Chapter 10
Oxidative Stress

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Abstract The oxidative damage theory has been the dominant paradigm in ageing research over the last 50 years. The versatile genetic nematode model *C. elegans* has been used by many to put this theory to the test. *C. elegans* is an attractive model as it ages fast, it has an elaborate antioxidant system which can be easily manipulated, and many long-lived mutants are available. Recently, it became possible to visualize reactive oxygen species (ROS) in vivo and in real-time in this transparent animal by using genetically encoded biosensors. The data generated in *C. elegans* to test the oxidative damage theory is often ambiguous and of mere correlative nature. Experimental manipulation of the antioxidant system most often disproves this theory. Over the years, it became clear that ROS, when present at normal physiological levels, are important signalling molecules. Interference with this ROS signal may elicit a cytoprotective programme that, in many cases, extends lifespan. It is still an open question whether the molecular underpinnings of this hormetic response is also of importance to the normal ageing process. Alternatives to the oxidative damage theory, such as the hypertrophy hypothesis, are currently gaining wider attention.

Keywords Reactive oxygen species • Genetically encoded sensors • Oxidative damage • Antioxidants • Hormesis • ROS signalling

10.1 Reactive Oxygen Species (ROS)

Oxygen became an important constituent of the Earth's atmosphere when the process of photosynthesis evolved in cyanobacteria about 2.2 billion years ago [1]. Although today O_2 is essential to support energy metabolism in the majority of species, it is essentially a toxic, mutagenic gas which requires appropriate cellular protection via antioxidant defences.

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Molecular oxygen is a free radical – a molecule that can exist freely with one or more unpaired electrons – and it can generate various reactive oxygen species (ROS) by single electron transfers, usually from transition metals. The group of reactive oxygen species contains oxygen radicals as well as non-radicals that are oxidizing agents and/or are easily converted into radicals. Besides ROS, also reactive nitrogen, sulphur and halogen species exist [2]. Molecular oxygen can be reduced to water by four single electron transfers, generating the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\bullet}$), and finally, water (H_2O). ROS may also be generated in other ways, such as homolytic fission of water via background ionizing radiation, generating two hydroxyl radicals. The reactivity of each of these species towards biological molecules varies widely but these uncontrolled reactions result in oxidative damage that may impair or alter the function of the molecule.

Superoxide can be formed at several sites in the cell by reduction of O_2 with one electron. The predominant source of superoxide in aerobic animals is the mitochondrial electron transport chain [3, 4]. The rate at which electrons leak from the electron transport chain to molecular oxygen is determined by the mitochondrial membrane potential, which in turn depends on mitochondrial activity and coupling efficiency. This way, active mitochondria may produce less $O_2^{\bullet -}$ than resting mitochondria [5–7]. Due to its negative charge, the superoxide anion cannot readily cross lipid membranes although transport through anion channels has been described [8]. Superoxide does not react with most biological molecules in aqueous solution but it can quickly react with other radicals or enzymatic Fe-S clusters. Despite its low reactivity, superoxide is an important ROS as it is the primary precursor of many other reactive species [2].

Hydrogen peroxide (H_2O_2) may be generated in the cell by spontaneous or enzyme-catalysed dismutation of $O_2^{\bullet -}$. Also, some enzyme systems such as oxygenases are known to produce hydrogen peroxide. This ROS is more stable than superoxide but it is also poorly reactive. Hydrogen peroxide is a potent but slow oxidizer: DNA, lipids and most proteins are not oxidized directly by H_2O_2, even at millimolar levels. This species can, however, inactivate some enzymes directly by oxidizing hyper-reactive thiols necessary for catalysis [9, 10]. The biological importance of hydrogen peroxide should not be underestimated as it can act as a signalling molecule and it is the source of hydroxyl radicals [11].

The hydroxyl radical $OH^{\bullet}$ is one of the most potent oxidizing agents known to chemistry. Immediately after its formation it reacts non-selectively with molecules such as DNA, lipids or proteins [12] and therefore is the most damaging ROS in biological systems. It is generated by homolytic fission of H_2O_2 by UV light, by reaction of HOCl with $O_2^{\bullet -}$, or most often by Fenton reactions. In these reactions, hydrogen peroxide oxidizes a reduced metal ion, usually Cu^{+} or Fe^{2+} to produce OH^{-} and $OH^{\bullet}$. The oxidized transition metal can return to its reduced state, possibly by aid of intracellular reductants such as ascorbate, quinines or semiquinones, cysteine, flavins and NAD(P)H [13–15]. The availability of free iron and copper in the cell is strictly regulated to minimize $OH^{\bullet}$ formation by Fenton chemistry. However, superoxide may cause the release of iron from Fe-S clusters or ferritin [2].

Besides these well-studied forms of ROS, other reactive species, such as carbonate, peroxyl, alkoxyl and sulphur radicals, singlet oxygen and ozone, may also be involved in oxidative damage.

10.2 Antioxidants

In living organisms, intracellular ROS levels are kept low because of reasons ranging from habitat choice to intracellular molecular architecture. Many small organisms avoid oxygen-rich environments (e.g. *C. elegans* prefers 5–12 % O_2 [16]) while larger animals only expose their epithelia to atmospheric oxygen levels. Another way to reduce ROS formation is the organization of electron transport chain components into an efficient respirasome [17], minimizing electron leakage to O_2. However, ROS levels and ROS-induced damage are, above all, restrained by antioxidants; substances that, by definition, delay, prevent, or remove oxidative damage to a target molecule [2]. These include enzymes and other proteins as well as small organic molecules.

Superoxide dismutases (SODs), first discovered in 1969 [18], catalytically remove superoxide by dismutation. These enzymes have been found in all organisms and are grouped according to their metal cofactor. MnSODs and FeSODs are found in prokaryotes and plants while animals possess MnSODs and Cu/ZnSODs. A nickel-containing SOD (NiSOD) was found in *Streptomyces* and cyanobacteria [19]. In animals, MnSOD is localized in the mitochondria, in agreement with the prokaryotic ancestry of these organelles. Cu/ZnSOD is found in the cytoplasm or extracellular. While most eukaryotes only have two SODs, the *C. elegans* genome encodes five *sod* genes [20]. Two cytosolic Cu/ZnSODs are represented by *sod-1* and *sod-5* and the MnSODs are *sod-2* and *sod-3*. *sod-4* encodes two Cu/ZnSOD isoforms resulting from alternative splicing: SOD-4.1 is a homologue of the mammalian extracellular Cu/ZnSOD while SOD-4.2 contains a C-terminal sequence resembling a transmembrane domain and hence this unique isoform is probably attached to the membrane [21]. SOD-1 is the most abundant *C. elegans* SOD transcript – making up about 75 % of all SOD transcripts – and it contributes most to total SOD activity in normal worms [22]. In mitochondria, SOD-2 is the predominant isoform [22] and this MnSOD has, together with SOD-3, been localized to the I:III:IV supercomplex of the electron transport chain, where it may stabilize the complex and/or reduce local superoxide formation [23]. Finally, SOD-3, SOD-4 and SOD-5 are expressed at low levels in normal worms but are strongly induced in dauers, probably via the Ins/IGF-1 like signalling pathway [20, 22]. Loss of SOD-1 activity may lead to compensatory induction of SOD-5 [24] although this was not confirmed by another study [25].

SODs convert $O_2^{\bullet-}$ into H_2O_2, which in turn can be eliminated by catalases and peroxidases. Catalases are homotetramers of haem-bearing subunits, each of which can catalyse the dismutation reaction of two H_2O_2 molecules into H_2O and O_2 [26]. As this reaction requires two hydrogen peroxide molecules at a single active site,

catalases are only efficient at high substrate levels. Catalases are found in prokaryotes and eukaryotes but have been lost during evolution in a few species [27, 28]. Catalase resides in the peroxisomes where it scavenges the hydrogen peroxide that is produced during fatty acid β-oxidation, but cytosolic catalases are also known. The *C. elegans* genome contains a tandem array of three catalase genes (*ctl-1*, *ctl-2* and *ctl-3*) with very high sequence similarity [29]. CTL-2 is a peroxisomal catalase that contributes up to 80 % of the total catalase activity in the worm. CTL-1 has been described as a cytosolic catalase [29, 30]. The details of CTL-3 are less clear but it appears to be expressed in the pharyngeal muscle and neurons.

Peroxidases are a class of enzymes that convert H_2O_2 to water or hydroperoxides (ROOH) to the corresponding alcohol (ROH) by oxidizing another substrate (e.g. NADPH or GSH). Glutathione peroxidase (GPX) is a Se-bearing enzyme that occurs as a monomer or homotetramer, depending on the isoform. The *C. elegans* GPX family contains at least 8 members although no enzymatic GPX activity could be detected when applying a standard assay using *tert*-butyl-hydroperoxide as a substrate [31], suggesting narrow substrate specificity of the *C. elegans* GPXs. *C. elegans* GPX-1 is a homologue of the mammalian phospholipid hydroperoxide GPX and interacts with dipeptide transport [32]. Other *C. elegans* GPX family members await detailed study. A second class of peroxidases contains the peroxiredoxins (PRDXs), which are also H_2O_2 scavenging enzymes that occur as homodimers with cysteines at their active sites. They are very abundant, localized in most intracellular and extracellular compartments and can constitute 0.1–0.8 % of the total soluble protein content. PRDX reduces H_2O_2 or ROOH by oxidation of a cysteine to a sulphenic acid (cys-SOH). The PRDX can be reduced to its original state by thioredoxins (TRXs) or glutaredoxins (GLRXs). The *C. elegans* genome encodes for two PRDXs: *prdx-2* and *prdx-3*. PRDX-2 appears to be expressed in the cytosol of the intestine, gonads and neurons. Intestinal expression of *prdx-2* is sufficient to support resistance against hydrogen peroxide treatment. However, loss of PRDX-2 activates the DAF-16 and SKN-1-dependent stress resistance programmes [33] (see also Chap. 9). The mitochondrial PRDX-3 does not protect against hydrogen peroxide insult [34].

An overview of reactive species and antioxidant systems in *C. elegans* is given in Fig. 10.1.

