Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans

Ryan Doonan, Joshua J. McElwee, Filip Matthijssens, et al.

Genes Dev. 2008 22: 3236-3241
Access the most recent version at doi:10.1101/gad.504808
Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans

Ryan Doonan,1,3 Joshua J. McElwee,1,3 Filip Matthijssens,2,3 Glenda A. Walker,1 Koen Houthoofd,2 Patricia Back,2 Andrea Matscheski,1 Jacques R. Vanfleteren,2 and David Gems1,4

1Institute of Healthy Ageing and Research Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, United Kingdom; 2Department of Biology, Ghent University, B-9000 Ghent, Belgium

The superoxide radical (O2−) has long been considered a major cause of aging. O2− in cytosolic, extracellular, and mitochondrial pools is detoxified by dedicated superoxide dismutase [SOD] isoforms. We tested the impact of each SOD isoform in Caenorhabditis elegans by manipulating its five sod genes and saw no major effects on life span. sod genes are not required for daf-2 insulin/IGF-1 receptor mutant longevity. However, loss of the extracellular Cu/ZnSOD sod-4 enhances daf-2 longevity and constitutive diapause, suggesting a signaling role for sod-4. Overall, these findings imply that O2− is not a major determinant of aging in C. elegans.

Supplemental material is available at http://www.genesdev.org.

Received April 11, 2008; revised version accepted September 29, 2008.

Many forms of pathology lead to elevated levels of damage to biological macromolecules [Halliwell and Gutteridge 2007]. This is also true of aging, the poorly understood biological process that leads to progressive deterioration and death. One strategy to discover the underlying mechanisms of aging has been to seek the causes of its associated molecular damage. An important early theory, proposed by Harman [1956, postulates that the cause might be oxygen free radicals. Harman later developed the theory, proposing a central role for the superoxide [O2−] radical, issuing from the mitochondrial electron transport chain [Harman 1972]. During the last few decades, much effort has been invested in tests of this nexus of theories [for review, see Muller et al. 2007]. Despite this, the importance of O2− as a cause of aging remains uncertain.

In this study, we take a genetic approach to critically test the role of O2− in aging in a short-lived animal, the nematode Caenorhabditis elegans, by manipulating expression of genes encoding the antioxidant enzyme superoxide dismutase [SOD]. This enzyme catalyzes the dismutation reaction

\[ 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2. \]

H2O2 [hydrogen peroxide] may then be broken down into H2O and O2 by catalase or glutathione peroxidase. O2− does not readily cross cellular membranes and, consequently, there are distinct extracellular, cytosolic, and mitochondrial O2− pools [Missirlis et al. 2003; Muller et al. 2004]. In principle, one or more of these three O2− pools may play a role in aging.

Instrumental in lowering levels of O2− in each pool is a dedicated, compartment-specific SOD isoform. Cytosolic and extracellular O2− is consumed by distinct Cu/ZnSOD isoforms, while O2− in the mitochondrial matrix is consumed by MnSOD [Weisiger and Fridovich 1973]. Most eukaryotes have a single SOD isoform for each compartment, but C. elegans, unusually, possesses two isoforms for each compartment. The two cytosolic Cu/ZnSOD isoforms are encoded by sod-1 and sod-5 [Larsen 1993; Giglio et al. 1994; Jensen and Culotta 2005]. The sod-4 gene encodes two predicted extracellular Cu/ZnSOD isoforms, products of alternative splicing of mRNA [Fujii et al. 1998]. Two mitochondrial MnSOD isoforms are encoded by sod-2 and sod-3 [Giglio et al. 1994; Suzuki et al. 1996; Hunter et al. 1997].

This superabundance of SOD isoforms has been a technical hurdle to investigations of the role of SOD and O2− in aging in C. elegans, and some of the sod genes have been barely studied. In situ gel SOD activity assays of a sod-1 deletion mutant imply that this gene encodes the major cytosolic Cu/ZnSOD [Jensen and Culotta 2005], leaving the function of sod-5 unclear. sod-3 mRNA levels are elevated in the dauer larva [Honda and Honda 1999], suggesting that this gene may play a special role in antioxidant defense in this long-lived, stress-resistant diapausal stage, but the role of sod-2 has remained obscure. In this study, we describe in detail the function of each of the five sod genes, characterizing their expression, and the phenotypic effects of manipulating their expression. This has allowed us to assess the effect on life history, especially aging, of each of the three major O2− pools, thereby critically testing the role of SOD and, by inference, O2−, in longevity assurance and aging.

