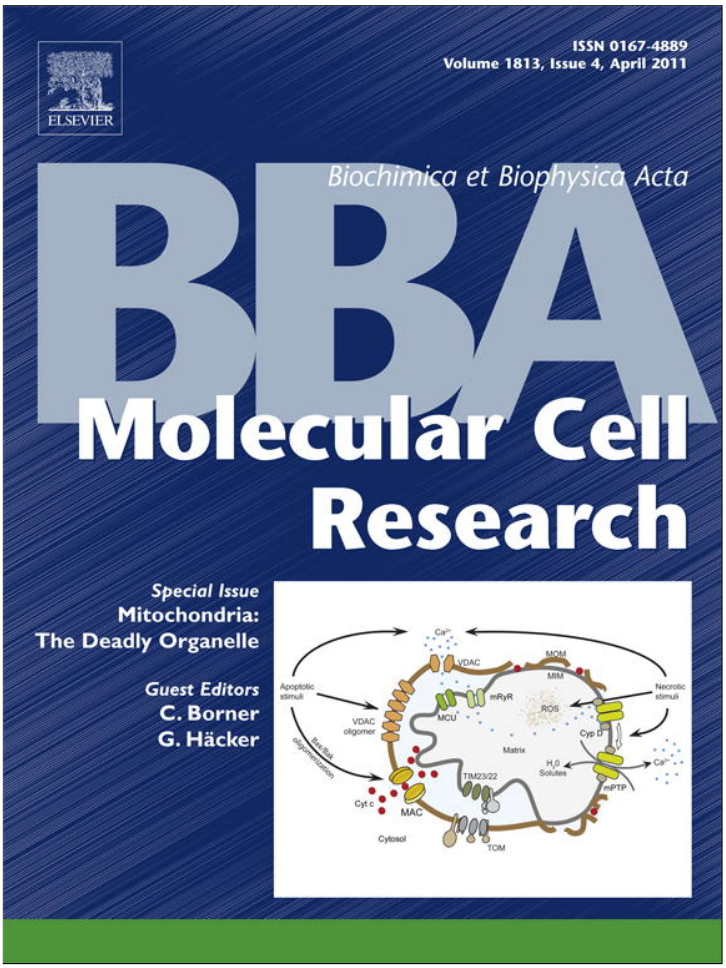

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Review

Apoptosis-induced changes in mitochondrial lipids ☆

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ABSTRACT

Apoptosis is an active and tightly regulated form of cell death, which can also be considered a stress-induced process of cellular communication. Recent studies reveal that the lipid network within cells is involved in the regulation and propagation of death signalling. Despite the vast growth of our current knowledge on apoptosis, little is known of the specific role played by lipid molecules in the central event of apoptosis—the piercing of mitochondrial membranes. Here we review the information regarding changes in mitochondrial lipids that are associated with apoptosis and discuss whether they may be involved in the permeabilization of mitochondria to release their apoptogenic factors, or just lie downstream of this permeabilization leading to the amplification of caspase activation. We focus on the earliest changes that physiological apoptosis induces in mitochondrial membranes, which may derive from an upstream alteration of phospholipid metabolism that reverberates on the mitochondrial re-modelling of their characteristic lipid, cardiolipin. Hopefully, this review will lead to an increased understanding of the role of mitochondrial lipids in apoptosis and also help revealing new stress sensing mechanisms in cells. This article is part of a Special Issue entitled Mitochondria: the deadly organelle.

1. Background and scope

It is now established that mitochondria play a central role in all major pathways of cell death. The best characterized programs of cell death are autophagy and apoptosis, with its two intersecting major pathways of death receptor-mediated and mitochondria-mediated signalling. There is an intriguing inter-dependence of death programs, so that cells in which apoptosis is impaired often die by autophagy, and vice-versa [1]. The major link that connects both programs seems to be the mitochondrion and, in particular, its membranes [1,2]. The purpose of this article is to provide an updated overview of how the lipid components of mitochondrial membranes become involved in apoptosis and may contribute to the regulation and propagation of death signalling. Besides the complex protein network that contributes to death signalling, there is also the network formed by the metabolic and traffic connections of membrane lipids. While the study of the protein network is advanced, the understanding of the role of the lipid network is limited, even if its importance is evident. Indeed, both apoptosis and autophagy require specific lipids in upstream reactions of their pathways and subsequent processes that ultimately characterize cell destruction. For instance, macroscopic changes of cellular lipids sustain the sequence of blebbing and formation of apoptotic bodies in cells dying by apoptosis, whereas proliferation of intracellular lipids drives the formation of autophagosomal membranes in autophagy—as well as other form of death not requiring caspases [1,3]. Mitochondria play a central role in the network of cellular lipids, especially at the level of their outer membrane (OM), which is a crucial crossover in lipid metabolism. Here the key steps of lipid degradation and fatty acid synthesis occur, together with reactions that are essential for the biosynthesis of phosphatidyl-ethanolamine (PE), of ceramide-based lipids and also of mature cardiolipin (CL). Notably, CL is the only membrane component that is synthesized by mitochondria, but in immature forms that are then rapidly re-modelled by reactions pivoting on the OM [3,4].

