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Invited mini-review

Mitochondria-targeted redox probes as tools in the study of oxidative damage and ageing

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Abstract

Mitochondrial reactive oxygen species (ROS) and oxidative damage are associated with a range of age-related human pathologies. It is also likely that mitochondrial ROS generation is a factor in stress response and signal transduction pathways. However, current methods for measuring and influencing mitochondrial ROS production in vivo often lack the desired specificity. To help elucidate the potential role of mitochondrial ROS production in ageing, we have developed a range of mitochondria-targeted ROS probes that may be useful in vivo. This was achieved by covalently attaching a lipophilic cation to a ROS-reactive moiety causing its membrane potential-dependent accumulation within mitochondria. Mitochondria-targeted molecules developed so far include antioxidants that detoxify mitochondrial ROS, probes that react with mitochondrial ROS, and reagents that specifically label mitochondrial protein thiols. Here, we outline how the formation and consequences of mitochondrial ROS production can be investigated using these probes.

Keywords: Coenzyme Q; Mitochondria; Reactive oxygen species; Antioxidants; Oxidative stress; Lipophilic cations; Redox signalling

1. Introduction

An unavoidable fact of life is eventual death, caused by a progressive degeneration of our biological functions with age, yet how ageing occurs is still poorly understood. One mechanism that is thought to contribute to this age-associated degeneration is the production of free radicals by the mitochondrial respiratory chain, which cause oxidative damage to mitochondrial DNA (mtDNA), protein and lipid, and consequent progressive cellular dysfunction. The mitochondrial free radical theory of ageing is an attractive hypothesis with much correlative experimental evidence supporting it. For example, there are a wide range of age-dependent human pathologies that are associated with oxidative damage to protein, DNA and lipid (Beal, 2003; Green et al., 2004; Sastre et al., 2003; Vina et al., 2004). The maximum lifespan of higher mammals and birds inversely correlates with the rate of mitochondrial ROS generation as well as oxidative damage to mtDNA but not nuclear DNA (Barja, 2004a,b; Barja and Herrero, 2000). A defective mitochondrial polymerase that generates mtDNA mutations at a higher rate accelerates ageing in mice (Trifunovic et al., 2004). However, despite these findings, there are ongoing disagreements in the literature about whether oxidative damage to mitochondria is a major cause of ageing or whether it simply correlates with ageing (Jacobs, 2003; Bokov et al., 2004).

If mitochondrial oxidative damage is central to ageing, then it is likely to play a role in the most robust mechanism for increasing lifespan in mammals, which is calorie restriction (CR). In the 1930s, it was recognised that a diet low in calories (25–60% below ad libitum fed controls), but that does not induce malnutrition, extends the lifespan of rodents (McCay et al., 1935). Today, CR is reported to extend the maximum lifespan of organisms ranging from yeast to rats (Koubova and Guarente, 2003) and may also do so in primates (Roth et al., 2004). How CR extends lifespan remains unclear (Koubova and Guarente, 2003), but CR reportedly decreases oxidative

Abbreviations: CR, calorie restriction; GPx, glutathione peroxidase; mtDNA, mitochondrial DNA; PBN, phenylbutylnitrone; ROS, reactive oxygen species; SOD, superoxide dismutase; TPP, triphenylphosphonium cation

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damage and mitochondrial ROS production, at least in some systems (Lee and Yu, 1990). Therefore, it is possible that some of the effects of CR are due to changes in mitochondrial oxidative damage. Since there is evidence associating increased mitochondrial ROS and oxidative damage with ageing, decreased mitochondrial ROS and oxidative damage with CR, and increased maximum lifespan with CR, why has it been so hard to determine whether mitochondrial ROS contribute to normal ageing and whether a decrease in mitochondrial oxidative damage underlies life extension by CR? The rest of this review tries to answer this question and to suggest possible ways forward in unravelling the role of mitochondrial ROS in ageing and CR.

