Apoptosis (2007) 12:951–968
DOI 10.1007/s10495-007-0719-7

IP_3 receptors in cell survival and apoptosis: Ca^{2+} release and beyond

Suresh K. Joseph · György Hajnóczky

Published online: 6 February 2007
© Springer Science + Business Media, LLC 2007

Abstract Inositol 1,4,5-trisphosphate receptors (IP_3Rs) serve to discharge Ca^{2+} from ER stores in response to agonist stimulation. The present review summarizes the role of these receptors in models of Ca^{2+}-dependent apoptosis. In particular we focus on the regulation of IP_3Rs by caspase-3 cleavage, cytochrome *c*, anti-apoptotic proteins and Akt kinase. We also address the evidence that some of the effects of IP_3Rs in apoptosis may be independent of their ion-channel function. The role of IP_3Rs in delivering Ca^{2+} to the mitochondria is discussed from the perspective of the factors determining inter-organellar dynamics and the spatial proximity of mitochondria and ER membranes.

Keywords Apoptosis · Calcium · Endoplasmic reticulum · IP_3 · IP_3 receptor · Mitochondria

Abbreviations

NFAT	Nuclear factor of activated T-cells
ER	endoplasmic reticulum
MEF	murine embryo fibroblasts
HIV	Human immunodeficiency virus
STS	staurosporine
Bcl-2	B cell lymphoma 2
Bcl-xL	B cell lymphoma xL
IP_3R	Inositol 1,4,5-trisphosphate receptors
RyR	Ryanodine receptor
FasL	Ligand for Fas receptor (CD95)
VDAC	Voltage dependent anion selective channel
$[Ca^{2+}]_c$	cytoplasmic free $[Ca^{2+}]$
$[Ca^{2+}]_m$	mitochondrial matrix free $[Ca^{2+}]$
OMM	outer mitochondrial membrane
IMM	inner mitochondrial membrane
PTP	permeability transition pore

S. K. Joseph · G. Hajnóczky (✉)
Department of Pathology & Cell Biology,
Thomas Jefferson University,
1020 Locust Street, Philadelphia, PA 19107, USA
e-mail: gyorgy.hajnoczky@jefferson.edu

S. K. Joseph
e-mail: suresh.joseph@mail.tju.edu

1 Introduction

IP_3 receptors are ligand-gated channels that function to release Ca^{2+} from intracellular Ca^{2+} stores (predominantly the endoplasmic reticulum (ER)) in response to IP_3 generation initiated by agonist binding to cell-surface receptors (reviewed in Refs. [1–3]). The ensuing Ca^{2+} signal plays a fundamental role in modulating a diverse range of cellular responses including muscle contraction, exocytosis, motility, fertilization, proliferation and gene expression. The increased energy demand required for carrying out these processes is met, in part, from the accumulation of some of the released Ca^{2+} by the mitochondria with the resulting Ca^{2+} -dependent activation of key intramitochondrial enzymes linked to ATP production [4, 5]. The efficiency of this process is greatly enhanced by placement of ER and mitochondrial membranes in close proximity [6, 7]. Thus the physiological release of Ca^{2+} through IP_3Rs serves important signaling and "housekeeping" roles in maintaining normal function during agonist-mediated cell activation. However, in the presence of an apoptotic stimulus, the IP_3R-mediated Ca^{2+} release can activate apoptotic pathways by inducing the release from mitochondria of a number of pro-apoptotic factors including cytochrome *c*. The present article is focused on reviewing recent studies that implicate the IP_3R as an important molecular component regulating apoptosis induced by a number

of different stimuli. In particular, we have emphasized the role of the IP_3R in establishing macromolecular complexes on the surface of the ER membranes and in modulating the linkage between ER and mitochondrial membranes.

2 The pro-apoptotic role of IP_3Rs

There is general agreement in the literature that Ca^{2+} efflux from the ER and Ca^{2+} accumulation into the mitochondria is linked to the effects of various apoptotic stimuli [6–10]. With certain stimuli, such as ligation of T-cell [11, 12] or B-cell [13] receptors, this process clearly involves stimulation of phospholipase-C activity and generation of IP_3. The functional outcome in lymphocytes may result in either enhanced proliferation or apoptosis, depending on the maturation status of the cells or the intensity of the stimulation [14]. Other classic death ligands such as TNFα or Fas can also promote IP_3 generation in some but not all cells [15–17]. Yet other apoptotic stimuli such as ceramide [18], cytotoxic drugs (e.g. STS [19, 20] and cisplatin [21]), or manipulations such as withdrawal of trophic factors [22], can also cause ER Ca^{2+} release. In these cases an initiating role for IP_3 elevation in these processes has not been demonstrated and the ER Ca^{2+} release could be precipitated secondarily to changes in mitochondrial permeability (Fig. 1) or activation of ER stress responses [23, 24]. Thus although a role for ER to mitochondrial redistribution of Ca^{2+} has been implicated in many models of apoptosis, a primary role for IP_3 generation and activation of IP_3Rs in this process has been examined in only a few instances.

The magnitude of the Ca^{2+} translocated from the ER will be determined by the levels of ER Ca^{2+} and the activity of the efflux and influx pathways that cycle Ca^{2+} across ER membranes. The primary influx pathway of the ER are the SERCA pumps. The primary efflux pathway across the ER membranes (in the presence of IP_3) is the IP_3R but additional poorly characterized 'leak' pathways are also believed to play important roles (reviewed in Ref. [25]). Figure 1 depicts a number of known apoptotic regulatory feed-back loops that involve Ca^{2+}. Examination of this complex regulatory network clearly indicates that IP_3Rs could potentially play a central role in the initiation and/or progression of the apoptotic cascade.

The experimental evidence for an involvement of IP_3Rs in apoptosis have predominantly come from experiments in which the expression level of the receptor protein has been manipulated. There are three isoforms of IP_3Rs that are expressed in differing amounts in various cell types. A functional IP_3R Ca^{2+} channel is composed of tetramers that can be either homotetrameric or, to a lesser extent, heterotetramers of different isoforms [1–3]. In initial studies, it was shown that the levels of the type-III IP_3R protein were increased during apoptosis in T-lymphocytes and that apoptosis could be prevented by antisense-RNA to the type-III but not the type-I isoform [26]. These observations were later replicated in other models of apoptosis [27]. A role for the type-III isoform has also been proposed recently based on selective siRNA knockdown studies of IP_3R isoforms in CHO cells [28]. This preferential role was suggested to be the result of enhanced colocalization of the type-III isoform adjacent to mitochondrial membranes [28]. However, there is also evidence that stable knockdown of the type-I IP_3R in Jurkat T-lymphocytes can inhibit responsiveness to different apoptotic stimuli [29]. siRNA for the type-I IP_3R also diminishes apoptosis in response to FasL in Jurkat lymphocytes [17]. The introduction of constitutively active calcineurin restored the sensitivity of the type-I deficient cells and also restored the ability of the cells to dephosphorylate and generate the pro-apoptotic factor Bad [30], supporting the view that this is one pathway that is downstream of cytosolic Ca^{2+} elevation (c.f. Fig. 1). Immunoblotting of the type-I deficient Jurkat cells also showed decreased levels of the type-II and type-III isoforms [29], so it is difficult to conclude from these particular studies that only the type-I IP_3R plays a unique role. Studies on the T-cells of mice deficient in the type-I IP_3R did not reveal any abnormalities in T-cell function or development [31]. Similarly, mice deleted for both type-II and III IP_3R isoforms showed severe defects in the digestive system but were able to survive with appropriate feeding regimens, indicating that a single IP_3R isoform may be sufficient to sustain many essential signaling requirements [32].

The issue of isoform specificity has also been addressed using chicken B-lymphocyte DT-40 cell lines in which the genes for individual IP_3R isoforms have been selectively deleted by homologous recombination [33]. The loss of any one isoform did not affect B-cell receptor mediated apoptosis whereas the loss of all three isoforms (TKO cells) led to marked inhibition [34]. These data suggest that there is considerable redundancy in the ability of each IP_3R isoform to participate in the apoptotic process. The extent of the inhibition of apoptosis reported for TKO DT-40 cells in various studies has been variable. In the original report, 30% of the TKO cells stimulated with 10 μg/ml anti-IgM showed an apoptotic phenotype in 24h compared to 74% in WT cells. A similar magnitude of inhibition was also reported by Assefa et al. for both anti-IgM and STS [20]. Others have found a much larger suppression of apoptosis under similar experimental conditions, although the amount of cell death does increase in the TKO cells as exposure to the apoptotic stimulus is prolonged [35]. Overall, the data suggest that IP_3R-dependent apoptotic pathways play a major role, particularly in the early stages of the apoptotic response, but that alternative less efficient apoptotic pathways can be recruited in the absence of IP_3Rs.

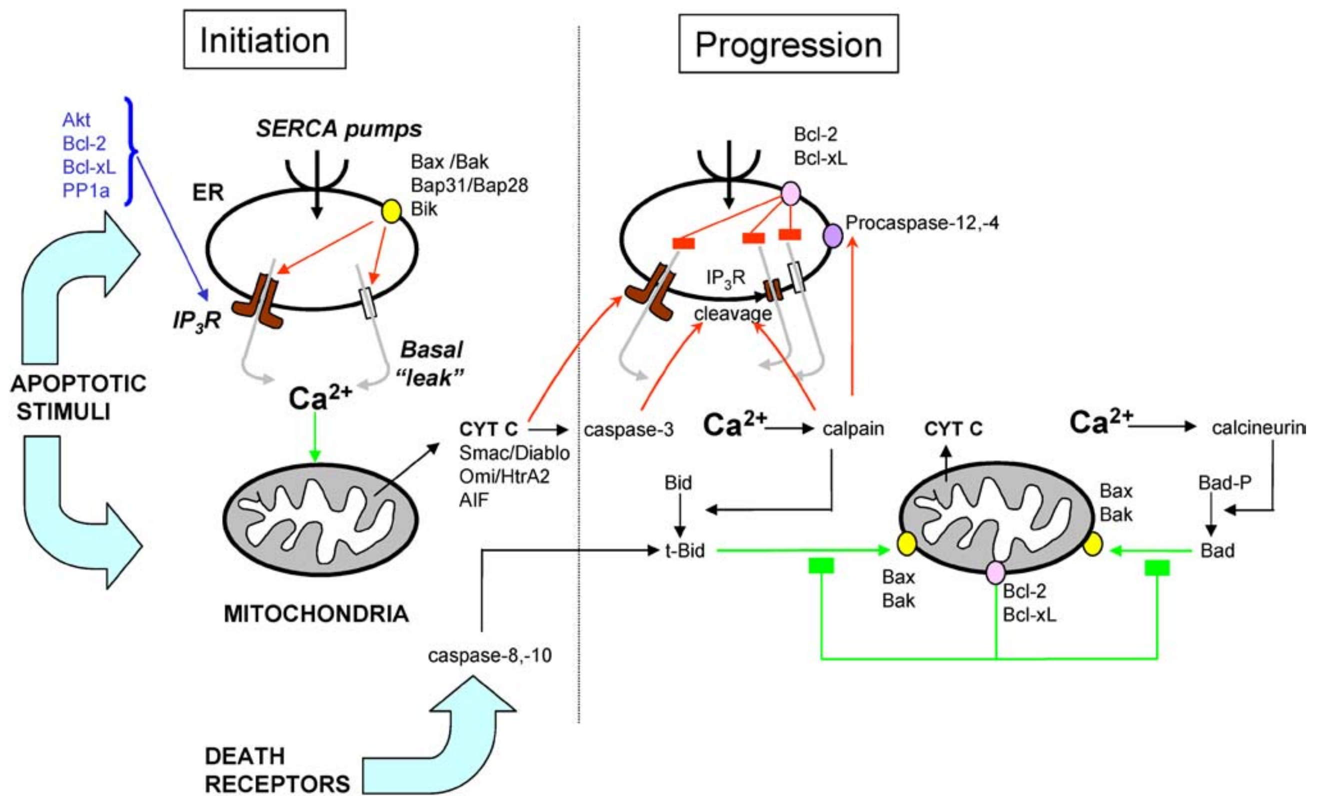

Fig. 1 Ca^{2+} dependent networks regulating apoptosis. The scheme incorporates some of the prevailing hypothesis regarding Ca^{2+}-dependent steps in apoptotic cascades. The initiating step induced by divergent stimuli is depicted as an enhanced translocation of Ca^{2+} from the ER to the mitochondria resulting in mitochondrial Ca^{2+} overload provoking the release of a number of pro-apoptotic proteins into the cytoplasm, including cytochrome *c* (CYT C). This intrinsic pathway is activated in response to STS and by many other cytotoxic agents including those that cause ER stress. The extrinsic pathway is activated by cell surface death receptors of the TNF-α superfamily. Downstream pathways which target steps occurring in the ER are depicted in red and those that target the mitochondria are depicted in green. Arrowheads and bars indicate activation and inhibitory steps respectively. Enhanced Ca^{2+} efflux from the ER can be promoted by cytochrome *c* directly activating IP_3Rs or indirectly by caspase or calpain cleavage of IP_3Rs generating "leaky" ion channels. The Ca^{2+} leak pathways in the ER membrane can be positively or negatively modulated by a number of Bcl-2 family of proteins. Proteins that bind to IP_3Rs but whose functional effects have not been firmly established are shown in blue. Ca^{2+} dependent steps that target the mitochondria include calpain cleavage of Bid and the calcineurin dependent dephosphorylation of Bad. For additional details see text

3 Molecular mechanism(s) of IP_3R function in apoptosis

In addition to the direct role of IP_3Rs in the initiaton of apoptosis by providing a conduit for ER-to-mitochondria Ca^{2+} transfer, there are several additional feedback mechanisms that have been proposed which allow IP_3Rs to play a role in amplifying Ca^{2+} dependent apoptotic pathways. These include the cleavage of the receptor by caspases (and possibly calpains) which is proposed to provide an enhanced ER Ca^{2+} leak pathway, and the direct binding and regulation of the IP_3R Ca^{2+} channel by several key players in apoptosis including cytochrome *c* and the anti-apototic proteins Bcl-2 and Bcl-xL. In addition, the IP_3R can be directly phosphorylated by Akt kinase. Current information on these regulatory mechanisms are summarized below.