Herein, we will focus on the changes of mitochondrial lipids–especially phospholipids–that occur during apoptosis, which could affect the physico-chemical properties of the OM and the pro-apoptotic action of Bcl-2 proteins that specifically interact with this membrane, leading to its permeabilization (mitochondrial outer membrane permeabilization, MOMP). We will discuss selected new points regarding CL, since previous reviews have amply covered various aspects of its involvement in cell death [3–5]. To sustain these points, we will present unpublished data from our ongoing research, illustrating how little we still know about the involvement of mitochondrial lipids in cell death.

2. Changes in mitochondrial membrane lipids during apoptosis

Several membranes actively participate in the process of cell death through changes in their lipid composition and potentially also after

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intermixing of different organelles [3,6]. The first lipid identified to have a role in apoptosis is phosphatidylserine (PS), which is now established to act as a "eat me" signal for macrophages once exposed at the surface of apoptotic cells [7]. Externalization of PS is predominantly driven by activated caspases and therefore is frequently used as an apoptotic marker of caspase-dependent death. However, transient PS exposure can occur independently of caspase activation, for instance in cell motility [8] and platelet activation [9]. Although cellular PS is predominantly synthesized in the mitochondria-associated membranes of the endoplasmic reticulum (ER), there seems to be no evidence of significant changes in mitochondrial PS during apoptosis, except for situations of oxidative stress.

Oxidative stress is one of the most used triggers of apoptosis because it bears relevance to a number of pathological conditions, for instance neurodegeneration [1]. Conditions of oxidative stress invariably increase the peroxidation of mitochondrial lipids [10,11]. In addition, apoptosis induced by diverse stimuli is frequently accompanied by the generation of peroxidized lipids, generally grouped within reactive oxygen species (ROS). Some of these peroxidized phospholipids, especially the truncated forms of phosphatidylcholine (PC), directly induce apoptosis in certain cellular contexts [12]. Preferential oxidation of PS has been shown to occur after apoptosis induction with pro-oxidants, including organic hydroperoxides, paraquat, and azo-initiators of peroxyl radicals (for a review, see [11]). A likely reason for this preferential peroxidation of PS, which is frequently correlated to its externalization during apoptosis [13], is its high content of poly-unsaturated fatty acids [14,15]. Here we will not further discuss oxidative changes of mitochondrial lipids, which are amply reviewed by Kagan and co-workers [11], also because they occur downstream MOMP and the activation of executioner caspases during death receptor-mediated apoptosis [3,16–19]. Ceramide is another lipid that has been extensively studied for its implications in signalling and apoptosis [20,21]. Cellular ceramide levels increase in response to a variety of apoptotic stimuli (e.g. TNFα, Fas ligand, IFNγ, UV irradiation and DNA damage) and have been reported to produce alterations in bioenergetics, ROS generation and also permeabilization of the mitochondrial OM [20,21]. However, the latter effect is unclear since activation of sphingomyelinases, the upstream event responsible for the transient accumulation of ceramide during apoptosis, has been shown to be caspase-dependent [22]. In contrast with the reports of others [20,21], our studies have indeed failed to detect a significant increase in the levels of major ceramide species in mitochondria before the activation of executioner caspases [23,24]. Conversely GD3, a ceramide-based glycolipid, has been reported to accumulate in mitochondria (Table 1, see also [25]).

