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REVIEW

Mitochondrial Medicine: Pharmacological targeting of mitochondria in disease

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Mitochondria play a central role in cell life and death and are known to be important in a wide range of diseases including the cancer, diabetes, cardiovascular disease, and the age-related neurodegenerative diseases. The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain. This review discusses mitochondrial drug targeting strategies and a variety of novel mitochondrial drug targets including the electron transport chain, mitochondrial permeability transition, Bcl-2 family proteins and mitochondrial DNA. Mitochondrial drug-targeting strategies will open up avenues for manipulating mitochondrial functions and allow for selective protection or eradication of cells for therapeutic gain in a variety of diseases.
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Abbreviations: $\Delta\mu_{H+}$, mitochondrial proton gradient; $\Delta\psi_m$, mitochondrial membrane potential; AD, Alzheimer's disease; ALS, amytrophic lateral sclerosis; ANT, adenine nucleotide translocator; bc_1, complex III; BR, benzodiazepine receptor; CD437, 6[3-adamantyl-4-hydroxyphenyl]-2-naphthalene carboxylic acid; CK, creatine kinase; complex I, NADH dehydrogenase; CsA, cyclosporin A; CyP-D, cyclophilin-D; DLC, delocalized lipophilic cation; DQAsome, dequalinium liposome; Drp-1, dynamin-related protein; ETC, electron transport chain; F0F1ATPase, ATP synthase; FRDA, Friedreichs ataxia; GFP, green fluorescent protein; HD, Huntington's disease; hFis1, mitochondrial fission protein; HK, hexokinase; IR, ischaemia and reperfusion; LND, lonidamine; MI, myocardial infarction; MitoPBN, triphenyl-phosphonium cation (TPP^+)-linked phenyl tert-butylnitrone; MitoPeroxidase, TPP^+-linked peroxidase (ebselen); MitoQ, TPP^+-linked coenzyme Q; MnSOD, manganese superoxide dismutase; MPT, mitochondrial permeability transition; MSP, mitochondria signal peptide; mtDNA, mitochondrial DNA; $O_2^{\bullet-}$, superoxide anion; OPA1, optic atrophy protein; OX-PHOS, oxidative phosphorylation; PD, Parkinson's disease; Rh123, rhodamine 123; ROS, reactive oxygen species; SOD1, superoxide dismutase; SS peptides, Szeto–Schiller peptides; SS01, tyrosine-linked peptide; SS31, dimethyltyrosine-linked peptide; THG, thapsigargin; UCP, uncoupling protein; VDAC, voltage-dependent anion channel

Introduction

After the landmark discovery of the regulation of mitochondrial energy production by chemiosmosis (Mitchell and Moyle, 1967) many scientists considered the role and function of the mitochondrion solved. Mitochondria again took the spotlight in the 1980s, with the breakthrough that certain diseases are caused by mutations in mitochondrial DNA (mtDNA) (Wallace *et al*., 1988) as well as by the seminal findings of Liu *et al*. (1996)that mitochondria are key regulators of programmed cell death by apoptosis. These discoveries rekindled scientific interest in the mitochondrion, and in its potential role in a variety of diseases including cancer, cardiovascular disease, diabetes and neurodegenerative diseases all of which have a significant mitochondrial component. Advances in mitochondrial research and in medical technology have been a major impetus behind the desire to design and develop drugs specifically targeting mitochondria for therapeutic gain. The main focus of this review is to discuss mitochondrial drug targeting strategies and novel mitochondrial targets that will, in the future, promote the development of mitochondrial-directed therapeutics. Targeting of biologically active molecules to the mitochondria of living cells will open up avenues for manipulating mitochondrial functions and allow for selective protection or eradication of cells for therapeutic gain in a variety of diseases.

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Mitochondrial drug targeting rationale

The rationale for targeting drugs to mitochondria for therapeutic gain lies in the fact that mitochondria play a key role in the regulation of energy metabolism, reactive oxygen species (ROS) production and apoptosis. Therefore, the specific delivery of drugs to mitochondria may provide the foundation to treat a variety of diseases wherein these functions are deregulated. The potential therapeutic applications of mitochondrial targeting include: (1) the delivery of antioxidants to mitochondria to prevent oxidative damage associated with the neurodegenerative diseases, ischaemia and reperfusion (IR) tissue injury and diabetes; (2) the targeting of toxic drugs or Bcl-2 proteins to mitochondria to trigger apoptosis in cancer therapy; (3) the delivery of drugs to mitochondria to inhibit the mitochondrial permeability transition (MPT) in IR-related tissue injury, for example, in heart attack and stroke; and (4) the targeting of drugs to either uncouple the electron transport chain (ETC), or activate the uncoupling proteins (UCPs), in obesity and diabetes (Table 1).

The benefits of targeting drugs to specific subcellular sites

Systemic drug administration is problematic because of the need to use a high concentration of drug to achieve an effective local concentration at the disease site because this often results in accompanying nonspecific toxic side effects. The idea of targeting drugs to the specific site of the disease was recognized by Ehrlich in the early 1900s whose 'Magic Bullet' approach remains the goal of pharmaceutical scientists worldwide. A variety of methods have been developed to achieve the selective targeting of drugs *in vivo* including the use of soluble polymer carrier systems, micelles and liposome-based strategies (Torchilin, 2000). While at the subcellular level, potential drug targets include the nucleus for gene therapy, mitochondria for proapoptotic cancer therapies and replacement enzyme therapies for the lysosomal storage diseases (Torchilin, 2006). For example, Gaucher disease is a lipid storage disease that has been successfully treated by replacing the lysosomal enzyme glucocerebrosidase and proapoptotic Bcl-2 family proteins have been

Table 1 Pharmacological targeting of mitochondria in disease

Molecule targeted	*Target*	*Desired effect*	*Potential disease treated*
MitoQ	$\Delta\psi_m$	Antioxidant[a]	Neurodegenerative disease,
MitoPBN	$\Delta\psi_m$	Antioxidant[a]	IR injury and diabetes
MitoPeroxidase	$\Delta\psi_m$	Antioxidant[a]	
GSH-choline ester	$\Delta\psi_m$	Antioxidant[a]	
NAC-choline ester	$\Delta\psi_m$	Antioxidant[a]	
SS31	MSP inner membrane	Antioxidant[a]	
SS01	MSP inner membrane	Antioxidant[a]	
mtDNA?	DQAsome-protein import pathway?	Replacement for mutant mtDNA	mtDNA-associated diseases
Cyanine dyes – MKT-077	$\Delta\psi_m$	Apoptosis[b]	Photochemotherapy
Rhodamine 123	$\Delta\psi_m$	Apoptosis[b]	Cancer therapy
DLC-AA1	$\Delta\psi_m$	Apoptosis[b]	
Paclitaxel	DQAsome-protein import pathway	Apoptosis[b]	
	Drp-1, hFis1, OPA1	Apoptosis[b]	
Ciprofloxacin	mtDNA-ETC	Apoptosis[b]	
Diamide-GSH depletion	Redox-Bcl-2	Apoptosis[b]	
Antisense oligonucleotides	A1-Bcl-XL Bcl-2	Apoptosis[b]	
BK11195	Benzodiazepine receptor-Bcl-2	Apoptosis[b]	
SMAC-DIABLO mimetic	Inhibit XIAP	Apoptosis[b]	
BH3 mimetic (SAHB)	Activate Bax	Apoptosis[b]	
Arsenite	Redox-MPT	Apoptosis[b]	
LND	ETC	Apoptosis[b]	
Betulinic acid	MPT	Apoptosis[b]	
CD437	MPT	Apoptosis[b]	
Mastoparan	$\Delta\psi_m$	Apoptosis[b]	
CyP-D overexpression?	MPT?	Necrosis/apoptosis	
CsA	CyP-D-MPT	↓ Necrosis/apoptosis[c]	IR injury in heart and brain attack
Ruthenium analogues	Ca^{2+} uniporter	↓ Necrosis/apoptosis[c]	
4-methyl-val-CsA	CyP-D-MPT	↓ Necrosis/apoptosis[c]	
Sangliferin	MPT	↓ Necrosis/apoptosis[c]	
Ro 68–3400	MPT	↓ Necrosis/apoptosis[c]	
UCP activators	UCP1	$\Delta\mu_{H+}$ to block ATP and ROS[d]	Diabetes/obesity
MnSOD	Matrix	Reduce ROS[d]	