3.1 Regulation by proteolytic cleavage

Mikoshiba and colleagues first demonstrated that the type-I IP_3R was cleaved by caspase-3 in Jurkat T-cells in response to STS or activation of the Fas pathway [36]. Cleavage *in vitro* of the cerebellum IP_3R was reported to inactivate IP_3-mediated Ca^{2+} release. The basic observation of caspase-3 mediated IP_3R proteolysis has since been confirmed in Jurkat T-cells [37], DT-40 B lymphocytes [20], SH-SY5Y neuroblastoma cells [38] and PC-12 cells [39]. The cleavage site was localized to aspartate 1892 within a DEVD caspase-3 proteolytic site. Proteolysis would be expected to generate a 95 kDa C-terminal fragment containing the six transmembrane domains that form the ion channel, and a 215 kDa N-terminal fragment that includes the IP_3 binding region of the receptor (Fig. 2(A)). Although the 95 kDa C-terminal band is experimentally observed [20, 36–38], the status of the

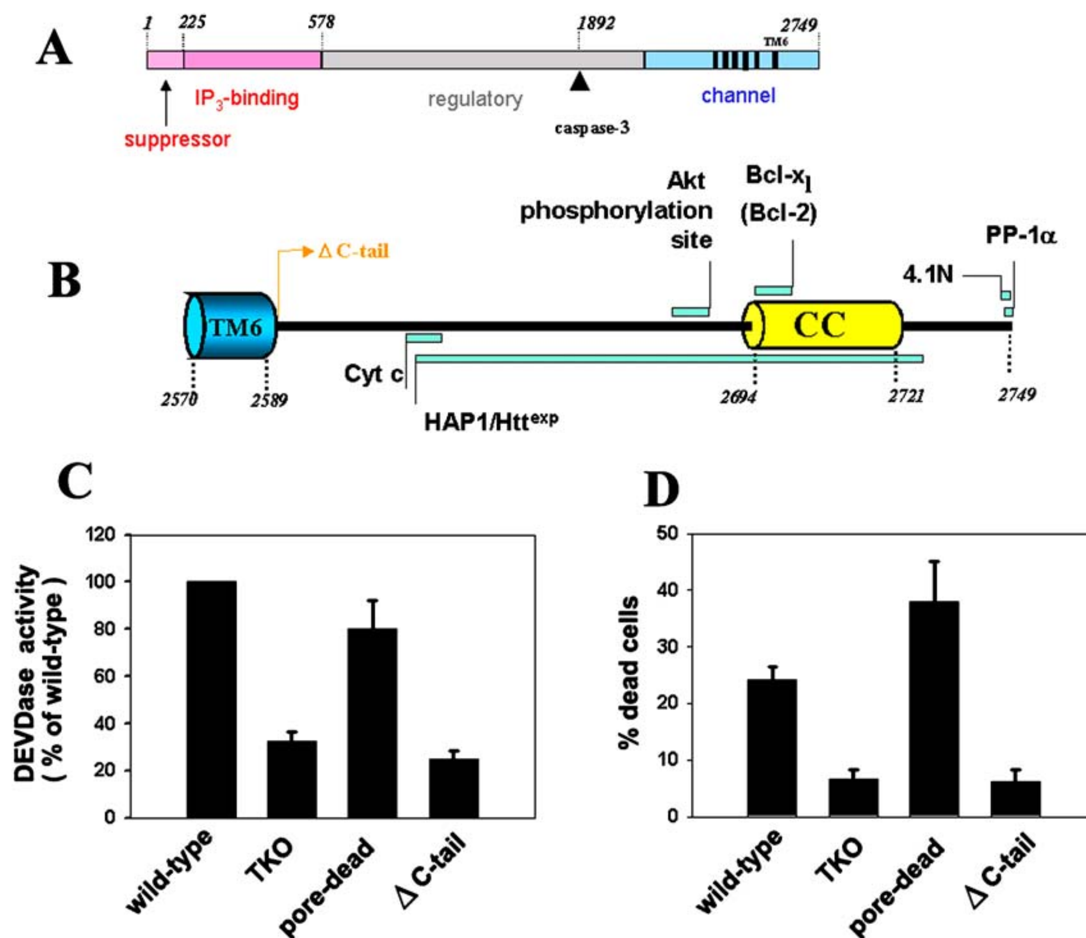

Fig. 2 Domain organization and protein/protein associations in the C-terminal tail of IP_3Rs. *Panel A* shows the domain organization of IP_3Rs with the N-terminal binding domain, C-terminal channel domain and intervening regulatory domain. The small region at the N-terminus which acts to suppress IP_3 binding and the predominant site of caspase-3 cleavage is also shown. *Panel B* shows in more detail the 159 amino acid, cytosol-exposed, C-terminal tail which contains interaction sites for several proteins that are key regulators of apoptosis. Abbreviations are CC, coiled-coil domain; TM6, transmembrane domain 6; PP-1α protein phosphatase-1α. Other abbreviations are defined in the text. *Panel C*: DT-40 chicken *B*-lymphocytes containing or lacking all 3 IP_3R isoforms (wild-type and TKO respectively) were incubated with 0.5 μM STS for 6 h and caspase-3 activity was measured in cell lysates with a fluorometric assay measuring the hydrolysis of DEVD-AMC. The data was compared to stable DT-40 cell lines made in the TKO background which contained the D2550A pore-dead mutant or a construct of the type-I IP_3R missing the 159 amino-acid tail (ΔC-tail; see Panel B). *Panel D*: The conditions were the same as for Panel D except that cell viability was measured with an MTS assay. The data in Panels D & E are the mean + S.E.M. of 3–5 independent experiments (M.T. Khan & S.K. Joseph, unpublished). Technical details of the preparation of stable DT-40 cell lines, cell culture and caspase-3 assay conditions are as described in Ref. [96]

N-terminal segment of the receptor after caspase-3 cleavage *in vivo* has not been examined. At least *in vitro*, the N-terminal segment formed is smaller than the expected 215 kDa, suggesting that caspase-3 may cleave at multiple sites [40]. Each IP_3R isoform contains multiple DXXD consensus caspase-3 cleavage sites, although the DEVD site at position 1892 is unique to the type-I isoform. In the initial study using Jurkat T-cells only the type-I isoform was reported to be a substrate [36]. However, in another study also using Jurkat T-cells, all 3 isoforms were degraded in response to TNFα stimulation but only the type-I and type-II IP_3R degradation was sensitive to the pan-caspase inhibitor z-VAD-fmk whereas type-III IP_3R degradation was sensitive to a calpain inhibitor [37].

Two key questions regarding the caspase-3 cleavage of IP_3R receptors remain to be addressed. Is proteolysis of IP_3Rs an essential step in the pathway of Ca^{2+}-dependent apoptosis and what are the functional consequences of generating cleaved forms of the IP_3R? MCF-7 breast carcinoma cells which lack caspase-3 do not degrade IP_3Rs in response to TNFα or STS [36]. This indicates that IP_3R cleavage cannot be mediated by other effector caspases present in MCF-7 cells, including caspase-7, which has the same substrate specificity as caspase-3. Yet, MCF-7 cells undergo Ca^{2+}-dependent apoptosis [41] and show a number of features common to other cells, including a Bcl-2 mediated decrease in ER Ca^{2+} [42, 43]. Boehning et al. observed minimal cleavage of IP_3Rs during the early stages of apoptosis induction by

STS in HeLa or PC12 cells when compared to the more rapid and extensive cleavage of established substrates such as poly (ADP-ribose) polymerase [39]. However, complete loss of IP_3Rs was observed at 24–48 h and this loss was blocked by caspase-3 inhibitors. Thus IP_3R cleavage may reflect a very distal event in apoptosis/necrosis that may be associated with an inactivation of Ca^{2+} channel function [36].

The strongest evidence in favor of an essential role of caspase-3 proteolysis comes from the finding that a non-cleavable DEVD mutant, in contrast to the wild-type type-I IP_3R, was unable to support apoptosis when introduced into the TKO DT-40 cells [20]. In this study, the addition of STS to wild-type cells caused a prolonged increase of cytosolic free Ca^{2+} which was blocked by caspase-3 inhibition. This increase was absent in the DEVD mutant. The interpretation of the data is consistent with the view that caspase-3 cleaves the IP_3R and the resulting 95 kDa "channel-only" fragment is responsible for the persistent cytosolic Ca^{2+} increase. The GFP-tagged version of the 95 kDa "channel-only" fragment induces an ER Ca^{2+} leak when expressed in COS-7 or HeLa cells [44] and this has lent support to the idea that the "channel-only" IP_3R fragment is a constitutively leaky Ca^{2+} channel (reviewed in Ref. [45]). However, expression of the untagged version of the 95 kDa "channel-only" domain in TKO DT-40 cells does not by itself induce ER Ca^{2+} depletion [20] (Bhanumathy & Joseph; unpublished observations). An enhanced leak in the presence of an apoptotic stimulus could arise if the truncated channel is gated open by some change induced by the stimulus e.g. alteration in the channel thiol redox state as a consequence of increased ROS generation. Alternatively, the "channel-only" or the N-terminal fragment could indirectly regulate the activity of another Ca^{2+} leak pathway in the ER. If the caspase-3 cleavage of IP_3Rs were critical for causing the Ca^{2+} changes observed in the cytosol and ER in response to various apoptotic stimuli, then it would be expected that these changes would be blocked by caspase-3 inhibitors. This is indeed the case for the STS-induced Ca^{2+} changes reported by Assefa et al. [20] but this is not a general finding. For example, the prolonged oscillations in cytosolic Ca^{2+} induced by STS in HeLa cells [39] or the STS-mediated depletion of ER Ca^{2+} in PC-3 prostate adenocarcinoma cells [19] are both insensitive to caspase-3 inhibitors. Thus although the data for caspase-3 IP_3R cleavage appears strong in the DT-40 B-cell model, a general involvement of caspase-3 cleavage of IP_3Rs in the early stages of the apoptotic cascade and a role for leaky channels formed from proteolytic fragments remain to be firmly established.

Activation of calpains have been observed in many models of apoptosis [46] and calpains have also been reported to cleave IP_3Rs [47, 48]. The Ca^{2+} dependent cleavage of the type-I IP_3R in cerebellum membranes produced C-terminal fragments of 95 and 130 kDa [47]. However, in those models of apoptosis where a degradation of IP_3Rs can be observed, the inclusion of calpain inhibitors does not prevent IP_3R proteolysis ([36, 39]; but c.f. [37]). Even if calpain activation is not important for IP_3R degradation, there is strong evidence that activation of calpain plays important roles in regulating Ca^{2+}-dependent steps involved in apoptosis. For example μ-calpain is involved in the cleavage and activation of *t*-Bid which, in turn, plays a prominent role in the release of pro-apoptotic factors from the mitochondria [49, 50]. In addition the stimulation of calpain activity has been linked to the activation of ER-associated caspase-12 [51], the activation of calcineurin [52] and the elevation of cytosolic Ca^{2+} as a result of the cleavage of the plasma membrane Na^+/Ca^{2+} exchanger [53].

