Biochimica et Biophysica Acta 1788 (2009) 2022–2031

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Cardiolipin acts as a mitochondrial signalling platform to launch apoptosis

Zachary T. Schug, Eyal Gottlieb *

Cancer Research UK, The Beatson Institute for Cancer Research, Glasgow, G61 1BD, UK

ARTICLE INFO

Article history:
Received 4 October 2008
Received in revised form 6 May 2009
Accepted 8 May 2009
Available online 18 May 2009

Keywords:
Cardiolipin
Apoptosis
Caspase-8
Bid
Cytochrome *c*
Mitochondrion

ABSTRACT

Cardiolipin (CL) is a unique anionic phospholipid specific to the mitochondria. CL influences the activity of electron transport chain enzyme complexes as well as members of the Bcl-2 family. Interactions between Bcl-2 family members and other pro-apoptotic enzymes have been shown to be crucial for the transduction of the apoptotic signalling cascades during programmed cell death. Targeting of tBid to the mitochondria, which is necessary for Bax/Bak oligomerization and cristae remodelling, is dependent on the exposure of CL at contact sites between the inner and outer mitochondrial membranes. Also, the mobilization of cytochrome *c*, another key apoptotic event, is tightly regulated by the oxidative state of cardiolipin. Moreover, CL has been shown to be essential for translocation and autoprocessing of caspase-8 on the mitochondria after death receptor stimulation. Deficiencies in CL inhibit the formation of tBid and prevent apoptosis by removing an essential activation platform for the autoprocessing of caspase-8. It is now apparent that CL acts as a crucial signalling platform from which it orchestrates apoptosis by integrating signals from a variety of death inducing proteins.

Contents

Abbreviations: CL, cardiolipin; MLCL, monolyso cardiolipin; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PA, phosphatidic acid; PS, phosphatidylserine; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; IMS, mitochondrial inter-membrane space; tBid, truncated Bid; NAO, 10-*N*-nonyl acridine orange; ER, endoplasmic reticulum; $[Ca^{2+}]_m$, mitochondrial calcium concentration; ROS, reactive oxygen species; uMtCK, ubiquitous form of creatine kinase; DISC, death inducing signalling complex; PLS3, phospholipid scramblase 3; PLA_2, phospholipase A_2; MEFs, mouse embryonic fibroblasts; VDAC1, voltage-dependent anion channel 1; p55, full length pro-caspase-8; p43/p10, first cleaved form of caspase-8; p18/p10, fully cleaved form of caspase-8; Cardif, caspase activation and recruitment domain inducing interferon-β; Drp1, dynamin-related protein 1; FADD, Fas-associated death domain; Bif-1, Endophilin B1/Bax Interacting Factor 1

* Corresponding author. Tel.: +44 141 3303981; fax: +44 141 9426521.
E-mail address: e.gottlieb@beatson.gla.ac.uk (E. Gottlieb).

0005-2736/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2009.05.004

1. Introduction

1.1. Apoptosis

Programmed cell death, or apoptosis, is a coordinated process encompassing a series of biochemical events which culminate in the death of the cell [1–3]. Apoptotic cells are characterized by specific morphological changes including chromatin condensation and fragmentation and plasma membrane blebbing [2]. In the late stages of apoptosis the cell becomes fragmented into vesicles called apoptotic bodies, which contain cytosolic, nuclear and organelle material [2]. The apoptotic bodies are recognized by macrophages and cleared from the tissue to avoid inflammatory responses. Apoptosis is perpetrated by the activation of endonucleases and a family of cysteine-dependent aspartate-specific proteases, called 'caspases'. Caspases are activated by a variety of pathological and cytotoxic stimuli such as death receptor engagement, cytoskeletal damage, loss of adhesion, DNA damage, oncogene activation, ER stress, growth factor deprivation, and oxidative stress amongst others. Mitochondria play a pivotal role in the execution of apoptotic cell death [4–7]. The transduction of apoptotic signalling cascades and the execution of cell death requires permeabilization of the outer mitochondrial membrane (OMM) and the subsequent release of apoptogenic factors from the mitochondrial inter-membrane space (IMS) (i.e. cytochrome *c*, Smac/Diablo, EndoG, Omi/HtrA2, AIF and cytochrome *b*) [4,8–11]. The release of cytochrome *c* leads to the ATP-dependent oligomerization of Apaf-1 and the formation of the 'apoptosome'. The apoptosome, a multimeric complex consisting of cytochrome *c*, Apaf-1 and ATP, facilitates the clustering and activation of caspase-9 which, in turn, cleaves and activates caspase-3 and caspase-7 leading to many of the morphological changes associated with apoptosis [4,12].

The permeabilization of the OMM is tightly regulated by the Bcl-2 family of proteins. Bcl-2 family members are evolutionarily conserved and divided into three subfamilies according to their function and degree of homology shared within four Bcl-2 homology domains (BH1-4). In general, the anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-X_L, Bcl-w, Mcl-1 and A1) contain BH domains 1–4 [13] and need to be neutralized by the other Bcl-2 family members or down regulated for apoptosis to progress. The pro-apoptotic members can be subdivided into two groups. The first group, often called the multidomain pro-apoptotic members, contains BH domains 1–3 and includes Bax and Bak (and possibly Bok). The second group is made up of the BH3-only proteins (Bid, Bim, Bad, Bik, Noxa, BMF, bNIP3, HRK and PUMA) which act as sentinels upstream of the multidomain pro-apoptotic proteins [14]. The BH3 domain inserts directly into a hydrophobic groove formed by the BH1-3 domains of multidomain proteins. There must be a coordinated effort between the different Bcl-2 family members in order for permeabilization of the OMM to occur. Despite being constitutively expressed, Bax and/or Bak will only induce permeabilization of the OMM following apoptotic stimuli, suggesting that they are inactive in non-apoptotic cells [15,16]. The specific mechanism by which the Bcl-2 proteins induce OMM permeabilization remains controversial (for a recent review see [14]). Nevertheless, two models have been proposed to explain the "life/death" switch controlled by the Bcl-2 family of proteins [17]. In the first model, following apoptotic stimuli specific BH3-only proteins directly induce Bax or Bak to homo-oligomerize and stably insert into the OMM, an important prerequisite leading to the permeabilization of the OMM [14]. In the second model, certain BH3-only proteins may act to sensitize or "de-repress" Bax and Bak by binding to the anti-apoptotic (BH1-4) proteins, thereby sequestering and neutralizing the ability of anti-apoptotic proteins to bind and inhibit the multidomain pro-apoptotic proteins [14–16]. Cancer cells have developed several strategies to evade apoptosis by either negatively regulating caspases, BH3-only and multidomain pro-apoptotic proteins or conversely, by upregulating anti-apoptotic proteins. Apoptosis also plays a key role in embryonic development, the immune response, viral defence and normal tissue homeostasis.

1.2. Cardioliopin and apoptosis

Cardiolipin (CL), also known as diphosphatidylglycerol, is an anionic phospholipid predominantly located in the inner mitochondrial membrane (IMM) [18]. However, CL has also been reported to reside in the OMM and is enriched at contact sites that are formed between the inner and outer membranes of the mitochondria [19–23]. CL is a unique phospholipid which contains four acyl chains, most of which are highly unsaturated. CL has two negative charges on the headgroup and is found almost exclusively in mitochondrial membranes. CL associates with numerous proteins of the electron transport chain, often acting as a crucial component for enzymatic function. CL–protein interactions also help to orient many receptors, channels and enzymes on the outer face of the OMM and within the intermembrane space (IMS).

CL has been shown to associate with members of the apoptotic machinery [24]. Specific interactions between CL and cytochrome *c*, Bid and more recently caspase-8 have now been established. It is becoming apparent that CL is a versatile phospholipid which participates not only in membrane fluidity and normal electron transport chain activity, but plays a major role in cell signalling through CL–protein interactions. This review aims to summarize the recent advances made in understanding the role of CL as a signal integrator for multiple proteins during the execution of programmed cell death with an emphasis on three CL-binding proteins: cytochrome *c*, Bid and caspase-8. For a comprehensive review on CL metabolism, function and the role of CL in different pathologies please see [25].

2. Cardiolipin and cytochrome *c*

Cytochrome *c* plays a pivotal role in oxidative phophosphorylation by transferring electrons from Complex III to Complex IV of the electron transport chain. However, during apoptotic signalling, cytochrome *c* is released from the IMS into the cytosol, where it becomes a potent inducer of the caspase cascade. Within the mitochondria a substantial portion (~85%) of the cytochrome *c* is trapped within the cristae while the remaining cytochrome *c* is free in the IMS [26–29]. In line with this, selective permeabilization of the OMM with digitonin was not sufficient for complete cytochrome *c* release and additionally required cristae remodelling [29–31]. Initial studies showed that a decrease in total CL levels or oxidation of the unsaturated acyl chains of CL led to a decrease in membrane-bound cytochrome *c* [32–35]. Similarly, the liberation of cytochrome *c* from the mitochondria was prevented by antioxidant treatment [36–39] or by overexpression of redox regulators such as mitochondrial glutaredoxin 2 or glutathione peroxidase 4 [34,40,41]. These reports indicated that a shift in the oxidation state of CL was a coordinated event involving the production of reactive oxygen species (ROS) which promoted efficient cytochrome *c* release. Cytochrome *c* contained within the mitochondrial cristae has been shown to interact with CL in the outer leaflet of the IMM [35]. Further studies have shown that cytochrome *c* does indeed have a much higher affinity for CL in comparison to other phospholipids such as phosphatidylcholine (PC), phosphatidylserine (PS) or phosphatidic acid (PA) [42]. A very limited portion of CL is available for cytochrome *c* binding because of the high percentage of CL found in the inner leaflet of the IMM (~60%) and the association of CL with many of the electron transport chain supercomplexes. Studies have approximated that <15% of the total cytochrome *c* is bound to CL and <5% of the total mitochondrial CL is bound to cytochrome *c* [35,43,44]. Cytochrome *c* interacts with CL through two separate binding sites [45]. The first binding site most likely involves weak electrostatic interactions between the phosphate groups of CL and lysine residues of

cytochrome *c* [43]. The second is a more stable interaction which may involve hydrogen bonding between one of the polyunsaturated acyl chains of CL and Asn_{52} of cytochrome *c* [43]. It has also been postulated that the hydrophobic interaction may include the insertion of one of the acyl chains of CL into a pocket of cytochrome *c* lined by hydrophobic residues, such that one of the CL acyl chains protrudes out of the membrane bilayer to interact with cytochrome *c* while the other three acyl chains remain in the membrane [46,47].

