Role of Oxidative Stress Biology in Evolution of Life History Traits in *Drosophila Melanogaster*

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1. INTRODUCTION

The crucial molecule sustaining aerobic life on planet earth is oxygen. Apart from having essential role in energy metabolism and respiration it plays a fundamental role both in survival and death of an organism. Its role is linked to survival on account of its high redox potential, which makes it an excellent oxidizing agent capable of accepting electron easily from reduced substrate. In contrast, its role in death stems from its electronic structure. The identical spin state of its two outer orbital renders oxygen kinetically stable, except in presence of appropriate catalysts (e.g. transition metals) that scramble electron spin states to produce partially reduced form of oxygen. Partially reduced form of reactive oxygen species include superoxide, hydrogen peroxide and hydroxyl radical [1] .The mitochondrial respiratory chain is responsible for most of the oxygen reduction and energy production, also it is known to produce most cellular ROS, which have been thought of as a toxic byproduct of respiratory chain [2]. Excess of ROS causes many harmful effects like oxidation and damage of proteins, nucleic acid, polysaccharides and lipids. Alongwith this, high intracellular ROS level affect the redox state level of glutathione and thioredoxin, cofactor for antioxidant enzymes. Interestingly, in addition to producing ROS, the mitochondrial respiratory chain is capable of producing nitric oxide [3]. This oxide can react with superoxide to form peroxynitrite, a strong oxidant. This and other oxidants derived from nitric oxide are collectively referred to as reactive nitrogen species (RNS). These can result in cumulative damage to proteins and nucleic acids [4]. Accumulation of ROS and RNS and their uncontrolled oxidation of cellular cellular components is referred to as oxidative stress. Oxidative stress accompanies aging and several diseases like hypertension, Alzheimers, dementias and atherosclerosis [5]. Despite of the deleterious effects of ROS and RNS, it is known that low to moderate level of each acts as a physiological signal for various cellular and developmental processes [6]. Major site of intracellular ROS production is mitochondria (Barja 2007), however other sites may include chloroplast, endoplasmic reticulum, microbodies and plasma membrane. In mitochondria most of the energy production is via oxidative phoshorylation (OXPHOS), which entails five enzyme complexes [7]. During respiration most of the oxygen consumed is reduced to water. However, an estimated 1-2% of oxygen consumed during respiration is not reduced completely but is instead

reduced to superoxide , which can be converted to hydrogen peroxide and highly reactive hydroxyl radical. Two major sites of oxygen production in mitochondrial respiratory chain are complexes I and III. It is clearly seen mitochondrial generated ROS not only affects mitochondria but also other cell compartments (Diagram Robert *etal* 2009). Because ROS can affect a large number of signalling pathways it has become of great interest to look for the footprints of oxygen reactions . Organsims are equipped with an array of enzymatic as well as non- enzymatic processes to effectively manage the level of ROS in order to maintain active growth through appropriate metabolic processes [8]. The primary enzymatic process is scavenging that involves dismutating superoxide to hydrogen superoxide via enzyme superoxide dismutase. This peroxide is subsequently dismutated into water and oxygen by enzyme catalyse. SOD was first isolated by Mann and Keilis (1938) whereas its catalytic function was discovered by McCord and Fridovtch (1969). On the basis of associated metal co-factor there are 3 distinct types of SOD classified as Cu/Zn- SOD (cytosolic), Mn- SOD (mitochondria) and Fe-SOD (chloroplast). The prokaryotic Mn-SOD and FeSOD, and eukaryotic Cu/Zn SOD enzymes are dimmers, whereas the Mn-SOD of mitochondria are tetramers [9]. Catalase is another scavenging enzymatic molecule containing heme group. It plays an important role in removal of hydrogen peroxide generated in peroxisomes by oxidases involved in β-oxidation of fatty acids. It is mainly of three types Cat-1 and Cat-2 (localised in peroxisomes) and Cat-3 (mitochondrial). All forms of the enzymes are tetramers in excess of 220 kd of molecular weight. There are four NADPH binding sites per catalase tetramer (Fita and Rossmann 1985). The other non enzymatic processes includes Ascorbate, glutathione, tocopherol, alkanoids, flavonoids and carotenoids [8].

