et al. 1971). Hence, aging related structural changes could be easily and conclusively deduced by observing a set of synchronously aging cells. Moreover, due to the absence of blood vessels in insect brain, the pathological changes due to blood vasculature can be debarred. In view of above noted advantages, *Drosophila* has been widely utilized to decipher various aspects of aging. A brief overview of the history of *Drosophila* aging research has been provided below.

3 History of *Drosophila* Aging Research

For the first time Thomas H. Morgan used the small invertebrate, *Drosophila melanogaster*, to write the purpose of his research and this marks the beginning of an era of groundbreaking research utilizing this system in his "fly room" at Columbia, USA. This led to the discovery of the 'chromosomal theory of inheritance' and he was eventually awarded Nobel Prize in 1933 for his excellent finding. Following this, the researchers have come a long way in terms of exploiting the powerful genetics offered by this tiny fruit fly. Remarkably, Loeb and Northrop in 1916 reported the first use of *Drosophila* as a model system to study aging. They performed several experiments to demonstrate the effects of temperature and food on fly longevity (Loeb and Northrop 1916). They concluded that longevity of flies as poikilothermic organisms depends on the temperature of the environment (Loeb and Northrop 1916). In addition, they also examined the effect of starvation and sugar concentration on fly longevity (Loeb and Northrop 1917). Subsequently, Pearl and co-workers demonstrated that longevity in flies is hereditable (Pearl and Parker 1921, 1922). Consistent to Pearl's finding, the significance of genetic influence in regulation of life span of adult flies was further reported by Clark and Gould in 1970. By utilizing *Drosophila* as a model system, several small compounds such as biotin, pyridoxine and pantothenic acid were identified which

extend the life span upon regulated feeding (Gardner 1948). The effect of reproductive behavior on aging has been a topic of aging research since middle of 20th century when J. Maynard Smith and colleagues reported that longevity of flies could be affected by changing their reproductive behavior (Smith 1958). Their studies had established *Drosophila* as a good model system to study the fitness trade-offs and life span (Smith 1958). Since then, the mechanistic correlation between reproduction and longevity has been a topic of great interest in the aging research. Consistently, the plasticity behaviors between fly longevity and reproductive output was further confirmed by the selection experiments performed in the 1980s, which showed that longevity could be significantly extended when female flies were selected for late-life fertility (Rose and Charlesworth 1980, 1981; Luckinbill et al. 1984; Luckinbill and Clare 1985). Michael Rose has reviewed the history of laboratory-based evolution experiments and the use of different genomic technologies to comprehend genetics of longevity in *Drosophila* (Rose and Charlesworth 1980, 1981). Interestingly, independent studies performed during end of 20th century led to identification of two different life extending mutations, the *Methuselah (mth)* and *I'm not dead yet (Indy)* by performing random genetic alterations. It was demonstrated that partial loss-of-function mutation in either *mth* or *indy* extend the life span in both male and female flies, without loss of fertility (Lin et al. 1998; Rogina et al. 2000). In modern era of aging research, in addition to classical approaches several contemporary approaches and novel strategies are being adopted to decipher the mechanistic in-depth of aging and longevity. Some of the popular genetic approaches include selective breeding, mutagenesis followed by forward genetic analysis, cellular and molecular genetics and QTL analysis (Jazwinski 2000). These methods, so far, have allowed identification of numerous genes involved in diverse cellular functions including aging and longevity in *Drosophila*. Table 3 provides a brief collection of some genes and their assigned function(s) which have been found to be associated with longevity in *Drosophila*. An overview of various methods and approaches related to *Drosophila* aging research have been provided below.

4 Evaluating Aging in *Drosophila*: Methods and Approaches

Over the past decades understanding the complex mechanisms underlying the process of aging has emerged as a great frontier of biomedical research considering not only the welfare of humankind but also to overcome the challenges associated with this complex biological phenomenon. As discussed above, aging is a process of progressive, irreversible changes at the molecular and cellular level, which results in the decline of organismal performances. The stereotypic/phenotypic changes which are associated with aging in most of the organisms are the result of the changes at molecular, physiological and cellular levels. Therefore, due to the

Table 3 A brief collection of some genes found to expand life span in *Drosophila melanogaster*

Gene	Function in the cell	Longevity increases due to	References
14-3-3ε	Antagonist to dFoxo	Knockdown	Nielsen (2008)
chico	Insulin receptor substrate	Knockdown	Clancy et al. (2001)
dFoxo	*Drosophila* forkhead transcription factor	Over-expression	Giannakou et al. (2004)
dilps	*Drosophila* insulin-like peptides	Knockdown	Grönke et al. (2010)
dInR	*Drosophila* Insulin receptor	Knockdown	Tatar et al. (2001)
dPTEN	*Drosophila* phosphatase and tensin homolog, controls cell growth and proliferation	Over-expression	Hwangbo et al. (2004)
dS6K	Major downstream kinase of the TOR pathway	Knockdown	Kapahi et al. (2004)
dsir2	Histone and non-histone, NAD-dependent deacetylase	Over-expression	Rogina and Helfand (2004), Bauer et al. (2009)
dTOR	Serine/threonine protein kinase that regulates growth, proliferation, survival, transcription, etc. of the cell	Knockdown	Kapahi et al. (2004)
dTsc1, dTsc2	Act synergistically to inhibit TOR	Over-expression	Kapahi et al. (2004)
GSH	Antioxidant enzyme involved in formation of reduced glutathione	Over-expression	Mockett et al. (1999)
Hep	JNK kinase	Over-expression	Wang et al. (2003)
Hsp22	Stress response	Overexpression	Morrow et al. (2004b)
Hsp23	Stress response	Overexpression	Morrow and Tanguay (2003)
Hsp26	Stress response	Overexpression	Liao et al. (2008)
Hsp27	Stress response	Overexpression	Liao et al. (2008)
Hsp68	Stress response	Overexpression	Wang et al. (2003)
Hsp70	Stress response	Overexpression	Tatar et al. (1997)
Indy	Succinate and citrate transmembrane transporter	Knockdown	Rogina et al. (2000)
mth	G-protein coupled receptor	Knockdown	Lin et al. (1998)
puc	Inhibits JNK by its specific phosphatase activity	Knockdown	Zeitlinger and Bohmann (1999), Wang et al. (2003)
SOD	Antioxidant enzyme involved in degeneration of superoxide radicals to molecular oxygen	Over-expression	Parkes et al. (1998), Sun and Tower (1999)

fact that aging follows the normal laws of chemical, physical and several of the complex biological phenomenon; combined efforts of molecular, genetics, physiological, anatomical and behavioral approaches have been used to assess the mysteries behind aging. In the following text, details of the different approaches which have been used to assay the process of aging in *Drosophila* have been discussed.

