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Understanding the aging fly through physiological genetics

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1. Introduction

While multiple factors determine the course of an individual's longevity, increased quality of life, development of health services and absence of war have enabled human populations who profit from these social benefits to live longer. Nonetheless, late-onset deteriorating physical and physiologic changes eventually lead to pathology and death (Balcombe and Sinclair, 2001). To understand and intervene with the negative aspects of the aging process in the human population, biologists investigate the molecular pathways, physiologic changes, genetic regulation, environmental influence and evolutionary origins of aging in a variety of species ranging from unicellular yeast to rodents and mammals (Guarente and Kenyon, 2000).

Energy consumption and energy use is one of the hallmarks of all living organisms (Rolfe and Brown, 1997). Energy is required not only for development and

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maintenance of a living individual, but also for getting access to food, surviving through environmental stress and reproducing. Given the tight association between such energy exchanges and life, it is not surprising that most of the biology of aging uncovers links with the systems that control energy balance, stress resistance and reproduction (Guarente and Kenyon, 2000). The model organism *Drosophila melanogaster* has been instrumental in studies of aging, due to its genetic amenability, its relatively short life span and its wide spread use in the laboratory (Rose, 1999; Helfand and Rogina, 2003). Here, the author tries to integrate recent developments in the field of *Drosophila* aging research into a general conceptual framework for the aging process. The term aging is used to include the phenotypic changes both at early and at later stages of life. These encompass morphological changes in fine structure (Anton-Erxleben et al., 1983; Gartner, 1987) fertility and fecundity (Sgro and Partridge, 1999; Arking et al., 2002), climbing and flying activities (Minois et al., 2001; Marden et al., 2003), learning and memory (Fois et al., 1991; Guo et al., 1996; Savvateeva et al., 1999; Neckameyer et al., 2000). The author proposes that aging of individual multicellular organisms should not be viewed as a mere consequence of cellular aging, even though the latter may influence, necessitate and in some cases determine the aging process. Rather, the existence of systemic regulation, which arises through communication between different organs and is coupled to a genetically determined repertoire of responses, is paramount. In this perspective, physiologic control remains responsive to environmental cues and the extent of genetic determination is shaped during evolution.

2. *Drosophila melanogaster* as a model system to study aging: new technical advances

A large number of laboratories around the world are currently trying to understand the aging process in *Drosophila* (reviews in Rose, 1999; Partridge, 2001; Arking et al., 2002; Tatar et al., 2003) and some aim to extend fly life span, without a concomitant loss of fitness or reproductive potential (Parkes et al., 1998; Kang et al., 2002; Helfand and Inouye, 2002). Longevity is in part under genetic control, as demonstrated by selection experiments (Arking, 1987; Riha and Luckinbill, 1996; Sgro et al., 2000) and variations of longevity and other age-associated traits have been documented in natural populations (Draye et al., 1994; Draye and Lints, 1996). Analysis of the *Drosophila* genome sequence (Adams et al., 2000) is therefore invaluable in current investigations, especially as the existence of fly counterparts for most human disease genes reveal extensive molecular conservation between the two species (Reiter et al., 2001). The technical advancements of site-directed mutagenesis through homologous recombination (Rong and Golic, 2000; Rong et al., 2002) and double stranded RNA silencing (Piccin et al., 2001) enable direct genetic manipulations of candidate aging genes (Bernards and Hariharan, 2001) and are currently being implemented in aging research (Kirby et al., 2002; Egli et al., 2003). In addition, systematic P-element mutagenesis offers defined genetic material to search for candidate genes (Spradling et al., 1999; Peter et al., 2002). In fact two of

the *Drosophila* life-extending genes were isolated through such screens (Lin et al., 1998; Rogina et al., 2000). P-element transposition techniques can also be used to enhance transcription of neighboring genes (Tower, 2000) allowing genetic screening for activities that may extend life span (Seong et al., 2001; Landis et al., 2003) or the direct assessment of specific gene overexpression effects (Orr and Sohal, 1994; Tatar et al., 1997; Parkes et al., 1998; Sun and Tower, 1999; Mockett et al., 1999a,b; Minois et al., 2001; Ruan et al., 2002; Sun et al., 2002).