The binding of cytochrome *c* to CL leads to a major negative shift in the redox potential of cytochrome *c* which prevents its reduction by Complex III, superoxide or ascorbate and inhibits its ability to transfer electrons between Complexes III and IV [48]. In addition to these effects, Kagan et al. found that CL-bound cytochrome *c* also acted as a peroxidase capable of catalyzing H_2O_2-dependent peroxidation of CL and that CL oxidation was essential step in the release of cytochrome *c* during apoptosis [44]. CL stimulated the peroxidase activity of cytochrome *c* by destabilizing the tertiary structure of cytochrome *c* through the hydrophobic interaction(s) [49,50], a process which was further enhanced by increases in the degree of CL unsaturation [42,51]. Thus, in the CL-bound state cytochrome *c* adopts a non-native conformation which significantly alters the heme crevice region [45], thereby creating a "channel" which facilitates the access of H_2O_2 to the heme [49]. Interestingly, the signalling molecules nitric oxide and carbon monoxide can also associate with the heme of the cytochrome *c*–CL complex and may confer a level of apoptotic regulation by inhibiting the peroxidase activity of cytochrome *c* [52,53]. CL oxidation would be dependent on the amount of available H_2O_2 and therefore on an increase in the generation and dismutation of superoxide radicals. The juxtaposition between CL and the electron transport chain, a major source of ROS, strongly implicates the enzymes of the supercomplexes as the impetus for ROS-mediated CL peroxidation [54]. Treatment of mouse embryonic fibroblasts (MEFs) with actinomycin D caused Bax translocation to the mitochondria and a subsequent increase in superoxide generation and CL oxidation [55]. In line with this, actinomycin D treated Bax/Bak double knockout MEFs displayed significantly less superoxide and CL oxidation in comparison to WT MEFs [55]. However, generation of superoxide radicals by rotenone or succinate was not sufficient for activation of cytochrome *c* as a peroxidase or for its release from mitochondria [55]. These cumulative results implied that Bax/Bak insertion and activation was prerequisite for CL oxidation and cytochrome *c* release. Rises in ROS production due to oxidative stress have also been noted as an early apoptotic event following the activation of the Tumour Necrosis Factor (TNF) death receptors such as Fas and TNF-R [56,57] and there has been a suggestion that $p66^{shc}$, an adaptor protein of the IMS which becomes activated by proapoptotic signalling, can oxidize reduced cytochrome *c* and catalyze the reduction of O_2 to H_2O_2 [58]. However, the steps connecting death receptor activation and activation of $p66^{shc}$ are speculative, but may involve the dissociation of $p66^{shc}$ from TOM–TIM import complexes or remodelling of the inner membrane and an increase of free IMS cytochrome *c*.

CL remodelling involves the phospholipase-dependent deacylation of *de novo* synthesized CL to form monolyso-CL (MLCL) [25], most likely through a variant of PLA_2 [59–62] or MitoPLD [63]. The reacylation of MLCL has been suggested to be directed by the acyltransferase, tafazzin [64]. Tafazzin is able to transfer linoleic acid from PC to MLCL and is crucial for maintaining the relatively high degree of unsaturation noted for the acyl chains of CL [65]. An increase in CL hydrolysis has been shown during the early stages of apoptosis and would affect the cytochrome *c*-dependent catalytic oxidation of CL [43,51,66,67]. Death receptor stimulation of U937, HeLa or Jurkat cells caused early changes (1 h) in the acyl chain composition of CL, decreases in CL and increases in MLCL [67,68]. In summation, the specific depletion and remodelling of CL, the generation of ROS and the CL oxygenase capability of cytochrome *c* are indispensable components for efficient release of cytochrome *c* from the IMS.

3. Cardiolipin and Bid

After death receptor engagement, the activation of caspase-8 results in the cleavage of Bid (a BH3-only protein) to a truncated form, tBid [9]. tBid translocates to the OMM and induces the oligomerization of Bax and/or Bak, which leads to OMM permeabilization. Immunoelectron microscopy suggested that tBid was targeted in a CL-dependent manner to the mitochondrial contact sites [69,70]. The binding of tBid to CL-enriched contacts sites of mitochondria was shown to minimally require α-helices 4–6 of tBid and was completely independent of the BH3 domain (α-3 helix) [9]. In addition, tBid binding to the mitochondria was significantly decreased in mitochondria from cells deficient in CL [69]. However, we have noted that mitochondria isolated from lymphoblastoid cells derived from Barth syndrome patients, which are devoid of tafazzin activity and therefore deficient in mature CL, successfully bind tBid [71]. In this case, the levels of MLCL, which are increased in Barth syndrome lymphoblastoid cells, may be sufficient at directing tBid translocation to the OMM [67,68,72,73]. The interaction of tBid with the OMM may involve the negatively charged headgroup of CL and basic residues in helices 4–6 [73], but may also involve insertion into the bilayer [74]. However, it should be noted that other phospholipids, such as PC [75] phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), permit tBid incorporation [76].

The binding of tBid to mitochondria also plays critical roles in other apoptotic associated processes, such as cristae remodelling [30] and mitochondrial fragmentation [77]. Cristae remodelling during apoptosis, which facilitates the release of cytochrome *c*, is regulated by the rhomboid protease PARL and the dynamin-related protein OPA1 [30,31,78]. Mitochondrial PARL actively cleaves the OPA1 protein, which generates a soluble pool of truncated OPA1 in the IMS. Soluble OPA1 is crucial for the anti-apoptotic effects of PARL because it maintains the bottleneck configuration of cristae and the compartmentalization of cytochrome *c* [30,78,79]. The short and long forms of OPA1 form hetero-oligomers that are disrupted soon after the binding of tBid to the mitochondria enabling cytochrome *c* mobilization from the cristae [30,78,79].

Possibly the best described function of tBid is its ability to induce Bax/Bak oligomerization, a process which requires the BH3 domain of tBid. Mitochondrial fractionation studies have detected tBid in fractions representative of the non-contact site regions of the OMM [80], which is not unexpected considering tBid may target Bak in these areas. 30 nM myristoylated Bid (p7/myrp15) was shown to induce cytochrome *c* release much more effectively than 10 μM Bid–BH3 peptide and may reflect that an association between tBid and the OMM, and perhaps more specifically CL, is needed for concentrating tBid into certain microdomains (i.e. the contact sites) [15]. Microdomains would help in focusing tBid for proper presentation of the BH3 domain to Bak or Bax [15] and confer prime positioning for inducing IMM changes like cristae remodelling. In support of this notion, pre-treatment of mitochondria with the CL-specific dye, 10-*N*-nonyl acridine orange (NAO), caused significant inhibition of cristae remodelling and cytochrome *c* release after addition of recombinant tBid, while having minimal effects on Bak oligomerization [29,70,80]. It is important to note that NAO did not prevent tBid incorporation into the OMM [80]. Furthermore, it has been proposed that mitochondria may contain lipid microdomains, reminiscent of the lipid rafts of the plasma membrane [75], which contribute to apoptosis-associated modification of mitochondrial morphology [81]. The ganglioside GD3, VDAC1 and the fission protein hFis1 formed a protein complex at lipid microdomains on the OMM of Fas-activated lymphoblasts into which both tBid and Bax were recruited [81]. Similarly, during the early steps of staurosporine-induced apoptosis Bax relocated to lipid microdomains associated with mitochondrial fission sites [82]. This complex may be involved in regulating the activity of mitochondrial fission-related proteins,

whereby GD3 facilitates the transient and local formation of inverted hexagonal structures in mitochondrial membranes undergoing active fission [81]. Moreover, tBid has been suggested to directly alter membrane curvature [83] and regulate targets involved in cristae remodelling [29,30]. It seems plausible that targeting and recruitment of tBid into lipid microdomains on the OMM, most likely through CL binding, may be necessary for formation of multiprotein complex(es) which regulate changes in the mitochondrial morphology [81]. Thus, the BH3 domain of Bid seemed to be important in activating the pro-apoptotic proteins Bak and Bax, while the Bid–CL interaction played a functionally distinct role in cristae remodelling and in the formation of mitochondrial fission sites. Cristae remodelling was shown to be essential for full release of cytochrome *c* and the progression of apoptosis [29–31]. As mentioned above, cytochrome *c* release depended on the dissociation of OPA1 complexes at the cristae junctions [29–31]. Nevertheless, the question remains as to how binding of tBid to CL or how the BH3 domain (via Bax/Bak) activates a mechanism which culminates in OPA1 dissociation.

Bax and Bak are important regulators of the mitochondrial steps of apoptosis, since cells lacking Bax and Bak were resistant to a diverse array of apoptotic insults [10]. Activation of these proteins led to their oligomerization and OMM permeabilization. In vitro studies have suggested that PLA_2-sensitive phospholipids in the OMM were essential for Bax activation [84]. More recently, studies on the membrane binding properties of Bax reported that CL was required for the first α-helix of Bax to bind to liposomes which had a phospholipid composition similar to mitochondrial membrane contact sites [85]. Furthermore, CL was required for Bax insertion and oligomerization on liposomes, a process which was independent of the recruitment of tBid [84]. Bax insertion and oligomerization is thought to occur in a coordinated series of protein–protein and protein–lipid interactions which culminate in pore formation [86] and can be completely inhibited by Bcl-X_L [87] or Mcl-1 [88]. Liposomal studies have suggested that Bax initially binds membrane-bound tBid before inserting into the membrane [86]. The Bax–tBid interaction was dependent on the presence of membranes which contained CL. These initial steps were followed by further recruitment of soluble Bax by membrane-bound Bax and the formation of pores in the OMM [86]. Endophilin B1/Bax interacting factor 1 (Bif-1) typically modulates mitochondrial dynamics downstream of Drp1 [89]. However, a new role for Bif-1 was revealed when it was shown that Bif-1 directly interacted with Bax through a Bax Binding Motif (BBM) in the presence of membranes [90]. Bif-1 stimulated Bax oligomerization and potentiated cytochrome *c* release [90]. This enhancement was critically dependent on the presence of CL. Bif-1 may associate with CL via an N-BAR domain located in the C-terminal region, a motif which is known to bind to lipid bilayers and cause large scale morphological rearrangements [91]. The binding of CL to the N-BAR domain of Bif-1 may create CL-enriched microdomains on the OMM, thereby forming an activation platform upon which Bax can be concentrated and activated [90]. However, there were reports of Bax activation in the absence of CL [88,92,93]. In this case, it may not be CL which is important for the activation of Bax, but rather the modification of the properties of the membrane, which suggests that trans-membrane migration of CL to the OMM may play a dual role by creating a preferential lipidic environment while simultaneously establishing nucleation points for protein signalling cascades and enzymatic reactions. Alternatively, experiments using CL-deficient proteo-liposomes as an artificial model for pore formation suggested that an unknown OMM protein(s) facilitated tBid-induced Bax pore formation by substituting for CL [88].