2. What are ROS?

2.1. ROS generation

Although the precise mechanisms of mitochondrial superoxide ($O_2^{\bullet -}$) formation in vivo remain unclear, some conclusions are nevertheless possible (Brand et al., 2004; Green et al., 2004). Mitochondria are predisposed to $O_2^{\bullet -}$ production when they are respiring but not making ATP (state 4). Under these conditions electron carriers, such as the Coenzyme Q (CoQ) pool, become highly reduced and the large proton motive force causes reverse electron flow through complex I and may also prolong ubisemiquinone lifetime within complex III. This contrasts with mitochondria that are actively making ATP (state 3), where the lower proton motive force increases the oxidation of electron carriers thereby decreasing $O_2^{\bullet -}$ production.

2.2. ROS and their properties

ROS are not a single entity, instead they comprise a group of chemically diverse molecules (e.g. superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and peroxynitrite ($ONOO^-$)) that are grouped together for simplicity (Fig. 1). Although it is true that most originate from $O_2^{\bullet -}$ generated as a by-product of oxidative phosphorylation, they differ in their mechanism of production, cofactors, diffusion range, hydrophobicity, biological targets, detoxification pathways and breakdown products. $O_2^{\bullet -}$ is membrane impermeant and its damaging reactions largely involve disassembly of iron–sulphur clusters in proteins such as aconitase (Vasquez-Vivar et al., 2000). In contrast, its conjugate acid, the hydroperoxyl radical ($HO_2^{\bullet}$; $pK_a \sim 4.8$) is membrane permeable and sufficiently reactive to initiate lipid peroxidation (Antunes et al., 1996). $O_2^{\bullet -}$ is dismutated to H_2O_2 by cytosolic (Cu/ZnSOD) and mitochondrial (MnSOD) superoxide dismutases as well as spontaneously dismutating with $HO_2^{\bullet}$. $O_2^{\bullet -}$ can also reduce haem groups in proteins (Azzi et al., 1975) and rapidly react with nitric oxide ($NO^{\bullet}$) to produce $ONOO^-$ (Beckman et al., 1990). $NO^{\bullet}$ diffuses easily into mitochondria and may also

