Ca^{2+} Signaling, Mitochondria and Cell Death

Carlotta Giorgi[1,2], Anna Romagnoli[1], Paolo Pinton[1] and Rosario Rizzuto*[,1]

[1]Dept. of Experimental and Diagnostic Medicine, Section of General Pathology, Telethon Center for Cell Imaging (TCCI) and Interdisciplinary Center for the Study of Inflammation (ICSI), University of Ferrara, Via L. Borsari 46, I-44100 Ferrara, Italy; [2]Vita-Salute San Raffaele University, Center of Excellence in Cell Development, and IIT Network, Research Unit of Molecular Neuroscience, Via Olgettina 58, 20132 Milan, Italy

Abstract: In the complex interplay that allows different signals to be decoded into activation of cell death, calcium (Ca^{2+}) plays a significant role. In all eukaryotic cells, the cytosolic concentration of Ca^{2+} ions ($[Ca^{2+}]_c$) is tightly controlled by interactions among transporters, pumps, channels and binding proteins. Finely tuned changes in $[Ca^{2+}]_c$ modulate a variety of intracellular functions ranging from muscular contraction to secretion, and disruption of Ca^{2+} handling leads to cell death. In this context, Ca^{2+} signals have been shown to affect important checkpoints of the cell death process, such as mitochondria, thus tuning the sensitivity of cells to various challenges. In this contribution, we will review (i) the evidence supporting the involvement of Ca^{2+} in the three major process of cell death: apoptosis, necrosis and autophagy (ii) the complex signaling interplay that allows cell death signals to be decoded into mitochondria as messages controlling cell fate.

Keywords: Calcium, apoptosis, necrosis, autophagy, mitochondria.

INTRODUCTION

Although the possibility that calcium (Ca^{2+}) controls a variety of physiological functions gradually emerged at the end of 19th century [1], the general theory of Ca^{2+} as a universal second messenger was proposed half a century later by Lewis Victor Heilbrunn, who concluded from the analysis of experimental data that "the reaction of this Ca^{2+} with the protoplasm inside the cell is the most basic of all protoplasmic reactions" [2]. This theory, although almost completely ignored at the time of its appearance, brilliantly withstood the test of time and experimental efforts, and today Ca^{2+} signaling is generally regarded as the most ubiquitous and pluripotent system, involved in the regulation of almost all known cellular processes [3].

The universality of Ca^{2+} as a signaling molecule can be inferred on the one hand from the presence of cellular events controlled by Ca^{2+} throughout phylogenetic history, on the other from the broad diversity of the cellular functions controlled by Ca^{2+} in different spatial (exocytosis, myocyte contraction) and temporal (synaptic plasticity, memory, long-term adaptation or neuronal ageing) domains [4,5].

Such a complexity, in terms of defined localization of Ca^{2+} sensitive targets and of generation of precise spatio-temporal signaling patterns, is based on the presence of numerous types of channel, pumps and carriers and on the involvement of numerous cell domains and organelles in transmitting and transporting Ca^{2+} mediated signals [6].

A compartment with unique Ca^{2+} handling properties is represented by mitochondria and will be the focus of this review. These organelles have an intriguing connection to Ca^{2+} signaling. On one hand, they are characterized by a complex (and molecularly undefined) machinery for Ca^{2+} handling: an electrophoretic route accumulating Ca^{2+} in the matrix down the electrochemical gradient generated by the respiratory chain (the mitochondrial Ca^{2+} uniporter, MCU) and exchangers (with Na^+ or H^+) re-extruding Ca^{2+} into the cytosol. On the other, Ca^{2+} effects within this organelle include radically different process, such as the stimulation of dehydrogenases (and thus the up-regulation of aerobic metabolism) and organelle changes leading to apoptotic or necrotic cell death [7].

As a consequence of their signaling complexity, Ca^{2+} ions often play very opposite effects even within the same cell [8]. Not surprisingly, the versatility of Ca^{2+} signaling makes it an important player not only in normal conditions but also in pathological cellular reactions. Depriving the cells of Ca^{2+} ions (by removing extracellular Ca^{2+}, buffering Ca^{2+}, or depleting intracellular stores), results in rapid and inevitable cell death. At the same time excess of Ca^{2+} is absolutely toxic, and cell death from Ca^{2+} overload represents probably the most general mechanism of cell demise [7].

The aim of this review is to give some insight into the different cell death pathways, especially those mechanisms conjugated with Ca^{2+} and mitochondria.

CALCIUM AND CELL DEATH

Programmed cell death (PCD) is one of the important terminal paths for the cells, and plays a role in a variety of biological events that include morphogenesis, maintenance of tissue homeostasis, and elimination of harmful cells. Dysfunction of PCD leads to various diseases in humans, including cancer and several degenerative diseases [9]. Many studies have subdivided PCD into the three categories of apoptosis (type I), autophagy (type II), and necrosis (type III)

*Address correspondence to this author at the Department of Experimental and Diagnostic Medicine, Section of General Pathology, Via Borsari 46, 44100 Ferrara, Italy; Tel: +39 0532 455361; Fax: +39 0532 2472; E-mail: r.rizzuto@unife.it

1566-5240/08 $55.00+.00

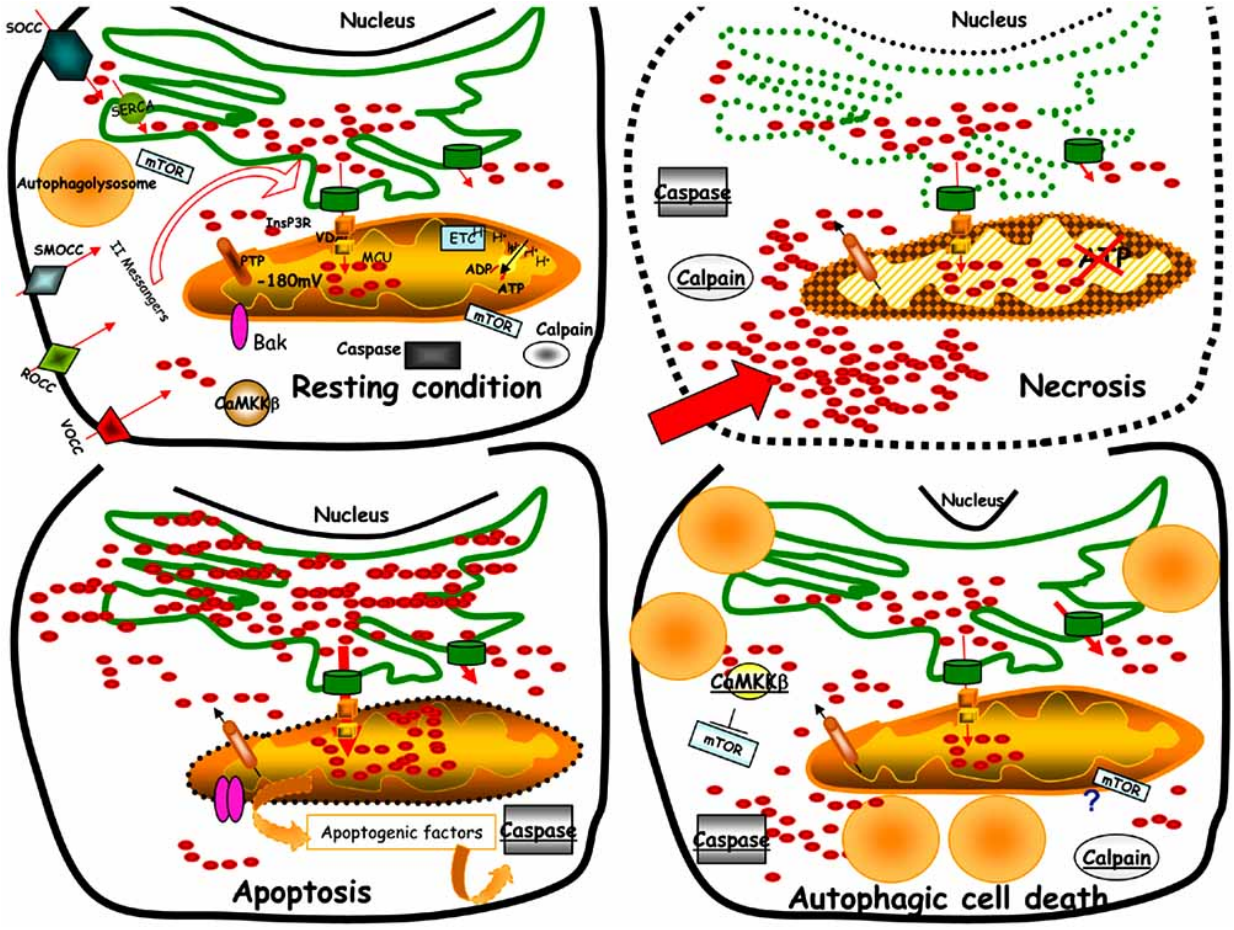

Fig. (1). Schematic representation of PCD. In necrosis an ionic alteration causes a major $[Ca^{2+}]_c$ rise, activation of calpains and caspases, PTP opening, impairement of ATP production and rupture of the plasma membrane. In apoptosis, the intrinsic pathway may involve mitochondrial Ca^{2+} overload, PTP opening, release of mitochondrial apoptogenic factors and activation of caspases. In autophagic cell death the increase of $[Ca^{2+}]_c$ causes CaMKKβ activation and mTOR inhibition, caspase and calpain activation and a greater number of autophagolysosomes.

based on criteria such as morphological alterations, initiating death signal, or the implication of caspases (see Fig. **1**).