Abbreviations: CsA, cyclosporin A; DQAsome, dequalinium liposome; ETC, electron transport chain; IR, ischaemia and reperfusion; LND, lonidamine; MnSOD, manganese superoxide dismutase; MPT, mitochondrial permeability transition; MSP, mitochondria signal peptide; mtDNA, mitochondrial DNA; NAC, *N*-acetylcysteine; ROS, reactive oxygen species; UCP, uncoupling protein; $\Delta\psi_m$, mitochondrial membrane potential; XIAP, inhibitor of apoptosis protein.
Table shows a variety of molecules that have been targeted to mitochondria for therapeutic gain and their putative mitochondrial targets. It also includes the desired effects of the drug targeting and the potential diseases in which these strategies could be applied. These include: (1) antioxidant effects for treatment of neurodegenerative disease, IR injury and diabetes[a]; (2) induction of apoptosis for cancer therapy[b]; (3) inhibition of MPT-related cell death to prevent IR-mediated tissue injury in brain and heart attack[c]; and (4) inhibition of the $\Delta\mu_{H+}$ by activation of UCP to block ATP production or ROS increase in obesity and diabetes, respectively[d] (see main text for detailed explanation).

successfully targeted to the mitochondrion to induce apoptosis in cancer therapy (Denicourt and Dowdy, 2004). It is also apparent that a number of other diseases, with a mitochondrial component, including the neurodegenerative diseases, IR tissue injury and diabetes, could offer similar opportunities for mitochondrial drug therapy. However, there has been a notable lack of progress in the development of mitochondria-specific drug delivery systems possibly due to a number of reasons including the notion that drugs targeted to the cell will eventually reach the mitochondrion by random interaction with subcellular components and also because important structural and functional knowledge of a variety of potential mitochondrial drug targets is lacking, for example, only about a third of the putative mitochondrial inner membrane ion transporters has been allocated a function. This is exemplified by the MPT, a major player in cell death, whose structure and mitochondrial location remains unknown after approximately 30 years of investigation (Haworth and Hunter, 1979). However, new ideas on mitochondrial targeting will become more important as scientific advances are made in mitochondrial structural biology, biochemistry and genetics which will identify new targets for therapeutic intervention. For example, the recent application of high-voltage electron tomography to study mitochondrial structure (Frey and Mannella, 2000) is providing important new information on the role of mitochondrial fission and fusion proteins in apoptotic cell death (Cipolat *et al*., 2006; Frezza *et al*., 2006).

Mitochondriotropic delivery devices

Delocalized lipophilic cations

Delocalized lipophilic cations (DLCs) have been used as carriers to deliver a variety of biologically active molecules to mitochondria because they target the mitochondrial inner membrane and accumulate in the matrix as a function of mitochondrial membrane potential ($\Delta\psi_m$). In this light, a variety of anticancer drugs strategies have been proposed based on the observation that DLC selectively targets cancer cells due to their higher $\Delta\psi_m$ compared to normal cells (Chen, 1988). Thus, rhodamine 123 (Rh123) has been used to direct a variety of anticancer drugs selectively to lung carcinoma cells (Teicher *et al*., 1987). In contrast to targeting apoptosis, DLCs are also being successfully used to deliver antioxidants to the mitochondria of cells to prevent cell death. For example, MitoQ, a triphenyl-phosphonium cation (TPP^+)-linked derivative, has been developed as mitochondriotropic antioxidant to directly target mitochondria to the site of ROS production in the cell while also circumventing poor solubility problems associated with the natural antioxidant coenzyme Q (CoQ10). MitoQ has been shown to concentrate several 100-fold within mitochondria and to be a significantly more potent antioxidant than the nontargeted CoQ10 analogue decylubiquinone (Jauslin *et al*., 2003). The efficacy of DLC-linked mitochondrial-targeted antioxidants has recently been shown in a study where MitoQ was found to be potently protective in an *ex vivo* model of IR injury (Adlam *et al*., 2005) and by the fact that it is currently undergoing clinical trials for Parkinson's disease (PD). Other antioxidant compounds that have been linked to DLC include the spin trap phenyl tert-butylnitrone (PBN) producing MitoPBN which was recently shown to protect animal models against IR tissue injury and neurodegenerative disease (Saito *et al*., 1998; Maples *et al*., 2004). DLC-conjugated compounds also include MitoPeroxidase, a derivative of ebselen, which possesses GSH peroxidase (Filipovska *et al*., 2005). In contrast to increasing GSH-peroxidase activity to break down mitochondrial peroxides, an alternative idea has been to specifically increase the mitochondrial GSH levels with the development of the mitochondria-targeted GSH-choline ester and *N*-acetyl-cysteine-choline ester (Sheu *et al*., 2006) (some of these targeting ideas are illustrated in Figure 1a). These compounds, although apparently effective as antioxidants in a variety of cell-based and animal studies suffer from the fact that at high concentrations they can depolarize the $\Delta\psi_m$ and lead to cell death. Thus, the use of DLC-linked antioxidants as cytoprotective agents must be accompanied by thorough studies of potential concentration-dependent side effects.

Szeto–Schiller peptides as mitochondriotropic agents

A novel class of cell-permeable antioxidant peptides that selectively partition into the inner mitochondrial membrane independent of the $\Delta\psi_m$ has recently been reported (Szeto, 2006a). These peptides, known as Szeto–Schiller (SS) peptides, possess a structurally similar aromatic–cationic motif in which the aromatic group, either tyrosine (SS01) or a dimethyltyrosine (SS31) group, alternates with a basic amino acid. SS peptides are rapidly taken up into cells, reach a steady-state concentration in minutes, and have a sequence motif that targets them to mitochondria in an energy-independent and nonsaturable manner (Szeto, 2006a) (illustrated in Figure 1b). Incubation of isolated mitochondria with [^{3}H]SS-02 or [^{3}H]SS-31 has shown that SS peptides are taken up and concentrated 1000–5000-fold in mitochondria and even though they are cationic, mitochondrial fractionation studies have shown that they are localized to the inner mitochondrial membrane and not the matrix (Zhao *et al*., 2004). Because SS peptides are not delivered into the matrix, their uptake is not self-limiting, and they do not cause mitochondrial depolarization even at high concentrations up to 1 mM, which is a potential benefit over DLC-linked antioxidants. Studies with isolated mitochondrial preparations and cell cultures have shown that SS peptides scavenge and reduce mitochondrial ROS production and block the MPT thereby inhibiting oxidative stress-induced apoptosis and necrosis. SS peptides are nontoxic and have been shown to protect against IR-mediated tissue injury (Cho *et al*., 2007) and neurodegenerative disease in animal models (Szeto, 2006b). In theory, SS peptides could be used as delivery devices for a variety of drugs including apoptosis activating drugs. Thus a variety of mitochondrial-targeted antioxidant strategies are currently being developed and it will be interesting to see how these approaches compare *in vivo* with nontargeted antioxidants. In addition, these approaches will provide useful information on cell-specific sites of toxic ROS generation during different disease states.

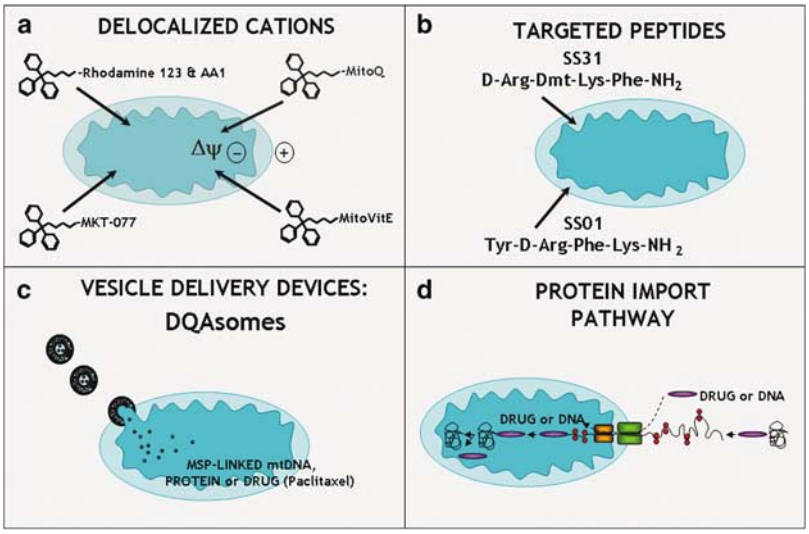

Figure 1 Title: pharmacological targeting of mitochondria in disease. (**a**) Mitochondria accumulate delocalized lipophilic cations (DLCs) because of the large membrane potential across their inner membrane (negative on the inside). DLCs can be linked to a variety of bioactive compounds including derivatives of antioxidants such as coenzyme Q and vitamin E yielding MitoQ and MitoVit E, respectively. Some DLCs are selectively toxic to cancer cells due to their increased $\Delta\psi_m$ including Rh123 or AA1, whereas others such as the cyanine dye MKT-077 can be photoexited to yield toxic species. (**b**) Szeto–Schiller (SS) peptides selectively partition into the inner mitochondrial membrane independent of the $\Delta\psi_m$ and possess intrinsic antioxidant and cytoprotective properties. They contain an aromatic–cationic sequence motif in which the aromatic group, either tyrosine (SS01) or a dimethyltyrosine group (SS31), alternates with a basic amino acid. (**c**) Dequalinium is a dicationic mitochondriotropic compound that self-assembles and forms vesicle-like aggregates called DQAsomes. These vesicles have been shown to be actively taken up by endocytosis and to fuse with the mitochondrial outer membrane allowing mitochondria signal peptide (MSP)-tagged cargo to enter the matrix via the protein import system. The anticancer drug paclitaxel has been successfully delivered to mitochondria using this approach. (**d**) Mitochondria can be targeted by linking a MSP to a nonmitochondrial protein to create a chimeric protein that is taken up in to the mitochondrial matrix via the protein import pathway. This strategy can target drugs, proteins and covalently linked MSP-DNA to mitochondria.