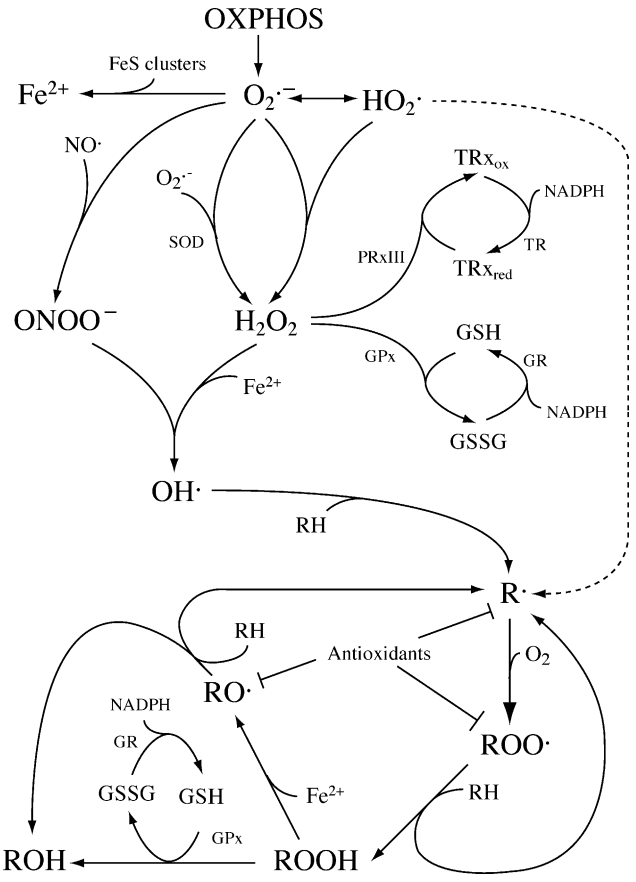

Fig. 1. Reactions of mitochondrial ROS. Superoxide ($O_2^{\bullet -}$) produced by the mitochondrial respiratory chain can react with iron–sulphur proteins to produce ferrous iron, react with nitric oxide ($NO^{\bullet}$) to form peroxynitrite ($ONOO^-$), be dismutated by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2) or be protonated to the hydroperoxyl radical ($HO_2^{\bullet}$). H_2O_2 can be degraded by peroxiredoxin III (PRxIII) or by glutathione peroxidases (GPx) which use thioredoxin (Trx) or glutathione (GSH) as electron donors, respectively. H_2O_2 breaks down to form the hydroxyl radical ($OH^{\bullet}$) in the presence of ferrous iron. The breakdown of $ONOO^-$ produces a species of equivalent reactivity to $OH^{\bullet}$. $OH^{\bullet}$ and possibly $HO_2^{\bullet}$ can remove $H^{\bullet}$ from lipids (RH) initiating formation of peroxolipids ($ROO^{\bullet}$) which can start a cycle of lipid peroxidation that includes the formation of peroxides (ROOH) and alkoxy radicals ($RO^{\bullet}$). These can be detoxified to alcohols (ROH) by the action of antioxidants and glutathione peroxidase. The dotted line indicates that while $HO_2^{\bullet}$ may be able to abstract $H^{\bullet}$ from lipids, strong experimental evidence for this occuring in vivo is lacking. GR, glutathione reductase; OXPHOS, oxidative phosphorylation; TR, thioredoxin reductase; TRx, thioredoxin.

be produced there (Ghafourifar and Colton, 2003; Ghafourifar and Richter, 1997; Tatoyan and Giulivi, 1998). The H_2O_2 produced by $O_2^{\bullet -}$ dismutation is membrane permeant and relatively unreactive. It is degraded by glutathione peroxidase (GPx) and peroxiredoxin III causing thiol oxidation on glutathione and thioredoxin, which may also be used as a regulatory signal (Finkel, 2003). H_2O_2 is also broken down in the presence of Fe^{2+} to form $OH^{\bullet}$, which is highly reactive and non-diffusible, causing non-specific damage to all forms of biological molecule near the site of its production. $OH^{\bullet}$ is not detoxified per se, rather its production is controlled by limiting the concentration of

free Fe^{2+}. $ONOO^-$ and $OH^\bullet$ are strong oxidants, leading to a chain reaction of lipid peroxidation, and protein and DNA modification. This chain reaction is broken by recyclable lipid- and water-soluble antioxidants, such as CoQ and Vitamin E. Thus, once $O_2^{\bullet -}$ is generated it has a number of fates, each affected by different cofactors and detoxification mechanisms with subtly different implications for the cell.

2.3. Problems with ROS detection

Reliable assays do not exist for all relevant species in all experimental systems leading to conclusions that are highly dependent on what was or could be measured. As an example, H_2O_2 is commonly measured as an indicator of ROS yet an increase in free Fe^{2+} or $NO^\bullet$, or a decrease in CoQ, could elevate oxidative damage without a rise in the concentration of $O_2^{\bullet -}$ or H_2O_2. The confusion is heightened by the techniques for measuring ROS, as they either depend on redox reactive probes that are somewhat non-specific and artefact-prone, or they rely on oxidative modifications or redox signalling that are several steps removed from the initial ROS generation event. For example, increased SOD or GPx mRNA or protein levels are frequently used to infer increased ROS generation. Yet this assumes that their expression is directly responsive to ROS, when in reality it is likely to be regulated via redox state and to be affected by a variety of other factors.

Furthermore, the location of ROS generation is important, as there is evidence that ROS produced in mitochondria may be of more importance for ageing than those formed in the cytosol. This primarily comes from the milder phenotype of mice and cells in which the cytosolic (Cu/Zn SOD) rather than the mitochondrial (MnSOD) forms of SOD are knocked out (Huang et al., 1997). However, most experimental techniques are incapable of measuring mitochondrial ROS or oxidative damage specifically without mitochondrial isolation. This also has the potential to produce artefacts, through oxidative damage during isolation and because dysfunctional mitochondria are swollen and rupture more easily.

