Review TRENDS in Neurosciences Vol.27 No.10 October 2004

Free radicals and aging

Gustavo Barja

Department of Animal Physiology-II, Faculty of Biology, Complutense University, Madrid 28040, Spain

Aging is characterized by decrements in maximum function and accumulation of mitochondrial DNA mutations, which are best observed in organs such as the brain that contain post-mitotic cells. Oxygen radicals are increasingly considered responsible for part of these aging changes. Comparative studies of animals with different aging rates have shown that the rate of mitochondrial oxygen radical generation is directly related to the steady-state level of oxidative damage to mitochondrial DNA and is inversely correlated with maximum longevity in higher vertebrates. The degree of unsaturation of tissue fatty acids also correlates inversely with maximum longevity. These are the two known traits connecting oxidative stress with aging. Furthermore, caloric restriction, which decreases the rate of aging, proportionately decreases mitochondrial oxygen radical generation, especially at complex I. These findings are reviewed, highlighting the results obtained in the brain.

The detrimental effects of aging are best observed in post-mitotic tissues, where cells that are irreversibly damaged or lost cannot be replaced by mitosis of intact ones. Among such tissues the brain is most important, owing to its main role in homeostasis of the organism. During normal aging, the brain suffers both morphological and functional modifications affecting dendritic trees and synapses, neurotransmitters, brain circulation and metabolism, motor and sensory systems, sleep, memory and learning, and lipofuscin accumulation [1]. The molecular basis of these changes is unknown but many studies implicate reactive oxygen species (ROS) and mitochondria [2].

In principle, oxidative stress could be related to aging through variations in ROS generation, ROS elimination, or both. However antioxidants, although possibly involved in protection against various age-related diseases, do not seem to control the rate of aging. There are four lines of evidence for this. First, contrary to early hypotheses, it is now well known that the endogenous levels of antioxidants in tissues including the brain do not decrease during aging [3,4]. Second, it is theoretically possible that the slow rate of aging of long-lived animals could be due to a constitutively higher antioxidant defense system. Surprisingly, when this was analyzed in detail the reverse was found. Most studies showed that the levels of antioxidant enzymes and low-molecular-weight antioxidants in the brain and other tissues correlate inversely with the species-specific maximum longevity of vertebrates [5]. The low antioxidant levels of long-lived animals indicate that their rate of ROS generation in *vivo* must also be low (and lower than that of short-lived animals), otherwise they could not maintain a level of oxidative stress homeostasis compatible with the maintenance of life.

A third source of information are studies in mammals in which levels of antioxidants are experimentally increased in the brain and other tissues through dietary supplementation, pharmacological induction or transgenic techniques [6–11]. The outcome of almost all such investigations, especially in the case of mammals, is that maximum longevity remains unaffected [4]. This is consistent with investigations performed in invertebrate models [12,13]. A recent re-evaluation of studies in insects concluded that increases in maximum longevity are observed only when the experiments are performed in short-lived in strains [13]. Thus, the effects observed are related to correction of a defect in each particular strain rather than to decreases in aging rate. A fourth line of evidence comes from studies in which genes encoding particular antioxidants are knocked out: the resulting animals can show different pathologies but their aging rates do not seem to be affected [14–16].

Increased mean lifespan is a much more frequent finding than increased maximum lifespan in antioxidant-treated or antioxidant-induced animals. These increases in mean lifespan suggest that antioxidants can non-specifically protect against many causes of early death – they can increase survival – especially when the experiments are performed under sub-optimum conditions. Part of this effect is related to the capacity of antioxidants to react inductively and then protect against increases in oxidative stress of exogenous origin. These protective effects can be very important in avoiding early death in human populations because they live in sub-optimum environmental conditions. But the general lack of effect of antioxidants on maximum longevity indicates that they do not slow down the endogenous aging process.

