Antioxidant defense and aging in *C. elegans*

Is the oxidative damage theory of aging wrong?

David Gems and Ryan Doonan

Institute of Healthy Ageing; and Research Department of Genetics, Evolution and Environment, University College London; London, UK

Abbreviations: Cu/ZnSOD, copper/zinc superoxide dismutase; DOG, 2-deoxy-D-glucose; DR, dietary restriction; GST, glutathione-S-transferase; IGF, insulin-like growth factor 1; IIS, insulin/IGF-1 signaling; MnSOD, manganese superoxide dismutase; O$_2^-$, superoxide radical; ROS, reactive oxygen species; SOD, superoxide dismutase

Key words: aging, antioxidant, *C. elegans*, catalase, free radical, nematode, oxidative stress, ROS, superoxide dismutase, theory

The oxidative damage theory of aging once seemed almost proven. Yet recently the buzzards have been assembling in the blue skies above it. New challenges to the theory from work using nematode worms seem set to bring them down to peck at its bones. But is the theory really dead, or does it just need to be modified?

What is the Oxidative Damage Theory of Aging?

The central idea of the oxidative damage (or oxidative stress) theory is that accumulation of molecular damage caused by reactive oxygen species (ROS) contributes significantly to aging. For example, aging has been viewed as the result of an increase with age of oxidative stress, resulting from increased ROS production and/or a decline in cellular antioxidant defenses. In particular, the superoxide radical, O$_2^-$, generated as a by-product of mitochondrial respiration, has been viewed as a potential major contributor to aging.

This influential theory has provided a useful starting point for investigations of aging, and has influenced many biogerontological studies. Some such studies have been performed using animal models, including the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus*. In some cases the results of such studies seem to support the oxidative damage theory, particularly those looking for correlations between aging and oxidative damage accumulation, and levels of antioxidant defense. For example, long-lived *C. elegans* mutants with reduced insulin/IGF-1 signaling (IIS) are resistant to oxidative stress, and selection for *C. elegans* mutants with increased resistance to a ROS-generating compound (juglone) lead to identification of long-lived mutants.

The most persuasive tests of the oxidative damage theory are those which aim to manipulate levels of ROS and observe the resulting effects on aging. Such intervention studies involve two approaches. In the first, the aim is to experimentally increase ROS levels to see whether this accelerates aging; in the second, to lower ROS levels to see whether this retards aging. A good way to achieve this is to manipulate genes encoding antioxidant defenses. Such studies were first performed using the fly, and then the mouse, and these results have been reviewed in detail by Muller et al.

Many studies using this approach have focused on superoxide dismutase (SOD) and, to a lesser extent, catalase. SOD converts O$_2^-$ into H$_2$O$_2$ (hydrogen peroxide), while catalase converts H$_2$O$_2$ into H$_2$O and O$_2$. Recently, a number of *C. elegans* studies have appeared describing the effects of manipulation of antioxidant genes. The frequent focus on SOD in these studies makes sense, given the traditional emphasis of the oxidative damage theory on O$_2^-$. A smaller number of *C. elegans* studies have begun to probe the effects on aging of manipulation of other elements of antioxidant defense, such as glutathione S-transferases, thioredoxin and peroxiredoxin.

In this review we will survey the results of these studies which, on the whole, fail to support the oxidative damage theory. For a comprehensive review of less recent *C. elegans* studies exploring oxidative stress and aging, see ref. 21. We will then ask where this leaves the oxidative damage theory, and briefly explore some possible alternative theories.

Interpreting Intervention Studies

One may imagine several alternative ways in which ROS might contribute to aging, depicted diagrammatically in Figure 1. ROS could be the primary cause of aging (Fig. 1A), or the primary...
cause of aging may be something else, with ROS playing a minor role (Fig. 1B). The process of aging involves progressive pathology and it is likely that this, like pathology generally,21 will result in increased levels of ROS. Such an increase in ROS may further exacerbate aging pathology, thereby hastening functional decline and death (Fig. 1B). A third possibility is that ROS plays little role in aging (Fig. 1C). Other possibilities may also be imagined, e.g., that ROS is a primary but minor determinant of aging, one among several such determinants.