O2− can affect living organisms in a variety of ways. It can cause molecular damage that might contribute to aging, thus, one expectation of our study was that lowering SOD activity and increasing O2− levels might accelerate aging, and vice versa. H2O2 derived from O2− can also act as a secondary messenger—for example, in receptor tyrosine kinase signaling pathways [Finkel 1998]—and as an activator of heat-shock factor. O2− can also be deployed as a chemical weapon in immune defense against bacterial pathogens in higher animals, and probably in C.
**Results and Discussion**

To understand the respective roles of the five *sod* genes, we characterized their expression using several techniques, including RT-PCR, Western blot analysis, SOD activity assays, and analysis of expression of *sod::gfp* transgenes. Expression was studied mainly in wild-type third-stage (L3) larvae and dauer larvae, *daf-2(m577)* mutants at the L3 stage, and mutants with deletions in each of the five *sod* genes (Supplemental Fig. S1A; for a detailed account of *sod* gene expression, see the Supplemental Material).

We report that *sod-1* and *sod-2* encode the major cytosolic Cu/ZnSOD and MnSOD isoforms, respectively. *sod-1* contributes ~80% of total SOD activity and is ubiquitously expressed (Supplemental Fig. S4C), and SOD-1 protein is localized to the cytosol and mitochondrial intermembrane space (Fig. 3A, below). By contrast, *sod-5* and *sod-3* are, respectively, minor cytosolic Cu/ZnSOD and MnSOD isoforms whose expression is up-regulated in dauer larvae (Supplemental Figs. S1B–E, S4D,F). In wild-type L3s, *sod-5::gfp* expression is largely restricted to the ASI, ASK, and ASG amphid neurons (Supplemental Fig. S3B,C), which influence longevity and dauer larva formation (Bargmann and Horvitz 1991; Alcedo and Kenyon 2004).

It has been shown previously that in *daf-2* mutants, there is an elevation in levels of SOD activity (Vanfleteren 1993) and large fold increase in *sod-3* mRNA (Honda and Honda 1999; Yanase et al. 2002). We confirmed this but also saw increases in expression of *sod-1* and *sod-5* (Supplemental Figs. S1B, S4B–D; Supplemental Material). Although *sod-3* is the most highly up-regulated of the *sod* genes in terms of fold change in expression, its relative contribution to SOD levels remains very small, both in terms of overall SOD activity and MnSOD protein levels (Supplemental Fig. S1B–E).

We examined the organismal effects of deletion alleles of *sod-2* and *sod-3* MnSOD genes on *C. elegans*. We find that *sod-2(0)* but not *sod-3(0)* results in delayed development (data not shown), delayed and reduced reproduction, and a slowed defecation cycle (Supplemental Fig. S5). However, *sod-3(0)* further reduces fertility of *sod-2(0)* animals (Supplemental Fig. S5B), implying functional redundancy between *sod-2* and *sod-3*.

As expected, loss of MnSOD increases sensitivity to oxidative stress. *sod-2(0)* causes a moderate reduction in resistance to the O$_2^-$ generator paraquat, while *sod-3(0)* does not, either alone or when added to *sod-2(0)* (Fig. 1A). In combination, *sod-2(0)* and *sod-3(0)* cause severe hypersensitivity to hyperoxia (60% oxygen), while each mutation alone has no effect (Fig. 1B). Thus, *sod-2* and *sod-3* are functionally redundant when defending against mild but not severe oxidative stress.

Next, we tested the effect of *sod-2(0)* and *sod-3(0)* on life span. Mean life span is unaffected by either mutation alone (Fig. 1C; Supplemental Table S1) or both mutations combined (Fig. 1D; Supplemental Table S1). Thus, surprisingly, complete absence of MnSOD has no effect on life span. We also find that activity of cytosolic and mitochondrial intermembrane space (Fig. 3A, below). By contrast, *sod-5* and *sod-3* are, respectively, minor cytosolic Cu/ZnSOD and MnSOD isoforms whose expression is up-regulated in dauer larvae (Supplemental Figs. S1B–E, S4D,F). In wild-type L3s, *sod-5::gfp* expression is largely restricted to the ASI, ASK, and ASG amphid neurons (Bargmann and Horvitz 1991; Alcedo and Kenyon 2004).