Mitochondrial oxygen radical generation

Although antioxidants do not determine the rate of aging, their negative correlation with maximum longevity indicates that the endogenous rate of free radical production should be lower in long-lived than in short-lived animals. Free radicals can be generated at many cellular sites but in healthy tissues a main free radical source is the mitochondrial respiratory chain. Thus, many studies have focused on the possible relationship between mitochondrial oxygen radical generation and maximum longevity.

Almost every investigation in this area has shown that the rate of ROS production of mitochondria isolated from

Corresponding author: Gustavo Barja (gbarja@bio.ucm.es).
Available online 27 July 2004

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.07.005

post-mitotic tissues including the brain is indeed lower in long-lived than in short-lived species [4]. This occurs in all kinds of long-lived homeothermic animals independently of their rates of oxygen consumption, which are low in mammals of large body size and high in birds of small size. This characteristic can explain why endogenous tissue antioxidants correlate negatively with maximum longevity: long-lived animals have constitutively low levels of antioxidants simply because they produce ROS at a low rate. The studies in birds are especially illustrative because these animals live much longer than mammals of similar body size and metabolic rate, which strongly disagrees with the rate-of-living theory of aging. However, in spite of their high rates of oxygen consumption, birds have low rates of mitochondrial free radical production in brain and other tissues. In many cases this is possible because the percentage of oxygen converted to ROS by their respiratory chain is lower than in short-lived mammals. Both small birds and large mammals have low rates of ROS production, in agreement with their slow aging rates, whereas metabolic rate is slow in large mammals but high in small birds. Thus, correlation of the mitochondrial rate of ROS generation with maximum longevity is better than that of metabolic rate with maximum longevity. Besides, the percentage of total electron flow directed to free radical generation is not a constant but can be different in different animals in relation to their longevity. This suggests that ROS generation is not a simple byproduct of mitochondrial respiration, as is frequently assumed. Instead, it is regulated in each species, as would be expected from a parameter controlling the endogenous rate of aging. A role for mitochondrial ROS production in the control of aging is also supported by current studies in invertebrates [17], fungi [18] and cultivated cells [19].

The site in the respiratory chain where ROS production is decreased in long-lived animals has been also studied. Oxygen radical generation at the respiratory chain has been classically attributed to complex III semiquinone [20]. However, in agreement with early information obtained in submitochondrial particles [21], complex I also contains an important ROS generator in intact functional heart and brain mitochondria [22–24]. This has been confirmed recently in rat brain and human parahippocampal gyrus [25]. Furthermore, the respiratory complex responsible for the lower mitochondrial ROS generation of a long-lived species (the pigeon) in relation to that of a short-lived one (the rat) is complex I, not complex III, because the difference in ROS production between both species using succinate as substrate disappears after the addition of rotenone [22].

Concerning the identity of the ROS generator inside complex I, some studies using the inhibitor diphenyliodonium are compatible with a role for flavin mononucleotide [25–27]. However, other investigations localize the ROS generator in the electron pathway inside complex I of heart and brain submitochondrial particles to between the ferricyanide reduction site and the rotenone-binding site. This discards flavin and suggests that the source of ROS might be the complex I FeS clusters situated in that region [28]. A similar conclusion was reached in studies from other laboratories using different approaches [24,29]. Treatment with diphenyliodonium interrupts electron flow to FeS clusters, which can explain the decreases in ROS generation observed. Because all FeS clusters of complex I are situated in the hydrophilic matrix domain of the complex, ROS arising from them will damage targets situated in the mitochondrial matrix, such as mitochondrial DNA (mtDNA), which is thought to be especially relevant for aging. Conversely, complex III ROS generation seems to be directed to the cytosolic side [30], mostly sparing mtDNA.

Oxidative damage to mtDNA

ROS can attack many different cellular macromolecules, including proteins, lipids and DNA. However damage to DNA must be most important for aging, especially in postmitotic cells such as neurons. mtDNA is situated very close to the site of mitochondrial ROS production. Because long-lived vertebrates have low rates of mitochondrial ROS generation, this should affect the level of oxidative damage in their mtDNA. In agreement with this (Figure 1), brain and heart mtDNA oxidative damage estimated by levels of 8-hydroxy-2′-deoxyguanosine (8-oxodG) correlates negatively with maximum longevity in mammals and birds, and this does not happen in the case of nuclear DNA (nDNA) [31]. In addition, the levels of 8-oxodG are higher in mtDNA than in nDNA in the brain and heart of many mammals and birds studied [4,31], also agreeing with the location of mtDNA very near to the sites of ROS production at mitochondria.