Colloidal drug delivery systems dequalinium liposomes

Liposomes, and other vesicular carrier systems, have been used for a number of years to deliver drugs to cells. Liposomal drug delivery systems offer specific advantages over non-encapsulated drug systems including: (1) improved drug release properties; (2) the ability to target cells and avoid problems associated with systemic toxicity; and (3) the ability to include several drugs into one delivery system (Minko *et al*., 2006). This method of drug targeting is currently being expanded to the subcellular level with the development of phospholipid and non-phospholipid-based mitochondriotropic drug carrier systems (Weissig *et al*., 2004, 2006; Boddapati *et al*., 2005). For example, dequalinium is a dicationic mitochondriotropic compound that self-assembles and forms vesicle-like aggregates called dequalinium liposomes (DQAsomes). These vesicles have been shown to be actively taken up by endocytosis and to fuse with the mitochondrial outer membrane allowing mitochondria signal peptide (MSP)-tagged cargo to enter the matrix via the protein import system (Weissig *et al*., 2004). This delivery system has been successfully shown to deliver paclitaxel to human colon carcinoma cells and activate apoptosis (Weissig *et al*., 2004) (this targeting idea is illustrated in Figure 1c). There are a number of potential benefits of DQAsome-mediated delivery systems. First, the therapeutic window for many drugs is relatively narrow and direct mitochondrial targeting should allow the delivery of a more precise drug dose to mitochondria while avoiding cellular toxicity. Second, the ability to hide a drug inside the vesicle should avoid the problems of potential drug resistance mediated by the P-glycoprotein multidrug efflux pump. Third, mitochondrial encapsulation of many drugs will avoid the need to prepare them as oil-based emulsions, for example, paclitaxel is administered as an emulsion of 'Cremophor EL' which is itself toxic (Seligson *et al*., 2001). Fourth, because cancer cells possess an increased $\Delta\psi_m$ compared to normal cells, vesicular mitochondriotropic delivery systems containing DLC-linked toxic drugs could selectively deliver drug to the mitochondria of tumour cells rather than normal cells. Fifth, DQAsome delivery systems could be used to transport drugs to the mitochondrial matrix that are otherwise precluded from use due to their nonspecific actions in the cytosol such as cyclosporin A (CsA), an inhibitor of the MPT.

The mitochondrial protein import machinery

An alternative mitochondrial targeting strategy that involves the delivery of macromolecules to mitochondria utilizing the mitochondrial protein import machinery has also been proposed. The MSP, an N-terminal-specific amino-acid sequence, can be linked to other nonmitochondrial proteins to create a chimeric protein that is taken up in to the mitochondrial matrix *via* the protein import pathway (this targeting idea is illustrated in Figure 1d). For example, a MSP has been linked to green fluorescent protein which has allowed the visualization of mitochondria in living cells (Murphy, 1997). The MSP has also been linked to aequorin, a calcium-sensitive protein, to determine mitochondrial

calcium levels in cells (Brini *et al*., 1995). Cytosolic enzymes such as dihydrofolate reductase and cytochrome *c* oxidase have also been successfully targeted to mitochondria by linking them to a MSP. However, it has been noted that the proteins conformation may significantly alter the efficiency of the protein import process (Verner and Lemire, 1989). The mitochondrial protein import pathway has also being used for mitochondrial gene replacement and, therefore, could potentially be used to correct a mutant mitochondrial genome similar to classical gene replacement therapies that have replaced a corrected copy of a defective nuclear gene. For example, the MSP for ornithine transcarbamylase was linked to a DNA molecule and the chimera was found to be efficiently translocated into the matrix of mitochondria (Seibel *et al*., 1995). Thus, the targeting of covalently linked MSP-DNA molecules to mitochondria via the protein import pathway may open up new ways for mtDNA delivery strategies.

Caveats concerning mitochondrial drug targeting

First, although studies have suggested that mitochondrial targeting ideas are effective in animal disease models, many of these investigations have been relatively short-term and currently there is very little data on the long-term side effects of using these compounds in animals. Another problem is the current lack of efficient methods to regulate the delivery of these drugs to the tissue of interest. For example, where MitoQ was shown to be protective in an *ex vivo* model of cardiac IR injury (Adlam *et al*., 2005), MitoVit E failed to protect rat neurons from acute hypoxia–ischaemia injury and at high concentration was neurotoxic (Covey *et al*., 2006). One possible idea to circumvent this problem might be to couple the mitochondrial drug to a carrier molecule to create an inactive prodrug that could be activated by a tissue-specific product, such as an enzyme, to release the mitochondrial drug for targeting. An example of this 'magic bullet' approach, has recently been shown with the SERCA-ATPase inhibitor thapsigargin (THG), which indirectly targets mitochondria through Bax activation and calcium signalling (Zhang and Armstrong, 2007). THG was linked to a peptide to create an inactive prodrug that was activated by the serine protease prostate-specific antigen specifically produced by prostate cancer cells (Denmeade and Isaacs, 2005).

Mitochondrial drug targets

Proteins of the mitochondrial outer membrane

The mitochondrial outer membrane encloses the entire organelle and contains porins or voltage-dependent anion channels (VDACs), which are small pore-forming proteins found in all eukaryotic cells. VDACs regulate the flux of metabolites between the cytosol and the mitochondrion and are also involved in the regulation of apoptosis by their interaction with proteins of the Bcl-2 family (Shimizu *et al*., 1999). In addition to the important role of VDAC regulating apoptosis, this protein channel is also a component of the MPT and is, therefore, a target for MPT modulation in diseases associated with IR injury such as heart attack and stroke (Crompton *et al*., 1987; Crompton, 1999; Armstrong, 2006). Recently, new potential apoptotic drug targets have been identified in the mitochondrial outer membrane that are also involved in the regulation of mitochondrial membrane dynamics including the dynamin-related protein (Drp-1) and the mitochondrial fission protein (hFis1) (Jagasia *et al*., 2005; Youle and Karbowski, 2005). Conversely, the proapoptotic proteins Bax and Bak have been shown to be involved in normal membrane dynamics processes including the regulated fusion of mitochondria illustrating that these proteins also appear to possess a dual function (Karbowski *et al*., 2006).

Proteins of the mitochondrial inner membrane and the ETC

The mitochondrial inner membrane contains the proteins of the ETC that regulate oxidative phosphorylation (OX-PHOS), transport metabolites between the mitochondrial matrix and the cytosol, and import nuclear-encoded proteins into the mitochondrion. The inner membrane has a high ψ_m, is rich in the lipid cardiolipin, and is compartmentalized into pleomorphic structures known as cristae. In addition to regulating ATP production, the ETC is an important source of mitochondrial ROS production that occurs due to the 'leakage' of electrons on to molecular oxygen. The principal mitochondrial ROS include the superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide which are continuously generated as byproducts of normal aerobic metabolism (Chance *et al*., 1979). Mitochondrial ROS are detoxified by the cooperative action of the matrix enzyme manganese superoxide dismutase (MnSOD) and mitochondrial GSH-dependent peroxidase that normally ensure that the ROS level is nontoxic. However, when ROS production exceeds the capacity of the cell's antioxidant defenses, the result is 'oxidative stress' which can cause mitochondrial damage and lead to cell death. The ETC sites and mechanisms involved in mitochondrial ROS production appear to vary under different conditions, but respiratory complex I, known as NADH dehydrogenase, generates significant levels of ROS, especially during reverse electron transport (Lambert and Brand, 2004), and is an important ETC target for antioxidant therapies in PD (Panov *et al*., 2005). Complex II, also known as succinate dehydrogenase, has been shown to generate ROS (Guo and Lemire, 2003), which appear to be involved in the regulation of glucose-induced molecular signalling in the diabetic endothelium suggesting that modulation of this site may be useful in preventing the long-term microvascular complications associated with diabetes (Nishikawa *et al*., 2000). On the other hand, complex III (bc_1) appears to have a variety of functions that make it a target for therapeutic gain including: (1) bc_1 is a key site of ROS production in isolated mitochondria (Muller *et al*., 2002); (2) bc_1 regulates the MPT and cell death by controlling mitochondrial calcium influx (Zhang and Armstrong, unpublished data); (3) bc_1 acts an oxygen sensor for hypoxia regulating the transcription factor HIF1α (Guzy *et al*., 2005). Another, potential target for cancer therapy is the ATP synthase (F0F1ATPase) because its inhibition would be expected to increase the ψ_m and facilitate the increased