Although ceramide and its related glycolipids have been reported to be involved in apoptosis [20–22,25], these lipids are only minor components of mitochondrial membranes that remain confined to the OM, together with lipids typical of the ER/plasmamembrane such as cholesterol [26]. The major components of mitochondrial membranes are phospholipids and numerous results document changes in their content or chemistry during death receptor-mediated apoptosis—the most physiological model of cell death. We have summarized these changes in Table 1, also in relation to the timing of the MOMP for outlining their possible involvement in this central event of apoptosis signalling. Some lipid changes associated with death receptor-mediated apoptosis occur also with intrinsic stimuli, in particular treatment with exogenous lipids (Table 1). For instance palmitate, a saturated (16:0) fatty acid found in plasma at high concentrations during ischemia and reperfusion, has been observed to induce apoptosis in many biological systems, including cardiomyocytes and breast cancer cells [27,28]. At the level of mitochondria, treatment with palmitate decreases the complement of CL, primarily due to a diminished concentration of fatty acid precursors needed for optimal

Table 1
Changes in mitochondrial lipids during apoptosis. In *italics* are those changes that appear to occur before or concomitantly with the onset of MOMP.

Lipid species	Cell system	Death stimulus	CHANGE	Timing vs. MOMP	Reference
Linoleyl-PC[a]	Mouse liver	Fas ligation	decrease	*Before*	[b]
Oleyl-PC[a]	Jurkat T	Fas ligation	decrease	*Before*	[24], [b]
LPC	Mouse liver	Fas ligation	increase	Before and after	[24]
LPC	Jurkat T	Fas ligation	increase	Before and after	[3,24]
LPC	Jurkat T	TRAIL	increase	Before and after	[23]
DAG	Mouse liver	Fas ligation	increase	Before and After	[3]
DAG	Jurkat T	Fas ligation	increase	*Before*	[24], [b]
DAG	U937	Fas ligation	increase	*Before*	[19]
MCL	Mouse liver	Fas ligation	increase	Before and after	[3,30]
MCL	Jurkat T	Fas ligation	increase	Before and after	[30]
MCL	Jurkat T	TRAIL	increase	Before and after	[23]
MCL	U937	Fas ligation	increase	Before and after	[19]
Cardiolipins	Mouse liver	Fas ligation	decrease	Before and after	[3,30]
Cardiolipins	Jurkat T	Fas ligation	decrease	Before and after	[30,100]
Cardiolipins	Jurkat T	TRAIL	decrease	Before and after	[23]
Cardiolipins	U937	Fas ligation	decrease	Before and after	[19]
Cardiolipins	Cardiomyocytes	palmitate	decrease	Not tested	[29]
Cardiolipins	Granulosa	stearate	decrease	Not tested	[29]
Cardiolipin	Neurones	PC starvation	decrease	Not tested	[10]
LPC	Jurkat T	Erucyl-PC	increase	*Before*	[b]
Oleyl-PC+	Jurkat T	Erucyl-PC	decrease	*Before*	[b]
Cardiolipins	Jurkat T	Erucyl-PC	decrease	Before and after	[b]
Phosporylated PI	Jurkat T	Erucyl-PC	increase	Before and after	[b]
Truncated ox PC	Mouse liver	Truncated ox PC	increase	Before and after	[12]
Phosporylated PI	Mouse liver	Fas ligation	increase	Before and after	[3]
Phosporylated PI	U937	Fas ligation	increase	Before and after	[19], [b]
Acyl-PG/SLBPA	Mouse liver	Fas ligation	increase	After	[19], [b]
Acyl-PG/SLBPA	Jurkat T	Fas ligation	increase	After	[30], [b]
Acyl-PG/SLBPA	U937	Fas ligation	increase	Before and after	[19]
Peroxidized CL	Various cells	Pro-oxidants	increase	Before and after	[11]
Peroxidized PS	A549 cells	Fas ligation	increase	After	[18]
GD3	Jurkat, U937	Fas ligation	increase	After?	[25]

[a] While in most mammalian tissues linoleyl (C18:2) is the predominant acyl species of CL, in haematopoietic cells like Jurkat oleyl (C18:1) is more common [4]; PC species reflect the same profile.
[b] M. Degli Esposti, unpublished.

CL synthesis [28]. Similar effects have been documented for the treatment of human granulosa cells with another saturated fatty acid, stearate (C18:0) [29].