Given these possibilities, how may the results of intervention studies be interpreted? First, let us consider the consequences of interventions that increase ROS. If these shorten lifespan, this supports scenarios A (ROS a primary cause of aging) and B (ROS a minor contributor), but not C (ROS not a cause). However, a flaw in such studies is that shortening of lifespan may result from pathology that is qualitatively different to that involved in normal aging. By contrast, if interventions that increase ROS do not accelerate aging, we may reliably deduce that scenario C is correct. Similarly, interventions that lower ROS levels may be more reliably interpreted if they fail to increase lifespan, and support scenario C. If increases in lifespan are seen, this could be because the oxidative damage theory is correct; alternatively, it may be the result of altered redox signaling. ROS such as O$_2^-$ and, particularly, H$_2$O$_2$ can act as second messengers in a variety of cellular signaling pathways.22 A final issue is establishing whether ROS is a primary determinant of aging, or a secondary determinant (distinguishing A and B). This is difficult but, arguably, if A is correct one would expect large effects on aging of manipulating ROS levels. By contrast, if B is correct, one would expect only small effects.

In studies of the fly and the mouse, a number of studies have shown that impairment of antioxidant defenses can shorten lifespan but, as we have seen, this is difficult to interpret. Of particular interest then, are antioxidant enzyme overexpression studies. In the fly, moderate increases in lifespan are produced by overexpression of either Cu/ZnSOD23,24 or MnSOD.25 By contrast, in the mouse, lifespan was unaffected by overexpression of Cu/ZnSOD, MnSOD, catalase or a combination of Cu/ZnSOD and MnSOD, or Cu/ZnSOD and catalase.26,27 However, one study did see a 21% increase in lifespan in mice expressing mitochondrially-targeted catalase.28 One interpretation of these findings is that ROS contributes to aging in the fly but not usually in the mouse. Supporting this, loss of one copy of the mouse Sod-2 MnSOD gene causes increased levels of DNA damage, but does not accelerate aging.29 Given the different findings from the fly and the mouse, it is of particular interest to know the outcome of ROS intervention studies in the worm.

Effects of Pro-Oxidants and Antioxidants on Aging in *C. elegans*

Prior to the recent series of antioxidant gene manipulation studies, several other approaches were used to manipulate ROS levels in *C. elegans*, including manipulation of ambient oxygen levels, and administration of free radical generators and antioxidants.

**Alterations in ambient oxygen.** *C. elegans* are relatively resistant to the effects of changes in ambient oxygen level. Aging was unaltered under 2%, 8% and 40% oxygen relative to normoxia.30 This could imply either that O$_2^-$ production is unaltered over this range of oxygen concentrations, or that O$_2^-$ is not a determinant of aging. More extreme changes in O$_2$ concentration did affect worm aging. Under 60% O$_2$, mean lifespan was reduced. Under 1% O$_2$, lifespan was increased, and demographic aging reduced.30,31 These effects may or may not be attributable to altered levels of ROS and molecular damage.

**Treatment with pro-oxidant chemicals.** *C. elegans* lifespan can be greatly shortened by high concentrations of pro-oxidants such as H$_2$O$_2$, tert-butylhydroperoxide32 and arsenite,33 and redox cycling agents such as paraquat and juglone.5,14 The level of O$_2^-$ production by redox cyclers is measurable as an increase in O$_2$ consumption in the presence of cyanide (to block respiratory oxygen consumption). 1 mM paraquat does not detectably increase this parameter in *C. elegans*,33 but 2 mM paraquat does, and the latter concentration is just sufficient to cause a moderate shortening of lifespan.34 Thus, the effects of paraquat on lifespan seem to be due to O$_2^-$. These results show that, ROS, probably including O$_2^-$, can shorten worm lifespan. This may or may not reflect an acceleration of the normal aging process.

**Antioxidant supplementation.** Effects on worm lifespan of various non-catalytic antioxidants have been tested, with mixed results. For example, vitamin E (α-tocopherol) increased lifespan in two studies,35,36 but not in a third.37 Likewise, trolox, an α-tocopherol derivative, increased lifespan in one study,38 but not in another.39 Lifespan was also slightly increased by a mixture of tococtrienols, antioxidants similar to α-tocopherol.37 α-lipoic acid increased lifespan (two studies),38,40 but N-acetylcysteine and vitamin C did not.35,39

The differences between reports might reflect differences in the way that compounds are administered, particularly dosage. Where

---

**Figure 1. Possible relationships between aging and accumulation of oxidative damage to molecular biological species.** (A) Oxidative damage as primary cause of aging, as might be the case in the filamentous fungus *Podospora anserina*.79 (B) Oxidative damage as a secondary effect of, and minor contributor to aging, as might be the case in *D. melanogaster*. (C) Oxidative damage as a mere correlate of aging, playing no significant role, as might be the case in *C. elegans* and *M. musculus*.
Is the oxidative damage theory of aging wrong?