4. Cardiolipin and caspase-8

There are two separate signalling pathways which lead to apoptotic cell death. The first is the intrinsic pathway, which is activated from inside the cell by the pro-apoptotic members of the Bcl-2 protein family. The second is the extrinsic pathway, which is activated from outside the cell by pro-apoptotic ligands and involves the clustering of death receptors at the cell surface. Stimulation of the Fas receptor (CD95/APO-1), a member of the death receptor family, induces clustering of the receptor and recruits pro-caspase-8 and Fas-associated death domain (FADD) into a death inducing signalling complex (DISC) at the plasma membrane [94]. DISC formation facilitates the clustering, dimerization and autoprocessing of pro-caspase-8 into an active form which, in turn, directly cleaves the effector caspases-3 and -7 (type I apoptosis) [94]. In some cell types (type II apoptosis) the level of caspase-8 activation is too low to substantially activate caspase-3 directly and requires a mitochondrial-dependent step to complete the execution of apoptosis. The mitochondrial step is dependent on the Bcl-2 family of proteins and can be inhibited by overexpression of anti-apoptotic proteins, such as Bcl-X_L or Bcl-2, or by deficiencies in the pro-apoptotic Bcl-2 family members (Bax, Bak or Bid) [10,95–99].

Pro-caspase-8 was shown to be predominantly localized within the cytosol, but has also been shown to be loosely associated with the OMM [100,101]. Recently, it was demonstrated that Fas receptor activation induced the translocation of active caspase-8 to the mitochondria, a step that was dependent on the presence of mature CL in the OMM [71]. Translocation of caspase-8 to the mitochondria was necessary for efficient caspase-8 processing and activation. The role for CL in these processes was identified by using immortalized lymphoblastoid cells from Barth syndrome patients. Barth syndrome is a genetic disorder often involving mutations in the *tafazzin* gene, a transacylase which catalyzes the transfer of an unsaturated acyl chain from PC to MLCL. Loss of tafazzin function leads to a decrease in total CL, a reduction in mature CL and an increase in MLCL. Barth syndrome lymphoblastoid cells displayed strong resistance to death receptor activation [71]. However, treatment of mitochondria isolated from Barth syndrome lymphoblasts with recombinant tBid-induced Bak oligomerization and cytochrome *c* release [71]. As stated previously, the abilities of tBid and Bax to promote OMM permeabilization and cytochrome *c* release in Barth syndrome lymphoblastoid cells may have been due to increased amounts of MLCL. Upon further inspection it was found that Fas-stimulated Barth syndrome-derived cells had significant inhibition of Bid cleavage suggesting a defect in caspase-8 activation. In addition, isolated mitochondria from tafazzin-knock-down HeLa cells displayed markedly reduced levels of the processed forms of caspase-8 and Bid [71]. Therefore mature CL must be critical for the activation of caspase-8 during Fas-induced apoptosis in lymphoblastoid and HeLa cells.

The activation of caspases often involves the formation of triggering complexes and many of these activation platforms have been described including the DISC for caspase-8 and -10 [102], the apoptosome for caspase-9 [103], the PIDDosome for caspase-2 [104] and the inflammasome for caspases-1 and -5 [105]. The autoprocessing of pro-caspase-8 at the DISC into the fully active caspase-8 involves two sequential cleavage events (Fig. 1). Upon homodimerization, pro-caspase-8 (p55) is first cleaved between the two active subunits p10 and p18 to generate the p43/p10 heterodimer. The second cleavage occurs in p43 between the death effector domain (DED) and the p18 subunit to produce the fully active p18/p10 form. p55 and the first cleavage product of caspase-8, p43/p10, associated with CL-containing membranes and were resistant to an alkaline or high salt wash, suggesting that caspase-8 partially inserted into the OMM [71]. This was the first proof of caspase-8 integration into the mitochondrial membrane. The resistance to alkali wash does suggest that hydrophobic interaction(s) with the unsaturated acyl chains of CL may contribute to the stability of the interaction. However, caspase-8 was sensitive to trypsin proteolysis suggesting that caspase-8 was still accessible from the cytosol and only partially inserted into the OMM [71]. A more detailed biophysical analysis of the CL–caspase-8

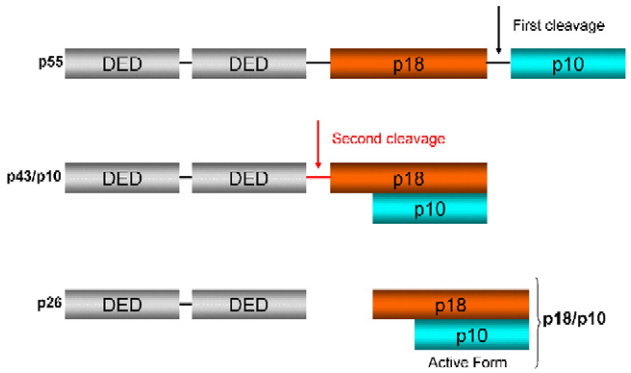

Fig. 1. Autoprocessing of caspase-8. After death receptor stimulation pro-caspase-8 (p55) is recruited to the DISC. The DISC facilitates the clustering of caspase-8. Caspase-8 undergoes two sequential cleavage events to create the active form. p55 is first cleaved between the p18 and p10 subunits. However, p18/p10 remains associated in non-peptidic bonds. The next cleavage separates p18 from the DED (p26) to create the fully active form of caspase-8 (p18/p10).

interaction may provide clues about the CL-binding domain of caspase-8. Additionally, an increased amount of stably inserted p43/p10 into liposomal membranes containing CL compared to liposomes lacking CL was noted [71]. Unfortunately, it was impossible to determine if the increase in p43/p10 had been the product of p55 clustering and processing on the membrane or a higher affinity of p43/p10 for CL-containing membranes. It will be interesting to determine if p43/p10 needs to be generated at the DISC before it can translocate to the mitochondria for further processing to p18/10 or whether pro-caspase-8 targets CL-enriched domains of the OMM where it can heterodimerize with other pro-caspase-8 molecules already present and undergo the cleavage events necessary for caspase-8 activation. An attractive idea is that CL provides an activation platform upon which pro-caspase-8 can cluster, oligomerize and process into p43/p10 and p18/p10. Peter et al. demonstrated that the amount of active caspase-8 generated at the DISC in type II cells was so small that it was not detected by Western blotting and suggested that a mitochondrial step was necessary for extrinsic apoptosis [94]. In support of this notion, p43/p10 was cross linked to other p43/p10 molecules on the OMM and formed high molecular weight oligomers [71]. This suggested that caspase-8 processing to its active form, p18/p10, occurs on the mitochondria through a CL-dependent clustering of p43/p10. If Bid were also targeted to CL-enriched regions of the OMM then CL could act as an activation platform for bringing together both the enzyme (caspase-8) and its substrate (Bid). It is plausible that caspase-8 binding to the mitochondria is regulated in a similar manner to other activating platforms and involves the formation of a 'mitosome' triggering complex.

It remains to be seen if other mitochondrial proteins could target caspase-8 to the mitochondria. Isolated mitochondria which had been trypsinized and then incubated with in vitro translated caspase-8 were unable to bind caspase-8, which suggested that a trypsin-sensitive protein in the OMM was responsible for the association of caspase-8 with mitochondria [100]. Potential caspase-8 binding proteins shown to be involved in caspase-8 translocation are BAR and FLASH [100,106]. Another more recent candidate is the mitochondrial membrane protein Cardif (also called IPS-1, MAVS and VISA), which contains an N-terminal caspase activation and recruitment domain and a C-terminal death effector domain [107,108]. Cardif was shown to interact with caspase-8, most likely through FADD, and to be targeted and inactivated by caspases [109,110].

The way in which caspase-8 associates with CL has not been determined, but seems to be dependent upon both the maturity (unsaturation) and total amount of CL. Unlike tBid, which can associate with either MLCL or CL, p43/p10 specifically interacted with mature CL. Our experiments did not determine whether caspase-8 was bound to CL or to peroxi-CL, though the liposomal studies would suggest peroxidation is not necessary. It would be interesting to see if caspase-8 insertion into the OMM is dependent upon or enhanced by peroxidation of CL. Peroxi-CL would then act to free cytochrome *c* from the IMM and as a signal to promote caspase-8 (and Bid) association with the OMM. However, gas chromatography, NAO staining and electron microscopy studies on NGF-deprived neurons suggested that the major ROS-induced CL peroxidation may be downstream of cytochrome *c* release and the activation of caspases, since it could be blocked by caspase inhibition [111]. NGF-deprivation also caused loss of mitochondrial mass in a oxidation-dependent manner, most likely by autophagocytosis of damaged mitochondria, suggesting that ROS-associated damage of CL leads to mitochondrial destruction via an autophagic pathway [111].

Similar to the multiple roles played by Bid/tBid (i.e. lipid transfer, membrane modifications, cristae remodelling, mitochondrial fission/fusion) perhaps there are other roles for caspase-8 in altering membrane structure upon CL binding or other substrates which play, as of yet, unidentified roles in the processes involved in the execution of type II apoptosis.

5. How does CL translocate to the outer mitochondrial membrane?

5.1. Contact sites

It has been over four decades since contact sites between the inner and outer mitochondrial membranes were first described [112]. These sites have been speculated to facilitate the transport of proteins and small molecules (i.e. metabolite and ions) between the cytosol and the inner mitochondrial matrix [113]. Further examination of the content of contact sites revealed that CL (25%) and PE (25%) constitute the two major phospholipids present within the contact site [22,114]. Although not yet described in vivo, in the presence of Ca^{2+} the IMM and OMM are predicted to form a non-bilayer hexagonal H_{II} structure at the contact sites given the high percentage of CL and PE [24,115–118]. CL and PE are non-lamellar lipids with a high propensity for negative membrane curvature which can spontaneously form inverted hexagonal lipid phases and cause significant stress in the lipid packing of the acyl chain region of the bilayer. The fusion of the IMM and OMM would allow CL to redistribute to the surface of the mitochondria and permit interactions with cytosolic proteins (Fig. 2).