It is now known that 8-oxodG is actively repaired not only in the nucleus but also in the mitochondria. Thus, the findings concerning mitochondrial ROS production and

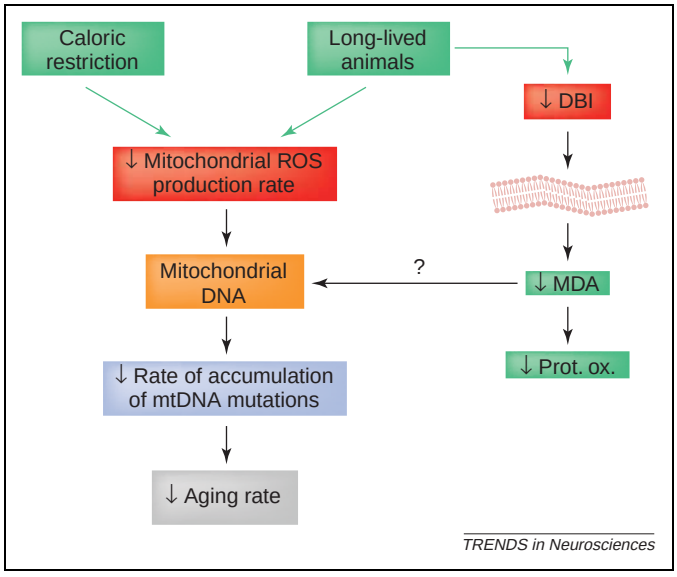

Figure 1. Summary of available results from mammals and birds relating to aging and oxidative stress. Both long-lived and calorie-restricted animals constitutively have low levels of production of mitochondrial reactive oxygen species (ROS), which could be responsible for their low rate of accumulation of mitochondrial DNA (mtDNA) mutations, and thus for their low rate of aging. Long-lived species also have low degrees of fatty acid unsaturation (DBI, double bond index) in their cellular membranes, and thus lower levels of lipid peroxidation (MDA, malondialdehyde) and lipoxidation-derived protein modification (Prot. ox.). This lower lipid peroxidation can also be partially responsible for the lower levels of oxidative damage in their mtDNA.

8-oxodG levels in mtDNA already described suggest that the flux of oxidative attack and repair through mtDNA is higher in short-lived than in long-lived animals, and is higher in the mtDNA of any species than in the nDNA [32]. Thus, the rate of oxygen radical attack to mtDNA is what seems to matter concerning differences in animal longevity [32]. Why would this be important if there is repair of 8-oxodG? It would be relevant because ROS can also cause many other kinds of DNA damage in addition to 8-oxodG generation, and some of these changes are not efficiently repaired and accumulate during aging. The higher rate of mitochondrial ROS production of short-lived animals can be an important cause of their much faster rate of accumulation of mtDNA mutations during aging (Figure 1); this accumulation occurs after 70–100 years in humans, but after 50 years in chimpanzees [33] and after only 2–3 years in mice [34].

Mutations in mtDNA, both deletions and point mutations, occur with aging in postmitotic tissues such as brain [35] and reach high levels in old individuals, especially in the control region responsible for mtDNA replication and transcription [36,37]. In these cells, the mutated mtDNA clonally expands towards predominance during aging. Pigmented neurons in aged substantia nigra can accumulate >50% deleted mtDNA [35]. Clonal expansion can explain why the mutations can have detrimental consequences for cellular function, despite the presence of many copies of each mitochondrial genome. These phenotypic alterations can include depressed respiration, enhanced radical formation and increased susceptibility to oxidative-stress-triggered apoptosis [38], segmental distribution of mutant mitochondria inside cells and secretion of extracellular ROS by mutated cells leading to damage of normal non-mutated cells [39]. Direct evidence that increases in mtDNA mutations increase aging rate has been recently obtained in mice [40]. In addition, because 8-oxodG is mutagenic, the higher 8-oxodG steady-state level present in the mtDNA of short-lived animals in relation to that of long-lived ones would also contribute to their higher rate of accumulation of mtDNA mutations. This is relevant because mitochondria continuously turnover even in post-mitotic quiescent cells [41,42]. Impaired lysosomal degradation of oxidatively damaged mitochondria can also contribute to aging [19].