accumulation of DLC-linked cancer drugs while also depleting the cells energy supplies. Other important target proteins of the mitochondrial inner membrane include the UCP which, when activated, dissipate the proton gradient ($\Delta\mu_{H+}$) without contributing to ATP synthesis. In addition to the role of the Drp-1 and hFis1 in apoptosis, mitochondrial dynamics proteins residing in the mitochondrial inner membrane also play an important role in regulating apoptosis including the dominant in optic atrophy protein (OPA1), a profusion GTPase, and the presenilin-associated rhomboid-like protease (Cipolat *et al*., 2006; Frezza *et al*., 2006). OPA1 has recently been identified as a regulator of cytochrome *c* release by controlling the remodelling of cristae junctions (CJs), a function that has been shown to be independent of its physiological role as a mitochondrial shaping protein (Frezza *et al*., 2006). Recent work from our laboratory has indicated that OPA1-mediated CJ remodelling is regulated by the MPT (Zhang and Armstrong, unpublished data). Thus, membrane dynamics proteins of the mitochondrial inner membrane are also intimately involved in apoptotic regulation and represent potential new targets for therapeutic intervention.

Protein targets involving both membranes

The MPT is a 'putative' polyprotein structure spanning both mitochondrial membranes and formed by the specific interaction between the adenine nucleotide translocator (ANT) (Woodfield *et al*., 1998), VDAC (Crompton *et al*., 1998) and the matrix protein cyclophilin-D (CyP-D), a peptidylprolyl *cis–trans* isomerase (Crompton *et al*., 1998; Halestrap *et al*., 1998; Woodfield *et al*., 1998). Two different models indicating how these proteins regulate the MPT have been proposed. First, increased calcium levels and redox stress have been shown to cause a CyP-D-dependent catalytic (peptidyl prolyl-isomerase; PPIase) change in the conformation of the ANT converting it to a nonspecific pore (Halestrap and Davidson, 1990; Halestrap *et al*., 1997, 2004). Second, CyP-D has been shown to bind to complexes of ANT and VDAC independent of its PPIase activity at mitochondrial 'contact sites' between the outer and inner membranes suggesting that VDAC-ANT and CyP-D complexes are normal structural components of the contact site which are deformed into the MPT by calcium and redox stress (Crompton *et al*., 1998, 2002; Crompton, 2000). Other proteins associated with the contact site also appear to be important regulators of the MPT including hexokinase, creatine kinase (CK) (Kottke *et al*., 1988) and the benzodiazepine receptor (BR) (McEnery *et al*., 1992; Beutner *et al*., 1998). This suggests that the MPT may be part of a 'multifunctional reaction center' involved in the regulation of a variety of normal cell functions including energy metabolism (Crompton, 1999). Over the years, this multiple protein model of the MPT has become widely accepted (Green and Reed, 1998) although genetic studies have shown that both the ANT and CyP-D are simply regulators of the MPT rather than its structural components (Kokoszka *et al*., 2004; Baines *et al*., 2005; Basso *et al*., 2005; Nakagawa *et al*., 2005; Bernardi *et al*., 2006). However, although the molecular structure of the MPT pore remains unknown, it is known to play an important role in necrosis associated with IR injury and may be important in the regulation of certain forms of apoptosis, especially that associated with increased cellular calcium levels, for example, during endoplasmic reticulum (ER) stress (Zhang and Armstrong, 2007). Thus, the MPT represents a key mitochondrial target for therapeutic intervention either to activate it to induce apoptosis for cancer therapy or inhibit it to protect against IR-related tissue during heart attack and stroke.

Mitochondrial DNA

The mitochondrial matrix is the space enclosed by the cristae membrane and contains a soup of metabolic enzymes, mitochondrial ribosomes and specialized transfer RNAs as well as several copies of circular nonchromosomal mtDNA. mtDNA codes for 13 subunits of enzyme complexes of the ETC including seven of NADH-Q reductase, one of cytochrome *c* reductase, three of cytochrome *c* oxidase, and two of F0F1ATPase (Attardi and Schatz, 1988). Mitochondrial function depends on proteins that are encoded by both nuclear DNA and mtDNA, indicating that mtDNA represents a potential target for therapeutic gain. For example, it may be possible to replace a defective mtDNA sequence to replace a faulty mitochondrial gene and thereby correct certain mtDNA-associated diseases. The protein import pathway has previously been used to direct the mitochondrial import of chimeric proteins to mitochondria and could also be used to direct the appropriate mtDNA sequences to mitochondria to correct for the defective function of a specific protein (Horwich *et al*., 1985). Patients with mtDNA diseases often have both wild-type and mutant mtDNA molecules and selectively blocking the replication of the mutant mtDNA to allow repopulation of the cell with wild-type DNA has also been suggested as a potential treatment strategy (Taylor *et al*., 1997). In contrast to improving mitochondrial function, the selective depletion of mtDNA could also be used to block OX-PHOS and induce cell death by ATP depletion for cancer therapy. For example, a variety of chemical agents are available that cause selective depletion of mtDNA in mammalian cells including ethidium bromide (Chua *et al*., 2005) and ciprofloxacin (Lawrence *et al*., 1996). Other mitochondrial targeting ideas include proteins of the mitochondrial matrix such as those regulating metabolic pathways including the citric acid cycle enzymes such as aconitase (Juang, 2004) and the mitochondrial NADH shuttle (Eto *et al*., 1999). One key matrix targets is the protein CyP-D which transduces calcium death signals via the MPT (Schneider, 2005). The targeted overexpression of CyP-D in cancer therapy or its selective inhibition using interference RNA strategies during IR-related injury could be potentially used for therapeutic gain.

Mitochondrial targeting in disease

Mitochondrial targeting in neurodegenerative disease

Age-related neurodegenerative diseases such as PD, Alzheimer's disease (AD), amytrophic lateral sclerosis (ALS), Huntington's disease (HD) and Friedreichs ataxia (FRDA) are multifactorial in nature and involve genetic,

environmental and endogenous factors. These diseases are commonly associated with mutations in mtDNA, impaired bioenergetics, increased ROS production and abnormal protein dynamics including the mitochondrial accumulation of disease-specific proteins such as amyloid-β in AD, α-synuclein and parkin in PD, mutant superoxide dismutase (SOD1) in ALS, Huntingtin in HD and Frataxin in FRDA (Lin and Beal, 2006). Although the exact cause of these diseases is unknown, the interplay of each of these factors is considered to contribute to the premature death of neurons and the progressive manifestation of clinical disease. Some neurodegenerative diseases are clearly associated with increased oxidative stress. For example, in AD increased ROS have been shown to precede the involvement of other changes such as senile plaque formation and increase formation of amyloid-β (Nunomura *et al*., 2001; Pratico *et al*., 2001). In other diseases, there is also a clear association between increased oxidative stress and disease pathology. For example, in PD the accumulation of α-synuclein in cultured human dopaminergic neurons has been shown to induce apoptosis mediated by increased ROS production (Xu *et al*., 2002). Also, while normal 'parkin' may limit ROS production, the mutant protein may increase ROS levels and induce apoptosis of dopaminergic neurons (Jiang *et al*., 2006). The overexpression of mutant SOD1 in ALS impairs ETC function, increases ROS production and decreases mitochondrial calcium loading which may cause the death of motor neurons via calcium-mediated excitotoxicity and apoptosis (Manfredi and Xu, 2005; Perry *et al*., 2007). In HD transgenic mice ROS have been suggested to play an active role on the onset and progression of the neurological phenotype (Perez-Severiano *et al*., 2004), whereas in FRDA, levels of frataxin, a mitochondrial iron chaperone, are reduced increasing mitochondrial iron levels, ETC dysfunction and oxidative stress (Pandolfo, 2006). Thus, it is clear that increased ROS production and apoptosis of neurons are cardinal features of the major neurodegenerative diseases and, as such, targeting of mitochondrial-specific antioxidants together with the use of specific anti-apoptotic drugs represents a strategy to prevent or delay disease progression.