10.3 ROS Quantification

ROS are key players in oxidative stress and can be generated by exogenous compounds as well as mitochondrial (dys)function. Their reactivity, ephemeral nature and local gradients make it very difficult to localize and quantify these molecules in vivo. The majority of *C. elegans* studies that analyse ROS make use of reduced dyes such as dihydrofluoresceins, lucigenins, MitoSOX and amplex red [35]. The problem with many dyes is that their uptake in live animals may vary, they often lack selectivity, they may need a catalyst to work, they may be metabolized or have

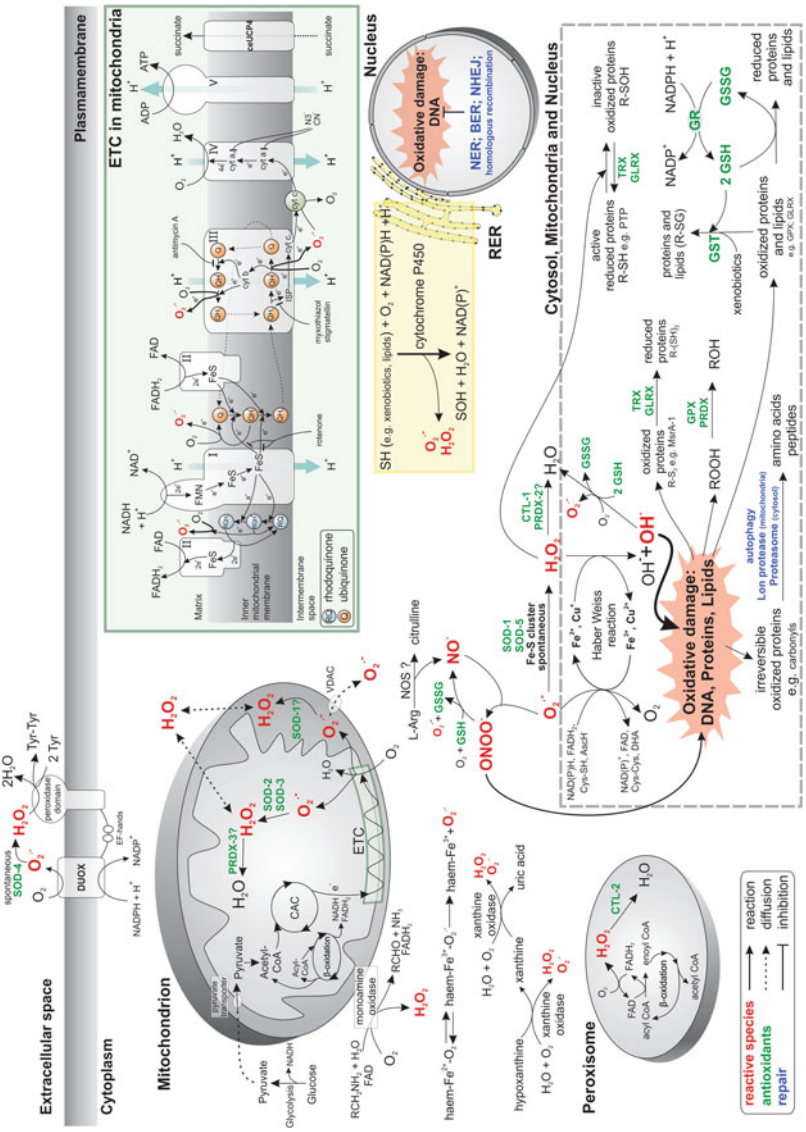

Fig. 10.1 Schematic representation of the production and removal of reactive oxygen species and the oxidative damage clearance and repair systems in the nematode *C. elegans*

poor stability, and some probes can even generate ROS by themselves and may disturb cellular physiology [36–38]. Moreover, many dyes react with ROS irreversibly, precluding dynamic measurements. Disruption of *C. elegans* for ROS quantitation may create oxidation artefacts as delicate cellular redox balances are disturbed. Hence, an ideal ROS probe should be selective, sensitive, instantaneous, reversible, compartment-specific, non-invasive and allow in vivo monitoring [39]. Some of the disadvantages of dyes have been overcome by designing protein-linked chemical reporters [40], or ratiometric mass spectrometry probes [41], but even these technologically advanced techniques cannot tackle every problem.

The introduction of genetically encoded ROS sensors has been a big leap forward in the search for reliable in vivo ROS detection. Wild-type GFP has two excitation peaks – 395 nm for the protonated and 475 nm for the deprotonated form of Y66 – while only one emission peak exists at 509 nm [42]. This dual excitation/single emission property of GFP can be exploited for ratiometric measurements in which emission intensity at one excitation wavelength is divided by the emission at the other excitation wavelength. This offers the advantage of being independent on probe expression levels and photobleaching, greatly simplifying comparison among samples. Fluorophore protonation is dependent on interactions with surrounding residues and therefore conformational alterations can cause a shift in fluorescence intensity. Based on these properties, several ROS-sensitive probes have been developed [43].

10.3.1 Superoxide

A circularly permutated yellow fluorescent protein (cpYFP), targeted to the mitochondria, has been used as a ROS biosensor to specifically detect superoxide bursts, called mitoflashes, in cardiomyocyte cell cultures after reoxygenation [44]. However, the specificity of this probe was heavily debated [45, 46]. Mitoflashes were also observed in *C. elegans* expressing the same cpYFP biosensor, with peaks of high frequency around the third day of adulthood, during active reproduction, and around adult day 9, at the time that worms started to die off. Interestingly, day-3 mitoflash frequency is negatively correlated with lifespan of individual animals [47]. This paper also got criticism as it was shown earlier that the cpYFP does react to pH differences rather than superoxide [48], which was in turn refuted [49]. It is clear that cpYFP is a very controversial sensor for the detection of superoxide and the research community is still awaiting a reliable alternative that allows specific, non-invasive, real-time in vivo detection of superoxide, preferably without the need of very specialized equipment.

10.3.2 Hydrogen Peroxide

The hydrogen peroxide-specific biosensor HyPer was engineered by inserting the H_2O_2-sensitive regulatory domain of the *Escherichia coli* transcription factor OxyR into cpYFP [50]. In the presence of H_2O_2, an intramolecular disulphide bridge is formed between two cysteins of the OxyR regulatory domain, causing a substantial conformational change close to the cYFP chromophore and hence a shift in the fluorescent properties of this probe. Upon H_2O_2 exposure, the 420-nm excitation peak decreases while the 500-nm excitation peak increases, yielding a maximal ratiometric shift of 3–4. Because of the use of the *E.coli* regulatory domain, the probe is highly selective and reacts within physiologically relevant ranges of H_2O_2 levels. The disulphide bridge in the oxidized HyPer is reduced by endogenous GSH and glutaredoxin (GLRX), allowing reversible shifts in HyPer fluorescence and dynamic measurements. By adding a single point mutation, the dynamic range was doubled and the modified sensor was called HyPer-2 [51]. However, the reaction kinetics were slowed down compared to the original HyPer probe. This problem was resolved with the development of Hyper-3 [52]. Despite these qualities, the HyPer biosensors have one major disadvantage: as it is based on cpYFP, this sensor is influenced by pH in the range between 6 and 10. Hence, the sensor is not reliable when comparing H_2O_2 levels in compartments that may differ in pH [53]. In that case an additional pH-sensor should be used with an emission wavelength other than that of HyPer, e.g. pHRed [54]. Alternatively, the percentage of HyPer oxidation can be calculated based on completely reduced and oxidized samples [55]. The HyPer biosensor has been expressed in *C. elegans* to analyse the real-time in vivo levels of hydrogen peroxide in developing and ageing worms. A gradual increase of hydrogen peroxide levels was observed in ageing individuals [56] although another study could also detect high H_2O_2 levels in larval stages [57]. This sensor was also used for in vivo H_2O_2 localization in *C. elegans*; high hydrogen peroxide levels were detected in the hypodermal cells, which is consistent with their role in cuticle biogenesis [56, 58].

Another hydrogen peroxide biosensor was built by fusing the yeast peroxidase Orp1 to roGFP2 [59]. roGFP2 is a redox-sensitive, ratiometric GFP with stable fluorescence output in a physiological pH range between 5.8 and 8.0 [60], making it a better alternative to HyPer. Hydrogen peroxide-specific oxidation of cysteine residues in the Orp1 moiety induces the formation of a disulphide bridge in roGFP2. This reaction is reversible as roGFP2-Orp1 can be reduced by endogenous thioredoxin or glutaredoxin [43]. The use of this sensor in *C. elegans* is currently limited to one study on the mitochondrial efficiency of axenically cultured worms [61].

The most recently developed H_2O_2-specific biosensors are chimeric proteins, OxyFRET and PerFRET, combining the yeast Orp1-Yap1 redox relay system with a Venus/Cerulean FRET couple [62]. Though insensitive to alkalinization, the properties of these sensors do change in acidifying cells. So far, these probes have not been applied in *C. elegans*.

Besides ROS-specific biosensors, some redox-sensing proteins have been developed as well. rxYFP was developed over a decade ago but, as it is also based on YFP, it suffers the same pH sensitivity problem as the HyPer sensors [43, 63]. The new generation ratiometric redox sensors are based on roGFP linked to the human glutaredoxin (Grx1) and can detect low levels of oxidized glutathione (GSSG) within a highly reduced GSH pool [64]. In *C. elegans*, the GSSG/GSH ratio is high in L1 larvae and tends to decrease during development, reaching a minimum at the L4-to-adult transition. During adult life the GSSG/GSH ratio rises again, mirroring the increasing hydrogen peroxide levels in ageing worms [56]. While the GSSG/GSH ratio is fairly constant over the whole body, it is particularly low in the spermatheca [56] possibly providing a low noise background for ROS signalling events or increased protection of gametes against oxidative stress. Peredox [65] and Frex [66] are another set of redox sensors that quantify the NAD^+/NADH ratio, but again, these sensors have not been applied in *C. elegans* yet.

10.4 The Oxidative Damage Theory

Oxidative stress is the disturbance of the prooxidant-antioxidant balance towards the prooxidant side, potentially leading to oxidative (and other) damage. Oxidative stress may result from decreased antioxidant capacity or an increase in reactive species [2].

In 1956, Denham Harman postulated the free radical theory of ageing [67], currently one of the most influential mechanistic theories of ageing. Free radicals, often considered to be produced as byproducts of normal oxidative metabolism, would cause molecular damage that accumulates over time. This in turn would result in the functional decline of cells, tissues and eventually the organism; a process which is called ageing. The theory was later fine-tuned by indicating the mitochondria as the major free radical source [68] and, as not all ROS are free radicals, it was referred to as the oxidative damage theory of ageing [69, 70]. The oxidative damage theory predicts that (1) the level of oxidative damage increases during ageing, and (2) lifespan extension is associated with a decrease of oxidative damage [69].