Another important advancement is the use of microarray chip technology (DeRisi et al., 1997) to monitor expression patterns of essentially all *Drosophila* mRNA transcripts during aging (Zou et al., 2000; Pletcher et al., 2002). This approach detected several classes of age-related expression profiles, supporting the earlier proposition that during aging gene expression remains controlled and regulated (Rogina et al., 1998). The observations argue against models of aging that solely implicate stochastic errors in metabolism accumulating with time, because such errors would increase the variability of gene expression at late age (not seen in Pletcher et al., 2002), in the absence of a possibly coupled, but regulated response. Development of a molecular signature for senescence will provide a general marker for the aging process, which can then be used to assess the impacts of treatments or genetic interventions in a definitive manner. Microarray studies also confirm that multiple molecular pathways are coordinately functioning in the aging process. Oxidative stress response, basal metabolism and reproduction-related genes alter their expression during aging in predictable ways. In addition, the immune and general detoxification responses are also regulated in an age-dependent manner, pointing to yet another energy requirement for organisms, namely defense against pathogens.

With whole genome analysis expanding in the near future, there is a need for software and databank development to analyze and better present the accumulating data (Hood, 2003). A second challenge is to employ appropriate statistical evaluation of these results (Rose and Long, 2002). As we enter into the genomics era, parallel technical advancements in 2D gel electrophoresis and mass spectrometry make it possible to monitor changes in expression of all proteins as well. However, pioneering use of proteomics in the field of human aging (Toda, 2001; Dierick et al., 2002) has not yet been matched by corresponding work in *Drosophila*.

Despite the astonishing evolutionary conservation of many molecular pathways, findings from *Drosophila* longevity research demand further qualifications before they can be transferred to the human case. *Drosophila* is a poikilotherm (i.e., changes its body temperature according to the environment), develops in discrete stages, each with different life strategies and metabolism, reproduces by large numbers of progeny, which do not require nurturing, and is post-mitotic in its adult stage. Human physiology is much more elaborate, with a bigger complex brain (Mattson et al., 2002), sophisticated systemic regulation (Schwartz et al., 2000; Baudry et al., 2002) and a significant part of energy use directed towards cultural evolution. However, lessons from *Drosophila* aging remain germain in both understanding the evolution of aging and the underlying pathways that control (or are associated) with it.

3. The basic metabolic pathways and their participation in energy allocations

Every cellular activity requires energy expenditure. Metabolism is the overall process through which living systems acquire free energy by nutrient oxidation, store it and utilize it. This process consists of interconnected pathways with many intermediate metabolites participating in multiple distinct reactions. Metabolic fates are not randomly determined, but rather depend on regulatory networks, which ensure the presence or absence of different enzymes, their activity or inhibition, their subcellular compartmentalization, as well as the availability of the intermediate moieties (Voet and Voet, 1990). Here, the author briefly discusses the major metabolic pathways, to facilitate examination of the changes that occur after genetic or environmental alterations in energy homeostasis. Such alterations significantly affect aging of the fly, as exemplified by the reverse correlation of external temperature and longevity (Miquel et al., 1976) or by life extension following caloric restriction (Pletcher et al., 2002; Rogina et al., 2002).

The digestive process is not well described in *Drosophila*. Large molecules in food are broken down into simpler units, such as amino acids, sugars, fatty acids, and glycerol. Midgut epithelium absorbs these nutrients and initiates catabolic reactions, in which citric acid cycle metabolites are produced and transported to fat bodies and oenocytes, the principal sites of intermediary metabolism (Rogina et al., 2000; Zinke et al., 2002). The transporter for citric acid cycle intermediates, *Indy* (Rogina et al., 2000; Inoue et al., 2002; Knauf et al., 2002), is expressed in the plasma membrane of these tissues. Intermediate metabolites enter into the citric acid cycle (Fig. 1), the primary pathway of carbohydrate and protein decarboxylation, yielding NADH and FADH2, which are then used in the mitochondrial electron transport chain to generate ATP (oxidative phosphorylation) (Voet and Voet, 1990). Therefore, continuous flux of the citric acid cycle coupled with oxidative phosphorylation contributes energy in the form of ATP during aerobic metabolism. In between meals, continuous flux of the cycle is ensured by anaplerotic functions (Owen et al., 2002). Glucose provides citric acid cycle intermediates, as it is broken down to pyruvate with concomitant energy release (glycolysis). Amino acids can also be used in the absence of glucose (e.g., during starvation).