Mitochondrial targeting in IR injury

Heart attack or myocardial infarction (MI) often results from coronary artery occlusion and is a predominant form of mortality. The survival of patients suffering from MI directly correlates with the extent of cardiomyocyte cell death and it is necessary to rapidly restore blood flow to preserve the function of the ischaemic myocardium. However, restoration of blood flow to the ischaemic tissue further damages the heart leading to arrhythmias, enzyme release and cell death by a complex process known as IR injury. IR injury involves the interplay of a variety of factors including: (1) the development of cardiomyocyte hypercontracture; (2) the generation of ROS; and (3) activation of the MPT, all of which contribute to cardiomyocyte cell death (Piper *et al*., 2004). Cardiac hypercontracture, that is, a shortening and stiffening of the myocardium, occurs when mitochondrial ATP production recovers during the reperfusion stage if IR injury and spreads between ischaemic cells via gap junctions to promote lethal cell injury. Strategies employed to control hypercontracture include the modulation of calcium levels, ROS production and the restoration of mitochondrial energy production (Kim *et al*., 2006). A role for ROS during IR-tissue injury is indicated by the observation that a variety of antioxidant strategies have been shown to reduce cardiomyocyte cell death (Ganote *et al*., 1982; Jolly *et al*., 1984; Zweier *et al*., 1987; Ambrosio *et al*., 1991). During IR mitochondrial ROS increase, during the ischaemic period as well as during the early reperfusion phase and the initiating ROS, signal can be amplified by ROS-induced ROS release leading to significant oxidative stress (Zorov *et al*., 2000). The site(s) of ROS formation during IR have been shown to involve the ETC during the ischaemic phase, but not during the reperfusion phase, because ROS production generated during reperfusion was not prevented by inhibitors of the ETC (Becker *et al*., 1999; Becker, 2004). The mechanism of ROS production during ischaemia has been suggested to depend on residual low oxygen levels in the presence of a reduced ETC (Vanden Hoek *et al*., 1997). Thus, the inhibition of ROS production during the ischaemic phase as well as during the reperfusion phase is desirable and it is considered that antioxidant therapy could benefit from mitochondrial targeting strategies. First, a mitochondrial-targeted antioxidant approach would prevent the potential antioxidant-induced deregulation of redox signalling pathways in the cytosol. Second, mitochondrial targeting would allow the antioxidant treatment to be precisely timed to coincide with the 'window' of ROS production, for example, the mitochondrial-targeted antioxidant should be most effective when administered during the ischaemic phase of the IR injury, that is, when mitochondria are the source of the increased ROS. In addition to the increased calcium levels and ROS production that occurs during IR injury, the 'reperfusion phase' of the tissue injury is associated with activation of the MPT. This occurs as a result of mitochondrial calcium loading, NADPH oxidation, decrease in adenine nucleotide levels and an increase in pH (Halestrap, 2004). These conditions favour activation of the MPT that leads to depolarization of the $\Delta\psi_m$, uncoupling of OX-PHOS and decreased mitochondrial energy production. In an attempt to maintain the $\Delta\psi_{m,}$ the F1F0ATPase reverses hydrolysing ATP and contributing to the further decline in ATP levels with loss of metabolic homeostasis, activation of enzymes and cell necrosis. If the MPT is activated together with proapoptotic Bax, cytochrome *c* can be released from the mitochondrial intermembrane space and lead to the induction of apoptosis (Halestrap *et al*., 2004). Protective strategies to inhibit the MPT may provide protection from IR-mediated cardiac injury and stroke and include: (1) inhibition of the MPT with CsA; (2) inhibition of mitochondrial calcium loading by blocking the calcium uniporter with ruthenium analogues such as ruthenium red or ruthenium 360 (Matlib *et al*., 1998; Zhang and Armstrong, 2007); and (3) inhibition of ROS production using mitochondrial-targeted antioxidants such as MitoQ. Problems with the nonspecificity of CsA could be potentially avoided by using vesicular systems such as DQAsomes to transport directly the drug to the mitochondrial compartment which would prevent the additional undesirable effects on the heart through

inhibition of calcineurin-mediated processes (Halestrap *et al*., 2004). Also, the drawback with CsA could be avoided by using CsA analogues that do not modulate calcineurin such as 4-methyl-val-CsA or by the use of alternative MPT inhibitors such as sangliferin (Halestrap *et al*., 2004). Because CsA only inhibits the MPT over a narrow concentration range, direct mitochondrial targeting would facilitate the delivery of precisely regulated doses of CsA to the mitochondrial compartment (Halestrap *et al*., 2004). Alternatively, it may be possible to inhibit CyP-D using antisense or interference RNA strategies because it is CyP-D that transduces MPT death signals. In this light, small-molecule inhibitors of CyP-D might be used for the treatment of acute MI and other ischaemic disorders. However, there remains much to be resolved regarding CyP-D, because it may have a crucial physiological role in the mitochondrial matrix in addition to its role in the MPT. Indeed, much effort is going into screening and identifying new MPT inhibitors for therapeutic gain. For example, the compound Ro 68–3400 was recently identified in a screen for MPT inhibitors and was initially thought to target specifically VDAC1 although it was later found that VDAC1 was not the target protein of the drug (Krauskopf *et al*., 2006). As with the case of the ANT and CyP-D, definitive evidence of a structural role for VDAC in the MPT is currently lacking, although future genetic work may provide the required information to more precisely classify its importance in the MPT as it has been recently done for CyP-D (Baines *et al*., 2005; Nakagawa *et al*., 2005).

Mitochondrial drug targeting to trigger apoptosis in cancer therapy

Mitochondria regulate cell death by apoptosis that has led to the development of mitochondria-directed drugs designed to trigger apoptosis in cancer cells. Although many conventional anticancer agents including doxorubicin, cisplatin and paclitaxel induce cell death by indirectly targeting mitochondria and activating apoptosis through signalling pathways such as p53, and Fas/FasL (Costantini *et al*., 2000), other agents directly target the membranes of mitochondria to bring about mitochondrial permeabilization and cell death (Armstrong, 2006).

In general, the aim of activating the Bcl-2 family proteins is to target them to the mitochondrial outer membrane where they induce membrane permeability and release apoptosis activating proteins into the cytosol. Because Bcl-2 is overexpressed in many solid organ tumours and increases the resistance to cell death by conventional cancer therapies, it has been the focus of intense research to design and develop Bcl-2 targeting strategies aimed at blocking its anti-apoptotic action (Miyashita and Reed, 1992). *In vitro* assays have shown that Bcl-2 does not protect against GSH-dependent loss of $\Delta\psi_m$ and cell death induced by the thiol-crosslinking agent diazenedicarboxylic acid bis 5*N*,*N*-dimethylamide or the GSH-depleting agent diethylmaleate (Zamzami *et al*., 1998; Armstrong and Jones, 2002). Thus, a potential way to overcome the anti-apoptotic action of Bcl-2 may be to develop drugs designed to deplete mitochondrial GSH levels and induce mitochondrial protein oxidation and cell death (Armstrong, 2006). A second strategy to overcome Bcl-2-mediated resistance to cell death has been to use ligands of the mitochondrial BR. For example, the BR ligand PK11195 has been found to reverse the resistance to apoptosis in cells that overexpress Bcl-2 by activating the MPT (Hirsch *et al*., 1998). Other approaches designed to block the action of Bcl-2 have included the use of antisense technology to inhibit A1 and Bcl-XL expression (Ackermann *et al*., 1999; Marcucci *et al*., 2005), whereas an alternative approach to inhibit Bcl-2 and activate apoptosis has been to target proapoptotic Bcl-2 proteins and peptides to mitochondria. For example, gene therapy employing adenoviral Bax-delivering vectors has been successful in activating mitochondrial apoptosis (Kagawa *et al*., 2000; Xiang *et al*., 2000; Li *et al*., 2001). A similar strategy used to induce apoptosis was taken by Walensky *et al*. (2004), who generated stable BH3 peptidomimetics designed to block the action of Bcl-2 and activate Bax and Bak. Li *et al*. (2004) developed mimetics of the protein Smac/DIABLO to block the action of the inhibitor of apoptosis protein. Although these approaches predominantly target the mitochondrial outer membrane, other agents directly target mitochondrial inner membrane including arsenite, lonidamine (LND), betulinic acid and 6[3-adamantyl-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) (Costantini *et al*., 2000; Armstrong, 2006). Arsenite induces cell death by a mechanism that involves modulation of mitochondrial protein thiol redox status (Zhu *et al*., 1999), whereas LND inhibits mitochondrial oxygen consumption blocking energy metabolism (Stryker and Gerweck, 1988). CD437 is a synthetic retinoic acid receptor agonist that has been shown to induce cell death by inducing the MPT (Marchetti *et al*., 1999) and betulinic acid, a pentacyclic triterpene, has been found to induce MPT and apoptosis via a direct effect on mitochondria in intact cells and in cell-free systems (Costantini *et al*., 2000). Other mitochondrial membrane targeting strategies have exploited the increased $\Delta\psi_m$ of tumour cells to target DLC including rhodamine and cyanine dyes to the mitochondrial matrix (Oseroff *et al*., 1986; Chen, 1988; Sun *et al*., 1994; Koya *et al*., 1996). The use of toxic peptides, such as mastoparan, which target the ψ_m and specific agents that deplete mtDNA has also been proposed as mitochondria-specific anticancer agents (Armstrong, 2006). Mitochondrial DQAsome-mediated delivery systems and the protein import pathway have been successfully used to target proapoptotic drugs such as paclitaxel to mitochondria (Weissig *et al*., 2004). In addition to direct and indirect targeting of specific mitochondrial membranes, certain drugs activate apoptotic pathways that involve both membranes. THG induces ER stress and apoptosis in cancer cells by targeting Bax to the outer membrane and activating the MPT (Zhang and Armstrong, 2007). Thus, the number of mitochondrial apoptotic targeting strategies used for cancer therapy are increasing and are likely to become more numerous in the future as research identifies new drugs and target molecules within the organelle.