Type I apoptotic cell death is characterized by cell shrinkage and extensive chromatin condensation. Formation of autophagic vacuoles inside the dying cell is typical of autophagic or type II cell death, whereas type III PCD is distinguished by rapid loss of plasma membrane integrity and spillage of the intracellular content. Undergoing a specific type of cell death depends on the stimulus and the cellular context. Indeed, every cell death program is a net result of self propagating signals and others that suppress the other death programs [10].

APOPTOSIS

The word 'apoptosis' is derived from a Greek word meaning 'the gentle falling of leaves' and is used to describe a form of cell death occurring in multicellular organisms. It is defined as a form of cell death that involves altruistic suicide of individual cells in favour of the organism as a whole. Apoptosis is a tightly regulated, highly efficient and energy requiring process which engages multiple cell signaling pathways. The apoptotic network components are genetically encoded and are usually in place in a cell ready to be activated by a death-inducing stimulus [11].

Apoptotic activity is desirable during organism development and morphological changes especially at the embryonic stage, as well as during the activation of the immune system [12]. Additionally, this process is essential for organ homeostasis by keeping under control cell number and tissue trophism. Defects in apoptosis can result in cancer, autoimmune diseases, neurodegenerative disorders, AIDS and ischemic diseases [13].

Apoptosis results in an orchestrated collapse of a cell, staging cell shrinkage, chromatin condensation, DNA and protein cleavage, fragmentation in the apoptotic-bodies followed by phagocytosis by neighbouring cells [11]. The apoptotic process can be driven by various stimuli from outside or inside the cell; in some cases, absence of survival factors is enough to drive a cell into apoptosis, but it can also be stimulated by DNA damage, oxidative stress, treatment with cytotoxic drugs or irradiation, interruption in cell cycle signaling and death receptor ligands (TNF and Fas ligand).

Apoptosis occurs through two types of pathways: the death receptor pathway (extrinsic apoptotic pathways) and the mitochondrial pathway (intrinsic apoptotic pathways), and requires the activity of dedicated enzymes (caspases) and regulatory proteins (such as the Bcl2-related family).

As to Ca^{2+}, experimental works of the past decade has highlighted its importance in the regulation of apoptosis [7]. However, the role of Ca^{2+} of apoptosis is very complex, given that in different systems Ca^{2+}-linked stimuli were shown to represent both survival signals and apoptosis inducers. This is not surprising, given the vast array of Ca^{2+} transducers present in the various compartments of the cell.

Ca^{2+} signaling is the focus of the regulation and activation of the multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaMK) family. This family, which phosphorylates a large variety of substrates, has activation properties that allows it to discriminate between Ca^{2+} signals that differ in spike frequency, amplitude and duration [14]. The CaMKI, II and IV subfamilies have been detected within the cell nucleus and suggested as mediators of nuclear Ca^{2+} signals. These kinases were implicated in control of gene transcription since they phosphorylate several transcription factors. Several reports have indicated that these kinases negatively modulate apoptosis [15]. Intracellular Ca^{2+} is a coordinating factor that positively regulates the activity of the nuclear transcription factor-kB (NF-kB) [16]. This transcription factor is considered an anti apoptotic agent and plays a key role in cell survival by up-regulating expression of several apoptosis inhibitor genes and negative regulating the activity of caspase-3 [17].

Cytosolic Ca^{2+} increase has a pivotal role in activating the serine threonine Ca^{2+}-calmodulin-regulated phosphatase calcineurin (also called protein phosphatase 2B). This phosphatase is a critical transducer of Ca^{2+} signals in most cell types particularly in the immune system and in the heart, due to its specific responsiveness to sustained low frequency Ca^{2+} signals [18]. Calcineurin was suggested to be both a promoter and a supportive agent during apoptosis. Some reports suggest that the Ca^{2+}-calcineurin pathway is critical in the progression of heart failure by regulating cardiomyocyte apoptosis [19]. On the other hand, Ca^{2+}-calcineurin activation by 2-deoxyglucose and staurosporine prevents apoptosis of cardiac myocytes. Calcineurin, which dephosphorylates the transcription factor NF-AT3, enables it to translocate into the nucleus, leading to prevention of apoptosis both *in vitro* and *in vivo* [20]. Another distinctive feature of apoptosis is the requirement for *de novo* RNA synthesis. A key transcription factor for apoptosis is c-Jun, an immediate-early gene. Ca^{2+} influx has been reported to be involved in c-jun N-terminal kinase (JNK) signaling pathway mediated IL-1ß-induced apoptosis [21]. Ca^{2+} mediated regulation, however, is not restricted to the cytosolic compartment. The switch into a death signal often involves the coincidental detection of Ca^{2+} and proapoptotic stimuli, and depends on the amplitude of the mitochondrial Ca^{2+} signal. Several studies indicate that the Ca^{2+} content of the Endoplasmic Reticulum (ER) determines the cell's sensitivity to apoptotic stress and perturbation of ER Ca^{2+} homeostasis appears to be a key component in the development of several pathological situations. The ER is the main intracellular agonist-sensitive Ca^{2+} store capable of rapid Ca^{2+} exchange [22].

The potential of the ER to function as a rapidly exchanging Ca^{2+} store is due to the presence of three main components: i) ATP dependent pumps for Ca^{2+} uptake (called SERCAs: Sarco/Endoplasmic Reticulum Ca^{2+} ATPases), ii) channels for Ca^{2+} release such as the ubiquitous inositol 1,4,5-trisphosphate receptor (IP_3R) and the ryanodine receptor (RyR), and iii) Ca^{2+} binding proteins for Ca^{2+} storage, the best characterized being calreticulin and calsequestrin [6].

Procedures that decrease the Ca^{2+} loading of the ER, such as genetic ablation of the ER Ca^{2+}-buffering protein calreticulin or overexpression of plasma membrane Ca^{2+} ATPases, protect cells from apoptosis. Conversely, procedures that increase the ER Ca^{2+} load, such as overexpression of SERCA or calreticulin, sensitize cells to apoptotic stress [23-25]. Sensitivity to apoptosis correlates with the total ER Ca^{2+} load, rather than with the free ER Ca^{2+} concentration, and depends on the ability of cells to transfer Ca^{2+} from the ER to the mitochondria. Accordingly, procedures that enhance the transfer of Ca^{2+} from the ER to mitochondria augment ceramide induced cell death ([23] and see below).

AUTOPHAGIC CELL DEATH

The Greek word autophagy means "self-eating", and indeed this form of cell death is characterized by pathways for the degradation of cytosolic constituents by the lysosome/vacuole. Autophagy has an essential role in differentiation and development, in addition to its role in cellular response to stress. It is activated upon amino acid deprivation and has been associated with neurodegenerative diseases, cancer, pathogen infections and myopathies. There are three main autophagic pathways: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [26].

Macroautophagy involves the sequestration of cytosolic portions, including proteins and organelles, through double-membrane structures, termed autophagosomes, to the degradation in lysosomes. In microautophagy, cytosolic constituents are engulfed directly by lysosomes through invaginations of the lysosomal membrane. Both these pathways are conserved from yeast to mammals. However, the latter pathway is far less characterized, especially in mammalian systems. The CMA is found only in mammalian cells and degrades selectively cytosolic proteins that contain a specific signal motif, KFERQ. This signal motif is recognized by a specific chaperone, which translocates the protein into the lysosome through the interaction with the Lamp2a receptor [27].

Although considered originally nonspecific, 'bulk' degradation pathways, it is now accepted that preferential autophagy of damaged or excess organelles, such as peroxisomes, ER and mitochondria, occurs both through micro- and macro-autophagy, under certain conditions. The selective degradation of damaged mitochondria through autophagy, termed mitophagy, occurs in response to various stimuli, both in yeast and in mammalian cells [28].

In mammalian cells, autophagosomes undergo a maturation process by fusing with endocytic compartments and lysosomes. Autophagy is active at a basal level in most cells, and this probably reflects its role in regulating the turnover of long-lived proteins, and getting rid of damaged structures. In the pathogenesis of various diseases, autophagy has been proposed to have both beneficial and harmful effects [29]. One aspect of this complexity probably reflects the dual role of autophagy, which is both cell-protective and -destructive. Indeed, controversy exists as to whether autophagy promotes or prevents cell death. If autophagy removes damaged mitochondria that would otherwise activate caspases and apoptosis, then autophagy should be protective. In agreement with these item, disruption of autophagic processing and/or lysosomal function promotes caspase-dependent cell death. In addition, enhanced availability of substrates may delay cell death upon starvation. However, excessive and deregulated autophagy may promote cell death, since enzymes leaking from lysosomes/autolysosomes, such as cathepsins and other hydrolases, can initiate mitochondrial permeabilization, caspase activation and apoptosis, and in certain instances deletion of autophagy genes decreases apoptosis [30].