2.4. Summary

In summary, many diverse pro-oxidant and antioxidant processes occur in mitochondria (Fig. 1). Oxidative damage takes place whenever the ROS produced by mitochondria evade detoxification and the steady state level of oxidative damage depends on the relative rates of damage accumulation, repair and degradation (James and Murphy, 2002; Sies, 1993). Although mitochondrial ROS production may be involved in redox signalling from mitochondria to the rest of the cell (Finkel, 2003), considerable unknowns remain about the nature and significance of individual mitochondrial ROS and their targets. Some of this uncertainty arises because current experimental techniques for measuring mitochondrial ROS are not specific to a type of ROS or a mitochondrial location. To help resolve these issues we have developed molecules that enable us to manipulate mitochondrial ROS production selectively and thereby infer their significance in biological processes. This is done by using antioxidants and other probe molecules that accumulate within mitochondria.

3. Mitochondria-targeted ROS probes and antioxidants

ROS probes and antioxidants can be delivered selectively to mitochondria by covalently attaching them to the lipophilic triphenylphosphonium cation (TPP) through an alkyl chain (Murphy, 1997; Murphy and Smith, 2000; Smith et al., 2003) (Fig. 2). The delocalised positive charge of lipophilic cations enables them to easily permeate phospholipid bilayers, leading to a several hundred-fold accumulation within mitochondria due to the large membrane potential (−150 to −170 mV, negative inside) generated by mitochondria during oxidative phosphorylation (Murphy and Smith, 2000; Smith et al., 2003). Cellular uptake from the extracellular medium is further driven by the plasma membrane potential (−30 to −60 mV, negative inside).

3.1. Mitochondria-targeted antioxidants

As the natural antioxidants Vitamin E and CoQ are thought to protect mitochondria from oxidative damage in vivo, mitochondria-targeted derivatives of these molecules were first developed. In vitro experiments show that MitoVit E and MitoQ are rapidly and selectively accumulated by isolated mitochondria, and by mitochondria within isolated

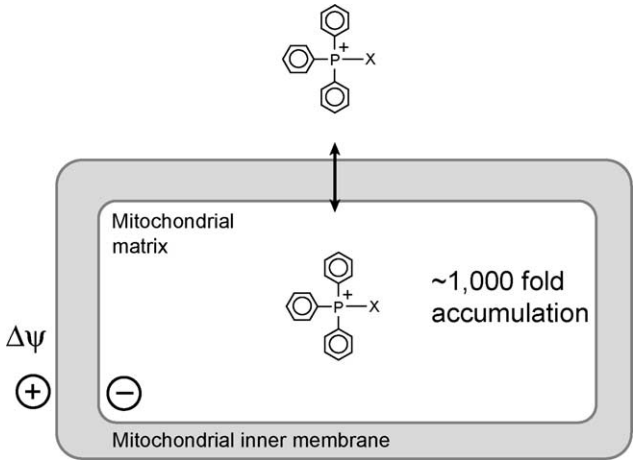

Fig. 2. Accumulation of mitochondria-targeted probes by energised mitochondria. The TPP cation is shown attached to a probe moiety (X) which could be an antioxidant such as ubiquinol, PBN, or a thiol probe such as an iodoalkyl moiety. These lipophilic cations easily permeate into mitochondria driven by the large membrane potential across the inner membrane ($\Delta\psi$).

cells (Echtay et al., 2002; Kelso et al., 2001; Smith et al., 1999). Importantly, the accumulation of these antioxidants by mitochondria protected them from oxidative damage far more effectively than untargeted antioxidants (Kelso et al., 2001). Furthermore, these compounds were several hundred-fold more effective at preventing cell death in fibroblasts from Friedreich's ataxia patients than non-targeted derivatives (Jauslin et al., 2003). This suggests that the accumulation of antioxidants within mitochondria does increase their efficacy. In addition, we have recently developed a mitochondria-targeted version of the carbon-centred radical spin trap phenylbutylnitrone (PBN) (Murphy et al., 2003). Furthermore, mitochondria-targeted antioxidants have also been shown to modulate the role of mitochondrial ROS production in putative redox signalling pathways (Bedogni et al., 2003; Chen et al., 2004; Dhanasekaran et al., 2004; Hwang et al., 2001; Schafer et al., 2003) and prevent telomere shortening (Saretzki et al., 2003).

