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Review

Flies, worms and the Free Radical Theory of ageing

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ABSTRACT

Drosophila and *Caenorhabditis elegans* have provided the largest body of evidence addressing the Free Radical Theory of ageing, however the evidence has not been unequivocally supportive. Oxidative damage to DNA is probably not a major contributor, damage to lipids is assuming greater importance and damage to proteins probably the source of pathology. On balance the evidence does not support a primary role of oxidative damage in ageing in *C. elegans*, perhaps because of its particular energy metabolic and stress resistance profile. Evidence is more numerous, varied and consistent and hence more compelling for *Drosophila*, although not conclusive. However there is good evidence for a role of oxidative damage in later life pathology. Future work should: 1/ make more use of protein oxidative damage measurements; 2/ use inducible transgenic systems or pharmacotherapy to ensure genetic equivalence of controls and avoid confounding effects during development; 3/ to try to delay ageing, target interventions which reduce and/or repair protein oxidative damage.

1. Introduction

The ground has recently shifted under the Free Radical Theory of ageing. There have been tremors for the past two decades and warnings sounded, but the degree to which these were heard and then further investigated has only recently increased to the extent that it is beginning to be heard by the mainstream.

The report of the proceedings of the Dahlem Workshop on molecular aspects of ageing, held in Berlin 1994 (Esser and Martin, 1995), was remarkable for one thing in particular. Amongst several chapters documenting evidence supporting the Free Radical Theory of ageing, Swartz and Maeder (1995) concluded that "The free radical theory of ageing . . . is not well supported by existing data" and that ". . . the evidence for oxidative damage being the principal cause of ageing is not strong and unambiguous".

In the same report, Sohal and Orr (1995) stated that "The most direct and probably the strongest supportive evidence (for oxidative stress causative of ageing) is that overexpression of Cu–Zn superoxide dismutase (SOD) and catalase genes increases the average and maximum lifespans of *Drosophila melanogaster* by up to one third and delays the age-related loss of function". Orr and Sohal (2003) presented data refuting the conclusions of their earlier work (Orr and Sohal, 1994).

However the Free Radical Theory of ageing was and is still accepted unquestioningly by many, especially in biomedical research. Whether it turns out to be true or not, such uncritical attitudes can be, and probably have been, detrimental to research.

Here we review the vital role played in the development of this field by studies using invertebrate model organisms, including their advantages, limitations and potential for future work. As well as covering work which has been most influential, we have tried also to include work which we think should have been, and should be, more influential. As has become almost inevitable in biology, this work has uncovered a daunting complexity beneath what began as attractive simplicity.

2. Free Radical Theory – early history

Connecting observations from comparative physiology (metabolic 'rate of living' theory) with radiation biology (oxyradical generation), Denham Harman proposed that functional decline in cells and tissues was due to the cumulative effects of macromolecular damage caused by oxygen radicals produced by respiratory enzymes (Harman, 1956). This was the Free Radical Theory of ageing.

It is an extraordinarily attractive theory. The mechanism of reactive oxygen species (ROS) production is universal among animals, as is the typical Gompertzian trajectory of population mortality. But most of all, it is a mechanism that causes damage as a by-product of normal living, damage which accumulates over time (Stadtman and Levine, 2000).

The links between normal ROS production, damage and ultimate effects on the organism are not conclusive. The main reason why such a link may be obscure is represented by much of the work that has occurred in the years since Harman's paper; that the steps from

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ROS production to organism health are many, often interlinked and involved in feedback systems, and admit a huge degree of variation, both environmental and genetic. This variation reflects, perhaps causes, the variation we see in lifespan within and between taxa.

3. ROS production

One- and two electron reductions of oxygen produce superoxide and peroxide respectively, largely at complexes I and III of the electron transport chain in the mitochondria, and can be produced at a rate of 1–4% of each oxygen molecule used (Brand et al., 2004; Kell, 2009). Typically production is highest when oxygen, the terminal electron acceptor, is limiting (e.g. hypoxia, anoxia, ischaemia), or when substrates are in excess. There are many immediate mechanisms to cope with these potentially damaging byproducts, inherited in common from ancient bacterial ancestors (Imlay, 2008), and each has, to a greater or lesser extent, been the subject of manipulation in invertebrate model organisms with the aim of reducing oxidative damage and extending lifespan.

3.1. The role of iron

Biochemical work showed that doses of superoxide or peroxide alone needed to damage biomolecules in vitro were far too high to be physiologically relevant, implicating other ROS species (reviewed in Imlay, 2008). In an extensive review of the possible role of poorly-liganded iron in ROS production, disease and ageing, Kell (2009) notes that the most damaging ROS species, the hydroxyl radical, is produced largely by conversion of peroxide by exposure to free or incompletely liganded Fe ions. In normal metabolism, the Fenton reaction of peroxide with unincorporated ferrous ion bound to lipids and DNA and bound to or constituent of proteins (iron–sulfur clusters) is probably the main source of oxidative damage to these macromolecules.