3.2 Regulation by cytochrome *c*

Interactions with cytochrome *c* were detected in a yeast two-hybrid screen using the C-terminal segment of the type-I IP_3R as bait [39]. The IP_3R Ca^{2+} channel exhibits a biphasic dependence on $[Ca^{2+}]$ with low concentrations of Ca^{2+} stimulating and high concentrations of Ca^{2+} inhibiting channel activity [54]. The functional effect of cytochrome *c* on the channel was to prevent the inhibitory effect of Ca^{2+} thereby disrupting the normal mechanism that acts to shut off the channel. The uptake of Ca^{2+} into mitochondria can also indirectly influence local feedback effects of Ca^{2+} on IP_3Rs [55, 56]. The exact mechanism by which Ca^{2+} activates or inhibits the IP_3R Ca^{2+} channel is not well understood. However, it is of interest that the site of cytochrome *c* binding on the IP_3R has been localized to a 16 amino-acid stretch (2621–2636) present within the short 160 amino acid C-terminal tail of the receptor [57]. This segment projects into the cytosol from the end of the transmembrane domain helix 6 which lines the ion-channel pore [58]. The C-terminal tail is also the binding site for Bcl-xL, protein phosphatase-1α and contains the phosphorylation site for Akt kinase (Fig. 2(B)). It has been assumed that these effector proteins must be able to easily access the cytosol-exposed C-terminal segment in the intact cell. The 3D conformation of the IP_3R has been reconstructed from EM studies but the limited resolution of the structures does not allow the location of the C-terminal tail to be identified (reviewed in Ref. [58]). The presence of Ca^{2+} causes a large conformational change in the IP_3R that has been visualized in EM as a transition from a "square" to a "wind-mill" structure [59]. In the native receptor, this conformational change could also alter the accessibility of the C-terminal tail to cytochrome *c* and to the other molecules that bind to this region. The finding that cytochrome *c* has its largest functional effect at concentrations of $Ca^{2+} > 1\ \mu M$ is consistent with this idea [39].

Analysis of ER enriched membrane fractions in cells undergoing apoptosis indicates that cytochrome *c* translocates

from the mitochondria to ER membranes [39]. Under these conditions, the 100,000×*g* cytosol fraction appears devoid of cytochrome *c* except in DT-40 cells lacking IP_3Rs [39]. This has led to the proposal that cytochrome *c* released from the mitochondria is sequestered at ER membranes by high-affinity binding to IP_3Rs. Recently, a translocation of cytochrome *c* to the ER was also noted in the hippocampus after ischemia/ reperfusion injury [60]. However, a principal function of cytochrome *c* is to associate with Apaf-1 and facilitate the formations of the apoptosome that ultimately leads to the activation of caspases. Since these complexes are formed in the cytosol, a minimal amount of cytochrome *c* must be released into this compartment. This has been confirmed in many studies which have used digitonin-permeabilization (e.g. [61]) or single cell imaging of GFP-cytochrome *c* [62].

Evaluation of the mitochondrial release of cytochrome *c* in single cells indicate that, once initiated, the process is rapid (<5 min) and encompasses all the mitochondria in the cell [62]. The cytochrome *c*/ IP_3R interaction has been suggested to co-ordinate the global release of cytochrome *c*. It is proposed that a partial release of cytochrome *c* would activate Ca^{2+} release from adjacent IP_3Rs that would feed back on the mitochondria to promote further permeabilization and additional cytochrome *c* release [39, 63]. A requirement for this feed-back loop is supported by studies which have employed the 16 amino-acid peptide encoding the highly conserved cytochrome *c* binding motif from IP_3Rs. Addition of a cell-permeant version of the peptide exerts dominant negative effects and inhibited apoptosis induced by either STS or FasL, presumably by competing for cytochrome *c* binding to the IP_3R [57].

In summary, the evidence points to an important role for the interaction of cytochrome *c* with IP_3Rs in the regulation of apoptosis. Further characterization of the cytochrome *c* effects on Ca^{2+} regulation of the IP_3R at the single channel level and identification of the sites on cytochrome *c* that are relevant for IP_3R interaction should provide additional mechanistic insights. An absolute requirement of IP_3Rs for global cytochrome *c* release would predict that cytochrome *c* release would be severely inhibited in the DT 40 TKO cell line from which IP_3Rs are absent. Although cytochrome *c* translocation to the ER membrane fraction is eliminated, a release into the cytosol is still observed in TKO cells stimulated with anti-IgM [39]. Thus, alternative compensatory mechanisms must be in place to facilitate limited mitochondrial permeabilization in the absence of IP_3Rs.

3.3 Regulation by pro- and anti-apoptotic proteins

It is increasingly recognized that a number of the key pro and anti-apoptotic Bcl-2 family of proteins in the cell are present in ER membranes and exert important functional effects from this subcellular location (reviewed in Refs. [23]). In particular, the anti-apoptotic proteins Bcl-2 / Bcl-xL and the pro-apoptotic proteins Bax/Bak have been found to alter the Ca^{2+} permeability of ER membranes [64–66]. Anti-apoptotic proteins can potentially attenuate the pro-apoptotic role of IP_3Rs in two distinct ways. Firstly, overexpression of Bcl-xL causes a dramatic decrease in IP_3R expression in a number of T-cell lines [67]. However, this has not been a general finding in cells over-expressing Bcl-2 anti-apoptotic proteins [35, 43, 68]. Secondly, the extent of agonist-mediated Ca^{2+} release from the ER is diminished when anti-apoptotic proteins are over-expressed. Although this is a fairly consistent observation in the literature [42, 43, 68–70] the underlying mechanism remains controversial. Several lines of evidence support the view that the anti-apoptotic proteins function to inhibit IP_3R channel activity. This has been documented by measuring the response of permeabilized cells over-expressing Bcl-2 to added IP_3 [43, 68, 70], or intact cells to a permeant form of IP_3 [68], or by monitoring IP_3R channel activity in planar lipid bilayers in response to recombinant Bcl-2 [68]. None of these studies found any changes in the content of ER Ca^{2+} stores induced by Bcl-2 overexpression. However, other studies have shown that Bcl-2 expression can induce a leak of Ca^{2+} from ER stores that would be expected to diminish the amount of Ca^{2+} available for release by apoptotic stimuli (reviewed in Ref. [64]). Recently White et al. [35] have reported that Bcl-xL activates IP_3Rs by allowing channels to remain open even at the very low IP_3 concentrations expected to be present under unstimulated conditions. They have made the interesting proposal that the enhanced leak of ER Ca^{2+} promoted by anti-apoptotic proteins, under some conditions, may just be a manifestation of an activation of IP_3R channels by endogenous levels of IP_3. This was supported by the finding that stable over-expression of Bcl-xL induced a decrease in the ER Ca^{2+} store in wild-type but not in the TKO IP_3R-deficient DT-40 cells [35].

At present the question of whether the Bcl-2 family of anti-apoptotic proteins inhibits or activates the IP_3R channel awaits further clarification. Some of the discrepant data may be related to the different type of complexes that Bcl-2/Bcl-xL could potentially form with IP_3Rs. Both proteins have been shown to bind to all three IP_3R isoforms using co-immunoprecipitation and fusion protein pull-down assays [35, 68, 71]. The site of interaction with IP_3Rs is in the C-terminal tail and has been further localized to a region in the proximal portion of a coiled-coil domain that may be critical for channel assembly and function (Kevin Foskett, personal communication) ([72]; c.f. Fig. 2(B)). The interactions with this site have been presumed to be direct but a recent report argues that only the type-I IP_3R isoform can associate with Bcl-2 and that this association is indirect, involving a ternary complex with protein phosphatase-1α (PP-1α) [43]. PP-1α associates with a site on the C-terminal tail and inhibits IP_3R channel activity [73] (Fig. 2(B)). The

binding of Bcl-2 to PP1α has also been observed in other experimental systems [74]. Another protein phosphatase, calcineurin, is also reported to form a ternary complex with Bcl-2 and IP_3Rs [75]. This raises the issue that the modulation of IP_3R function by anti-apoptotic proteins could be related to alterations in the phosphorylation state of the IP_3R and that the direct versus indirect modes of association of anti-apoptotic proteins with the receptor could have different functional effects on the IP_3R Ca^{2+} channel. In addition, the assumption that Bcl-2 and Bcl-xL function in exactly the same manner may not be warranted [76].

IP_3 receptors are phosphoproteins and have long been recognized as excellent substrates for A and G- kinases [3, 77, 78]. The major phosphorylation sites for A-kinase on the type-I IP_3R are serines 1589 and 1755 and the phosphorylation in most cases produces a potentiation of IP_3-mediated Ca^{2+} release [79, 80]. A notable exception is vascular smooth muscle where phosphorylation by G-kinase inhibits IP_3R-mediated Ca^{2+} release and promotes relaxation [81]. IP_3Rs form macromolecular complexes with both A-kinase, G-kinase and protein phosphatases [81–83]. The role of the pro-apoptotic proteins Bax and Bak in regulating ER Ca^{2+} dynamics have been studied in MEFs isolated from Bax(−/−)/Bak(−/−) mice [84]. These cells had a lower steady-state Ca^{2+} content in the ER, an enhanced basal leak of Ca^{2+} and were resistant to Ca^{2+} dependent apoptotic stimuli. These effects were attributed to the presence of unrestrained Bcl-2 in ER membranes. It was also noted that the type-I IP_3R in the Bax/Bak deficient cells showed increased phosphorylation at serine 1755 [71]. siRNA suppression of Bcl-2 or the type-I IP_3R (but not the type-III IP_3R) reduced the basal Ca^{2+} leak and partially restored the ER Ca^{2+} content of the Bax/Bak deficient cells. From this data it was suggested that the intrinsic leak of Ca^{2+} from the ER is related to the degree of phosphorylation of the IP_3R, with "hyperphosphorylation" of the receptor generating a leaky channel [71]. The exact mechanism by which unrestrained Bcl-2 activity may bring about enhanced phosphorylation of the type-I IP_3R was not defined but the ability of Bcl-2 to recruit calcineurin to the IP_3R was considered a possibility. However, enhanced calcineurin association would be expected to decrease (not increase) the phosphorylation state of the protein. Indeed, IP_3Rs have a reduced phosphorylation state in a T-cell line overexpressing Bcl-2 [85]. There is also no evidence that increasing the phosphorylation state of the receptor makes IP_3R channels intrinsically leaky. In contrast, there is clear evidence that increased phosphorylation at the A-kinase sites can markedly increase the IP_3 sensitivity of the channel [79, 80]. Therefore, the increased Ca^{2+} "leak" seen in the ER of Bax/Bak deficient cells could reflect an enhanced responsiveness of IP_3Rs to ambient levels of IP_3.

Although Bax and Bak are partly localized to ER membranes, there have been no reports that they directly interact with IP_3Rs. Therefore, their effects on ER Ca^{2+} handling are assumed to be entirely due to modulating the levels of IP_3R–associated anti-apoptotic proteins. A mutant form of Bcl-2 which is unable to bind pro-apoptotic proteins does not reduce the levels of ER Ca^{2+} stores [86]. Interestingly, a mutant form of Bcl-xl with impaired binding to Bax or Bak, but normal binding to BH3-only Bcl-2 family of proteins, continued to decrease ER Ca^{2+} stores [86]. It is unclear if any of these Bcl-2/Bcl-xL mutants have an altered ability to interact with IP_3Rs. The role of the BH3-only family of proteins in regulating ER Ca^{2+} dynamics has not been extensively explored. The addition of *t*-Bid had no effects by itself on IP_3R channel activity but did reverse the stimulatory effects of Bcl-xL [35]. The ER localized BH3-only protein Bik can also induce an ER Ca^{2+} leak and the effect requires the presence of Bax or Bak [87]. The Bcl-2 associated protein-31 (Bap31) is a polytopic integral membrane protein located in the ER which can be cleaved during apoptosis by caspase-8. The cleaved product p21 enhances ER Ca^{2+} leak and triggers apoptosis [88]. Whether any of these pathways of ER Ca^{2+} depletion also involves IP_3Rs or reflect separate mechanisms remains to be established.

An increased prevalence of neural stem cells has been noted in the adult brains of mice deficient in either Bax and/or Bak [89, 90]. Recently, it was reported that the stem cells in culture from Bax-deficient mice are more resistant to Ca^{2+}-dependent apoptosis and have a lower ER Ca^{2+} content than those from wild-type mice. siRNA knockdown of the type-I IP_3R in wild-type neural precursor cells mimics the phenotype of the precursor cells from Bax deficient mice [89]. Further studies of the interactions of IP_3Rs with anti-apoptotic proteins and molecular identification of the pathways that constitute an ER Ca^{2+} leak should provide important advances in our understanding of the changes occurring in ER Ca^{2+} handling during apoptosis.