In addition to providing reducing power for the generation of ATP, the principal immediate donor of free energy, metabolites of the citric acid cycle are also the biosynthetic precursors of molecules for long-term storage of cellular energy, such as glycogen and fat. Gluconeogenesis is the process by which glucose is formed in a reverse sequence of reactions used in glycolysis. Tissues that are "energy suppliers" (Fig. 1) synthesize and secrete glucose, which is used as an energy source in brain and muscle. Glucose is also used in the pentose phosphate pathway or can be stored as glycogen. The pentose phosphate pathway (pentose shunt) generates NADPH, which is essential for numerous biosynthetic reactions, including synthesis of fatty acids, amino acids and nucleotides. The pentose shunt also generates ribulose-5-phosphate, an essential precursor for DNA and RNA. Finally, citrate (another intermediate of the citric acid cycle) can be converted into fatty acids and stored in the form of lipid triglycerides. Systemic regulation of these reactions is

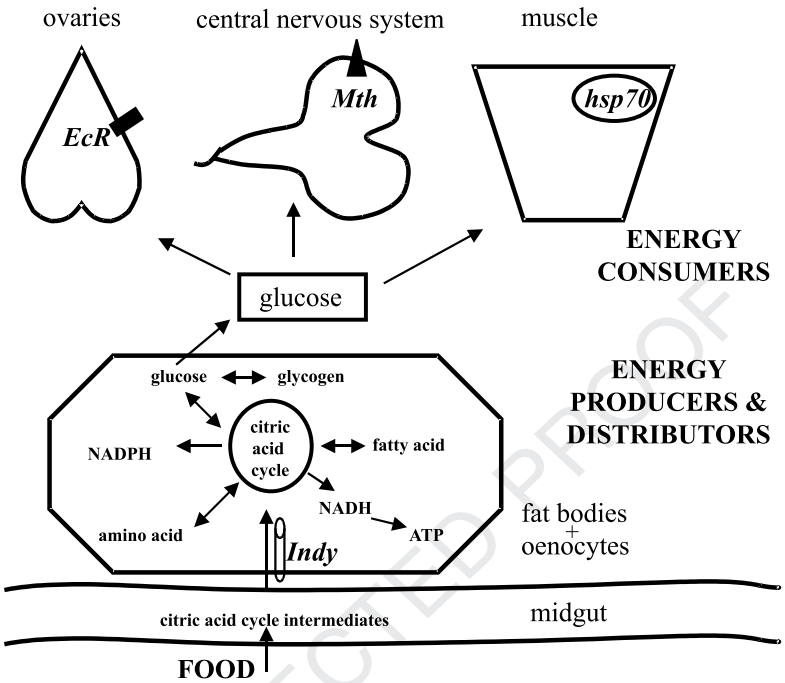

Fig. 1. Midgut, fat bodies and oenocytes are the principal sites of intermediate metabolism. The citric acid cycle allocates energy derived from nutrient oxidation to ATP (for direct use), glucose (to be used in muscle, brain and ovaries), glycogen and fat (storage molecules) and NADPH (reducing power), while generating biosynthetic precursors of the major biomolecules. Regulation of intermediary metabolism is tightly associated with the aging process, as exemplified by extension of life through caloric restriction or genetic interference with the insulin and ecdysone pathways (see text). Candidate "aging" genes are expressed in different tissues. Systemic signaling pathways, in addition to cellular aging, may control the aging process of the adult organism.

controlled by insulin (Baudry et al., 2002; Garofalo, 2002; Rulifson et al., 2002). Thus, the citric acid cycle is at the center of metabolic regulation because of its important roles in generating energy and allocating metabolites towards the different cellular needs.