Table 1  Influence of key antioxidant genes on C. elegans lifespan

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
<th>Effect on lifespan of gene knock out</th>
</tr>
</thead>
<tbody>
<tr>
<td>sod-2</td>
<td>Major MnSOD</td>
<td>None$^{10,12,13,15}$ or increased$^{14}$</td>
</tr>
<tr>
<td>sod-3</td>
<td>Minor MnSOD</td>
<td>None$^{11,15}$</td>
</tr>
<tr>
<td>sod-1</td>
<td>Major Cu/ZnSOD</td>
<td>Decreased$^{10,12,15,b}$ or none$^{14,15,b}$</td>
</tr>
<tr>
<td>sod-5</td>
<td>Minor Cu/ZnSOD</td>
<td>None$^{13,15}$</td>
</tr>
<tr>
<td>sod-4</td>
<td>Extracellular Cu/ZnSOD</td>
<td>None$^{13,15}$</td>
</tr>
<tr>
<td>ctl-1</td>
<td>Cytosolic catalase</td>
<td>None$^{9}$</td>
</tr>
<tr>
<td>ctl-2</td>
<td>Peroxisomal catalase</td>
<td>Decreased$^{9}$</td>
</tr>
<tr>
<td>prdx-2</td>
<td>Peroxiredoxin$^c$</td>
<td>Decreased$^{20}$</td>
</tr>
<tr>
<td>trx-1</td>
<td>Thioredoxin</td>
<td>Decreased$^{18,19}$</td>
</tr>
<tr>
<td>gst-10</td>
<td>Glutathione S-transferase</td>
<td>Decreased$^{17}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect on oxidative damage of gene knock out</th>
<th>Other effects, notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased$^{10,14}$</td>
<td>Clk/Mit phenotype$^{13,14}$</td>
</tr>
<tr>
<td>N.D.</td>
<td>Promotes immunity$^{11}$</td>
</tr>
<tr>
<td>Increased$^{10}$ or none$^{13}$</td>
<td>High expression in amphid neurons$^{13}$</td>
</tr>
<tr>
<td>N.D.</td>
<td>Two predicted isoforms</td>
</tr>
<tr>
<td>Decreased$^{9}$</td>
<td>Cytosolic catalase unusual in eukaryotes</td>
</tr>
<tr>
<td>Decreased$^{9}$</td>
<td></td>
</tr>
<tr>
<td>N.D.</td>
<td>Thioredoxin peroxidase, removes H$_2$O$_2$</td>
</tr>
<tr>
<td>Increased$^{17}$</td>
<td>Part of protein-disulphide reductase</td>
</tr>
</tbody>
</table>

$^a$Either by deletion or RNAi, in an otherwise wild-type background. $^b$Cold-sensitive effect of deletion of sod-1, with lifespan shortened at 15°C but not 25°C. $^c$Also a molecular chaperone, and regulator of expression of phase 2 detoxification genes.$^{20}$

Increases in lifespan are seen, the question remains: is the effect due to reduced oxidative damage? Interestingly, doses of trolox and α-lipoic acid that increase lifespan also increase resistance to heat shock, suggesting the possibility that these compounds might increase lifespan by inducing a stress response.$^{38}$

SOD mimetics. EUK-8 and EUK-134 are low molecular weight salen manganese compounds that possess SOD catalytic activity. Administration of these compounds to C. elegans increased in vivo SOD activity levels, up to five-fold in mitochondria,$^{34}$ and also increased paraquat resistance.$^{34,41-43}$ One group also reported that these compounds increase lifespan,$^{44,45}$ but four other groups did not see this effect.$^{34,42,43,46}$ Lifespan was not increased in Drosophila or houseflies either.$^{47,48}$ In fact, concentrations of EUK-8 that were optimal to protect against 2 mM paraquat had no effect on lifespan in the absence of paraquat.$^{34}$ Thus, if O$_2^-$ did limit worm lifespan under standard laboratory culture conditions, EUK-8 ought to be able to extend it, yet it does not. This implies that O$_2^-$ does not contribute to normal aging in C. elegans. However, it is worth noting here that a recent study saw increased lifespan in C. elegans treated with polymer-coated platinum nanoparticles (nano-pt), which have SOD, catalase and other catalytic activities.$^{43}$ Which of these activities causes the effect on lifespan remains to be determined.