3.4 Regulation by Akt-kinase

In addition to the phosphorylation by A-kinase and G-kinase discussed above, the IP_3R is also a substrate for several other protein kinases including calmodulin dependent protein kinase II [91], protein kinase-C [92], cdc2/cyclin B1 [93] and MAP kinases [94]. From the context of apoptosis, a particularly relevant observation is that the IP_3R is a substrate for Akt kinase [95]. This enzyme has a key role in promoting cell survival utilizing many independent mechanisms. These include regulating the activity of fork-head and NF-κB transcription factors and inactivation of the pro-apoptotic protein Bad (reviewed in Refs. [96, 97]). The phosphorylation of the type-I IP_3R occurs in the C-terminal tail at a single consensus Akt phosphorylation site (serine 2618) that is present in all

three IP_3R isoforms (Fig. 2(B)). IP_3R phosphorylation was demonstrated *in vitro* and *in vivo* in response to insulin activation of the PI-3 kinase pathway [94]. Some transformed cells which have a constitutively active Akt kinase, such as the prostate carcinoma cell line LnCaP, also have basally phosphorylated IP_3Rs which can be suppressed by the PI-3 kinase inhibitor LY294002 [95]. Only a small proportion of the total cellular pool of IP_3Rs appears to be a substrate for Akt kinase. This may be related to the fact that Akt kinase is normally activated in the plasma membrane and that subsequent translocation to the ER surface is limited and/or that the phosphorylation site in the C-terminal tail in the native protein is not readily accessible to the kinase. Potentially, changes in the phosphorylation state of a small pool of IP_3Rs at a critical location could still exert large effects on the initiation of the apoptotic cascade. The functional consequences of the phosphorylation have been studied by making phosphomimic mutants of the Akt phosphorylation site and examining their flux properties when expressed in TKO DT-40 cells or in COS cell microsomes. The data indicate that phosphorylation does not alter the flux properties of the channel [95]. A number of studies have shown that a requirement for IP_3Rs in obtaining optimal rates of apoptosis can be observed by monitoring the levels of caspase-3 activation. Thus, TKO DT-40 cells [20, 98] and T-cells deficient in the type-I IP_3R [99] have low rates of caspase-3 activation in response to an apoptotic stimulus when compared to the wild-type cells. In TKO DT-40 cells a non-phosphorylatable S2618A mutant was able to support almost 3.5-fold higher caspase-3 activation than the phosphomic S2618E which behaves similarly to wild-type receptors [95]. This suggests that there is some protective role of Akt phosphorylation of IP_3Rs that is independent of any inhibition of Ca^{2+} channel activity. Possibly, this could involve altered interactions of other regulatory proteins with the IP_3R such as those occurring in the C-terminal tail (Fig. 2(B)) but the nature of these alterations remain to be identified.

4 IP_3Rs as a macromolecular signaling complex at ER membranes

At the time of writing 55 proteins are reported as associating with IP_3Rs based on co-immunoprecipitation, yeast two-hybrid and fusion protein studies (see Ref. [3] for a partial list). The most recently identified interaction is with a novel protein called DANGER, which in contrast to cytochrome *c*, enhances the inhibitory effect of Ca^{2+} on IP_3-mediated Ca^{2+} release [100]. The multiplicity of associations displayed by the IP_3R has led several authors to propose that the receptor functions as a macromolecular signaling complex on the ER membrane [3, 101]. A role for IP_3Rs independent of their ability to mobilize intracellular stores has been observed previously. TKO DT-40 cells lack both agonist-mediated intracellular Ca^{2+} release and Ca^{2+} entry across the plasma membrane [102]. However, agonist-mediated Ca^{2+} entry can be restored by transfection with a functionally inactive IP_3R containing a point mutation (D2550A) in the selectivity filter of the ion channel pore, but not by IP_3R mutants defective in IP_3 binding [102]. Figures 2(D) and (E) show the effect of STS on caspase-3 activation and cell viability of stable cell lines containing the D2550A mutant. Surprisingly, this mutant functions to support STS-induced apoptosis. By contrast, another functionally inactive mutant in which the C-terminal tail was deleted [72] was unable to support apoptosis in DT-40 cells. Assefa et al. also showed that a functionally inactive mutant containing a 225 amino-acid deletion from the N-terminus was able to support caspase-3 activation in DT-40 cells [20]. The interpretation of this result was that the N-terminal deletion mutant could still be proteolytically cleaved by caspase-3 to generate the 95 kDa "channel-only" fragment, and would provide the ER Ca^{2+} leak necessary to initiate the apoptotic cascade. In our hands the "channel-only" domain containing the pore-inactivating D2550A mutant was also able to support STS-induced apoptosis in DT-40 cells (Khan & Joseph, data not shown).

It can be anticipated that multiple and redundant pathways to apoptosis exist in a cell. Functional IP_3Rs may provide one of several pathways of Ca^{2+} delivery to the mitochondria. A correlation between enhanced IP_3 sensitivity of IP_3Rs and STS sensitivity to apoptosis has indeed been noted in DT-40 cell lines [98]. However, the results also suggest that IP_3Rs can serve functions in apoptosis that go beyond simply supplying Ca^{2+} to sensitize the mitochondria to apoptotic insults. In the absence of functional IP_3Rs, this role could be subserved by Ca^{2+} entry across the plasma membrane. There is ample evidence for microdomains in which plasma membrane Ca^{2+} entry and mitochondrial Ca^{2+} uptake are coupled (reviewed in Ref. [103]). Recent studies suggest that a few IP_3Rs in the plasma membrane can themselves contribute substantially to Ca^{2+} entry in lymphocytes, but this activity requires a functional pore [104]. While the ion-channel function of IP_3Rs appears dispensable for apoptosis when plasma membrane Ca^{2+} entry mechanisms are operative, the C-terminal tail of the receptor still appears to be essential (Fig. 2(C) and (D)). This could reflect the necessity for interaction with one or more of the known proteins targeting this domain (Fig. 2(B)). It cannot presently be excluded that there are additional interactors remaining to be identified. As pointed out above, cell-permeant peptides encoding the IP_3R cytochrome *c* binding site can inhibit cell death [57]. Further mapping studies with additional C-terminal deletion mutants, a search for additional proteins targeting the C-terminal tail and investigation of the relative accessibility of this region of the native receptor should all provide insights into the ion-channel independent role of IP_3Rs.

5 Diseases linked to aberrant apoptotic signaling by IP_3Rs

Recent studies of several diseases linked to defects in apoptosis and Ca^{2+} signaling have converged on examining IP_3Rs as a possible molecular target. Four examples are briefly discussed. They are the HIV-1 associated Nef protein, presenilins, the polyglutamine repeat protein huntingtin, and the NF-κB signaling pathway.

Nef is an accessory protein encoded by the HIV-1 & 2 virus which functions to maintain infected T-cells in an activated state and to induce apoptosis in non-infected cells of the immune system [105]. Manninen and Saksela [106] found that Jurkat cells transfected with Nef showed a robust activation of NFAT which was dependent on Ca^{2+} entry across the plasma membrane. The authors also found that NFAT activation was attenuated in Jurkat cells deficient in the type-I IP_3R, that Nef and the IP_3R were co-immunoprecipitated, and that Nef did not modify the content of IP_3-sensitive stores [106]. It was concluded that Nef did not affect the channel function of IP_3Rs but altered its coupling to the plasma membrane Ca^{2+} entry mechanism(s) such that Ca^{2+} entry occurred independently of store depletion. Interestingly, another HIV encoded protein, HIV-Tat, whose neurotoxic effects are thought to contribute to AIDS related dementia also promotes apoptosis [107]. In this case the mechanism appears to involve a rapid mobilization of IP_3-sensitive stores resulting from an increased generation of IP_3 [108].

Presenilin-1 (PS-1) is a predominantly ER localized protein involved in the proteolysis of amyloid precursor protein. Mutations in PS-1 are associated with the majority of cases of early onset familial Alzheimer's disease (FAD). There have been a number of studies showing that cultured cells or primary neurons expressing mutant PS-1 display an enhanced response to caged-IP_3 with no changes in the expression level of IP_3Rs [109, 110]. This has led to the hypothesis that mutant PS-1 can cause an over-filling of IP_3-sensitive Ca^{2+} stores by an unidentified mechanism, and that an enhanced Ca^{2+} release may underlie a number of the pathological effects associated with Alzheimers disease [109, 110]. The expression of ryanodine receptors is increased in PS-1 expressing transgenic mouse models [110]. Recently, the mechanism of enhanced IP_3-mediated Ca^{2+} release has been suggested to be indirect, and to involve recruitment of ryanodine receptor channels activated by calcium-induced Ca^{2+} release [111]. Another possible explanation offered by Tu et al. is based on their observations that the wild-type (but not the mutant) presenilins can form low conductance, non-selective ion channels in planar lipid bilayers [112]. They suggest that wild-type PS may contribute to the basal ER Ca^{2+} leak and that the absence of this leak with mutant PS may cause the observed overfilling of Ca^{2+} stores. Although, this would be in agreement with the bulk of the experimental data, it should also be pointed out that data inconsistent with an enhanced ER Ca^{2+} leak mediated by PS-1 has been reported by Kasri et al. [113].

Presenilin-2 (PS-2) is closely related to PS-1 (67% identical) and has similar effects on the over-filling of ER Ca^{2+} stores [114]. Both normal and mutant PS-2 are pro-apoptotic proteins [115]. Proteolytic processing of PS-2 by caspase-3 generates a short 22 amino-acid peptide from the C-terminal fragment that has been shown to induce apoptosis when introduced into a prostate adenocarcinoma cell line [116]. This peptide depleted intracellular Ca^{2+} stores, co-immunoprecipitated with the type-I IP_3R, and increased IP_3R channel activity. The site of interaction with the IP_3R has not been identified. The corresponding peptide from PS-1 was inactive. Thus PS-1 and PS-2 appear to possess both similar and different modalities for augmenting IP_3-mediated Ca^{2+} release.

Huntington's disease is caused by a polyglutamine expansion in the N-terminal region of huntingtin (Htt^{exp}), a 350 kDa cytosolic protein. Both wild type Htt and the mutant Htt^{exp} can associate with the type-I IP_3R by direct interactions, as well as the formation of a ternary complex involving the Huntington associated protein HAP1A [117]. A high-affinity binding site was localized to the extreme C-terminal region of the IP_3R [117] (Fig. 2(B)). The addition of Htt^{exp}, but not Htt, caused an activation of IP_3R channel activity measured with suboptimal IP_3 concentrations in planar lipid bilayers [117, 118]. Repetitive glutamate stimulation of medium spiny neurons derived from a mouse model of Huntington's disease caused a persistent elevation of cytosolic Ca^{2+} that was associated with release of cytochrome *c* and apoptosis [119]. These changes were absent in the neurons derived from control mice. These studies have led to a model in which the activation of IP_3R by Htt^{exp}, or its proteolytically derived fragments, contributes to enhanced apoptosis and neuronal cell loss [119].

NF-κB is a transcription factor whose function has been implicated in a wide range of diseases including immune disorders, cancer and inflammatory conditions [120, 121]. NF-κB signaling functions to favor survival by regulating the expression of key proteins such as Bcl-2, inhibitor of apoptosis proteins and manganese superoxide dismutase [122]. Mobilization of intracellular Ca^{2+} stores can activate NF-κB by indirect mechanisms that are not fully understood. For example Glazner et al. [123] reported that depletion of ER stores with IP_3 or thapsigargin caused the release of a protein factor that could activate NF-κB when added to cytosol extracts. More recently, it has been shown that NF-κB activation can decrease type-I IP_3R expression probably through NF-κB-sensitive promoter elements present in the type-I IP_3R gene [123]. Thus a feed-back loop exists in which Ca^{2+} can activate NF-κB which in turn can suppress the Ca^{2+} signal by reducing IP_3R expression. The reduction of IP_3R expression

is believed to be one mechanism that would contribute to the pro-survival function of NF-κB [123].

6 Mechanisms underlying a pro-apoptotic shift in the IP_3R-mitochondrial Ca^{2+} transfer

In the 1990s, a breakthrough discovery revealed that permeabilization of the outer mitochondrial membrane (OMM) and the ensuing release of cytochrome *c* and other intermembrane space factors to the cytosol represents the commitment steps in a variety of apoptotic paradigms [124, 125]. The possible role of the IP_3-induced mitochondrial matrix $[Ca^{2+}]$ ($[Ca^{2+}]_m$) signal in the control of apoptosis has become a subject of immense interest since uptake of Ca^{2+} by mitochondria is one of the most effective triggers of the mitochondrial permeability transition [126]. First, IP_3R-mediated $[Ca^{2+}]_m$ spikes were shown to cooperate with some apoptotic stimuli to induce mitochondrial membrane permeabilization and apoptosis [127]. Subsequently, apoptotic stimuli were shown to target IP_3R-mediated Ca^{2+} mobilization, evoking a $[Ca^{2+}]_m$ signal that leads to mitochondrial membrane permeabilization [128]. These two mechanisms i.e. the coincidence of IP_3R-mediated $[Ca^{2+}]_m$ signal with some forms of mitochondrial stress and the augmentation and prolongation of IP_3R-mediated Ca^{2+} transfer to the mitochondria seem to represent the main pathways for inducing cell death by the IP_3R-linked $[Ca^{2+}]_m$ signal.