10.5 Oxidative Damage and Ageing in *C. elegans*

The oxidative damage theory has been tested in a plethora of species of wide phylogenetic diversity. In this chapter, we will focus on the work that has been carried out specifically in *C. elegans*, which has become a very prominent model species in biogerontology over the last few decades [71–73].

The predicted increase of oxidative damage with age has been supported by several *C. elegans* studies. Levels of protein carbonylation, the oxidation of amino acid side-chains to carbonyl residues, have been shown to increase over time in adult

worms [74, 75], at least in their mitochondria [76, 77]. A positive correlation was found between adult age and DNA damage such as single-strand DNA breaks and 5-methylcytosine [78] although the latter could not be confirmed in another study [79]. Also, the increased occurrence of mitochondrial DNA breaks in ageing *C. elegans* is ambiguous [80–82]. DNA damage and ageing in *C. elegans* is presented in detail in Chap. 11. 4-hydroxy-2-nonenal (4-HNE), a lipid peroxidation product that forms as a consequence of oxidative stress, can be conjugated to proteins by the action of glutathione S transferases. It was shown that 4-HNE protein adducts do indeed accumulate with age in the worm [83]. Lipofuscin is a heterogeneous cross-linked aggregate of oxidatively damaged lipids and proteins and tends to aggregate with age in vertebrates [84]. These aggregates are also called age pigments and tend to show a specific fluorescence spectrum. Autofluorescence with similar characteristics has been found to accumulate in gut granules of *C. elegans* populations over time and therefore has been referred to as lipofuscin and used as a biomarker of ageing [85–87]. However, more recently it was found that gut granule autofluorescence is caused by anthranilic acid glucosyl esters and that, at the individual worm level, this autofluorescence does not increase gradually with age but rather bursts at the time of death [88]. Overall, there are many indications that oxidative damage increases with age in *C. elegans*, as predicted by the oxidative damage theory, but not all studies are consistent. However, this correlation does not imply causation, just like greying hair in humans is not causal to ageing.

A tighter link between oxidative stress and ageing appeared when researchers started to analyse the oxidative damage and antioxidant capacity of *C. elegans* mutants with altered lifespan. Early studies showed that *age-1*, a long-lived Insulin/IGF-1 signalling pathway mutant (see Chap. 4), displays enhanced catalase and SOD activity compared to controls and antioxidant activity appeared to rise with age in the mutant [31, 89, 90]. This rise could not be confirmed in a later study although the levels of antioxidant enzymes were clearly increased in the long-lived mutants [91]. Most other long-lived mutants also show increased oxidative stress resistance [92–94]. This strong correlation has even been exploited in a screen for longevity mutants by using oxidative stress resistance as a rapid selection marker [95].

The relationship between oxidative damage and lifespan also extends in the opposite direction: the complex II mutant *mev-1* suffers excessive oxidative stress, has a higher load of protein carbonyls and lives shorter than the wild-type strain [74, 96]. However, in these cases, it is more difficult to distinguish between accelerated ageing or oxidative stress pathologies that are not linked to ageing [97].

10.6 Manipulating ROS and Its Effect on Ageing

Lifespan extension and oxidative stress resistance are strongly linked suggesting that both processes are causally related. However, this correlation does not provide sufficient proof that the theory is correct. Long-lived strains are usually resistant to

other types of stress as well, e.g. heat, UV and pathogenic bacteria [92, 98]. Hence, these data would equally support theories claiming that heat, UV or bacteria are primary causes of ageing.

A more direct approach to test the causal relation between ROS and ageing is to manipulate the intracellular ROS levels and examine its subsequent effect on lifespan. ROS levels can be changed by interfering with ROS generating systems or with antioxidant defence, either pharmacologically or genetically.

10.6.1 Genetic Interventions

As a prime genetic model, *C. elegans* provides ample of possibilities to study the effect of genetic alterations of the antioxidant system on ageing. Nearly all relevant enzymes of this system have been knocked out or overexpressed and the effect of these manipulations on lifespan has been scrutinized. RNAi knockdown of the major cytosolic and mitochondrial SOD isoforms (*sod-1* and *sod-2*, respectively) increases oxidative damage levels in *C. elegans* but does not affect lifespan [99]. Deletion of both mitochondrial SOD isoforms (*sod-2* and *sod-3*) renders worms hypersensitive to oxidative stress but, again, does not drastically shorten lifespan [100]. Mitochondrial SOD knockdown does not increase oxidative damage to the mitochondrial DNA [81]. Hence, oxidative stress or damage is not necessarily a limiting factor for normal lifespan [101]. Similar conclusions were drawn in a study that included all SOD isoforms, although here, inactivation of the most abundant superoxide dismutase, SOD-1, caused a small reduction of lifespan, confirmed by [24], while its overexpression increased lifespan [22]. However, this lifespan increase appeared to be an indirect effect of SOD-1, depending on DAF-16 activation [102]. In other studies, the lifespan-shortening effect of *sod-1* mutation was not clearly observed, but instead, deletion of *sod-2* caused lifespan extension and a Mit mutant phenotype [103, 104]. This corroborates with the finding that SOD-2 is associated with the electron transport chain [23]. Finally, a quintuple deletion mutant, deficient in all sod genes and lacking any SOD activity showed a normal lifespan but was hypersensitive to acute stresses. This convincingly demonstrates that *sod* genes are necessary for surviving stressors but dispensable for normal lifespan [104, 105].

In *C. elegans*, mutation of the peroxisomal catalase *ctl-2* (but not the cytosolic *ctl-1*) shortens lifespan, which seems in agreement with the oxidative damage theory. However, counter to prediction, catalase mutation leads to reduced levels of protein carbonyls at old age [29] and catalase overexpression reduces lifespan as well [22].

In summary, these studies make clear that there is no straightforward relation between SOD or catalase activity and lifespan and *C. elegans*. In many cases, effects opposite of what the oxidative damage theory predicts are observed. Genetic interventions in other antioxidant systems are less well studied in *C. elegans*. The peroxiredoxin *prdx-2* mutants have a reduced lifespan, but the effect of overexpression on

lifespan is still elusive [34]. Suppression of *prdx-3* during adulthood does not influence levels of oxidative damage to proteins, nor does it alter lifespan [106]. For the thioredoxin *trx-1*, mutation slightly reduces lifespan while overexpression increases lifespan to some extent, but the effect on oxidative damage accumulation was not tested [107, 108]. Finally, the lifespan and oxidative damage phenotypes obtained after knock-down and overexpression of the glutathione-S transferase *gst-10* are consistent with the predictions of the oxidative damage theory [83].

10.6.2 Pharmacological Interventions

Many studies have pointed out that addition of pro-oxidants shortens *C. elegans* lifespan. Although this may seem to agree with the oxidative damage theory, it supports this theory only very weakly as it may reflect a toxic effect rather than an acceleration of the ageing process [97]. Antioxidant treatments, which are supposed to extend lifespan, have been much more instructive. Numerous studies examined the effect of exogenous catalytic and non-catalytic antioxidants on *C. elegans* lifespan [109]. Many of the non-catalytic antioxidants, such as Vitamin E and C, trolox, α-tocopherol, and N-acetylcysteine, affected lifespan differently in distinct studies, probably because of differences in dose and method of delivery [110–116]. In some cases, the antioxidants increased oxidative stress without affecting lifespan [117].

According to the oxidative damage theory, sufficient dietary intake of these antioxidants should delay the ageing process. A more interesting approach would be the intake of catalytically active antioxidants that require much lower doses because of their catalytic rather than stoichiometric reaction properties. EUK-8 and EUK-134 are SOD/catalase mimetics that are readily taken up in *C. elegans* and tend to accumulate in mitochondria [118]. Initial lifespan analyses showed that both mimetics extend lifespan in *C elegans* by an average of 44 % [119]. However, these results could not be replicated in independent studies. On the contrary, the EUK compounds seemed to shorten lifespan with increasing dose [118, 120, 121]. However, these molecules directly protect against oxidative stress imposed by exogenous compounds [118, 121, 122].

Together, these studies do not convincingly show that feeding antioxidants to worms extends lifespan. The fact that various antioxidants can protect against exogenous oxidative stress without influencing lifespan suggests that oxidative stress has no causal relation with normal ageing.

10.7 The Oxidative Stress Response and Hormesis

Oxidative stress causes a hormetic effect on lifespan in *C. elegans*, i.e. low doses result in moderate lifespan extension while higher doses are harmful and shorten lifespan. This effect was observed for the oxidants juglone [123] and paraquat [105,

124, 125]. The hormetic lifespan increase is caused by activation of a genetic cytoprotective programme in response to the stressor (for more details, see Chap. 9). The major transcription factors involved in the response to oxidative stress are DAF-16 [90] and SKN-1 [126]. DAF-16 is a Fork head transcription factor which is part of the Insulin/IGF-1 like signalling pathway [127] involved in dauer formation, metabolism, innate immunity and stress resistance. SKN-1, the *C. elegans* Nrf2 homologue, is a transcription factor involved in gut development and oxidative stress resistance [126]. The actions of DAF-16 and SKN-1 are intertwined [128] and these transcription factors may interact with many other factors such as BAR-1, SIR-2.1, 14-3-3, SMK-1, and HSF-1, to elicit the expression of overlapping gene sets with protective functions [25]. Typical downstream genes in oxidative stress response are glutathione-S-transferases, catalases, and superoxide dismutases [126, 128, 129]. However, other cytoprotective genes, such as small heat shock proteins, are also activated by these transcription factors.

These hormetic effects are often at play in the beneficial effects of 'antioxidant' plant extracts on *C. elegans* lifespan. Such studies have become increasingly popular over the last few years but their innovative power and contribution to the understanding of the ageing process is usually very limited. In most cases, the studied extracts trigger well-known cytoprotective responses, often involving DAF-16 and/or SKN-1, resulting in lifespan extension at low sub-toxic doses [130–134]. In many of these studies, authors claim to have found promising anti-ageing chemicals, but essentially a very broad range of molecules may trigger this general hormetic effect. A similar effect has been observed with the addition of the antioxidants N-acetyl-L-cysteine [135] and S-linolenoyl glutathione [136]. However, not all plant extracts extend lifespan via the same genetic pathways [137].