Iron accumulates with age in *Drosophila* (Massie, 1984; Massie et al., 1985) and other species, including mammals (Massie et al., 1983). In fact, feeding flies with tea extracts reduced iron accumulation with age and was associated with lifespan extension of up to 21% (Massie et al., 1993). Investigating the effects on lifespan of more specific iron chelating agents, especially later in life, should shed light on this potentially important effect.

3.2. The relationship between ROS production and ageing

ROS production increases with age in mammals (e.g. Nabben et al., 2008) and in flies. Peroxide production doubled in mitochondria isolated from older houseflies, as did activities of a range of respiratory enzymes. Interestingly also, oxidative damage imposed in vitro to the isolated aged mitochondria led to an increased peroxide production (Sohal and Sohal, 1991). Similarly, peroxide production increases with age in *Drosophila*, but while both diet restriction (DR) and inactivity decrease oxidative damage (and increase lifespan), DR has no effect on, and inactivity increases, peroxide production (Cocheme et al., 2011) suggesting a significant potential disconnect between ROS production and oxidative damage.

Perhaps this is unsurprising. Fig. 1 shows a schema describing the process from ROS production to organism ageing, and lists, non-exhaustively, the large range of factors which can influence the steps of this process. Each one of these factors is subject to genetic and/or environmental influences.

The lifespan extension due to DR may be unrelated to ROS production in *Drosophila*. Although whole body mitochondrial extracts necessarily overrepresent flight muscle, lifespan increase with DR was not associated with reduced ROS (Partridge et al., 2005), and reducing ROS production by genetically lowering membrane potential failed to increase lifespan (Miwa et al., 2004). However in rats DR reduces membrane potential and does reduce peroxide production from isolated mitochondria (Ash and Merry, 2011), raising the issue of comparability of invertebrates to mammals in this area.

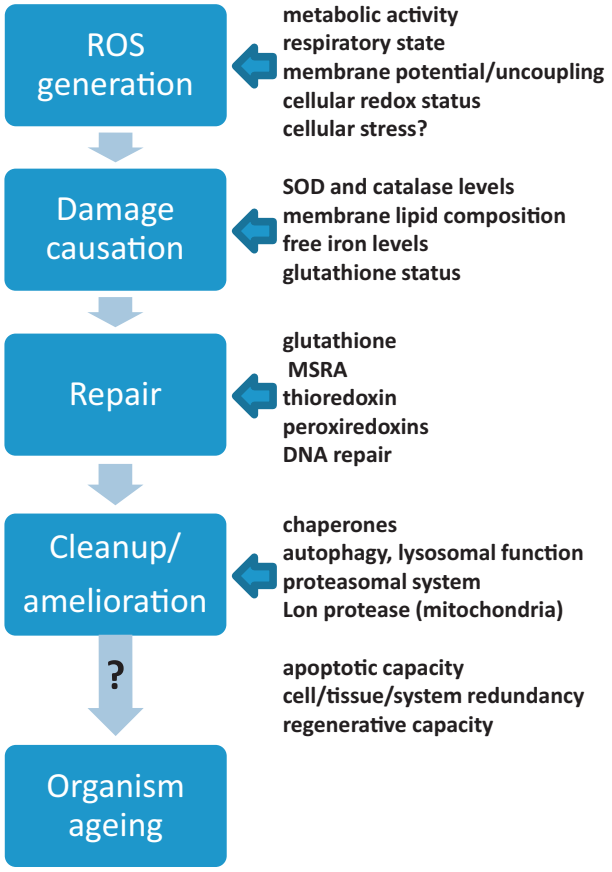

Fig. 1. Schema for stages of the process from ROS production to effects on organism health, with (non-exhaustive) lists of potential modulators at each stage.

Indeed, in the nematode worm *Caenorhabditis elegans*, metabolism is very different. They can tolerate very high oxygen tensions and are capable of significant anaerobic metabolism. Suppressing almost every respiratory chain gene during development (but not adulthood) significantly extends lifespan, possibly by upregulating non-aerobic metabolism and reducing superoxide production, although this is still a hypothesis (reviewed in Muller et al., 2007).

4. Oxidative damage: types, measurement, and ageing

The routes and reactions by which ROS damage lipids, proteins and DNA are well covered elsewhere (Halliwell and Gutteridge, 2007). We will discuss the common end-products, their measurement and use in ageing research.

Early work aimed to demonstrate that oxidative damage increases with age, by comparing groups with ostensibly different physiological ages. Physiological age refers to the amount of its ultimate lifespan an organism has lived. Typically this has been achieved by assaying young vs. older animals, or by using a measurable proxy of physiological age, or imposing dietary restriction or other methods to impose differential rates of mortality. Invertebrates have been especially useful for this.