It has been shown previously that in *daf-2* mutants, there is an elevation in levels of SOD activity (Vanfleteren 1993) and large fold increase in *sod-3* mRNA (Honda and Honda 1999; Yanase et al. 2002). We confirmed this but also saw increases in expression of *sod-1* and *sod-5* (Supplemental Figs. S1B, S4B–D; Supplemental Material). Although *sod-3* is the most highly up-regulated of the *sod* genes in terms of fold change in expression, its relative contribution to SOD levels remains very small, both in terms of overall SOD activity and MnSOD protein levels (Supplemental Fig. S1B–E).

We examined the organismal effects of deletion alleles of *sod-2* and *sod-3* MnSOD genes on *C. elegans*. We find that *sod-2(0)* but not *sod-3(0)* results in delayed development (data not shown), delayed and reduced reproduction, and a slowed defecation cycle (Supplemental Fig. S5). However, *sod-3(0)* further reduces fertility of *sod-2(0)* animals (Supplemental Fig. S5B), implying functional redundancy between *sod-2* and *sod-3*.

As expected, loss of MnSOD increases sensitivity to oxidative stress. *sod-2(0)* causes a moderate reduction in resistance to the O$_2^-$ generator paraquat, while *sod-3(0)* does not, either alone or when added to *sod-2(0)* (Fig. 1A). In combination, *sod-2(0)* and *sod-3(0)* cause severe hypersensitivity to hyperoxia (60% oxygen), while each mutation alone has no effect (Fig. 1B). Thus, *sod-2* and *sod-3* are functionally redundant when defending against mild but not severe oxidative stress.

Next, we tested the effect of *sod-2(0)* and *sod-3(0)* on life span. Mean life span is unaffected by either mutation alone (Fig. 1C; Supplemental Table S1) or both mutations combined (Fig. 1D; Supplemental Table S1). Thus, surprisingly, complete absence of MnSOD has no effect on life span. We also find that activity of cytosolic and mitochondrial intermembrane space (Fig. 3A, below). By contrast, *sod-5* and *sod-3* are, respectively, minor cytosolic Cu/ZnSOD and MnSOD isoforms whose expression is up-regulated in dauer larvae (Supplemental Figs. S1B–E, S4D,F). In wild-type L3s, *sod-5::gfp* expression is largely restricted to the ASI, ASK, and ASG amphid neurons (Bargmann and Horvitz 1991; Alcedo and Kenyon 2004).

It has been shown previously that in *daf-2* mutants, O$_2^-$ can increase life span, perhaps by activating stress defense processes (Cypser and Johnson 2002; Schulz et al. 2007).

One possibility is that increased SOD levels and reduced damage from O$_2^-$ contribute to the longevity of IIS mutants. *daf-2* mutants do show increased SOD and catalase activity levels, and resistance to oxidative stress (Vanfleteren 1993; Honda and Honda 1999). *sod-3* mRNA and protein levels are elevated in *daf-2* mutants (Honda and Honda 1999; Yanase et al. 2002, Dong et al. 2007), suggesting a possible role for mitochondrial MnSOD in longevity assurance. However, a more critical test is to examine the effects of alteration of SOD activity on life span.

In this study, we first examine the biology of the five *C. elegans sod* genes and show that *sod-1* and *sod-2* encode the major Cu/ZnSOD and MnSOD isoforms in reproductive development, while *sod-5* and *sod-3* encode minor, auxiliary isoforms mainly expressed in dauer larvae. We then critically test the importance of SOD and, by extension, O$_2^-$ in *C. elegans* aging and in the *daf-2* Age phenotype by means of *sod* gene deletion and over-expression.
mitochondrial aconitase (an oxidation-sensitive iron–sulfur protein) is not detectably reduced in sod-2, sod-3 animals [data not shown], suggesting no major increase in oxidative damage to protein. To test for a role in daf-2 mutant longevity, we examined the effect of accelerated aging, then overexpression of sod-1(0) on daf-2 longevity but there is none [Fig. 1E; Supplemental Table S2]. Altogether, these results strongly imply, against expectation, that mitochondrial matrix O$_2^-$ has no effect on aging and that MnSOD does not contribute to longevity assurance in C. elegans.