The summary presented in Table 1 indicates that only a few of the lipid changes associated with apoptosis appear to occur before MOMP. It is important to elaborate on these changes, because they could be instrumental in the process of MOMP itself, by "priming" for the pro-apoptotic action of Bcl-2 proteins [3,30,31]. Within the model of death receptor apoptosis, the most prominent early change is the increase in two common lysolipids, C16:0 and C18:1 lysophosphatidyl-choline (LPC) [3,24]. The majority of this LPC is likely to originate outside mitochondria and then accumulate in the OM. Even if detailed studies on the lipid composition of the OM are missing, this possibility is supported by robust data showing an early increase in LPC–with a parallel decrease of major PC species such as C16:0,C18:2 PC (linoleyl PC, Table 1)–in light membrane fractions comprising ER and endosomes. These changes occur within 30 min of Fas stimulation in both liver and haematopoietic cells and seem to derive from an early inhibition of phosphocholine cytidylyltransferase (CCT), the rate-limiting enzyme in PC biosynthesis [32]. Indeed, inhibition of CCT with alkyl-lysoPC derivatives such as erucyl-PC [33] reproduces, in exacerbated form, the same changes observed in intracellular membranes during Fas-mediated apoptosis (Table 1). Since diacyl-glycerol (DAG) is a substrate of CCT, its early accumulation in mitochondria (Table 1) is consistent with upstream inhibition of CCT activity in the ER [32]. The absence of phospholipase activation in mitochondria [3] further supports the extra-mitochondrial origin of the LPC species that accumulate in the mitochondrial membranes of apoptotic cells.

The other change that appears to occur before MOMP is an increase in the first metabolite of CL, monolysocardiolipin (MCL), often concomitantly with the initial loss of CL (Table 1 cf. [30]). Given that degradation of CL to MCL is normally catalyzed by lysosomal phospholipases, which are unlikely to be released from lysosomes before MOMP [34], the increase in MCL could be connected to that of LPC and the parallel decrease of parent CL and PC species by enhanced re-modelling of cardiolipin [3,4]. This process, which occurs predominantly in the OM [4], could well be stimulated by the upstream deficit in PC biosynthesis mentioned above, because PC is the fundamental donor of the unsaturated acyl groups that are required to re-model nascent CL into its mature, dominant forms present in cells [4,35]. The scenario that thus emerges is that death receptor activation may first induce a block in PC biosynthesis at the level of the plasmamembrane and its parts internalized within the endosomal and ER compartments, which then reverberates on the OM by dynamic transport of lipid species [36], as well as inter-mixing with the membranes of other organelles [6]. The latter possibility is supported by the early accumulation of protein markers of endosomes and the intermediate compartment ERGIC in mitochondria [3,6,37,38]. On the other hand, the transport of LPC to mitochondria could be catalyzed by Bid, a pro-apoptotic Bcl-2 protein which has intrinsic capacity of transferring this and other lipids between ER and mitochondrial membranes [24,36,39,40]. Strong evidence has been recently reported for the lipid transfer activity of Bid using truncated oxidized PC compounds, which structurally resemble LPC and accumulate in a Bid-dependent manner within mitochondria [12].

In summary, we are beginning to understand the early changes in mitochondrial lipids that occur before, or concomitantly with MOMP. They relate to upstream alterations in lipid metabolism that reverberate onto mitochondria via membrane traffic routes and the lipid transfer action of Bid, which indeed accumulates in mitochondria of apoptotic cells (reviewed in [3]). Thus, changes in lipid metabolism together with protein- and vesicles-mediated lipid transport can also act as a stress-sensing mechanism. Subsequent changes in mitochondrial lipids (Table 1) may amplify the damage to the organelle, or simply reflect downstream processes that spontaneously follow the early changes, but cannot substantially contribute to the MOMP.