Examples of antioxidants shortening lifespan. Several studies have also reported life-shortening effects of antioxidant supplementation in C. elegans. Higher doses of EUK-8 and EUK-134 shortened lifespan in a dose-dependent fashion.$^{34,42,43,46}$ A predicted effect of elevating SOD levels is that increased removal of O$_2^-$ due to conversion to H$_2$O$_2$ will cause a net increase in O$_2^-$ generation, potentially leading to high levels of H$_2$O$_2$ production.$^{49}$ Treatment of E. coli with EUK-8 did indeed have this effect.$^{50}$ Whether the deleterious effects of SOD mimetics on C. elegans are due to increased ROS production remains to be determined.

In another study, worms were subjected to dietary glucose restriction (a form of dietary restriction), imposed using an inhibitor of glycolysis, 2-deoxy-D-glucose (DOG). This treatment lead to increased production of ROS, increased oxidative stress resistance, and increased lifespan.$^{39}$ The authors surmised that DOG-induced ROS might trigger an adaptive stress response, or hormesis, thereby producing the protective effects against stress and aging. Consistent with this, treatment with the antioxidants N-acetylcysteine, vitamin E or vitamin C suppressed the beneficial effects of DOG. Thus, in this case at least, increased ROS appears to actually protect against aging, rather than contributing to it.

Manipulation of C. elegans Antioxidant Enzyme Genes

SOD and catalase. A number of recent reports describe in detail the effects on aging of manipulation of C. elegans genes encoding SOD$^{10-15}$ and catalase.$^{9,13}$ Despite its short lifespan, C. elegans has more than the usual complement of SOD and catalase isoforms (summarized in Table 1). Most eukaryotes possess three SOD isoforms: MnSOD within the mitochondrial matrix, an intracellular Cu/ZnSOD in the cytosol and mitochondrial intermembrane space, and a secreted, extracellular Cu/ZnSOD. In C. elegans there are two mitochondrial MnSODs, encoded by sod-2 and sod-3$^{51-53}$, two cytosolic Cu/ZnSODs, encoded by sod-1 and sod-5$^{54}$, and two predicted extracellular Cu/ZnSOD isoforms, both encoded by sod-4$^{55}$ sod-2 and sod-1 are highly expressed during normal development, while sod-3 and sod-5 are minor isoforms whose expression is increased in the diapausal dauer larva stage.$^{7,13,54,56}$ C. elegans also possesses three genes encoding catalases, ctl-3, ctl-1 and ctl-2, arranged in a tandem array.$^{9}$

Six recent reports from five independent research groups have tested the effects of manipulating sod gene expression, with largely consistent results. Three reports surveyed deletions of each of the five sod genes,$^{13,15}$ one report focused on deletion of the sod-2 and sod-3 mitochondrial isoforms,12 and another deletion of sod-3 alone.$^{11}$ In a sixth study, RNA-mediated interference (RNAi) was used to knock down expression of the two major isoforms, sod-1 and sod-2.$^{10}$

Loss of mitochondrial MnSOD does not accelerate aging. The occurrence in eukaryotes of multiple SOD isoforms reflects the relative inactivity of O$_2^-$ (unlike H$_2$O$_2$) to cross cell membranes. The existence of distinct O$_2^-$ pools adds another dimension of complexity to tests of the oxidative damage theory, as one has to
Is the oxidative damage theory of aging wrong?

ask: Does any particular \(O_2^-\) pool influence aging? The importance in aging of the mitochondrial matrix \(O_2^-\) pool can be probed by manipulating expression of sod-2 and sod-3. Of these, sod-2, the major isoform, is critical. Lifespan was not reduced by deletion of either gene.\(^{10,15}\) Strikingly, simultaneous deletion of sod-2 and sod-3 did not reduce lifespan either, despite a marked increase in sensitivity to oxidative stress.\(^{12,15}\) This confirms that antioxidant defense in the mitochondrial matrix is impaired in these mutants, and not rescued by compensatory activation of other antioxidant defenses.