Fatty acid unsaturation

In addition to ROS production, another constitutive characteristic of long-lived animals connects aging with oxidative stress: the degree of fatty acid unsaturation of tissue cellular membranes. Unsaturated fatty acids are the cellular macromolecules most sensitive to oxygen radical damage, owing to the presence of highly unstable electrons near their double bonds, and their sensitivity to lipid peroxidation exponentially increases as a function of the number of double bonds per molecule. Thus, a low level of fatty acid unsaturation will decrease cellular oxidative stress.

Many studies have shown that the degree of fatty acid unsaturation of mammalian tissues is indeed negatively correlated with maximum longevity [43] (Figure 1). It has been proposed that the low fatty acid unsaturation of large mammals would decrease their metabolic rates, because a low unsaturation will diminish the rates of passive ion leaks through membranes [44]. However, birds have low tissue fatty acid unsaturation despite their high metabolic rates [43]. Fatty acid unsaturation is low both in birds and in large mammals, in agreement with their high longevity. This characteristic constitutively protects them against lipid peroxidation [43,45]. Moreover, lipid peroxidation products can cause detrimental protein covalent modifications. In agreement with this, the low fatty acid unsaturation of long-lived mammals and birds is accompanied by low levels of malondialdehyde-lysine and carboxymethyl-lysine protein adducts in mitochondria [45] and tissues [46]. Recent studies indicate that experimentally induced increases in liver and brain fatty acid unsaturation also increase oxidative damage in mtDNA [47]. Further investigations are needed to establish whether the same occurs for mtDNA lipoxidation markers such as malondialdehyde-deoxyguanosine.

The low degree of fatty acid unsaturation of long-lived animals is not due to their diet. Instead, it is mainly a parameter homeostatically regulated at a different level in each species depending on its maximum longevity [43]. Detailed analysis of the fatty acid composition suggests that a main reason why the fatty acid unsaturation is low in slowly aging species is their possession of low tissue delta-5 and delta-6 desaturase activities. These enzymes are limiting for the biosynthesis of highly unsaturated long chain n-6 and n-3 fatty acids such as 20:4n-6 and 22:6n-3 from their precursors, 18:2n-6 and 18:3n-3. Thus, the tissues of long-lived animals typically have low 22:6n-3 and high 18:2n-6 levels, and sometimes low 20:4n-6 and high 18:3n-3 levels [43]. What differs between species with different longevities is not the total amount of unsaturated fatty acids but rather their degree of unsaturation. By maintaining unsaturation at a low level, oxidative damage to lipids, proteins and mtDNA is minimized, thus possibly contributing to decrease the aging rate.

Caloric restriction and mitochondrial oxidative stress

Although comparative studies are consistent with the mitochondrial free radical theory of aging, correlation does not necessarily indicate causation. Thus, studies in which the rate of aging is modified are needed to establish whether oxidative stress also changes in the expected direction. Caloric restriction (CR) is the only known experimental manipulation that decreases the rate of mammalian aging, and it has many beneficial effects on the brain of rodents and possibly of humans [48,49]. CR in laboratory rodents opposes the development of age-associated deficits in psychomotor and spatial memory tasks [50] and dendritic spine loss [51], and bolsters neuroprotective mechanisms [52]. Although fatty acid unsaturation does not seem to be consistently changed by CR, there is evidence that mitochondrial ROS production and oxidative damage to mtDNA are decreased by this anti-aging manipulation.