Ageing is a slow process best studied in intact organisms. Therefore for mitochondria-targeted molecules to be used as probes of mitochondrial ROS production in ageing, they must be accumulated by mitochondria in vivo. TPP cations accumulate in mitochondria of all tissues as they pass easily through phospholipid bilayers by non-carrier mediated transport. This contrasts with hydrophilic compounds, which often depend on tissue-specific transport pathways for uptake (Murphy, 2001). When mice were fed mitochondria-targeted antioxidants for several weeks, this lead to stable steady-state concentrations within all tissues assessed, including the brain, heart, liver and kidneys (Smith et al., 2003). Mass spectrometry showed that they distribute to tissues in their intact, active form. After several days of feeding, the cation concentration within mitochondria came to a steady state distribution with circulating blood levels. Furthermore, the rapid clearance of the simple lipophilic cation methyltriphenylphosphonium (TPMP) from all organs when oral administration stopped showed that this was reversible (Smith et al., 2003).

3.2. Mitochondria-targeted thiol probes

Changes to the redox state of mitochondrial thiol proteins are likely to be of significance in the response of mitochondria to oxidative stress (Beer et al., 2004). To explore this possibility, we developed mitochondria-targeted thiol reagents which comprise the triphenylphosphonium cation attached to a thiol-reactive moiety (Coulter et al., 2000). 4-Thiolbutyltriphenylphosphonium binds reversibly as a disulfide (Burns and Murphy, 1997; Burns et al., 1995) while 4-iodobutyltriphenylphosphonium binds permanently as a thioether (Lin et al., 2002) to thiols on mitochondrial proteins, enabling their detection using antiserum against TPP (Lin et al., 2002). This labelling is dramatically affected by redox alterations to thiols, enabling redox active thiol proteins to be assessed (Lin et al., 2002). So far this procedure has been used to localise redox active thiols on respiratory complex I (Lin et al., 2002; Taylor et al., 2003).

4. Conclusions

Probes for ROS frequently have specificity problems and technical limitations when used in cells and in vivo. The development of mitochondria-targeted reagents is at a preliminary stage, even so they have already proven useful tools in manipulating mitochondrial ROS production in isolated mitochondria and cells. Data suggest that it may also be possible to extend this approach to in vivo situations. However, much still needs to be determined about the basic chemistry and interactions with mitochondria of the small number of probes developed to date. In addition, a wide range of more specific antioxidants and ROS-reactive probes are under development. The hope is that these more specific tools will extend current work and help elucidate the nature and significance of particular ROS in mitochondrial oxidative damage and redox signalling and thereby help unravel the role of mitochondrial ROS in ageing. If it proves possible to manipulate mitochondrial oxidative damage in vivo by this procedure then it may be particularly informative to see if doing so affects lifespan and whether such effects are additive to those of CR. In addition, it will be interesting to see whether CR does affect mitochondrial ROS production measured in the intact organism.

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References

Antunes, F., Salvador, A., Marinho, H.S., Alves, R., Pinto, R.E., 1996. Lipid peroxidation in mitochondrial inner membranes I. An integrative kinetic model. Free Radic. Biol. Med. 21, 917–943.

Azzi, A., Montecucco, C., Richter, C., 1975. The use of acetylated ferricytochrome *c* for the detection of superoxide radicals produced in biological membranes. Biochem. Biophys. Res. Commun. 65, 597–603.

Barja, G., 2004a. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? Biol. Rev. Camb. Philos. Soc. 79, 235–251.

Barja, G., 2004b. Free radicals and aging. Trends Neurosci. 27, 595–600.

Barja, G., 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.

Beal, M.F., 2003. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. Ann. N.Y. Acad. Sci. 991, 120–131.

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., Freeman, B.A., 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. U.S.A. 87, 1620–1624.

