Advanced Drug Delivery Reviews 61 (2009) 1375–1385

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

# Mitochondrial targeting of electron scavenging antioxidants: Regulation of selective oxidation vs random chain reactions ☆

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ARTICLE INFO

*Article history:*
Received 4 June 2009
Accepted 8 June 2009
Available online 27 August 2009

*Keywords:*
Cytochrome *c*
Cardiolipin
Mitochondria
Apoptosis
Triphenylphosphonium
MnSOD
Peroxidation
Nitroxides
Gramicidin S-conjugates

ABSTRACT

Effective regulation of highly compartmentalized production of reactive oxygen species and peroxidation reactions in mitochondria requires targeting of small molecule antioxidants and antioxidant enzymes into the organelles. This review describes recently developed approaches to mitochondrial targeting of small biologically active molecules based on: (i) preferential accumulation in mitochondria because of their hydrophobicity and positive charge (hydrophobic cations), (ii) binding with high affinity to an intra-mitochondrial constituent, and (iii) metabolic conversions by specific mitochondrial enzymes to reveal an active entity. In addition, targeted delivery of antioxidant enzymes via expression of leader sequences directing the proteins into mitochondria is considered. Examples of successful antioxidant and anti-apoptotic protection based on the ability of targeted cargoes to inhibit cytochrome *c*-catalyzed peroxidation of a mitochondria-specific phospholipid cardiolipin, in vitro and in vivo are presented. Particular emphasis is placed on the employment of triphenylphosphonium- and hemi-gramicidin S-moieties as two effective vehicles for mitochondrial delivery of antioxidants.



**Contents**



☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Mitochondrial Medicine and Therapeutics, Part II".

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0169-409X/$ – see front matter 
doi:10.1016/j.addr.2009.06.008

"The proletarians have nothing to lose but their chains." Karl Marx

## 1. Introduction: selective oxidation vs random chain reactions of lipid peroxidation

The remarkable success of chemistry in understanding the mechanisms and kinetics of chain reactions in the gas phase [1,2] and the subsequent demonstration of these ideas for chemical oxidation reactions in the liquid phase [3] created a supposition that free radical chain oxidation reactions can also take place in biological systems. This resulted in the appearance of several novel hypotheses on the free radical mechanisms of aging [4,5] and radiation injury [6] as well as their role in major chronic cardiovascular and neurodegenerative diseases and cancer [7–12]. Reactive oxygen species (ROS) or oxygen radicals, particularly superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$) and subsequently formed highly reactive hydroxyl radicals, have been implicated in the oxidative modification of biological molecules and initiation of free radical chain reactions [13,14].

Logically, this has led to attempts to utilize free radical scavengers as therapeutic and/or preventive remedies. The initial optimism, however, faded over the years as many (if not most) antioxidant clinical trials have failed [15–27]. A skeptical view is that the major concept may be flawed: are there indeed (peroxidation) chain reactions in tissues and cells of our body normally or in disease conditions that develop as a random uncontrolled process? Is there solid experimental proof for hydroxyl radicals formed in vivo — based, for example, on spin trapping or other specific biomarkers [28–30]? Of course, there is a popular concept of chain breaking (sacrificial) water-soluble and lipid-soluble antioxidants such as vitamin C, vitamin E, ubiquinol, etc. But is antioxidant action their only or major function, and do they act as "random" scavengers of "random" radicals? Is it conceivable that old-fashioned classical biochemistry still works with the peroxidation reactions? If so — the attempts to use chain breaking antioxidants are destined to fail. The consequences and conclusions are simple — mechanisms of specific peroxidation reactions have to be revealed and interventions aimed at their regulation and/or inhibition have to take into consideration the compartmentalized nature of these reactions.

## 2. ROS reactivity: specific enzyme-dependent ROS signaling vs random free radical damage

ROS – formed during one-electron reduction of oxygen – are believed to be essential for the initiation of free radical reactions. They are commonly viewed as nonspecific oxidants capable of inducing oxidation of practically any biological molecule (proteins, lipids, DNA) via free radical pathways [31]. Yet, direct interactions of ROS (namely, $O_2^{\cdot-}$ and $H_2O_2$) with lipids and reactive groups of proteins are slow and inefficient. For example, the rate of the reaction of $H_2O_2$ with unsaturated lipids is negligible [14]; the rate of the reaction with thiols is usually below 30 $M^{-1}s^{-1}$ [32]. In addition, both species are effectively removed by antioxidant enzymes of cells — superoxide dismutases (SOD; the reaction rate with $O_2^{\cdot-}$ is ~$10^9$ $M^{-1}s^{-1}$ [33]), catalase (the reaction rate with $H_2O_2$ is ~$10^7$ $M^{-1}s^{-1}$) and glutathione peroxidases (the reaction rate with $H_2O_2$ is ~$10^7$ $M^{-1}s^{-1}$) [34–36].

To solve this conundrum, the role of ROS in direct oxidations, proposed chemical mechanisms that often include chain reactions catalyzed by redox active metal ions via the generation of highly reactive O-, S- and C-centered radicals [13,14]. However, detection of these radicals in vivo and in cells appears to be difficult. The generation of O- and S-centered radicals has been documented in cultured cells (but not in whole organisms) following exposures to high doses of toxicants under conditions incompatible with normal physiology. Experiments with a combination of primary and secondary radical-traps – dimethyl sulfoxide/α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone (POBN) – provided some evidence in favor of the formation of hydroxyl radicals in vivo in acute injury induced by cadmium poisoning and LPS-exposure [37,38]. Similarly, C-centered radicals were reported in vivo predominantly in acute injury (i.e., methanol intoxication, chromium poisoning, superantigen-induced toxic shock syndrome) [39–42]. Thus, the physiological relevance of random free radical reactions requires further investigation.

## 3. Mitochondrial peroxidation reactions — catalysis and role of the electron transport chain (ETC)

An alternative view on the ROS production and functions in cells suggests that they are involved in specific, compartmentalized and controlled catalytic reactions. What are the known major sites of radical production and oxidative stress? There are multiple possible site-specific sources of oxidizing equivalents and enzymes with high oxidizing potential that may participate in the generation of oxygen radicals. NADPH oxidases in the plasma membrane of inflammatory cells are potent producers of $O_2^{\cdot-}$ and $H_2O_2$. The generated ROS are believed to play a significant role in inflammation. "Friendly fire" produced by activated immune cells can induce growth arrest, apoptosis or necrotic death in off-target cells contributing to and modifying the inflammatory response [43–46]. Thus generated oxidized epitopes on cell surfaces and in the extracellular matrix enhance immune reactions and trigger autoimmune response [47–49].

Among potent catalysts of peroxidation reactions are heme-containing proteins, particularly heme-peroxidases. These enzymes can effectively utilize $H_2O_2$ (with rate constants in the range of $10^4$–$10^7$ $M^{-1}s^{-1}$) and oxidize specific substrates and generate reactive intermediates at extremely high rates (up to $10^8$ $M^{-1}s^{-1}$) [50]. In particular, cyclooxygenase can oxidize arachidonic acid upon reaction with peroxides at rates up to ~$10^7$ $M^{-1}s^{-1}$ and produce arachidonic acid hydroperoxides and endoperoxides, prostaglandins G2 and H2, which possess specific biological activity [51,52]. Neutrophil myeloperoxidase and the peroxidase activity of cyt *c* complexes with mitochondria-specific phospholipid cardiolipin (CL) in the intermembrane space of mitochondria add to the list of examples of enzymatic systems involved in the compartmentalized generation of oxidative stress.

It is commonly accepted that mitochondria and their electron transport chains (ETCs) – if and when de-regulated – act as the major source of oxygen radicals in cells. Initial estimates suggested that during the normal transfer of electrons, 2–5% of total molecular oxygen consumed by mitochondria is converted into superoxide due to its incomplete reduction and electron escape during the process coupled with oxidative phosphorylation [53]. In subsequent studies, arguments were presented that at physiological level of tissue oxygenation only 0.2% oxygen is converted to superoxide [54]. The sites of superoxide production in the mitochondrial ETC have been mostly associated with Complexes I and III in the mitochondrial inner membrane [55–59]. Since the superoxide does not freely diffuse across membranes, the location of superoxide within mitochondria is important. It has been suggested that Complex I generates superoxide within the mitochondrial matrix. In contrast, Complex III can release superoxide both into the intermembrane space and matrix [60]. Dysfunctional ETC, resulting from either genetic mutations or the action of toxic chemicals or environmental factors, may lead to enhanced production of ROS via facilitated deviation of electron flow to molecular oxygen causing its univalent reductions [61,62].

A typical example is the disruption of electron transport in cells undergoing apoptosis [63]. Until recently, the role of ROS production in the execution of the apoptotic program has not been elucidated. Establishment of the important role of oxidation of a mitochondrial-specific phospholipid, CL, in the permeability transition and release of pro-apoptotic factors [64] pointed to a possible connection of this

reaction with the enhanced production of ROS. Indeed, it has been discovered that early apoptotic events, including ROS generation, are associated with the migration of CL from the inner to the outer mitochondrial membrane [65]. As a consequence, cyt *c* forms a complex with CL that is non-productive in shuttling electrons but instead acts as a potent CL-specific oxygenase/peroxidase [66]. CL oxidation products play an important role in the detachment of cyt *c* from the mitochondrial membranes and release of pro-apoptotic factors into the cytosol [67,68]. Based on the recent understanding of the specific role that ROS play as activators of cyt *c*/CL-mediated oxidative phospholipid signaling in apoptosis, the current review will focus on new concepts in mitochondrial targeting of regulators of free radical reactions and their possible role in cell and tissue protection.

There are several possible routes through which the peroxidase activity of cyt *c*/CL complexes towards CL oxidation can be avoided or suppressed: i) prevention of $O_2^{\cdot-}/H_2O_2$ generation or elimination of these ROS as sources of oxidizing equivalents for the cyt *c*/CL peroxidase; ii) inhibition of the cyt *c*/CL peroxidase activity via quenching of its reactive intermediates; and iii) manipulations of CL oxidizability by integrating into the molecule mono-unsaturated or saturated fatty acid residues. In this review, we will illustrate new opportunities of the mitochondria-targeted delivery of these different inhibitors of CL peroxidation as a prototypical example for discovery of new anti-apoptotic agents/drugs.

"Fine art and pizza delivery, what we do falls neatly in between!"
David Letterman

## 4. Mitochondrial targeted delivery of oxidation regulators: major principles

Because a large number of human diseases may be associated with mitochondrial dysfunction [69,70], there is an emerging field of biomedical research – "mitochondrial medicine" – that includes pharmacological approaches to control and correct de-regulated mitochondria [71,72]. This research stimulated the development of methods for mitochondrial drug delivery for selective protection, repair, or even eradication (in cases of irreparable damage) of mitochondria.