Hormesis has also been described in cases of mild mitochondrial dysfunction. Incremental reduction of mitochondrial electron transport chain (ETC) activity by RNAi dilution showed that lifespan is extended by mild ETC inhibition while more severe inhibition reduces lifespan [138] (see also Chap. 5). Interestingly, no direct correlation could be found between levels of oxidative damage and lifespan in this study. Some mitochondrial (Mit) mutants show increased ROS production [124, 125] and enhanced expression of antioxidant enzymes [99, 139], but the latter is dispensable for longevity [29, 99]. However, ROS generation is required to support lifespan extension in Mit mutants such as *isp-1* and *nuo-6* [125]. In the Mit mutant *clk-1* the prolongevity effect of excessive ROS production is compartment-specific [140].

The hormetic effect of ROS generated in the mitochondria is called mitohormesis [141, 142]. In the mitohormetic theory, ROS are not only damaging agents, but instead can act as signalling molecules that initiate cell-protective programmes of which some key players have been identified [141, 143, 144]. In the Mit mutants *clk-1* and *isp-1*, the hypoxia-inducible factor HIF-1 is required for longevity. Hence, respiratory stress and increased ROS production are linked to a nuclear transcriptional response that promotes longevity [124]. Inhibition of mitochondrial respiration by RNAi triggers the mitochondrial unfolded protein response (UPR^{mt}), which is also required for longevity of these animals. However, this response does not

occur in long-lived worms bearing mutations in the ETC genes, suggesting that there are at least two classes of Mit mutants – genetic and RNAi - each showing lifespan extension by independent molecular mechanisms [145]. The UPR^{mt} is a cell-non-autonomous response as mitochondrial perturbation in one tissue can elicit the UPR^{mt} in another [146]. Yet in the frataxin mutant, another Mit mutant, it was shown that lifespan extension is mediated by the *C. elegans* p53 homologue *cep-1* and not by *skn-1* or *daf-16* [147]. Although several molecular mechanisms of Mit longevity have recently been discovered, still many gaps remain on their relative importance and interactions [143, 148, 149].

10.8 New Horizons

C. elegans may survive a wide variety of stressors in its natural environment by activating specific cytoprotective programmes. These programmes have an ancient evolutionary history (e.g. detoxification, innate immunity, proteostasis, oxidative stress response) and all of them have been associated with longevity. Moreover, these programmes largely overlap and induction by one stressor may protect against another [150, 151]. Although activation of a general cytoprotective programme may add several days to a worm's life it may not be very informative about the underlying causes of the normal ageing process under unstressed conditions. Alternatively, one may assume that ageing is essentially the gradual loss of the ability to respond to stress and that therefore this response is a major determinant of longevity [152, 153]. It is also possible that ROS are linked to ageing because they mediate the stress response to age-dependent damage [154]. However, it is important to realize that lifespan extension is not specifically linked to the oxidative stress response only, as was thought in the past [155]. On the contrary, knockout of specific oxidative stress response genes only seems to affect oxidative stress resistance, but not lifespan [22, 100, 105]. Vice versa, altering oxidative stress resistance does not always affect ageing in *C. elegans* [156]. With this in mind, several interesting alternative views on ROS and ageing have been formulated over the last few years.

10.8.1 ROS Are Signalling Molecules

The notion that ROS act as signalling molecules rather than being damaging byproducts of oxidative metabolism is not entirely new [157]. In *C. elegans*, several ROS-mediated biological processes have been described (for a overview, see [39]). Reduced glycolysis [115] or mild mitochondrial dysfunction [138] increase mitochondrial superoxide production which acts as a signal triggering a protective response that extends lifespan (mitohormesis, see Sect. 10.7). Intracellular SOD may convert the short-lived superoxide into the more stable hydrogen peroxide which can oxidize cysteins of PRDX-2 monomers, forming activated homodimers.

Subsequently, PRDX-2 can activate SKN-1 via a MAPK pathway, resulting in the expression of a cytoprotective programme [158]. Interestingly, DAF-16 can be directly oxidized by ROS, linking it to the importin IMB-2 with a cysteine disulphide bridge [159], enabling it to enter the nucleus. This mechanism links oxidative stress or ROS signals directly to DAF-16 activation. The redox control of DAF-16 and PRDX-2 may be a response to relatively large cellular redox imbalances that require the acute activation of stress programmes to maintain cellular homeostasis and avoid cell death. However, ROS signalling also occurs on a much smaller spatial scale to regulate normal household functions such as reproduction. The *C. elegans* globin GLB-12 was recently identified as a membrane-bound superoxide generator, which, in concert with the intracellular SOD-1 and extracellular SOD-4, creates a hydrogen peroxide gradient over the plasma membrane of the somatic gonad. This gradient is required for normal gonad function and the control of germline apoptosis [160]. Loss of this redox signal results in complete sterility. This indicates that, rather than being omnipresent scavengers of superoxide, SODs are part of local signalling cascades, an idea that was already put forward earlier [100]. Despite the general notion that hydrogen peroxide easily crosses lipid bilayers, redox signals may act very locally as was shown in mammalian cells by means of membrane-anchored ROS biosensors [161]. These local signals may be propagated throughout the cell by GSH, formerly considered as an omnipresent cellular redox buffer, but now believed to be a redox signal amplifier [162].

As an alternative to the oxidative damage theory, the redox stress hypothesis states that functional loss during ageing is caused by a progressing pro-oxidizing shift in the cellular redox state, leading to the disruption of redox-regulated signalling mechanisms [163]. This would better explain the wide-spread cellular deterioration with age than does the relatively small accrual of structural oxidative damage. However, the cause of the pro-oxidizing shift with age is still unexplained. In the same vein, analysis of lifespan and hydrogen peroxide level in over 40 long-lived *C. elegans* strains led to the conclusion that not the absolute levels but rather the fluctuation of hydrogen peroxide correlates to lifespan [164]. This suggests that tight control of ROS fluctuation is more vital than minimizing ROS levels, hinting at the importance of redox signalling in lifespan determination.

10.8.2 Developmental Programmes Gone Wild

Taking together the (lack of) evidence for the oxidative damage theory in *C. elegans*, it seems that this theory is ageing badly and the call for paradigm shifts is getting louder. One such radically different view is that of Mikhail V. Blagosklonny, who proposed that ageing is a quasi-programme, a continuation of the developmental programme that is not switched off, becoming hyperfunctional and damaging [165]. A central player in this theory is the TOR (target of rapamycin) nutrient and mitogen-sensing pathway, a central pathway in development and anabolic growth. Inhibiting TOR activity by mutation or caloric restriction indeed increases lifespan in *C. elegans* [166, 167]. TOR-inhibition by rapamycin also increases lifespan

although this effect is dependent on the SKN-1-mediated stress response [168]. Ageing worms show several forms of hypertrophy at advanced age, such as post-reproductive yolk accumulation, oocyte stacking and endoreduplication, ectopic lipid deposition, excessive neurite outgrowths, cuticle hypertrophy, and excessive germline apoptosis [169]. These phenotypes are clearly in favour of the hyperfunction theory. A very related concept to this theory is developmental drift [170]. It is very likely that these theories will attract more experimental attention in the next few years.

Besides exploring alternative views on ageing, many researchers still attach to the oxidative damage theory, refining it according to the latest experimental evidence [171, 172]. An updated version of the oxidative stress theory, a number of misconceptions and rebuttals to criticisms are given in [173]. Others conclude that there is not enough evidence yet to accept or reject the oxidative damage theory and call for more rigorous testing in a broader range of species [174].

10.9 Relevance to Human Ageing

There is no doubt that *C. elegans* research has pushed forward molecular biogerontology over the last three decades. As a prime genetic model that ages fast and that is easily subjected to large-scale genetic screens, this species enabled us to track down genetic pathways that influence lifespan [175]. In many cases, these pathways appeared to be conserved and relate to ageing in other species as well [176]. Due to its complete transparency and the availability of strains expressing genetically encoded biosensors, *C. elegans* is currently the most accessible organism to study the role of ROS, in vivo and in real-time, in the ageing process of a multicellular organism. Hence, there are many reasons to continue *C. elegans* ageing research and undoubtedly thrilling discoveries about the molecular mechanisms of ageing lie ahead of us. Yet, this optimism should go hand in hand with necessary caution. We always need to bear in mind that some mechanisms may be private to *C. elegans* (or by extension, to nematodes) rather than public (i.e. valid for every animal species). Being an euryoxic ectotherm, *C. elegans* can cope well with changing environments and has a much more flexible metabolic network than mammals. For example, *C. elegans* has a fully functional glyoxylate cycle (specific to nematodes in the animal kingdom) and this pathway seems to be important in lifespan extension of Mit mutants and Insulin/IGF signalling mutants [47, 177, 178]. Also trehalose, a disaccharide absent in vertebrates [179], was shown to support lifespan extension in Insulin/IGF mutants [180]. Besides differences in biochemistry, *C. elegans* also lacks several systems such as the cardiovascular and adaptive immune system, that have been linked to age-related diseases in humans.

In conclusion, it is clear that *C. elegans* is not just a 1-mm human that ages 1300 times faster than us. Nevertheless, it is an ideal system for making very fast progress in the search for important molecular determinants of the animal ageing process that may serve as candidates for follow up studies in other models that are closer related to humans.