Upon exposure of mitochondria to trains of Ca^{2+} pulses (micromolar or larger), Ca^{2+} uptake triggers opening of the permeability transition pore (PTP) and in turn, permeabilization of both the outer and inner mitochondrial membranes [126]. Permeation of solutes (with molecular masses below approximately 1500 Da) across the PTP may cause mitochondrial swelling and rupture of the OMM by itself. However, in several Ca^{2+}-induced apoptosis paradigms the release of intermembrane space proteins to the cytosol occurs in the absence of large scale swelling, indicating that the PTP also recruits alternative mechanisms of OMM permeabilization. The Ca^{2+}-induced PTP opening can be promoted by increasing either the size of the individual pulses or the frequency of the pulsing, illustrating possible mechanisms for a pro-death shift during $[Ca^{2+}]_c$ oscillations [129]. Furthermore, a prolonged, submicromolar Ca^{2+} increase may evoke gradual sensitization of the mitochondrial Ca^{2+} uptake sites resulting in a slower and more sustained stimulation of mitochondrial Ca^{2+} accumulation [130, 131]. Over a long time, this may lead to mitochondrial Ca^{2+} overloading and PTP-dependent permeabilization of mitochondria. Therefore, an enhancement of the ER-mitochondrial transfer may cause a pro-death shift in calcium signaling both during IP_3-induced $[Ca^{2+}]_c$ spikes/oscillations or as a result of a slow and persistent leak from the ER. Although the PTP opening has been considered as the central mechanism for the $[Ca^{2+}]_m$-dependent mitochondrial membrane permeabilization and cell death, the diversity of mitochondrial Ca^{2+} effectors (reviewed in Refs. [9, 132]) suggests the possible involvement of additional mechanisms. The factors determining the extent of the Ca^{2+} transfer from IP_3Rs to the mitochondria are discussed below.

6.1 Ca^{2+} storage in the ER

During $[Ca^{2+}]_c$ oscillations, mitochondrial Ca^{2+} uptake is closely coupled to the IP_3R-dependent Ca^{2+} release that is driven by the $[Ca^{2+}]$ gradient between the ER lumen and the cytosol. Studies evaluating the connection between the ER Ca^{2+} storage and cell survival have reported that fluctuations of ER luminal $[Ca^{2+}]$ ($[Ca^{2+}]_{ER}$) in a narrow range (approx. 30%) cause massive changes in apoptosis. A clue to the mechanism of this effect is that in cells where the apoptotic agents or Bcl-2 family proteins induced moderate differences in the $[Ca^{2+}]_{ER}$ and in the IP_3-linked $[Ca^{2+}]_c$ rise, relatively large changes occurred in the $[Ca^{2+}]_m$ signal. For example, Scorrano et al. reported that in the Bax/Bak knockout cells the IP_3-linked $[Ca^{2+}]_c$ signal was partially inhibited but the $[Ca^{2+}]_m$ signal was essentially abolished [84]. Quantitative analysis of ER loading and IP_3-induced mitochondrial Ca^{2+} transport indicates a supralinear relationship between these parameters (Csordás and Hajnóczky, unpublished). This complex relationship seems to reflect the sensitization of the mitochondrial Ca^{2+} uptake sites exposed to the high $[Ca^{2+}]_c$ microdomains close to the IP_3Rs. Patches of calreticulin, a high capacity Ca^{2+} binding protein in the ER lumen and IP_3Rs were shown in close association with mitochondria along cell processes in cultured rat oligodendrocytes, illustrating the morphological basis for a local interaction between ER Ca^{2+} storage, IP_3R-mediated Ca^{2+} release and mitochondrial Ca^{2+} uptake [133]. Local ER Ca^{2+} filling also seems to be supported by concentration of SERCA pumps in proximity to these sites [134]. Interestingly, overexpression of calreticulin in HEK-293 cells led to an increase in ER Ca^{2+} storage and in the IP_3-linked $[Ca^{2+}]_c$ signal but caused a decrease in the concomitant $[Ca^{2+}]_m$ signal [135]. However, the calreticulin-overexpressing cells also showed low $\Delta\Psi_m$, indicating that a decrease in the driving force was responsible for the attenuated mitochondrial Ca^{2+} uptake. Overexpression of the Ca^{2+} binding C domain of calreticulin was sufficient to reproduce the effect of the full length calreticulin. It was suggested that a prolonged increase in Ca^{2+} turnover between ER and mitochondria evoked mitochondrial injury and in turn, promoted apoptosis in the calreticulin-overexpressing cells [135]. Thus, an increase in the ER Ca^{2+} storage may support mitochondrial membrane permeabilization by both rapidly and slowly acting mechanisms.

6.2 IP_3R-dependent Ca^{2+} permeation across the ER membrane

During stimulation with varying concentrations of IP_3-linked agonists or IP_3, the $[Ca^{2+}]_c$ transients are usually associated with a $[Ca^{2+}]_m$ rise that is in proportion to the magnitude of the $[Ca^{2+}]_c$ signal. However, quantitation of the $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ responses has revealed a rightward shifted IP_3 dose-response for the $[Ca^{2+}]_m$ signal [136]. Furthermore, the $[Ca^{2+}]_m$ signal was disproportionately suppressed by overexpression of IP_3-binding proteins that serve as IP_3 buffers [137] and was also small when complete mobilization of the ER Ca^{2+} store was achieved slowly by a gradual increase in $[IP_3]$ [136]. These data indicated that optimal activation of the rapid Ca^{2+} transfer to the mitochondria is dependent on concurrent activation of the IP_3Rs during Ca^{2+} mobilization. Therefore, any factors that alter the IP_3/Ca^{2+} sensitivity, Ca^{2+} permeability, or the number of available IP_3Rs are expected to affect the efficacy of the $[Ca^{2+}]_c$ signal propagation to the mitochondria. However, among the numerous factors that have been described to interact with the IP_3Rs (see Section 4), only Bcl-2/Bcl-xL has been evaluated for modulation of the IP_3-linked $[Ca^{2+}]_m$ signal or the mitochondrial calcium content. Both overexpression of Bcl-2 [69] and knockout of Bax and Bak, which results in a relative abundance of Bcl-2/Bcl-xL [84] have been reported to cause suppression of both the $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ signal evoked by IP_3-linked agonists. In these cases, attenuation of Ca^{2+} release through the IP_3R accounts for the suboptimal Ca^{2+} delivery to the mitochondria. The decrease in the Ca^{2+} release may reflect either a Bcl-2-mediated inhibition of the IP_3R Ca^{2+} channel [68] or reduction in the ER Ca^{2+} load [69]. Notably, in the study that described Bcl-xL-dependent sensitization of the IP_3R to IP_3 [35], the IP_3R-mediated Ca^{2+} leakage from the ER was proposed to support a permanent Ca^{2+} transfer to the mitochondria, stimulating oxidative metabolism. Based on the amount of the FCCP-releasable Ca^{2+}, an increase in mitochondrial Ca^{2+} storage has also been reported in unstimulated Bcl-2-overexpressing cells [138]. Generally, prolonged mitochondrial Ca^{2+} storage would enhance the vulnerability of the mitochondria but the mitochondria localized fraction of Bcl-2/Bcl-xL in the overexpression model exerts protection against membrane permeabilization.

As described earlier (Section 2), the siRNA-mediated depletion of the type-III IP_3R isoform had a more marked effect on the $[Ca^{2+}]_m$ signal and bile-acid or STS-induced apoptosis in CHO cells than depletion of the other isoforms [28]. These results suggested that type-III IP_3Rs preferentially transmit Ca^{2+} to the mitochondria at least in some cell types and that the maximal strength of the IP_3R-dependent $[Ca^{2+}]_m$ signaling is needed for the apoptotic cascade to prevail. Localization studies indicated that the type-III isoform may be preferentially localized adjacent to mitochondria [28]. Hence, another potentially important factor dictating the magnitude of the $[Ca^{2+}]_m$ signal may be the amount and spatial localization of individual IP_3R isoforms.

6.3 Strategic positioning of mitochondria close to the sites of IP_3R-mediated Ca^{2+} release

Mitochondria are distributed at various subcellular locations and show functional heterogeneity but in most paradigms, at least a fraction of mitochondria rapidly respond to IP_3-mediated Ca^{2+} mobilization [139–141]. The bulk $[Ca^{2+}]_c$ rise seems to be too low to activate the uniporter [142, 143], although a “rapid mode” of mitochondrial Ca^{2+} uptake has been claimed to respond to submicromolar $[Ca^{2+}]_c$ increases [144]. A compelling piece of evidence for the role of a local IP_3R-mitochondrial Ca^{2+} transfer in the rapid mitochondrial Ca^{2+} uptake is the observation that buffering of the global $[Ca^{2+}]_c$ signal by Ca^{2+} chelators (EGTA or BAPTA 200 μM) fails to abolish the IP_3-induced $[Ca^{2+}]_m$ signal [136, 145]. While the bulk $[Ca^{2+}]_c$ rises to approx. 1 μM, the $[Ca^{2+}]_c$ at a 100 nm distance from an open IP_3R channel is estimated to reach ≥ 10 μM and in the immediate vicinity of the IP_3R (10 nm distance) would attain ≥ 100 μM [146]. Since the mitochondrial Ca^{2+} uptake is greatly facilitated in the 10 μM $[Ca^{2+}]$ range [142] the uniporter-mediated Ca^{2+} current would be expected to steeply increase in the micromolar to millimolar $[Ca^{2+}]$ range [147]. In addition, the distance between the IP_3R and the uniporter is expected to affect the relay of the IP_3-dependent $[Ca^{2+}]_c$ signal to the mitochondria. When bifunctional linkers (5 nm in length) that anchor ER to the OMM were transiently overexpressed in RBL-2H3 cells in order to narrow the ER-mitochondrial gap, an enhancement of the IP_3R-mitochondrial Ca^{2+} transfer and an increased sensitivity to ER stress-induced apoptosis was observed [148].

In light of the above data, a change in mitochondrial distribution might be expected to cause a change in the IP_3R-mediated Ca^{2+} transfer to the mitochondria. However, the movement of mitochondria to the perinuclear region, induced by overexpression of proteins that control mitochondrial morphology and anchorage did not seem to affect the IP_3-linked $[Ca^{2+}]_m$ signal [149, 150]. In this case, mitochondria were clearly separated from the peripherial domains of the ER. However, mitochondria could form interactions with the copious perinuclear ER to sustain the IP_3-dependent $[Ca^{2+}]_m$ signal. Also, formation of a physical linkage between ER and mitochondria (see below) could still hold some ER domains close even during repositioning of the mitochondria.

6.4 Physical linkage between ER and mitochondria

The existence of anchorage sites between ER and mitochondria have been suggested based on co-sedimentation of ER

particles with mitochondria and electron microscopic observations of close associations between mitochondria and ER vesicles [151, 152]. Recent studies of the ER-mitochondrial interface by electron tomography and by limited proteolysis provided evidence for direct protein linkage (tethers) between both rough and smooth ER and mitochondria. The tethers range in size from 6 to 15 nm at the smooth ER, and 19 to 30 nm at the rough ER [148]. The variations in the tether length indicate diversity of the protein components that may be involved in the linkage. The specific tether-forming proteins are unknown. Tethers originated at the rough ER appear close to the ribosomes, raising the possible role of proteins that leave the ER to enter the mitochondria. In addition, several ER or mitochondrial membrane or membrane-associated proteins have been considered. Participation of the IP_3Rs in the formation of the links would localize the Ca^{2+} release site to the anchorage points of the ER-mitochondrial interface. Indeed, the IP_3Rs seem to act as an anchor for >50 proteins (see Section 4) and show high density at the ER-mitochondrial associations [133, 153]. Formation of a possible ER-mitochondrial "Ca^{2+} tunnel" has been supported by data on a mitochondria-associated macromolecular complex that would include IP_3R and VDAC [154]. Furthermore, overexpression of VDAC has been shown to shorten the coupling time between IP_3R-mediated $[Ca^{2+}]_c$ rise and $[Ca^{2+}]_m$ elevation and led to an increased susceptibility to a Ca^{2+}-dependent form of apoptosis [155]. The IP_3R domains that may participate in ER-mitochondrial linkages are unknown. Szabadkai et al. [154] have described an unexpected stimulatory effect of the ligand binding domain of the IP_3R (LBD_{IP3R}) on the IP_3-linked $[Ca^{2+}]_m$ rise in HeLa cells when overexpressed in the cytoplasm or membrane-anchored either to the ER or OMM. It remains to be seen whether these constructs enhance the physical coupling between ER and mitochondria. Also, the cytosolic LBD_{IP3R} caused suppression of the IP_3-dependent $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ signals in COS-7 cells, which seems to reflect the IP_3-buffering effect of the construct [137]. If the LBD_{IP3R} increases both IP_3 buffering and the ER-mitochondrial coupling, depending on the strength of each effect, the $[Ca^{2+}]_m$ signal may be either increased or suppressed. Ryanodine receptors (RyRs) also provide for rapid Ca^{2+} transfer to the mitochondria, supporting the possibility that an IP_3R-like domain for interaction with the mitochondria may also be present in the RyR. The IP_3Rs and RyRs share the highest sequence homology within the N-terminal and transmembrane regions [156]. Conservation in the transmembrane region may be due to their common conductance of Ca^{2+} ions, whereas the homology within the N terminus is unclear since RyR lacks the ability to bind IP_3. Structural analysis and structure-based sequence analysis of IP_3Rs and RyRs suggest that the N-terminal portion of RyRs is comprised of two β-trefoil domains in a similar manner as IP_3Rs [157]. IP_3R knockouts have also been used to determine the role of IP_3R in the coupling of ER to the mitochondria. Electron microscopy and tomography studies have shown the presence of tight ER-mitochondrial associations and tethers in both IP_3R-expressing and IP_3R-deficient TKO DT40 cells [148]. Thus, an IP_3R-and VDAC-containing complex may support both the functional and structural aspects of the ER-mitochondrial interface but it has no exclusive role in formation of the linker.