The role of mitochondrial ROS production in the effect of CR has been investigated in rat tissue [53–55]. Such studies show that CR significantly decreases the rate of

mitochondrial ROS generation in brain, heart and liver. This generally agrees with a previous report in mouse tissues, including brain [56]. By contrast, neither the expression [57] nor the activity [56] of the antioxidant enzymes superoxide dismutase (SOD), catalase or glutathione (GSH)-peroxidase are modified consistently by CR. In contrast to the situation in mice [56], the effect of CR in rats is not limited to suppression of increases in ROS production with age. Instead, mitochondrial ROS generation is strongly decreased by CR below the basal levels of young animals fed *ad libitum* [53]. Therefore, this decrease could be a basic mechanism responsible for the slow aging rate of calorie-restricted animals. Interestingly, the decrease in mitochondrial ROS generation observed in rats is accompanied by significant decreases in 8-oxodG levels in mtDNA, whereas levels in nDNA remain unchanged [53].

Although mitochondria can produce ROS at complexes I and III, CR decreases ROS production exclusively at complex I, because the decrease in oxygen radical generation occurs with pyruvate plus malate, but not with succinate plus rotenone, as substrate [53]. Furthermore, the mechanism allowing the decrease in ROS production during CR is not a simple decline in mitochondrial oxygen consumption because it stays unchanged. Instead, the percentage of total electron flow directed to ROS generation (the free radical leak) is decreased in CR. This shows that calorie-restricted mitochondria, similar to those of long-lived animal species, avoid more efficiently the generation of ROS. That capacity seems related to the degree of electronic reduction of the complex I ROS generator in the steady state. This is based on the observation that the lower ROS production of the restricted animals is observed with pyruvate plus malate alone (a condition leading to partial reduction of complex I) but not with pyruvate and malate plus rotenone (which fully reduces this respiratory complex).

ROS in neural systems and degenerative diseases

In agreement with a crucial role for ROS in brain aging [58], numerous studies currently highlight their relevance in neural cells and neurodegenerative diseases. It is well known that ROS can cause neuron [59] and astrocyte [60] death by both apoptosis and necrosis. Mitochondria are involved in excitotoxic nerve cell death through Ca^{2+}-related bursts of ROS production and opening of permeability transition pores [61]. Oxidative stress is also related to release of glutamate and continuous NMDA receptor activation during cerebral ischemia-reperfusion [62], production of superoxide radicals in neurons and brain macrophages [60], and glutamine-induced ROS production in astrocytes [63].

Evidence implicating ROS in major degenerative diseases is also consistent with their role in brain aging. Most studies agree that oxidative stress contributes to dopaminergic cell degeneration in Parkinson's disease [64]. It is most interesting in relation to findings, as already described here, that the pathogenesis of this illness includes impairment of mitochondrial function and deficiency of complex I activity [65]. Furthermore, the 1-methyl-4-phenylpyridinium ion (MPP+), which induces a Parkinson's-disease-like syndrome, is a complex I inhibitor that increases ROS generation in this complex [66]. Increases in levels of neuromelanin-associated redox-active iron and ROS production by microglial NADPH oxidase seem also to be involved in this disease. Many lines of evidence indicate that oxidative stress is also one of the earliest events in Alzheimer's disease [67]. Links between ROS and this illness include iron deposition in senile plaques and neurofibrillary tangles [67], iron-induced modification of tau phosphorylation by ROS [68], and direct association between amyloid-β deposits and ROS production *in vivo* [69]. Oxidative stress seems to be involved also in other neurodegenerative diseases, such as amyotrophic lateral sclerosis [70].