References

1. De Marais DJ (2000) Evolution. When did photosynthesis emerge on Earth? Science 289(5485):1703–1705
2. Halliwell B, Gutteridge J (2007) Free radicals in biology and medicine, 4th edn. Oxford University Press, Oxford
3. Muller F (2000) The nature and mechanism of superoxide production by the electron transport chain: its relevance to aging. J Am Aging Assoc 23(4):227–253. doi:10.1007/s11357-000-0022-9 22 [pii]
4. Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. Cell 120(4):483–495
5. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD (2002) Topology of superoxide production from different sites in the mitochondrial electron transport chain. J Biol Chem 277(47):44784–44790. doi:10.1074/jbc.M207217200 M207217200 [pii]
6. Liu Y, Fiskum G, Schubert D (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 80(5):780–787
7. Brand MD (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Exp Gerontol 35(6–7):811–820, doi:S0531-5565(00)00135-2 [pii]
8. Fisher AB (2009) Redox signaling across cell membranes. Antioxid Redox Signal 11(6):1349–1356. doi:10.1089/ARS.2008.2378
9. Brodie AE, Reed DJ (1987) Reversible oxidation of glyceraldehyde 3-phosphate dehydrogenase thiols in human lung carcinoma cells by hydrogen peroxide. Biochem Biophys Res Commun 148(1):120–125, doi:0006-291X(87)91084-9 [pii]
10. Mahadev K, Zilbering A, Zhu L, Goldstein BJ (2001) Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. J Biol Chem 276(24):21938–21942. doi:10.1074/jbc.C100109200 C100109200 [pii]
11. Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1):47–95. doi:10.1152/physrev.00018.2001
12. Czapski G (1984) Reaction of.OH. Methods Enzymol 105:209–215
13. Park S, Imlay JA (2003) High levels of intracellular cysteine promote oxidative DNA damage by driving the fenton reaction. J Bacteriol 185(6):1942–1950
14. Rowley DA, Halliwell B (1982) Superoxide-dependent formation of hydroxyl radicals from NADH and NADPH in the presence of iron salts. FEBS Lett 142(1):39–41. doi:0014-5793(82)80214-7 [pii]
15. Woodmansee AN, Imlay JA (2002) Reduced flavins promote oxidative DNA damage in non-respiring Escherichia coli by delivering electrons to intracellular free iron. J Biol Chem 277(37):34055–34066. doi:10.1074/jbc.M203977200 M203977200 [pii]
16. Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, Ellis RE, Marletta MA, Bargmann CI (2004) Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature 430(6997):317–322. doi:10.1038/nature02714 nature02714 [pii]
17. Schagger H, Pfeiffer K (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J 19(8):1777–1783. doi:10.1093/emboj/19.8.1777
18. McCord JM, Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244(22):6049–6055
19. Wuerges J, Lee JW, Yim YI, Yim HS, Kang SO, Djinovic Carugo K (2004) Crystal structure of nickel-containing superoxide dismutase reveals another type of active site. Proc Natl Acad Sci U S A 101(23):8569–8574. doi:10.1073/pnas.0308514101 0308514101 [pii]
20. Hoogewijs D, Houthoofd K, Matthijssens F, Vandesompele J, Vanfleteren JR (2008) Selection and validation of a set of reliable reference genes for quantitative sod gene expression analysis in *C. elegans*. BMC Mol Biol 9:9. doi:1471-2199-9-9 [pii] 10.1186/1471-2199-9-9

21. Fujii M, Ishii N, Joguchi A, Yasuda K, Ayusawa D (1998) A novel superoxide dismutase gene encoding membrane-bound and extracellular isoforms by alternative splicing in *C. elegans*. DNA Res: Int J Rapid Publ Rep Genes Genomes 5(1):25–30
22. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, Back P, Matscheski A, Vanfleteren JR, Gems D (2008) Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *C. elegans*. Genes Dev 22(23):3236–3241. doi:10.1101/gad.504808
23. Suthammarak W, Somerlot BH, Opheim E, Sedensky M, Morgan PG (2013) Novel interactions between mitochondrial superoxide dismutases and the electron transport chain. Aging Cell 12(6):1132–1140. doi:10.1111/acel.12144
24. Yanase S, Onodera A, Tedesco P, Johnson TE, Ishii N (2009) SOD-1 deletions in *C. elegans* alter the localization of intracellular reactive oxygen species and show molecular compensation. J Gerontol 64(5):530–539. doi:10.1093/gerona/glp020
25. Back P, Matthijssens F, Vlaeminck C, Braeckman BP, Vanfleteren JR (2010) Effects of sod gene overexpression and deletion mutation on the expression profiles of reporter genes of major detoxification pathways in *C. elegans*. Exp Gerontol 45(7–8):603–610. doi:S0531-5565(10)00048-3 [pii] 10.1016/j.exger.2010.01.014
26. Reid TJ 3rd, Murthy MR, Sicignano A, Tanaka N, Musick WD, Rossmann MG (1981) Structure and heme environment of beef liver catalase at 2.5 A resolution. Proc Natl Acad Sci U S A 78(8):4767–4771
27. Barrett J, Beis I (1982) Catalase in free-living and parasitic platyhelminthes. Experientia 38(5):536
28. Ishikawa T, Tajima N, Nishikawa H, Gao Y, Rapolu M, Shibata H, Sawa Y, Shigeoka S (2010) Euglena gracilis ascorbate peroxidase forms an intramolecular dimeric structure: its unique molecular characterization. Biochem J 426(2):125–134. doi:BJ20091406 [pii] 10.1042/BJ20091406
29. Petriv OI, Rachubinski RA (2004) Lack of peroxisomal catalase causes a progeric phenotype in *C. elegans*. J Biol Chem 279(19):19996–20001. doi:10.1074/jbc.M400207200
30. Togo SH, Maebuchi M, Yokota S, Bun-Ya M, Kawahara A, Kamiryo T (2000) Immunological detection of alkaline-diaminobenzidine-negative peroxisomes of the nematode *C. elegans* purification and unique pH optima of peroxisomal catalase. Eur J Biochem/FEBS 267(5):1307–1312
31. Vanfleteren JR (1993) Oxidative stress and ageing in *C. elegans*. Biochem J 292(Pt 2):605–608
32. Benner J, Daniel H, Spanier B (2011) A glutathione peroxidase, intracellular peptidases and the TOR complexes regulate peptide transporter PEPT-1 in *C. elegans*. PLoS ONE 6(9):e25624. doi:10.1371/journal.pone.0025624
33. Olahova M, Veal EA (2015) A peroxiredoxin, PRDX-2, is required for insulin secretion and insulin/IIS-dependent regulation of stress resistance and longevity. Aging Cell 14(4):558–568. doi:10.1111/acel.12321
34. Olahova M, Taylor SR, Khazaipoul S, Wang J, Morgan BA, Matsumoto K, Blackwell TK, Veal EA (2008) A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. Proc Natl Acad Sci U S A 105(50):19839–19844. doi:10.1073/pnas.0805507105
35. Labuschagne CF, Brenkman AB (2013) Current methods in quantifying ROS and oxidative damage in *C. elegans* and other model organism of aging. Ageing Res Rev 12(4):918–930. doi:S1568-1637(13)00066-4 [pii] 10.1016/j.arr.2013.09.003
36. Bartosz G (2006) Use of spectroscopic probes for detection of reactive oxygen species. Clin Chim Acta 368(1–2):53–76. doi:10.1016/j.cca.2005.12.039
37. Gomes A, Fernandes E, Lima JL (2005) Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods 65(2–3):45–80. doi:10.1016/j.jbbm.2005.10.003

38. Wardman P (2007) Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic Biol Med 43(7):995–1022. doi:10.1016/j.freeradbiomed.2007.06.026
39. Back P, Braeckman BP, Matthijssens F (2012) ROS in aging *C. elegans*: damage or signaling? Oxidative Med Cell Longev 2012:608478. doi:10.1155/2012/608478
40. Srikun D, Albers AE, Nam CI, Iavarone AT, Chang CJ (2010) Organelle-targetable fluorescent probes for imaging hydrogen peroxide in living cells via SNAP-Tag protein labeling. J Am Chem Soc 132(12):4455–4465. doi:10.1021/ja100117u
41. Cocheme HM, Quin C, McQuaker SJ, Cabreiro F, Logan A, Prime TA, Abakumova I, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RA, Saeed S, Carre JE, Singer M, Gems D, Hartley RC, Partridge L, Murphy MP (2011) Measurement of H2O2 within living Drosophila during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. Cell Metab 13(3):340–350. doi:10.1016/j.cmet.2011.02.003
42. Chattoraj M, King BA, Bublitz GU, Boxer SG (1996) Ultra-fast excited state dynamics in green fluorescent protein: multiple states and proton transfer. Proc Natl Acad Sci U S A 93(16):8362–8367
43. Meyer AJ, Dick TP (2010) Fluorescent protein-based redox probes. Antioxid Redox Signal 13(5):621–650. doi:10.1089/ars.2009.2948
44. Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, Wang X, Li K, Han P, Zheng M, Yin J, Mattson MP, Kao JP, Lakatta EG, Sheu SS, Ouyang K, Chen J, Dirksen RT, Cheng H (2008) Superoxide flashes in single mitochondria. Cell 134(2):279–290. doi:S0092-8674(08)00769-1 [pii] 10.1016/j.cell.2008.06.017
45. Huang Z, Zhang W, Fang H, Zheng M, Wang X, Xu J, Cheng H, Gong G, Wang W, Dirksen RT, Sheu SS (2011) Response to "A critical evaluation of cpYFP as a probe for superoxide". Free Radic Biol Med 51(10):1937–1940. doi:S0891-5849(11)00537-5 [pii] 10.1016/j.freeradbiomed.2011.08.024
46. Muller FL (2009) A critical evaluation of cpYFP as a probe for superoxide. Free Radic Biol Med 47(12):1779–1780. doi:S0891-5849(09)00545-0 [pii] 10.1016/j.freeradbiomed.2009.09.019
47. Shen EZ, Song CQ, Lin Y, Zhang WH, Su PF, Liu WY, Zhang P, Xu J, Lin N, Zhan C, Wang X, Shyr Y, Cheng H, Dong MQ (2014) Mitoflash frequency in early adulthood predicts lifespan in *C. elegans*. Nature 508(7494):128–132. doi:nature13012 [pii] 10.1038/nature13012
48. Schwarzlander M, Wagner S, Ermakova YG, Belousov VV, Radi R, Beckman JS, Buettner GR, Demaurex N, Duchen MR, Forman HJ, Fricker MD, Gems D, Halestrap AP, Halliwell B, Jakob U, Johnston IG, Jones NS, Logan DC, Morgan B, Muller FL, Nicholls DG, Remington SJ, Schumacker PT, Winterbourn CC, Sweetlove LJ, Meyer AJ, Dick TP, Murphy MP (2014) The 'mitoflash' probe cpYFP does not respond to superoxide. Nature 514(7523):E12–E14. doi:nature13858 [pii] 10.1038/nature13858
49. Cheng H, Wang W, Wang X, Sheu SS, Dirksen RT, Dong MQ (2014) Cheng et al. reply. Nature 514(7523):E14–E15. doi:nature13859 [pii] 10.1038/nature13859
50. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Terskikh AV, Lukyanov S (2006) Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. Nat Methods 3(4):281–286. doi:nmeth866 [pii] 10.1038/nmeth866
51. Markvicheva KN, Bilan DS, Mishina NM, Gorokhovatsky AY, Vinokurov LM, Lukyanov S, Belousov VV (2011) A genetically encoded sensor for H2O2 with expanded dynamic range. Bioorg Med Chem 19(3):1079–1084. doi:S0968-0896(10)00657-7 [pii] 10.1016/j.bmc.2010.07.014
52. Bilan DS, Pase L, Joosen L, Gorokhovatsky AY, Ermakova YG, Gadella TW, Grabher C, Schultz C, Lukyanov S, Belousov VV (2013) HyPer-3: a genetically encoded H(2)O(2) probe with improved performance for ratiometric and fluorescence lifetime imaging. ACS Chem Biol 8(3):535–542. doi:10.1021/cb300625g