In the past years, several ER-or mitochondria-resident proteins have been shown to be important for maintaining the spatial relationship between ER and mitochondria and hence, have also been implicated as possible ER-mitochondrial linking elements. These proteins include dynamin-related protein (DLP-1/DRP1-1; [158, 159]), tumor autocrine motility factor receptor (AMF-R; [160]) and PACS-2 (phosphofurin acidic cluster sorting protein-2) and BAP31 (B cell antigen receptor-associated protein 31) [161]. Overexpression of DLP-1/DRP-1 that mediates OMM fission has been reported to cause attenuation of the IP_3R-mediated $[Ca^{2+}]_m$ signal evoked by suboptimal stimulation. Attenuation of the $[Ca^{2+}]_m$ signal was also associated with suppression of ceramide-induced apoptosis, whereas other apoptotic mechanisms were augmented [162]. However, in this case mitochondrial fragmentation seems to hinder the spreading of the $[Ca^{2+}]_m$ rise among the mitochondria rather than the Ca^{2+} transfer between ER and mitochondria [162]. Immunofluorescence studies of the smooth ER resident AMF-R revealed a domain of the ER that shows Ca^{2+} dependent associations with the mitochondria, illuminating a possible mechanism for the recruitment of mitochondrial Ca^{2+} uptake to the sites of Ca^{2+} release [160]. Further investigation is needed however, to determine the significance of this mechanism for the Ca^{2+} transfer to the mitochondria and for the control of apoptosis. Similar to the DLP-1/DRP-1 overexpression, depletion of the ER/cytoplasmic sorting protein PACS-2 has been reported to cause mitochondrial fragmentation and localization in the perinuclear area and suppression of apoptosis [161]. The PACS-2-depletion induced mitochondrial fragmentation required the cleavage of BAP31 by caspase-8 in the ER to yield p20. In turn, p20 would cause ER Ca^{2+} release and ER-mitochondrial Ca^{2+} transfer to facilitate DLP1/DRP1-mediated mitochondrial fission [23]. A similar mechanism has also been reported for another ER-linked protein, the BH3-only Bik [163]. Thus several ER factors converge on the DLP-1/DRP-1-mediated mitochondrial fission to evoke a change in the relative distribution of mitochondria and ER and to impact on cell survival. The change in mitochondrial distribution is expected to yield some rearrangements of the ER-mitochondrial interface, but the area of the ER-mitochondrial interface does not necessarily decrease and its organization does not necessarily change. At least, Ca^{2+} transfer at the ER-mitochondrial interface is

needed to activate DLP-1/DRP-1 and seems to be sustained in some situations where mitochondria have changed their distribution. Most studies of ER-mitochondrial coupling described conditions that caused increased separation of ER from mitochondria. In a recent study, a narrowing of the ER-mitochondrial gap and an increase in the area of the interface was observed in cells exposed to ER stress conditions (tunicamycin treatment and serum starvation) [148]. It has been speculated that these changes support mitochondrial Ca^{2+} uptake during slow ER Ca^{2+} release that normally would be excluded from the mitochondria, making mitochondria more prone to Ca^{2+} overloading and PTP opening. Although a change in the gross ER and mitochondrial morphology did not seem occur, the role of tethers in shortening the ER-mitochondrial gap needs further investigation. Identifying the tether forming proteins and imaging the tethers in live cells, where the ER and mitochondria also move and undergo fusion-fission, will offer clues about how the ER-mitochondrial interface is controlled.

6.5 Dynamic aspects of the ER-mitochondrial distribution

In many cell types, mitochondria are highly dynamic organelles that undergo fusion and fission and display both short and long range movements. Mitochondrial fragmentation has been observed as an obligatory early event in several apoptotic paradigms [164, 165]. Mitochondrial fragmentation may be both a cause and a consequence of a change in the IP_3R-mitochondrial calcium transfer (reviewed in Ref. [166]). Recently, mitochondrial and ER motility has also been implicated in the control of ER-mitochondrial calcium signaling. Mitochondria are commonly bound and move along microtubules utilizing kinesin and dynein motor proteins but in other instances mitochondrial association with microfilament and intermediate filament tracks have also been documented [167]. ER morphology is less dependent on the cytoskeletal structures [168] but domains of the ER are also linked to and may move along the microtubules or microfilaments. However, it has not been determined whether the mitochondrial and ER movements are coordinated with each other. The ER-mitochondrial tethers may allow ER stacks to piggy-back on moving mitochondria and vice versa. Alternatively, rapid turnover of the tether-forming structures may ensure movement of mitochondria along the ER while a permanent linkage is maintained. Pretreatment of H9c2 cells with a microtubule-disrupting agent, to inhibit the mitochondrial movements, resulted in an improvement in IP_3R induced $[Ca^{2+}]_m$ rise although the $[Ca^{2+}]_c$ increase was not enhanced [169]. Thus organellar movements may interfere with some aspects of the local ER-mitochondrial Ca^{2+} transfer. Recently, $[Ca^{2+}]_c$ elevations have been shown to cause mitochondrial motility inhibition in several cell types [169–171] and a homeostatic circuit has been proposed whereby mitochondria are retained at the sites of the IP_3-induced Ca^{2+} release to enhance the local ATP supply and Ca^{2+} buffering and become free to move after the decay of the $[Ca^{2+}]_c$ rise [169]. The relevance of mitochondrial motility in the context of apoptosis has not yet been explored. Nevertheless, when cells were treated with NO or H_2O_2 to induce cell death, suppression of the mitochondrial motility was observed as an early event ([172]; Saotome and Hajnóczky, unpublished). Furthermore, several studies support a link between impairment of the mitochondrial-cytoskeletal interactions and cell death [173].

7 Conclusions and future perpectives

A considerable redundancy is apparent in apoptotic pathways and this has been a complicating feature of attempts to therapeutically modify this process in the treatment of diseases such as cancer or neurodegenerative disorders. An important goal has been the identification of critical control points in apoptotic pathways that could potentially be used as drug targets. The present review has summarized the data indicating that IP_3Rs may be critical regulators of apoptosis triggered by stimuli that engage either ER and/or mitochondrial mechanisms. IP_3Rs may serve not only to funnel Ca^{2+} into the mitochondria but may have other effects on apoptosis that are independent of their ion-channel function, such as their role as adaptor proteins. Since IP_3Rs also have critical house-keeping roles in cell survival an indiscriminate targeting of these proteins would seem undesirable. The selective targeting of spatially and functionally distinct pools of IP_3Rs would be more profitable, but this would require much more knowledge of the full range of IP_3R interactions with other proteins. Another critical control factor is the spatial relationship and dynamics of ER and mitochondrial membranes. The possibility of manipulating the apoptotic process at this level awaits the challenging task of identifying the molecular machinery involved in inter-organellar dynamics.

Acknowledgments This work was supported by NIH grants DK34804 & NS51822 (SKJ) and DK51526 & GM59419 (GH). We would like to thank Georgia Anyatonwu, Tariq Khan, John Pastorino and Darren Boehning for reading the manuscript. We apologize to those of our colleagues whose work could not be cited because of space restrictions.

References

1. Mikoshiba K (2006) Inositol 1,4,5-trisphosphate IP(3) receptors and their role in neuronal cell function. J Neurochem 97:1627–1633
2. Patel S, Joseph SK, Thomas AP (1999) Molecular properties of inositol 1,4,5-trisphosphate receptors. Cell Calcium 25:247–264

3. Patterson RL, Boehning D, Snyder SH (2004) Inositol 1,4,5-trisphosphate receptors as signal integrators. Annual reviews in Biochemistry 73:437–465
4. McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70:391–425
5. Robb-Gaspers LD, Rutter GA, Burnett P, Hajnoczky G, Denton RM, Thomas AP (1998) Coupling between cytosolic and mitochondrial calcium oscillations: role in the regulation of hepatic metabolism. Biochim Biophys Acta 1366:17–32
6. Walter L, Hajnoczky G (2005) Mitochondria and endoplasmic reticulum: the lethal interorganelle cross-talk. J Bioenerg Biomembr 37:191–206
7. Rizzuto R, Pozzan T (2006) Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. Physiol Rev 86:369–408
8. Hanson CJ, Bootman MD, Roderick HL (2004) Cell signalling: IP3 receptors channel calcium into cell death. Curr Biol 14:R933–R935
9. Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. Nature review Molecular Cell Biology 4:552–565
10. Demaurex N, Distelhorst C (2003) Cell biology. Apoptosis—the calcium connection. Science 300:65–67
11. Randriamampita C, Trautmann A (2004) Ca^{2+} signals and T lymphocytes; "New mechanisms and functions in Ca^{2+} signalling". Biol Cell 96:69–78
12. Bhakta NR, Lewis RS (2005) Real-time measurement of signaling and motility during T cell development in the thymus. Semin Immunol 17:411–420
13. Kurosaki T (2002) Regulation of B-cell signal transduction by adaptor proteins. Nat Rev Immunol 2:354–363
14. Zhong F, Davis MC, McColl KS, Distelhorst CW (2006) Bcl-2 differentially regulates Ca^{2+} signals according to the strength of T cell receptor activation. J Cell Biol 172:127–137
15. McFarlane SM, Anderson HM, Tucker SJ, Jupp OJ, MacEwan DJ (2000) Unmodified calcium concentrations in tumour necrosis factor receptor subtype-mediated apoptotic cell death. Mol Cell Biochem 211:19–26
16. Binah O, Shilkrut M, Yaniv G, Larisch S (2004) The Fas receptor-1,4,5-IP3 cascade: a potential target for treating heart failure and arrhythmias. Ann NY Acad Sci 1015:338–350
17. Wozniak AL, Wang X, Stieren ES, Scarbrough SG, Elferink CJ, Boehning D (2006) Fas apoptosis is mediated by phospholipase C-gamma1 activation and cytosolic Ca^{2+} elevation. J Cell Biol
18. Pinton P, Ferrari D, Rapizzi E, Di Virgilio F, Pozzan T, Rizzuto R (2001) The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. EMBO J 20:2690–2701
19. Nutt LK, Chandra J, Pataer A et al (2002) Bax-mediated Ca^{2+} mobilization promotes cytochrome *c* release during apoptosis. J Biol Chem 277:20301–20308
20. Assefa Z, Bultynck G, Szlufcik K et al (2004) Caspase-3-induced Truncation of Type 1 Inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis. J Biol Chem 279:43227–43236
21. Nawrocki ST, Carew JS, Dunner K Jr et al (2005) Bortezomib inhibits PKR-like endoplasmic reticulum (ER) kinase and induces apoptosis via ER stress in human pancreatic cancer cells. Cancer Res 65:11510–11519
22. Baffy G, Miyashita T, Williamson JR, Reed JC (1993) Apoptosis induced by withdrawal of Interleukin-3 from an IL-3 dependent hematopoetic cell line is associated with repartitioning of intracellular calcium and is blocked by enforced bcl-2 oncoprotein production. J Biol Chem 268:6511–6519
23. Breckenridge DG, Germain M, Mathai JP, Nguyen M, Shore GC (2003) Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene 22:8608–8618
24. Boyce M, Yuan J (2006) Cellular response to endoplasmic reticulum stress: a matter of life or death. Cell Death Differ 13:363–373
25. Camello C, Lomax R, Petersen OH, Tepikin AV (2002) Calcium leak from intracellular stores—the enigma of calcium signalling. Cell Calcium 32:355–361
26. Khan AA, Soloski MJ, Sharp AH et al (1996) Lymphocyte apoptosis: Mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. Science 273:503–506
27. Blackshaw S, Sawa A, Sharp AH, Ross CA, Snyder SH, Khan AA (2000) Type 3 inositol 1,4,5-trisphosphate receptor modulates cell death. FASEB J 14:1375–1379
28. Mendes CC, Gomes DA, Thompson M et al (2005) The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca^{2+} signals into mitochondria. J Biol Chem 280:40892–40900
29. Jayaraman T, Marks AR (1997) T cells deficient in Inositol 1,4,5-trisphosphate receptor are resistant to apoptosis. Mol Cell Biol 17:3005–3012
30. Jayaraman T, Marks AR (2000) Calcineurin is downstream of the inositol 1,4,5-trisphosphate receptor in the apoptotic and cell growth pathways. J Biol Chem 275:6417–6420
31. Hirota J, Baba M, Matsumoto M, Furuichi T, Takatsu K, Mikoshiba K (1998) T-cell-receptor signalling in inositol 1,4,5-trisphosphate receptor (IP3R) type-1-deficient mice: is IP3R type 1 essential for T-cell-receptor signalling? Biochem J 333:615–619
32. Futatsugi A, Nakamura T, Yamada MK et al (2005) IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism. Science 309:2232–2234
33. Miyakawa T, Maeda A, Yamazawa T, Hirose K, Kurasaki T, Iino M (1999) Encoding of calcium signals by differential expression of IP3 receptor subtypes. EMBO J 18:1303–1308
34. Sugawara H, Kurosaki M, Takata M, Kurosaki T (1997) Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. EMBO J 16:3078–3088
35. White C, Li C, Yang J et al (2005) The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. Nat Cell Biol 7:1021–1028
36. Hirota J, Furuichi T, Mikoshiba K (1999) Inositol 1,4,5-trisphosphate receptor type-I is a substrate for caspase-3 and is cleaved in apoptosis in a caspase-3 dependent manner. J Biol Chem 274:34433–34437
37. Diaz F, Bourguignon LY (2000) Selective down-regulation of IP3 receptor subtypes by caspases and calpains during TNFalpha apoptosis of human T-lymphoma cells. Cell Calcium 27:315–328
38. Haug LS, Walaas I, Ostvold AC (2000) Degradation of the type-I Inositol 1,4,5-trsiphosphate receptor by caspase-3 in SH-SY5Y Neuroblastoma cells undergoing apoptosis. J Neurochem 75:1852–1861
39. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH (2003) Cytochrome *c* binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat Cell Biol 5:1051–1061
40. Bhanumathy CD, Nakao SK, Joseph SK (2006) Mechanism of proteasomal degradation of inositol trisphosphate receptors in CHO-K1 cells. J Biol Chem 281:3722–3730
41. Sergeev IN (2004) Genistein induces Ca^{2+} -mediated, calpain/caspase-12-dependent apoptosis in breast cancer cells. Biochem Biophys Res Commun 321:462–467