Autophagic cell death is mainly a morphologic definition (i.e. cell death associated with autophagosomes/autolysosomes, autophagic degradation of cytoplasmic structures preceding nuclear collapse), and the specific mechanism is still under debate. The regulation of autophagy is a very complex process; together with mTOR (mammalian target of rapamycin), GTPases and a wide range of kinases, Ca^{2+} is a controller of autophagy. Indeed, different works [31;32] suggest that the depletion of Ca^{2+} pools is responsible for the inhibitory effect on autophagy, and that elements that modify lysosomal Ca^{2+} levels, also modify the total volume of autophagic vacuoles, as well as glycogen degradation. These notions should be revised in the light of recent work published by Hoyer-Hansen and co-workers [33], that demonstrated that various, related Ca^{2+} mobilizing stimuli (vitamin D_3 compounds, ATP, thapsigargin and ionomycin) inhibit the activity of mTOR and induce massive accumulation of autophagosomes in a Beclin-1 and ATG-dependent manner. The paper concluded that a rise in the free cytosolic [Ca^{2+}] rather than [Ca^{2+}] alterations in other cellular compartments, is responsible for the induction of autophagy. This notion is supported by the identification of cytosolic Ca^{2+}-activated kinase, CaMKKβ, as an essential mediator of Ca^{2+} induced autophagy, and by the finding that inhibition of autophagy by targeted Bcl-2 constructs was related to they effect on agonist-induced Ca^{2+} release from the ER and not to the steady state of ER [Ca^{2+}] ([Ca^{2+}]$_{ER}$). The studies in which [Ca^{2+}]$_{ER}$ has been measured directly by ER-targeted Ca^{2+}-sensitive probes support the view that Bcl-2 mainly acts on [Ca^{2+}]$_{ER}$ by increasing the Ca^{2+} permeability of the ER membrane [34]. Bcl-2 might inhibit the autophagic response by lowering the amount of free ER Ca^{2+} available for release. Such a combined anti- and proautophagy function of Bcl-2 may help to maintain autophagy at level that is compatible with cell survival, rather than death [35].

NECROSIS

Up until 1971, the term "necrosis" was used for all types of cell death. A common definition of necrosis is that of a catastrophic derangement of cell integrity following exposure to different types of cell injury and leading to the activation of Ca^{2+}-activated hydrolysing enzymes. The concept that necrosis is unprogrammed was reinforced by the fact that necrotic cell death can be caused by exposures of cells to supraphysiological conditions such as mechanical force, heat, or cold. Thus, necrosis has long been described as a consequence of physico-chemical stress, accidental and uncontrolled.

Recently, it is becoming clear that necrotic cell death is as well controlled and programmed as caspase-dependent apoptosis, and that it may be an important cell death mode that is both pathologically and physiologically relevant [36]. Necrotic cell death is not the result of a single well-described signaling cascade but is the consequence of extensive crosstalk between several biochemical and molecular events at different cellular levels. Necrosis is characterized by cytoplasmic swelling, irreversible plasma membrane damage, and organelle breakdown. In necrosis, the cellular content leaks into the extracellular environment, where it may act as a "danger signal". Consequently, necrosis is usually associated with inflammation.

Intracellular Ca^{2+} is an important signaling molecule also in necrosis. Indeed, in certain pathological conditions, extracellular ligands can induce Ca^{2+}-dependent necrosis. A good example is the excitotoxic neuronal cell death, triggered by excitatory amino acids such as NMDA [37].

Ca^{2+} is involved in necrotic cell death also by modulating the translocation of Phospholipase A2 (PLA2) and the activity of calpains. PLA2 encompasses a family of esterases that are responsible for the liberation of fatty acids from membrane phospholipids. Several distinct mammalian PLA2 enzymes have been identified, which are classified into three major subfamilies: Ca^{2+}-independent PLA2 (iPLA2), secretory PLA2 (sPLA2), cytosolic PLA2 (cPLA2) [38]. Translocation of cPLA2 to the membranes, which enables it to interact with its substrates, is essential for the release of AA from

membrane. This process is Ca^{2+}-dependent and might hasten the progression to necrotic cell death [39].

Calpains participate in various signaling pathways mediated by Ca^{2+} by modulating the activities and/or functions of other proteins. They are present in the cytosol as inactive precursors that are activated by increased cytosolic Ca^{2+}. Severe stress conditions often result in elevation of cytosolic Ca^{2+} levels and overactivation of calpains. In turn, the latter have been shown to cleave Ca^{2+} extrusion machinery, such as the plasma membrane Ca^{2+} ATPases, PMCA [40], and the Na^{+}/Ca^{2+} exchanger [41], thus leading to a sustained increase in intracellular Ca^{2+}. Calpains were shown to fulfil important roles in necrotic cell death in neurons of C. elegans and in necrosis of dystrophin deficient muscles [42]. Yamashima and colleagues [43] therefore postulated a "calpain-cathepsin hypothesis", suggesting that necrotic insults causing excessive Ca^{2+} overload lead to calpain-mediated lysosomal disruption with succeeding release of cathepsins B and L. Cathepsin B was also shown to be involved in caspase-independent cell death induced by death receptor ligands. Increased activity of calpains is also observed in certain neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [44]. Whether this activity is strictly linked to the occurrence of necrosis during these pathologies remains unclear.

At this point a question remains unresolved: what is the correlation between these three different pathways of cell death? Is there a check point in which they converge? A broad body of experimental evidence suggests that mitochondria are the most likely candidate to this role.

THE MITOCHONDRIA

Mitochondria are intriguing subcellular organelles (see Fig. **2**). With a bacterial evolutionary origin, they have become perhaps the ultimate symbiont, maintaining its own DNA while also deriving many important proteins from the nuclear DNA of the host cell. While they may maintain a modicum of independence from the host cell in some respects, they nevertheless lie at the heart of the life of almost all eukaryotic cells. The primary function of the mitochondrion is oxidative phosphorylation (ox-phos) and ATP supply, i.e. the function upon which all cellular activities depend [45]. The mitochondria are the "energy powerhouse of the cell" generating approximately 90% of cellular energy and consuming about 98% of the total O_2 we breathe [46]. Multicellular organism have indeed high-energy requirements necessary to carry out complex function, such as muscle contraction, hormones and neurotransmitters synthesis and secretion, in addition to ba-

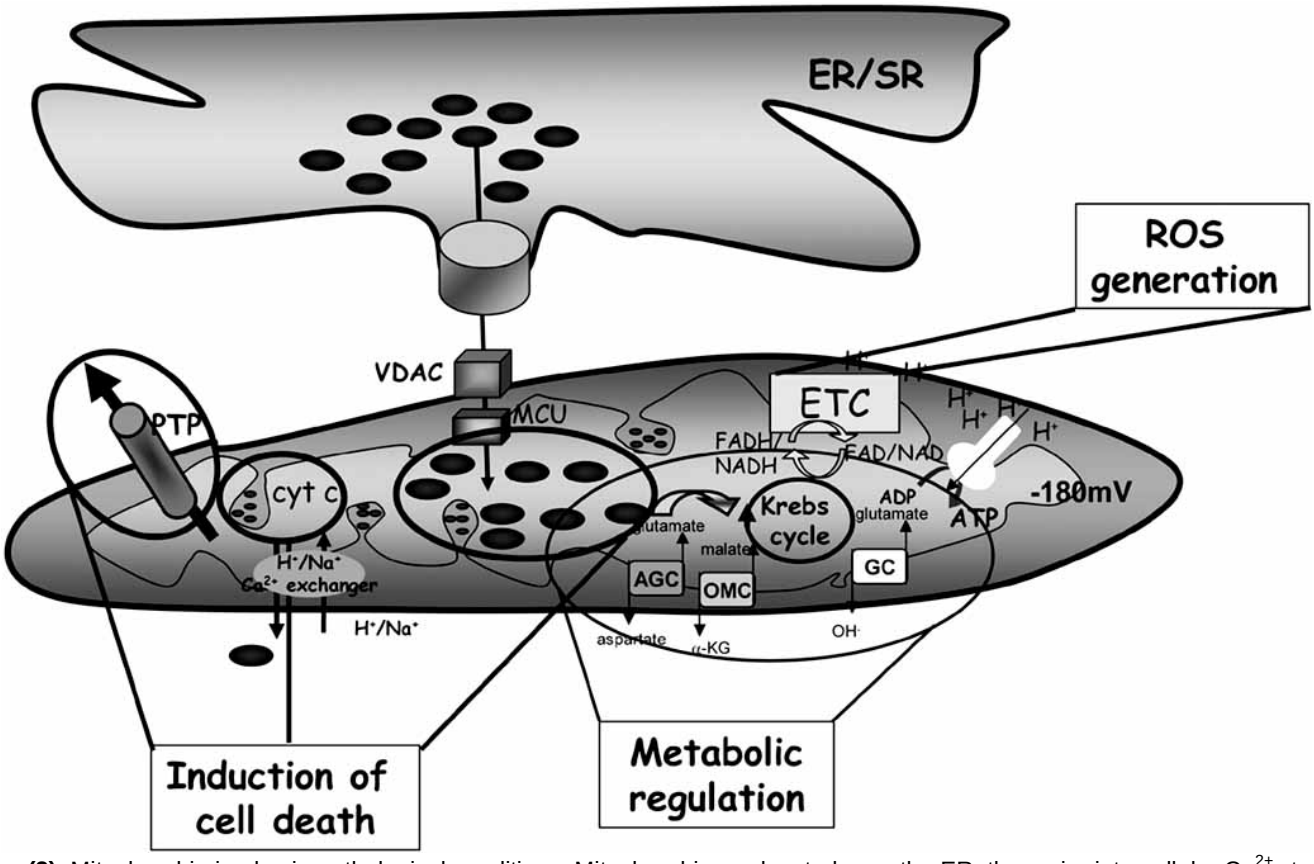

Fig. (2). Mitochondria in physio-pathological conditions. Mitochondria are located near the ER, the major intracellular Ca^{2+} store. In physiological conditions Ca^{2+} activates enzymes of Krebs cycle within mitochondria and stimulates ATP production. Mitochondria are also the principle site of ROS generation, that act as second messenger (e.g. possibly in autophagy) and damage inducers to DNA, membranes and proteins. Pathological stimuli enhancing the release of Ca^{2+} from the ER cause mitochondrial Ca^{2+} overload, PTP opening and release of apoptogenic factors, such as cytochrome c.

sal cellular metabolism (biomolecules synthesis and transformation, maintenance of ionic gradients across membrane, cell division) [47].