4.1 Assessing Life Expectancy

It is difficult to measure how an individual changes with age, but demographic assay such as age of the dead individuals in a cohort can be easily measured. Even though the age of the dead individual does not provide any direct information on what causes death, it does signify some important aspects of the aging process including the stochastic nature of the life span and relationship of mortality to age. In contemporary *Drosophila* aging research, determination of life span and progression of aging is performed and compared by analyzing survivorship curves. Figure 2 provides a representative survivorship graph of aging over time in wild type and a symbolic mutant strain of *Drosophila*. Assuming the fact that shortening or lengthening of life span of an organism is the result of relative aging, comparative analyses among mean, median and maximum life span of different populations under different conditions could be treated as one of the factors to measure aging process. Considering the primary potential role of life span assay in aging research, it is also important to consider the interventions such as genetic and environmental factors during the analysis because in *Drosophila* a mild change will affect the age of the individuals.

4.2 Behavioral Assay

Several of the behaviors including locomotor activities, circadian rhythm, sleep patterns and even cognitive functions can be quantitatively assessed in *Drosophila* and functional deficits could be clearly observed and recorded in aging adults. Behavioral activities of *Drosophila* could be studied with two widely used simple methods, i.e. Rapid Iterative Negative Geotaxis (RING) and *Drosophila* activity monitoring (DAM) system (Nichols et al. 2012; Sun et al. 2013). RING is one of

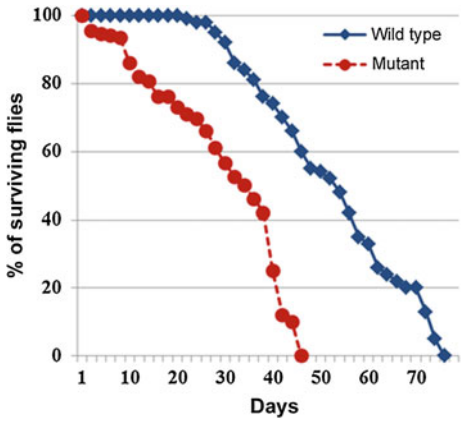

Fig. 2 Comparative survival curve of wild type and a symbolic mutant line of *D. melanogaster*. With advancing age, increased mortality and drastic decrease in the life span of the mutant population could be observed

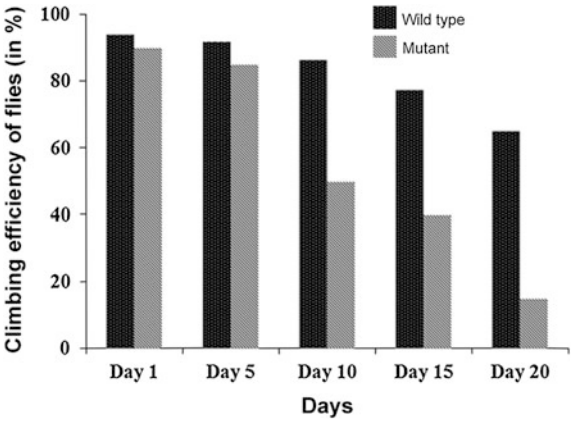

Fig. 3 Comparative climbing behavior of wild type and a symbolic mutant line of *D. melanogaster* with age. A decline in climbing activity in wild type population could be observed with increasing age, which is radically affected in mutant population

the most commonly used systems to assess the locomotor behavior of flies. Taking the advantage of inherent negative geotaxis response of the flies, this assay records the climbing ability of the flies against the gravity on the wall of empty vial after being tapped on the bottom of the container. Aging studies in *Drosophila* had reported gradual decline in the locomotor activities in almost all the species that have been studied (Iliadi and Boulianne 2010). The functional decline in the locomotor activity of the flies with age in wild type and in a symbolic mutant strain is depicted in Fig. 3. In the case of DAM based analysis, flies are kept individually in sealed activity tubes of DAM system and activity of the fly is measured based on the frequency of the event recorded each time a fly breaks an infrared light beam across the middle of the activity tube (Pfeiffenberger et al. 2010). It is mostly used to study circadian rhythm, sleep patterns, hypo and hyperactivity of flies. Moreover, more sophisticated video based tracking systems have been developed to analyze various fly behaviors including movement pattern, courting behavior etc. (Branson et al. 2009).

4.3 Assessing Aging on the Basis of Dietary Composition

Similar to other organisms, major environmental factors such as diet or food has a huge impact on the lifespan in *Drosophila* (Tatar et al. 2014). Thus, calorie or dietary restriction based studies is also among the important methods used to study aging in flies. Dietary restriction, by diluting all or specific components of food ingredients has two important impacts on physiology of flies: life span extension and reduction in the reproductive ability (Partridge et al. 2005). Intriguingly, studies based on dietary restriction allowed discovery of some fundamental regulators of aging (Partridge et al. 2005). It has been found that dietary restriction mediated life span extension is primarily controlled by some major metabolic pathways such as insulin/IGF-1 signaling, TOR (Target of Rapamycin) pathway etc. (Partridge et al.

2005). Studies on the effect of dietary restriction on aging and longevity have contributed enormously in understanding the in-depth of aging related pathways and their mechanistic details. Taken together, the powerful molecular genetic system present with *Drosophila* allows dissecting out the relationship between food intake, its utilization and its potential impact on the life span of the organism.

4.4 Reproductive Output: Measure to Evaluate Aging

Measurement of the lifetime reproductive output is another aspect of lifespan related physiological assay. The concept "cost of reproduction" in aging signifies a negative correlation between reproductive output and longevity of the organism (Tatar 2010). To measure the reproductive output of the flies, lifetime egg production in once mated female or number of progeny from the mating of male and female are measured. Selection experiments in *Drosophila* has resulted selection of long lived flies with decreased early reproduction and selection of the late life reproduction leads to the identification of lines with increased life span; moreover, long lived mutant females have reduced fecundity or fertility (Iliadi et al. 2012). Considering the cost of reproduction in *Drosophila* system, virgin/sterile females live longer than fertile control ones and fertile flies with increased reproduction results in increased susceptibility to stress (Salmon et al. 2001). The reason behind the extended life span with reduced reproduction may be probably the energy cost from lower or delayed egg production, as well as reduced cost of mating.