Next, we describe the effects of deletion of sod-1 and sod-5 cytosolic Cu/ZnSOD genes. sod-1(0) increases sensitivity to paraquat, but sod-5(0) does not, either alone or when added to sod-1(0) [Fig. 2A]. By contrast, neither sod-1(0) nor sod-5(0) has a marked effect on sensitivity to mild hyperoxia [Fig. 2B]. sod-1(0) also decreases mean life span, by 15%–31%, while sod-5(0) has no effect, either alone or when added to sod-1(0) [Fig. 2C,D, Supplemental Table S1]. Moreover, addition of sod-2(0) does not further reduce the life span of sod-1(0) mutants [data not shown], supporting the view that O$_2^-$ does not move between mitochondrial and cytosolic pools. Potentially, sod-1(0) shortens life span by accelerating the age increase in molecular damage. We therefore examined the effect of sod-1(0) on the age increase in damage to protein and lipid but could not detect any acceleration [Supplemental Material], perhaps because the impact of sod-1(0) is relatively subtle and difficult to detect.

If the shorter life span of sod-1 mutants is due to accelerated aging, then overexpression of sod-1 should increase life span. To test this, we first examined the effect of expression of the sod-1::gfp transgene on life span but saw no effect [data not shown]. However, we subsequently discovered that fusion of GFP to SOD-1 reduces Cu/ZnSOD-specific activity [Supplemental Fig. S7; Supplemental Material]. Next, we generated transgenic lines with multiple copies of the sod-1 gene, focusing initially on two lines bearing integrated transgenic arrays, wu152 and wu154. Overall SOD activity is increased approximately twofold by wu152 [Fig. 3B] and wu154 [data not shown], and both lines show increased Cu/ZnSOD immunoreactivity [Fig. 3A; data not shown].

Against expectation, sod-1 overexpression increases sensitivity to paraquat [Fig. 3E]. Potentially, this could result from elevated levels of H$_2$O$_2$ due to faster conversion of paraquat-generated O$_2^-$ into H$_2$O$_2$. To test this, we generated an integrated transgene array, wu151, with multiple copies of the entire ctl-1 ctl-2 ctl-3 gene cluster, which produces a 10-fold increase in catalase activity [Fig. 3C]. Catalase overexpression suppresses the increased paraquat sensitivity resulting from sod-1 overexpression [Fig. 3E], implying that this hypersensitivity is indeed due to elevated H$_2$O$_2$ levels.

wu152 caused a statistically significant ($P < 0.05$) increase in life span in five out of 11 trials, and in no instance did it decrease life span [Supplemental Table S3]. Combined data for these 11 trials defines a 21.5% increase in mean life span [Fig. 3F, $P < 0.0001$]. Although wu154 did not increase life span, increased life span was seen in three further lines with extrachromosomal arrays [wuEx125, wuEx122, and wuEx123] [Supplemental Table S3]. From this we conclude that elevated SOD-1 can slightly increase life span and that the absence of an effect in wu154 likely reflects a life-shortening mutation associated with chromosomal insertion of the transgene array.

Given that SOD converts O$_2^-$ into H$_2$O$_2$, it is possible that increased levels of SOD-1 lowers cytosolic O$_2^-$ but increases cytosolic H$_2$O$_2$, replacing damage from O$_2^-$ with damage from H$_2$O$_2$. To test this, we compared the effect on life span of elevated SOD-1, catalase, or both. Overexpression of catalase alone results in a high level of mortality due to internal hitching of larvae [bagging] [Fig. 3D], perhaps reflecting H$_2$O$_2$ deficiency. This bagging is suppressed by overexpression of sod-1 [Fig. 3D], perhaps due to restoration of H$_2$O$_2$. In the absence of bagging [prevented by the inhibitor of DNA replication fluorodeoxyuridine, FUdR] overexpression of catalase slightly shortens life span, either alone or in addition to overexpression of sod-1 [Fig. 3G; Supplemental Table S3]. Taken together, these results imply that cytosolic Cu/ ZnSOD and, by implication, cytosolic O$_2^-$, are weak determinants of longevity and aging, respectively. By contrast, H$_2$O$_2$ does not seem to contribute to aging.