Cells routinely utilize mitochondrial targeting of proteins — only 13 out of more than 2000 proteins employed for mitochondrial functions are encoded by the mitochondrial genome. The rest of them are expressed from nuclear DNA and then processed and delivered into the mitochondria. These proteins have similar features: (i) moderate trans-membrane domain hydrophobicity (required for mitochondria targeting as well as membrane anchoring) and (ii) a net positive charge within the trans-membrane domain. Known presequences do not share a common primary structure, so targeting information is encoded in structural elements rather than in a specific primary sequence [73].

Common approaches to mitochondrial targeting of small biologically active molecules are based on several principles: (i) preferential accumulation in mitochondria because of their hydrophobicity and positive charge (hydrophobic cations), (ii) ability to enter mitochondria via carrier proteins unique to the organelle, (iii) binding with high affinity to an intra-mitochondrial constituent (e.g., cardiolipin) and (iv) metabolic conversions by specific mitochondrial enzymes to reveal an active entity [74–76]. The chemistry of targeted as well as targeting molecules may be very different: from (poly)peptides to small molecules of non-peptide nature.

Although phospholipid membranes are impermeable to ions, certain amphiphiles can cross mitochondrial membranes and accumulate in the mitochondrial matrix driven by its negative membrane potential. Skrede and Liberman et al. were the first to report that positively charged disulfides and triphenylphosphonium (TPP) cations are rapidly taken up by mitochondria [77,78]. Follow up studies led to the identification of a series of positively charged organic compounds that compartmentalize into mitochondria (e.g. rhodamine [79], cyanine dyes [80], and dequalinium cation [81]). Common characteristics for these compounds include amphiphilicity and a delocalized positive charge. In recent years, the uptake of amphiphilic organic cations by mitochondria has been used by several groups for targeted delivery of anti-cancer drugs [81–83] and antioxidants [84,85] in these organelles.

In spite of significant success of this approach there are still controversies in the field. Horobin [86] analyzed >100 available "mitochondria-tropics" and concluded that there are no simple correlations between mitochondria targeting capacity and physical–chemical properties. Contrary to general opinion, only one third of mitochondria-tropics were hydrophobic cations. Acids and anions were in the same proportions as electrically neutral compounds. Two thirds of known mitochondria-targeted molecules are lipophilic, one third is hydrophilic. Amphiphilicity is not a general property of mitochondria-tropics.

## 5. Mitochondrial targeted delivery of antioxidant enzymes

As mentioned above, targeted mitochondrial delivery of proteins, including antioxidant enzymes, can be achieved via expression of leader sequences directing the proteins into mitochondria. Herein, we will describe a series of elegant experiments illustrating the successful application of this principle with regards to protection of cells and animals by SOD and catalase against oxidative stress induced by ionizing irradiation.

Mitochondrial targeting of both transgene products [79,87] and small molecules [88] has been shown to be highly effective in protecting cells from ionizing radiation damage. Ionizing irradiation through radiolysis of water rapidly generates radicals that attack vital biomolecules. Irreparable damage to DNA triggers the mitochondria-mediated intrinsic apoptotic program. The latter includes mitochondrial production of ROS, particularly superoxide radicals [87]. Mammalian cells overexpressing the mitochondrial localized transgene for MnSOD show protection from ionizing irradiation lethality. Overexpression of MnSOD has been shown to ameliorate ionizing irradiation-induced cell death. In contrast, cells overexpressing the transgene for Cu/ZnSOD, which is naturally expressed in the cytoplasm or extracellular space, had no significant radiation protection under the same assay conditions [87].

Experiments converting Cu/ZnSOD to a mitochondrial targeted protein, by attaching the mitochondrial localization peptide sequence to the transgene, showed that cells overexpressing this protein were radioprotected as effectively as were those overexpressing MnSOD [87] (Fig. 1). The SOD activity measured in the mitochondria was, however, a Cu/Zn-metalloenzyme rather than one requiring Mn, confirming that mitochondrial localization conferred the mechanism of radioprotection [87]. When the mitochondrial localization sequence of MnSOD was removed from the transgene product, cells overexpressing this construct demonstrated cytoplasmic localization of a manganese metalloenzyme and were not radioprotected.

Mitochondrial localization of transgene products was recently shown to extend to the enzyme catalase [89]. Dismutation of $O_2^{\cdot-}$ by MnSOD in mitochondria leads to the reaction product, $H_2O_2$, which can produce mitochondrial damage leading to cell death. Cells overexpressing a mitochondrial targeted catalase transgene (by adding the mitochondrial localization peptide sequence from MnSOD to the catalase transgene) demonstrated significant radioprotection [89] while cells overexpressing native cytoplasmic catalase were not protected from ionizing irradiation. Furthermore, cells overexpressing both MnSOD and mitochondrial targeted catalase showed the greatest radioprotection [89].

Administration of either MnSOD [79,90] or mitochondria-targeted catalase [89] confers effective in vivo organ specific radioprotection, as

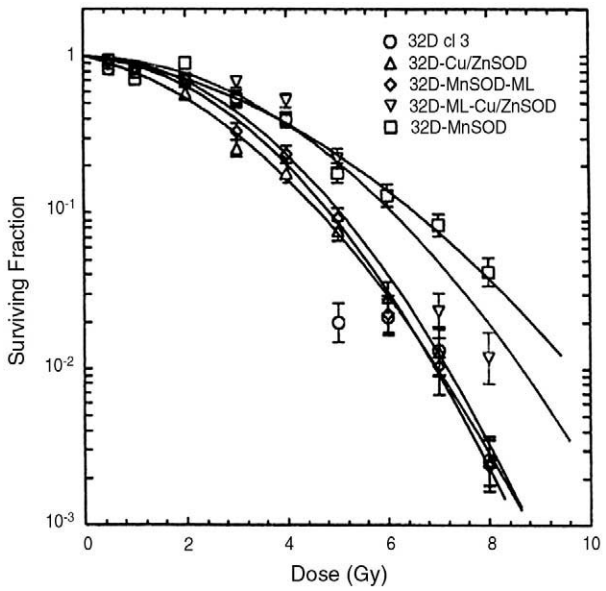



**Fig. 1.** Increased survival of 32D cl 3 cells with elevated mitochondrial SOD activity. Cells from 32D cl 3, 32D-Cu/ZnSOD, 32D-MnSOD, 32D-MnSOD-ML, and 32D-ML-Cu/ZnSOD were irradiated with doses ranging from 0 to 8 Gy. The cells were plated in 4% methylcellulose and colonies of >50 cells were counted 7 days later. The data were analyzed using linear-quadratic and single-hit, multitarget models. 32D-MnSOD and 32D-ML-Cu/ZnSOD cells having increased mitochondrial SOD activity were more resistant to radiation [$D_0 = 2.10 \pm 0.10$ ($P = 0.001$) and $1.97 \pm 0.17$ ($P = 0.0127$) Gy, respectively] than 32D cl 3, 32D-Cu/ZnSOD, or 32D-MnSOD-ML cells ($D_0 = 1.15 \pm 0.11$, $0.89 \pm 0.01$, and $1.08 \pm 0.02$ Gy, respectively) ($P < 0.195$ and $P < 0.673$). Results are presented as the means ± SEM with an $n$ of 3. $P$ values were determined using a Student's $t$ test. *Represents a statistically significant difference from 32D cl 3 cells. (Reproduced from M.W. Epperly et al., Radiat Res 160 (2003) 568-78, with permission of the publisher.)

demonstrated using assays against radiation damage to esophagus, oral cavity, lung, and also in total body radiation [88]. Overexpression in vitro of both mitochondria-targeted catalase and MnSOD enhanced radioprotection above the level seen with overexpression of one transgene [89]. Since in vivo transfection efficiency rarely exceeds 50%, experiments to test the effect of both transgene products will require the construction of a transgene vector containing both expressed transgenes on the same plasmid. The construction of a plasmid containing both transgenes has been achieved, and these studies are in progress.

Success with both MnSOD integrated plasmid in phospholipid liposomes (MnSOD-PL) and mitochondria-targeted catalase-PL radiation protection strategies (overexpressing transgene product prior to irradiation) suggests that studies in the radiation mitigation paradigm (adding transgene product after irradiation) might also be effective.

Initial results were encouraging with respect to MnSOD; however, mitigation did not achieve the levels seen with the addition of transgene prior to irradiation [91]. One possible explanation for the decreased effect of adding transgene after irradiation may have involved the lag time between transfection and expression of transgene product [87]. Studies determining the time of detectable expression of transgene product indicated that 24 h were required for plasma integration into cellular DNA, transcription of RNA, and translation of protein. These data were acquired in healthy cells. Since irradiation of cells is known to induce both nuclear DNA fragmentation, as well as transport to the mitochondria of stress activated protein kinases, p53, and Bcl-2 family pro-apoptotic protein [90], it is not known how these rapid radiation-induced events might affect the time course and effectiveness of the expression of an added MnSOD transgene by plasmid liposome, retro-virus, or other vector moiety. In addition, the complexity and size of transgene product, and concern for effectiveness, possible need for multiple administrations, and cellular bioavailability suggested that small molecule mitochondrial targeted antioxidant might be attractive alternative approach to radioprotection.

## 6. Chemistry of small molecule targeted delivery into mitochondria

Selective delivery is key to eliciting desirable therapeutic effects in diseases that originate from mitochondrial dysfunction [92]. Most targeting agents profit from the negative potential of mitochondria. The process of electron transfer to $O_2$ is coupled to a proton gradient that drives ATP production and generates a negative potential of −150 to −180 mV across the inner mitochondrial membrane [93]. Furthermore, the permeability of the outer membrane to small organic molecules facilitates the recruitment of cationic agents to the mitochondrial matrix. Several classes of lipophilic cations (triarylphosphonium salts and rhodamine) have been found to accumulate in mitochondria with an enrichment factor of up to several hundred over the cytosol, and, more significantly, serve as transporters for the selective delivery of therapeutic species to mitochondria (Fig. 2).

### 6.1. Delivery of GS-nitroxides and TPP-nitroxides

Triphenylphosphonium (TPP) salts offer a general platform to shuttle antioxidants into the mitochondrial matrix and have been conjugated to ubiquinols, tocopherols, lipoic acid, nitroxides and other electron- and radical scavengers [94–99]. A useful extension of this concept was cationic, arginine-rich oligopeptides such as the Szeto-Schiller tetrapeptides (Fig. 3) [100]. The peptide sequences Dmt-D-Arg-Phe-Lys-$NH_2$ and D-Arg-Dmt-Lys-Phe-$NH_2$ have been shown to be very effective in inhibiting mitochondrial swelling, oxidative cell death, and reperfusion injury [101]. Notably, these small peptides were concentrated 1000-fold across the inner mitochondrial membrane and also readily crossed the cell membrane. This work supports the hypothesis that small peptides, or peptide mimetics, that contain appropriate antioxidants can be used to counteract the overproduction of ROS.