This constant production of energy involves costs and toxic by-products generated in the electron transport chain of mitochondria (Mandavilli et al., 2002). The superoxide radical is such an example. Superoxide attacks iron sulfur clusters of the very enzymes that perform the citric acid intermediate reactions (Kirby et al., 2002). Effective detoxification rests on the first line antioxidant enzymes superoxide dismutase (Sod) (Phillips et al., 1989), which converts superoxide into hydrogen peroxide, and catalase (Cat) (Griswold et al., 1993), which breaks down hydrogen peroxide into oxygen and water. Hydrogen peroxide is readily diffusible and highly reactive in the presence of iron or copper. Cells employ thiol-dependent antioxidant systems, resting on glutathione and thioredoxins, as a second line of defense against hydrogen peroxide and consequent

metal-catalyzed lipid peroxidation (Girotti, 1998; Missirlis et al., 2001). Interestingly, unlike other taxa, insects do not directly depend on glutathione, but rather employ thioredoxin reductase (TrxR) and thioredoxin peroxidases (TPx, Radyuk et al., 2001 and GTPx, Missirlis et al., 2003) as their second line of antioxidant defenses (Kanzok et al., 2001). The thioredoxin system requires NADPH to function (Carmel-Harel and Storz, 2000). Thus, part of the energy captured from the pentose shunt is used for self-defense.

Oxidative phosphorylation occurs in the highly specialized inner membrane of mitochondria. Citric acid cycle reactions are also predominantly mitochondrial. In contrast, gluconeogenesis and glycolysis, fatty and amino acid metabolism occur in the cytoplasm. Compartmentalization of the different metabolic pathways necessitates the presence of transport systems for the common intermediates in the mitochondrial membrane (Kaplan et al., 1995; Kakhniashvili et al., 1997). Interestingly, many of the citric acid cycle enzymatic activities are present in both the cytosolic and mitochondrial compartments. This raises the intriguing possibility that the enzymes functioning in the mitochondria are geared towards ATP production (NADH and FADH2), while their cytosolic counterparts may be primarily involved in cataplerotic and anaplerotic functions (Owen et al., 2002). In view of the unanswered questions regarding the involvement of mitochondria in aging (see below), intracellular sites of metabolic regulation will require further clarification.

Antioxidant defense enzymes also reside both in the mitochondria and in the cytoplasm of cells. *Drosophila* possesses two *Sod* genes, a cytosolic copper–zinc *Sod1* (Phillips et al., 1989) and a mitochondrial manganese *Sod2* (Duttaroy et al., 1997). *Drosophila* also possess a single *Trxr-1* gene, which encodes two alternative transcripts, one giving rise to a mitochondrial isoform and the other encoding a cytosolic enzyme with identical biochemical properties (Missirlis et al., 2002). Each of the two enzymes provides an essential function; overexpression of TrxR-1 in cytosol cannot compensate for lack of TrxR-1 in mitochondria and vice versa (Missirlis et al., 2002). Thus, in addition to citric acid metabolites, the redox state in cytosol or mitochondria of cells is independently regulated.

4. Oxidative stress and aging

Superoxide and other reactive oxygen species (ROS) lead to DNA damage, lipid peroxidation and protein oxidation (Halliwell and Gutteridge, 1999). Due to these straightforward deleterious effects, ROS are implicated as a causal factor of aging (Harman, 1956; Beckman and Ames, 1998). *Drosophila* has been extensively used to test this theory. Despite recent criticisms (Le Bourg, 2001; Sohal et al., 2002), there is strong supportive evidence for at least some of the theory's proposals. First, mutations in several antioxidant genes greatly impact longevity. This has been demonstrated for cytosolic *Sod1* (Phillips et al., 1989) and mitochondrial *Sod2* (Kirby et al., 2002), cytosolic (Missirlis et al., 2001) and mitochondrial (Missirlis et al., 2002) *TrxR-1*, *catalase* (Griswold et al., 1993) and *glutathione-S-transferase* (Toba and Aigaki, 2000). Furthermore, studies of three marker genes, namely *heat*

shock protein 70, *cytochrome oxidase c* and *wingless*, that generally follow an age-specific expression pattern suggest that the actual rate of aging is increased in *Sod1* and *Cat* mutants (Wheeler et al., 1995; Schwarze et al., 1998a; Rogina and Helfand, 2000). A decrease in life span, accompanied by age-related changes in fine structure is also observed if flies are exposed to 50% oxygen levels (Miquel et al., 1975). In summary, ROS overproduction leads to a dramatic acceleration of the aging process.