INTRACELLULAR Ca^{2+} IN MITOCHONDRIAL PHYSIOLOGY

Ca^{2+} is a key regulator of mitochondrial function and acts at several levels within the organelle to stimulate ATP synthesis. Most of the mitochondrial effects of Ca^{2+} require its entry across the double membrane into the matrix. Although the mitochondrial outer membrane was thought to be permeable to Ca^{2+}, recent studies suggest that the outer membrane voltage-dependent anion channel (VDAC) serves to regulate Ca^{2+} entry to mitochondrial intermembrane space [48].

Then, its transport across the inner membrane is highly regulated. The accumulation of Ca^{2+} within the mitochondrial matrix is driven by membrane potential and depends primarily on the existence of an electrogenic transport mechanism, the MCU located in the organelle's inner membrane. MCU was recently demonstrated to be a highly selective Ca^{2+} channel (K_d < 2nM) [49] in agreement with the conclusions of classical studies carried out in isolated mitochondria [50]. As to the molecular nature, the attempts to identify the gene or the protein have been so far unsuccessful. An intriguing hypothesis is that, at least an excitable cells MCU is the type I Ryanodine Receptor (RyR1), partly localized to the inner mitochondria membrane and termed mRyR [51]. Finally, it has been proposed that alternative uptake routes could be operative, that may represented different molecular mechanisms on operative modes of MCU. Namely “rapid-mode” uptake (RaM), occurs on a millisecond timescale and allows fast changes in mitochondrial matrix Ca^{2+} concentration ($[Ca^{2+}]_m$) to mirror changes in the cytosol ($[Ca^{2+}]_c$) [52].

The primary role of mitochondrial Ca^{2+} is the stimulation of oxidative phosphorylation and the control of organelle metabolic activity. Three crucial metabolic enzymes within the matrix, pyruvate, α-ketoglutarate and isocitrate dehydrogenases, are activated by Ca^{2+}, by two distinct mechanisms. In the case of pyruvate dehydrogenase, a Ca^{2+}-dependent dephosphorylation step is involved; in the other two cases, activation occurs through the direct binding of Ca^{2+} to the enzyme complex [53]. Given that these enzymes represent the rate limiting step for feeding electrons into the respiratory chain, Ca^{2+} within the matrix is ultimately the positive modulator of mitochondrial ATP synthesis. This aspect was directly addressed by our group a few years ago using targeted recombinant luciferase to monitor, in living cells, the ATP concentration within the cytoplasm and the mitochondrial matrix [54]. Interestingly, recent works indicate that other Ca^{2+}-dependent metabolic checkpoints are operative. Namely, the aspartate/glutamate metabolite carriers (AGCs) were shown to be activated by Ca^{2+} and in turn, recombinant expression of wild type AGCs enhanced ATP production upon cell stimulation [55]. Other mitochondrial functions are also regulated by Ca^{2+}. For example, Ca^{2+}activation of *N*-acetylglutamine synthetase generates *N*-acetylglutamine, a potent allosteric activator of carbamoyl-phosphate synthetase, the rate-limiting enzyme in the urea cycle [56]. Moreover, different mechanisms can finely tune amplitude and kinetics of the mitochondrial Ca^{2+} responses. For example, Ca^{2+} uptake can be increased or decreased by protein kinases, such as protein kinase C [57] or p38 MAP kinases [58].

In endocrine pancreas, mitochondrial Ca^{2+} modulates insulin secretion and in the granulosa cells of the adrenal gland, it controls a key step in aldosterone synthesis [59;60]. Mitochondria can also act as Ca^{2+} buffers, thus regulating the spatio-temporal patterning of Ca^{2+} signals. Petersen and co-workers demonstrated that in pancreatic acinar cells mitochondria strategically located beneath the granular region prevent the spreading of a Ca^{2+} wave from the secretory pole towards the basolateral region by accumulating Ca^{2+} [61]. In neurons, mitochondria buffer $[Ca^{2+}]$ increases in the presynaptic motoneuron ending [62]. Moreover, the local buffering of $[Ca^{2+}]_c$ by mitochondria has been shown to have an important role in chromaffin cells in the modulation of catecholamine secretion. By using aequorin mutants with different affinities, Alvarez and co-workers have shown that inhibition of Ca^{2+} sequestration by mitochondria causes a large increase in the secretion of catecholamine, suggesting that mitochondrial buffering of Ca^{2+} represents a way to control the recruitment or the fusion of catecholamine-containing secretory vesicles [63].

A fast response of $[Ca^{2+}]_m$ to $[Ca^{2+}]_c$ requires, to be efficient and productive, a rapid Ca^{2+} efflux from the mitochondrial matrix, and several mechanisms exist for this purpose. Primarily, Ca^{2+} efflux is achieved in exchange with Na^+, which is in turn re-extruded out of the matrix in exchange with protons. Thus both Ca^{2+} uptake and efflux from mitochondria consume mitochondrial membrane potential ($\Delta\psi_m$) and are therefore reliant on H^+ pumping by the respiratory chain to maintain this driving force (Na^+/ Ca^{2+} and H^+/ Ca^{2+} uniporters) [64].

In addition to these pathways of Ca^{2+} efflux, an additional mechanism exists in the form of the permeability transition pore (PTP). The PTP is assembled from a group of preexisting proteins in the mitochondrial inner and outer membranes [65,66] with Ca^{2+} binding sites on the matrix side of the inner membrane believed to regulate pore activity. These non selective route for ions equilibration may allow to rapidly release Ca^{2+} from the matrix in conditions in which electrochemical equilibrium requires Ca^{2+} efflux, i.e. when a major drop in $\Delta\psi_m$ has occurred. Obviously, this situation may occur in pathophysiological conditions and adds complexity to the emerging role of PTP in the pathogenesis of numerous disease states [67].

Mitochondrial Ca^{2+} accumulation, amplitude and duration is influenced by Ca^{2+} released from ER store so by the ER loading [6]. The ER supplies Ca^{2+} directly to mitochondria *via* IP_3Rs. It was postulated that Ca^{2+} re-

leased from the ER *via* IP_3Rs may generate microdomains of high [Ca^{2+}] at focal contacts between ER and mitochondria, providing sufficient [Ca^{2+}] for the activation of uniporters [68]. These intimate connection are called mitochondria-associated ER membranes (MAM) [69]. Importantly, Ca^{2+}-handling proteins such as IP_3Rs (especially type 3 IP_3Rs) are highly compartmentalized at MAM, providing a direct and proper mitochondrial Ca^{2+} signaling and playing a special role in induction of apoptosis [70].

MITOCHONDRIA: REGULATORS OF DEATH PATHWAYS

Given the key energetic and signaling function of mitochondria, it comes as no surprise that mitochondrial dysfunction and failure leads to cell death. Indeed, much evidence indicates mitochondria are the main target of stresses leading to cell death. Mitochondrial alterations such as the release of sequestered apoptogenic proteins, loss of transmembrane potential, production of ROS, disruption of the electron transport chain, and decreases in ATP synthesis have been shown to be involved in, and possibly responsible for, the different manifestations of cell death. Here we will discuss the role of the mitochondria in integrating of the three different cell death pathways.

Mitochondria and Apoptosis

Apoptosis is triggered by two major initiating pathways, designated as the inner/intrinsic/mitochondria-mediated and outer/extrinsic/receptor-mediated route, respectively [71]. Both pathways converge to a final apoptosis execution step resulting in the cleavage of cell regulatory and structural molecules. Both the extrinsic and the intrinsic pathways are interconnected at the mitochondrial checkpoint.

A well characterized role of mitochondria in apoptosis is the release into the cytosol of pro apoptotic proteins, i.e. proteins that are normally retained in the space (IMS) between the inner (IM) and outer (OM) mitochondrial membranes. These IMS proteins include caspase-independent death effectors such as nucleases and/or proteases, as well as caspase activators. The pivotal events that initiate the intrinsic apoptotic pathway are the mitochondrial outer membrane permeabilization (MOMP) and release of pro-apoptotic factors. However, the precise molecular mechanism responsible for MOMP is still controversial. In fact different models have been proposed. One model involves opening of PTP. Opening of the PTP can be triggered by multiple stimuli and leads to (i) $\Delta\Psi_m$ loss as ions equilibrate across this membrane, and (ii) swelling of the matrix as water enters. The latter can result in breakage of the OM, thus leading to the nonspecific release of proteins from the IMS [72].

The second mechanism for MOMP appears to be mediated by members of the Bcl-2 family acting directly on the mitochondrial OM. It has been proposed that a direct interaction of the pro-apoptotic Bcl-2 family members, such as Bax, with Adenine Nucleotide Translocator (ANT) and VDAC induces mitochondria permeabilization. In a different model, release of pro-apoptotic proteins exclusively depends on the balance between pro and anti-apoptotic Bcl-2 family members. In this model BH3-only proteins (such as Bid, Bim, Puma and Noxa) play a crucial role in pro-apoptotic Bax and Bak activation that results in their homo-oligomerization within the OM. This leads to the formation of a pore with subsequent release of apoptotgenic proteins [71]. The first protein shown to be released from mitochondria upon apoptotic stimuli is cytochrome c followed by other pro-apoptotic molecules such as Smac/DIABLO (second mitochondria derived activator of caspase/direct IAP-binding protein with low pl), AIF (apoptosis-inducing factor) and endonucleaseG are released from the disrupted mitochondria to the cytoplasm [73].