Several "classical" lipophillic antioxidants – vitamin E, coenzyme Q, plastoquinones – have been utilized as cargoes for mitochondrial targeting and demonstrated useful protective propensities [98]. TPP-conjugated homologues of vitamin E were among the first mitochondria-targeted lipid antioxidants that were shown to be effectively taken up and protect mitochondrial functions from oxidative damage far more effectively than vitamin E itself [98]. Another lipid-soluble antioxidant, MitoQ represents a mixture of 10-(6′-ubiquinolyl)-decyltriphenylphosphonium bromide and 10-(6′-ubiquinonyl)-decyltriphenylphosphonium bromide and was found to exert biochemical evidence of protection against oxidative stress in vitro [102] and to lessen dysfunction and augmented mitochondrial membrane potential in a rat model of lipopolysaccharide-peptidoglycan-induced organ failure [85]. Currently, MitoQ is under development by Antipodean Pharmaceuticals, Inc. in phase II clinical trials for Parkinson's disease and liver damage associated with HCV infection (reviewed by [103]). However, it should be noted that multifunctional modes of action of coenzyme Q and a risk of its involvement in

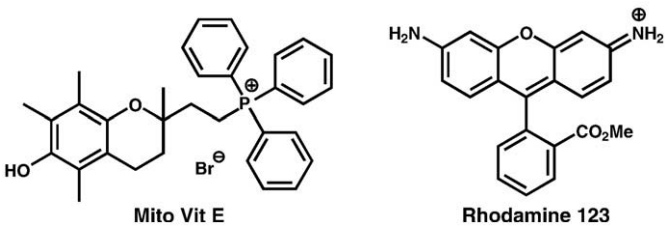



**Fig. 2.** Mito Vit E and rhodamine localize in the mitochondria due to the negatively charged membrane potential across the inner mitochondrial membrane.

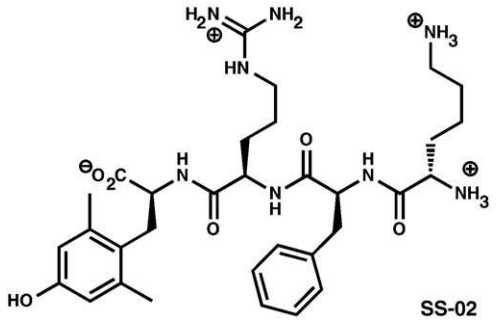



**Fig. 3.** A charge-targeted peptide antioxidant.

the generation of oxygen radicals have been also associated with its potential to cause damage to mitochondria and cells [104].

Similar quinone-based mitochondria-targeted protectors – SkQs – employ cationic plastoquinone derivatives. These compounds were demonstrated to be effective in preventing CL peroxidation and apoptosis in cell culture models [96]. Moreover, SkQs prolonged lifespan in different species, being especially effective at early and middle stages of aging in different species. Most notably, in mammals, the effect of SkQs on aging was accompanied by inhibition of development of such age-related diseases and traits as cataract, retinopathy, glaucoma, balding, osteoporosis, involution of the thymus, hypothermia, torpor, peroxidation of lipids and proteins, etc. [96].

An alternative concept to targeting ROS scavengers to mitochondria was based on the affinity of certain antibiotics to microbial cell membranes [105]. Due to the close relationship between bacterial membrane and the mitochondrial inner membrane structure, in particular their lipid composition, the antibacterial membrane disruptor, gramicidin S (GS), could be re-engineered as a mitochondrial targeting agent (Fig. 4). The uncharged XJB-5-131 contains a hemi-GS sequence, specifically the pentapeptide Leu-D-Phe-Pro-Val-Orn, attached to a stable nitroxide, 4-amino-Tempo (4-AT). The peptide bond between Leu and D-Phe was replaced with an alkene peptide isostere in order to increase the metabolic stability of the compound.

The major advantage of sterically hindered free radicals such as 4-AT is their ability to accept and donate electrons depending on the redox potential of the environment [106]. Upon loss of an electron, the nitroxide radical is converted to an oxoammonium cation, which is readily reduced by ascorbate (in biofluids) or via NAD(P)H-driven electron transport (in cells) to the hydroxylamine (Fig. 5). Nitroxides can be also directly reduced by ascorbate and electron transporting enzymes to hydroxylamines [107]. The hydroxylamine is by itself a powerful reducing agent, and can regenerate the nitroxide radical or scavenge a reactive oxygen species by electron or hydrogen atom transfer, respectively. The 4-AT subunit acts as a scavenger of electrons and antioxidant, whereas the peptidic component of XJB-5-131 targets the mitochondrial lipid, possibly CL, and thus enriches the agent ca. 600-fold over the cytosol [65,108,109]. XJB-5-131 was shown to prolong survival in a rodent model of hemorrhagic shock, mitigate oxidative stress, and preempt the mitochondrial pathway toward apoptosis [110–112]. Furthermore, a related compound in this series, XJB-5-125, demonstrated substantive in vivo radioprotective effects [113]. Detailed structure–activity studies revealed that the presence of the GS-moiety is a necessary but not sufficient pre-requisite for the effective prevention of ROS formation, oxidative stress and anti-apoptotic effects of the GS-nitroxide conjugates in mitochondria [109]. Monte Carlo simulations showed that active nitroxide conjugates of hemi-GS peptides with intact β-turn structures were positioned at the interface between polar and nonpolar regions of the lipid membrane. Thus not only the presence but also the optimized localization in the membrane needs to be taken into consideration to design hemi-GS-nitroxide conjugates that can successfully compete with $O_2$ for electrons from ETC to prevent $O_2^{\cdot-}$ and $H_2O_2$ production.

In the course of optimizing the pharmacokinetic properties of XJB-5-131 and XJB-5-125, JP4-039 was identified as a small molecular weight analog with impressive anti-inflammatory and radioprotective properties (Fig. 6) (Pierce et al., unpublished data). While JP4-039 does not have the complete hemi-GS derived mitochondrial targeting sequence and therefore does not reach the high mitochondrial enrichment factor of XJB-5-131, it is similarly uncharged and therefore readily passes through mammalian cell membranes.

As an alternative to hemi-GS as a vehicle used for delivery into mitochondria, we and others have also employed TPP to deliver nitroxide cargoes into mitochondria using “electrophoresis” due to a gradient of membrane potential [114]. We tested the potential of

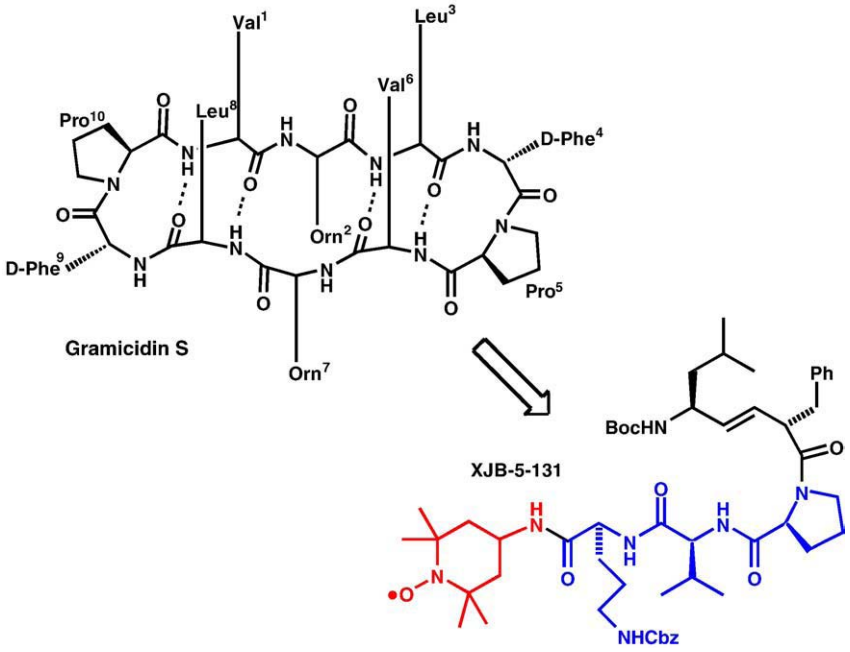



**Fig. 4.** The neutral, 4-AT conjugated mitochondrial targeting agent XJB-5-131 was designed based on the molecular structure of the antibiotic gramicidin S.

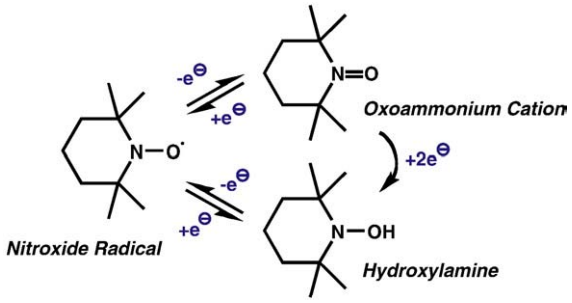



**Fig. 5.** Nitroxide radicals such as 4-AT can redox cycle between several states.

several TPP-nitroxide homologues to affect CL peroxidation and inhibit apoptosis in cells. Interestingly, of several nitroxide homologues studied, only TPEY-Tempo (Fig. 7) was taken up by cells, concentrated in mitochondria and exhibited both anti-apoptotic and radioprotective properties (Jiang et al., 2009, submitted). However, the inability of other homologues to accumulate in cells indicates that an important pre-requisite for the process is penetration of TPP-nitroxide's effective integration into mitochondria and anti-apoptotic protection. Further studies delineating containing molecules into cells, a process possibly dependent on chemical structure of structural requirements and the role of linkers connecting TPP with nitroxides in their penetration into cells are essential. Interestingly, in several nitroxide homologues studied, only TPEY-Tempo was taken up by cells, concentrated in mitochondria and exhibited both anti-apoptotic and radioprotective properties (Jiang et al., 2009, submitted).