Mitochondria are key sites of ROS generation and energy production (see above) and show distinct age-related morphological changes in flies (Anton-Erxleben et al., 1983). Aged flies show a marked decrease in their ability to produce ATP (Vann and Webster, 1977), but increase hydrogen peroxide production (Ross, 2000) which leads to lipid peroxidation of the membranes (Schwarze et al., 1998b). An interesting linear correlation was noted between mean life span and mitochondrial ROS production between five insect species, including *Drosophila* (Sohal et al., 1995). Mitochondrial DNA remains intact in older flies, but, curiously, mitochondrial transcripts decline sharply with age (Calleja et al., 1993; Schwarze et al., 1998b). What causes this decline remains unknown, but they may involve signaling from the cytoplasm, because cytoplasm derived from old flies and incubated with mitochondria from young individuals has inhibitory effects on ATP production (Vann and Webster, 1977). Thus, disfunction of mitochondria contributes to cellular and animal aging (Miquel, 1998), though in the case of *Drosophila* not primarily due to DNA mutations.

The question that has proven more frustrating to answer conclusively is whether enhanced protection from ROS by overexpression of antioxidant enzymes decelerates aging or not. Some studies confirmed such predictions for both Sods (Parkes et al., 1998; Sun and Tower, 1999; Sun et al., 2002), but in other similar experimental setups life span extension was not observed (Seto et al., 1990; Orr and Sohal, 1993; Mockett et al., 1999a). In addition, overexpression of catalase in an otherwise wild type background did not show positive effects on life span (Orr and Sohal, 1992; Griswold et al., 1993; Sun and Tower 1999; Phillips et al., 2000). Overexpression of a mitochondrially targeted catalase (Mockett et al., 2003) and of thioredoxin reductase (Mockett et al., 1999b) also failed to show any positive effects. In contrast, life span extension was achieved by overexpression of protein repair enzymes, which reduce oxidized methionines (Ruan et al., 2002) and asparagines (Chavous et al., 2001). A correlation of increased antioxidant enzyme activities and longevity was observed in one study of populations that were selected for extended longevities (Arking et al., 2000), but adding to the confusion, inbred lines from the same founding populations exhibited no difference in antioxidant defenses, while retaining their increased longevity, when tested independently (Mockett et al., 2001). These, overall contradicting results may underscore the importance of genetic background differences, tissue specifities, levels of over-expression, subcellular compartmentalization and cofactor requirements of the antioxidant enzyme activities. In other words, negative results from overexpression experiments are not sufficient to discard the otherwise well-documented causal role of ROS in the aging process. Conversely, regarding ROS as the sole contributor to

the process is an oversimplification, as many apparently unrelated factors (see below) influence aging as well, and potent detoxification machineries can be employed for protection.

5. Intermediate metabolism and aging

Intermediate metabolism allocates energy towards biosynthesis, ATP production or maintenance of an intracellular reduced redox environment (i.e., protection from oxidative stress). Genetic evidence for a tight association between intermediate metabolism and the rate of aging emerges from the analysis of mutations in the *Indy*, *InR* and *chico* genes (Rogina et al., 2000; Clancy et al., 2001; Tatar et al., 2001). *Indy* encodes a membrane transporter for citrate and other dicarboxylic acid cycle intermediates (Inoue et al., 2002; Knauf et al., 2002). Reduced expression of *Indy*, caused by P-element insertions in this locus, results in *Drosophila* strains with extended longevity (Rogina et al., 2000). This observation persists in different genetic backgrounds. Basal metabolic rate as measured by CO_2 emission appears unaltered and no associated decrease in fecundity or locomotion is observed for adequately fed flies (Marden et al., 2003). In contrast, nutritional restriction resulted in a significant downregulation of early fecundity in *Indy* mutants compared to respective wild type controls, demonstrating the importance of environmental variables when evaluating complex phenotypes (see below).