It has become evident that there is an intensive cross-talk between the extrinsic (death receptors) and intrinsic (mitochondrial) pathways [74]. The best characterized connection from the extrinsic to the intrinsic pathway is associated with the Bcl-2 family member Bid (BH3- interacting DD agonist). Bid can be cleaved by activated caspase-8, 3 and 10 (induced by DISC, death-inducing signaling complex, formation) to yield truncated Bid (tBid). tBid then translocates to the mitochondrion where it binds and activates the pro-apoptotic Bcl-2 members Bax/Bak [75]. Another recently described cross-talk mechanism between the extrinsic and intrinsic apoptotic pathways is ceramide generation by death receptor activated sphingomyelinases. Apart from triggering various signaling pathways, ceramide directly acts on mitochondria, favoring PTP activation and release of caspase cofactors [76]. In addition to tBid and ceramide, apoptotic pathways can be influenced by death receptors triggering parallel signaling cascades. Such pathways are initiated by distinct adaptor molecules that lead to activation of protein kinases and/or transcription factors pathway (i.e. MAPK). Indeed, evidence suggests that the JNK cascade promotes Bax translocation to mitochondria through phosphorylation of the 14-3-3 protein, a cytoplasmic anchor of Bax [77].

Finally, many factors that trigger the inner apoptotic cascade modulate to a certain degree the receptor-mediated pathway. The tumor suppressor protein p53 is a typical example of such intrinsic-to-extrinsic cross-talk [78]. Apart from binding directly to the mitochondrial membrane, p53 enhances transcription of genes coding for death receptors, which increases susceptibility of the cells to receptor-mediated programmed death [79].

Inappropriate apoptosis is a factor in many human pathological conditions. Many disease may involve enhanced apoptosis (e.g. neurodegenerative disease or ischemia) or repressed apoptosis (e.g. cancer due to mutations of p53 or overexpression of many anti-apoptotic proteins). Tumor cells can acquire resistance to apoptosis by up-regulating or activating anti-

apoptotic proteins or by the down-regulating or inactivating pro-apoptotic proteins that in many cases are mitochondrial proteins. The enhanced expression level of the Bcl-2 protein in some tumor cells correlates with malignancy of tumors and reduced response to chemotherapy. Moreover, the strong pro-survival PI-3K-AKT pathway in many tumors is over activated by loss-of-function mutations of the PTEN tumor suppressor gene [80] or gain-of-function mutations of oncogenes such as Ras, BCR-ABL or growth factor receptors such as EGFR. Regarding pro-apoptotic pathways, p53 is very frequently mutated gene in cancer resulting in loss or down-regulation of protein function. Other key mediators of the intrinsic apoptotic pathway including Bax, Bak or Apaf-1 are also functionally altered in cancer cells. Reduced Bax expression has been associated with a poor response rate to chemotherapy and shorter survival in some situations [81]. In neurodegenerative diseases, the scenario appears specular. Indeed, under normal conditions, neuron possess strong intrinsic anti-apoptotic factors and survive for the life-time of the organism. Pathological PCD occurs if metabolic stress, damage or genetic abnormalities overwhelm these survival factors. Premature death of adult neurons leads to irreversible functional deficits and neuro-degeneration. The remaining neurons have no capacity for regeneration to compensate for the loss [82]. A mitochondrial hypothesis of Parkinson's disease has been proposed by Park and co-workers that was put on even firmer footing with the discoveries of causative mutations in the DJ-1and PINK1genes. The DJ-1 protein has a role in the oxidative stress response; under oxidative conditions, DJ-1 partially translocates to mitochondria where its function remains to be elucidated. PINK1 is found primarily in mitochondria, and it is predicted act as a kinase although its substrates are unknown. PINK1-mutant flies show loss of dopaminergic neurons in a degenerative disorder characterized by mitochondrial swelling [83]. Moreover, mutations in the recently discovered mitochondrial protein REEP1 cause hereditary spastic paraplegia, further stressing the importance of mitochondrial function in neurodegenerative disease [84].

Evidence has been accumulating to suggest that dysregulation of apoptosis may also contribute to age-associated changes such as the progressive decline of physiological functions and significant increases in the incidence of cancer and degenerative disease (see Fig. **3**). This notion is supported by recent works on the role of p66shc, the mutation of which was demonstrated to increase resistance to oxidative stress and to prolong life span [85]. Intriguingly, following an activation process that includes phosphorylation by protein kinase C β and peptidyl-prolyl-isomerase Pin1 recognition, p66shc translocates to mitochondria [86] where it exerts an oxidoreductase activity [87]. Indeed, p66shc directly oxidizes cytochrome c and generates H_2O_2, leading to the opening of PTP and cell death. While the example of p66shc highlights important signaling pathways operating in age-related organ deterioration, the complete scenario of the changes in the apoptotic process and the positive and negative regulatory mechanism affected by ageing remains to be unravelled.

Mitochondria and Autophagic Cell Death

Interesting, while the central role of mitochondria in apoptosis is well established, recent evidence strongly suggests that also in autophagy this organelles act as intracellular check points. Autophagy was recently established as a novel tumor suppression mechanism [88], which stimulated a wave of investigations aimed at understanding its regulatory mechanism and its importance in the development of human cancers. Autophagy was first linked to cancer following the identification and characterization of the Beclin 1 gene. Beclin 1 is a tumor-suppressor gene that is frequently monoallelically deleted in human sporadic cancer [89]. Beclin 1 co-localizes with Bcl-2 in mitochondria and Bcl-2 inhibits Beclin 1-dependent autophagy [90]. HSpin 1 is a transmembrane protein that is localized primarly in the mitochondria. HSpin 1 has been shown to bind Bcl-2 and Bcl-xL (but not Bax and Bak) influencing mitochondrial autophagy in a caspase independent manner [91]. Moreover, autophagy is negatively regulated by mTOR, a key signaling protein that integrates signals from nutrients and growth factors. The pathways leading to mTOR are often altered in human cancer. The common consequence of these alterations is up-regulation of mTOR, which in turn inhibits autophagy and contributes to tumorigenesis. mTOR has been shown to be partly associated with the mitochondrial OM [92] and the functional significance of this observation is currently investigated by many laboratories, including ours. Finally, recent work revealed that autophagy is regulated also by p53 as a result of its activation [93,94]. In light of the finding that in some systems autophagy and apoptosis seem to be interdependent phenomena, molecular switches are likely to link the two types of cell death.

Mitochondria and Necrosis

The core events of necrosis are bioenergetic failure and rapid loss of plasma membrane integrity. Perturbation of intracellular ion homeostasis can result in mitochondria dysfunction, opening of PTP, loss of $\Delta\psi_m$ and diminished ATP production.

PTP opening is a common pathway leading to both necrotic and apoptotic cell death. In contrast to necrotic cell death which is a consequence of ATP depletion, ATP is required for the development of apoptosis [95]. Thus the balance between ATP depletion after the PTP opening and ATP generation by glycolysis determines whether the fate of cells will be apoptotic or necrotic death [96]. During stress conditions (such as anoxia, ischemia), mitochondrial ATP formation is impaired and the cell becomes profoundly ATP depleted, which leads to necrotic cell death. By contrast, if the MOMP does not involve all mitochondria within a cell or progresses slowly as may occur after less severe ischemia, then ATP levels may recover, at least in part (i.e. after reper-