Another important issue is the appropriate topography of mitochondria-targeted cargoes and their proximity to the cyt *c*/CL complex and its catalytic domains participating in CL oxidation and subsequent release of pro-apoptotic factors [65]. The peroxidase activity of cyt *c* is triggered by binding of CL itself, probably involving residues K72, K73, K86, and K87 [115,116] which are believed to partially unfold, hence activate the protein. Free fatty acid hydroperoxides (FFA-OOH) are $10^2$–$10^3$ times more effective as sources of oxidizing equivalents for the peroxidase activity of cyt *c*/CL than $H_2O_2$ or other small organic hydroperoxides (Belikova et al., 2009, submitted). This suggests that FFA-OOH rather than $H_2O_2$ may be endogenous substrates for CL peroxidation. Computer modeling including docking experiments implicated residues V20, L32, H33, L35, F36, R38, L98, K99, K100, A101, T102 and N103 in the FFA-OOH binding. In contrast, small organic hydroperoxides such as *tert*-butyl hydroperoxide (*t*-BuOOH) were predicted to associate with cyt *c* near residues V11, C14, T19, V20, E21, L22, G23, and Y97 (Fig. 8). Thus, the enhanced peroxidase activity in the presence of FFA-OOH as compared to small organic hydroperoxides is likely related to differences in binding site. Furthermore, it is clear from these studies that the CL and FFA-OOH interaction sites on

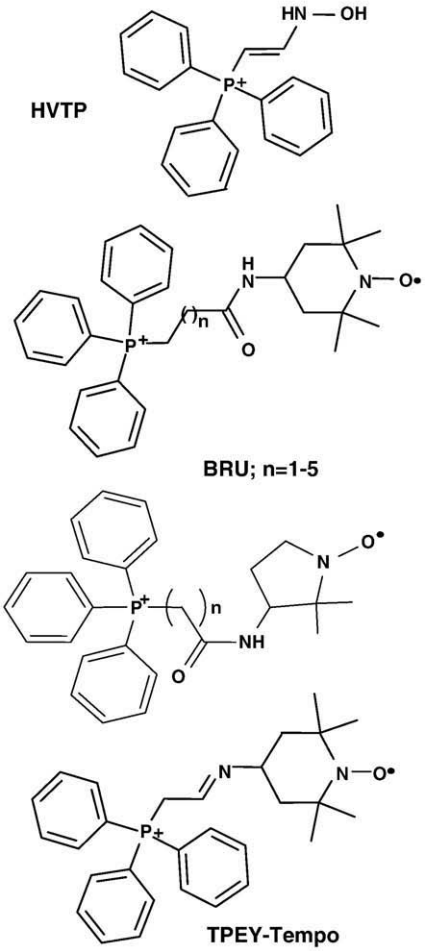



**Fig. 7.** TPP-conjugates of aminoxyls.

cyt *c* are also distinct. This indicates that cyt *c* contains multiple binding pockets, interaction with which results in different effects on cyt *c* structure and activity. Furthermore, ATP may act as a regulatory effector in modulating structural transitions of cyt *c* and its activity [117,118]. Since ATP can displace the binding of oleic acid to cyt *c*, it has been proposed that FFAs can reverse a non-native conformation (induced by CL) of the protein to the native (non-lipid bound) one. However, the peroxidase activity of cyt *c* is retained in the presence of ATP [117] suggesting that the cyt *c* native structure was not recovered in the presence of excess ATP. It is possible that the ATP-bound state of the protein is different from its native conformation, albeit not as extensively altered as the lipid-bound form. Docking

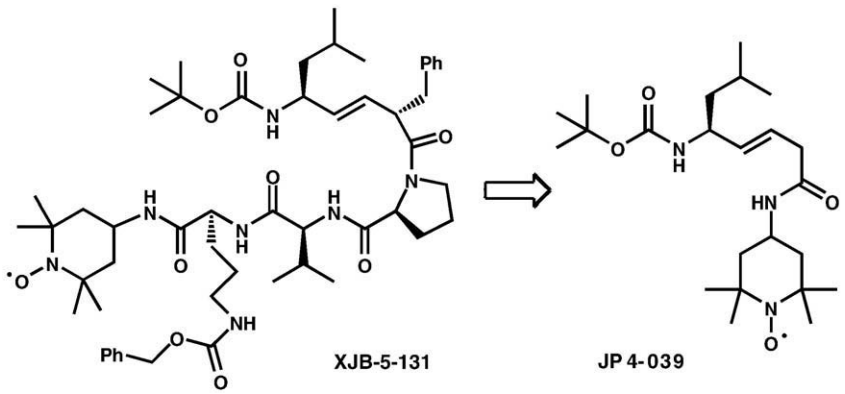



**Fig. 6.** The radioprotective and anti-inflammatory agent JP4-039 was designed based on the minimum pharmacophore of XJB-5-131.

simulations indicate that ATP was preferentially bound at two sites, one involving residues Q16, T19, K22, and K27 and another composed of residues F36, K60, E61, E62, T89, E92, D93, I95, A96, K99, and K100. Of the two predicted binding sites, the latter was the preferred, based on energetic considerations as well as the largest number of docked conformations at this site. This preferred ATP binding site and the FFA-OOH predicted binding site slightly overlap with each other, most importantly at the positively charged residues K99 and K100. The predicted overlap in the binding site region thus provides a plausible explanation for the experimental finding why ATP interferes with oleic acid interaction with cyt *c* [118]. These studies further suggest that ATP can also be used as a regulator of the binding of FFA-OOH to cyt *c* and may be used to avoid or decrease peroxidase activity of cyt *c*. These structural studies indicate new important strategies in optimizing mitochondria-targeted regulators of peroxidase function of cyt *c*/CL complexes as potential inhibitors of CL oxidation with anti-apoptotic protective properties.

In summary, we utilized three major approaches for mitochondria targeting of antioxidant agents (enzymes, small molecule electron scavengers/antioxidants) to achieve significant protection against apoptosis induced by either chemical agents or physical factors (irradiation): i) creation of protein constructs with mitochondria leader sequences, ii) synthesis of conjugates in which the targeting moiety – TPP – facilitated "electrophoresis" of the cargo into mitochondria, and iii) use of fragments of natural compounds, antibiotics such as hemi-GS, with high affinity to one or more intra-mitochondrial constituents. In all cases, we were able to localize/enrich the vehicle/cargo conjugates in mitochondria and achieve significant protective effects against ROS production and CL oxidation that associated with anti-apoptotic action. As exemplified by radiation protection, all of these mitochondria-targeted molecules (enzymes, nitroxides) demonstrated superior effect in vitro and in vivo compared to non-targeted counterparts (enzymes without mitochondria leader sequences, non-conjugated 4-AT). Since mitochondrial targeted transgene products including MnSOD and catalase, as well as small molecule nitroxides, are effective radioprotectors, we suggest that enhanced methods for the smart delivery of these agents and/or combinations thereof to mitochondria should further improve their effectiveness in both clinical radiation protection in cancer patients as well as systemic radiation protection and radiation damage mitigation in individuals exposed to accidental or radiological terrorism mediated total body irradiation exposure.

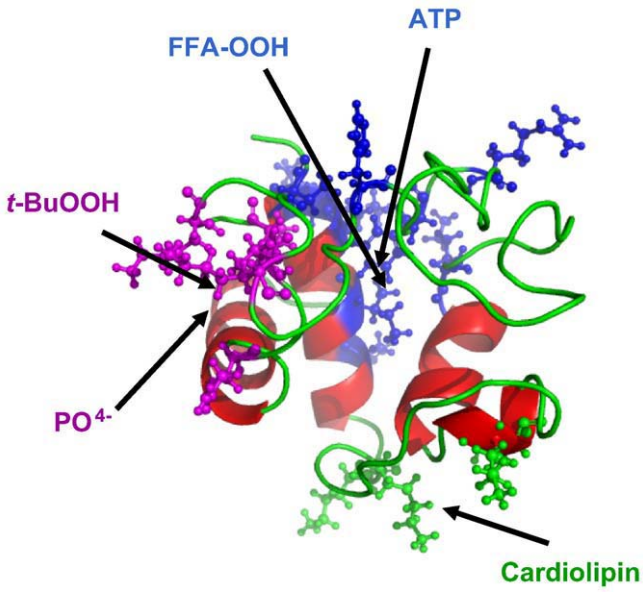



**Fig. 8.** Cartoon representation of cytochrome *c* (cyt *c*) with the predicted binding sites for cardiolipin (CL), free fatty acid hydroperoxides (FFA-OOH), adenosine triphosphate (ATP), *tert*-butyl hydroperoxide (*t*-BuOOH), and phosphate ($PO_4^-$). The helices of cyt *c* are colored in red and loops in green. The predicted binding site residues that are within 5 Å distance in each case are represented by arrows and rendered in spheres. The site for CL is colored in green, for FFA-OOH and ATP in blue, and for the *t*-BuOOH and $PO_4^-$ binding sites in magenta.

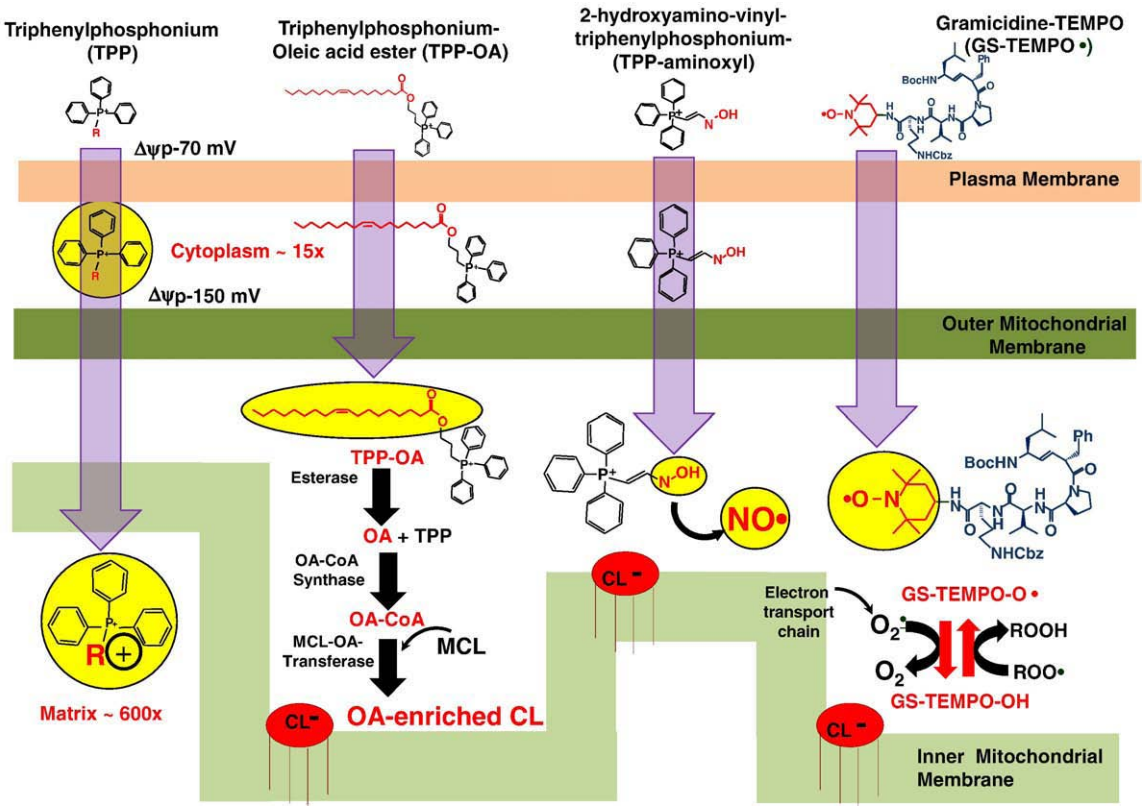
### 6.2. TPP-aminoxyls suppress the peroxidase activity of cyt c/CL complexes

Selective peroxidation of CL is an early event in mitochondrial apoptosis catalyzed by the peroxidase activity of cyt *c*/CL complex [65]. This suggests that inhibition of the peroxidase activity may be a novel approach to regulation of apoptotic cell death. Nitric oxide ($NO^{\cdot}$) inhibits peroxidases mainly due to its ability to quench the reactive intermediates at the heme-site or by interacting with protein immobilized radicals [65,119]. This competitive "substrate-like" function of $NO^{\cdot}$ requires its relatively high concentrations in mitochondria preferably in close proximity to catalytically competent cyt *c*/CL complexes. To this end, we synthesized a series of compounds containing both TPP and an aminoxyl function (TPP–$C_n$–X; X = N–$O^{\cdot}$; NH–OH) (Fig. 7). The rationale for this structural design is based on previous studies demonstrating the ability of aminoxyls to undergo one-electron oxidation by peroxidases with concomitant release of $NO^{\cdot}$ [88,120,121].