3. Lipid changes vs. membrane structure (curvature and lipid pores)

As we have seen, apoptosis induces specific changes in the lipid composition of the plasma membrane and various intracellular membranes. However, the most critical changes during apoptosis occur in mitochondria, where they favour the permeabilization of the OM to release apoptogenic factors into the cytoplasm. While membrane-associated proteins induce and regulate structural changes in intracellular membranes, lipids play a fundamental role in facilitating these changes within the membrane architecture. The chemical properties of different lipid acyl chains or headgroups can favour different membrane curvatures: for example, lysophosphatidic acid (LPA) and phosphatidic acid (PA), which are converted by LPA:acyl transferase and phospholipase A2 activity, respectively, favour opposite curvatures [41–43]. Non-lamellar lipids with a large effective hydrophilic part relative to the effective hydrophobic part, exemplified by LPC, possess positive intrinsic curvature, whereas non-lamellar lipids with a small effective hydrophilic part relative to their hydrophobic part, such as unsaturated species of PE and DAG, possess negative intrinsic curvature [44,45]. Notably, both LPC and DAG increase early in mitochondria after stimulation of death receptors (Table 1). Interaction of Bax with lipid vesicles is partially modulated by changes in membrane curvature due to different combinations of the above lipids [31,44–46]. It is also known that mitochondrial lipids will convert from a lamellar to a hexagonal phase in the presence of Ca^{+2} [47]. However, on balance the effect of membrane curvature seems to be less important than other aspects of membrane lipids for generating MOMP *in vivo* [48].

How do lipids contribute to MOMP then? The best models are bacterial toxins that permeate lipid membranes, given the structural similarity of Bcl-2 proteins with some of these toxins [50]. Although ceramide has been shown to form channels in the OM [21], pure lipid pores are rare in nature. Conversely, various bacterial toxins recruit specific lipids to form proteolipid aggregates that produce discontinuities in the lipid bi-layer (reviewed in [3,51]). Whenever these discontinuities are maintained for a time longer than that taken by the later mobility of the lipids to fill the gap, the permeability barrier is lost and diverse molecules can cross the membrane in both directions. In analogy with bacterial toxins, conformational changes and oligomerization of pro-apoptotic Bid [52,53] and Bax [54] could provide the driving force to produce discontinuities leading to membrane permeabilization—namely "proteolipid pores" [31,49]. These structural changes have been documented to occur with detergents (reviewed in the article of Lindsay et al in this BBA issue) and membrane lipids [40,45,52,55–62]. By considering the early changes observed in mitochondrial membranes (Table 1), CL, DAG and lysolipids are the membrane lipids most likely to be involved in the piercing of the OM by activated Bcl-2 proteins. As we will now see, these lipids are interconnected by metabolic pathways of CL re-modelling. While CL has been extensively studied, little is known of the potential roles of lysolipids in MOMP. We will thus review existing information on lysolipids in cell death, before discussing recent literature on CL.

4. Lysolipids and cell death

Lysolipids not only are essential metabolites of membrane lipids, but also natural detergents. Ceramides have detergent-like properties too [21]. As detergents, these natural lipids can disrupt the integrity of the lipid bi-layer and participate to dynamic changes in the organization of intracellular membrane organelles, including mitochondria. Concentrations of lysolipids, or ceramides, above their critical micellar concentration can be cytotoxic, as they break the plasmamembrane like detergents [63]. This effect would apply also to various cell-free assays that use isolated mitochondria and preparations of recombinant tBid that are kept in solution with non-ionic

detergents in micellar state [52,64–67]. However, truncated lysoPC [12] and alkyl-lysoPC derivatives [33] can be cytotoxic even at sub-micellar concentrations, or in the presence of medium albumin that normally sequesters most of the lipid in solution. They are incorporated by endocytosis in the traffic of cellular membranes and can interfere with the activity of lipid degrading enzymes [68,69], or the structure and function of mitochondrial membranes [12].

Conversely, natural LPC can be beneficial to cells in culture, which rely in precursors obtained from the growth medium for the essential *de novo* biosynthesis of PC [10,32,69,70]. Exogenous LPC, once incorporated within cellular membrane, becomes immediately acylated by acyl-transferase enzymes using endogenous lipids as donors, thereby producing new PC molecules by scavenging other phospholipids, or triglycerides [32]. Indeed, exogenous LPC can correct the PC deficiency produced by CCT inhibition [69]. The same PC deficiency, when not supported by exogenous biosynthetic sources, leads to rapid accumulation of LPC within cells, as in the case of Fas-mediated apoptosis mentioned earlier (Table 1). Another way of increasing LPC is by the enhanced activity of phospholipases that generally occurs downstream of caspase activation [71–73]. Some of the LPC produced in this way is then secreted outside cells, providing an important long distance signal to attract phagocytes towards apoptotic cells [72,73].