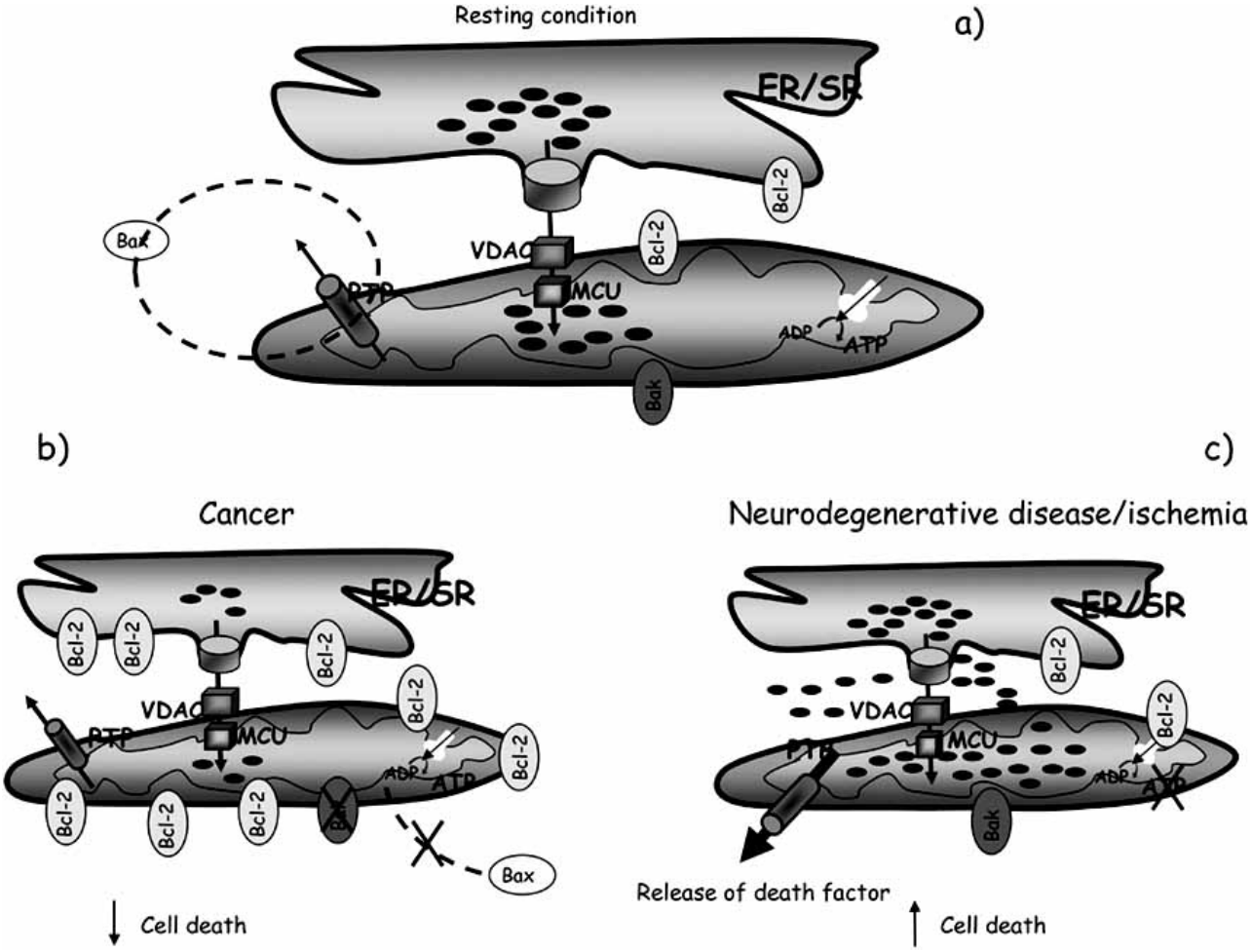

Fig. (3). Alterations of mitochondrial cell death in common pathologies. (**A**) In normal conditions the rate of cell death is finely regulated to guarantee the correct tissue homeostasis. (**B**) In cancer cells the cell death process is downregulated (e.g. Bcl-2 overexpression and thus apoptotic inhibition), on the contrary (**C**) in neurodegenerative diseases the cell death program is upregulated e.g. due to excessive PTP opening and in turn apoptosis challenge, or massive ATP depletion such as after ischemic injury.

fusion), especially if glycolytic substrate is abundant. Partial ATP recovery prevents necrotic cell killing and ATP supply can mediate a switch from necrosis to apoptosis. Even after initiation of apoptosis, necrosis may supervene if ATP levels subsequently fall [97]. Moreover, is important to remember that also the extent of PTP opening probably determines the balance between apoptosis and necrosis.

During a mild stress, transient opening of the PTP can occur in highly Ca^{2+}-sensitive mitochondrial population, leading to the release of pro-apoptotic proteins from intermembrane space and the induction of apoptosis. However, the majority of mitochondria must remain competent for ATP synthesis, otherwise the energy requiring processes of apoptosis could not occur. More severe insults, on the other hand, cause depletion of Ca^{2+} stores with the induction of sustained global Ca^{2+} elevations that inhibit mitochondrial function with a consequent irreversible PTP opening, drastic fall of ATP production, prematurely activate digestive enzymes and cell death occur *via* necrosis [98].

Many human tumors carry mutations that inactivate apoptotic pathways. Inactivation of apoptosis allows tumor cells to proliferate beyond normal homeostatic control. Necrosis represents an alternative pathway for tumor cells to be eliminated. The inflammatory component of necrotic death has the potential advantage of stimulating an immune response that could increase the efficacy of tumor cell killing. The balance between apoptotic and necrotic cell death may be modulated to potentiate a patient's immune response to a tumor. However, inflammatory responses induced by necrosis may also be associated with systemic toxicity. Cell necrosis plays a role in a number of disease processes including vascular-occlusive disease, neurodegenerative diseases, infection, inflammatory diseases, exposures to toxins, and cancer [36].

CALCIUM AND MITOCHONDRIAL CELL DEATH

Ca^{2+} is a global positive effector of mitochondrial function, and thus any perturbation in mitochondrial or

cytosolic Ca^{2+} homeostasis will have profound implications for cell function, for example, at the level of ATP synthesis.

At the same time, Ca^{2+} overload, frequently observed in a variety of pathological condition, adversely affects mitochondrial function, and Ca^{2+} signals have been implicated in triggering the various forms of cell death. Such a dual function, i.e. the control of the two extremes of cell fate decision requires a tight signaling control and the activity of the finely timed decoding mechanisms. Mitochondrial Ca^{2+} homeostasis appears to be an important check point. Indeed, $[Ca^{2+}]_m$ favors the opening of PTP, one of the main mechanism for mitochondria shape changes and ensuring release of caspase cofactors residing in the organelles. In addition, the activity of apoptotic agents and cellular stresses sensitizes the organelles, causing the translation of a Ca^{2+} signal into a death promoting process. At least two conceptual mechanisms have been described. In the first, a larger Ca^{2+} load into the organelle is triggered, with a variety of molecular mechanisms and cellular targets: from the caspase induced cleavage of the Ca^{2+} cleaning machinery (e.g. the PMCA in neurons [40] and in hepatocytes [99]) to the enhanced loading of ER Ca^{2+} stores [100] to the induction of Ca^{2+} release of internal stores [23]. The second, non alternative route, is the sensitization of the mitochondrial targets, such as ATP, to the effect of Ca^{2+}. In an elegant series of experiments, Hajnoczky and co-workers, showed that the lipid mediator of apoptosis, ceramide, enhances the Ca^{2+} sensitivity of PTP. Thus, in the presence of sub-threshold concentrations of ceramide (incapable per se of triggering cell death), stimulation with physiological doses of an IP_3 generating agonist causes a Ca^{2+} wave that is immediately followed by a wave of mitochondrial depolarization, indicative of PTP opening [101]. In their model prolonged exposure to pro-apoptotic stimuli or large Ca^{2+} overload are not necessary to induce cell death, but rather PTP opening is the consequence of a coincident detection of short-living signals: it is dependent on a privileged Ca^{2+} signal transmission between IP_3 receptor and mitochondria, in addition to a yet unidentified direct effect of the pro-apoptotic lipid on mitochondria. In this contest, it is not surprising that important oncogenes act as Ca^{2+} modulators [102]. The original observation was made with Bcl-2. Its overexpression (i.e. the event occurring in a variety of human cancers) causes a Ca^{2+} leak from the ER, and thus reduces the steady state ER Ca^{2+} levels (and the net amount of Ca^{2+} that can be released from the organelle upon stimulation [34]). As a consequence, mitochondrial Ca^{2+} loading is reduced and pro-apoptotic organelle changes prevented. This scenario is supported by the observation that any experimental manoeuvre that reduces ER Ca^{2+} filling mimics the anti-apoptotic effect of Bcl-2 and protects from a variety of apoptotic challenges [23]. Accordingly, genetic ablation of the pro-apoptotic proteins Bax and Bak (frequently observed in cancer and associated to increased resistance to apoptotic death) causes a large reduction in $[Ca^{2+}]$ within the ER and a drastic reduction in the transfer of Ca^{2+} from ER to mitochondria [100,103]. On the other hand, treatments that increased $[Ca^{2+}]$ within the stores have the opposite effect on the susceptibility of cells to the apoptotic stimulus inducing mitochondrial Ca^{2+} overload. Overall, up-regulation of mitochondrial Ca^{2+} homeostasis is now recognized to play an active role in apoptosis by switching the mitochondrial Ca^{2+} signaling to facilitate Ca^{2+}-induced opening of the PTP and release of apoptogenic factors such as cytochrome c. A role of Ca^{2+} in cell death, however, is not restricted to the mechanism of this family of regulators of apoptosis.

Cardiac ischemia-reperfusion injury and neuronal excitotoxicity are two example in which overload of $[Ca^{2+}]_m$ induce cells to undergo apoptosis or necrosis in response to different stimuli [104,105]. Excitotoxicity results from the release of excess neurotransmitters and the engagement of cell membrane receptors with excitatory amino acids (EAA) such as ionotropic N-methyl-D-aspartate (NMDA), kainate, and 2-amino propionate (AMPA). Excitotoxins share the property of inducing increased intracellular Ca^{2+} *via* L-type Ca^{2+} channel, transient receptor potential (TRP) cation channels, or ASIC [106]. These initial Ca^{2+} entries activate Ca^{2+}-dependent proteases such as calpains to cleave the Na^{+}/Ca^{2+} exchanger NCX that is required for extrusion of Ca^{2+}. This leads to impairment of Ca^{2} extrusion, hence to a sustained intracellular Ca^{2+} increase [41]. Thus, an important role can be attributed to a "self-amplification" mechanism for cytosolic Ca^{2+} spikes under these conditions. A prolonged increase in cytosolic Ca^{2+} can trigger mitochondrial Ca^{2+} overload that leads to opening of PTP and ATP depletion, as well as the activation of Ca^{2+}-dependent proteases. Like many other insults, increased cytosolic Ca^{2+} can initiate either apoptosis or necrosis. The outcome of cell death is probably determined by the concentration of cytoplasmic Ca^{2+}. Whereas low to moderate Ca^{2+} (200–400 nM) triggers apoptosis, higher concentration of Ca^{2+} (>1 μM) is associated with necrosis [107]. This may explain why Ca^{2+} released from the ER is mostly apoptotic, whereas Ca^{2+} influx through the plasma membrane is associated with necrosis.