53. Lukyanov KA, Belousov VV (2014) Genetically encoded fluorescent redox sensors. Biochim Biophys Acta 1840(2):745–756. doi:S0304-4165(13)00226-2 [pii] 10.1016/j.bbagen.2013.05.030
54. Tantama M, Hung YP, Yellen G (2011) Imaging intracellular pH in live cells with a genetically encoded red fluorescent protein sensor. J Am Chem Soc 133(26):10034–10037. doi:10.1021/ja202902d
55. Malinouski M, Zhou Y, Belousov VV, Hatfield DL, Gladyshev VN (2011) Hydrogen peroxide probes directed to different cellular compartments. PLoS ONE 6(1):e14564. doi:10.1371/journal.pone.0014564
56. Back P, De Vos WH, Depuydt GG, Matthijssens F, Vanfleteren JR, Braeckman BP (2012) Exploring real-time in vivo redox biology of developing and aging *C. elegans*. Free Radic Biol Med 52(5):850–859. doi:10.1016/j.freeradbiomed.2011.11.037
57. Knoefler D, Thamsen M, Koniczek M, Niemuth NJ, Diederich AK, Jakob U (2012) Quantitative in vivo redox sensors uncover oxidative stress as an early event in life. Mol Cell 47(5):767–776. doi:10.1016/j.molcel.2012.06.016
58. Edens WA, Sharling L, Cheng G, Shapira R, Kinkade JM, Lee T, Edens HA, Tang X, Sullards C, Flaherty DB, Benian GM, Lambeth JD (2001) Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. J Cell Biol 154(4):879–891. doi:10.1083/jcb.200103132 154/4/879 [pii]
59. Gutscher M, Sobotta MC, Wabnitz GH, Ballikaya S, Meyer AJ, Samstag Y, Dick TP (2009) Proximity-based protein thiol oxidation by H2O2-scavenging peroxidases. J Biol Chem 284(46):31532–31540. doi:M109.059246 [pii] 10.1074/jbc.M109.059246
60. Schwarzlander M, Fricker MD, Muller C, Marty L, Brach T, Novak J, Sweetlove LJ, Hell R, Meyer AJ (2008) Confocal imaging of glutathione redox potential in living plant cells. J Microsc 231(2):299–316. doi:JMI2030 [pii] 10.1111/j.1365-2818.2008.02030.x
61. Castelein N, Muschol M, Dhondt I, Cai H, De Vos WH, Dencher NA, Braeckman BP (2014) Mitochondrial efficiency is increased in axenically cultured *C. elegans*. Exp Gerontol 56:26–36. doi:S0531-5565(14)00057-6 [pii] 10.1016/j.exger.2014.02.009
62. Enyedi B, Zana M, Donko A, Geiszt M (2013) Spatial and temporal analysis of NADPH oxidase-generated hydrogen peroxide signals by novel fluorescent reporter proteins. Antioxid Redox Signal 19(6):523–534. doi:10.1089/ars.2012.4594
63. Ostergaard H, Henriksen A, Hansen FG, Winther JR (2001) Shedding light on disulfide bond formation: engineering a redox switch in green fluorescent protein. EMBO J 20(21):5853–5862. doi:10.1093/emboj/20.21.5853
64. Gutscher M, Pauleau AL, Marty L, Brach T, Wabnitz GH, Samstag Y, Meyer AJ, Dick TP (2008) Real-time imaging of the intracellular glutathione redox potential. Nat Methods 5(6):553–559. doi:nmeth.1212 [pii] 10.1038/nmeth.1212
65. Hung YP, Albeck JG, Tantama M, Yellen G (2011) Imaging cytosolic NADH-NAD(+) redox state with a genetically encoded fluorescent biosensor. Cell Metab 14(4):545–554. doi:S1550-4131(11)00342-1 [pii] 10.1016/j.cmet.2011.08.012
66. Zhao Y, Jin J, Hu Q, Zhou HM, Yi J, Yu Z, Xu L, Wang X, Yang Y, Loscalzo J (2011) Genetically encoded fluorescent sensors for intracellular NADH detection. Cell Metab 14(4):555–566. doi:S1550-4131(11)00350-0 [pii] 10.1016/j.cmet.2011.09.004
67. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11(3):298–300
68. Harman D (1972) The biologic clock: the mitochondria? J Am Geriatr Soc 20(4):145–147
69. Sohal RS, Weindruch R (1996) Oxidative stress, caloric restriction, and aging. Science 273(5271):59–63
70. Sohal RS (2002) Role of oxidative stress and protein oxidation in the aging process. Free Radic Biol Med 33(1):37–44, doi:S0891584902008560 [pii]
71. Johnson TE (2003) Advantages and disadvantages of *C. elegans* for aging research. Exp Gerontol 38(11–12):1329–1332, doi:S0531556503002870 [pii]

72. Johnson TE (2013) 25 years after age-1: genes, interventions and the revolution in aging research. Exp Gerontol 48(7):640–643. doi:S0531-5565(13)00063-6 [pii] 10.1016/j.exger.2013.02.023
73. Tissenbaum HA (2015) Using for aging research. Invertebr Reprod Dev 59(sup1):59–63. doi :10.1080/07924259.2014.940470 940470 [pii]
74. Adachi H, Fujiwara Y, Ishii N (1998) Effects of oxygen on protein carbonyl and aging in *C. elegans* mutants with long (age-1) and short (mev-1) life spans. J Gerontol A Biol Sci Med Sci 53(4):B240–B244
75. Yasuda K, Adachi H, Fujiwara Y, Ishii N (1999) Protein carbonyl accumulation in aging dauer formation-defective (daf) mutants of *C. elegans*. J Gerontol A Biol Sci Med Sci 54(2):B47–B51, discussion B52-43
76. Matthijssens F, Braeckman BP, Vanfleteren JR (2007) Evaluation of different methods for assaying protein carbonylation. Curr Anal Chem 3(2):93–102. doi:10.2174/157341107780361727
77. Yasuda K, Ishii T, Suda H, Akatsuka A, Hartman PS, Goto S, Miyazawa M, Ishii N (2006) Age-related changes of mitochondrial structure and function in *C. elegans*. Mech Ageing Dev 127(10):763–770. doi:S0047-6374(06)00166-7 [pii] 10.1016/j.mad.2006.07.002
78. Klass M, Nguyen PN, Dechavigny A (1983) Age-correlated changes in the DNA template in the nematode *C. elegans*. Mech Ageing Dev 22(3–4):253–263
79. Simpson VJ, Johnson TE, Hammen RF (1986) *C. elegans* DNA does not contain 5-methylcytosine at any time during development or aging. Nucleic Acids Res 14(16):6711–6719
80. Brys K, Castelein N, Matthijssens F, Vanfleteren JR, Braeckman BP (2010) Disruption of insulin signalling preserves bioenergetic competence of mitochondria in ageing *C. elegans*. BMC Biol 8:91. doi:1741-7007-8-91 [pii] 10.1186/1741-7007-8-91
81. Gruber J, Ng LF, Fong S, Wong YT, Koh SA, Chen CB, Shui G, Cheong WF, Schaffer S, Wenk MR, Halliwell B (2011) Mitochondrial changes in ageing *C. elegans* – what do we learn from superoxide dismutase knockouts? PLoS ONE 6(5):e19444. doi:10.1371/journal.pone.0019444 PONE-D-11-04291 [pii]
82. Melov S, Lithgow GJ, Fischer DR, Tedesco PM, Johnson TE (1995) Increased frequency of deletions in the mitochondrial genome with age of *C. elegans*. Nucleic Acids Res 23(8):1419–1425, doi:4a0746 [pii]
83. Ayyadevara S, Dandapat A, Singh SP, Siegel ER, Shmookler Reis RJ, Zimniak L, Zimniak P (2007) Life span and stress resistance of *C. elegans* are differentially affected by glutathione transferases metabolizing 4-hydroxynon-2-enal. Mech Ageing Dev 128(2):196–205. doi:10.1016/j.mad.2006.11.025
84. Tonna EA (1975) Accumulation of lipofuscin (age pigment) in aging skeletal connective tissues as revealed by electron microscopy. J Gerontol 30(1):3–8
85. Davis BO Jr, Anderson GL, Dusenbery DB (1982) Total luminescence spectroscopy of fluorescence changes during aging in *C. elegans*. Biochemistry 21(17):4089–4095
86. Gerstbrein B, Stamatas G, Kollias N, Driscoll M (2005) In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *C. elegans*. Aging Cell 4(3):127–137
87. Houthoofd K, Braeckman BP, Lenaerts I, Brys K, De Vreese A, Van Eygen S, Vanfleteren JR (2002) Ageing is reversed, and metabolism is reset to young levels in recovering dauer larvae of *C. elegans*. Exp Gerontol 37(8–9):1015–1021, doi:S0531556502000633 [pii]
88. Coburn C, Allman E, Mahanti P, Benedetto A, Cabreiro F, Pincus Z, Matthijssens F, Araiz C, Mandel A, Vlachos M, Edwards SA, Fischer G, Davidson A, Pryor RE, Stevens A, Slack FJ, Tavernarakis N, Braeckman BP, Schroeder FC, Nehrke K, Gems D (2013) Anthranilate fluorescence marks a calcium-propagated necrotic wave that promotes organismal death in *C. elegans*. PLoS Biol 11(7):e1001613. doi:10.1371/journal.pbio.1001613
89. Larsen PL (1993) Aging and resistance to oxidative damage in *C. elegans*. Proc Natl Acad Sci U S A 90(19):8905–8909