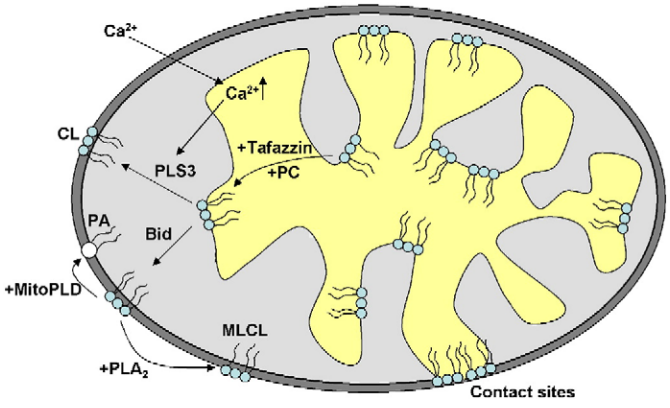

Fig. 2. Trans-membrane migration and remodelling of cardiolipin. CL may be relocated from the IMM to the OMM by PLS3 (in Ca^{2+}-dependent manner) or by lipid transferase activity of Bid and/or tBid. Alternatively, hexagonal H_{II} phase transition between the IMM and OMM would create contact sites which would allow CL to diffuse to the OMM from the IMM. CL may be hydrolyzed by a variant of PLA_2 or MitoPLD to create MLCL in the OMM. CL may also undergo MitoPLD-dependent hydrolysis to form phosphatidic acid (PA). MLCL can be reacylated by tafazzin, an acyl-transferase which catalyzes the transfer of an unsaturated acyl chain from PC to MLCL.

5.2. Protein-mediated contact site formation and stabilization

The physiological presence of contact sites has been under debate for many years. Nevertheless, it seems that the number and stability of contact sites may be strongly influenced by a number of different proteins, although the exact mechanism which triggers the formation of the contact site is still unknown. A role for mitochondrial creatine kinase (MtCK) in the formation and stabilization of contact sites has recently been suggested [119,120]. Epand et al. described an interaction between the octameric ubiquitous form of mitochondrial creatine kinase (uMtCK) and CL which potently induced the lipid phase separation of CL into discrete domains in vitro [121]. Transgenic mice expressing the uMtCK displayed a 3-fold increase in the number of contact sites compared to WT control mice [122]. Furthermore, within the contact sites uMtCK may bind to CL on the IMM and VDAC on the OMM to form a bridge across the double membrane of the mitochondria which stabilizes the CL-enriched contact site [121]. Interestingly, the same study showed that both cytochrome *c* and tBid were capable of producing CL clustering, although to a lesser extent [121].

5.3. CL trans-membrane migration

Recently, there have been investigations into the trans-bilayer movement and intracellular distribution of CL. In cells undergoing staurosporine-induced apoptosis the CL content in the OMM was significantly increased [44,123] while the CL (mol%) distribution of the outer leaflet to the inner leaflet of the IMM went from 40:60 to 70:30, respectively [44]. The intra-mitochondrial movements of CL occur very early during apoptosis, well before changes in mitochondrial membrane potential or nuclear condensation and fragmentation, but after the production of ROS [124]. These changes in CL distribution during apoptosis could be directed by Bid. There is evidence that Bid, and to a greater extent tBid, possesses lipid transfer activity (Fig. 2) [67,76,125]. Bid may act to translocate CL to the OMM surface which would then promote further caspase-8 binding and Bid binding, as well as disruption of the CL–cytochrome *c* interaction. However, the exact physiological function of Bid and tBid lipid transfer activity is not well understood leading others to suggest that tBid may act by augmenting a Bax-dependent CL redistribution between the mitochondrial membranes. Studies using liposomes suggested that the rate at which the translocation of CL to the OMM occurs was enhanced by oligomeric Bax and that liposomes containing CL were more susceptible to leakage in the presence of Bax than those containing the CL precursor PG [16,126]. Furthermore, Bid association with the mitochondria would have to occur prior to caspase-8 cleavage of Bid to tBid and after death receptor activation. There are reports of recombinant Bid binding to liposomes containing CL or MLCL [72,76]. In mouse liver, Fas-induced apoptosis caused an early increase in mitochondrial-bound Bid which was concomitant with the appearance tBid and cytochrome *c* release [72]. It is important to note that Bid was detectable in untreated mitochondria isolated by subcellular fractionation from mouse livers [72]. There are also reports of Bid inserting specific lysolipids into the mitochondrial membrane, similar to the lipid transfer activity of Bid described above for CL [67,76,125], thereby priming mitochondria for the release of apoptogenic factors [125,127]. Similarly, incubation of mouse liver mitochondria with tBid-induced PLA_2 activation and the trans-membrane migration of CL and its hydrolysis products [125]. Overall, the tBid-induced translocation of CL between all four leaflets has many consequences i) increased cytochrome *c*–CL interactions, ii) enhanced peroxidation of CL, iii) enhanced release of cytochrome *c*, iv) mitochondrial bioenergetic disruption and v) large membrane perturbing effects such as the formation of CL-enriched microdomains, cristae remodelling and the accumulation of lyso-phospholipids [125,128].

5.4. Ca^{2+} and CL

Increases in the $[Ca^{2+}]_m$ occur after death receptor stimulation and stimulate multiple mitochondrial enzymes, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and isocitrate dehydrogenase whose increased activity results in higher ROS production unless accompanied by a concomitant increase in ADP. Ca^{2+}-induced oxidative stress generates superoxide radicals which are promptly dismutated to H_2O_2. As discussed above, the formation of H_2O_2 promotes the cytochrome *c*-dependent peroxidation of CL. Peroxi-CL has a different molecular conformation and altered packing characteristics which can promote formation of a non-bilayer hexagonal structure and exposure of CL to the cytosolic surface of the mitochondria [116].

Phospholipid translocators are highly regulated enzymes which play vital roles in membrane biogenesis and in the maintenance of membrane asymmetry [129]. Phospholipid scramblases (PLS) are a small family of phospholipid translocators which contain a single pass trans-membrane domain and an EF-hand-like Ca^{2+}-binding motif. PLS induce non-specific bi-directional movement of phospholipids across the membrane during cell activation in response to rises in $[Ca^{2+}]_c$. PLS3 is localized to the mitochondria and has been shown to translocate CL from the IMM to the OMM during apoptosis induced by UV-irradiation [130,131] (Fig. 2). Overexpressing PLS3 in HeLa cells led to an increase in ROS production by an unknown mechanism [130,131]. These processes may be enhanced by the lipid transfer activity of tBid. Furthermore, it has been shown that stimulation of the Fas receptor in Jurkat cells led to the activation of PLCγ1, which in turn generated IP_3 receptor-mediated Ca^{2+} release [132]. IP_3-mediated Ca^{2+} release caused a rise in $[Ca^{2+}]_m$ due to a local Ca^{2+} transfer between IP_3 receptors and mitochondrial Ca^{2+} uptake sites [133,134] Rises of $[Ca^{2+}]_m$ during the early stages of type II extrinsic apoptosis may trigger the activation of PLS3 which translocates CL to the OMM where it would have the opportunity to interact with caspase-8 and Bid. Indeed, PLS3 overexpression has been shown to enhance the level of TRAIL-induced caspase-8 processing in Jurkat cells [135].

Ca^{2+} has long been known to play a crucial role in apoptosis [136,137]. Ca^{2+} has the ability to bind to CL thereby neutralizing the negatively charged headgroup of CL. The Ca^{2+}–CL interaction has been suggested to be an early and fundamental step by which Ca^{2+} triggers OMM permeabilization. The effects of the Ca^{2+}–CL interaction include the induction of lateral phase separation and formation of CL-enriched domains, protein aggregation, increased membrane permeability, and promotion of lipid peroxidation by optimizing the configuration of proteins and lipids for attack by ROS [117]. CL is associated with numerous proteins within the mitochondria, many of which are associated with the electron transport chain supercomplexes [35,138–140]. The formation of Ca^{2+}-induced CL-enriched domains would be expected not only to affect the lipid microenvironment, but also to influence the lateral motion, conformation and function of membrane proteins of the electron transport chain supercomplexes. Together these data would suggest that an early and initial rise in $[Ca^{2+}]_m$ after induction of apoptosis could be important for the distribution of CL into discreet functional domains thereby concentrating particular CL-binding enzymes and proteins and thus behaving as a signal integrator. Moreover, it is plausible that the binding of tBid, and potentially caspase-8, further induces lateral phase separation in a feed-forward mechanism in order to create a signalling platform from which OMM permeabilization could manifest.

After death receptor engagement activated caspase-8 cleaves Bid to promote mitochondrial release of cytochrome *c*, Smac/Diablo [97,141], the death domain kinase RIP to prevent the activation of NF-κB survival responses [142] and the cytolinker plectin for disassembly of microfilaments [101]. Recently, knockdown of Apaf-1 or cytochrome *c* did not completely prevent Fas-induced cell death in HeLa cells [11]. In this scenario, Fas receptor activation triggered intra-

mitochondrial cleavage of cytochrome *b* which was directly dependent on caspase-8 (and Bid) activity and suggested that caspase-8 may activate an unidentified intra-mitochondrial protease. Once cleaved cytochrome *b* translocated to the cytosol and activated a caspase-dependent (apoptosome-independent) cell death. Caspase-8 also cleaves BAP31, an integral protein of the ER membrane [143], which generates a membrane-bound fragment, called p20, that is capable of inducing apoptosis when expressed ectopically [144,145]. A caspase-resistant mutant of BAP31 inhibited Fas-mediated apoptosis by preventing mitochondrial remodelling and the release of cytochrome *c* despite normal caspase-8 activation [145]. The BAP31 cleavage product, p20, stimulated a direct release of ER Ca^{2+} into the mitochondria which sensitized the mitochondria to OMM permeabilization [146]. The uptake of Ca^{2+} by mitochondria also triggered the recruitment of Drp1, a key protein involved in the induction of mitochondrial fission, to the OMM [147]. It should be noted that a mechanism was recently proposed which described mitochondrial fragmentation as an occurrence that accompanied OMM permeabilization (Bax/Bak activation) and that cytochrome *c* release and mitochondrial fission are two separable events [148]. Overexpression of the BH3-only ER-membrane protein BIK was also capable of inducing an early and robust release of Ca^{2+} from the ER stores in a Bax/Bak-dependent manner as evidenced by the ability of Bik to release Ca^{2+} from ER stores in transformed kidney epithelial cells derived from wild-type, but not from Bax/Bak double knockout mice [149]. Furthermore, Bik-mediated cell death was dependent on Drp1 and caspase activation [149]. Could Bik- or p20-mediated rises in $[Ca^{2+}]_m$ lead to the activation of the Ca^{2+}-dependent PLS3, the generation of ROS through enhancement of matrix dehydrogenase activity, disruption of CL–cytochrome *c* interactions, lateral phase transitions and promotion of bilayer to hexagonal H_{II} phase transition? In summary, these above findings support the idea of a "two hit" model in which the transfer of an ER Ca^{2+} signal to the mitochondria and a parallel BH3-dependent hit collaborate to promote the mitochondrial phase of apoptosis [146,150,151]. All of these factors together suggest that major perturbations in the CL distribution, metabolism and redox status play major roles in apoptosis. However, at the moment these connections remain largely speculative and only additional research will explain how changes in the location and state of CL affect the different steps of programmed cell death.