Mitochondrial targeting of the ETC and the UCP to treat diabetes and obesity

It is, perhaps, not surprising that mitochondria are important in diabetes given their crucial role in the regulation of

metabolism. In the developed world, increased access to food together with a sedentary lifestyle is leading to increased obesity and a 'pandemic' in diabetes (Naser *et al*., 2006; Smyth and Heron, 2006). This suggests that increased research into metabolic diseases is warranted to discover potential new drugs and targets designed to modulate metabolism for therapeutic gain. In this light, recent research into the causes of reduced insulin secretion in diabetes has underscored the role of increased levels of mitochondrial ROS and the UCP in this disease (Brownlee, 2005; Lowell and Shulman, 2005). For example, the exposure of pancreatic islet beta cells to increased levels of glucose has been shown to (1) reduce insulin gene expression due to loss of transcription factors PDX-1 and MafA (Robertson, 2004); (2) reduce insulin secretion via ROS-mediated activation of the UCP and decrease in ATP levels (Krauss *et al*., 2003); and (3) reduce numbers of functional beta cells due to increased apoptosis (Federici *et al*., 2001). These three events, often coupled with increased insulin resistance in adipose tissue and muscle, lead to a state of chronic hyperglycaemia and long-term microvascular pathologies such as retinopathy, nephropathy and neurological damage (Brownlee, 2005). At the molecular level, hyperglycaemia increases mitochondrial ROS production and activates key biochemical pathways including: (1) the polyol and hexosamine pathways; (2) the protein kinase C pathway; and (3) the formation of advanced glycation end products that are considered to play a key role in diabetic microvascular pathology (Nishikawa *et al*., 2000; Du *et al*., 2003; Brownlee, 2005). The principal mitochondrial ROS involved has been proposed to be mitochondrial $O_2^{\bullet -}$ because the overexpression of either MnSOD or UCP1 was shown to block these pathways (Nishikawa *et al*., 2000). The UCP are members of a family of mitochondrial carrier proteins that regulate proton leak across the mitochondrial inner membrane and, in doing so, dissipate the mitochondrial proton gradient ($\Delta\mu_{H+}$) thereby reducing the amount of ATP generated by OX-PHOS (Echtay *et al*., 2002). UCP1 is involved in the regulation of nonshivering thermogenesis, whereas UCP2 and UCP3 are more ubiquitously expressed and may play a role in the regulation of ROS production (Schrauwen and Hesselink, 2002). Because the overexpression of UCP1 was shown to reduce the glucose-induced $\Delta\mu_{H+}$ and $O_2^{\bullet -}$ production in endothelial cells, UCPs are considered potential mitochondrial targets for intervention in diabetes (Nishikawa *et al*., 2000). However, it should be noted that none of the UCPs, with the exception of UCP1, have ever been actually shown to uncouple mitochondria or to regulate mitochondrial ROS production. Their functions are currently unknown despite intense research into this aspect of mitochondrial biology (Starkov, 2006). Also, there are important factors to consider regarding modulation of UCP function for therapeutic gain. For example, while the overexpression of UCP might be expected to reduce ROS levels and diabetic signalling in the endothelium it could reduce ATP production in beta cells and, thereby, inhibit normal insulin secretion that depends on ATP levels. This suggests that modulation of UCP function for therapeutic gain would have to be done in a highly tissue-specific manner (Mattiasson and Sullivan, 2006). UCPs are also candidate genes for the treatment of obesity because (1) chemical uncoupling of the ETC reduces body adiposity and (2) animal studies have shown that the overexpression of different UCP homologues causes mice to be lean and resistant to diet-induced obesity (Dalgaard and Pedersen, 2001; Kopecky *et al*., 2001; Krauss *et al*., 2003). Advances in our knowledge of the regulation of UCP function together with the development of more efficient drugs designed to modulate mitochondrial ROS production will be expected to expedite progress in health and disease management in this area.

Conclusions

Mitochondrial medicine is a unique discipline that is evolving owing to advances in technology and in our knowledge of the role of the mitochondrion in disease. The unique structural and functional characteristics of mitochondria enable selective intracellular targeting of drugs designed to modulate mitochondrial function for therapeutic gain. However, these ideas are still largely in the developmental stage and currently there are a number of caveats associated with mitochondrial drug targeting which include: (1) a lack of knowledge of the potential long-term toxic effects of using these compounds in animals; and (2) a current lack of efficient methods to regulate drug delivery to the tissue of interest. Future developments in mitochondria-specific drug delivery technologies will be expected to solve these problems and to promote the development of mitochondrial medicine for improved disease treatment.

Conflict of interest

The author states no conflict of interest.

References

Ackermann EJ, Taylor JK, Narayana R, Bennett CF (1999). The role of antiapoptotic Bcl-2 family members in endothelial apoptosis elucidated with antisense oligonucleotides. *J Biol Chem* **274**: 11245–11252.

Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP *et al*. (2005). Targeting an antioxidant to mitochondria decreased cardiac ischemia–reperfusion injury. *FASEB J* **19**: 1088–1095.

Ambrosio G, Zweier JL, Flaherty JT (1991). The relationship between oxygen radical generation and impairment of myocardial energy metabolism following post-ischemic reperfusion. *J Mol Cell Cardiol* **23**: 1359–1374.

Armstrong JS (2006). The role of the mitochondrial permeability transition in cell death. *Mitochondrion* **6**: 225–234.

Armstrong JS, Jones DP (2002). Glutathione depletion enforces the mitochondrial permeability transition and causes cell death in Bcl-2 overexpressing HL60 cells. *FASEB J* **16**: 1263–1265.

Attardi G, Schatz G (1988). Biogenesis of mitochondria. *Annu Rev Cell Biol* **4**: 289–333.

Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA *et al*. (2005). Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**: 658–662.

Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P (2005). Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem* **280**: 18558–18561.