InR encodes an insulin receptor and *chico* is part of the *InR* signaling cascade (Garofalo, 2002). Reduced expression of either gene also results in extended life span phenotypes (Clancy et al., 2001; Tatar et al., 2001). Insulin signaling is best known for its role in glucose homeostasis (Baudry et al., 2002) and therefore *InR* and *chico* may be part of a control mechanism regulating nutrient availability, which also involves *Indy*. Indeed, caloric restriction extends longevity by an unknown mechanism (Pletcher et al., 2002; Rogina et al., 2002). Microarray analysis from caloric restricted flies suggests a slower progression of the rate of aging, as assessed by a delayed, yet characteristic genome-wide age-dependent expression pattern (Pletcher et al., 2002). Asking if slowing the rate of aging by caloric restriction and by the *chico* mutation occurs by overlapping mechanisms, Clancy et al. reported that *chico* mutants exhibit an optimum life span at a higher food concentration than that of wild type flies (Clancy et al., 2002). The same study concluded that *chico* mutants starve faster and their enhanced longevity respective to wild type strains depends on food availability, arguing for an overlapping mechanism of the genetic and environmental manipulations. Regulation of aging by insulin-like signals extends to other species as well (Tatar et al., 2003).

If modulating intermediate metabolism affects aging, the question arises as to whether aging in turn impacts metabolic efficiency. Mitochondrial aconitase, a key iron–sulfur cluster enzyme of the citric acid cycle, is the predominant protein that undergoes oxidative carbonylation with age (Das et al., 2001). This oxidation inactivated the enzyme, an effect also observed in flies with silenced *Sod2*, which have a dramatically reduced life span (Kirby et al., 2002). In addition to these observations, a key step in the electron transport chain mediated by cytochrome *c*

oxidase shows age- and oxidative stress-dependent loss in function (Schwarze et al., 1998a). In summary, both citric acid cycle and oxidative phosphorylation functionally decline in aging *Drosophila*.

Finally, classic selection experiments also asserted a tight association between metabolism and longevity (Riha and Luckinbill, 1996; Arking et al., 2002). Analysis of larval metabolism by tracing radioactive glucose incorporated into proteins and lipids shows a directly proportional change in the amount of metabolized food relative to mean life span (Riha and Luckinbill, 1996). In addition, a conspicuous decrease in life spans of previously selected long-lived strains when reared at low population densities can be attributed to greater nutrient intake by those animals. Furthermore, extensive analysis of different longevity phenotypes that have been obtained through various selection regimes has led to the formulation of an integrated interpretation of the changes that eventually lead to extension of life (Arking et al., 2002). The proposed steps include an initial upregulation of antioxidant defenses coupled to an increase in the use of the pentose shunt. This is later followed by alterations in mitochondrial fatty acid composition and other changes necessary to reduce the leakage of hydrogen peroxide from mitochondria into the cytosol. The recaptured energy can be diverted from somatic maintenance back into reproduction. This is an elegant proposal that is consistent with our current understanding of the process and corroborated by many experimental observations mentioned above and below; further investigations are expected to unravel orchestrated pathways that bring about these changes.

6. The interplay between different organs during aging

In multicellular organisms, different cell types are not equally responsive to environmental, genetic or hormonal alterations. Some cells differentiate to perform highly specialized tasks and cannot be replaced if lost or injured. Neurons, for instance, remain highly active, transfer material and electric signals over great distances and sometimes continuously respond to hazardous stimuli, such as UV light. Whether all tissues age at the same time and the extent to which they contribute to senescence and death is not known.

In *Drosophila*, genes that when mutated extend life span are preferentially expressed in a variety of different tissues (Fig. 1). Reduced expression levels of a novel G protein-coupled receptor *methuselah* (*mth*) significantly extend fly life span and resistance to stress (Lin et al., 1998). Intriguingly, this receptor is localized at the synapse of fly motorneurons (Song et al., 2002). Human Sod1 overexpression in fly motorneurons is also associated with life span extension, implicating these cells in adult life span control of *Drosophila* (Parkes et al., 1998). Mth functions as a positive regulator of pre-synaptic transmission (Song et al., 2002). This provides an elegant mechanism, whereby *mth* could act as a classic antagonistic pleiotropy gene (see next section). In young flies Mth upregulates neurotrasmitter release, presumably enhancing neuronal responsiveness, which may eventually lead to damage and premature degeneration. This hypothesis predicts that overexpression of *mth* should decrease life span. Song et al. performed an experiment using the *elav*

pan-neuronal driver line to induce *mth* expression from a *UAS*-transgene and did not observe an impact on fly life span. However, in view of the observation that when the *elav* driver line was used to overexpress human Sod1, a life span increase was not reproduced (Phillips et al., 2000) it seems important to test the flies which overexpress *mth* via the *D-42* driver line, which expresses specifically in motorneurons. Further investigations of possible genetic interactions between *mth* and *Sod1* will address if the two genes act in the same process within motorneurons (Parkes et al., 1999).