6. Conclusions

The identification of the CL–caspase-8 interaction has further emphasized the critical importance of CL distribution within the mitochondrial membranes during apoptosis. CL seems to act as a platform upon which multiple apoptotic signals converge to coordinate programmed cell death. There is a need to identify if the amount of CL found at the contact sites is sufficient to support the dynamic processes involved in mitochondria remodelling, fission and OMM permeabilization or if there is a regulated mechanism needed which causes CL migration from the inner leaflet of the IMM to the outer leaflet of the OMM. Possible candidates for CL alterations include Bid lipid transfer activity, acyl chain remodelling, PLA_2 activation and MLCL formation, PLS3 activation, Ca^{2+}-induced hexagonal H_{II} phase non-lamellar membrane formation and ROS production. Also, at the present it is not known if changes in mitochondria are needed before translocation of caspase-8 and/or Bid.

7. Perspectives

Biomedical research on the roles of biological membranes has revealed them as signalling and trafficking platforms for many fundamental processes involved in cellular growth, maintenance, metabolism, and homeostasis. Moreover, genetic abnormalities in lipid metabolism are often associated with diseases such as cancer, arthritis, diabetes and in the case of CL, Barth syndrome. Therefore, there is a need to comprehensively understand protein–lipid interactions for the identification of practical targets for drug development. Protein–protein interactions have dominated the field for many years, but more recently the significance of protein–lipid interactions has received more attention. The lipid microenvironment has long been known to affect receptors, ion channels and scaffolding complexes and more recently membrane recruitment of peripheral proteins. It seems that the spatial and temporal interactions between caspases and Bcl-2 family members with mitochondrial CL are vital to the progression of apoptosis. However, the mechanisms by which the pro- and anti-apoptotic protein family are recruited to and interact with the mitochondrial membranes remain poorly characterized. Nevertheless, there is now an unquestionable need to exploit lipid-binding proteins in drug development targeted at cellular membranes.

Acknowledgements

We would like to thank Francois Gonzalvez and Christian Frezza for helpful discussions. This work was funded by the Cancer Research UK.

References

[1] R.A. Lockshin, C.M. Williams, Programmed cell death. V. Cytolytic enzymes in relation to the breakdown of the intersegmental muscles of silkmoths, J. Insect Physiol. 11 (1965) 831–844.
[2] G. Kroemer, W.S. El-Deiry, P. Golstein, M.E. Peter, D. Vaux, P. Vandenabeele, B. Zhivotovsky, M.V. Blagosklonny, W. Malorni, R.A. Knight, M. Piacentini, S. Nagata, G. Melino, Classification of cell death: recommendations of the nomenclature committee on cell death, Cell Death Differ. 12 (Suppl. 2) (2005) 1463–1467.
[3] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[4] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*, Cell 86 (1996) 147–157.
[5] N. Zamzami, P. Marchetti, M. Castedo, D. Decaudin, A. Macho, T. Hirsch, S.A. Susin, P.X. Petit, B. Mignotte, G. Kroemer, Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death, J. Exp. Med. 182 (1995) 367–377.
[6] N. Zamzami, P. Marchetti, M. Castedo, C. Zanin, J.L. Vayssiere, P.X. Petit, G. Kroemer, Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo, J. Exp. Med. 181 (1995) 1661–1672.
[7] D.D. Newmeyer, D.M. Farschon, J.C. Reed, Cell-free apoptosis in *Xenopus* egg extracts: inhibition by Bcl-2 and requirement for an organelle fraction enriched in mitochondria, Cell 79 (1994) 353–364.
[8] R.M. Kluck, E. Bossy-Wetzel, D.R. Green, D.D. Newmeyer, The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis, Science 275 (1997) 1132–1136.
[9] A. Gross, X.-M. Yin, K. Wang, M.C. Wei, J. Jockel, C. Milliman, H. Erdjument-Bromage, P. Tempst, S.J. Korsmeyer, Caspase cleaved BID targets mitochondria and is required for cytochrome *c* release, while BCL-xl prevents this release but not tumor necrosis factor-R1/Fas death, J. Biol. Chem. 274 (1999) 1156–1163.
[10] M.C. Wei, W.-X. Zong, E.H.Y. Cheng, T. Lindsten, V. Panoutsakopoulou, A.J. Ross, K.A. Roth, G.R. MacGregor, C.B. Thompson, S.J. Korsmeyer, Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death, Science 292 (2001) 727–730.
[11] A.P. Komarov, O.W. Rokhlin, C.A. Yu, A.V. Gudkov, Functional genetic screening reveals the role of mitochondrial cytochrome *b* as a mediator of FAS-induced apoptosis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14453–14458.
[12] J. Rodriguez, Y. Lazebnik, Caspase-9 and APAF-1 form an active holoenzyme, Genes Dev. 13 (1999) 3179–3184.
[13] J.E. Chipuk, L. Bouchier-Hayes, D.R. Green, Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario, Cell Death Differ. 13 (2006) 1396–1402.
[14] J.E. Chipuk, D.R. Green, How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 18 (2008) 157–164.
[15] A. Letai, M.C. Bassik, L.D. Walensky, M.D. Sorcinelli, S. Weiler, S.J. Korsmeyer, Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics, Cancer Cell 2 (2002) 183–192.
[16] T. Kuwana, M.R. Mackey, G. Perkins, M.H. Ellisman, M. Latterich, R. Schneiter, D.R. Green, D.D. Newmeyer, Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane, Cell 111 (2002) 331–342.
[17] J.M. Adams, S. Cory, The Bcl-2 protein family: arbiters of cell survival, Science 281 (1998) 1322–1326.
[18] J.J. Krebs, H. Hauser, E. Carafoli, Asymmetric distribution of phospholipids in the inner membrane of beef heart mitochondria, J. Biol. Chem. 254 (1979) 5308–5316.

[19] A.I.P.M. de Kroon, D. Dolis, A. Mayer, R. Lill, B. de Kruijff, Phospholipid composition of highly purified mitochondrial outer membranes of rat liver and *Neurospora crassa*. Is cardiolipin present in the mitochondrial outer membrane? Biochim. Biophys. Acta (BBA)- Biomembranes 1325 (1997) 108–116.

[20] G. Daum, Lipids of mitochondria, Biochim. Biophys. Acta (BBA)- Rev. Biomembr. 822 (1985) 1–42.

[21] E. Zinser, C.D. Sperka-Gottlieb, E.V. Fasch, S.D. Kohlwein, F. Paltauf, G. Daum, Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*, J. Bacteriol. 173 (1991) 2026–2034.

[22] R. Simbeni, L. Pon, E. Zinser, F. Paltauf, G. Daum, Mitochondrial membrane contact sites of yeast. Characterization of lipid components and possible involvement in intramitochondrial translocation of phospholipids, J. Biol. Chem. 266 (1991) 10047–10049.

[23] R. Hovius, H. Lambrechts, K. Nicolay, B. de Kruijff, Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane,, Biochim. Biophys. Acta (BBA)- Biomembranes 1021 (1990) 217–226.

[24] F. Gonzalvez, E. Gottlieb, Cardiolipin: setting the beat of apoptosis, Apoptosis 12 (2007) 877–885.

[25] R. Houtkooper, F. Vaz, Cardiolipin, the heart of mitochondrial metabolism, Cell. Mol. Life Sci. (CMLS) 65 (2008) 2493–2506.

[26] E.K.J. Tuominen, C.J.A. Wallace, P.K.J. Kinnunen, Phospholipid–cytochrome *c* interaction. evidence for extensive lipid anchorage, J. Biol. Chem. 277 (2002) 8822–8826.

[27] E.K.J. Tuominen, K. Zhu, C.J.A. Wallace, I. Clark-Lewis, D.B. Craig, M. Rytomaa, P.K.J. Kinnunen, ATP induces a conformational change in lipid-bound cytochrome *c*, J. Biol. Chem. 276 (2001) 19356–19362.

[28] M. Subramanian, A. Jutila, P.K.J. Kinnunen, Binding and dissociation of cytochrome *c* to and from membranes containing acidic phospholipids, Biochemistry 37 (1998) 1394–1402.

[29] L. Scorrano, M. Ashiya, K. Buttle, S. Weiler, S.A. Oakes, C.A. Mannella, S.J. Korsmeyer, A Distinct pathway remodels mitochondrial cristae and mobilizes cytochrome *c* during apoptosis, Dev. Cell 2 (2002) 55–67.

[30] C. Frezza, S. Cipolat, O. Martins de Brito, M. Micaroni, G.V. Beznoussenko, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, L. Scorrano, OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion, Cell 126 (2006) 177–189.

[31] R. Yamaguchi, L. Lartigue, G. Perkins, R.T. Scott, A. Dixit, Y. Kushnareva, T. Kuwana, M.H. Ellisman, D.D. Newmeyer, Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization, Mol. Cell 31 (2008) 557–569.

[32] S.L. Iverson, M. Enoksson, V. Gogvadze, M. Ott, S. Orrenius, Cardiolipin is not required for Bax-mediated cytochrome *c* release from yeast mitochondria, J. Biol. Chem. 279 (2004) 1100–1107.

[33] D.B. Ostrander, G.C. Sparagna, A.A. Amoscato, J.B. McMillin, W. Dowhan, Decreased cardiolipin synthesis corresponds with cytochrome *c* release in palmitate-induced cardiomyocyte apoptosis, J. Biol. Chem. 276 (2001) 38061–38067.

[34] K. Nomura, H. Imai, T. Koumura, T. Kobayashi, Y. Nakagawa, Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome *c* from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia-induced apoptosis, Biochem. J. 351 (2000) 183–193.

[35] M. Ott, J.D. Robertson, V. Gogvadze, B. Zhivotovsky, S. Orrenius, Cytochrome *c* release from mitochondria proceeds by a two-step process, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 1259–1263.

[36] G. Paradies, G. Petrosillo, M. Pistolese, F.M. Ruggiero, Reactive oxygen species generated by the mitochondrial respiratory chain affect the complex III activity via cardiolipin peroxidation in beef-heart submitochondrial particles, Mitochondrion 1 (2001) 151–159.