42. Palmer AE, Jin C, Reed JC, Tsien RY (2004) Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor. Proc Natl Acad Sci USA 101:17404–17409
43. Xu L, Kong D, Zhu L, Zhu W, Andrews DW, Kuo TH (2006) Suppression of IP3-mediated calcium release and apoptosis by Bcl-2 involves the participation of protein phosphatase 1. Mol Cell Biochem
44. Nakayama T, Hattori M, Uchida K et al (2004) The regulatory domain of the inositol 1,4,5-trisphosphate receptor is necessary to keep the channel domain closed: possible physiological significance of specific cleavage by caspase 3. Biochem J 377:299–307
45. Szlufcik K, Missiaen L, Parys JB, Callewaert G, De Smedt H (2006) Uncoupled IP3 receptor can function as a Ca^{2+}-leak channel: cell biological and pathological consequences. Biol Cell 98:1–14
46. Harwood SM, Yaqoob MM, Allen DA (2005) Caspase and calpain function in cell death: bridging the gap between apoptosis and necrosis. Ann Clin Biochem 42:415–431
47. Magnusson A, Haug L, Walaas S, Ostvold A (1993) Calcium-induced degradation of the inositol (1,4,5)-trisphosphate receptor/$Ca^{(2+)}$-channel. FEBS Lett 323:229–232
48. Wojcikiewicz RJH, Oberdorf JA (1996) Degradation of inositol 1,4,5-trisphosphate receptors during cell stimulation is a specific process mediated by cysteine protease. J Biol Chem 271:16652–16655
49. Chen M, He H, Zhan S, Krajewski S, Reed JC, Gottlieb RA (2001) Bid is cleaved by calpain to an active fragment in vitro and during myocardial ischemia/reperfusion. J Biol Chem 276:30724–30728
50. Shulga N, Pastorino JG (2006) Acyl coenzyme a binding protein augments bid induced mitochondrial damage and cell death by activating mu-calpain. J Biol Chem
51. Tan Y, Dourdin N, Wu C, De Veyra T, Elce JS, Greer PA (2006) Ubiquitous calpains promote caspase-12 and JNK activation during endoplasmic reticulum stress-induced apoptosis. J Biol Chem 281:16016–16024
52. Shioda N, Moriguchi S, Shirasaki Y, Fukunaga K (2006) Generation of constitutively active calcineurin by calpain contributes to delayed neuronal death following mouse brain ischemia. J Neurochem 98:310–320
53. Bano D, Young KW, Guerin CJ et al (2005) Cleavage of the plasma membrane Na^+/Ca^{2+}exchanger in excitotoxicity. Cell 120:275–285
54. Bezprozvanny I, Watras J, Ehrlich BE (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature 351:751–754
55. Hajnoczky G, Hager R, Thomas AP (1999) Mitochondria suppress local feedback activation of inositol 1,4, 5-trisphosphate receptors by Ca^{2+}. J Biol Chem 274:14157–14162
56. Jouaville LS, Ichas F, Holmuhamedov EL, Camacho P, Lechleiter JD (1995) Synchronization of calcium waves by mitochondrial substrates in Xenopus laevis oocytes. Nature 377:438–441
57. Boehning D, van Rossum DB, Patterson RL, Snyder SH (2005) A peptide inhibitor of cytochrome *c*/inositol 1,4,5-trisphosphate receptor binding blocks intrinsic and extrinsic cell death pathways. Proc Natl Acad Sci USA
58. Taylor CW, da Fonseca PC, Morris EP (2004) IP(3) receptors: the search for structure. Trends Biochem Sci 29:210–219
59. Hamada K, Terauchi A, Mikoshiba K (2003) Three-dimensional rearrangements within inositol 1,4,5-trisphosphate receptor by calcium. J Biol Chem 278:52881–52889
60. Beresewicz M, Kowalczyk JE, Zablocka B (2006) Cytochrome *c* binds to inositol (1,4,5) trisphosphate and ryanodine receptors *in vivo* after transient brain ischemia in gerbils. Neurochem Int 48:568–571
61. Csordas G, Madesh M, Antonsson B, Hajnoczky G (2002) tcBid promotes $Ca^{(2+)}$ signal propagation to the mitochondria: control of $Ca^{(2+)}$ permeation through the outer mitochondrial membrane. EMBO J 21:2198–2206
62. Munoz-Pinedo C, Guio-Carrion A, Goldstein JC, Fitzgerald P, Newmeyer DD, Green DR (2006) Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. Proc Natl Acad Sci USA 103:11573–11578
63. Sedlak TW, Snyder SH (2006) Messenger molecules and cell death: therapeutic implications. JAMA 295:81–89
64. Pinton P, Rizzuto R (2006) Bcl-2 and Ca^{2+} homeostasis in the endoplasmic reticulum. Cell Death Differ 13:1409–1418
65. Distelhorst CW, Shore GC (2004) Bcl-2 and calcium: controversy beneath the surface. Oncogene 23:2875–2880
66. Oakes SA, Opferman JT, Pozzan T, Korsmeyer SJ, Scorrano L (2003) Regulation of endoplasmic reticulum Ca^{2+} dynamics by proapoptotic BCL-2 family members. Biochem Pharmacol 66:1335–1340
67. Li C, Fox CJ, Master SR, Bindokas VP, Chodosh LA, Thompson CB (2002) Bcl-X(L) affects $Ca^{(2+)}$ homeostasis by altering expression of inositol 1,4,5-trisphosphate receptors. Proc Natl Acad Sci USA 99:9830–9835
68. Chen R, Valencia I, Zhong F et al (2004) Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. J Cell Biol 166:193–203
69. Pinton P, Ferrari D, Magalhaes P et al (2000) Reduced loading of intracellular $Ca^{(2+)}$ stores and downregulation of capacitative $Ca^{(2+)}$ influx in Bcl-2-overexpressing cells. J Cell Biol 148:857–862
70. Basset O, Boittin FX, Cognard C, Constantin B, Ruegg UT (2006) Bcl-2 overexpression prevents calcium overload and subsequent apoptosis in dystrophic myotubes. Biochem J 395:267–276
71. Oakes SA, Scorrano L, Opferman JT et al (2005) Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. Proc Natl Acad Sci USA 102:105–110
72. Schug ZT, Joseph SK (2006) The role of the S4–S5 linker and C-terminal tail in inositol 1,4,5-trisphosphate receptor function. J Biol Chem 281:24431–24440
73. Tang TS, Tu H, Wang Z, Bezprozvanny I (2003) Modulation of type 1 inositol (1,4,5)-trisphosphate receptor function by protein kinase a and protein phosphatase 1alpha. J Neurosci 23:403–415
74. Malissein E, Verdier M, Ratinaud MH, Troutaud D (2006) Activation of Bad trafficking is involved in the BCR-mediated apoptosis of immature B cells. Apoptosis 11:1003–1012
75. Erin N, Billingsley ML (2004) Domoic acid enhances Bcl-2-calcineurin-inositol-1,4,5-trisphosphate receptor interactions and delayed neuronal death in rat brain slices. Brain Res 1014:45–52
76. Fiebig AA, Zhu W, Hollerbach C, Leber B, Andrews DW (2006) Bcl-XL is qualitatively different from and ten times more effective than Bcl-2 when expressed in a breast cancer cell line. BMC Cancer 6:213
77. Yamamoto H, Maeda N, Niinobe M, Miyamoto E, Mikoshiba K (1989) Phosphorylation of P400 protein by cyclic AMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II. J Neurochem 53:917–923
78. Soulsby MD, Wojcikiewicz RJ (2005) The type III inositol 1,4,5-trisphosphate receptor is phosphorylated by cAMP-dependent protein kinase at three sites. Biochem J 392:493–497
79. Wagner LE, Li WH, Yule DI (2003) Phosphorylation of type-1 inositol 1,4,5-trisphosphate receptors by cyclic nucleotide-dependent protein kinases: a mutational analysis of the