We next investigated whether sod-1 and sod-5 might contribute to the daf-2(m577) longevity [Age] phenotype. daf-2(m577) is a weak, temperature-sensitive [ts] allele resulting in a moderate increase in life span at 20°C and a large increase at 25°C [Gems et al. 1998; Patel et al. 2008]. m577 was selected because it is a class 1 allele showing fewer pleiotropic effects than class 2 alleles such as e1370, making epistasis results easier to interpret. sod-1(0) slightly shortens daf-2 life span at 25°C, perhaps reflecting a minor contribution of sod-1 to daf-2 Age, but not 20°C [Fig. 2E; Supplemental Table S2], sod-5(0) had little consistent effect [Supplemental Table S2].
Figure 3. Overexpression of sod-1 cytosolic Cu/ZnSOD increases life span. [A] Western blots of protein extracts from wild-type, sod-1 mutant, and sod-1 overexpression lines using anti-Cu/ZnSOD antibodies. Cytochrome C (Cyt-C) and actin were used as mitochondrion- and cytosol-specific markers, respectively. Increased SOD-1 protein was also detected in wuIs154 transgenics (data not shown). [B] Total SOD activity in protein extracts from lines overexpressing sod-1. **P < 0.01, Student’s t-test. (C) Total catalase activity in line overexpressing catalase. **P < 0.01, Student’s t-test. [D] Catalase overexpression causes high levels of mortality from internal hatching of larvae (hagging), and this is suppressed by overexpression of sod-1. (***) P < 0.001, Student’s t-test. [E] sod-1 overexpression increases sensitivity to oxidative stress (40 mM paraquat), and this is suppressed by overexpression of catalase. [F] Overexpression of sod-1 increases life span. [G] Elevated catalase does not further extend longevity of the sod-1 overexpression.

Finally, we examined the phenotypic effects of loss of the sod-4 extracellular Cu/ZnSOD. sod-4(0) does not affect sensitivity to oxidative stress [Fig. 2A,B] or life span in otherwise wild-type animals [Fig. 2C]. Surprisingly, sod-4(0) enhances daf-2 Age at both 20°C and 25°C [Fig. 2E, Supplemental Table S2]. One possibility is that this reflects an effect on signaling. SOD-4, like mammalian Cu/ZnSOD, might generate H₂O₂, which then crosses into the cell and promotes insulin signaling by inhibiting redox-sensitive, signal-quenching phosphatases [Goldstein et al. 2005]. In C. elegans, treatment with H₂O₂ increases PIP₃ levels and promotes cytosolic retention of DAF-16 [Weinkove et al. 2006]. If sod-4(0) does reduce IIS, then it should enhance other daf-2 mutant traits, including constitutive dauer larva formation [Daf-c]. We therefore tested the effect of loss of each sod gene on daf-2(m577) Daf-c and report that sod-4(0) significantly enhances Daf-c [Figs. 1F, 2F]. Thus, sod-4 may contribute to IIS.

O₂⁻ has long been viewed as a possible major determinant of aging [Harman 1956, 1972]. In this study, we explored the importance of the three major O₂⁻ pools on aging in C. elegans. Overall, our results imply that O₂⁻ is not a major determinant of aging in this model organism, either in wild-type animals or long-lived daf-2(tm577) mutants; however, it remains possible that SOD contributes substantially to longevity in other contexts (e.g., under dietary restriction). Our findings point to the novel conclusion that each O₂⁻ pool is different in terms of its effect on aging. In wild-type C. elegans, the cytosolic O₂⁻ pool contributes weakly to aging while mitochondrial O₂⁻, long considered a likely determinant of aging, and extracellular O₂⁻ have no detectable effect on normal aging. In daf-2 mutants, extracellular O₂⁻ appears to promote longevity; however, it is more likely that SOD-4 converts O₂⁻ into H₂O₂, which then weakly activates IIS, thereby shortening life span. The effects of SOD and O₂⁻ on aging therefore vary according to cell compartment and to genotype [summarized in Fig. 4].

Our findings paint a clearer picture of the role of the various SOD isoforms in C. elegans. sod-1 and sod-2 encode the major Cu/ZnSOD and MnSOD isoforms, corresponding to the equivalent isoforms in other eukaryotes. By contrast, sod-5 and sod-3 encode inducible, auxiliary Cu/ZnSOD and MnSOD isoforms. The presence of these supernumerary isoforms, like that of the stress-resistant dauer larva stage in which they are up-regulated, may reflect the hostile soil environment in which C. elegans has evolved.