[37] G. Petrosillo, F.M. Ruggiero, G. Paradies, Role of reactive oxygen species and cardiolipin in the release of cytochrome *c* from mitochondria, FASEB J. 17 (2003) 2202–2208.

[38] Y.Y. Tyurina, V. Kini, V.A. Tyurin, I.I. Vlasova, J. Jiang, A.A. Kapralov, N.A. Belikova, J. C. Yalowich, I.V. Kurnikov, V.E. Kagan, Mechanisms of cardiolipin oxidation by cytochrome *c*: relevance to pro- and antiapoptotic functions of etoposide, Mol. Pharmacol. 70 (2006) 706–717.

[39] T. Kriska, W. Korytowski, A.W. Girotti, Role of mitochondrial cardiolipin peroxidation in apoptotic photokilling of 5-aminolevulinate-treated tumor cells, Arch. Biochem. Biophys. 433 (2005) 435–446.

[40] M. Enoksson, A.P. Fernandes, S. Prast, C.H. Lillig, A. Holmgren, S. Orrenius, Overexpression of glutaredoxin 2 attenuates apoptosis by preventing cytochrome *c* release, Biochem. Biophys. Res. Commun. 327 (2005) 774–779.

[41] Q. Ran, H. Liang, M. Gu, W. Qi, C.A. Walter, L.J. Roberts Roberts, B. Herman, A. Richardson, H. Van Remmen, Transgenic mice overexpressing glutathione peroxidase 4 are protected against oxidative stress-induced apoptosis, J. Biol. Chem. 279 (2004) 55137–55146.

[42] G. Stepanov, O. Gnedenko, A. Mol'nar, A. Ivanov, Y. Vladimirov, A. Osipov, Evaluation of cytochrome *c* affinity to anionic phospholipids by means of surface plasmon resonance, FEBS Lett. 583 (2009) 97–100.

[43] H. Bayir, B. Fadeel, M.J. Palladino, E. Witasp, I.V. Kurnikov, Y.Y. Tyurina, V.A. Tyurin, A.A. Amoscato, J. Jiang, P.M. Kochanek, S.T. DeKosky, J.S. Greenberger, A.A. Shvedova, V.E. Kagan, Apoptotic interactions of cytochrome *c*: redox flirting with anionic phospholipids within and outside of mitochondria, Biochim. Biophys. Acta (BBA)- Bioenergetics 1757 (2006) 648–659.

[44] V.E. Kagan, V.A. Tyurin, J. Jiang, Y.Y. Tyurina, V.B. Ritov, A.A. Amoscato, A.N. Osipov, N.A. Belikova, A.A. Kapralov, V. Kini, I.I. Vlasova, Q. Zhao, M. Zou, P. Di, D.A. Svistunenko, I.V. Kurnikov, G.G. Borisenko, Cytochrome *c* acts as a cardiolipin oxygenase required for release of proapoptotic factors, Nat. Chem. Biol. 1 (2005) 223–232.

[45] F. Sinibaldi, L. Fiorucci, A. Patriarca, R. Lauceri, T. Ferri, M. Coletta, R. Santucci, Insights into cytochrome *c*–cardiolipin interaction. Role played by ionic strength, Biochemistry 47 (2008) 6928–6935.

[46] M. Ott, B. Zhivotovsky, S. Orrenius, Role of cardiolipin in cytochrome *c* release from mitochondria, Cell Death Differ. 14 (2007) 1243–1247.

[47] E. Kalanxhi, C.J. Wallace, Cytochrome *c* impaled: investigation of the extended lipid anchorage of a soluble protein to mitochondrial membrane models, Biochem. J. 407 (2007) 179–187.

[48] H. Bayir, V.A. Tyurin, Y.Y. Tyurina, R. Viner, V. Ritov, A.A. Amoscato, Q. Zhao, X.J. Zhang, K.L. Janesko-Feldman, H. Alexander, L.V. Basova, R.S. Clark, P.M. Kochanek, V.E. Kagan, Selective early cardiolipin peroxidation after traumatic brain injury: an oxidative lipidomics analysis, Ann. Neurol. 62 (2007) 154–169.

[49] A.A. Kapralov, I.V. Kurnikov, VlasovaII , N.A. Belikova, V.A. Tyurin, L.V. Basova, Q. Zhao, Y.Y. Tyurina, J. Jiang, H. Bayir, Y.A. Vladimirov, V.E. Kagan, The hierarchy of structural transitions induced in cytochrome *c* by anionic phospholipids determines its peroxidase activation and selective peroxidation during apoptosis in cells, Biochemistry 46 (2007) 14232–14244.

[50] N.A. Belikova, Y.A. Vladimirov, A.N. Osipov, A.A. Kapralov, V.A. Tyurin, M.V. Potapovich, L.V. Basova, J. Peterson, I.V. Kurnikov, V.E. Kagan, Peroxidase activity and structural transitions of cytochrome *c* bound to cardiolipin-containing membranes, Biochemistry 45 (2006) 4998–5009.

[51] V.A. Tyurin, Y.Y. Tyurina, W. Feng, A. Mnuskin, J. Jiang, M. Tang, X. Zhang, Q. Zhao, P.M. Kochanek, R.S. Clark, H. Bayir, V.E. Kagan, Mass-spectrometric characterization of phospholipids and their primary peroxidation products in rat cortical neurons during staurosporine-induced apoptosis, J. Neurochem. 107 (2008) 1614–1633.

[52] S.M. Kapetanaki, G. Silkstone, I. Husu, U. Liebl, M.T. Wilson, M.H. Vos, Interaction of carbon monoxide with the apoptosis-inducing cytochrome *c*–cardiolipin complex, Biochemistry 48 (2009) 1613–1619.

[53] VlasovaII , V.A. Tyurin, A.A. Kapralov, I.V. Kurnikov, A.N. Osipov, M.V. Potapovich, D.A. Stoyanovsky, V.E. Kagan, Nitric oxide inhibits peroxidase activity of cytochrome *c*/cardiolipin complex and blocks cardiolipin oxidation, J. Biol. Chem. 281 (2006) 14554–14562.

[54] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, Physiol. Rev. 59 (1979) 527–605.

[55] J. Jiang, Z. Huang, Q. Zhao, W. Feng, N.A. Belikova, V.E. Kagan, Interplay between bax, reactive oxygen species production, and cardiolipin oxidation during apoptosis, Biochem. Biophys. Res. Commun. 368 (2008) 145–150.

[56] S. Devadas, J.A. Hinshaw, L. Zaritskaya, M.S. Williams, Fas-stimulated generation of reactive oxygen species or exogenous oxidative stress sensitize cells to Fas-mediated apoptosis, Free Radic. Biol. Med. 35 (2003) 648–661.

[57] E. Gottlieb, M.G. Vander Heiden, C.B. Thompson, Bcl-xL prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis, Mol. Cell Biol. 20 (2000) 5680–5689.

[58] M. Giorgio, E. Migliaccio, F. Orsini, D. Paolucci, M. Moroni, C. Contursi, G. Pelliccia, L. Luzi, S. Minucci, M. Marcaccio, P. Pinton, R. Rizzuto, P. Bernardi, F. Paolucci, P.G. Pelicci, Electron transfer between cytochrome *c* and $p66^{Shc}$ generates reactive oxygen species that trigger mitochondrial apoptosis, Cell 122 (2005) 221–233.

[59] J.M. de Winter, H.B. Lenting, F.W. Neys, H. van den Bosch, Hydrolysis of membrane-associated phosphoglycerides by mitochondrial phospholipase A2, Biochim. Biophys. Acta 917 (1987) 169–177.

[60] A.G. Buckland, A.R. Kinkaid, D.C. Wilton, Cardiolipin hydrolysis by human phospholipases A2. The multiple enzymatic activities of human cytosolic phospholipase A2, Biochim. Biophys. Acta 1390 (1998) 65–72.

[61] M. Ghosh, R. Loper, M.H. Gelb, C.C. Leslie, Identification of the expressed form of human cytosolic phospholipase A2beta (cPLA2beta): cPLA2beta3 is a novel variant localized to mitochondria and early endosomes, J. Biol. Chem. 281 (2006) 16615–16624.

[62] D.J. Mancuso, X. Han, C.M. Jenkins, J.J. Lehman, N. Sambandam, H.F. Sims, J. Yang, W. Yan, K. Yang, K. Green, D.R. Abendschein, J.E. Saffitz, R.W. Gross, Dramatic accumulation of triglycerides and precipitation of cardiac hemodynamic dysfunction during brief caloric restriction in transgenic myocardium expressing human calcium-independent phospholipase A2gamma, J. Biol. Chem. 282 (2007) 9216–9227.

[63] S.Y. Choi, P. Huang, G.M. Jenkins, D.C. Chan, J. Schiller, M.A. Frohman, A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis, Nat. Cell Biol. 8 (2006) 1255–1262.

[64] S. Bione, P. D'Adamo, E. Maestrini, A.K. Gedeon, P.A. Bolhuis, D. Toniolo, A novel X-linked gene, G4.5. is responsible for Barth syndrome, Nat. Genet. 12 (1996) 385–389.

[65] Y. Xu, A. Malhotra, M. Ren, M. Schlame, The enzymatic function of tafazzin, J. Biol. Chem. 281 (2006) 39217–39224.

[66] F. Gonzalvez, F. Pariselli, P. Dupaigne, I. Budihardjo, M. Lutter, B. Antonsson, P. Diolez, S. Manon, J.C. Martinou, M. Goubern, X. Wang, S. Bernard, P.X. Petit, tBid interaction with cardiolipin primarily orchestrates mitochondrial dysfunctions and subsequently activates Bax and Bak, Cell Death Differ. 12 (2005) 614–626.

[67] M. Sorice, A. Circella, I.M. Cristea, T. Garofalo, L.D. Renzo, C. Alessandri, G. Valesini, M.D. Esposti, Cardiolipin and its metabolites move from mitochondria to other cellular membranes during death receptor-mediated apoptosis, Cell Death Differ. 11 (2004) 1133–1145.

[68] F. Sandra, M. Degli Esposti, K. Ndebele, P. Gona, D. Knight, M. Rosenquist, R. Khosravi-Far, Tumor necrosis factor-related apoptosis-inducing ligand alters mitochondrial membrane lipids, Cancer Res. 65 (2005) 8286–8297.