Overload of $[Ca^{2+}]_m$ is a key event in ischemia/reperfusion (IR) injury with the end point being opening of the PTP, cytochrome c release, and apoptosis/necrosis. After IR, the fate of the cell is determined by potency of the effect. If minimal, the cell may recover; if moderate, the cell may undergo programmed cell death; if severe, the cell may die from necrosis due to inadequate energy production. Mitochondria thus can serve as arbiter of cell fate in response to stress.

Finally, the cross-talk between Ca^{2+}, mitochondria and autophagic cell death is still largely unclear. The involvement of different mitochondrial proteins, important for Ca^{2+} homeostasis, in several crucial steps of the autophagic process, suggests a possible alteration of $[Ca^{2+}]_m$ as key step in triggering this type of PCD. In this case, however, our knowledge is still superficial,

and the direct investigation of Ca^{2+} signaling, and its cellular targets in autophagy, represents a fascinating challenge for the years to come.

ACKNOWLEDGEMENTS

The authors are deeply indebted to past and present collaborators. This work was supported by Telethon grant GGP05284, the Italian Association for Cancer Research (AIRC), local funds from the University of Ferrara, the Italian University Ministry, the EU (fondi strutturali Obiettivo 2), the PRRIITT program of the Emilia Romagna Region**,** the Italian Space Agency (ASI), NIH (Grant #1P01AG025532-01A1) and the United Mitochondrial Disease Foundation (UMDF).

REFERENCES

[1] Ringer, S. (1883) *J. Physiol.*, **4,** 29-42.
[2] Heilbrunn, L.V. (1956) *Fed. Proc.*, **15,** 948-953.
[3] Carafoli, E. (1987) *Annu. Rev. Biochem.*, **56,** 395-433.
[4] Berridge, M.J., Lipp,P., and Bootman,M.D. (2000) *Nat. Rev. Mol. Cell Biol.*, **1,** 11-21.
[5] Clapham, D.E. (2007) *Cell*, **131,** 1047-1058.
[6] Rizzuto, R. and Pozzan, T. (2006) *Physiol. Rev.*, **86,** 369-408.
[7] Rizzuto, R., Pinton, P., Ferrari, D., Chami, M., Szabadkai, G., Magalhaes, P.J., Di Virgilio, F., and Pozzan, T. (2003) *Oncogene*, **22,** 8619-8627.
[8] Wellman, G.C., Nathan, D.J., Saundry, C.M., Perez, G., Bonev, A.D., Penar, P.L., Tranmer, B.I., and Nelson, M.T. (2002) *Stroke*, **33,** 802-808.
[9] Lockshin, R.A. and Zakeri, Z. (2004) *Int. J. Biochem. Cell Biol.*, **36,** 2405-2419.
[10] Clarke, P.G. (2002) *Trends Pharmacol. Sci.*, **23,** 308-309.
[11] Ellis, R.E., Yuan, J.Y., and Horvitz, H.R. (1991) *Annu. Rev. Cell Biol.*, **7,** 663-698.
[12] Rathmell, J.C., Fournier, S., Weintraub, B.C., Allison, J.P., and Goodnow, C.C. (1998) *J. Exp. Med.*, **188,** 651-659.
[13] Rathmell, J.C. and Thompson, C.B. (2002) *Cell*, **109** Suppl, S97-107.
[14] Corcoran, E.E. and Means, A.R. (2001) *J. Biol. Chem.*, **276,** 2975-2978.
[15] Bok, J., Wang, Q., Huang, J., and Green, S.H. (2007) *Mol. Cell Neurosci.*, **36,** 13-26.
[16] Mattson, M.P., Culmsee, C., Yu, Z., and Camandola, S. (2000) *J. Neurochem.*, **74,** 443-456.
[17] Sun, L. and Carpenter, G. (1998) *Oncogene*, **16,** 2095-2102.
[18] Wang, H.G., Pathan, N., Ethell, I.M., Krajewski, S., Yamaguchi, Y., Shibasaki, F., McKeon, F., Bobo, T., Franke, T.F., and Reed, J.C. (1999) *Science*, **284,** 339-343.
[19] Saito, S., Hiroi, Y., Zou, Y., Aikawa, R., Toko, H., Shibasaki, F., Yazaki, Y., Nagai, R., and Komuro, I. (2000) *J. Biol. Chem.*, **275,** 34528-34533.
[20] Molkentin, J.D., Lu, J.R., Antos, C.L., Markham, B., Richardson, J., Robbins, J., Grant, S.R., and Olson, E.N. (1998) *Cell*, **93,** 215-228.
[21] Storling, J., Zaitsev, S.V., Kapelioukh, I.L., Karlsen, A.E., Billestrup, N., Berggren, P.O., and Mandrup-Poulsen, T. (2005) *Endocrinology*, **146,** 3026-3036.
[22] Pozzan, T., Rizzuto, R., Volpe, P., and Meldolesi, J. (1994) *Physiol. Rev.*, **74,** 595-636.
[23] Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F.D., Pozzan, T., and Rizzuto, R. (2001) *EMBO J.*, **20,** 2690-2701.
[24] Arnaudeau, S., Frieden, M., Nakamura, K., Castelbou, C., Michalak, M., and Demaurex, N. (2002) *J. Biol. Chem.*, **277,** 46696-46705.
[25] Campanella, M., de Jong, A.S., Lanke, K.W., Melchers, W.J., Willems, P.H., Pinton, P., Rizzuto, R., and van Kuppeveld, F.J. (2004) *J. Biol. Chem.*, **279,** 18440-18450.
[26] Cuervo, A.M. (2004) *Mol. Cell Biochem.*, **263,** 55-72.
[27] Dice, J.F. (2007) *Autophagy*, **3,** 295-299.
[28] Kim, I., Rodriguez-Enriquez, S., and Lemasters, J.J. (2007) *Arch. Biochem. Biophys.*, **462,** 245-253.
[29] Rubinsztein, D.C., Gestwicki, J.E., Murphy, L.O., and Klionsky, D.J. (2007) *Nat. Rev. Drug Discov.*, **6,** 304-312.
[30] Maiuri, M.C., Zalckvar, E., Kimchi, A., and Kroemer, G. (2007) *Nat. Rev. Mol. Cell Biol.*, **8,** 741-752.
[31] Gordon, P.B., Holen, I., Fosse, M., Rotnes, J.S., and Seglen, P.O. (1993) *J. Biol. Chem.*, **268,** 26107-26112.
[32] Kalamidas, S.A., Kotoulas, O.B., and Hann, A.C. (2002) *Microsc. Res. Tech.*, **57,** 507-511.
[33] Hoyer-Hansen, M., Bastholm, L., Szyniarowski, P., Campanella, M., Szabadkai, G., Farkas, T., Bianchi, K., Fehrenbacher, N., Elling, F., Rizzuto, R., Mathiasen, I.S., and Jaattela, M. (2007) *Mol. Cell*, **25,** 193-205.
[34] Pinton, P., Ferrari, D., Magalhaes, P., Schulze-Osthoff, K., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2000) *J. Cell Biol.*, **148,** 857-862.
[35] Ferraro, E. and Cecconi, F. (2007) *Arch. Biochem. Biophys.*, **462,** 210-219.
[36] Zong, W.X. and Thompson, C.B. (2006) *Genes Dev.*, **20,** 1-15.
[37] Sattler, R. and Tymianski, M. (2000) *J. Mol. Med.*, **78,** 3-13.
[38] Kudo, I. and Murakami, M. (2002) *Prostaglandins Other Lipid Mediat.*, **68-69,** 3-58.
[39] Sapirstein, A., Spech, R.A., Witzgall, R., and Bonventre, J.V. (1996) *J. Biol. Chem.*, **271,** 21505-21513.
[40] Schwab, B.L., Guerini, D., Didszun, C., Bano, D., Ferrando-May, E., Fava, E., Tam, J., Xu, D., Xanthoudakis, S., Nicholson, D.W., Carafoli, E., and Nicotera, P. (2002) *Cell Death. Differ.*, **9,** 818-831.
[41] Bano, D., Young, K.W., Guerin, C.J., Lefeuvre, R., Rothwell, N.J., Naldini, L., Rizzuto, R., Carafoli, E., and Nicotera, P. (2005) *Cell*, **120,** 275-285.
[42] Syntichaki, P., Xu, K., Driscoll, M., and Tavernarakis, N. (2002) *Nature*, **419,** 939-944.
[43] Yamashima, T., Kohda,Y., Tsuchiya, K., Ueno,T., Yamashita, J., Yoshioka, T., and Kominami, E. (1998) *Eur. J. Neurosci.*, **10,** 1723-1733.
[44] Camins, A., Verdaguer, E., Folch, J., and Pallas, M. (2006) *CNS Drug Rev.*, **12,** 135-148.
[45] Lardy,H.A. and Ferguson, S.M. (1969) *Annu. Rev. Biochem.*, **38,** 991-1034.
[46] Nicholls, D. (2002) *Sci. Aging Knowledge. Environ.*, **2002,** e12.
[47] Duchen, M.R. (2004) *Diabetes*, **53** Suppl 1, S96-102.
[48] Rapizzi, E., Pinton, P., Szabadkai, G., Wieckowski ,M.R., Vandecasteele, G., Baird, G., Tuft, R.A., Fogarty, K.E., and Rizzuto, R. (2002) *J. Cell Biol.*, **159,** 613-624.
[49] Kirichok, Y., Krapivinsky, G., and Clapham, D.E. (2004) *Nature*, **427,** 360-364.
[50] Nicholls, D. and Akerman, K. (1982) *Biochim. Biophys. Acta*, **683,** 57-88.
[51] Beutner, G., Sharma, V.K., Lin, L., Ryu,S .Y., Dirksen, R.T., and Sheu, S.S. (2005) *Biochim. Biophys. Acta*, **1717,** 1-10.
[52] Gunter, T.E., Buntinas, L., Sparagna, G., Eliseev, R., and Gunter, K. (2000) *Cell Calcium*, **28,** 285-296.
[53] Denton, R.M. and McCormack, J.G. (1986) *Cell Calcium*, **7,** 377-386.
[54] Jouaville, L.S., Pinton, P., Bastianutto, C., Rutter, G.A., and Rizzuto, R. (1999) *Proc. Natl. Acad. Sci. U S A*, **96,** 13807-13812.
[55] Lasorsa, F.M., Pinton, P., Palmieri, L., Fiermonte,G., Rizzuto,R., and Palmieri,F. (2003) *J. Biol. Chem.*, **278,** 38686-38692.
[56] Johnston, J.D. and Brand, M.D. (1990) *Biochim. Biophys. Acta*, **1033,** 85-90.
[57] Pinton, P., Leo, S., Wieckowski, M.R., Di Benedetto, G., and Rizzuto, R. (2004) *J. Cell Biol.*, **165,** 223-232.
[58] Montero, M., Lobaton, C.D., Moreno, A., and Alvarez, J. (2002) *FASEB J.*, **16,** 1955-1957.
[59] Maechler, P., Kennedy, E.D., Pozzan, T., and Wollheim, C.B. (1997) *EMBO J.*, **16,** 3833-3841.