Concluding remarks

In summary, many lines of evidence suggest that ROS are involved in brain aging, neural cell death and neurodegenerative diseases. Only two characteristics controlling the level of oxidative stress correlate appropriately with the maximum longevity of animals: the rate of mitochondrial oxygen radical generation and the degree of unsaturation of membrane fatty acids. These two characteristics are maintained at comparatively low levels in long-lived birds and mammals, and might be major causes of their low rate of aging. The low rate of mitochondrial ROS production of long-lived animals is accompanied by low levels of steady-state oxidative damage in mtDNA. Calorie-restricted animals also show decreased levels of both mitochondrial ROS production and oxidative damage in mtDNA, although changes in fatty acid unsaturation are not implicated in the mechanism of action of this anti-aging dietary manipulation. The site and mechanism responsible for the decrease in mitochondrial ROS production seems to be strikingly similar in calorie-restricted and long-lived animals and implicates mitochondrial complex I. An unanswered question is whether experimentally decreasing ROS generation and/or fatty acid unsaturation increases maximum longevity. The first of these two manipulations seems more difficult to achieve at present without perturbing ATP production and other physiological mitochondrial functions. However, such further investigations are necessary to demonstrate the causal role of ROS in aging.

Acknowledgements

Supported in part by an I+D grant (SAF2002–01635) from the Spanish Ministry of Science and Technology.

References

1 Timiras, P.S. (2003) *Physiological Basis of Aging and Geriatrics*, CRC Press
2 Harman, D. (1999) Free radical theory of aging: increasing the average life expectancy at birth and the maximum life span. *J. Anti-aging Med.* 2, 199–208
3 Benzi, G. and Moretti, A. (1995) Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system. *Free Radic. Biol. Med.* 19, 77–101
4 Barja, G. (2004) Aging in vertebrates and the effect of caloric restriction: a mitochondrial free radical production–DNA damage mechanism? *Biol. Rev.* 79, 235–251

5 Pérez-Campo, R. *et al*. (1998) The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J. Comp. Physiol. [B]* 168, 149–158

6 Harris, S.B. *et al*. (1990) Dietary restriction alone and in combination with oral ethoxyquine/2-mercaptoethylamine in mice. *J. Gerontol.* 45, B141–B147

7 López-Torres, M. *et al*. (1993) Simultaneous induction of SOD, glutathione reductase, GSH and ascorbate in liver and kidney correlates with survival during aging. *Free Radic. Biol. Med.* 15, 133–142

8 Huang, T.T. *et al*. (2000) Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. *J. Gerontol.* 55, B5–B9

9 Jaarsma, D. *et al*. (2000) Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. *Neurobiol. Dis.* 7, 623–643

10 Mockett, R.J. *et al*. (2003) Ectopic expression of catalase in *Drosophila* mitochondria increases stress resistance but not longevity. *Free Radic. Biol. Med.* 34, 207–217

11 Orr, W.C. *et al*. (2003) Effects of overexpression of copper–zinc and manganese superoxide dismutases, catalase, and thioredoxin reductase genes on longevity in *Drosophila melanogaster*. *J. Biol. Chem.* 278, 26418–26422

12 Le Bourg, E. (2001) Oxidative stress, aging and longevity in *Drosophila melanogaster*. *FEBS Lett.* 498, 183–186

13 Orr, W.C. and Sohal, R.S. (2003) Does overexpression of Cu,Zn–SOD extend life span in *Drosophila melanogaster*? *Exp. Gerontol.* 38, 227–230

14 Shefner, J.M. *et al*. (1999) Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy. *Neurology* 53, 1239–1246

15 Ho, Y.S. *et al*. (1997) Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. *J. Biol. Chem.* 272, 16644–16651

16 Melov, S. *et al*. (1999) Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 846–851

17 Hekimi, S. and Guarente, L. (2003) Genetics and the specificity of the aging process. *Science* 299, 1351–1354

18 Osiewacz, H.D. (2002) Mitochondrial functions and aging. *Gene* 286, 65–71

19 Brunk, U.T. and Terman, A. (2002) The mitochondrial–lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. *Eur. J. Biochem.* 269, 1996–2002

20 Boveris, A. and Cadenas, E. (2000) Mitochondrial production of hydrogen peroxide regulation by nitric oxide and the role of ubisemiquinone. *IUBMB Life* 50, 245–250