[69] M. Lutter, M. Fang, X. Luo, M. Nishijima, X.-s. Xie, X. Wang, Cardiolipin provides specificity for targeting of tBid to mitochondria, Nat. Cell Biol. 2 (2000) 754–761.
[70] J. Liu, A. Weiss, D. Durrant, N.w. Chi, R.M. Lee, The cardiolipin-binding domain of Bid affects mitochondrial respiration and enhances cytochrome *c* release, Apoptosis 9 (2004) 533–541.
[71] F. Gonzalvez, Z.T. Schug, R.H. Houtkooper, E.D. MacKenzie, D.G. Brooks, R.J. Wanders, P.X. Petit, F.M. Vaz, E. Gottlieb, Cardiolipin provides an essential activating platform for caspase-8 on mitochondria, J. Cell Biol. 183 (2008) 681–696.
[72] M.D. Esposti, I.M. Cristea, S.J. Gaskell, Y. Nakao, C. Dive, Proapoptotic Bid binds to monolysocardiolipin, a new molecular connection between mitochondrial membranes and cell death, Cell Death Differ. 10 (2003) 1300–1309.
[73] J. Liu, D. Durrant, H.-S. Yang, Y. He, F.G. Whitby, D.G. Myszka, R.M. Lee, The interaction between tBid and cardiolipin or monolysocardiolipin, Biochem. Biophys. Res. Commun. 330 (2005) 865–870.
[74] N. Van Mau, A.V. Kajava, C. Bonfils, J.C. Martinou, M.C. Harricane, Interactions of Bax and tBid with lipid monolayers, J. Membr. Biol. 207 (2005) 1–9.
[75] T. Garofalo, R. Misasi, V. Mattei, A.M. Giammarioli, W. Malorni, G.M. Pontieri, A. Pavan, M. Sorice, Association of the death-inducing signaling complex with microdomains after triggering through CD95/Fas. Evidence for caspase-8–ganglioside interaction in T cells, J. Biol. Chem. 278 (2003) 8309–8315.
[76] M.D. Esposti, J.T. Erler, J.A. Hickman, C. Dive, Bid, a widely expressed proapoptotic protein of the Bcl-2 family, displays lipid transfer activity, Mol. Cell Biol. 21 (2001) 7268–7276.
[77] R.J. Youle, M. Karbowski, Mitochondrial fission in apoptosis, Nat. Rev., Mol. Cell Biol. 6 (2005) 657–663.
[78] S. Cipolat, T. Rudka, D. Hartmann, V. Costa, L. Serneels, K. Craessaerts, K. Metzger, C. Frezza, W. Annaert, L. D'Adamio, C. Derks, T. Dejaegere, L. Pellegrini, R. D'Hooge, L. Scorrano, B. De Strooper, Mitochondrial rhomboid PARL regulates cytochrome *c* release during apoptosis via OPA1-dependent cristae remodeling, Cell 126 (2006) 163–175.
[79] E. Gottlieb, OPA1 and PARL keep a lid on apoptosis, Cell 126 (2006) 27–29.
[80] T.-H. Kim, Y. Zhao, W.-X. Ding, J.N. Shin, X. He, Y.-W. Seo, J. Chen, H. Rabinowich, A.A. Amoscato, X.-M. Yin, Bid–cardiolipin interaction at mitochondrial contact site contributes to mitochondrial cristae reorganization and cytochrome *c* release, Mol. Biol. Cell 15 (2004) 3061–3072.
[81] T. Garofalo, A.M. Giammarioli, R. Misasi, A. Tinari, V. Manganelli, L. Gambardella, A. Pavan, W. Malorni, M. Sorice, Lipid microdomains contribute to apoptosis-associated modifications of mitochondria in T cells, Cell Death Differ. 12 (2005) 1378–1389.
[82] M. Karbowski, Y.-J. Lee, B. Gaume, S.-Y. Jeong, S. Frank, A. Nechushtan, A. Santel, M. Fuller, C.L. Smith, R.J. Youle, Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis, J. Cell Biol. 159 (2002) 931–938.
[83] R.F. Epand, J.-C. Martinou, M. Fornallaz-Mulhauser, D.W. Hughes, R.M. Epand, The apoptotic protein tBid promotes leakage by altering membrane curvature, J. Biol. Chem. 277 (2002) 32632–32639.
[84] S. Lucken-Ardjomande, S. Montessuit, J.C. Martinou, Contributions to Bax insertion and oligomerization of lipids of the mitochondrial outer membrane, Cell Death Differ. 15 (2008) 929–937.
[85] M.A. Sani, E.J. Dufourc, G. Grobner, How does the Bax-alpha1 targeting sequence interact with mitochondrial membranes? The role of cardiolipin, Biochim. Biophys. Acta 1788 (2009) 623–631.
[86] J.F. Lovell, L.P. Billen, S. Bindner, A. Shamas-Din, C. Fradin, B. Leber, D.W. Andrews, Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax, Cell 135 (2008) 1074–1084.
[87] L.P. Billen, C.L. Kokoski, J.F. Lovell, B. Leber, D.W. Andrews, Bcl-XL inhibits membrane permeabilization by competing with Bax, PLoS Biol. 6 (2008) e147.
[88] B. Schafer, J. Quispe, V. Choudhary, J.E. Chipuk, T.G. Ajero, H. Du, R. Schneiter, T. Kuwana, Mitochondrial outer membrane proteins assist Bid in Bax-mediated lipidic pore formation, Mol. Biol. Cell 20 (2009) 2276–2285.
[89] M. Karbowski, S.Y. Jeong, R.J. Youle, Endophilin B1 is required for the maintenance of mitochondrial morphology, J. Cell Biol. 166 (2004) 1027–1039.
[90] A. Etxebarria, O. Terrones, H. Yamaguchi, A. Landajuela, O. Landeta, B. Antonsson, H.G. Wang, G. Basanez, Endophilin B1/Bif-1 stimulates BAX activation independently from its capacity to produce large scale membrane morphological rearrangements, J. Biol. Chem. 284 (2009) 4200–4212.
[91] B. Habermann, The BAR-domain family of proteins: a case of bending and binding? EMBO Rep. 5 (2004) 250–255.
[92] N.W. Werneburg, M.E. Guicciardi, S.F. Bronk, S.H. Kaufmann, G.J. Gores, Tumor necrosis factor-related apoptosis-inducing ligand activates a lysosomal pathway of apoptosis that is regulated by Bcl-2 proteins, J. Biol. Chem. 282 (2007) 28960–28970.
[93] A.B. Hehl, A. Regos, E. Schraner, A. Schneider, Bax function in the absence of mitochondria in the primitive protozoan *Giardia lamblia*, PLoS ONE 2 (2007) e488.
[94] C. Scaffidi, S. Fulda, A. Srinivasan, C. Friesen, F. Li, K.J. Tomaselli, K.M. Debatin, P.H. Krammer, M.E. Peter, Two CD95 (APO-1/Fas) signaling pathways, EMBO J. 17 (1998) 1675–1687.
[95] V. Lacronique, A. Mignon, M. Fabre, B. Viollet, N. Rouquet, T. Molina, A. Porteu, A. Henrion, D. Bouscary, P. Varlet, V. Joulin, A. Kahn, Bcl-2 protects from lethal hepatic apoptosis induced by an ant-Fas antibody in mice, Nat. Med. 2 (1996) 80–86.
[96] A. Strasser, A.W. Harris, D.C. Huang, P.H. Krammer, S. Cory, Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis, EMBO J. 14 (1995) 6136–6137.
[97] X.-M. Yin, K. Wang, A. Gross, Y. Zhao, S. Zinkel, B. Klocke, K.A. Roth, S.J. Korsmeyer, Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis, Nature 400 (1999) 886–891.
[98] T. Lindsten, A.J. Ross, A. King, W.-X. Zong, J.C. Rathmell, H.A. Shiels, E. Ulrich, K.G. Waymire, P. Mahar, K. Frauwirth, Y. Chen, M. Wei, V.M. Eng, D.M. Adelman, M.C. Simon, A. Ma, J.A. Golden, G. Evan, S.J. Korsmeyer, G.R. MacGregor, C.B. Thompson, The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues, Mol. Cell 6 (2000) 1389–1399.
[99] T.J. Schneider, D. Grillot, L.C. Foote, G.E. Nunez, T.L. Rothstein, Bcl-x protects primary B cells against Fas-mediated apoptosis, J. Immunol. 159 (1997) 4834–4839.
[100] A.H. Stegh, B.C. Barnhart, J. Volkland, A. Algeciras-Schimnich, N. Ke, J.C. Reed, M.E. Peter, Inactivation of caspase-8 on mitochondria of Bcl-xL-expressing MCF7-Fas cells. Role for the bifunctional apoptosis regulator protein, J. Biol. Chem. 277 (2002) 4351–4360.
[101] A.H. Stegh, H. Herrmann, S. Lampel, D. Weisenberger, K. Andra, M. Seper, G. Wiche, P.H. Krammer, M.E. Peter, Identification of the cytolinker plectin as a major early in vivo substrate for caspase 8 during CD95- and tumor necrosis factor receptor-mediated apoptosis, Mol. Cell Biol. 20 (2000) 5665–5679.
[102] F.C. Kischkel, S. Hellbardt, I. Behrmann, M. Germer, M. Pawlita, P.H. Krammer, M. E. Peter, Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor, EMBO J. 14 (1995) 5579–5588.
[103] H. Zou, Y. Li, X. Liu, X. Wang, An APAF-1.cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9, J. Biol. Chem. 274 (1999) 11549–11556.
[104] A. Tinel, J. Tschopp, The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress, Science 304 (2004) 843–846.
[105] F. Martinon, K. Burns, J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta, Mol. Cell 10 (2002) 417–426.
[106] K. Milovic-Holm, E. Krieghoff, K. Jensen, H. Will, T.G. Hofmann, FLASH links the CD95 signaling pathway to the cell nucleus and nuclear bodies, EMBO J. 26 (2007) 391–401.
[107] J. Hiscott, R. Lin, P. Nakhaei, S. Paz, MasterCARD: a priceless link to innate immunity, Trends Mol. Med. 12 (2006) 53–56.
[108] C.L. Johnson, M. Gale Jr, CARD games between virus and host get a new player, Trends Immunol. 27 (2006) 1–4.
[109] M. Rebsamen, E. Meylan, J. Curran, J. Tschopp, The antiviral adaptor proteins Cardif and Trif are processed and inactivated by caspases, Cell Death Differ. (2008).
[110] K. Takahashi, T. Kawai, H. Kumar, S. Sato, S. Yonehara, S. Akira, Roles of caspase-8 and caspase-10 in innate immune responses to double-stranded RNA, J. Immunol. 176 (2006) 4520–4524.
[111] R.A. Kirkland, R.M. Adibhatla, J.F. Hatcher, J.L. Franklin, Loss of cardiolipin and mitochondria during programmed neuronal death: evidence of a role for lipid peroxidation and autophagy, Neuroscience 115 (2002) 587–602.
[112] C.R. Hackenbrock, Chemical and physical fixation of isolated mitochondria in low-energy and high-energy states, Proc. Natl. Acad. Sci. 61 (1968) 598–605.
[113] A.S. Reichert, W. Neupert, Contact sites between the outer and inner membrane of mitochondria-role in protein transport, Biochim. Biophys. Acta (BBA)- Mol. Cell Res. 1592 (2002) 41–49.
[114] D. Ardail, J.P. Privat, M. Egret-Charlier, C. Levrat, F. Lerme, P. Louisot, Mitochondrial contact sites. Lipid composition and dynamics, J. Biol. Chem. 265 (1990) 18797–18802.
[115] A. Ortiz, J.A. Killian, A.J. Verkleij, J. Wilschut, Membrane fusion and the lamellar-to-inverted-hexagonal phase transition in cardiolipin vesicle systems induced by divalent cations, Biophys. J. 77 (1999) 2003–2014.
[116] L. Aguilar, G. Ortega-Pierres, R. Fonseca, M. Ibanez, C. Wong, N. Farfan, J.M. Naciff, M.A. Kaetzel, J.R. Dedman, I. Baeza, Phospholipid membranes form specific nonbilayer molecular arrangements that are antigenic, J. Biol. Chem. 274 (1999) 25193–25196.
[117] M.T. Grijalba, A.E. Vercesi, S. Schreier, Ca^{2+}-induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca^{2+}-stimulated generation of reactive oxygen species by the respiratory chain,, Biochemistry 38 (1999) 13279–13287.
[118] J. Antoinette Killian, M.C. Koorengevel, J.A. Bouwstra, G. Gooris, W. Dowhan, B. de Kruijff, Effect of divalent cations on lipid organization of cardiolipin isolated from *Escherichia coli* strain AH930, Biochim. Biophys. Acta (BBA)- Biomembr. 1189 (1994) 225–232.
[119] U. Schlattner, F. Gehring, N. Vernoux, M. Tokarska-Schlattner, D. Neumann, O. Marcillat, C. Vial, T. Wallimann, C-terminal lysines determine phospholipid interaction of sarcomeric mitochondrial creatine kinase, J. Biol. Chem. 279 (2004) 24334–24342.
[120] O. Maniti, M.F. Lecompte, O. Marcillat, B. Desbat, R. Buchet, C. Vial, T. Granjon, Mitochondrial creatine kinase binding to phospholipid monolayers induces cardiolipin segregation, Biophys. J. 96 (2009) 2428–2438.
[121] R.F. Epand, M. Tokarska-Schlattner, U. Schlattner, T. Wallimann, R.M. Epand, Cardiolipin clusters and membrane domain formation induced by mitochondrial proteins, J. Mol. Biol. 365 (2007) 968–980.
[122] O. Speer, N. Back, T. Buerklen, D. Brdiczka, A. Koretsky, T. Wallimann, O. Eriksson, Octameric mitochondrial creatine kinase induces and stabilizes contact sites between the inner and outer membrane, Biochem. J. 385 (2005) 445–450.
[123] V.E. Kagan, Y.Y. Tyurina, H. Bayir, C.T. Chu, A.A. Kapralov, I.I. Vlasova, N.A. Belikova, V.A. Tyurin, A. Amoscato, M. Epperly, J. Greenberger, S. DeKosky, A.A.