4.5 Stress as a Measure to Study Aging

Certain environmental stresses such as oxidative stress, starvation, crowded culture condition, heat or cold shock etc. have profound effect on aging and can be evaluated in *Drosophila*. According to the free radical theory of aging, an accumulative damage to the major biological macromolecules is the result of increasing level of cellular Reactive Oxygen species (ROS) (Harman 1992). In *Drosophila* system also, measurement of resistance against different stresses is another widely used method to study aging. Survival in the presence of a strong oxidizing agent like Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride), an organic compound widely used as herbicide has been used to assess resistance against stress (Vermeulen et al. 2005). Paraquat feeding in flies induces various ROS and consequently, due to increased oxidative damage survival of flies declines. Starvation resistance is another interesting aspect which has been found to extend life span in *Drosophila* as it deals with their ability to manage with energy shortage (Minois and Le Bourg 1999). Moreover, stresses such as extremes of temperature have

significant impact on *Drosophila* aging as both adversely affect their survival and life span (Minois and Le Bourg 1999).

4.6 Aging Analysis Utilizing Genetic Approaches

Since 1920 when Pearl's studies demonstrated for the first time that longevity in flies is heritable, genetic approaches remain as invaluable method for identifying the physiological mechanism of aging process. It includes alternation of single genes and careful analysis of the resultant phenotypes which affect the longevity and behavioral response of the flies. This method can be adapted to confirm any of the existing hypotheses based on the candidate gene approach or to explore new genes using the random gene alternation approach (Helfand and Rogina 2003b). In *Drosophila* system, a number of genetic approaches have been developed to generate mutation and to manipulate gene expression for aging studies. Some of such popular approaches include insertional mutagenesis by P-element, gene expression alternation by UAS-Gal4 system, inducible gene expression by Gene-switch Gal4 (GSG-UAS system) and gene knockdown by RNA interference (RNAi) strategy (Sun et al. 2013).

5 Contribution of *Drosophila* in Excavating Molecular and Genetic Mechanisms of Aging

As discussed earlier, *Drosophila* has been extensively utilized to unravel the molecular and genetic aspects of aging and longevity. In addition to genetic factors, environmental stresses which deteriorate cellular functions are largely known to be instrumental in instigating the process of aging. Therefore, several approaches have been undertaken to modify the genetic makeup of flies to promote extension of life span by modulating the cellular response to environmental stresses. Some of them have been briefly addressed in following texts:

5.1 Oxidative Stress

About a century ago the observation that animals with higher metabolic rates generally exhibit shorter life span led to the foundation of "Rate-of-living Hypothesis"; though the mechanistic association between metabolic rate and life expectancy was unknown during that period. Interestingly, in contrast to this theory, some species don't exhibit any strict inverse correlation between metabolic rate and longevity, particularly in birds and primates (Finkel and Holbrook 2000). In

1956, Denham Harman proposed mechanistically stronger theory of aging known as "Free-radical theory of aging"; according to which cumulative oxidative damage to biological macromolecules, brought about by ROS over the time results in deterioration of cellular function and stability, which ultimately act as a driving force for progression of aging (Harman 1956; Yadav et al. 2015). It was a decade later when enzyme superoxide dismutase (SOD) (enzyme with sole function of degeneration of superoxide anions) was discovered and first compelling evidence in the support of Harman's theory was presented (McCord and Fridovich 1969). Later in 1985 extensive research in redox biology concept of oxidative stress was used to symbolize the damage incurred in biological systems due to excessive ROS production and/or inadequate antioxidant defense (Sies and Cadenas 1985). Subsequently, the free-radical theory of aging was revised to the Oxidative stress theory of aging which subsequently emerged as the most persuasive theory in aging research (Pérez et al. 2009). A great deal of research work was performed to substantiate this theory but the results were inconsistent and partially challenging as well (Lapointe and Hekimi 2010). However, large number of findings from various organisms including *Drosophila* is reminiscent that decline in oxidative stress level is directly associated with increased life expectancy (Bokov et al. 2004). Therefore, intricate balance in the production of oxidants along with the capability of the organism to counteract the oxidative stress is critically linked to the progression of aging.

D. melanogaster has been widely used at the forefront to examine the oxidative stress hypothesis. The elementary idea behind such studies reside on the assumption that factors which aid in decreasing oxidative stress should have beneficial effects against aging and hence should result in enhancement of life expectancy. In support to this claim, linear correlation between oxidative stress resistance and longevity has been found in *Drosophila* utilizing various strains (Dudas and Arking 1995). In such cases, strains with extended life span exhibited either higher resistance to oxidative stress or had enhanced level of antioxidant enzymes (Dudas and Arking 1995; Harshman and Haberer 2000). For instance, reduced function of *Methuselah* (*mth*) gene which is a G-protein coupled receptor results in increase in life span. P-element insertion line of *mth* enhances longevity of the flies by approximately 35 % (Lin et al. 1998). In addition to increase in life span, this gene also provides tolerance against several stresses including high temperature, dietary paraquat (intracellular free radical generator) and starvation (Lin et al. 1998). Though, the explicit function of the *mth* is still unknown but it has been proposed to be involved in transmitting cues for regulating stress response pathways (Lin et al. 1998). Figure 4 attempts to provide a schematic representation of various signaling cascades which are known to modulate aging and longevity in *D. melanogaster*.

Relationship between oxidative stress tolerance and longevity has been tested in *Drosophila* by overexpressing antioxidant genes, utilizing transgenic approaches. Increase in the expression of glutathione reductase (GSH) antioxidant enzyme (involved in formation of reduced glutathione) results in high level of oxidative stress tolerance and prolonged life span in flies exposed to hyperoxic conditions, though no effect on the longevity was observed when the flies were reared at