Mutation of sod-2 also resulted in slow growth, reduced and delayed fecundity and defection rate.\(^{13,14}\) This set of traits is typical of long-lived strains with defects in mitochondrial function, called Clk (clock) or Mit mutants.\(^{57,58}\) Consistent with this, sod-2 RNAi enhanced the longevity of a clk-1 mutant,\(^{10}\) and in one study a marked increase in lifespan resulted from deletion of sod-2 alone.\(^{14}\) Although this increase was not seen in four other studies,\(^{10,12,13,15}\) Van Raamsdonk and Hekimi\(^{14}\) saw this effect using two different sod-2 alleles. Given that loss of sod-2 does not reduce lifespan, and can even increase it, it is notable that sod-2 mutants show elevated levels of oxidative damage.\(^{14}\)

These findings strongly imply that damage caused by \(O_2^-\) within the mitochondrial matrix does not contribute to organismal aging in \(C.\) elegans. Here the fact that sod-2; sod-3 double mutants do not show reduced lifespan is particularly persuasive. As discussed above, there is no ambiguity in the interpretation of results of this form (see Fig. 1C).

Weak effects of cytosolic Cu/ZnSOD on aging. Manipulation of expression of sod-1 and sod-5 give insight into the importance of \(O_2^-\) in the cytosol, and also the mitochondrial inter-membrane space (Table 1). Of these, SOD-1, the major isoform, is critical, and demonstrably present in mitochondria, presumably in the inter-membrane space.\(^{13}\) In two studies, loss of sod-1 slightly reduced lifespan,\(^{10,13}\) in a third it did not,\(^{14}\) while in a fourth loss of sod-1 had a cold-sensitive effect, shortening lifespan at 15°C, but not at 25°C.\(^{15}\) Overexpression of sod-1 caused a small increase in lifespan.\(^{13}\) This could imply that \(O_2^-\) in the cytosol and/or mitochondrial inter-membrane space contributes slightly to aging; however, there are reasons for doubting this (see below). Loss of sod-5, either alone or in combination with loss of sod-1, had no effect on lifespan;\(^{13,15}\) sod-5 is discussed further below.

Little effect of sod gene inactivation in long-lived \(C.\) elegans. Reduced insulin/IGF-1 signaling (IIS) can greatly increase \(C.\) elegans lifespan.\(^{59,60}\) It was initially suggested that this might be attributable to the elevated levels of SOD activity seen in IIS mutants.\(^{5,6,61}\) However, the effects of loss of sod genes on daf-2 insulin/IGF-1 receptor mutants provide little support for this view. Loss of sod-2 had no effect on daf-2 longevity (Age) in two studies,\(^{13,14}\) but slightly reduced it in a third.\(^{12}\) Deletion of sod-3 had no effect on daf-2 longevity in one study,\(^{13}\) but markedly increased it in another,\(^{12}\) perhaps reflecting differences in the daf-2 allele used in each study. sod-3 RNAi slightly reduced daf-2 mutant lifespan in a third study,\(^{62}\) and we saw this effect too (Weinkove D and Gems D, unpublished data). This is possibly because off-target effects reduce sod-2 mRNA levels as well. Loss of sod-1 slightly reduced daf-2 longevity,\(^{10,13}\) while sod-5 had little effect.\(^{13}\) Thus, removal of cytosolic \(O_2^-\) (but not mitochondrial matrix \(O_2^-\)) might contribute to daf-2 longevity a little, perhaps consistent with a secondary role of \(O_2^-\) in aging (see Fig. 1B).

Dietary restriction (DR) increases \(C.\) elegans lifespan and can increase SOD activity levels.\(^{63}\) However, deletion of each of the five sod genes did not suppress longevity induced by DR (axenic culture).\(^{15}\) This suggests that effects of DR on aging in \(C.\) elegans are not attributable to lower ROS levels.