functionally important sites in the S^{2+} and S^{2-} splice variants. J Biol Chem 278:45811–45817
80. Wagner LE, Li WH, Joseph SK, Yule DI (2004) Functional consequences of phosphomimetic mutations at key cAMP-dependent protein kinase phosphorylation sites in the type 1 inositol 1,4,5-trisphosphate receptor. J Biol Chem 279:46242–46252
81. Schlossmann J, Ammendola A, Ashman K et al (2000) Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta. Nature 404:197–201
82. DeSouza N, Reiken S, Ondrias K, Yang YM, Matkovich S, Marks AR (2002) Protein kinase A and two phosphatases are components of the inositol 1,4,5-trisphosphate receptor macromolecular signaling complex. J Biol Chem 277:39397–39400
83. Tu H, Tang TS, Wang Z, Bezprozvanny I (2004) Association of type 1 inositol 1,4,5-trisphosphate receptor with AKAP9 (Yotiao) and protein kinase A. J Biol Chem 279:19375–19382
84. Scorrano L, Oakes SA, Opferman JT et al (2003) BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. Science 300:135–139
85. Zhong F, Davis MC, McColl KS, Distelhorst CW (2006) Bcl-2 differentially regulates Ca^{2+} signals according to the strength of T cell receptor activation. J Cell Biol 172:127–137
86. Bassik MC, Scorrano L, Oakes SA, Pozzan T, Korsmeyer SJ (2004) Phosphorylation of BCL-2 regulates ER Ca^{2+} homeostasis and apoptosis. EMBO J 23:1207–1216
87. Mathai JP, Germain M, Shore GC (2005) BH3-only BIK regulates BAX,BAK-dependent release of Ca^{2+} from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death. J Biol Chem 280:23829–23836
88. Breckenridge DG, Stojanovic M, Marcellus RC, Shore GC (2003) Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome *c* release to the cytosol. J Cell Biol 160:1115–1127
89. Shi J, Parada LF, Kernie SG (2005) Bax limits adult neural stem cell persistence through caspase and IP3 receptor activation. Cell Death Differ
90. Lindsten T, Golden JA, Zong WX, Minarcik J, Harris MH, Thompson CB (2003) The proapoptotic activities of Bax and Bak limit the size of the neural stem cell pool. J Neurosci 23:11112–11119
91. Bare DJ, Kettlun CS, Liang M, Bers DM, Mignery GA (2005) Cardiac type 2 inositol 1,4,5-trisphosphate receptor: interaction and modulation by calcium/calmodulin-dependent protein kinase II. J Biol Chem 280:15912–15920
92. Vermassen E, Fissore RA, Nadif KN et al (2004) Regulation of the phosphorylation of the inositol 1,4,5-trisphosphate receptor by protein kinase C. Biochem Biophys Res Commun 319:888–893
93. Malathi K, Li X, Krizanova O et al (2005) Cdc2/cyclin B1 interacts with and modulates inositol 1,4,5-trisphosphate receptor (type 1) functions. J Immunol 175:6205–6210
94. Bai GR, Yang LH, Huang XY, Sun FZ (2006) Inositol 1,4,5-trisphosphate receptor type 1 phosphorylation and regulation by extracellular signal-regulated kinase. Biochem Biophys Res Commun 348:1319–1327
95. Khan MT, Wagner L, Yule DI, Bhanumathy C, Joseph SK (2006) Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors. J Biol Chem 281:3731–3737
96. Brazil DP, Yang ZZ, Hemmings BA (2004) Advances in protein kinase B signalling: AKTion on multiple fronts. Trends Biochem Sci 29:233–242
97. Kim D, Chung J (2002) Akt: versatile mediator of cell survival and beyond. J Biochem Mol Biol 35:106–115
98. Szlufcik K, Bultynck G, Callewaert G, Missiaen L, Parys JB, De Smedt H (2006) The suppressor domain of inositol 1,4,5-trisphosphate receptor plays an essential role in the protection against apoptosis. Cell Calcium 39:325–336
99. Tantral L, Malathi K, Kohyama S, Silane M, Berenstein A, Jayaraman T (2004) Intracellular calcium release is required for caspase-3 and -9 activation. Cell Biochem Funct 22:35–40
100. van Rossum DB, Patterson RL, Cheung KH et al (2006) DANGER: A novel regulatory protein of IP3-receptor activity. J Biol Chem
101. Mikoshiba K (2006) Inositol 1,4,5-trisphosphate IP(3) receptors and their role in neuronal cell function. J Neurochem 97:1627–1633
102. van Rossum DB, Patterson RL, Kiselyov K et al (2004) Agonist-induced Ca^{2+} entry determined by inositol 1,4,5-trisphosphate recognition. Proc Natl Acad Sci USA 101:2323–2327
103. Parekh AB, Putney JW, Jr (2005) Store-operated calcium channels. Physiol Rev 85:757–810
104. Dellis O, Dedos SG, Tovey SC, Taufiq UR, Dubel SJ, Taylor CW (2006) Ca^{2+} entry through plasma membrane IP3 receptors. Science 313:229–233
105. Greenway AL, Holloway G, McPhee DA, Ellis P, Cornall A, Lidman M (2003) HIV-1 Nef control of cell signalling molecules: multiple strategies to promote virus replication. J Biosci 28:323–335
106. Manninen A, Saksela K (2002) HIV-1 Nef interacts with inositol trisphosphate receptor to activate calcium signaling in T cells. J Exp Med 195:1023–1032
107. Mattson MP, Haughey NJ, Nath A (2005) Cell death in HIV dementia. Cell Death Differ 12(Suppl 1):893–904
108. Haughey NJ, Holden CP, Nath A, Geiger JD (1999) Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. J Neurochem 73:1363–1374
109. Stutzmann GE (2005) Calcium dysregulation, IP3 signaling, and Alzheimer's disease. Neuroscientist 11:110–115
110. Smith IF, Green KN, Laferla FM (2005) Calcium dysregulation in Alzheimer's disease: recent advances gained from genetically modified animals. Cell Calcium 38:427–437
111. Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, Parker I (2006) Enhanced ryanodine receptor recruitment contributes to Ca^{2+} disruptions in young, adult, and aged Alzheimer's disease mice. J Neurosci 26:5180–5189
112. Tu H, Nelson O, Bezprozvanny A et al (2006) Presenilins form ER $Ca^{(2+)}$ leak channels, a function disrupted by familial alzheimer's disease-linked mutations. Cell 126:981–993
113. Kasri NN, Kocks SL, Verbert L et al (2006) Up-regulation of inositol 1,4,5-trisphosphate receptor type 1 is responsible for a decreased endoplasmic-reticulum Ca^{2+} content in presenilin double knock-out cells. Cell Calcium 40:41–51
114. Leissring MA, Parker I, Laferla FM (1999) Presenilin-2 mutations modulate amplitude and kinetics of inositol 1,4,5-trisphosphate-mediated calcium signals. J Biol Chem 274:32535–32538
115. Wolozin B, Iwasaki K, Vito P et al (1996) Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. Science 274:1710–1713
116. Cai C, Lin P, Cheung KH et al (2006) The presenilin-2 loop peptide perturbs intracellular Ca^{2+} homeostasis and accelerates apoptosis. J Biol Chem 281:16649–16655
117. Tang TS, Tu H, Chan EY et al (2003) Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. Neuron 39:227–239
118. Tang TS, Tu H, Orban PC, Chan EY, Hayden MR, Bezprozvanny I (2004) HAP1 facilitates effects of mutant huntingtin on inositol 1,4,5-trisphosphate-induced Ca release in primary culture of striatal medium spiny neurons. Eur J Neurosci 20:1779–1787

119. Tang TS, Slow E, Lupu V et al (2005) Disturbed Ca^{2+} signaling and apoptosis of medium spiny neurons in Huntington's disease. Proc Natl Acad Sci USA 102:2602–2607
120. Li Q, Verma IM (2002) NF-kappaB regulation in the immune system. Nat Rev Immunol 2:725–734
121. Karin M (2006) Nuclear factor-kappaB in cancer development and progression. Nature 441:431–436
122. Burstein E, Duckett CS (2003) Dying for NF-kappaB? Control of cell death by transcriptional regulation of the apoptotic machinery. Curr Opin Cell Biol 15:732–737
123. Glazner GW, Camandola S, Geiger JD, Mattson MP (2001) Endoplasmic reticulum D-myo-inositol 1,4,5-trisphosphate-sensitive stores regulate nuclear factor-kappaB binding activity in a calcium-independent manner. J Biol Chem 276:22461–22467
124. 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
125. Green DR, Kroemer G (2004) The pathophysiology of mitochondrial cell death. Science 305:626–629
126. Haworth RA, Hunter DR (1979) The Ca^{2+}-induced membrane transition in mitochondria. II. Nature of the Ca^{2+} trigger site. Arch Biochem Biophys 195:460–467
127. Szalai G, Krishnamurthy R, Hajnoczky G (1999) Apoptosis driven by IP(3)-linked mitochondrial calcium signals. EMBO J 18:6349–6361
128. Pinton P, Ferrari D, Rapizzi E, Di Virgilio F, Pozzan T, Rizzuto R (2001) The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: Significance for the molecular mechanism of Bcl-2 action. EMBO J 20:2690–2701
129. Ichas F, Jouaville LS, Sidash SS, Mazat JP, Holmuhamedov EL (1994) Mitochondrial calcium spiking: A transduction mechanism based on calcium-induced permeability transition involved in cell calcium signalling. FEBS Lett 348:211–215
130. Kroner H (1986) Ca^{2+} ions, an allosteric activator of calcium uptake in rat liver mitochondria. Arch Biochem Biophys 251:525–535
131. Csordas G, Hajnoczky G (2003) Plasticity of mitochondrial calcium signaling. J Biol Chem 278:42273–42282
132. Hajnoczky G, Davies E, Madesh M (2003) Calcium signaling and apoptosis. Biochem Biophys Res Commun 304:445–454
133. Simpson PB, Mehotra S, Lange GD, Russell JT (1997) High density distribution of endoplasmic reticulum proteins and mitochondria at specialized Ca^{2+} release sites in oligodendrocyte processes. J Biol Chem 272:22654–22661
134. Csordas G, Hajnoczky G (2001) Sorting of calcium signals at the junctions of endoplasmic reticulum and mitochondria. Cell Calcium 29:249–262
135. Arnaudeau S, Frieden M, Nakamura K, Castelbou C, Michalak M, Demaurex N (2002) Calreticulin differentially modulates calcium uptake and release in the endoplasmic reticulum and mitochondria. J Biol Chem 277:46696–46705
136. Csordas G, Thomas AP, Hajnoczky G (1999) Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. EMBO J 18:96–108
137. Lin X, Varnai P, Csordas G et al (2005) Control of calcium signal propagation to the mitochondria by inositol 1,4,5-trisphosphate-binding proteins. J Biol Chem 280:12820–12832
138. Zhu L, Ling S, Yu XD et al (1999) Modulation of mitochondrial $Ca^{(2+)}$ homeostasis by Bcl-2. J Biol Chem 274:33267–33273
139. Park MK, Ashby MC, Erdemli G, Petersen OH, Tepikin AV (2001) Perinuclear, perigranular and sub-plasmalemmal mitochondria have distinct functions in the regulation of cellular calcium transport. EMBO J 20:1863–1874
140. Collins TJ, Lipp P, Berridge MJ, Bootman MD (2001) Mitochondrial $Ca^{(2+)}$ uptake depends on the spatial and temporal profile of cytosolic $Ca^{(2+)}$ signals. J Biol Chem 276:26411–26420
141. Rizzuto R, Pinton P, Carrington W et al (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science 280:1763–1766
142. Nicholls DG (2005) Mitochondria and calcium signaling. Cell Calcium 38:311–317
143. Putney JW, Jr., Thomas AP (2006) Calcium signaling: double duty for calcium at the mitochondrial uniporter. Curr Biol 16:R812–R815
144. Gunter TE, Yule DI, Gunter KK, Eliseev RA, Salter JD (2004) Calcium and mitochondria. FEBS Lett 567:96–102
145. Rizzuto R, Brini M, Murgia M, Pozzan T (1993) Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science 262:744–747
146. Neher E (1998) Usefulness and limitations of linear approximations to the understanding of Ca^{++} signals. Cell Calcium 24:345–357
147. Kirichok Y, Krapivinsky G, Clapham DE (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427:360–364
148. Csordas G, Renken C, Varnai P et al (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol 174:915–921
149. Frieden M, James D, Castelbou C, Danckaert A, Martinou JC, Demaurex N (2004) $Ca^{(2+)}$ homeostasis during mitochondrial fragmentation and perinuclear clustering induced by hFis1. J Biol Chem 279:22704–22714
150. Rutter GA (2006) Moving Ca^{2+} from the endoplasmic reticulum to mitochondria: is spatial intimacy enough? Biochem Soc Trans 34:351–355
151. Shore GC, Tata JR (1977) Two fractions of rough endoplasmic reticulum from rat liver. I. Recovery of rapidly sedimenting endoplasmic reticulum in association with mitochondria. J Cell Biol 72:714–725
152. Mannella CA, Buttle K, Rath BK, Marko M (1998) Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. Biofactors 8:225–828
153. Maeda N, Niinobe M, Inoue Y, Mikoshiba K (1989) Developmental expression and intracellular location of P400 protein. Dev Biol 133:67–76
154. Szabadkai G, Bianchi K, Varnai P et al (2006) Chaperone mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. J Cell Biol
155. Rapizzi E, Pinton P, Szabadkai G et al (2002) Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. J Cell Biol 159:613–624
156. Furuichi T, Kohda K, Miyawaki A, Mikoshiba K (1994) Intracellular channels. Curr Opin Neurobiol 4:294–303
157. Bosanac I, Yamazaki H, Matsu-ura T, Michikawa T, Mikoshiba K, Ikura M (2005) Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor. Mol Cell 17:193–203
158. Pitts KR, Yoon Y, Krueger EW, McNiven MA (1999) The dynamin-like protein DLP1 is essential for normal distribution and morphology of the endoplasmic reticulum and mitochondria in mammalian cells. Mol Biol Cell 10:4403–4417
159. Varadi A, Cirulli V, Rutter GA (2004) Mitochondrial localization as a determinant of capacitative Ca^{2+} entry in HeLa cells. Cell Calcium 36:499–508
160. Wang HJ, Guay G, Pogan L, Sauve R, Nabi IR (2000) Calcium regulates the association between mitochondria and a smooth subdomain of the endoplasmic reticulum. J Cell Biol 150:1489–1498

161. Simmen T, Aslan JE, Blagoveshchenskaya AD et al (2005) PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. EMBO J 24:717–729
162. Szabadkai G, Simoni AM, Chami M, Wieckowski MR, Youle RJ, Rizzuto R (2004) Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca^{2+} waves and protects against Ca^{2+}-mediated apoptosis. Mol Cell 16:59–68
163. Germain M, Mathai JP, McBride HM, Shore GC (2005) Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. EMBO J 24:1546–1556
164. Frank S, Gaume B, Bergmann-Leitner ES et al (2001) The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1:515–525
165. Karbowski M, Lee YJ, Gaume B et al (2002) Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. J Cell Biol 159:931–938
166. Szabadkai G, Simoni AM, Bianchi K et al (2006) Mitochondrial dynamics and Ca^{2+} signaling. Biochim Biophys Acta 1763:442–449
167. Hollenbeck PJ, Saxton WM (2005) The axonal transport of mitochondria. J Cell Sci 118:5411–5419
168. Shibata Y, Voeltz GK, Rapoport TA (2006) Rough sheets and smooth tubules. Cell 126:435–439
169. Yi M, Weaver D, Hajnoczky G (2004) Control of mitochondrial motility and distribution by the calcium signal: a homeostatic circuit. J Cell Biol 167:661–672
170. Rintoul GL, Filiano AJ, Brocard JB, Kress GJ, Reynolds IJ (2003) Glutamate decreases mitochondrial size and movement in primary forebrain neurons. J Neurosci 23:7881–7888
171. Brough D, Schell MJ, Irvine RF (2005) Agonist-induced regulation of mitochondrial and endoplasmic reticulum motility. Biochem J 392:291–297
172. Rintoul GL, Bennett VJ, Papaconstandinou NA, Reynolds IJ (2006) Nitric oxide inhibits mitochondrial movement in forebrain neurons associated with disruption of mitochondrial membrane potential. J Neurochem 97:800–806
173. Boldogh IR, Pon LA (2006) Interactions of mitochondria with the actin cytoskeleton. Biochim Biophys Acta 1763:450–462