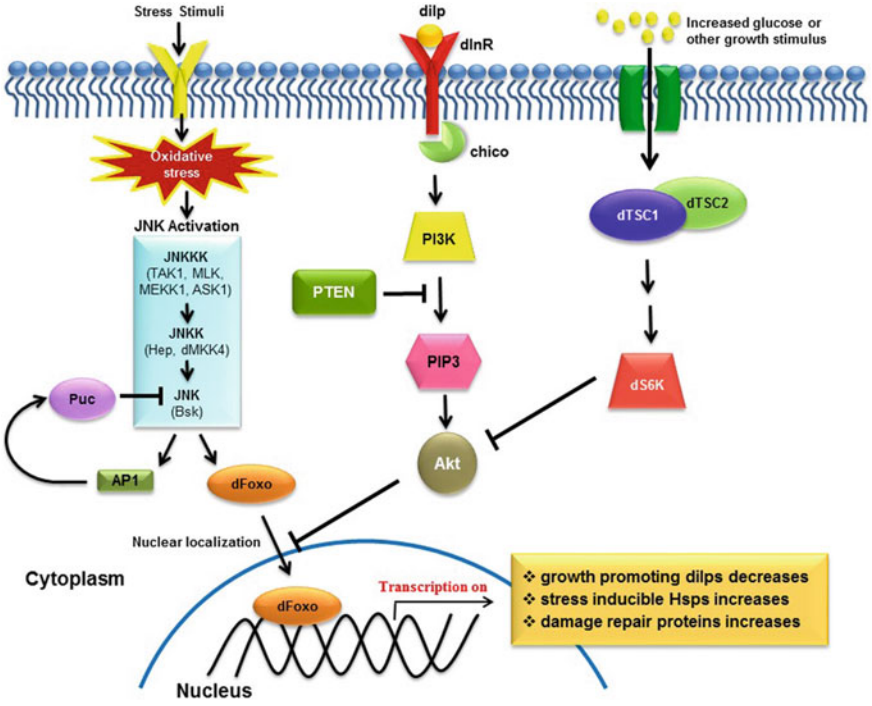

Fig. 4 Schematic representation of various stimulus and signaling pathways which modulate progression of aging and longevity in *D. melanogaster* (please refer text for detail)

normoxic condition (Mockett et al. 1999). In addition, it has also been demonstrated that decrease in expression of antioxidant enzymes such as superoxide dismutase (SOD) (scavenges superoxide anion radicals) and catalase (involved in eradication of H_2O_2), shortens the life span indicating the significance of ROS detoxification on life expectancy (Phillips et al. 1989; Phillips and Hilliker 1990; Missirlis et al. 2001; Kirby et al. 2002). However, in this context it is also important to note that since the mutation was prevalent even during the fly development, and therefore, a decrease in life span could also be partially attributed to the damage accumulated during development and might not be solely due to oxidative stress. To overcome this discrepancy, studies were focused on increasing the fly expectancy by over-expressing the antioxidant enzymes. In this respect numerous studies displayed higher oxidative stress resistance and modest enhancement in life span, either by combined overexpression of SOD and catalase or SOD alone (Orr and Sohal 1994; Parkes et al. 1998; Sun and Tower 1999). A noteworthy report in these findings was 40–50 % increase in life span by overexpressing human SOD in motor neurons of fly (Parkes et al. 1998). Achieving modest increase in life span by overexpression of antioxidant enzymes supports oxidative stress theory of aging. However, on the other side some studies reported slight or insignificant enhancement of oxidative

stress resistance and life span by overexpression of SOD (Seto et al. 1990; Orr and Sohal 1993). However, the root cause of these inconsistencies is largely unknown.

5.1.1 Proposed Mechanisms of Oxidative Stress Mediated Aging

Mitochondria being the principle source of energy in cell via aerobic respiration consume majority of the cellular oxygen and therefore, are the prime source of ROS. Irrespective of source or how ROS is generated inside a cell, enhanced level of oxidants broadly effect organisms by incurring oxidative damage to cellular components and/or by eliciting the activation of oxidative stress responsive signaling cascades. Prevalence of these phenomenon due to oxidative stress over continuous periods of time stimulates aging associated cellular processes (Finkel and Holbrook 2000). A brief overview of the above aspects have been discussed below:

Oxidative Damage to Cellular Components Due to Enhanced Level of ROS

An enhanced level of ROS causes oxidative damage to all the macromolecules (nucleic acids, proteins and lipids) present in a cell. Progressive accumulation of damaged macromolecules contributes to imbalance in cellular homeostasis, thereby instigating aging process (Le Bourg 2001). Interestingly amongst all the cellular organelles, mitochondria in spite of being the major source of ROS, are also the key targets of oxidants. Moreover, due to the close proximity of mitochondrial elements to ROS production site, they are more susceptible to the damage by ROS. Further, lack of histone protection and repair mechanism in mitochondrial DNA aggravates their susceptibility to ROS mediated damages. All this cumulatively results in mitochondrial dysfunctioning and has been profoundly linked to manifestation of aging progression (Sohal 2002; Wallace 2005; Yadav et al. 2013).

There have been several studies carried out in *Drosophila* where correlative data on age associated changes in the structure and functions of mitochondria, are suggestive of the idea that gradual mitochondrial dysfunctioning is associated with aging process (Wallace 2005). One such study investigating the effect of aging on *Drosophila* flight muscles reported a specific reorganization of mitochondrial cristae under oxidative stress, with aging (Walker and Benzer 2004). Aging induces local rearrangement of the cristae in a “swirl” like pattern in individual fly mitochondria (Walker and Benzer 2004). Rapid and extensive accrual of the same pathological condition was witnessed even in young flies under the condition of severe oxidative stress. From functional aspect of this pathological condition, cristae associated with swirling pattern were found to have reduced enzymatic activity of cytochrome c (COX) or complex IV, which is an important respiratory enzyme present in mitochondria. Furthermore, occurrence of swirls is accompanied by alteration in the structural conformation of the cytochrome c and extensive apoptosis of the cells present in the tissue of flight muscles in *Drosophila* (Wallace 2005; Cho et al. 2011).

Electron transport chain (ETC) occurring in mitochondria is one of the most vital processes which is essential for cellular homeostasis; primarily because this process is associated with energy production in the cell. A comprehensive study examining the ETC functioning with aging in *Drosophila* reported a decrease in several aspects of ETC such as electron transport and respiration with gradual increase in aging (Ferguson et al. 2005). Interestingly, compared to the other mitochondrial ETC enzymes which were examined, age-associated reduction was predominantly found in the activity of COX (Ferguson et al. 2005). Also, drug mediated inactivation of COX in mitochondria obtained from young flies result in enhanced ROS production. These observations suggest that ROS induced mitochondrial impairment results in further enhanced production of ROS which exaggerates the mitochondrial damages, forming a "vicious cycle" and thereby acting as driving force in aging and age associated impairments (McCarroll et al. 2004).

ROS Mediated Activation of Oxidative Stress Response Signaling Cascades

Oxidative stress triggers activation of several signaling cascades such as extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), p53 activation, nuclear factor (NF)-kB signaling cascade, c-Jun amino-terminal kinase (JNK), the phosphoinositide 3-kinase (PI(3)K)/Akt pathway etc. (Fig. 4) as a mechanism to combat stress (Finkel and Holbrook 2000). Amongst them, JNK pathway activated by ROS or other stimuli has been recognized as an evolutionarily conserved cascade which can potentially increase life span in flies by triggering protective gene expression program to alleviate toxic effects of oxidative stress (Wang et al. 2003; 2005).