2. REACTIVE OXYGEN SPECIES AND EVOLUTION OF AGING

The central idea on which life history theory is based is that evolution of fitness related traits will be constrained due to presence of tradeoffs between them [10]. Such tradeoffs are ubiquitous in nature. One of such an important tradeoff is ROS generation. It is produced as a consequence of performance of one activity generating negative consequences for other trait. All organisms are dependent on biological processes for their growth, repair and metabolism, for which they have evolved the capacity to utilize oxygen for the efficient release of energy. They also need to prevent the oxidation of body components due to reactive substances produced in process of oxidative stress mechanism. The need for energy efficiency therefore needs to be balanced against potential by product toxicity. This basis suggests that oxidative stress management mediates life history trade offs. During past decades there had been an explosive research in biology of ROS extending into evolutionary and ecological sciences [8,11]. As first proposed by The Free Radical Theory of Aging (Harman, 1956) there exists a conceptual link in between ROS production and process of aging. Aging can be defined as general deterioration of cells, tissues and organs of an organism,

ultimately reducing reproductive fitness, normal functioning and increasing probability of mortality [13]. It is multitude of both genetic and environmental factors that determines lifespan of an organism. Slight alterations in these factors might help us to achieve significantly increased lifespan.

ROS are being generated as an inevitable byproduct of normal aerobic metabolism in mitochondria and also at other cellular .These are majorily known to cause age related oxidative damage. Organisms have evolved protective mechanisms to combat the harmful effects of ROS. Homeostasis of ROS helps us to maintain static balance between its production and elimination.

Using mammals for oxidative stress related aging studies is not only expensive but also raises thical issues. *Drosophila* is widely used research model on aging [14]. because of its short generation time, high reproduction rate, ease of animal husbandry, the availability of sophisticated genetic tools and well measurable age related parameters viz- Longevity and fertility. Longevity extension by oxidative stress management relied on prevention of ROS generation or Reducing/Scavenging of free ROS level. The potential complication with above strategies is that the cause of death of an organism cannot be easily determined and the limiting factors for the survival under normal experimental conditions is not clear. Moreover it is not known if similar factors are known to affect both aging and lifespan in laboratory cultures as well as in wild.

3. REDUCING ROS LEVEL

The electrons that leaks out during electron chain transport leads to the formation of ROS. It must be prevented from causing cellular by action of scavengers/ detoxifying molecule.

By Antioxidants: The postulate associated with this type of prevention is that amount of ingested food amount is not known and at higher doses its lethality may reflect feeding rejection causing starvation [15]. Variable set of results are reported when the food is supplied with antioxidants, it might increase longevity in some cases whereas not in rest. Unfortunately deleterious affects associated with longevity increase are often not taken care of. There exists a dose dependant action of antioxidants, as vitamin E is known to extend or shorten the lifespan suggesting that a small optimal concentration range exists for lifespan extension [16]. This variability can be resultant of numerous redox dependent cellular signaling cascades. Also compounds other than antioxidants can also help in increasing lifespan like urea, a toxic metabolic waste of flies increased longevity of mated flies but not virgins. As urea is known to decrease fecundity, i.e. a tradeoff between reproduction and longevity is there. These tradeoffs reflect some beneficial effects of antioxidants. Before reaching a conclusion of increased longevity by antioxidants alone it should be discovered if that affects longevity alone or side effects are also there

By modifying activity of detoxifying/antioxidant enzymes: In accordance with free radical theory increasing or decreasing activities of enzymes should affect longevity. Long living organisms might posses greater oxidative stress resistance and displays lesser signs of oxidative damage. Moderate increase in activity of catalase or Cu/Zn SOD alone had no effect on lifespan of male *drosophila* [17]. However in combined insertion of SOD gene and CAT gene by P-element transformation extended mean lifespan by 15 % and maximum lifespan by 34% [18]. Molecular and physiological parameters based studies revealed that extended longevity is related with significant reduction of accumulated oxidative proteins and DNA damage, diminished age related H_2O_2 generation in mitochondria, an increase in later age oxygen consumption and higher physical activity [19].

By overexpression of detoxifying/antioxidant enzymes: Overexpression results of different lifespan trials involving transgenic flies suggested that its effect may depend greatly on experimental conditions which is negligible under set of more physiological conditions[20]. Overexpression of antioxidative enzymes demonstrated an inverse relationship between lifespan extended and control flies. Recent studies have shown that long lived genetic backgrounds failed to extend longevity. However there is a situation when overexpression successfully prolongs lifespan of even long lived genetic makeup. Under oxidative stress induced by 100% oxygen (hyperoxia), H_2O_2 (73.5 mM) or paraquat (20 mM).

The failure of overexpression to extended lifespan can be explained on the ground that their endogenous activities, atleast in long lived and non-stressed flies, are normally adequate to control normal ROS level . But during stressful conditions these antioxidants can have beneficial effects. Another possible cause can be related to optimal concentration of ROS showing spatial variations, its excess or deficiency are equally important. ROS acts as a signaling molecule in various physiological phenomena . and if its normal level is perturbed by increasing massive supplies of scavengers, it may not be beneficial for cellular metabolism.

These considerations suggest that modulation of repair and degradation of ROS damaged protein could be an alternative to this.

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