The alternative mechanism explaining the increased levels of LPC (and other lysolipids, cf. Table 1) in mitochondria would be an enhanced re-modelling of CL, which primarily requires PC species as acyl donors [4,74]. It is now established that this remodelling fundamentally follows a transacylation reaction, which uses PC species resident in the OM or arriving from the ER to re-acylate MCL formed by hydrolysis of the immature CL species that are produced in the inner mitochondrial membranes [4]. LPC is the product of the transacylation of MCL to CL and is normally removed from the reaction by re-acylation by acyl-CoA-dependent acyl-transferases [4]. However, upstream deficiency in PC production and subsequent accumulation of extra-mitochondrial LPC in the OM will inevitably shift the equilibrium of the transacylation towards the formation of MCL and PC. The pattern of changes observed in mitochondria of Fas-stimulated cells seems to match the expected alteration in this transacylation reaction of CL re-modelling, because LPC increases in parallel with both an initial decrease in CL and an increase in MCL (Table 1). The consequence of a reverse transacylation of CL to LPC would also be a diverse spectrum of PC species in mitochondria, a situation that is found in several models of apoptosis (Table 1 and M. Degli Esposti, unpublished data). An additional increase in MCL species may derive from its limited re-acylation by other routes, e.g. those present in the ER [3]. Furthermore, the increased levels of DAG may stimulate the biosynthesis of phosphatidyl-glycerol (PG) and its acyl-derivatives, which indeed accumulate in mitochondria of apoptotic cells (Table 1, cf. [19]).

Intriguingly, Bid binds to both LPC and CL as well as MCL, thereby providing a link between metabolic alterations in PC biosynthesis and CL re-modelling in the OM [30]. This link has also a catalytic dimension because Bid is capable of transporting LPC between different membranes [24,36,39]. The Bid-mediated insertion of LPC into membranes produces transient perturbation of the lipid bi-layer [24], similarly to the effect of activated Bax [45,46,48,75]. However, this perturbation is generally insufficient to produce enough membrane de-stabilization to abrogate the permeability barrier of liposomes or mitochondria. Something else has to be present in the membrane to allow Bid to bind firmly and then facilitate the interaction of Bax or Bak with the lipid bi-layer leading to the disruption of its continuity. CL and/or MCL can constitute this essential component because: a) Bid binds to these lipids, also in exchange with LPC (as shown in Fig. 1, cf. [24,40]); b) binding to mitochondrial CL/MCL induces stable oligomerization of Bid [30]. LPC and certain detergents such as SDS also induce some oligomerization of Bid, but only at micellar concentrations [40,52]), similarly to Bax [54,76] and Bcl-xL [77]. In contrast, CL and MCL produce stable, discrete oligomers of Bid that match those found after adding the protein to mitochondria or their lipid extracts (M. Degli Esposti, unpublished data, cf. Ref. [30]). Of note, other lysolipids do not induce the same oligomerization of Bid, indicating biochemical specificity like a lipid-interacting enzyme [24,30,36,40].