We examined the effect on life span of loss of SOD in each of the three major cellular compartments. The oxidative damage theory of aging predicts that loss of SOD should cause accelerated aging, particularly cytosolic Cu/ZnSOD and MnSOD, both of which contribute to scavenging of mitochondrially generated O₂⁻ [the former in the mitochondrial intermembrane space]. In fact, only loss of sod-1 shortened life span, and then only modestly. However, overexpression of sod-1 did increase life span slightly [Fig. 3F, Supplemental Table S3], implying a small contributory role of O₂⁻ to aging.

Loss of MnSOD isoforms had no effect on life span, either in a daf-2(+) or daf-2(m577) background [Fig. 1C-E, Supplemental Tables S1, S2]. This strongly implies that O₂⁻ within the mitochondrial matrix is not a significant cause of aging in C. elegans. An alternative possibility is that other mechanisms protect C. elegans mitochondria against O₂⁻; however, the oxygen hypersensitivity of sod-2; sod-3 mutants argues against this.

**Figure 4.** Influence of SOD and O₂⁻ on aging. This scheme shows a synthesis of conclusions drawn from the present study. Different SOD isoforms [and by deduction, the corresponding O₂⁻ pools] have different effects on aging. Extracellular Cu/ZnSOD weakly inhibits dauer formation and promotes aging, potentially by generating H₂O₂, which crosses into the cell and stimulates insulin/IGF-1 signaling by inhibiting redox-sensitive phosphatases. Cytosolic Cu/ZnSOD weakly promotes longevity, perhaps by protecting against molecular damage [sod-1 does not influence daf-2 Daf-c]. Mitochondrial MnSOD has no detectable effect on aging. Arrow with dotted line implies a weak effect.
Very recently, another study also reported, using different mutant alleles, that sod-2(0); sod-3(0) does not affect life span in an otherwise wild-type background [Honda et al. 2008], confirming our findings. These investigators also observed that in a daf-2(e1370) mutant, sod-2(0) slightly shortened life span and lessened Daf-c while sod-3(0) had the opposite effect. daf-2 alleles fall into two phenotypic classes: Class 2 alleles are more pleiotropic than class 1 alleles and show more complex epistatic interactions with other mutations [Gems et al. 1998; Patel et al. 2008]. The difference in effects of loss of MnSOD on daf-2(m577) [this study] and daf-2(e1370) [Honda et al. 2008] is interesting since m577 is a class 1 allele and e1370 a class 2 allele and suggests that MnSOD exerts a selective influence on class 2-specific defects.

Loss of function of many genes involved in mitochondrial function result in a Clk or Mit phenotype, which includes a delayed reproductive schedule, lowered fertility, slowed defecation cycle, and increased life span [Wong et al. 1995; Rea 2005]. In this study, we found that sod-2(0) slightly shortened life span in all those traits [Supplemental Fig. S5] except the increase in life span [Fig. 1C,D]. RNAi of sod-2 enhances clk-1(qm30) mutant longevity [Yang et al. 2007], suggesting a possible cryptic effect of sod-2 on aging via a Clk/Mit-type mechanism.

Loss of sod-4 enhances the daf-2 Age and Daf-c phenotypes [Fig. 2E,F], suggesting that sod-4 may play a role in IIS regulation of dauer formation and life span. We postulate that SOD-4-generated H2O2 promotes IIS by inhibiting IIS antagonistic phosphatase enzymes, as occurs in mammals [Goldstein et al. 2005].

While our findings imply that SOD and O2\(^-\) have, at most, minor effects on aging in C. elegans, the question remains: What is the relevance of these findings to higher animals? The effects on life span of altering SOD gene expression vary between C. elegans, Drosophila, and the mouse [for review, see Muller et al. 2007]. Loss of MnSOD causes early lethality in the fly and the mouse but not the worm, while loss of cytosolic Cu/ZnSOD causes only small decreases life span in the worm and the mouse but a large (~80%) decrease in life span in the fly. Overexpressing cytosolic Cu/ZnSOD slightly increases life span in C. elegans [this study], and in Drosophila overexpression of cytosolic Cu/ZnSOD and MnSOD seem to increase life span, although findings vary [Muller et al. 2007]; however, overexpression of cytosolic Cu/ZnSOD does not increase life span in the mouse [Huang et al. 2000]. By contrast, in the filamentous fungus Podospora anserina, reduction of mitochondrial ROS production results in a dramatic retardation of aging. Instead of aging rapidly after a brief period of growth, hyphae grow continuously and, apparently, indefinitely [Dufour et al. 2000].