In vertebrates, each components of JNK pathway is represented by huge gene families; however in *Drosophila,* JNK signaling is significantly less complicated thereby making its genetic analysis much simpler than other model organisms (Fig. 4) (Johnson and Nakamura 2007; Igaki 2009). JNK pathway is a part of MAPK signaling cascade which in *Drosophila* constitutes several JNK kinase kinase (JNKKK) [i.e. TGF-β Activated Kinase 1 (TAK1), Mixed Lineage Protein Kinase 2/Slipper (MLK), MEK Kinase1 (MEKK1) and Apoptotic signal-regulating Kinase 1 (ASK1)], two JNK kinase (JNKK) [i.e. Hemipterous (Hep) and dMKK4)] and one JNK [Basket(Bsk)] (Boutros et al. 2002; Chen et al. 2002; Geuking et al. 2009; Biteau et al. 2011). Activation of the pathway by stress stimuli including ROS results in activation of transcription factors AP-1 and dFoxo (*Drosophila* forkhead transcription factor) by Bsk phosphorylation which instigates changes in gene expression resulting in stress specific cellular response. JNK pathway is regulated by negative feedback loop where puckered (*puc*), one of the target gene of AP-1, dampens JNK signaling by its specific JNK phosphatase activity (Wang et al. 2005; Biteau et al. 2011). Studies in *Drosophila* revealed that either reduction in the dosage of *puc* gene product or overexpression of JNKK/Hep in neuronal tissues enhances basal JNK signaling levels resulting in heightened oxidative stress tolerance and increased life span (Zeitlinger and Bohmann 1999; Wang et al. 2003).

Along the same lines, mutant flies for JNKK/Hep gene displayed higher sensitivity towards oxidative stress and were observed incapable of eliciting JNK signaling dependent transcriptional factor induced stress response (Fig. 4) (Wang et al. 2003).

It has been demonstrated that availability of dFoxo transcription factor is essential to achieve JNK signaling mediated increased longevity in *Drosophila*. There is an antagonistic relationship between JNK and insulin/insulin-like growth factor (IGF)-like signaling (IIS) pathways (Wang et al. 2005). JNK inhibits IIS both cell autonomously and systemically (endocrine mechanism) to control life expectancy in *Drosophila*. Cell autonomously, JNK inhibits IIS by promoting nuclear localization of dFoxo, inducing transcription of genes involved in growth control, stress defense and damage repair (Wang et al. 2005; Hotamisligil 2006; Biteau et al. 2011). JNK inhibits IIS signaling systemically by repressing the expression of IIS ligand, *Drosophila* insulin-like peptide (dilp2) in insulin-producing neuroendocrine cells present in the fly brain (Karpac and Jasper 2009; Wang et al. 2005). Therefore, dFoxo and dilp2 dependent antagonistic relationship between JNK and IIS fairly explains the effect of oxidative stress on aging phenomenon.

5.2 Molecular Chaperones

As discussed earlier, aging is a complex process involving both genetic and non-genetic factors. In natural populations, several of the environmental factors including extreme temperatures, starvation, oxidative stress and other stresses etc. influence the survival capacity of organisms. Life span of an individual is the ability to withstand against these stresses which result in irreversible cellular damages; therefore, longevity of the organism is largely determined by the stress response of the individual. According to several proposed theories, aging is the result of an imbalance between damage and repair of cellular macromolecules (Vijg 2008), and moreover, with increasing age the response of the organism and its cells towards such damage tends to decline (Campisi and Vijg 2009). Generally, proteins are particularly more subjected to aging-related damages, including cleavage of the polypeptide chain, covalent modification of amino acid side chains, oxidative lesions, crosslinking, and denaturation (Stadtman 2006). Therefore, correct synthesis, proper folding of nascent and denatured protein, and turnover of proteins become one of the most crucial functions in cellular physiology, and failure of the stringent regulation of the cellular protein quality control system results in proteotoxicity, which is a key component of aging and aging related diseases (Morimoto and Cuervo 2009). Cellular protein quality control system is a combined network of molecular chaperones and their regulators, the ubiquitin-proteasome system (UPS) and autophagy system, allowing the proper folding, timely removal of the misfolded and aggregated proteins (Chen et al. 2011; Amm et al. 2014). Interestingly, despite of the unclear molecular mechanism(s) of aging process, increase in the damage of cellular macromolecules including proteins, nucleic acids, lipid etc. due to the upsurge of cellular oxidative stress stands one of the most

accepted theories of aging. Aging dependent progressive accumulation of abnormal mitochondria in several *Drosophila* tissues further supports the view of increased production of ROS with advancing age (Walker and Benzer 2004; Chistiakov et al. 2014). It appears that with increased oxidative stress and reduction in ATP synthesis, mitochondrial dysfunction stands as one of the core reasons behind aging related abnormal protein creation and accumulation.

Since the time heat shock response and genes encoding for molecular chaperones were discovered for the first time in *Drosophila*, the functions of these proteins, their regulation in heat shock response and their potential correlation with aging and longevity has been a topic of immense interest (Ritossa 1962, 1996). Despite their constitutive expression during normal homeostasis, molecular chaperones are also known as stress proteins or Heat shock proteins (Hsps) because of their induced expression during stress condition(s). Based on the sequence conservation and molecular weight, Hsps have been divided into several families. Conventionally, principal Hsps range in molecular mass from 15 to 110 kDa which are grouped into 5 major families, viz. Hsp100 (100–104 kDa), Hsp90 (82–90 kDa), Hsp70 (68–75 kDa), Hsp60 (58–65 kDa) and the small Hsp (15–30 kDa) (sHsps) families (Sarkar et al. 2011). Similar to other organisms, *Drosophila* also harbors homologs of the Hsp families which include Hsp83 (Hsp90), Hsp/Hsc70 complex (Hsp70 family), Hsp60, Hsp40 and sHsp (Hsp22, Hsp23, Hsp26 etc.) (Morrow and Tanguay 2003). In addition to its role in assisting denovo folding of the nascent polypeptide chains, Hsps are also defined by their ability to bind and refold the denatured proteins (Morrow et al. 2006). Therefore, induced expressions of Hsps are found in response to stresses that cause protein denaturation, such as heat and oxidative stresses (Morimoto 2008). Expression of the Hsps is mediated by binding of heat shock transcription factor (HSF) to heat shock response elements (HSEs) localizing at promoters of the heat shock genes, and activates their high-level transcription (Voellmy 2004). Interestingly, subsets of heat shock genes are also induced by oxidative stress through the JNK pathway and the transcription factor dFoxo (Wang et al. 2005).