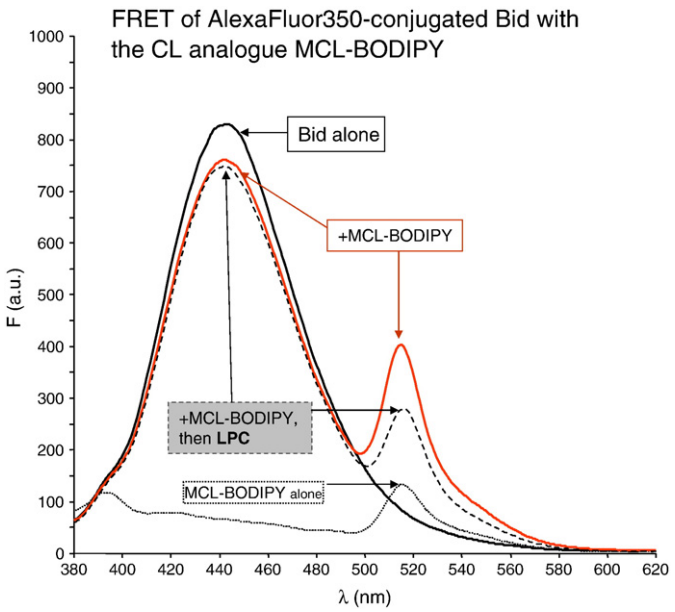

Fig. 1. Bid binds to a fluorescent analogue of CL in competition with LPC. Recombinant mouse Bid [40] was conjugated to AlexaFluor350, a blue fluorescent dye, and then purified by size-exclusion chromatography. Spectra were obtained in PBS with excitation at 346 nm, before (black solid line) and after (red line) equilibration with 50 nM of MCL-BODIPY, a green fluorescent derivative of cardiolipin [93]. Note the clear increase in BODIPY fluorescence at around 520 nm, which indicates energy transfer from binding to the Bid conjugate (FRET) (cf. [59]). The subsequent addition of 5 μM LPC (16:0, dotted line) removes this increase in green fluorescence, but not the corresponding quenching in blue fluorescence around 440 nm, indicating that binding competition between CL and micellar LPC could be incomplete, or involves some conformational changes.

5. Cardiolipin in apoptosis—an update

Cardiolipin (CL) is a unique glycerol-based phospholipid, which is composed of four acyl groups and two phosphate moieties. Unlike other phospholipids, the acyl groups of mammalian CL are essentially restricted to C18:2 chains (linoleic acid) that are required for optimal function of many enzyme complexes involved in oxidative phosphorylation [36,78]. CL is considered to be a specific component of mitochondria, since it is synthesized within the inner mitochondrial membrane. However, it can exit mitochondria and reach the plasmamembrane in some models of apoptosis [19], thereby suggesting that its intracellular location is dynamic and not confined within the organelle of its origin [3]. Even if CL is present almost exclusively within the inner mitochondrial membrane, it is also found in the OM (approximately 4% of the lipid content of mammals) and even more at the contact sites connecting the OM with the inner membrane [79]. Recent findings have suggested two additional routes by which CL can reach the OM from the inner mitochondrial membrane: (1) via the catalytic activity of phospholipid scramblase 3 [80]; (2) via transient association with oligomeric kinases in the inter-membrane space [81]. How the inter-membrane transfer of CL is integrated with its re-modelling remains unclear, given that the major re-modelling enzyme, tafazzin, seems to be predominantly located in the inner membrane [4]. Several independent studies suggest that CL has either survival- or death-supporting roles in cells (for recent reviews on various aspects of cardiolipin, see [4,5]). In addition to

the previous discussion, we will now review recent advances on CL involvement in apoptosis.

It is basically established that CL does play a role in the concerted pro-apoptotic action of Bcl-2 family proteins. Regarding model systems with artificial liposomes, recent prominent studies have clarified that there is no effective release of trapped indicators [59], or permeation of externally added indicators [67] without CL in the liposomal membranes. In biological systems including mitochondria, of particular note is the recent work of Martinou's group showing that selective degradation of mitochondrial CL impairs the pro-apoptotic action of tBid plus Bax [60]. All these recent reports support earlier works indicating a role of CL in the modulation of the pro-apoptotic action of Bid and other Bcl-2 proteins [3,82].

Besides CL, some membrane proteins have been implicated in the mitochondrial association of tBid and Bax [49,83] (reviewed in the article of Lindsay et al in this BBA issue). The cumulative evidence thus points to a combination of lipid-protein mixtures, i.e. microdomains containing CL, in the expression and regulation of the pro-apoptotic action of Bcl-2 proteins. Several aspects of putative mitochondrial microdomains have been reviewed recently [5,25]. Here we mention them in connection with the involvement of Bcl-2 proteins in the dynamic changes of mitochondrial morphology due to fusion and fission of their membranes [84–88]. Of note, CL accumulates at the cell poles of bacteria before their division [89–92], contributing to the structural changes associated with the process of cell membrane fission [89,92]. Because mitochondria divide by a fission mechanism that is comparable to that of their bacterial ancestors, microdomains enriched in CL may accumulate at the membrane poles in the elongation process, contributing to the coordinated fission of their inner and outer membranes [84]. This process involves membrane tubulation, a stretching of the bi-layer in which CL clusters seem to participate, probably because they can provide transient stabilization of the elongated membrane structures that then lead to organelle fission [88]. Similar membrane alterations have been reported in liposomes supplemented with tBid and Bax [49].

Intriguingly, we have recently observed a striking accumulation of a fluorescent CL analogue in long membrane protrusions which connect T cells, called membranes nanotubes (Fig. 2, cf. [93]). It is thus possible that the clustering of activated Bax and Bak in foci of mitochondrial fission (for a review, see [84,94]) reflects an underlying accumulation of CL-enriched microdomains in the OM, which in turn may drive the oligomerization and pro-apoptotic action of the same proteins. Bid or tBid may facilitate the assembly of these microdomains by aggregating together with specific CL and MCL molecules, as observed with different experimental approaches [30,40,52,55].