It has been suggested that damage from reactive oxygen species might represent a public [i.e., evolutionarily conserved] rather than a private [i.e., lineage-specific] mechanism of aging [Martin et al. 1996]. Our findings imply that the role of O2\(^-\) in aging is to some extent public and to some extent private: Cytosolic O2\(^-\) appears to contribute to aging in C. elegans and Drosophila but not mice. By contrast, loss of MnSOD is lethal to the fly and the mouse but has no effect on aging in the worm. Thus, O2\(^-\) in some cellular compartments seems to contribute to some degree to aging in some species, but in other contexts [e.g., C. elegans] it appears unimportant to aging.

Materials and methods

Nematode culture

Nematodes were cultured on NGM agar seeded with E. coli OP50 as described previously [Sulston and Hodgkin 1988]. Strains were maintained at 20°C unless otherwise noted. Mutant alleles and transgenic arrays used or generated in this study are listed in the Supplemental Material.

Oxidative stress resistance assays

For paraquat, young adults were placed overnight on agar plates containing 40 μM fluorodeoxyuridine (FUdR), and then picked into microtiter wells with 100 μl of 40 mM paraquat (Sigma, 856177) in M9 buffer. Viability was assayed over a 20-h period. For hypoxia, young adults were picked onto plates containing 40 μM FUdR and placed in a sealed chamber under 60% O2 [22°C]. Animals were briefly removed from the O2 chamber to score viability every 2–3 d.

Life span measurements

Life span assays were performed as described previously [Gems et al. 1998], at either 20°C or 25°C (see Supplemental Tables S1–S3). Survivorship of populations was compared statistically using the log rank test, performed using JMP 7.0.1 (SAS).

Construction of transgenic lines

Reporter constructs for each of the sod genes are full-length, translational fusions of GFP to the C terminus (see Supplemental Figure S2). For sod and cit (catalase) overexpression, gDNA fragments were generated by PCR and microinjected directly. For primer sequences for reporter and overexpression constructs, see the Supplemental Material. Unless otherwise noted, pRF4 [rol-6(sa1006)] was used as a marker of transformation. Integrated lines were generated by X-irradiation, and backcrossed to wild type [N2] at least five times before further study.

SOD and catalase activity assays

SOD activity was measured using an assay involving the inhibition of superoxide-induced lucigenin chemiluminescence by SOD, as described previously [Lenaerts et al. 2002]. Catalase activity was assayed at 25°C according to a standard method [Aebi 1984], adapted for use in microtiter plate format.

Dauer formation assays

Eggs from strains bearing daf-2(m577) were collected by hypochlorite lysis and cultured at 23.5°C. The proportion of dauer larvae was scored 72 h later.

Acknowledgements

We thank David Hoogewijs for assistance with mRNA level estimations, and Peter Piper, Jennifer Tullet, and David Weinkove for useful discussion and comments on the manuscript. This work was supported by grants from the Wellcome Trust and the European Union [to D.G.], and Ghent University [GOA 12050101], the Fund for Scientific Research-Flanders [G.0025.06], and the European Community [LSHM-CT-2004-512020] (to J.R.V.). P.B. acknowledges a grant from the Institute for the Promotion of Innovation by Science and Technology in Flanders [IWT]. Some strains were obtained from the Caenorhabditis Genetics Center, which is supported by the National Institutes of Health National Center for Research Resources.

References

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. 105: 121–126.

Alcedo, J. and Kenyon, C. 2004. Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron 41: 45–55.

Bargmann, C.I. and Horvitz, H.R. 1991. Control of larval development by chemosensory neurons in Caenorhabditis elegans. Science 251: 1243–1246.

Chavez, V., Mohri-Shiomi, A., Maadani, A., Vega, L.A., and Garsin, D.A. 2007. Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by Caenorhabditis elegans. Genetics 176: 1567–1577.

Cypher, J.R. and Johnson, T.E. 2002. Multiple stressors in Caenorhabditis
Harman, D. 1956. Aging: A theory based on free radical and radiation chemistry.