90. Honda Y, Honda S (1999) The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *C. elegans*. FASEB J 13(11):1385–1393
91. Houthoofd K, Fidalgo MA, Hoogewijs D, Braeckman BP, Lenaerts I, Brys K, Matthijssens F, De Vreese A, Van Eygen S, Munoz MJ, Vanfleteren JR (2005) Metabolism, physiology and stress defense in three aging Ins/IGF-1 mutants of the nematode *C. elegans*. Aging Cell 4(2):87–95. doi:ACE150 [pii] 10.1111/j.1474-9726.2005.00150.x
92. Johnson TE, de Castro E, Hegi de Castro S, Cypser J, Henderson S, Tedesco P (2001) Relationship between increased longevity and stress resistance as assessed through gerontogene mutations in *C. elegans*. Exp Gerontol 36(10):1609–1617, doi:S0531556501001449 [pii]
93. Stuart JA, Brown MF (2006) Energy, quiescence and the cellular basis of animal life spans. Comp Biochem Physiol A Mol Integr Physiol 143(1):12–23. doi:S1095-6433(05)00370-3 [pii] 10.1016/j.cbpa.2005.11.002
94. Lithgow GJ, Walker GA (2002) Stress resistance as a determinate of *C. elegans* lifespan. Mech Ageing Dev 123(7):765–771, doi:S0047637401004225 [pii]
95. de Castro E, Hegi de Castro S, Johnson TE (2004) Isolation of long-lived mutants in *C. elegans* using selection for resistance to juglone. Free Radic Biol Med 37(2):139–145. doi:10.1016/j.freeradbiomed.2004.04.021
96. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394(6694):694–697
97. Gems D, Doonan R (2009) Antioxidant defense and aging in *C. elegans*: is the oxidative damage theory of aging wrong? Cell Cycle 8(11):1681–1687, doi:8595 [pii]
98. Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, Calderwood SB, Ruvkun G, Ausubel FM (2003) Long-lived *C. elegans* daf-2 mutants are resistant to bacterial pathogens. Science 300(5627):1921
99. Yang W, Li J, Hekimi S (2007) A Measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of *C. elegans*. Genetics 177(4):2063–2074. doi:177/4/2063 [pii] 10.1534/genetics.107.080788
100. Honda Y, Tanaka M, Honda S (2008) Modulation of longevity and diapause by redox regulation mechanisms under the insulin-like signaling control in *C. elegans*. Exp Gerontol 43(6):520–529. doi:10.1016/j.exger.2008.02.009
101. Van Raamsdonk JM, Hekimi S (2010) Reactive oxygen species and aging in *C. elegans*: causal or casual relationship? Antioxid Redox Signal 13(12):1911–1953. doi:10.1089/ars.2010.3215
102. Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, Gems D (2011) Increased life span from overexpression of superoxide dismutase in *C. elegans* is not caused by decreased oxidative damage. Free Radic Biol Med 51(8):1575–1582. doi:10.1016/j.freeradbiomed.2011.07.020
103. Van Raamsdonk JM, Hekimi S (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *C. elegans*. PLoS Genet 5(2):e1000361. doi:10.1371/journal.pgen.1000361
104. Yen K, Patel HB, Lublin AL, Mobbs CV (2009) SOD isoforms play no role in lifespan in ad lib or dietary restricted conditions, but mutational inactivation of SOD-1 reduces life extension by cold. Mech Ageing Dev 130(3):173–178. doi:S0047-6374(08)00207-8 [pii] 10.1016/j.mad.2008.11.003
105. Van Raamsdonk JM, Hekimi S (2012) Superoxide dismutase is dispensable for normal animal lifespan. Proc Natl Acad Sci U S A 109(15):5785–5790. doi:1116158109 [pii] 10.1073/pnas.1116158109
106. Ranjan M, Gruber J, Ng LF, Halliwell B (2013) Repression of the mitochondrial peroxiredoxin antioxidant system does not shorten life span but causes reduced fitness in *C. elegans*.

Free Radic Biol Med 63:381–389. doi:S0891-5849(13)00235-9 [pii] 10.1016/j.freeradbiomed.2013.05.025
107. Jee C, Vanoaica L, Lee J, Park BJ, Ahnn J (2005) Thioredoxin is related to life span regulation and oxidative stress response in *C. elegans*. Genes Cells 10(12):1203–1210. doi:GTC913 [pii] 10.1111/j.1365-2443.2005.00913.x
108. Miranda-Vizuete A, Fierro Gonzalez JC, Gahmon G, Burghoorn J, Navas P, Swoboda P (2006) Lifespan decrease in a *C. elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons. FEBS Lett 580(2):484–490. doi:10.1016/j.febslet.2005.12.046
109. Collins JJ, Evason K, Kornfeld K (2006) Pharmacology of delayed aging and extended lifespan of *C. elegans*. Exp Gerontol 41(10):1032–1039. doi:S0531-5565(06)00221-X [pii] 10.1016/j.exger.2006.06.038
110. Adachi H, Ishii N (2000) Effects of tocotrienols on life span and protein carbonylation in *C. elegans*. J Gerontol 55(6):B280–B285
111. Benedetti MG, Foster AL, Vantipalli MC, White MP, Sampayo JN, Gill MS, Olsen A, Lithgow GJ (2008) Compounds that confer thermal stress resistance and extended lifespan. Exp Gerontol 43(10):882–891. doi:10.1016/j.exger.2008.08.049
112. Brown MK, Evans JL, Luo Y (2006) Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in *C. elegans*. Pharmacol Biochem Behav 85(3):620–628. doi:S0091-3057(06)00352-2 [pii]. 10.1016/j.pbb.2006.10.017
113. Harrington LA, Harley CB (1988) Effect of vitamin E on lifespan and reproduction in *C. elegans*. Mech Ageing Dev 43(1):71–78, doi:0047-6374(88)90098-X [pii]
114. Ishii N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS, Furukawa S (2004) Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. Mech Ageing Dev 125(1):41–46, doi:S0047637403001982 [pii]
115. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *C. elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6(4):280–293
116. Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, Guthke R, Platzer M, Kahn CR, Ristow M (2012) Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab 15(4):451–465. doi:10.1016/j.cmet.2012.02.013
117. Pun PB, Gruber J, Tang SY, Schaffer S, Ong RL, Fong S, Ng LF, Cheah I, Halliwell B (2010) Ageing in nematodes: do antioxidants extend lifespan in *C. elegans*? Biogerontology 11(1):17–30. doi:10.1007/s10522-009-9223-5
118. Keaney M, Matthijssens F, Sharpe M, Vanfleteren J, Gems D (2004) Superoxide dismutase mimetics elevate superoxide dismutase activity in vivo but do not retard aging in the nematode *C. elegans*. Free Radic Biol Med 37(2):239–250. doi:10.1016/j.freeradbiomed.2004.04.005 S0891584904003089 [pii]
119. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000) Extension of life-span with superoxide dismutase/catalase mimetics. Science 289(5484):1567–1569
120. Keaney M, Gems D (2003) No increase in lifespan in *C. elegans* upon treatment with the superoxide dismutase mimetic EUK-8. Free Radic Biol Med 34(2):277–282, doi:S089158490201290X [pii]
121. Kim J, Takahashi M, Shimizu T, Shirasawa T, Kajita M, Kanayama A, Miyamoto Y (2008) Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of *C. elegans*. Mech Ageing Dev 129(6):322–331. doi:S0047-6374(08)00050-X [pii] 10.1016/j.mad.2008.02.011
122. Sampayo JN, Olsen A, Lithgow GJ (2003) Oxidative stress in *C. elegans*: protective effects of superoxide dismutase/catalase mimetics. Aging Cell 2(6):319–326
123. Heidler T, Hartwig K, Daniel H, Wenzel U (2010) *C. elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. Biogerontology 11(2):183–195. doi:10.1007/s10522-009-9239-x

124. Lee SJ, Hwang AB, Kenyon C (2010) Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. Curr Biol 20(23):2131–2136. doi:S0960-9822(10)01374-6 [pii] 10.1016/j.cub.2010.10.057
125. Yang W, Hekimi S (2010) A mitochondrial superoxide signal triggers increased longevity in *C. elegans*. PLoS Biol 8(12):e1000556. doi:10.1371/journal.pbio.1000556
126. An JH, Blackwell TK (2003) SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. Genes Dev 17(15):1882–1893
127. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 389(6654):994–999
128. Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK (2008) Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132(6):1025–1038. doi:10.1016/j.cell.2008.01.030
129. Park SK, Tedesco PM, Johnson TE (2009) Oxidative stress and longevity in *C. elegans* as mediated by SKN-1. Aging Cell 8(3):258–269. doi:ACE473 [pii] 10.1111/j.1474-9726.2009.00473.x
130. Buchter C, Ackermann D, Honnen S, Arnold N, Havermann S, Koch K, Watjen W (2015) Methylated derivatives of myricetin enhance life span in *C. elegans* dependent on the transcription factor DAF-16. Food Funct. doi:10.1039/c5fo00463b
131. Pant A, Asthana J, Yadav AK, Rathor L, Srivastava S, Gupta MM, Pandey R (2015) Verminoside mediates life span extension and alleviates stress in *C. elegans*. Free Radic Res:1–9. doi:10.3109/10715762.2015.1075017.
132. Seo HW, Cheon SM, Lee MH, Kim HJ, Jeon H, Cha DS (2015) Catalpol modulates lifespan via DAF-16/FOXO and SKN-1/Nrf2 activation in *C. elegans*. Evid Based Complement Alternat Med 2015:524878. doi:10.1155/2015/524878
133. Su S, Wink M (2015) Natural lignans from Arctium lappa as antiaging agents in *C. elegans*. Phytochemistry 117:340–350. doi:S0031-9422(15)30033-9 [pii] 10.1016/j.phytochem.2015.06.021
134. Zhang Y, Lv T, Li M, Xue T, Liu H, Zhang W, Ding X, Zhuang Z (2015) Anti-aging effect of polysaccharide from Bletilla striata on nematode *C. elegans*. Pharmacogn Mag 11(43):449–454. doi:10.4103/0973-1296.160447 PM-11-449 [pii]
135. Oh SI, Park JK, Park SK (2015) Lifespan extension and increased resistance to environmental stressors by N-acetyl-L-cysteine in *C. elegans*. Clinics (Sao Paulo) 70(5):380–386. doi:S1807-59322015000500380 [pii] 10.6061/clinics/2015(05)13
136. Cascella R, Evangelisti E, Zampagni M, Becatti M, D'Adamio G, Goti A, Liguri G, Fiorillo C, Cecchi C (2014) S-linolenoyl glutathione intake extends life-span and stress resistance via Sir-2.1 upregulation in *C. elegans*. Free Radic Biol Med 73:127–135. doi:S0891-5849(14)00220-2 [pii] 10.1016/j.freeradbiomed.2014.05.004
137. Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow CA (2006) Blueberry polyphenols increase lifespan and thermotolerance in *C. elegans*. Aging Cell 5(1):59–68. doi:ACE192 [pii] 10.1111/j.1474-9726.2006.00192.x
138. Rea SL, Ventura N, Johnson TE (2007) Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *C. elegans*. PLoS Biol 5(10):e259
139. Feng J, Bussiere F, Hekimi S (2001) Mitochondrial electron transport is a key determinant of life span in *C. elegans*. Dev Cell 1(5):633–644, doi:S1534-5807(01)00071-5 [pii]
140. Schaar CE, Dues DJ, Spielbauer KK, Machiela E, Cooper JF, Senchuk M, Hekimi S, Van Raamsdonk JM (2015) Mitochondrial and cytoplasmic ROS have opposing effects on lifespan. PLoS Genet 11(2):e1004972. doi:10.1371/journal.pgen.1004972 PGENETICS-D-14-02541 [pii]
141. Ristow M, Zarse K (2010) How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Exp Gerontol 45(6):410–418. doi:S0531-5565(10)00128-2 [pii] 10.1016/j.exger.2010.03.014