During development and throughout the life span of *Drosophila*; Hsps, especially sHsps exhibit well-regulated, distinct and stage specific expression dynamics, though, upon exposure to environmental stresses like heat, Hsps shows upregulated expression (Morrow and Tanguay 2003). Interestingly, definite role of Hsps in aging and increased sensitivity of the aged flies to environmental stress have emerged from the comparative analysis of the heat shock response between young and old flies (Fleming et al. 1988). Comparative analysis of the expression profile of old and young flies revealed a greater abundance of damaged proteins in the old flies. Interestingly, detection of the same set of induced proteins in young flies fed with canavanine (an arginine analogue used to mimic accumulation of damaged proteins) as otherwise found only in old flies suggests increased sensitivity due to accumulation of aging mediated damaged proteins (Fleming et al. 1988; Niedzwiecki et al. 1991). Consistent to the above conclusion, even in unstressed flies, enhanced expression of Hsps has been found during normal fly aging in tissue-specific patterns (Morrow and Tanguay 2003). For instance, up-regulation of

hsp22 and *hsp70* at both, RNA and protein level while hsp23 at RNA level could be observed during normal *Drosophila* aging (Morrow and Tanguay 2003). With the aim of elucidating transcriptional changes accompanying the aging process, studies based on genome wide gene profiling in *Drosophila* have revealed aging associated upregulation of Hsps in aged flies (Curtis et al. 2007; Pletcher et al. 2002; Zou et al. 2000). Interestingly, dramatic upregulation of subset of Hsps including Hsp70 and sHsps and the genes for innate immune response was reported in old flies, in contrast, down-regulation of genes involved in energy synthesis and electron transport chain was found in same set of flies (Curtis et al. 2007; Pletcher et al. 2002; Zou et al. 2000). Moreover, extensive overlap between the gene expression profile of aged flies and young flies exposed to oxidative stress, further suggests the potential relationship between aging and oxidative stress (Zou et al. 2000).

The beneficial effect of Hsps on longevity is also evident from mild stress experiments known as "hormesis" in *Drosophila*, which activates the stress response without causing cellular damages (Minois 2000). Exposing the organisms to sub-lethal stress, induces hormetic effect through the modulation of heat shock response and helps the animals to live longer by counteracting negative effects of aging (Minois 2000). Besides, young adults of *Drosophila* strains with increased life span also exhibit intrinsic increased expression of sHsps, further suggesting that enhanced expression of Hsp might have a role in favoring longevity (Kurapati et al. 2000). Consistent to the above observation, mutation in *hsp70* or *hsp22* shows reduction in the adult fly survival, and these mutants with decreased lifespan also become more sensitive to stress. The role of Hsps in longevity was further confirmed by HDAC inhibitors mediated enhanced expression of hsp70 and sHsp, which in turn increase the life span of adult flies (Zhao et al. 2005). Remarkably, several of the independent studies have revealed decreased survival of the flies against heat and other stresses in the *Drosophila* mutants of *hsp22* (Morrow et al. 2004a) and all six copies of the *hsp70* (Gong and Golic 2006). In addition, *hsp83* mutant flies become more sensitive to the toxic effects of stresses like sleep deprivation (Shaw et al. 2002).

Interestingly, unlike the sHsps, major Hsps like Hsp70, Hsp60 etc. have failed to demonstrate any substantial effect on longevity, except reduced mortality rates upon mild stress, enhanced heat tolerance and a small increase in overall life span (Tatar et al. 1997; Minois et al. 2001). Among several of the sHsp in *Drosophila*, four of the sHsps i.e. Hsp27, Hsp26, Hsp23 and Hsp22 are well characterized and result in substantial life span extension upon tissue specific over-expression (Morrow and Tanguay 2003; Wang et al. 2003; Liao et al. 2008; Tower 2011). For instance, ubiquitous expression of Hsp22 in motor neuron increases the life span by 30 % and these flies exhibit increase resistance against stress and improved locomotor activity (Morrow et al. 2004b). Therefore, because of the ubiquitous nature of Hsps and its crucial role in variety of cellular processes by interacting with many different proteins, it can be concluded that the widespread outcome of aging is the

consequence of the aging associated chaperone failure, and therefore, molecular chaperones itself represent one of the vital intrinsic components to govern the aging process in the living system.

5.3 *Insulin/Insulin-like Growth Factor (IGF)-like Signaling (IIS)/TOR Pathway in Regulation of Longevity and Aging*

The Insulin/insulin-like growth factor (IGF)-like signaling (IIS) pathway has been long known to serve an established role of regulating somatic growth and development (Butler and Le Roith 2001), reproduction (Netchine et al. 2011), stress resistance (Holzenberger et al. 2003), metabolic homeostasis (Vowels and Thomas 1992; Saltiel and Kahn 2001) and even in aging and longevity (Partridge and Gems 2002; Tatar 2003; Kenyon 2005) in most organisms. There has been substantial evidences which suggest that compromised IIS signaling by introducing mutation (s) in the component(s) of the IIS pathway increase lifespan. On contrary, mutations that tend to shorten lifespan, have been proposed to do so by introducing pathological changes in the cell rather than by speeding up the process of normal aging (Giannakou and Partridge 2007).

The IIS pathway was first elucidated in *Drosophila* as one of the major pathways regulating growth and size of cells (Leevers et al. 1996). However, a plausible link between IIS pathway and longevity originated from studies on *C. elegans* when *daf-2* (a homolog of the insulin/IGF-1 receptor) mutants were found to extend lifespan (Kimura et al. 1997). Later, similar findings were reported in *Drosophila* when null mutants of insulin receptor substrate gene *chico* were found to be responsible for lifespan extension to as much as 48 % in homozygous female flies (Clancy et al. 2001). Interestingly, *chico* heterozygous female flies, though, exhibit an increase of 31 % in median lifespan, but their capacity to resist paraquat-induced acute oxidative stress was found more than their homozygous counterparts. As opposed to females, homozygous *chico* males are short-lived as compared to heterozygous males. Notably, long-lived homozygous mutants displayed higher levels of lipid and SOD activity (Böhni et al. 1999; Clancy et al. 2001; Kabil et al. 2007). Such contradictory observations, therefore, suggest that the trait of stress resistance may not contribute to the phenomenon of longevity via IIS signaling in flies. This can be justified by the fact that free radical generation and oxidative stress responses could be associated with a host of other reasons than just IIS signaling.