21 Takeshige, K. and Minakami, S. (1979) NADH- and NADPH-dependent formation of superoxide anions by bovine heart submitochondrial particles and NAD–ubiquinone reductase preparation. *Biochem. J.* 180, 129–135

22 Herrero, A. and Barja, G. (1997) Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon. *Mech. Ageing Dev.* 98, 95–111

23 Barja, G. and Herrero, A. (1998) Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon. *J. Bioenerg. Biomembr.* 30, 235–243

24 Genova, M.L. *et al*. (2001) The site of production of superoxide radical in mitochondrial complex I is not a bound ubisemiquinone but presumably iron–sulphur cluster N2. *FEBS Lett.* 505, 364–368

25 Kudin, A.P. *et al*. (2004) Characterization of superoxide-producing sites in isolated brain mitochondria. *J. Biol. Chem.* 279, 4127–4135

26 Young, T.A. *et al*. (2002) Reactive oxygen species production by the mitochondrial respiratory chain in isolated rat hepatocytes and liver mitochondria: studies using myxothiazol. *Arch. Biochem. Biophys.* 405, 65–72

27 Liu, Y. *et al*. (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. *J. Neurochem.* 80, 780–787

28 Herrero, A. and Barja, G. (2000) Localization of the site of oxygen radical generation inside the complex I of heart and nonsynaptic brain mammalian mitochondria. *J. Bioenerg. Biomembr.* 32, 609–615

29 Kushnareva, Y. *et al*. (2002) Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state. *Biochem. J.* 368, 545–553

30 St-Pierre, J. *et al*. (2002) Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J. Biol. Chem.* 277, 44784–44790

31 Barja, G. and Herrero, A. (2000) Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J.* 14, 312–318

32 Barja, G. (2000) The flux of free radical attack through mitochondrial DNA is related to aging rate. *Aging Clin. Exp. Res.* 12, 342–355

33 Melov, S. *et al*. (1999) Mouse models of mitochondrial disease, oxidative stress, and senescence. *Mutat. Res.* 434, 233–242

34 Wang, E. *et al*. (1997) The rate of mitochondrial mutagenesis is faster in mice than in humans. *Mutat. Res.* 377, 157–166

35 Nekhaeva, E. *et al*. (2002) Clonally expanded mtDNA mutations are abundant in individual cells of human tissues. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5521–5526

36 Kraytsberg, Y. *et al*. (2003) Mutation and intracellular clonal expansion of mitochondrial genomes: two synergistic components of the aging process. *Mech. Ageing Dev.* 124, 49–53

37 Chomyn, A. and Attardi, G. (2003) MtDNA mutations in aging and apoptosis. *Biochem. Biophys. Res. Commun.* 304, 519–529

38 Szibor, M. and Holtz, J. (2003) Mitochondrial aging. *Basic Res. Cardiol.* 98, 210–218

39 de Grey, A.D. (2002) The reductive hot spot hypothesis of mammalian aging: membrane metabolism magnifies mutant mitochondrial mischief. *Eur. J. Biochem.* 269, 2003–2009

40 Trifunovic, A. *et al*. (2004) Premature aging in mice expressing defective mitochondrial DNA polymerase. *Nature* 429, 417–423

41 de Grey, A.D. (1999) Mitochondrial turnover. In *The Mitochondrial Free Radical Theory of Aging*. pp. 30–33, R.G. Landes Co., Austin, TX, USA

42 Sullivan, P.G. *et al*. (2004) Proteasome inhibition alters neural mitochondrial homeostasis and mitochondrial turnover. *J. Biol. Chem.* 279, 20699–20707

43 Pamplona, R. *et al*. (2002) Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span. *Ann. N. Y. Acad. Sci.* 959, 475–490

44 Hulbert, A.J. *et al*. (2002) The acyl composition of mammalian phospholipids: an allometric analysis. *Comp. Biochem. Physiol.* 132, 515–527

45 Pamplona, R. *et al*. (1999) A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. *Mech. Ageing Dev.* 106, 283–296

46 Pamplona, R. *et al*. (2000) Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life spans. *J. Gerontol.* 55, B286–B291