142. Tapia PC (2006) Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: "Mitohormesis" for health and vitality. Med Hypotheses 66(4):832–843. doi:S0306-9877(05)00467-6 [pii] 10.1016/j.mehy.2005.09.009
143. Munkacsy E, Rea SL (2014) The paradox of mitochondrial dysfunction and extended longevity. Exp Gerontol 56:221–233. doi:S0531-5565(14)00088-6 [pii] 10.1016/j.exger.2014.03.016
144. Ristow M, Schmeisser S (2011) Extending life span by increasing oxidative stress. Free Radic Biol Med 51(2):327–336. doi:S0891-5849(11)00312-1 [pii] 10.1016/j.freeradbiomed.2011.05.010
145. Yang W, Hekimi S (2010) Two modes of mitochondrial dysfunction lead independently to lifespan extension in *C. elegans*. Aging Cell 9(3):433–447. doi:10.1111/j.1474-9726.2010.00571.x
146. Durieux J, Wolff S, Dillin A (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 144(1):79–91. doi:10.1016/j.cell.2010.12.016
147. Ventura N, Rea SL, Schiavi A, Torgovnick A, Testi R, Johnson TE (2009) p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress. Aging Cell 8(4):380–393. doi:10.1111/j.1474-9726.2009.00482.x
148. Bennett CF, Kaeberlein M (2014) The mitochondrial unfolded protein response and increased longevity: cause, consequence, or correlation? Exp Gerontol 56:142–146. doi:S0531-5565(14)00050-3 [pii] 10.1016/j.exger.2014.02.002
149. Dancy BM, Sedensky MM, Morgan PG (2014) Effects of the mitochondrial respiratory chain on longevity in *C. elegans*. Exp Gerontol 56:245–255. doi:S0531-5565(14)00119-3 [pii] 10.1016/j.exger.2014.03.028
150. Shore DE, Ruvkun G (2013) A cytoprotective perspective on longevity regulation. Trends Cell Biol 23(9):409–420. doi:10.1016/j.tcb.2013.04.007
151. Cypser JR, Johnson TE (2002) Multiple stressors in *C. elegans* induce stress hormesis and extended longevity. J Gerontol A Biol Sci Med Sci 57(3):B109–B114
152. Johnson TE, Henderson S, Murakami S, de Castro E, de Castro SH, Cypser J, Rikke B, Tedesco P, Link C (2002) Longevity genes in the nematode *C. elegans* also mediate increased resistance to stress and prevent disease. J Inherit Metab Dis 25(3):197–206
153. Darr D, Fridovich I (1995) Adaptation to oxidative stress in young, but not in mature or old, *C. elegans*. Free Radic Biol Med 18(2):195–201, doi:0891584994001184 [pii]
154. Hekimi S, Lapointe J, Wen Y (2011) Taking a "good" look at free radicals in the aging process. Trends Cell Biol 21(10):569–576. doi:S0962-8924(11)00134-6 [pii] 10.1016/j.tcb.2011.06.008
155. Honda Y, Honda S (2002) Life span extensions associated with upregulation of gene expression of antioxidant enzymes in *C. elegans*; studies of mutation in the age-1, PI3 kinase homologue and short-term exposure to hyperoxia. J Am Aging Assoc 25(1):21–28. doi:10.1007/s11357-002-0003-2 3 [pii]
156. Valentini S, Cabreiro F, Ackerman D, Alam MM, Kunze MB, Kay CW, Gems D (2013) Manipulation of in vivo iron levels can alter resistance to oxidative stress without affecting ageing in the nematode *C. elegans*. Mech Ageing Dev 133(5):282–290. doi:S0047-6374(12)00038-3 [pii] 10.1016/j.mad.2012.03.003
157. Droge W (2003) Oxidative stress and aging. Adv Exp Med Biol 543:191–200
158. De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, Braeckman BP, Schoofs L, Temmerman L (2014) Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc Natl Acad Sci U S A 111(24):E2501–2509. doi:1321776111 [pii] 10.1073/pnas.1321776111
159. Putker M, Madl T, Vos HR, de Ruiter H, Visscher M, van den Berg MC, Kaplan M, Korswagen HC, Boelens R, Vermeulen M, Burgering BM, Dansen TB (2013) Redox-dependent control of FOXO/DAF-16 by transportin-1. Mol Cell 49(4):730–742. doi:S1097-2765(12)01050-7 [pii] 10.1016/j.molcel.2012.12.014

160. De Henau S, Tilleman L, Vangheel M, Evi Luyckx E, Trashin S, Pauwels M, Germani F, Vlaeminck C, Vanfleteren JR, Bert W, Pesce A, Nardini M, Bolognesi M, De Wael K, Moens L, Dewilde S, Braeckman BP (2015) A redox signalling globin is essential for reproduction in *C. elegans*. Nature Commun 6:8782
161. Mishina NM, Tyurin-Kuzmin PA, Markvicheva KN, Vorotnikov AV, Tkachuk VA, Laketa V, Schultz C, Lukyanov S, Belousov VV (2011) Does cellular hydrogen peroxide diffuse or act locally? Antioxid Redox Signal 14(1):1–7. doi:10.1089/ars.2010.3539
162. Romero-Aristizabal C, Marks DS, Fontana W, Apfeld J (2014) Regulated spatial organization and sensitivity of cytosolic protein oxidation in *C. elegans*. Nat Commun 5:5020. doi:ncomms6020 [pii] 10.1038/ncomms6020
163. Sohal RS, Orr WC (2012) The redox stress hypothesis of aging. Free Radic Biol Med 52(3):539–555. doi:S0891-5849(11)01109-9 [pii] 10.1016/j.freeradbiomed.2011.10.445
164. Fu X, Tang Y, Dickinson BC, Chang CJ, Chang Z (2015) An oxidative fluctuation hypothesis of aging generated by imaging H(2)O(2) levels in live *C. elegans* with altered lifespans. Biochem Biophys Res Commun 458(4):896–900. doi:S0006-291X(15)00283-1 [pii] 10.1016/j.bbrc.2015.02.055
165. Blagosklonny MV (2006) Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. Cell Cycle 5(18):2087–2102, doi:3288 [pii]
166. Klass MR (1977) Aging in the nematode *C. elegans*: major biological and environmental factors influencing life span. Mech Ageing Dev 6(6):413–429
167. Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F (2003) Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature 426(6967):620. doi:10.1038/426620a 426620a [pii]
168. Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, Sabatini DM, Blackwell TK (2012) TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab 15(5):713–724. doi:S1550-4131(12)00147-7 [pii] 10.1016/j.cmet.2012.04.007
169. Gems D, de la Guardia Y (2013) Alternative perspectives on aging in *C. elegans*: reactive oxygen species or hyperfunction? Antioxid Redox Signal 19(3):321–329. doi:10.1089/ars.2012.4840
170. Lezzerini M, Smith RL, Budovskaya Y (2013) Developmental drift as a mechanism for aging: lessons from nematodes. Biogerontology 14(6):693–701. doi:10.1007/s10522-013-9462-3
171. Prasad KN, Bondy SC (2013) Evaluation of role of oxidative stress on aging in *C. elegans*: a brief review. Curr Aging Sci 6(3):215–219, doi:53119 [pii]
172. Liochev SI (2013) Free radical paradoxes. Free Radic Biol Med 65:232–233. doi:S0891-5849(13)00308-0 [pii] 10.1016/j.freeradbiomed.2013.06.027
173. Barja G (2013) Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. Antioxid Redox Signal 19(12):1420–1445. doi:10.1089/ars.2012.5148
174. Shi Y, Buffenstein R, Pulliam DA, Van Remmen H (2010) Comparative studies of oxidative stress and mitochondrial function in aging. Integr Comp Biol 50(5):869–879. doi:icq079 [pii] 10.1093/icb/icq079
175. Rodriguez M, Snoek LB, De Bono M, Kammenga JE (2013) Worms under stress: *C. elegans* stress response and its relevance to complex human disease and aging. Trends Genet 29(6):367–374. doi:S0168-9525(13)00022-X [pii]
176. Lapierre LR, Hansen M (2012) Lessons from *C. elegans*: signaling pathways for longevity. Trends Endocrinol Metab: TEM 23(12):637–644. doi:10.1016/j.tem.2012.07.007
177. Cristina D, Cary M, Lunceford A, Clarke C, Kenyon C (2009) A regulated response to impaired respiration slows behavioral rates and increases lifespan in *C. elegans*. PLoS Genet 5(4):e1000450. doi:10.1371/journal.pgen.1000450

178. Gallo M, Park D, Riddle DL (2011) Increased longevity of some *C. elegans* mitochondrial mutants explained by activation of an alternative energy-producing pathway. Mech Ageing Dev 132(10):515–518. doi:S0047-6374(11)00123-0 [pii] 10.1016/j.mad.2011.08.004
179. Danchin EG, Gouret P, Pontarotti P (2006) Eleven ancestral gene families lost in mammals and vertebrates while otherwise universally conserved in animals. BMC Evol Biol 6:5. doi:1471-2148-6-5 [pii] 10.1186/1471-2148-6-5
180. Honda Y, Tanaka M, Honda S (2010) Trehalose extends longevity in the nematode *C. elegans*. Aging Cell 9 (4):558–569. doi:ACE582 [pii] 10.1111/j.1474-9726.2010.00582.x