Dufour, E., Boulaj, J., Rincheval, V., and Sainsard-Chanet, A. 2000. A causal link between respiration and senescence in Podospora anserina. Proc. Natl. Acad. Sci. 97: 4138–4143.

Finkel, T. 1998. Oxygen radicals and signaling. Curr. Opin. Cell Biol. 10: 248–253.

Fujii, M., Ishii, N., Joguchi, A., Yasuda, K., and Ayusawa, D. 1998. Novel superoxide dismutase gene encoding membrane-bound and extracellular isozymes by alternative splicing in Caenorhabditis elegans. DNA Res. 5: 25–30.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Larson, P.L., Albert, P.S., King, K.V., Edgley, M., and Riddle, D.L. 1998. Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. Genetics 150: 129–155.

Giglio, M.-P., Hunter, T., Bannister, J.V., Bannister, W.H., and Hunter, G.J. 1994. The manganese superoxide dismutase gene of Caenorhabditis elegans. Biochem. Mol. Biol. Int. 33: 87–40.

Halliwell, B. and Gutteridge, J.M.C. 2007. Free radicals in biology and medicine. Oxford University Press, Oxford, UK.

Harman, D. 1956. Aging: A theory based on free radical and radiation chemistry. J. Gerontol. 11: 298–300.

Harman, D. 1972. The biologic clock: The mitochondrial J. Am. Geriatr. Soc. 20: 145–147.

Honda, Y. and Honda, S. 1999. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. FEBS Lett. 431: 1385–1393.

Honda, Y., Tanaka, M., and Honda, S. 2008. Modulation of longevity and diapause by redox regulation mechanisms under the insulin-like signaling control in Caenorhabditis elegans. Exp. Gerontol. 43: 520–529.

Huang, T., Carlson, E., Gillespie, A., Shi, Y., and Epstein, C. 2000. Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. J. Gerontol. 55: 85–89.

Hunter, T., Bannister, W.H., and Hunter, G.J. 1997. Cloning, expression, and characterization of two manganese superoxide dismutases from Caenorhabditis elegans. J. Biol. Chem. 272: 28652–28659.

Jensen, L.T. and Culotta, V.C. 2005. Activation of CuZn superoxide dismutase from Caenorhabditis elegans does not require the copper chaperone CCS. J. Biol. Chem. 280: 41373–41379.

Kenyon, C. 2005. The plasticity of aging: Insights from long-lived mutants. Cell 120: 449–460.

Larsen, P.L. 1993. Aging and resistance to oxidative stress in Caenorhabditis elegans. Proc. Natl. Acad. Sci. 90: 8905–8909.

Lenaerts, I., Braeckman, B., Matthijssens, F., and Vanfleteren, J. 2002. A high-throughput microtiter plate assay for superoxide dismutase based on lucigenin chemiluminescence. Anal. Biochem. 311: 90–92.

Martin, G.M., Austad, S.N., and Johnson, T.E. 1996. Genetic analysis of ageing: Role of oxidative damage and environmental stresses. Nat. Genet. 13: 25–34.

Missirlis, F., Hu, J., Kirby, K., Hilliker, A., Rouault, T., and Phillips, J. 2003. Compartment-specific protection of iron-sulfur proteins by superoxide dismutase. J. Biol. Chem. 278: 47365–47369.

Muller, F.L., Liu, Y., and Van Remmen, H. 2004. Complex III releases superoxide to both sides of the inner mitochondrial membrane. J. Biol. Chem. 279: 49064–49073.

Muller, F.L., Lastgarten, M., Jiang, Y., Richardson, A., and Van Remmen, H. 2007. Trends in oxidative aging theories. Free Radic. Biol. Med. 43: 477–503.

Patel, D.S., Garza-Garcia, A., Nanji, M., McElwee, J.J., Ackerman, D., Driscoll, P.C., and Gems, D. 2008. Clustering of genetically defined allele classes in the Caenorhabditis elegans DAF-2 insulin/IGF-1 receptor. Genetics 178: 931–946.

Rea, S.L. 2005. Metabolism in the Caenorhabditis elegans Mit mutants. Exp. Gerontol. 40: 841–849.

Riddle, D.L. and Albert, P.S., eds. 1997. Genetic and environmental regu-