Detailed studies in model systems and in mouse embryonic cells (MEC) established that cyt *c*/CL complexes (but not cyt *c*) indeed effectively catalyzed the oxidation of (2-hydroxyamino-vinyl)-triphenylphosphonium (HVTP) with concomitant release of $NO^{\cdot}$. HVTP compartmentalized preferentially into mitochondria of MEC and inhibited mitochondrial peroxidase activity [122]. These effects were accompanied by significant protection of MEC against actinomycin D (ActD) — and gamma irradiation-induced apoptosis [122,123]. $NO^{\cdot}$ reacts with $O_2^{\cdot-}$ at diffusion controlled rates to form a highly reactive oxidant and nitrating agent, peroxynitrite (ONOO–) [124,125]. Therefore, incomplete elimination of $O_2^{\cdot-}$ and simultaneous employment of donors generating $NO^{\cdot}$ within the diffusible distances may be detrimental to mitochondria [126]. It is possible that undesirable interactions of $NO^{\cdot}$ with $O_2^{\cdot-}$ may be minimized by co-administration of TPP-aminoxyls and TPP-nitroxides, thus attaining a safe superoxide-free redox environment.

### 6.3. Modulation of CL unsaturation leads to protection against apoptosis

Previous work has established that saturated and mono-unsaturated molecular species of CL do not undergo peroxidation by the peroxidase activity of cyt *c*/CL complexes (Tyurina et al., unpublished data). This indicates that manipulations of the unsaturation levels of mitochondrial CLs may represent an interesting opportunity to modulate the sensitivity of cells to undergo apoptosis, provided that the required fatty acid precursors for CL synthesis are available. To experimentally test this opportunity, we synthesized a TPP-conjugate with oleic acid (TPP-OA, Scheme 1) and tested its ability to affect the apoptotic response of MECs. Importantly, pre-incubation of cells with TPP-OA, indeed was associated with decreased levels of apoptosis induced by ActD (Tyurina et al., unpublished data). This indicates that the cell's sensitivity to apoptosis may be manipulated via the delivery into mitochondria of either non-oxidizable precursors (oleic, stearic, palmitic) or readily oxidizable polyunsaturated fatty acids (such as docosapenta- and docosahexaenoic acids) that may be integrated into CL species. This approach illustrates an important principle whereby one or more metabolic reactions involved in synthesis or remodeling of CL may be contributory to the realization of the protective effects.




**Scheme 1.** This illustrates the major mitochondria-targeted compounds with antioxidant and anti-apoptotic effects described in the review. The triphenylphosphonium (TPP) moiety is targeted ("electrophoresed") into the mitochondria due to their lipophilic nature and positive charge. Triphenylphosphonium oleic acid ester (TPP-OA) accumulates in the mitochondria and is acted upon by esterase releasing oleic acid at the site of cardiolipin (CL) synthesis and remodeling. Through activation by oleic acid-CoA synthase, followed by integration into CL by monolyso-cardiolipin transferase, CL unsaturation decreases. This limits both oxidation and further downstream apoptotic events. 2-hydroxyamino-vinyl-triphenylphosphonium (TPP-aminoxyl) interacts with cytochrome *c*/CL complexes liberating nitric oxide, which interacts with reactive peroxidase intermediates thus preventing CL peroxidation and apoptosis. Gramicidin S-TEMPO conjugates (GS-TEMPO-O˙), preferentially accumulates in the mitochondria due to high-affinity GS binding with the inner mitochondrial membranes; the nitroxide cargo of TEMPO-O˙ undergoes cyclic reduction and oxidation by acting as a scavenger of electrons thus preventing superoxide generation.

### *6.4. Targeted avoidance of specific compartments such as mitochondria: delivery of NBD-CL*

Liposomes are common vehicles for the delivery of various compounds into cells. In particular, liposomes have been successfully used for the incorporation of phospholipids in cell membranes. Liposomes with signaling phospholipids, such as phosphatidylserine (PS), are known to be selectively taken up by professional phagocytes, such as macrophages, dendritic cells and microglial cells and are utilized for selective delivery of their contents into phagocytozing cells [127]. Recently, PS has been successfully utilized as a vector for delivery of PS-coated nano-particles with other types of cargo into phagocytozing cells [128]. After integration into cells, PS-containing liposomes are found in plasma membrane as well in endosomes from which they slowly exchange with other intracellular membranes. An interesting opportunity may be associated with organelle-specific phospholipids. Cardiolipin is not normally found in any other intracellular organelle or plasma membranes [129]. This suggests that specific mechanisms restricting CL translocation to other membranes are implemented precluding its intracellular distribution. Surprisingly, incubation of cells with fluorescently labeled CL – 7-nitro-2-1,3-benzoxadiazol (NBD) group (NBD-CL) – exogenously added to cells revealed its avoidance of mitochondria (Fig. 9). More detailed analysis of intracellular trafficking of exogenous NBD-CL showed that in contrast to other NBD-labeled phospholipids, NBD-CL, when delivered to mouse embryonic cells by using liposomes, was internalized via the endocytotic pathway, but was not transferred between cell membranes. Even endosomal disruptor chloroquine did not facilitate redistribution of NBD-CL to other intracellular membranes. Moreover, CL and a combination of CL with cationic phospholipids (e.g., dioleoyl-ethylphosphocholine) drastically enhanced uptake of the liposomes by cells. It is tempting to speculate that CL is recognized by specific receptors, which enhance uptake of CL-containing vehicles, and that CL trafficking in cells is tightly controlled.

These unique properties of CL can be used for the delivery of liposomes into cells and for the targeted avoidance of the unwanted compartmentalization of the liposomal content. This approach may be

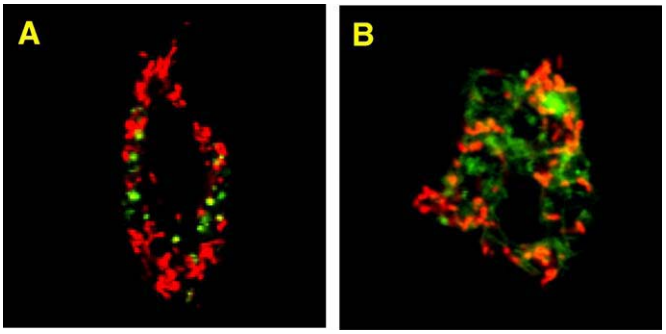



**Fig. 9.** Microphotographs of intracellular distribution of NBD-labeled phospholipids in mouse embryonic cells obtained by confocal fluorescence microscopy. $C_{12}$-NBD-CL (A) and $C_{12}$-NBD-phosphotidylcoline (B) were delivered in liposomes. Mitochondria were stained with Mitotracker Red CMX Ros (red color); NBD-labeled phospholipids are shown in green color. A — Note a punctuate pattern of intracellular distribution of $C_{12}$-NBD-CL characteristic for endosomes; NBD-CL and mitochondrial staining are not colocalized. B — Diffuse staining with $C_{12}$-NBD-phosphotidylcoline.

particularly useful for the delivery of cationic drugs such antracycline antibiotics. One of the antracyclines, doxorubicin, has found outstanding clinical utility as an anti-cancer drug. The antitumor activity of doxorubicin is mainly attributed to a direct binding to DNA. In addition, this compound causes cytotoxic effects via free radical generation, metal chelation, bioalkylation and disordering of the membrane (reviewed in [130]). The major side effect of doxorubicin that significantly limits its application is cardiotoxicity, which is mainly due to the targeting of cardiomyocyte mitochondria. In mitochondria, doxorubicin binds with high affinity to cardiolipin, inhibits Complex I, deviates electron flow from the respiratory chain to molecular oxygen yielding ROS ($O_2^{\cdot-}$ and $H_2O_2$) and stimulating peroxidation of lipids [131]. Therefore, targeting of doxorubicin to other compartments than mitochondria is of importance. Cationic anthracyclines form high-affinity complexes with anionic phospholipids. For example, the affinity constant for the interaction between doxorubicin and cardiolipin is $1.6\times10^6\,M^{-1}$ [132]. Application of CL-containing liposomes for doxorubicin delivery can limit its free diffusion through the cell membranes and avoid integration of the drug into the mitochondria.

The further fate of the drug in the liposomes is likely to be governed by changes in pH and by CL metabolism. The pH in late endosomes and lysosomes is acidic. At acidic conditions, the headgroup of CL bears only one negative charge ($pK_2>8.0$), while the amino group of doxorubicin is deprotonated (pK 8.2) and, hence, less than 1% of drug molecules is in the cationic state when the pH drops from below 6. This should lead to the loss of electrostatic interactions between doxorubicin and CL that are important for the formation of CL/doxorubicin complex [133]. The fast decomposition of CL in liposomes (that we recently documented by analyzing NBD-CL and its metabolites in cells) is the second factor, which will effectively liberate doxorubicin from lysosomes to cytosol. Overall, employment of CL-containing liposomes with inclusions of antitumor tetracycline antibiotics, such as doxorubicine, may be viewed as a useful idea in the development of approaches to targeted avoidance in selective drug delivery to specific intracellular compartments.

## 7. Concluding remarks

A diversified set of principles and chemically or biosynthetically synthesized delivery tools offers broad opportunities to achieving differential levels of enrichment or impoverishment of regulators of choice in different types of cells — tumor cells, surrounding normal cells as well as in their compartments. This may be important not only for stimulated sensitization of tumor cells towards pro-apoptotic agents (chemotherapeutics, irradiation) but also for increased resistance of surrounding normal tissues to them. As a result, escalation of radiation/chemotherapy regimens may be elaborated based on protection of the most sensitive normal cells to improve the clinical outcomes of treatment. This differential smart targeting may capitalize on known dramatic differences in metabolic energy profiles between normal and malignant cells. Indeed, tumor cells predominantly utilize glycolysis for their bioenergetic and metabolic demands whereas non-transformed cell rely mostly on mitochondrial respiration. Targeted delivery of mitochondrial protectors into normal cells with simultaneous accumulation of glycolytic poisons in cancers may generate a clinically appreciable difference for employment with traditional or optimized radio/chemotherapy regimens.