A second single-gene mutant with a dramatic effect on longevity is *Indy* (Rogina et al., 2000). As mentioned above, *Indy* is regulating transport of citric acid cycle intermediates in midgut, fat bodies and oenocytes, therefore acting in organs with very distinct physiological roles from motorneurons (Fig. 1). Another candidate "aging" gene, *heat shock protein 70*, is induced by oxidative stress or aging predominantly in thorax flight muscle (Wheeler et al., 1995), although the role of heat shock proteins in aging still remains controversial (Tatar et al., 1997; King and Tower, 1999; Kurapati et al., 2000; Minois et al., 2001).

Flies selected for reproduction at a late age give rise to long-lived populations (Arking, 1987; Sgro et al., 2000). Sterilizing the long-lived strain by X-rays or through a dominant female-sterile genetic mutation will abolish these effects (Sgro and Partridge, 1999). Oxidative stress susceptibility is also associated with increased egg production (Wang et al., 2001), suggesting a trade-off mechanism in energy use. Therefore, reproductive organs may send signals that systemically regulate the individual's fitness and/or energy use. Interference with systemic signaling pathways can influence longevity, as exemplified by the evolutionarily conserved insulin pathway regulating aging (Tatar et al., 2003). Interestingly, the life span extention of *InR* mutants was abrogated by supplementation of junevile hormone (Tatar et al., 2001), low levels of which direct the fly into a diapause state (Tatar and Yin, 2001). Moreover, flies with reduced ecdysone synthesis, or ecdysone receptor also live longer, without an apparent deficit in fertility or activity (Simon et al., 2003). Ecdysone is the main steroid hormone acting in flies and could serve as a signal, sent by the gonads, to sustain the organism in good health (Tatar et al., 2003). In support of this proposal, mutations in histone deacetylase *Rpd3*, a downstream target of ecdysone (Tsai et al., 1999), also extend life span (Rogina et al., 2002). In addition, administration of a drug which induces histone acetylation, 4-phenylbutyrate, also confers extended longevity (Kang et al., 2002). Microarray analysis of flies treated with 4-phenylbutyrate showed a conspicuous 50-fold increase in *Sod1* levels and moderate increase in other heat shock proteins and antioxidants (Kang et al., 2002). Furthermore, *Sod1* levels were elevated in *InR* and *chico* mutants (Clancy et al., 2001; Tatar et al., 2001). Therefore, we can suggest a model to explain trade-offs between fertility and oxidative stress susceptibility (Wang et al., 2001), in which sterile flies have lower ecdysone levels, reduced expression of *Rpd3* and consequently higher levels of *Sod1*.

This model adds another example to the emerging picture that systemic regulation of the aging organism works at a higher organizational level than cellular aging, through the networking of these cells. The fly genome may also contain a program

for coordinating energy metabolism, antioxidant defenses and reproduction to support perpetuation of life. As work in the field of developmental biology unraveled a highly complex process of development, similar complexity can be expected during the process of aging.

7. Evolutionary considerations

The data presented above suggest that coordination of metabolism is tightly associated with the aging process. This may imply that there are genes that will function to increase or decrease the rate of aging. However, current evolutionary theory refutes the existence of genes that evolve to regulate aging and considers aging as a side effect of vital functions (Kirkwood and Austad, 2000; Kirkwood, 2002; Partridge and Gems, 2002). The mutation-accumulation theory (Medawar, 1952) states that the force of counter-selection for deleterious alleles that manifest their phenotypes post-reproductively decreases at that very time and therefore such alleles accumulate in the genome. Thus, after reproductive stages a combination of deleterious genetic mutations contributes to death. The antagonistic pleiotropy theory (Williams, 1957) states that there is a number of genes (or genetic pathways) that can prove beneficial at early stages of life, but the same genes (or the causes of their early actions) may later negatively impact the organism. Such genes would be favored by selection because of the advantages they provide to young individuals seeking to reproduce outweighing the debilitating effects that follow.