Becker LB (2004). New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* **61**: 461–470.
Becker LB, Vanden Hoek TL, Shao ZH, Li CQ, Schumacker PT (1999). Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *Am J Physiol* **277**: H2240–H2246.
Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F *et al*. (2006). The mitochondrial permeability transition from *in vitro* artifact to disease target. *FEBS J* **273**: 2077–2099.
Beutner G, Ruck A, Riede B, Brdiczka D (1998). Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition. *Biochim Biophys Acta* **1368**: 7–18.
Boddapati SV, Tongcharoensirikul P, Hanson RN, D'Souza GG, Torchilin VP, Weissig V (2005). Mitochondriotropic liposomes. *J Liposome Res* **15**: 49–58.
Brini M, Marsault R, Bastianutto C, Alvarez J, Pozzan T, Rizzuto R (1995). Transfected aequorin in the measurement of cytosolic Ca2+ concentration ([Ca2+]c). A critical evaluation. *J Biol Chem* **270**: 9896–9903.
Brownlee M (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* **54**: 1615–1625.
Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. *Physiol Rev* **59**: 527–605.
Chen LB (1988). Mitochondrial membrane potential in living cells. *Annu Rev Cell Biol* **4**: 155–181.
Cho S, Szeto HH, Kim E, Kim H, Tolhurst AT, Pinto JT (2007). Novel cell-permeable antioxidant peptide, SS31, attenuates ischemic brain injury by down-regulating CD36. *J Biol Chem* **282**: 4634–4642.
Chua YL, Zhang D, Boelsterli U, Moore PK, Whiteman M, Armstrong JS (2005). Oltipraz-induced phase 2 enzyme response conserved in cells lacking mitochondrial DNA. *Biochem Biophys Res Commun* **337**: 375–381.
Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K *et al*. (2006). Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* **126**: 163–175.
Costantini P, Belzacq AS, Vieira HL, Larochette N, De Pablo MA, Zamzami N *et al*. (2000). Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. *Oncogene* **19**: 307–314.
Covey MV, Murphy MP, Hobbs CE, Smith RA, Oorschot DE (2006). Effect of the mitochondrial antioxidant, Mito Vitamin E, on hypoxic-ischemic striatal injury in neonatal rats: a dose-response and stereological study. *Exp Neurol* **199**: 513–519.
Crompton M (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochem J* **341**: 233–249.
Crompton M (2000). Mitochondrial intermembrane junctional complexes and their role in cell death. *J Physiol* **529**: 11–21.
Crompton M, Barksby E, Johnson N, Capano M (2002). Mitochondrial intermembrane junctional complexes and their involvement in cell death. *Biochimie* **84**: 143–152.
Crompton M, Costi A, Hayat L (1987). Evidence for the presence of a reversible Ca2+-dependent pore activated by oxidative stress in heart mitochondria. *Biochem J* **245**: 915–918.
Crompton M, Virji S, Ward JM (1998). Cyclophilin-D binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore. *Eur J Biochem* **258**: 729–735.
Dalgaard LT, Pedersen O (2001). Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and Type II diabetes. *Diabetologia* **44**: 946–965.
Denicourt C, Dowdy SF (2004). Medicine. Targeting apoptotic pathways in cancer cells. *Science* **305**: 1411–1413.
Denmeade SR, Isaacs JT (2005). The SERCA pump as a therapeutic target: making a 'smart bomb' for prostate cancer. *Cancer Biol Ther* **4**: 14–22.
Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C *et al*. (2003). Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* **112**: 1049–1057.
Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA *et al*. (2002). Superoxide activates mitochondrial uncoupling proteins. *Nature* **415**: 96–99.
Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N *et al*. (1999). Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. *Science* **283**: 981–985.
Federici M, Hribal M, Perego L, Ranalli M, Caradonna Z, Perego C *et al*. (2001). High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes* **50**: 1290–1301.
Filipovska A, Kelso GF, Brown SE, Beer SM, Smith RA, Murphy MP (2005). Synthesis and characterization of a triphenylphosphonium-conjugated peroxidase mimetic. Insights into the interaction of ebselen with mitochondria. *J Biol Chem* **280**: 24113–24126.
Frey TG, Mannella CA (2000). The internal structure of mitochondria. *Trends Biochem Sci* **25**: 319–324.
Frezza C, Cipolat S, Martins de Brito O, Micaroni M, Beznoussenko GV, Rudka T *et al*. (2006). OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* **126**: 177–189.
Ganote CE, Simms MA, Safavi S (1982). Effects of dimethylsulfoxide (DMSO) on the oxygen paradox in perfused rat hearts. *Am J Pathol* **109**: 270–276.
Green DR, Reed JC (1998). Mitochondria and apoptosis. *Science* **281**: 1309–1312.
Guo J, Lemire BD (2003). The ubiquinone-binding site of the Saccharomyces cerevisiae succinate-ubiquinone oxidoreductase is a source of superoxide. *J Biol Chem* **278**: 47629–47635.
Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD *et al*. (2005). Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab* **1**: 401–408.
Halestrap AP (2004). Mitochondrial permeability: dual role for the ADP/ATP translocator? *Nature* **430**: 984.
Halestrap AP, Clarke SJ, Javadov SA (2004). Mitochondrial permeability transition pore opening during myocardial reperfusion – a target for cardioprotection. *Cardiovasc Res* **61**: 372–385.
Halestrap AP, Davidson AM (1990). Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl *cis–trans* isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J* **268**: 153–160.
Halestrap AP, Kerr PM, Javadov S, Woodfield KY (1998). Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochim Biophys Acta* **1366**: 79–94.
Halestrap AP, Woodfield KY, Connern CP (1997). Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *J Biol Chem* **272**: 3346–3354.
Haworth RA, Hunter DR (1979). The Ca2+-induced membrane transition in mitochondria II Nature of the Ca^{2+} trigger site. *Arch Biochem Biophys* **195**: 460–467.
Hirsch T, Decaudin D, Susin SA, Marchetti P, Larochette N, Resche-Rigon M *et al*. (1998). PK11195, a ligand of the mitochondrial benzodiazepine receptor, facilitates the induction of apoptosis and reverses Bcl-2-mediated cytoprotection. *Exp Cell Res* **241**: 426–434.
Horwich AL, Kalousek F, Mellman I, Rosenberg LE (1985). A leader peptide is sufficient to direct mitochondrial import of a chimeric protein. *EMBO J* **4**: 1129–1135.
Jagasia R, Grote P, Westermann B, Conradt B (2005). DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* **433**: 754–760.
Jauslin ML, Meier T, Smith RA, Murphy MP (2003). Mitochondria-targeted antioxidants protect Friedreich Ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. *FASEB J* **17**: 1972–1974.
Jiang H, Jiang Q, Liu W, Feng J (2006). Parkin suppresses the expression of monoamine oxidases. *J Biol Chem* **281**: 8591–8599.
Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR (1984). Canine myocardial reperfusion injury. Its reduction by the combined