Finally, recent developments of nano-particle based protocols open fantastic new opportunities for temporally- and spatially-selective delivery and release of specified regulators at the site of their immediate action. This is achieved through the use of linkers sensitive to specific enzymatic activities highly expressed in different cell compartments (e.g., esterases, phosphatases, peptidases) or pH and redox environments in cells and in close proximity to the cell surface (e.g., acidified pH around tumor cells, high concentrations of $O_2^{\cdot-}$ in proximity to NADPH-sites on macrophages). As a result, one can imagine a nano-particle that will be similar to a multi-stage rocket, reaching the cell surface with a tool-set of regulators each of which will be released at pre-set intracellular compartments. While this idea may seem futuristic, the work in this field has already begun.

## Acknowledgements

This work was supported by NIH Grants U19 AIO68021, HL70755, HD057587, NS061817, NORA 927Z1LU, PittGrid (http://www.pittgrid.pitt.edu) and la Junta de Extremadura -Consejeria de Infraestructuras y Desarrollo Tecnologico- y el Fondo Social Europeo (Orden 2008050288).

## References

[1] C. Nihshelwood, Chemical kinetics in the past few decades, Nobel Lecture, December 11 1956.
[2] N. Semenov, Some problems relating to chain reactions and to the theory of combustion, Nobel Lecture, December 11, 1956.
[3] N. Emanuel, Free radicals and the action of inhibitors of radical processes under pathological states and ageing in living organisms and in man, Q. Rev. Biophys. 9 (1976).
[4] D. Harman, Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11 (1956) 298–300.
[5] D. Harman, A biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20 (1972) 145–147.
[6] B. Tarusov, Primary physico-chemical mechanisms of radiation injury, Radiobiologiia 7 (1967) 670–677 Sep–Oct.
[7] C. Ceconi, A. Boraso, A. Cargnoni, R. Ferrari, Oxidative stress in cardiovascular disease: myth or fact? Arch. Biochem. Biophys. 420 (2003) 217–221.
[8] R. Ferrari, G. Guardigli, D. Mele, G.F. Percoco, C. Ceconi, S. Curello, Oxidative stress during myocardial ischaemia and heart failure, Curr. Pharm. Des. 10 (2004) 1699–1711.
[9] E. Hervouet, H. Simonnet, C. Godinot, Mitochondria and reactive oxygen species in renal cancer, Biochimie 89 (2007) 1080–1088.
[10] T.M. Paravicini, G.R. Drummond, C.G. Sobey, Reactive oxygen species in the cerebral circulation: physiological roles and therapeutic implications for hypertension and stroke, Drugs 64 (2004) 2143–2157.
[11] H. Pelicano, D. Carney, P. Huang, ROS stress in cancer cells and therapeutic implications, Drug Resist. Updat. 7 (2004) 97–110.
[12] E. Trushina, C.T. McMurray, Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases, Neuroscience 145 (2007) 1233–1248.
[13] H. Forman, Hydrogen peroxide: the good, the bad, and the ugly, in: G. Valacchi (Ed.), Oxidants in Biology. A Question of Balance, Springer Science, 2008, pp. 1–18.
[14] B. Halliwell, J. Gutteridge, Free Radicals in Biology and Medicine, Clarendon Press, Oxford, U.K, 1999.
[15] Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. The Parkinson Study Group, N. Engl. J. Med. 328 (1993) 176–183.
[16] The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, N. Engl. J. Med. 330 (1994) 1029–1035.
[17] MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial, Lancet 360 (2002) 7–22.
[18] R. Clarke, J. Armitage, Antioxidant vitamins and risk of cardiovascular disease. Review of large-scale randomised trials, Cardiovasc. Drugs Ther. 16 (2002) 411–415.
[19] S. Devaraj, R. Tang, B. Adams-Huet, A. Harris, T. Seenivasan, J.A. de Lemos, I. Jialal, Effect of high-dose alpha-tocopherol supplementation on biomarkers of oxidative stress and inflammation and carotid atherosclerosis in patients with coronary artery disease, Am. J. Clin. Nutr. 86 (2007) 1392–1398.
[20] C.H. Hennekens, J.E. Buring, J.E. Manson, M. Stampfer, B. Rosner, N.R. Cook, C. Belanger, F. LaMotte, J.M. Gaziano, P.M. Ridker, W. Willett, R. Peto, Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease, N. Engl. J. Med. 334 (1996) 1145–1149.
[21] J.M. Leppala, J. Virtamo, R. Fogelholm, J.K. Huttunen, D. Albanes, P.R. Taylor, O.P. Heinonen, Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 230–235.
[22] I. Miklya, B. Knoll, J. Knoll, A pharmacological analysis elucidating why, in contrast to (−)-deprenyl (selegiline), alpha-tocopherol was ineffective in the DATATOP study, Life Sci. 72 (2003) 2641–2648.
[23] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart, S. Hammar, Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease, N. Engl. J. Med. 334 (1996) 1150–1155.
[24] D. Pratico, Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows, Ann N Y Acad Sci. 1147 (2008) 70–78.
[25] N.G. Stephens, A. Parsons, P.M. Schofield, F. Kelly, K. Cheeseman, M.J. Mitchinson, Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS), Lancet 347 (1996) 781–786.