Although the two theories lead to some opposing predictions (Shaw et al., 1999), they are not mutually exclusive, as presence of one type of alleles does not prohibit simultaneous actions of the second type of alleles and both explain aging as a consequence of declining selection pressures after successful reproduction. Evidence in support of both theories is reviewed by Rose (1999) and they are powerful in explaining why in a given species there are limitations to extreme fluctuations in maximum longevity. The two theories are based on the more general theory of natural selection (Darwin, 1859) in its modern synthesis form (Fisher, 1930; Haldane, 1941). Natural selection is undisputedly the strongest force contributing to the evolution of species and genomes within a species. Nonetheless, it requires a pre-existing variation of genetic populations on which it can act. How this variation is achieved in the first place is also an important fundamental process in evolution. Despite his denial of natural selection altogether, Kimura remains the main contributor to the generally accepted notion that radiation and other causes lead to random mutations, which is how variation arises (Kimura, 1968). However, presuming that biological systems are changing randomly neglects the role of environmental effects on evolving variability (besides those explained by natural selection) and the developmental and historical constraints on how genomes (to give one example) may or may not change. A second presumption made by the theories mentioned above, is that natural selection works only at the level of individual organisms, coupled to the premise that it is simultaneously functioning at the level of single genes. This is clearly not sufficient to describe evolution, as selection of species

or even ecosystems occurs in nature, for instance in the event of dinosaur extinction, during speciation or during expansion of a desert (Gould, 2002).

How are these criticisms of evolutionary theory related to the question *why* do *Drosophila* age? Imagine a scenario in which flies could not age. If they continue to multiply, they would soon consume the resources of their habitat (as happens with bacteria in a culture). For them to survive, they would have to reproduce less. This would result in diminished variability within the species (because reproduction allows recombination and genetic shuffling). It would increase the chances of being eaten by a bird before reproducing. It would also limit the energy resources of the species, as larvae feed within a fruit and have a metabolism geared towards growth in contrast to the adult imago which searches an appropriate partner and habitat to deposit its eggs. Aging may have evolved exactly to tune such problems of ecological balance and may in this respect be a programmed event, subject to selection, rather than solely a by-product of other functions. In such a context phenomena greatly influencing aging such as diapause (Tatar and Yin, 2001), can be viewed as switches of the metabolic program that will or will not lead to senescence (Kenyon, 2001).

The notion that there is a need to transcend (but not refute) current theory was recently proposed by scientists actively working on this field (Promislow and Pletcher, 2002). These authors present other evolutionary parameters that need to be incorporated in theoretical models, such as conflict of sexes or social behavior and discuss the corresponding progress in mathematical modeling. Evolutionary debates set aside, there is a general consensus that organisms have to deal with costs when using their energy sources for their metabolic maintenance and reproduction, and failure to do so is the universal trademark of senescence. Which genetic factors determine different life expectancies of the various species remains at this point obscure. As a more thorough investigation of the biology of aging proceeds and efforts to postpone its manifestations of senescence succeed, we will hopefully gain insight on their origin and purpose.

8. Conclusions

Aging experiments in *Drosophila* have shown:

1. ROS are a major causal factor of the aging process.
2. Decreased metabolic rate (as a result of caloric restriction, temperature drop or genetic mutation) extends life span.
3. Suppression of fertility extends life span.
4. Extension of life span is commonly accompanied with corresponding trade-offs in fecundity or metabolic activity.
5. Selection experiments can result in incremental physiologic changes (observed at different generations) which are heritable and extend life span without apparent trade-offs in fecundity or metabolic activity.
6. Genetic manipulations of single genes can result in extended life span without apparent trade-offs in fecundity or metabolic activity.

7. Aging is in part under neuroendocrine and hormonal control and gene expression remains regulated even in old animals.

These conclusions imply that the aging process is much more complex and interconnected with other physiological functions, than previously anticipated. The roles of different tissues and cellular pathways that are directly affecting the aging individual are just starting to be recognized.

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