administration of superoxide dismutase and catalase. *Circ Res* **543**: 277–285.
Juang HH (2004). Modulation of mitochondrial aconitase on the bioenergy of human prostate carcinoma cells. *Mol Genet Metab* **81**: 244–252.
Kagawa S, Pearson SA, Ji L, Xu K, Mcdonnell TJ, Swisher SG *et al*. (2000). A binary adenoviral vector system for expressing high levels of the proapoptotic gene bax. *Gene Ther* **7**: 75–79.
Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ (2006). Role of Bax and Bak in mitochondrial morphogenesis. *Nature* **443**: 658–662.
Kim JS, Jin Y, Lemasters JJ (2006). Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia–reperfusion. *Am J Physiol Heart Circ Physiol* **290**: H2024–H2034.
Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP *et al*. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**: 461–465.
Kopecky J, Rossmeisl M, Flachs P, Bardova K, Brauner P (2001). Mitochondrial uncoupling and lipid metabolism in adipocytes. *Biochem Soc Trans* **29**: 791–797.
Kottke M, Adam V, Riesinger I, Bremm G, Bosch W, Brdiczka D *et al*. (1988). Mitochondrial boundary membrane contact sites in brain: points of hexokinase and creatine kinase location, and control of Ca^{2+} transport. *Biochim Biophys Acta* **935**: 87–102.
Koya K, Li Y, Wang H, Ukai T, Tatsuta N, Kawakami M *et al*. (1996). MKT-077, a novel rhodacyanine dye in clinical trials, exhibits anticarcinoma activity in preclinical studies based on selective mitochondrial accumulation. *Cancer Res* **56**: 538–543.
Krauskopf A, Eriksson O, Craigen WJ, Forte MA, Bernardi P (2006). Properties of the permeability transition in VDAC1(−/−) mitochondria. *Biochim Biophys Acta* **1757**: 590–595.
Krauss S, Zhang CY, Scorrano L, Dalgaard LT, St-Pierre J, Grey ST *et al*. (2003). Superoxide-mediated activation of uncoupling protein 2 causes pancreatic beta cell dysfunction. *J Clin Invest* **112**: 1831–1842.
Lambert AJ, Brand MD (2004). Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I). *J Biol Chem* **279**: 39414–39420.
Lawrence JW, Claire DC, Weissig V, Rowe TC (1996). Delayed cytotoxicity and cleavage of mitochondrial DNA in ciprofloxacin-treated mammalian cells. *Mol Pharmacol* **50**: 1178–1188.
Li L, Thomas RM, Suzuki H, De Brabander JK, Wang X, Harran PG (2004). A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. *Science* **305**: 1471–1474.
Li X, Marani M, Yu J, Nan B, Roth JA, Kagawa S *et al*. (2001). Adenovirus-mediated Bax overexpression for the induction of therapeutic apoptosis in prostate cancer. *Cancer Res* **61**: 186–191.
Lin MT, Beal MF (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **443**: 787–795.
Liu X, Kim CN, Yang J, Jemmerson R, Wang X (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. *Cell* **86**: 147–157.
Lowell BB, Shulman GI (2005). Mitochondrial dysfunction and type 2 diabetes. *Science* **307**: 384–387.
Manfredi G, Xu Z (2005). Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. *Mitochondrion* **5**: 77–87.
Maples KR, Green AR, Floyd RA (2004). Nitrone-related therapeutics: potential of NXY-059 for the treatment of acute ischaemic stroke. *CNS Drugs* **18**: 1071–1084.
Marchetti P, Zamzami N, Joseph B, Schraen-Maschke S, Mereau-Richard C, Costantini P *et al*. (1999). The novel retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphtalene carboxylic acid can trigger apoptosis through a mitochondrial pathway independent of the nucleus. *Cancer Res* **54**: 6257–6266.
Marcucci G, Stock W, Dai G, Klisovic RB, Liu S, Klisovic MI *et al*. (2005). Phase I study of oblimersen sodium, an antisense to Bcl-2, in untreated older patients with acute myeloid leukemia: pharmacokinetics, pharmacodynamics, and clinical activity. *J Clin Oncol* **23**: 3404–3411.
Matlib MA, Zhou Z, Knight S, Ahmed S, Choi KM, Krause-Bauer J *et al*. (1998). Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca^{2+} uptake into mitochondria *in vitro* and *in situ* in single cardiac myocytes. *J Biol Chem* **273**: 10223–10231.
Mattiasson G, Sullivan PG (2006). The emerging functions of UCP2 in health, disease, and therapeutics. *Antioxid Redox Signal* **8**: 1–38.
Mcenery MW, Snowman AM, Trifiletti RR, Snyder SH (1992). Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* **89**: 3170–3174.
Minko T, Pakunlu RI, Wang Y, Khandare JJ, Saad M (2006). New generation of liposomal drugs for cancer. *Anticancer Agents Med Chem* **6**: 537–552.
Mitchell P, Moyle J (1967). Chemiosmotic hypothesis of oxidative phosphorylation. *Nature* **213**: 137–139.
Miyashita T, Reed JC (1992). Bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. *Cancer Res* **52**: 5407–5411.
Muller F, Crofts AR, Kramer DM (2002). Multiple Q-cycle bypass reactions at the Qo site of the cytochrome bc1 complex. *Biochemistry* **41**: 7866–7874.
Murphy MP (1997). Selective targeting of bioactive compounds to mitochondria. *Trends Biotechnol* **15**: 326–330.
Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H *et al*. (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* **434**: 652–658.
Naser KA, Gruber A, Thomson GA (2006). The emerging pandemic of obesity and diabetes: are we doing enough to prevent a disaster? *Int J Clin Pract* **60**: 1093–1097.
Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y *et al*. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **404**: 787–790.
Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK *et al*. (2001). Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* **60**: 759–767.
Oseroff AR, Ohuoha D, Ara G, McAuliffe D, Foley J, Cincotta L (1986). Intramitochondrial dyes allow selective *in vitro* photolysis of carcinoma cells. *Proc Natl Acad Sci USA* **83**: 9729–9733.
Pandolfo M (2006). Iron and Friedreich ataxia. *J Neural Transm Suppl* **70**: 143–146.
Panov A, Dikalov S, Shalbuyeva N, Taylor G, Sherer T, Greenamyre JT (2005). Rotenone model of Parkinson disease: multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication. *J Biol Chem* **280**: 42026–42035.
Perez-Severiano F, Santamaria A, Pedraza-Chaverri J, Medina-Campos ON, Rios C, Segovia J (2004). Increased formation of ROS, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the Huntington's disease mutation. *Neurochem Res* **29**: 729–733.
Perry JJ, Fan L, Tainer JA (2007). Developing master keys to brain pathology, cancer and aging from the structural biology of proteins controlling reactive oxygen species and DNA repair. *Neuroscience Dec* **145**: 1280–1299.
Piper HM, Abdallah Y, Schäfer C (2004). The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovasc Res* **15**: 365–371.
Pratico D, Uryu K, Leight S, Trojanoswki JQ, Lee VM (2001). Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J Neurosci* **21**: 4183–4187.
Robertson RP (2004). Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* **279**: 42351–42354.
Saito K, Yoshioka H, Cutler RG (1998). A spin trap, *N*-tert-butyl-alpha-phenylnitrone extends the life span of mice. *Biosci Biotechnol Biochem* **62**: 792–794.
Schneider MD (2005). Cyclophilin D: knocking on death's door. *Sci STKE* **2005**: 26.
Schrauwen P, Hesselink M (2002). UCP2 and UCP3 in muscle controlling body metabolism. *J Exp Biol* **205**: 2275–2285.
Seibel P, Trappe J, Villani G, Klopstock T, Papa S, Reichmann H (1995). Transfection of mitochondria: strategy towards a

gene therapy of mitochondrial DNA diseases. *Nucleic Acids Res* **23**: 10–17.

Seligson AL, Terry RC, Bressi JC, Douglass III JG, Sovak M (2001). A new prodrug of paclitaxel: synthesis of Protaxel. *Anticancer Drugs* **12**: 305–313.

Sheu SS, Nauduri D, Anders MW (2006). Targeting antioxidants to mitochondria: a new therapeutic direction. *Biochim Biophys Acta* **1762**: 256–265.

Shimizu S, Narita M, Tsujimoto Y (1999). Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature* **399**: 483–487.

Smyth S, Heron A (2006). Diabetes and obesity: the twin epidemics. *Nat Med* **12**: 75–80.

Starkov AA (2006). Protein-mediated energy-dissipating pathways in mitochondria. *Chem Biol Interact* **163**: 133–144.

Stryker JA, Gerweck LE (1988). Lonidamine-induced, pH dependent inhibition of cellular oxygen utilization. *Radiat Res* **113**: 356–361.

Sun X, Wong JR, Song K, Hu J, Garlid KD, Chen LB (1994). AA1, a newly synthesized monovalent lipophilic cation, expresses potent *in vivo* antitumor activity. *Cancer Res* **54**: 1465–1471.

Szeto HH (2006a). Cell-permeable, mitochondrial-targeted, peptide antioxidants. *AAPS J* **8**: E277–E283.

Szeto HH (2006b). Mitochondria-targeted peptide antioxidants: novel neuroprotective agents. *AAPS J* **8**: E521–E531.

Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN (1997). Selective inhibition of mutant human mitochondrial DNA replication *in vitro* by peptide nucleic acids. *Nat Genet* **15**: 212–215.

Teicher BA, Holden SA, Cathcart KN (1987). Efficacy of Pt(Rh-123)2 as a radiosensitizer with fractionated X rays. *Int J Radiat Oncol Biol Phys* **13**: 1217–1224.

Torchilin VP (2000). Drug targeting. *Eur J Pharm Sci* **11** (Suppl 2): S81–S91.

Torchilin VP (2006). Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annu Rev Biomed Eng* **8**: 343–375.

Vanden Hoek TL, Shao Z, Li C, Schumacker PT, Becker LB (1997). Mitochondrial electron transport can become a significant source of oxidative injury in cardiomyocytes. *J Mol Cell Cardiol* **29**: 2441–2450.

Verner K, Lemire BD (1989). Tight folding of a passenger protein can interfere with the targeting function of a mitochondrial presequence. *EMBO J* **8**: 1491–1495.

Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD *et al*. (2004). Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. *Science* **305**: 1466–1470.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM *et al*. (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* **242**: 1427–1430.

Weissig V, Boddapati SV, Cheng SM, D'Souza GG (2006). Liposomes and liposome-like vesicles for drug and DNA delivery to mitochondria. *J Liposome Res* **16**: 249–264.

Weissig V, Cheng SM, D'Souza GG (2004). Mitochondrial pharmaceutics. *Mitochondrion* **3**: 229–244.

Woodfield K, Ruck A, Brdiczka D, Halestrap AP (1998). Direct demonstration of a specific interaction between cyclophilin-D and the adenine nucleotide translocase confirms their role in the mitochondrial permeability transition. *Biochem J* **336**: 287–290.

Xiang J, Gomez-navarro J, Arafat W, Liu B, Barker SD, Alvarez RD *et al*. (2000). Pro-apoptotic treatment with an adenovirus encoding Bax enhances the effect of chemotherapy in ovarian cancer. *J Gene Med* **2**: 97–106.

Xu J, Kao SY, Lee FJ, Song W, Jin LW, Yankner BA (2002). Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. *Nat Med* **8**: 600–606.

Youle RJ, Karbowski M (2005). Mitochondrial fission in apoptosis. *Nat Rev Mol Cell Biol* **6**: 657–663.

Zamzami N, Marzo I, Susin SA, Brenner C, Larochette N, Marchetti P *et al*. (1998). The thiol crosslinking agent diamide overcomes the apoptosis-inhibitory effect of Bcl-2 by enforcing mitochondrial permeability transition. *Oncogene* **16**: 1055–1063.

Zhang D, Armstrong JS (2007). Bax and the mitochondrial permeability transition cooperate in the release of cytochrome *c* during endoplasmic reticulum-stress-induced apoptosis. *Cell Death Differ* **14**: 703–715.

Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW *et al*. (2004). Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J Biol Chem* **279**: 34682–34690.

Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y *et al*. (1999). Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. *J Natl Cancer Inst* **91**: 772–778.

Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ (2000). Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med* **192**: 1001–1014.

Zweier JL, Flaherty JT, Weisfeldt ML (1987). Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci USA* **84**: 1404–1407.