Another noteworthy IIS-linked mutation found to increase life span in *Drosophila* was that of the insulin like receptor (dInR). Adult flies with a mutated copy of the dInR gene tend to live longer than their wild type counterparts (Tatar et al. 2001). In this case as well, long-lived *inr* mutants exhibit higher triglyceride content and SOD activity. However, level of lipid content and SOD activity has also been found to be raised in some short lived mutants. In view of above, it may

be postulated that in addition to increase in SOD activity and lipid levels, other pleiotropic effects are necessarily involved in fly longevity. Furthermore, reduced expression of *Drosophila* insulin-like peptides (*dilps*), the ligands for dInR (Grönke et al. 2010) or increased expression of dPTEN (*Drosophila* phosphatase and tensin homolog), the negative regulator of insulin pathway (Hwangbo et al. 2004), also results in lifespan extension. dPTEN was shown to be doing so by antagonizing the action of the signal transducer PI3K (phosphatidylinositol-3-kinase) leading to nuclear localization of dFoxo, which in turn downregulates the expression of tissue specific chaperones and *dilps*, thereby completing the loop. Interestingly, increase in lifespan by activation of JNK signaling in response to various stresses as discussed above, also mediates its effect via dFoxo. The mechanism that dFoxo follows in this case comprises at-least in part of reduced IIS, since upregulation of JNK signaling in brain median neurosecretory cells (MNCs) has been shown to be linked with reduced transcript levels of *dilps* 2 and 5 (Wang et al. 2005). This is an interesting finding owing to the fact that MNCs are the site of *dilp* 2, 3 and 5 in the brain. Moreover, low levels of *dilp* 5 and subsequent lifespan extension had also been demonstrated in flies subjected to dietary restrictions (Min et al. 2008). Moreover, upregulation of dFoxo itself has been found to increase lifespan in *Drosophila* (Giannakou et al. 2004).

Furthermore, *dilp*-producing MNCs in adult *Drosophila* brain that integrate external signals to the IIS have also been implicated in influencing longevity. Flies carrying ablated MNCs exhibited up to 33.5 % increase in lifespan which was however accompanied by an age-related reduction in egg laying capacity (Broughton et al. 2005). These flies also demonstrated enhanced levels of circulating glucose along with stored carbohydrates and lipids. They could also resist paraquat- and starvation-induced stresses more efficiently as compared to wild type, though such flies are more sensitive to heat and cold stresses (Broughton et al. 2005). In view of above findings, it may be postulated that some compensatory alterations of related pathways which interact with IIS might be needed in order to balance out the undesirable effects of reduced IIS, so that longevity can be increased without any fitness cost.

One of the major pathways that interact with IIS to regulate growth and longevity in *Drosophila* is TOR pathway (Oldham and Hafen 2003). Two major complexes instigate the TOR pathway—TOR complex 1 (TORC1) and TORC2. TORC1 is sensitive to rapamycin and is implicated in controlling the temporal aspects of growth within a cell (Um et al. 2006) whereas TORC2 is insensitive to rapamycin and is involved in controlling the spatial facets of cellular growth (Jacinto et al. 2004). Reduction in TOR signaling by ubiquitously upregulating dTsc1 and dTsc2, or expression of a dominant negative variant of TOR or expression of a mutated dS6K, a major downstream kinase of the TOR pathway, led to substantial increases in *Drosophila* lifespan (Kapahi et al. 2004). Moreover, rapamycin-mediated inhibition of TOR signaling was also shown to prolong lifespan in flies by as much as 10 % (Bjedov et al. 2010). Rapamycin has been suggested to do so by inactivating TORC1, and by lowering the rate of protein translation in the cell and inducing autophagy (Bjedov et al. 2010). Notably, the

long lifespan of *dtsc2* mutants cannot be further extended by subjecting the flies to caloric restriction (Kapahi et al. 2004). Thus, the mechanisms of life extension by inhibited TOR signaling and dietary restriction could be overlapping in nature.

Some of the most apparent evidences of interaction between the IIS and TOR pathway have been elucidated in *Drosophila* models of neurodegenerative disorders. Reduced activity of the IIS/TOR pathway has been found to suppress mutant proteins mediated neurotoxicity in a variety of neurodegenerative disease models (Hirth 2010). Though, the precise modulations required for IIS/TOR signaling to bring about neuroprotection remain elusive. It also remains uncertain whether specific modulations protect against specific forms of neurotoxicity or there is a common link between neuroprotection and IIS/TOR pathways.

5.4 Dietary Restriction

As mentioned earlier, dietary restriction is a phenomenon linked to increase in life expectancy by limiting the nutrient intake. The process of dietary restriction controlling aging is conserved across the species (Piper and Partridge 2007). Influence of dietary restriction on aging has been center of curiosity among the researchers for deciphering the underlying genetic and molecular mechanism(s) involved. One of the hypotheses to explain the role of dietary restriction on aging states that it reduces the body metabolic rate thereby decreasing ROS generation which in turn slows down the aging process. Though this ideology is consistent with the existing relationship between oxidative damage and aging but experimental validation is still awaited. However, several nutrient sensing pathways such as Sirtuin (Sir2) and TOR signaling which operate under indirect control of IIS signaling have been identified to be crucial for dietary restriction mediated life span enhancement.

Sir2 are the members of highly conserved protein family which act as NAD-dependent deacetylases and target both, histone as well as non-histone proteins. They have been implicated as one of the key mediators in dietary restriction triggered increased life expectancy (Dali-Youcef et al. 2007). Subsequently several studies have demonstrated that flies overexpressing dSir2 proteins have higher life expectancy (Rogina and Helfand 2004; Bauer et al. 2009). The maximum increase in mean life span in flies was 57 %, which was achieved by ubiquitous overexpression of dSir2 under the influence of tubulin-Gal4 driver (Rogina and Helfand 2004). Above studies highlight the conserved role of dsir2 in facilitating the favorable effect of dietary restriction on fly life expectancy.

Similar to Sir2, IIS and TOR signaling pathway have been well characterized in demonstrating their noteworthy contribution in fly aging process by coupling growth to nutrition (Tatar et al. 2001; Kapahi et al. 2004; Broughton et al. 2005). It has been proposed that mutants of these signaling pathways extend the life span primarily by slowing down the growth and rate of metabolism (Tatar et al. 2001;

Broughton et al. 2005). Remarkably, it has been shown that reduction in life expectancy due to dFoxo mutation in flies can be compensated by dietary restriction, which further highlights the crosstalk between IIS and TOR pathway in regulating aging (Giannakou et al. 2008). However, despite availability of large information, the accurate role of TOR and dietary restriction in aging is still illusive and further investigations are expected to generate novel insights.