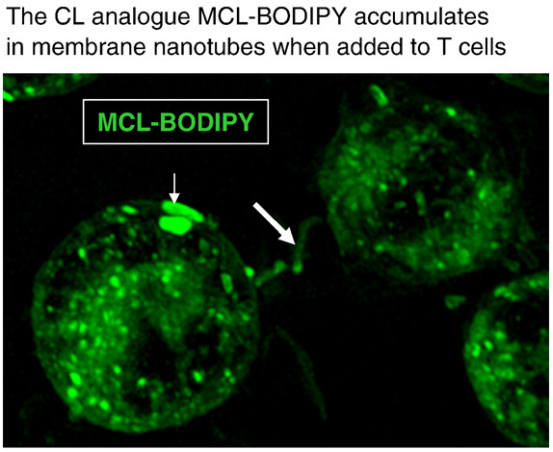

Fig. 2. Accumulation of exogenous CL in membrane nanotubes. Jurkat T cells were attached to coverslip after incubation with lipid vesicles containing the same fluorescent analogue of CL as that used in Fig. 1, then fixed and imaged by deconvolution microscopy [93]. The small arrow indicates vesicles that have not yet been internalized within the cell, while the large arrow indicates a membrane nanotube connecting two cells that appears enriched in MCL-BODIPY staining.

Although the caspase-cleaved form of Bid, tBid, is predominantly used in model systems for studying MOMP [52,55,59,67,82,83], it is worth noting that also the full length protein binds to CL (Fig. 1) and displays pro-apoptotic capacity with mitochondria [19,39,47]. It is just less catalytically efficient than tBid, which has a higher affinity for CL and MCL than the full length protein [30]. Although tBid has a much larger hydrophobic surface than the full-length protein, the tight packing of the N-terminal region cleaved by caspases would cover most of it [64,95]. Yet, caspase-cleaved Bid (namely "cut" Bid, even if often it is called tBid) acquires the propensity of self-aggregation even in the absence of lipids or detergents [30]. Thus caspase-cleaved Bid is actually a suspension of protein aggregates under the usual conditions of the *in vitro* assays, a property that may be instrumental for accelerating its pro-apoptotic activity. Even in this self-aggregated state, tBid retains the capacity of binding tightly to CL and its metabolites [30], as well as interacting with other Bcl-2 proteins and lipid membranes [53,59]. However, it loses the capacity of forming oligomers with LPC and detergents [30,39]. This seems to be a biophysical property that clearly distinguishes caspase-cleaved Bid from its parent protein and could represent a biological switch to irreversibly associate it to mitochondria during apoptosis [96].

6. Conclusions and perspectives

In this review we hope to have clarified the following issues:

(1) After apoptosis induction, the initial lipid changes that are observed in mitochondria appear to reflect an upstream deficiency in the biosynthesis of PC impacting on the re-modelling of CL;
(2) lysolipids are prominent among these initial changes and may play a crucial, yet largely unexplored role in facilitating the pro-apoptotic action of Bax and Bak and the onset of MOMP;
(3) Bid provides a link between upstream PC deficiency and mitochondrial membranes by transporting LPC to the OM; thus, probably it contributes to disequilibrium in the transacylation of CL re-modelling;
(4) binding to CL or MCL produce stable oligomers of (t)Bid that fix the protein onto the membrane surface, thereby providing the attractor for Bax and Bak action on the same membrane;
(5) peroxidation of CL and other lipid changes observed in mitochondria (Table 1) essentially occur after MOMP; hence, they may only amplify the release of cytochrome *c* and consequent caspase cascade.

In perspective, it can be considered that specific lipid metabolites and the binding of these molecules by proteins controlling apoptotic signalling may form a new stress sensing mechanism that act prior to the involvement of mitochondrial membranes. Lipid molecules, and in particular lysolipids, may thus have unexplored signalling roles in sensing death stimuli and other developmental cues within cells. In the plant kingdom, lysolipids have already been reported to be directly involved in signalling pathways of mycorrhizal and rhizobial symbiosis [97,98]. Perhaps new signalling routes involving lysolipids are about to be discovered in animals too, following the precedent of LPA [99].

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