[60] Brandenburger, Y., Kennedy, E.D., Python, C.P., Rossier, M.F., Vallotton, M.B., Wollheim, C.B., and Capponi, A.M. (1996) *Endocrinology*, **137,** 5544-5551.
[61] Tinel, H., Cancela, J.M., Mogami, H., Gerasimenko, J.V., Gerasimenko, O.V., Tepikin, A.V., and Petersen, O.H. (1999) *EMBO J.*, **18,** 4999-5008.
[62] Rizzuto, R. (2003) *J. Cell Biol.*, **163,** 441-443.
[63] Moreno, A., Lobaton, C.D., Santodomingo, J., Vay, L., Hernandez-SanMiguel, E., Rizzuto, R., Montero, M., and Alvarez, J. (2005) *Cell Calcium*, **37,** 555-564.
[64] Gunter, K.K. and Gunter, T.E. (1994) *J. Bioenerg. Biomembr.*, **26,** 471-485.
[65] Brenner, C. and Grimm, S. (2006) *Oncogene*, **25,** 4744-4756.
[66] Rasola, A. and Bernardi, P. (2007) *Apoptosis.*, **12,** 815-833.
[67] Bernardi, P. (1999) *Physiol Rev.*, **79,** 1127-1155.
[68] Rizzuto, R., Pinton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.M., Tuft, R.A., and Pozzan, T. (1998) *Science*, **280,** 1763-1766.
[69] Vance, J.E. (1990) *J. Biol. Chem.,* **265**, 7248-7256.
[70] Mendes, C.C., Gomes, D.A., Thompson, M., Souto, N.C., Goes, T.S., Goes, A.M., Rodrigues, M.A., Gomez, M.V., Nathanson, M.H., Leite, M.F. (2005) *J. Biol. Chem.*, **280**, 40892-40900.
[71] Green, D.R. and Kroemer, G. (2004) *Science*, **305,** 626-629.
[72] Kroemer, G., Galluzzi, L., and Brenner, C. (2007) *Physiol. Rev.*, **87,** 99-163.
[73] Riedl, S.J. and Salvesen, G.S. (2007) *Nat. Rev. Mol. Cell Biol.*, **8,** 405-413.
[74] Jin, Z. and El Deiry, W.S. (2005) *Cancer Biol. Ther.*, **4,** 139-163.
[75] Ruffolo, S.C., Breckenridge, D.G., Nguyen, M., Goping, I.S., Gross, A., Korsmeyer, S.J., Li, H., Yuan, J., and Shore, G.C. (2000) *Cell Death Differ.*, **7,** 1101-1108.
[76] Yuan, H., Williams, S.D., Adachi, S., Oltersdorf, T., and Gottlieb, R.A. (2003) *Mitochondrion*, **2,** 237-244.
[77] Tsuruta, F., Sunayama, J., Mori, Y., Hattori, S., Shimizu, S., Tsujimoto, Y., Yoshioka, K., Masuyama, N., and Gotoh, Y. (2004) *EMBO J.*, **23,** 1889-1899.
[78] Haupt, S., Berger, M., Goldberg, Z., and Haupt, Y. (2003) *J. Cell Sci.*, **116,** 4077-4085.
[79] Karawajew, L., Rhein, P., Czerwony, G., and Ludwig, W.D. (2005) *Blood*, **105**, 4767-4775.
[80] Chow, L.M. and Baker, S.J. (2006) *Cancer Lett.*, **241,** 184-196.
[81] Igney, F.H. and Krammer, P.H. (2002) *Nat. Rev. Cancer*, **2,** 277-288.
[82] Okouchi, M., Ekshyyan, O., Maracine, M., and Aw, T.Y. (2007) *Antioxid. Redox. Signal.*, **9,** 1059-1096.
[83] Park, J., Lee, S.B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J.M., and Chung, J. (2006) *Nature*, **441,** 1157-1161.
[84] Zuchner, S., Wang, G., Tran-Viet, K.N., Nance, M.A., Gaskell, P.C., Vance, J.M., Ashley-Koch, A.E., and Pericak-Vance, M.A. (2006) *Am. J. Hum. Genet.*, **79,** 365-369.
[85] Pelicci, G., Lanfrancone, L., Grignani, F., McGlade, J., Cavallo, F., Forni, G., Nicoletti, I., Grignani, F., Pawson, T., and Pelicci, P.G. (1992) *Cell*, **70,** 93-104.
[86] Pinton, P., Rimessi, A., Marchi, S., Orsini, F., Migliaccio, E., Giorgio, M., Contursi, C., Minucci, S., Mantovani, F., Wieckowski, M.R., Del, S.G., Pelicci, P.G., and Rizzuto, R. (2007) *Science*, **315,** 659-663.
[87] Giorgio, M., Migliaccio, E., Orsini, F., Paolucci, D., Moroni, M., Contursi, C., Pelliccia,G., Luzi,L., Minucci, S., Marcaccio, M., Pinton, P., Rizzuto, R., Bernardi, P., Paolucci, F., and Pelicci, P.G. (2005) *Cell*, **122,** 221-233.
[88] Jin, S. (2005) *Autophagy*, **1,** 171-173.
[89] Levine, B. and Klionsky, D.J. (2004) *Dev. Cell*, **6,** 463-477.
[90] Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X.H., Mizushima, N., Packer, M., Schneider, M.D., and Levine, B. (2005) *Cell*, **122,** 927-939.
[91] Yu, L., Alva, A., Su, H., Dutt, P., Freundt, E., Welsh, S., Baehrecke, E.H., and Lenardo, M.J. (2004) *Science*, **304,** 1500-1502.
[92] Meijer, A.J. and Codogno, P. (2004) *Int. J. Biochem. Cell Biol.*, **36,** 2445-2462.
[93] Feng, Z., Zhang, H., Levine, A.J., and Jin, S. (2005) *Proc. Natl. Acad. Sci. USA*, **102,** 8204-8209.
[94] Murphy, M.E., Leu, J.I., and George, D.L. (2004) *Cell Cycle*, **3,** 836-839.
[95] Kim, J.S., He,L., Qian, T., and Lemasters, J.J. (2003) *Curr. Mol. Med.*, **3,** 527-535.
[96] Leist, M., Single, B., Castoldi, A.F., Kuhnle,S., and Nicotera,P. (1997) *J. Exp. Med.*, **185,** 1481-1486.
[97] Borutaite, V., Budriunaite, A., Morkuniene, R., and Brown, G.C. (2001) *Biochim. Biophys. Acta*, **1537,** 101-109.
[98] Javadov, S. and Karmazyn, M. (2007) *Cell Physiol Biochem.*, **20,** 1-22.
[99] Delgado-Coello, B., Trejo, R., and Mas-Oliva, J. (2006) *Mol. Cell Biochem.*, **285,** 1-15.
[100] Chami, M., Prandini, A., Campanella, M., Pinton, P., Szabadkai, G., Reed, J.C., and Rizzuto, R. (2004) *J. Biol. Chem.*, **279**, 54581-54589.
[101] Szalai, G., Krishnamurthy, R., and Hajnoczky, G. (1999) *EMBO J.*, **18,** 6349-6361.
[102] Pinton, P. and Rizzuto, R. (2006) *Cell Death. Differ.*, **13,** 1409-1418.
[103] Scorrano, L., Oakes, S.A., Opferman, J.T., Cheng, E.H., Sorcinelli, M.D., Pozzan, T., and Korsmeyer, S.J. (2003) *Science*, **300,** 135-139.
[104] Ferrari, R., Pedersini, P., Bongrazio, M., Gaia, G., Bernocchi, P., Di Lisa, F., and Visioli, O. (1993) *Basic Res. Cardiol.*, **88,** 495-512.
[105] Reynolds, I.J. (1999) *Ann. N. Y. Acad. Sci.*, **893,** 33-41.
[106] Nicotera, P., Leist, M., and Manzo, L. (1999) *Trends Pharmacol. Sci.*, **20,** 46-51.
[107] McConkey, D.J. and Orrenius, S. (1996) *J. Leukoc. Biol.*, **59,** 775-783.

Received: December 15, 2007 Revised: January 05, 2008 Accepted: January 14, 2008