Evidence that extracellular Cu/ZnSOD influences IIS. Loss of sod-4 alone has no effect on lifespan.\(^{12,15}\) This implies that damage caused by extracellular \(O_2^-\) does not contribute significantly to aging. However, in a long-lived daf-2 mutant background with reduced IIS, loss of sod-4 increased lifespan.\(^{13}\) It also enhanced the daf-2 constitutive dauer arrest (Daf-c) phenotype. This suggests that sod-4 influences IIS. In mammals, extracellular Cu/ZnSOD generates \(H_2O_2\) which crosses into the cytosol and promotes insulin signaling by inhibiting signal-quenching phosphatase enzymes.\(^{64}\) The effects of sod-4 on daf-2 mutant traits imply that the equivalent mechanism exists in \(C.\) elegans. Thus, rather than protecting against aging by reducing damaging ROS, extracellular Cu/ZnSOD appears to promote aging by activating IIS.

A proposed role for sod-5. Of the five \(C.\) elegans sod genes, the most mysterious is sod-5, encoding the minor isoform of cytosolic Cu/ZnSOD. Here we propose a hypothetical function for this gene based on our recent findings. Like sod-4, loss of sod-5 enhances the daf-2 dauer constitutive phenotype. Expression of sod-5 is strong and under dynamic IIS-regulation in the gustatory amphid neurons ASI, ASK and ASG, and this expression is dependent on the IIS-regulated FoxO transcription factor DAF-16.\(^{13}\) These neurons also influence dauer formation.\(^{65}\)

We suggest that SOD-5-generated \(H_2O_2\) promotes IIS by inhibiting signal-quenching phosphatases in the cytosol of amphid neurons. This could potentially explain the existence of sod-5. In dauer larvae in the absence of food, levels of IIS are likely to be low due to absence of insulin-like ligands for DAF-2. This will lead to increased DAF-16 activity, increasing SOD-5 and, consequently, local \(H_2O_2\) levels. This will inactivate IIS-antagonizing phosphatases (e.g., DAF-18/PTEN), priming the IIS pathway for a rapid response to increased food availability and increased insulin ligand levels. According to this view, the evolution of sod-5, presumably by duplication of sod-1, enhanced fitness by increasing efficiency of exit from dauer and resumption of reproductive growth. Winning the race to reach reproductive maturity is critical to fitness in \(C.\) elegans,\(^{66}\) and we suggest that sod-5 helps dayers to win this race.

Catalase and other \(H_2O_2\) sinks. The major sinks for \(H_2O_2\) in \(C.\) elegans appear to be catalase\(^9\) and the thioredoxin peroxiredoxin PRDX-2,\(^{20}\) The worm lacks \(H_2O_2\)-scavenging glutathione peroxidase activity,\(^5\) which might explain why \(C.\) elegans, unlike most eukaryotes, has a cytosolic catalase, ctl-1. The peroxisomal catalase is ctl-2, while ctl-3 remains uncharacterized. Deletion of ctl-2 or prdx-2, but not ctl-1, shortens lifespan.\(^{9,20}\) This might suggest that \(H_2O_2\) detoxification by ctl-2 and/or prdx-2 protect against aging. However, other observations argue against this. For example, protein oxidation levels increase more slowly with age in ctl-2 mutants than in wild type.\(^9\)

© 2009 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.
Figure 2. Beyond the oxidative damage theory. The failure of experimental tests require modification of the theory. Three possible modifications are shown here. (A) Assumes the theory is essentially correct, generating a neo-oxidative damage theory. (B) Views the theory as a half truth, generating the molecular damage theory of aging. (C) Views the theory as wholly false, requiring some form of supramolecular dysfunction theory.

Moreover, overexpression of catalase by increasing copy number of the cti gene cluster did not increase lifespan, either alone, or in the presence of increased levels of SOD-1. Also, increased intestinal expression of prdx-2 increased resistance to H$_2$O$_2$ but did not increase lifespan.

Summary. Overall, these studies do not support a major, primary role of either O$_2^\cdot$ or H$_2$O$_2$ in aging in C. elegans. However, the fact that lifespan is shortened by loss of sod-1, cti-2 and prdx-2 is consistent with the view that antioxidant defense might play a minor contributory role to longevity assurance. That enhancement of antioxidant defense against molecular damage is usually not sufficient to increase lifespan suggests that, to some degree, antioxidant activity may be necessary but not sufficient to protect against aging.

How Wrong is the Oxidative Damage Theory?

The results of C. elegans studies surveyed here join a growing body of findings that argue against the oxidative damage theory. The discovery that elevation of antioxidant activity can increase pro-oxidant levels, and that antioxidants can block beneficial hormetic effects of ROS turn topsy-turvy key tenets of the theory. This is consistent with recent meta-analyses showing that dietary supplements of various antioxidants not only fail to increase lifespan, either alone, or in the presence of increased levels of SOD-1.