Bedogni, B., Pani, G., Colavitti, R., Riccio, A., Borrello, S., Murphy, M., Smith, R., Eboli, M.L., Galeotti, T., 2003. Redox regulation of cAMP-responsive element-binding protein and induction of manganous superoxide dismutase in nerve growth factor-dependent cell survival. J. Biol. Chem. 278, 16510–16519.

Beer, S.M., Taylor, E.R., Brown, S.E., Dahm, C.C., Costa, N.J., Runswick, M.J., Murphy, M.P., 2004. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant defense. J. Biol. Chem. 279, 47939–47951.

Bokov, A., Chaudhuri, A., Richardson, A., 2004. The role of oxidative damage and stress in aging. Mech. Ageing Dev. 125, 811–826.

Brand, M.D., Affourtit, C., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Pakay, J.L., Parker, N., 2004. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic. Biol. Med. 37, 755–767.

Burns, R.J., Murphy, M.P., 1997. Labeling of mitochondrial proteins in living cells by the thiol probe thiobutyltriphenylphosphonium bromide. Arch. Biochem. Biophys. 339, 33–39.

Burns, R.J., Smith, R.A., Murphy, M.P., 1995. Synthesis and characterization of thiobutyltriphenylphosphonium bromide, a novel thiol reagent targeted to the mitochondrial matrix. Arch. Biochem. Biophys. 322, 60–68.

Chen, K., Thomas, S.R., Albano, A., Murphy, M.P., Keaney Jr., J.F., 2004. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. J. Biol. Chem. 279, 35079–35086.

Coulter, C.V., Kelso, G.F., Lin, T.K., Smith, R.A., Murphy, M.P., 2000. Mitochondrially targeted antioxidants and thiol reagents. Free Radic. Biol. Med. 28, 1547–1554.

Dhanasekaran, A., Kotamraju, S., Kalivendi, S.V., Matsunaga, T., Shang, T., Keszler, A., Joseph, J., Kalyanaraman, B., 2004. Supplementation of endothelial cells with mitochondria-targeted antioxidants inhibit peroxide-induced mitochondrial iron uptake, oxidative damage, and apoptosis. J. Biol. Chem. 279, 37575–37587.

Echtay, K.S., Murphy, M.P., Smith, R.A., Talbot, D.A., Brand, M.D., 2002. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. J. Biol. Chem. 277, 47129–47135.

Finkel, T., 2003. Oxidant signals and oxidative stress. Curr. Opin. Cell Biol. 15, 247–254.

Ghafourifar, P., Colton, C.A., 2003. Mitochondria and nitric oxide. Antioxid. Redox Signal 5, 249–250.

Ghafourifar, P., Richter, C., 1997. Nitric oxide synthase activity in mitochondria. FEBS Lett. 418, 291–296.

Green, K., Brand, M.D., Murphy, M.P., 2004. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. Diabetes 53 (Suppl. 1), S110–S118.

Huang, T.T., Yasunami, M., Carlson, E.J., Gillespie, A.M., Reaume, A.G., Hoffman, E.K., Chan, P.H., Scott, R.W., Epstein, C.J., 1997. Superoxide-mediated cytotoxicity in superoxide dismutase-deficient fetal fibroblasts. Arch. Biochem. Biophys. 344, 424–432.

Hwang, P.M., Bunz, F., Yu, J., Rago, C., Chan, T.A., Murphy, M.P., Kelso, G.F., Smith, R.A., Kinzler, K.W., Vogelstein, B., 2001. Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. Nat. Med. 7, 1111–1117.

Jacobs, H.T., 2003. The mitochondrial theory of aging: dead or alive? Aging Cell 2, 11–17.

James, A.M., Murphy, M.P., 2002. How mitochondrial damage affects cell function. J. Biomed. Sci. 9, 475–487.

Jauslin, M.L., Meier, T., Smith, R.A., Murphy, M.P., 2003. Mitochondria-targeted antioxidants protect Friedreich Ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. FASEB J. 17, 1972–1974.

Kelso, G.F., Porteous, C.M., Coulter, C.V., Hughes, G., Porteous, W.K., Ledgerwood, E.C., Smith, R.A., Murphy, M.P., 2001. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J. Biol. Chem. 276, 4588–4596.