Shvedova, J. Jiang, The "pro-apoptotic genies" get out of mitochondria: oxidative lipidomics and redox activity of cytochrome *c*/cardiolipin complexes, Chemico-Biological Interact. 163 (2006) 15–28.
[124] M.G. Fernandez, L. Troiano, L. Moretti, M. Nasi, M. Pinti, S. Salvioli, J. Dobrucki, A. Cossarizza, Early changes in intramitochondrial cardiolipin distribution during apoptosis, Cell Growth Differ. 13 (2002) 449–455.
[125] V.A. Tyurin, Y.Y. Tyurina, A.N. Osipov, N.A. Belikova, L.V. Basova, A.A. Kapralov, H. Bayir, V.E. Kagan, Interactions of cardiolipin and lyso-cardiolipins with cytochrome *c* and tBid: conflict or assistance in apoptosis, Cell Death Differ. 14 (2006) 872–875.
[126] R.F. Epand, J.-C. Martinou, S. Montessuit, R.M. Epand, Membrane perturbations induced by the apoptotic Bax protein, Biochem. J. 367 (2002) 849–855.
[127] A. Goonesinghe, E.S. Mundy, M. Smith, R. Khosravi-Far, J.C. Martinou, M.D. Esposti, Pro-apoptotic Bid induces membrane perturbation by inserting selected lysolipids into the bilayer, Biochem. J. 387 (2005) 109–118.
[128] V.E. Kagan, H.A. Bayir, N.A. Belikova, O. Kapralov, Y.Y. Tyurina, V.A. Tyurin, J. Jiang, D.A. Stoyanovsky, P. Wipf, P.M. Kochanek, J.S. Greenberger, B. Pitt, A.A. Shvedova, G. Borisenko, Cytochrome *c*/cardiolipin relations in mitochondria: a kiss of death, Free Radic. Biol. Med. 46 (11) (2009) 1439–1453.
[129] S.K. Sahu, S.N. Gummadi, N. Manoj, G.K. Aradhyam, Phospholipid scramblases: an overview, Arch. Biochem. Biophys. 462 (2007) 103–114.
[130] J. Liu, J. Chen, Q. Dai, R.M. Lee, Phospholipid scramblase 3 is the mitochondrial target of protein kinase C {delta}-induced apoptosis, Cancer Res. 63 (2003) 1153–1156.
[131] J. Liu, Q. Dai, J. Chen, D. Durrant, A. Freeman, T. Liu, D. Grossman, R.M. Lee, Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response, Mol. Cancer Res. 1 (2003) 892–902.
[132] A.L. Wozniak, X. Wang, E.S. Stieren, S.G. Scarbrough, C.J. Elferink, D. Boehning, Requirement of biphasic calcium release from the endoplasmic reticulum for Fas-mediated apoptosis, J. Cell Biol. 175 (2006) 709–714.
[133] G. Csordás, M. Madesh, B. Antonsson, G. Hajnóczky, tcBid promotes Ca^{2+} signal propagation to the mitochondria: control of Ca^{2+} permeation through the outer mitochondrial membrane, EMBO J. 21 (2002) 2198–2206.
[134] R. Rizzuto, P. Pinton, W. Carrington, F.S. Fay, K.E. Fogarty, L.M. Lifshitz, R.A. Tuft, T. Pozzan, Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses, Science 280 (1998) 1763–1766.
[135] K. Ndebele, P. Gona, T.-G. Jin, N. Benhaga, A. Chalah, M. Degli-Esposti, R. Khosravi-Far, Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) induced mitochondrial pathway to apoptosis and caspase activation is potentiated by phospholipid scramblase-3, Apoptosis 13 (2008) 845–856.
[136] S. Orrenius, B. Zhivotovsky, P. Nicotera, Regulation of cell death: the calcium-apoptosis link, Nat. Rev., Mol. Cell Biol. 4 (2003) 552–565.
[137] S. Joseph, G. Hajnóczky, IP3 receptors in cell survival and apoptosis: Ca^{2+} release and beyond, Apoptosis 12 (2007) 951–968.
[138] N.C. Robinson, J. Zborowski, L.H. Talbert, Cardiolipin-depleted bovine heart cytochrome *c* oxidase: binding stoichiometry and affinity for cardiolipin derivatives, Biochemistry 29 (1990) 8962–8969.
[139] V. Yankovskaya, R. Horsefield, S. Tornroth, C. Luna-Chavez, H. Miyoshi, C. Leger, B. Byrne, G. Cecchini, S. Iwata, Architecture of succinate dehydrogenase and reactive oxygen species generation, Science 299 (2003) 700–704.
[140] K.S. Eble, W.B. Coleman, R.R. Hantgan, C.C. Cunningham, Tightly associated cardiolipin in the bovine heart mitochondrial ATP synthase as analyzed by 31P nuclear magnetic resonance spectroscopy, J. Biol. Chem. 265 (1990) 19434–19440.
[141] S. Li, Y. Zhao, X. He, T.-H. Kim, D.K. Kuharsky, H. Rabinowich, J. Chen, C. Du, X.-M. Yin, Relief of extrinsic pathway inhibition by the Bid-dependent mitochondrial release of Smac in Fas-mediated hepatocyte apoptosis, J. Biol. Chem. 277 (2002) 26912–26920.
[142] Y. Lin, A. Devin, Y. Rodriguez, Z.-g. Liu, Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis, Genes Dev. 13 (1999) 2514–2526.
[143] T. Adachi, W.W. Schamel, K.M. Kim, T. Watanabe, B. Becker, P.J. Nielsen, M. Reth, The specificity of association of the IgD molecule with the accessory proteins BAP31/BAP29 lies in the IgD transmembrane sequence, EMBO J. 15 (1996) 1534–1541.
[144] F.W.H. Ng, M. Nguyen, T. Kwan, P.E. Branton, D.W. Nicholson, J.A. Cromlish, G.C. Shore, p28 Bap31, a Bcl-2/Bcl-XL- and procaspase-8-associated protein in the endoplasmic reticulum, J. Cell Biol. 139 (1997) 327–338.
[145] M. Nguyen, D.G. Breckenridge, A. Ducret, G.C. Shore, Caspase-resistant BAP31 inhibits Fas-mediated apoptotic membrane fragmentation and release of cytochrome *c* from mitochondria, Mol. Cell Biol. 20 (2000) 6731–6740.
[146] D.G. Breckenridge, M. Stojanovic, R.C. Marcellus, G.C. Shore, Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome *c* release to the cytosol, J. Cell Biol. 160 (2003) 1115–1127.
[147] S. Frank, B. Gaume, E.S. Bergmann-Leitner, W.W. Leitner, E.G. Robert, F. Catez, C.L. Smith, R.J. Youle, The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis, Dev. Cell 1 (2001) 515–525.
[148] C. Sheridan, P. Delivani, S.P. Cullen, S.J. Martin, Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome *c* release, Mol. Cell 31 (2008) 570–585.
[149] J.P. Mathai, M. Germain, G.C. Shore, 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 (2005) 23829–23836.
[150] G. Szalai, R. Krishnamurthy, G. Hajnoczky, Apoptosis driven by IP(3)-linked mitochondrial calcium signals, EMBO J. 18 (1999) 6349–6361.
[151] P. Pinton, D. Ferrari, E. Rapizzi, F. Di Virgilio, T. Pozzan, R. Rizzuto, 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 (2001) 2690–2701.