[26] M.E. Tornwall, J. Virtamo, J.K. Haukka, D. Albanes, J.K. Huttunen, Alpha-tocopherol (vitamin E) and beta-carotene supplementation does not affect the risk for large abdominal aortic aneurysm in a controlled trial, Atherosclerosis 157 (2001) 167–173.
[27] S. Yusuf, G. Dagenais, J. Pogue, J. Bosch, P. Sleight, Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators, N. Engl. J. Med. 342 (2000) 154–160.
[28] K.T. Knecht, R.P. Mason, In vivo spin trapping of xenobiotic free radical metabolites, Arch. Biochem. Biophys. 303 (1993) 185–194.
[29] R.P. Mason, P.M. Hanna, M.J. Burkitt, M.B. Kadiiska, Detection of oxygen-derived radicals in biological systems using electron spin resonance, Environ. Health Perspect. 102 (Suppl 10) (1994) 33–36.
[30] R.P. Mason, K.T. Knecht, In vivo detection of radical adducts by electron spin resonance, Methods Enzymol. 233 (1994) 112–117.
[31] C. Richter, V. Gogvadze, R. Laffranchi, R. Schlapbach, M. Schweizer, M. Suter, P. Walter, M. Yaffee, Oxidants in mitochondria: from physiology to diseases, Biochim. Biophys. Acta 1271 (1995) 67–74.
[32] C.C. Winterbourn, D. Metodiewa, Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide, Free Radic. Biol. Med. 27 (1999) 322–328.
[33] P. Lester, E. Cadenas, Handbook of Synthetic Antioxidants, CRC Press, New York–Basel–Hong Kong, 1997, p. 442.
[34] B. Chance, N. Oshino, Kinetics and mechanisms of catalase in peroxisomes of the mitochondrial fraction, Biochem. J. 122 (1971) 225–233.
[35] B. Gray, A.J. Carmichael, Kinetics of superoxide scavenging by dismutase enzymes and manganese mimics determined by electron spin resonance, Biochem. J. 281 (Pt 3) (1992) 795–802.
[36] B. Halliwell, J. Gutteridge, Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity, Free Radic Biol Med, Clarendon Press, Oxford, 1989.
[37] J. Liu, S.Y. Qian, Q. Guo, J. Jiang, M.P. Waalkes, R.P. Mason, M.B. Kadiiska, Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies, Free Radic. Biol. Med. 45 (2008) 475–481.
[38] K. Nakai, M.B. Kadiiska, J.J. Jiang, K. Stadler, R.P. Mason, Free radical production requires both inducible nitric oxide synthase and xanthine oxidase in LPS-treated skin, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 4616–4621.
[39] E.G. Janzen, R.A. Towner, S. Yamashiro, The effect of phenyl tert-butyl nitrone (PBN) on CCl4-induced rat liver injury detected by proton magnetic resonance imaging (MRI) in vivo and electron microscopy (EM)Free Radic. Res. Commun. 9 (1990) 325–335.
[40] M.B. Kadiiska, R.P. Mason, Acute methanol intoxication generates free radicals in rats: an ESR spin trapping investigation, Free Radic. Biol. Med. 28 (2000) 1106–1114.
[41] M.B. Kadiiska, J.D. Morrow, J.A. Awad, L.J. Roberts II, R.P. Mason, Identification of free radical formation and F2-isoprostanes in vivo by acute Cr(VI) poisoning, Chem. Res. Toxicol. 11 (1998) 1516–1520.
[42] H. Miyakawa, R.P. Mason, J. Jiang, M.B. Kadiiska, Lipid-derived free radical production in superantigen-induced interstitial pneumonia, Free Radic. Biol. Med. 47 (2009) 241–249.
[43] S. Chatterjee, S. Kundu, S. Sengupta, A. Bhattacharyya, Divergence to apoptosis from ROS induced cell cycle arrest: effect of cadmium, Mutat. Res. 663 (2009) 22–31.
[44] E. Esposito, S. Cuzzocrea, Superoxide, NO, peroxynitrite and PARP in circulatory shock and inflammation, Front Biosci. 14 (2009) 263–296.
[45] H.R. Griffiths, ROS as signalling molecules in T cells—evidence for abnormal redox signalling in the autoimmune disease, rheumatoid arthritis, Redox Rep. 10 (2005) 273–280.
[46] A. Larbi, J. Kempf, G. Pawelec, Oxidative stress modulation and T cell activation, Exp. Gerontol. 42 (2007) 852–858.
[47] H. Ahsan, A. Ali, R. Ali, Oxygen free radicals and systemic autoimmunity, Clin. Exp. Immunol. 131 (2003) 398–404.
[48] H.R. Griffiths, Is the generation of neo-antigenic determinants by free radicals central to the development of autoimmune rheumatoid disease? Autoimmun. Rev. 7 (2008) 544–549.
[49] Z. Wu, I.A. MacPhee, D.B. Oliveira, Reactive oxygen species in the initiation of IL-4 driven autoimmunity as a potential therapeutic target, Curr. Pharm. Des. 10 (2004) 899–913.
[50] J. Everse, K.E. Everse, M.B. Grisham, Peroxidases in Chemistry and Biology, vol. I, CRC Press, Boca Raton, 1991.
[51] S. Peng, N.M. Okeley, A.L. Tsai, G. Wu, R.J. Kulmacz, W.A. van der Donk, Synthesis of isotopically labeled arachidonic acids to probe the reaction mechanism of prostaglandin H synthase, J. Am. Chem. Soc. 124 (2002) 10785–10796.
[52] C.A. Rouzer, L.J. Marnett, Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases, Chem. Rev. 103 (2003) 2239–2304.
[53] B. Sjodin, Y. Hellsten Westing, F.S. Apple, Biochemical mechanisms for oxygen free radical formation during exercise, Sports Med. 10 (1990) 236–254.
[54] J. St-Pierre, J.A. Buckingham, S.J. Roebuck, M.D. Brand, Topology of superoxide production from different sites in the mitochondrial electron transport chain, J. Biol. Chem. 277 (2002) 44784–44790.
[55] A. Boveris, E. Cadenas, A.O. Stoppani, Role of ubiquinone in the mitochondrial generation of hydrogen peroxide, Biochem. J. 156 (1976) 435–444.
[56] E. Cadenas, A. Boveris, C.I. Ragan, A.O. Stoppani, Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome *c* reductase from beef-heart mitochondria, Arch. Biochem. Biophys. 180 (1977) 248–257.
[57] F.L. Muller, Y. Liu, M.A. Abdul-Ghani, M.S. Lustgarten, A. Bhattacharya, Y.C. Jang, H. Van Remmen, High rates of superoxide production in skeletal-muscle mitochondria respiring on both complex I- and complex II-linked substrates, Biochem. J. 409 (2008) 491–499.
[58] J.F. Turrens, A. Alexandre, A.L. Lehninger, Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria, Arch. Biochem. Biophys. 237 (1985) 408–414.
[59] J.F. Turrens, A. Boveris, Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria, Biochem. J. 191 (1980) 421–427.
[60] F.L. Muller, Y. Liu, H. Van Remmen, Complex III releases superoxide to both sides of the inner mitochondrial membrane, J. Biol. Chem. 279 (2004) 49064–49073.
[61] H.P. Indo, M. Davidson, H.C. Yen, S. Suenaga, K. Tomita, T. Nishii, M. Higuchi, Y. Koga, T. Ozawa, H.J. Majima, Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage, Mitochondrion 7 (2007) 106–118.
[62] J.J. Palacino, D. Sagi, M.S. Goldberg, S. Krauss, C. Motz, M. Wacker, J. Klose, J. Shen, Mitochondrial dysfunction and oxidative damage in parkin-deficient mice, J. Biol. Chem. 279 (2004) 18614–18622.
[63] J.E. Ricci, C. Munoz-Pinedo, P. Fitzgerald, B. Bailly-Maitre, G.A. Perkins, N. Yadava, I.E. Scheffler, M.H. Ellisman, D.R. Green, Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain, Cell 117 (2004) 773–786.
[64] S. Orrenius, Reactive oxygen species in mitochondria-mediated cell death, Drug Metab. Rev. 39 (2007) 443–455.
[65] V.E. Kagan, V.A. Tyurin, J. Jiang, Y.Y. Tyurina, V.B. Ritov, A.A. Amoscato, A.N. Osipov, N.A. Belikova, A.A. Kapralov, V. Kini, Vlasova II, Q. Zhao, M. Zou, P. Di, D.A. Svistunenko, I.V. Kurnikov, G.G. Borisenko, Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors, Nat. Chem. Biol. 1 (2005) 223–232.
[66] L.V. Basova, I.V. Kurnikov, L. Wang, V.B. Ritov, N.A. Belikova, Vlasova II, A.A. Pacheco, D.E. Winnica, J. Peterson, H. Bayir, D.H. Waldeck, V.E. Kagan, Cardiolipin switch in mitochondria: shutting off the reduction of cytochrome *c* and turning on the peroxidase activity, Biochemistry 46 (2007) 3423–3434.
[67] N.A. Belikova, J. Jiang, Y.Y. Tyurina, Q. Zhao, M.W. Epperly, J. Greenberger, V.E. Kagan, Cardiolipin-specific peroxidase reactions of cytochrome *C* in mitochondria during irradiation-induced apoptosis, Int. J. Radiat. Oncol. Biol. Phys. 69 (2007) 176–186.
[68] J. Jiang, Z. Huang, Q. Zhao, W. Feng, N.A. Belikova, V.E. Kagan, Interplay between bax, reactive oxygen species production, and cardiolipin oxidation during apoptosis, Biochem. Biophys. Res. Commun. 368 (2008) 145–150.
[69] C.D. Berdanier, Mitochondria in health and disease, Oxidative Stress and Disease, vol. 16, CRC Group, 2005.
[70] N. Lane, Mitochondrial disease: powerhouse of disease, Nature 440 (2006) 600–602.
[71] J. Dessolin, M. Schuler, A. Quinart, F. De Giorgi, L. Ghosez, F. Ichas, Selective targeting of synthetic antioxidants to mitochondria: towards a mitochondrial medicine for neurodegenerative diseases? Eur. J. Pharmacol. 447 (2002) 155–161.
[72] J.S. Armstrong, D.P. Jones, Glutathione depletion enforces the mitochondrial permeability transition and causes cell death in Bcl-2 overexpressing HL60 cells, Faseb J. 16 (2002) 1263–1265.
[73] L.A. Pon, E.A. Schon, 2nd Ed., Mitochondria, vol. 80, Academic Press, 2007.
[74] K. Brandner, D.U. Mick, A.E. Frazier, R.D. Taylor, C. Meisinger, P. Rehling, Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth Syndrome, Mol. Biol. Cell 16 (2005) 5202–5214.
[75] G.M. Hatch, Cardiolipin: biosynthesis, remodeling and trafficking in the heart and mammalian cells, Int. J. Mol. Med. 1 (1998) 33–41 Review.
[76] Y. Xu, R.I. Kelley, T.J. Blanck, M. Schlame, Remodeling of cardiolipin by phospholipid transacylation, J. Biol. Chem. 278 (2003) 51380–51385.
[77] S. Skrede, A permeability barrier in mitochondria in; high-energy states' against positively charged disulphides, Biochem. J. 107 (1968) 645–654.
[78] E.A. Liberman, V.P. Topaly, L.M. Tsofina, A.A. Jasaitis, V.P. Skulachev, Mechanism of coupling of oxidative phosphorylation and the membrane potential of mitochondria, Nature 222 (1969) 1076–1078.
[79] X. Zhang, M.W. Epperly, M.A. Kay, Z.Y. Chen, T. Dixon, D. Franicola, B.A. Greenberger, P. Komanduri, J.S. Greenberger, Radioprotection in vitro and in vivo by minicircle plasmid carrying the human manganese superoxide dismutase transgene, Hum. Gene Ther. 19 (2008) 820–826.
[80] A.R. Oseroff, D. Ohuoha, G. Ara, D. McAuliffe, J. Foley, L. Cincotta, Intramitochondrial dyes allow selective in vitro photolysis of carcinoma cells, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 9729–9733.
[81] R. Bleday, M.J. Weiss, R.R. Salem, R.E. Wilson, L.B. Chen, G. Steele Jr., Inhibition of rat colon tumor isograft growth with dequalinium chloride, Arch. Surg. 121 (1986) 1272–1275.
[82] R. Paddenberg, B. Ishaq, A. Goldenberg, P. Faulhammer, F. Rose, N. Weissmann, R.C. Braun-Dullaeus, W. Kummer, Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature, Am. J. Physiol. Lung Cell Mol. Physiol. 284 (2003) L710–L719.
[83] X. Sun, et al., AA1, a newly synthesized monovalent lipophilic cation, expresses potent in vivo antitumor activity, Cancer Res. 54 (1994) 1465–1471.
[84] R.J. Burns, R.A. Smith, M.P. Murphy, Synthesis and characterization of thiobutyltriphenylphosphonium bromide, a novel thiol reagent targeted to the mitochondrial matrix, Arch. Biochem. Biophys. 322 (1995) 60–68.
[85] D.A. Lowes, B.M. Thottakam, N.R. Webster, M.P. Murphy, H.F. Galley, The mitochondria-targeted antioxidant MitoQ protects against organ damage in a lipopolysaccharide-peptidoglycan model of sepsis, Free Radic. Biol. Med. 45 (2008) 1559–1565.
[86] R.W. Horobin, S. Trapp, V. Weissig, Mitochondriotropics: a review of their mode of action, and their applications for drug and DNA delivery to mammalian mitochondria, J. Control Release 121 (2007) 125–136.
[87] M.W. Epperly, J.E. Gretton, C.A. Sikora, M. Jefferson, M. Bernarding, S. Nie, J.S. Greenberger, Mitochondrial localization of superoxide dismutase is required for decreasing radiation-induced cellular damage, Radiat. Res. 160 (2003) 568–578.