An alternative view of the oxidative damage theory of aging in C. elegans, HNE is detoxified by several GSTs, including GST-10. Reduction or elevation of GST-10 levels slightly decreases and increases lifespan, respectively.

In mammals, expression of phase 2 enzymes is induced by the Nrf2 transcription factor, whose equivalent in C. elegans is SKN-1. Reduction or elevation of SKN-1 levels decreases and increases lifespan, respectively; however, whether the effect of SKN-1 on lifespan is mediated by SKN-1-induced phase 2 enzymes remains to be determined. GSTs also show increased expression in long-lived mutant worms, flies and mice with reduced IIS. Thus, GSTs might form part of a front line defense against diverse causes of oxidative damage, including electrophilic stress, that contribute to aging.

Another contributor to antioxidant defense that is not directed purely against O$_2^\cdot$ and its derivatives is the thioredoxin system, which is comprised of thioredoxin, thioredoxin reductase and NADPH. It acts as a general protein-disulphide reductase, contributing to maintenance of a reduced redox state in the cell. Deletion of the thioredoxin gene trx-1 reduces lifespan in C. elegans. In one study, increased expression of trx-1 caused a slight increase in mean but not maximum lifespan, suggesting a weak protective effect against aging.

These findings can be reconciled with those reviewed above by modifying the oxidative damage theory of aging (Fig. 2A). According to the proposed neo-oxidative damage theory, oxidative damage is a major contributor to aging, but O$_2^\cdot$ and H$_2$O$_2$ are not the main causes of this damage. This theory implies that one element of the older oxidative damage theory, that mitochondrial O$_2^\cdot$ and H$_2$O$_2$ cause aging, is incorrect.

The oxidative damage theory is a half truth. An alternative possibility is that the real truth contained in Harman’s original...
theory is that molecular damage. Oxidative damage is clearly not the only form of molecular damage to accumulate during aging. Advanced glycation end-products, for example, also accumulate, with detrimental effects. One possibility is that it is the informatic challenge of the extreme structural diversity of harmful reactive molecular species that is particularly difficult for the cell to cope with. This middle way implies that the narrow oxidative damage theory of aging should be succeeded with a broader molecular damage theory of aging (Fig. 2B). A challenge for the future is to establish the extent to which aging is really caused by molecular damage, rather than something else.

The oxidative damage theory is wholly wrong. A third possibility is that molecular damage is not the primary cause of aging. Not to consider this likelihood would be a failure of the imagination. The causal role of molecular damage in aging remains to be convincingly proven. An alternative possibility is that molecular damage is a correlate of aging, but that other mechanisms actually cause the gross manifestations of aging. This would imply that the malfunctions that drive the degenerative process of aging occur at a supramolecular level (Fig. 2C), i.e., at the cell, tissue or organ level. This is a possibility that some are beginning to consider, and might involve, for example, insulin resistance and cellular overgrowth resulting from Tor signaling. A plausible fourth scenario is that aging is caused by a combination of broad spectrum molecular damage and supra-molecular dysfunction. It also remains possible that there are some organisms for which the oxidative damage theory is true.

Conclusions

A growing number of reports have failed to validate key predictions of the oxidative damage theory of aging. Whether it will ever be possible to entirely refute the theory is debatable. Arguably, this is not a critical issue. Theories are sometimes fully refuted, but sometimes instead they are merely weakened, and then succeeded by new ones that are better able to explain what is observed. If there is one lesson from these studies, it is that the time is ripe to seek alternatives to the oxidative damage theory, at least in its standard form. We have suggested several possibilities here. As far as the worm, at least, is concerned, the oxidative damage theory has been not so much refuted as—like Napoleon on St. Helena—relegated. The decline of the oxidative damage theory represents an exciting departure, marking a new beginning for biogerontology. It is time to start thinking about aging in new ways.

Acknowledgements

We thank Filipe Cabreiro, Linda Partridge, Jennifer Tullet, Jeremy Van Raamsdonk and Elizabeth Veal for critical comments on the manuscript. This work was funded by the European Union and the Wellcome Trust.

References

Is the oxidative damage theory of aging wrong?