6 Aging and Neurodegeneration

Aging is one of the major risk factors for onset of brain related neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) etc. Neuronal loss, shrinkage of cell bodies and axons of neuronal cells and loss of synapse collectively leads to reduced brain volume and weight in aging individuals (Reiter et al. 2001). Subsequently, progressive deterioration of brain function leads to cognitive decline, memory loss, movement disorders and finally to functional decline and death. With a hastily increasing aging population and due to lack of effective treatment measures, these disorders have emerged as major economic and social burden. Therefore, in view of the fact that these disorders show substantial interference with aging; in-depth investigation on age-related molecular mechanisms or pathways may potentially help in developing novel therapeutic strategies.

Although, it appears quite rational to hypothesize that disease related proteins enhance disease toxicity by accelerating the aging process, however, it is still unclear whether aging related changes are responsible for driving neuronal pathology or both aging and disease associated proteins act synergistically to develop neuronal dysfunction. For instance, in *C. elegans*, mutation that extends longevity in poly(Q) disease reveals age dependent reduction in protein aggregate formation and toxicity, consequently testifying the effect of aging in poly(Q) mediated cellular dysfunction (Morley et al. 2002). Several reports including our own findings demonstrate progressive aggravation of poly(Q) mediated neurotoxicity in age dependent manner. Targeted expression of Htt-93(Q) in *Drosophila* eye exhibits cellular degeneration characterized by retinal depigmentation and cellular toxicity. Our studies on individual flies expressing Htt-93(Q) transgene during aging suggest that the magnitude of retinal depigmentation and cellular toxicity progressively increases with age (Fig. 5). Moreover, involvement of common signaling networks in longevity and mitigation of poly(Q) toxicity raises the prospect that slowing down aging may act as a neuroprotective measure. Therefore, in order to cultivate novel strategies to prevent onset and progression of such deadly disorders, it will be interesting to explore how aging dysfunction and poly (Q)mediated neuropathology are interlinked and how they interact during disease pathogenesis.

As stated earlier, all eukaryotic life forms have well evolved protein quality control machinery, which includes chaperone network, ubiquitin-proteosome and

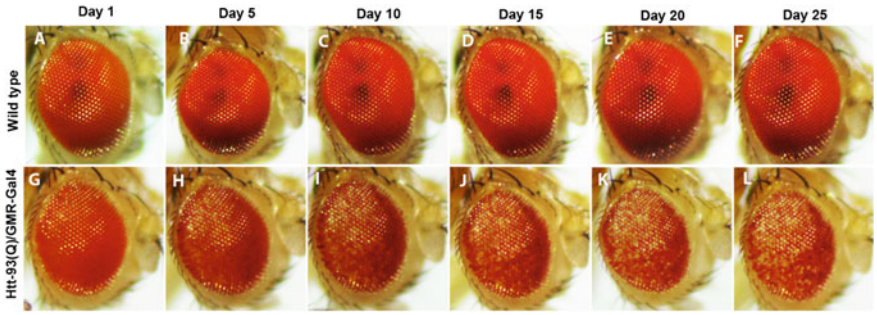

Fig. 5 Progressive increase in poly(Q) mediated neurodegeneration in *Drosophila* eye with advancing age. Adult wild type and poly(Q) diseased fly have been chased from day 1 to day 25. With aging, while wild type eye appears continuously normal (A-F), *GMR-Gal4* driven eye specific expression of Htt-93(Q) exhibits progressive increase in eye depigmentation and cellular degeneration (G-L)

lysosome-mediated autophagy system. Stringency of these systems is essential for post translational modifications, protein folding, stress response and clearance/translocation of damaged proteins (Soti and Csermely 2003; Arslan et al. 2006). Induction and functional capacity of chaperones and cellular proteasome system gets distorted during aging and disease stress condition; therefore, the post mitotic neurons become susceptible to toxic protein aggregates and ultimately, leads to neurodegeneration. Therefore, it is not surprising that overexpression of Hsps ameliorates the neurotoxicity and age related cellular impairments. Overexpression of Hsp70 in *Drosophila* poly(Q) disease models suppresses neurotoxicity by restoring axonal transport, cell death and ultimately extends the life span (Muchowski and Wacker 2005). In addition, role of Hsp70 and Hsp40 in regulating poly(Q) aggregation and toxicity has also been demonstrated in poly(Q) models of *S. cerevisiae*, *C. elegans* and mouse (Muchowski et al. 2000; Cummings et al. 2001). Several mechanisms have been proposed to explain progressive decline in the level of Hsps in neurodegenerative diseases, including transcriptional deficit of Hsps expression via the toxic misfolded protein and sequestration of cellular soluble Hsps along with the toxic aggregates to form IBs. Evidences like CBP mediated transcriptional impairment of Hsp70 in *Drosophila* via reduction of HSF-1 activity further support the transcriptional deficit hypothesis (Hands et al. 2008). Therefore, it appears that mis-regulation of molecular pathways and several factors which are responsible for cellular protein quality control might be the risk factor for disease occurrence, which could be considered while designing novel therapeutic strategies.

In addition to molecular chaperones, potential involvement of insulin/IGF-1 signaling in protein aggregation and toxicity has also been reported. Studies on *C. elegans* suggested a direct link of insulin/IGF-1 signaling in protein aggregation for the first time, when it was demonstrated that insulin/IGF-1 also protects the worms from motility impairment by neutralizing the poly(Q) aggregation and toxicity in

HSF-1 and DAF-16 mediated manner (Teixeira-Castro et al. 2011). Subsequently, downregulation of insulin/IGF-1 signaling pathway was demonstrated to reduce the level of toxic aggregates in poly(Q) mediated Machado-Joseph disease (MJD) (Cohen 2012). Several studies performed on mouse HD and AD models also suggest that insulin/IGF-1 signaling has remarkable neuroprotective capacity. Mouse knockout models for IGF-1 receptor and Insulin Receptor Substrate (IRS) have shown rescue the animals from poly(Q) induced behavioral impairments along with learning and memory deficit (Raj et al. 2012). Collectively, it is increasingly clear now that insulin/IGF-1 signaling plays an essential role in neuroprotective function via modulation of aging processes and could be exploited as a novel pathway to develop new therapeutic strategies.

7 Concluding Remarks

Though it is increasingly clear now that aging is regulated by explicit signaling pathways, however, whether the influence of these signals is applicable to an organism "as whole" or operating at tissue specific manner, which then affects aging systemically remains to be determined. In this context it is also interesting to note that a number of genetic manipulations which extend life span in *Drosophila* and other species have sex-specific preferences. Also, dietary restriction results in a greater extension of life span in female versus male flies. Therefore, exactly how these various pathways/factors control life span and influence the phenomenon of aging is still a "great scientific mystery". The dramatic progress made in recent years utilizing various model organisms has demonstrated the feasibility of decoding this mystery and further studies are expected to reveal the insights of the biological aging and longevity.

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