[88] M.W. Epperly, T. Dixon, H. Wang, J. Schlesselman, D. Franicola, J.S. Greenberger, Modulation of radiation-induced life shortening by systemic intravenous MnSOD-plasmid liposome gene therapy, Radiat. Res. 170 (2008) 437–443.
[89] M.W. Epperly, Mitochondrial targetting of a catalase transgene product by plasmid liposomes increases radioresistance in vitro and in vivo, Radiat. Res. 171 (2009) 588–595.
[90] M.W. Epperly, C.A. Sikora, S.J. DeFilippi, J.A. Gretton, Q. Zhan, D.W. Kufe, J.S. Greenberger, Manganese superoxide dismutase (SOD2) inhibits radiation-induced apoptosis by stabilization of the mitochondrial membrane, Radiat. Res. 157 (2002) 568–577.
[91] M.W. Epperly, Delayed intratracheal injection of manganese superoxide dismutase (MnSOD)-plasmid/liposomes provides suboptimal protection against irradiation-induced pulmonary injury compared to treatment before irradiation, Gene Ther. Mol. Biol. 7 (2003) 61–68.
[92] S. Koene, J. Smeitink, Mitochondrial medicine: entering the era of treatment, J. Intern. Med. 265 (2009) 193–209.
[93] D. Nelson, M. Cox, Lehninger Principles of Biochemistry, 2004.
[94] L. Biasutto, A. Mattarei, E. Marotta, A. Bradaschia, N. Sassi, S. Garbisa, M. Zoratti, C. Paradisi, Development of mitochondria-targeted derivatives of resveratrol, Bioorg. Med. Chem. Lett. 18 (2008) 5594–5597.
[95] S. Leo, G. Szabadkai, R. Rizzuto, The mitochondrial antioxidants MitoE(2) and MitoQ(10) increase mitochondrial Ca(2+) load upon cell stimulation by inhibiting Ca(2+) efflux from the organelle, Ann. N. Y. Acad. Sci. 1147 (2008) 264–274.
[96] V.P. Skulachev, V.N. Anisimov, Y.N. Antonenko, L.E. Bakeeva, B.V. Chernyak, V.P. Erichev, O.F. Filenko, N.I. Kalinina, V.I. Kapelko, N.G. Kolosova, B.P. Kopnin, G.A. Korshunova, M.R. Lichinitser, L.A. Obukhova, E.G. Pasyukova, O.I. Pisarenko, V.A. Roginsky, E.K. Ruuge, Senin II, Severina II, M.V. Skulachev, I.M. Spivak, V.N. Tashlitsky, V.A. Tkachuk, M.Y. Vyssokikh, L.S. Yaguzhinsky, D.B. Zorov, An attempt to prevent senescence: a mitochondrial approach, Biochim. Biophys. Acta 1787 (2009) 437–461.
[97] R.A. Smith, V.J. Adlam, F.H. Blaikie, A.R. Manas, C.M. Porteous, A.M. James, M. F. Ross, A. Logan, H.M. Cocheme, J. Trnka, T.A. Prime, I. Abakumova, B.A. Jones, A. Filipovska, M.P. Murphy, Mitochondria-targeted antioxidants in the treatment of disease, Ann. N. Y. Acad. Sci. 1147 (2008) 105–111.
[98] R.A. Smith, C.M. Porteous, C.V. Coulter, M.P. Murphy, Selective targeting of an antioxidant to mitochondria, Eur. J. Biochem. 263 (1999) 709–716.
[99] J. Trnka, F.H. Blaikie, R.A. Smith, M.P. Murphy, A mitochondria-targeted nitroxide is reduced to its hydroxylamine by ubiquinol in mitochondria, Free Radic. Biol. Med. 44 (2008) 1406–1419.
[100] H.H. Szeto, Cell-permeable, mitochondrial-targeted, peptide antioxidants, Aaps J. 8 (2006) E277–E283.
[101] K. Zhao, G.M. Zhao, D. Wu, Y. Soong, A.V. Birk, P.W. Schiller, H.H. Szeto, Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury, J. Biol. Chem. 279 (2004) 34682–34690.
[102] V.J. Adlam, J.C. Harrison, C.M. Porteous, A.M. James, R.A. Smith, M.P. Murphy, I.A. Sammut, Targeting an antioxidant to mitochondria decreases cardiac ischemia–reperfusion injury, Faseb J. 19 (2005) 1088–1095.
[103] J.S. Tauskela, MitoQ—a mitochondria-targeted antioxidant, IDrugs 10 (2007) 399–412.
[104] A.K. Doughan, S.I. Dikalov, Mitochondrial redox cycling of mitoquinone leads to superoxide production and cellular apoptosis, Antioxid. Redox Signal. 9 (2007) 1825–1836.
[105] A.T. Hoye, J.E. Davoren, P. Wipf, M.P. Fink, V.E. Kagan, Targeting mitochondria, Acc. Chem. Res. 41 (2008) 87–97.
[106] B.P. Soule, F. Hyodo, K. Matsumoto, N.L. Simone, J.A. Cook, M.C. Krishna, J.B. Mitchell, The chemistry and biology of nitroxide compounds, Free Radic. Biol. Med. 42 (2007) 1632–1650.
[107] J.B. Mitchell, A. Russo, P. Kuppusamy, M.C. Krishna, Radiation, radicals, and images, Ann. N. Y. Acad. Sci. 899 (2000) 28–43.
[108] P. Wipf, J. Xiao, J. Jiang, N.A. Belikova, V.A. Tyurin, M.P. Fink, V.E. Kagan, Mitochondrial targeting of selective electron scavengers: synthesis and biological analysis of hemigramicidin-TEMPO conjugates, J. Am. Chem. Soc. 127 (2005) 12460–12461.
[109] J. Jiang, I. Kurnikov, N.A. Belikova, J. Xiao, Q. Zhao, A.A. Amoscato, R. Braslau, A. Studer, M.P. Fink, J.S. Greenberger, P. Wipf, V.E. Kagan, Structural requirements for optimized delivery, inhibition of oxidative stress, and antiapoptotic activity of targeted nitroxides, J. Pharmacol. Exp. Ther. 320 (2007) 1050–1060.
[110] C.A. Macias, J.W. Chiao, J. Xiao, D.S. Arora, Y.Y. Tyurina, R.L. Delude, P. Wipf, V.E. Kagan, M.P. Fink, Treatment with a novel hemigramicidin-TEMPO conjugate prolongs survival in a rat model of lethal hemorrhagic shock, Ann. Surg. 245 (2007) 305–314.
[111] V.E. Kagan, A. Bayir, H. Bayir, D. Stoyanovsky, G.G. Borisenko, Y.Y. Tyurina, P. Wipf, J. Atkinson, J.S. Greenberger, R.S. Chapkin, N.A. Belikova, Mitochondria-targeted disruptors and inhibitors of cytochrome *c*/cardiolipin peroxidase complexes: a new strategy in anti-apoptotic drug discovery, Mol. Nutr. Food Res. 53 (2009) 104–114.
[112] M.P. Fink, C.A. Macias, J. Xiao, Y.Y. Tyurina, J. Jiang, N. Belikova, R.L. Delude, J.S. Greenberger, V.E. Kagan, P. Wipf, Hemigramicidin-TEMPO conjugates: novel mitochondria-targeted anti-oxidants, Biochem. Pharmacol. 74 (2007) 801–809.
[113] J. Jiang, N.A. Belikova, A.T. Hoye, Q. Zhao, M.W. Epperly, J.S. Greenberger, P. Wipf, V.E. Kagan, A mitochondria-targeted nitroxide/hemigramicidin S conjugate protects mouse embryonic cells against gamma irradiation, Int. J. Radiat. Oncol. Biol. Phys. 70 (2008) 816–825.
[114] A. Dhanasekaran, S. Kotamraju, C. Karunakaran, S.V. Kalivendi, S. Thomas, J. Joseph, B. Kalyanaraman, Mitochondria superoxide dismutase mimetic inhibits peroxide-induced oxidative damage and apoptosis: role of mitochondrial superoxide, Free Radic. Biol. Med. 39 (2005) 567–583.
[115] E. Kalanxhi, C.J. Wallace, Cytochrome *c* impaled: investigation of the extended lipid anchorage of a soluble protein to mitochondrial membrane models, Biochem. J. 407 (2007) 179–187.
[116] C. Kawai, F.M. Prado, G.L. Nunes, P. Di Mascio, A.M. Carmona-Ribeiro, I.L. Nantes, pH-Dependent interaction of cytochrome *c* with mitochondrial mimetic membranes: the role of an array of positively charged amino acids, J. Biol. Chem. 280 (2005) 34709–34717.
[117] A. Patriarca, T. Eliseo, F. Sinibaldi, M.C. Piro, R. Melis, M. Paci, D.O. Cicero, F. Polticelli, R. Santucci, L. Fiorucci, ATP acts as a regulatory effector in modulating structural transitions of cytochrome *c*: implications for apoptotic activity, Biochemistry 48 (2009) 3279–3287.
[118] F. Sinibaldi, G. Mei, F. Polticelli, M.C. Piro, B.D. Howes, G. Smulevich, R. Santucci, F. Ascoli, L. Fiorucci, ATP specifically drives refolding of non-native conformations of cytochrome *c*, Protein Sci. 14 (2005) 1049–1058.
[119] J. Kanner, et al., Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin, Lipids 27 (1992) 46–49.
[120] A.A. Caro, et al., Oxidation of the ketoxime acetoxime to nitric oxidde by oxygen radical-generating systems, Nitric Oxide 5 (2001) 413–424.
[121] A.D. McGill, et al., para-Substituted N-nitroso-N-oxybenzenamine ammonium salts: a new class of redox-sensitive nitric oxide releasing compounds, Bioorg. Med. Chem. 8 (2000) 405–412.
[122] D.A. Stoyanovsky, et al., Activation of NO donors in mitochondria: peroxidase metabolism of (2-hydroxyamino-vinyl)-triphenyl-phosphonium by cytochrome *c* releases NO and protects cells against apoptosis, FEBS Lett. 582 (2008) 725–728.
[123] N.A. Belikova, J. Jiang, D.A. Stoyanovsky, A. Glumac, H. Bayir, J.S. Greenberger, V.E. Kagan, Mitochondria-targeted (2-hydroxyamino-vinyl)-triphenyl-phosphonium releases NO(.) and protects mouse embryonic cells against irradiation-induced apoptosis, FEBS Lett. 583 (2009) 1945–1950.
[124] R. Kissner, T. Nauser, P. Bugnon, P.G. Lye, W.H. Koppenol, Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis, Chem. Res. Toxicol. 10 (1997) 1285–1292.
[125] T. Nauser, W.H. Koppenol, The rate constant of the reaction of superoxide with nitrogen monoxide: approaching the diffusion limit, J. Phys. Chem. A 106 (2002) 4084–4086.
[126] R. Radi, A. Cassina, R. Hodara, C. Quijano, L. Castro, Peroxynitrite reactions and formation in mitochondria, Free Radic. Biol. Med. 33 (2002) 1451–1464.
[127] Y. Aramaki, Liposomes as immunomodulator—inhibitory effect of liposomes on NO production from macrophages, Biol. Pharm. Bull. 23 (2000) 1267–1274.
[128] N.V. Konduru, Y.Y. Tyurina, W. Feng, L.V. Basova, N.A. Belikova, H. Bayir, K. Clark, M. Rubin, D. Stolz, H. Vallhov, A. Scheynius, E. Witasp, B. Fadeel, P.D. Kichambare, A. Star, E.R. Kisin, A.R. Murray, A.A. Shvedova, V.E. Kagan, Phosphatidylserine targets single-walled carbon nanotubes to professional phagocytes in vitro and in vivo, PLoS ONE 4 (2009) e4398.
[129] M. Schlame, D. Rua, M.L. Greenberg, The biosynthesis and functional role of cardiolipin, Prog. Lipid Res. 39 (2000) 257–288.
[130] K. Jung, R. Reszka, Mitochondria as subcellular targets for clinically useful anthracyclines, Adv Drug Deliv Rev. 49 (2001) 87–105.
[131] M. Kitada, T. Horie, S. Awazu, Chemiluminescence associated with doxorubicin-induced lipid peroxidation in rat heart mitochondria, Biochem. Pharmacol. 48 (1994) 93–99.
[132] E. Goormaghtigh, P. Chatelain, J. Caspers, J.M. Ruysschaert, Evidence of a specific complex between adriamycin and negatively-charged phospholipids, Biochim. Biophys. Acta 597 (1980) 1–14.
[133] K. Nicolay, A.M. Sautereau, J.F. Tocanne, R. Brasseur, P. Huart, J.M. Ruysschaert, B. de Kruijff, A comparative model membrane study on structural effects of membrane-active positively charged anti-tumor drugs, Biochim. Biophys. Acta 940 (1988) 197–208.