47 Pamplona, R. *et al*. (2004) Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain. *Exp. Gerontol.* 39, 725–733

48 Mattson, M.P. *et al.* (2003) Cellular and molecular mechanisms whereby dietary restriction extends healthspan: a beneficial type of stress. In *Energy Metabolism and Lifespan Determination. Advances in Cell Aging and Gerontology.* (Vol. 14) (Mattson, M.P. ed.), pp. 87–103, Elsevier

49 Weindruch, R. (2003) Caloric restriction: life span extension and retardation of brain aging. *Clin. Neurosci. Res.* 2, 279–284

50 Ingram, D.K. *et al*. (1987) Dietary restriction benefits learning and motor performance of aged mice. *J. Gerontol.* 42, 78–81

51 Moroi-Fetters, S.E. (1989) Dietary restriction suppresses age-related changes in dendritic spines. *Neurobiol. Aging* 10, 317–322

52 Mattson, M.P. *et al*. (2002) Modification of brain aging and neurodegenerative disorders by genes, diet and behavior. *Physiol. Rev.* 82, 637–672

53 Gredilla, R. *et al*. (2001) Caloric restriction decreases mitochondrial free radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat heart. *FASEB J.* 15, 1589–1591

54 Gredilla, R. *et al*. (2001) Effect of short-term caloric restriction on H_2O_2 production and oxidative DNA damage in rat liver mitochondria, and location of the free radical source. *J. Bioenerg. Biomembr.* 33, 279–287

55 López-Torres, M. *et al*. (2002) Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. *Free Radic. Biol. Med.* 32, 882–889
56 Sohal, R.S. *et al*. (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech. Ageing Dev.* 74, 121–133
57 Weindruch, R. *et al*. (2001) Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J. Nutr.* 131, 918S–923S
58 Poon, H.F. *et al*. (2004) Free radicals: key to brain aging and heme oxygenase as a cellular response to oxidative stress. *J. Gerontol.* 59, 478–493
59 Sonee, M. (2003) The effect of tertiary butilhydroperoxide and nicotinamide on human cortical neurons. *Neurotoxicology* 24, 443–448
60 Rouach, N. *et al*. (2004) Hydrogen peroxide increases gap junctional communication and induces a astrocyte toxicity: regulation by brain macrophages. *Glia* 45, 28–38
61 Hattori, F. *et al*. (2003) Mitochondrial peroxiredoxin-3 protects hippocampal neurons from excitotoxic injury *in vivo*. *J. Neurochem.* 86, 860–868
62 Milusheva, E. *et al*. (2003) Non-synaptic release of [3H]noradrenaline in response to oxidative stress combined with mitochondrial dysfunction in rat hippocampal slices. *Neuroscience* 120, 177–181
63 Jayakumar, A.R. *et al*. (2004) Glutamine-induced free radical production in cultured astrocytes. *Glia* 46, 296–301
64 Jenner, P. (2003) Oxidative stress in Parkinson's disease. *Ann. Neurol.* 53, S26–S36
65 Beal, M.F. (2003) Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann. N. Y. Acad. Sci.* 991, 120–131
66 Hoglinger, G.U. *et al*. (2003) Dysfunction of mitochondrial complex I and the proteasome: interactions between two biochemical deficits in a cellular model of Parkinson's disease. *J. Neurochem.* 86, 1297–1307
67 Honda, K. *et al*. (2004) Oxidative stress and redox-active iron in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 1012, 179–182
68 Egana, J.T. *et al*. (2003) Iron-induced oxidative stress modify tau phosphorylation patterns in hippocampal cell cultures. *Biometals* 16, 215–223
69 McLellan, M.E. (2003) *In vivo* imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. *J. Neurosci.* 23, 2212–2217
70 Liochev, S.I. and Fridovich, I. (2003) Mutant Cu,Zn superoxide dismutases and familial amyotrophic lateral sclerosis: evaluation of oxidative hypotheses. *Free Radic. Biol. Med.* 34, 1383–1389