Koubova, J., Guarente, L., 2003. How does calorie restriction work? Genes Dev. 17, 313–321.

Lee, D.W., Yu, B.P., 1990. Modulation of free radicals and superoxide dismutases by age and dietary restriction. Aging (Milano) 2, 357–362.

Lin, T.K., Hughes, G., Muratovska, A., Blaikie, F.H., Brookes, P.S., Darley-Usmar, V., Smith, R.A., Murphy, M.P., 2002. Specific modification of mitochondrial protein thiols in response to oxidative stress: a proteomics approach. J. Biol. Chem. 277, 17048–17056.

McCay, C.M., Cromwell, M.F., Maynard, L.A., 1935. The effect of retarded growth upon the length of life span and upon ultimate body size. J. Nutr. 10, 63–79.

Murphy, M.P., 1997. Selective targeting of bioactive compounds to mitochondria. Trends Biotechnol. 15, 326–330.

Murphy, M.P., 2001. Development of lipophilic cations as therapies for disorders due to mitochondrial dysfunction. Expert Opin. Biol. Ther. 1, 753–764.

Murphy, M.P., Smith, R.A., 2000. Drug delivery to mitochondria: the key to mitochondrial medicine. Adv. Drug Deliv. Rev. 41, 235–250.

Murphy, M.P., Echtay, K.S., Blaikie, F.H., Asin-Cayuela, J., Cochemé, H.M., Green, K., Buckingham, J., Taylor, E.R., Hurrell, F., Hughes, G., Miwa, S., Cooper, C.E., Svistunenko, D.A., Smith, R.A.J., Brand, M.D., 2003. Superoxide activates uncoupling proteins by generating carbon-centred radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from -phenyl-*N*-tert-butylnitrone. J. Biol. Chem. 278, 48534–48545.

Roth, G.S., Mattison, J.A., Ottinger, M.A., Chachich, M.E., Lane, M.A., Ingram, D.K., 2004. Aging in rhesus monkeys: relevance to human health interventions. Science 305, 1423–1426.

Saretzki, G., Murphy, M.P., von Zglinicki, T., 2003. MitoQ counteracts telomere shortening and elongates lifespan of fibroblasts under mild oxidative stress. Aging Cell 2, 141–143.

Sastre, J., Pallardo, F.V., Vina, J., 2003. The role of mitochondrial oxidative stress in aging. Free Radic. Biol. Med. 35, 1–8.

Schafer, M., Schafer, C., Ewald, N., Piper, H.M., Noll, T., 2003. Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia. Circ. Res. 92, 1010–1015.

Sies, H., 1993. Strategies of antioxidant defense. Eur. J. Biochem. 215, 213–219.

Smith, R.A., Porteous, C.M., Coulter, C.V., Murphy, M.P., 1999. Selective targeting of an antioxidant to mitochondria. Eur. J. Biochem. 263, 709–716.

Smith, R.A., Porteous, C.M., Gane, A.M., Murphy, M.P., 2003. Delivery of bioactive molecules to mitochondria in vivo. Proc. Natl. Acad. Sci. U.S.A. 100, 5407–5412.

Tatoyan, A., Giulivi, C., 1998. Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. J. Biol. Chem. 273, 11044–11048.

Taylor, E.R., Hurrell, F., Shannon, R.J., Lin, T.K., Hirst, J., Murphy, M.P., 2003. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. J. Biol. Chem. 278, 19603–19610.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E., Bohlooly, Y.M., Gidlof, S., Oldfors, A., Wibom, R., Tornell, J., Jacobs, H.T., Larsson, N.G., 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417–423.

Vasquez-Vivar, J., Kalyanaraman, B., Kennedy, M.C., 2000. Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation. J. Biol. Chem. 275, 14064–14069.

Vina, J., Lloret, A., Orti, R., Alonso, D., 2004. Molecular bases of the treatment of Alzheimer's disease with antioxidants: prevention of oxidative stress. Mol. Aspects Med. 25, 117–123.