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Review

Putative partners in Bax mediated cytochrome-*c* release: ANT, CypD, VDAC or none of them?

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ABSTRACT

Release of cytochrome-*c* from mitochondria is a key regulatory event in the intrinsic pathway of apoptosis, and its mechanism has been the subject of extensive debate with investigators proposing different and contrasting models. While some models suggest that cytochrome-*c* release can occur in absence of permeability transition and is mediated by the pro-apoptotic protein Bax, some suggest involvement of various components of permeability transition pore with or without cooperative action of Bax. Various models of PTP-dependent or -independent cytochrome-*c* release are discussed in this review with special emphasis on all the independent/cooperative roles of Bax evidenced so far.

1. Introduction

Apoptosis, which was originally described by Kerr et al. (1972), is a morphologically defined and highly regulated form of programmed cell death. Apoptosis can be categorized into at least two types viz., extrinsic and intrinsic pathways based on the origin of apoptotic signal. In the extrinsic pathway of apoptosis, the signal is delivered through cell surface transmembrane receptor-mediated interactions. Extrinsic or death receptor mediated pathways of apoptosis are well studied using FasL/FasR and TNF-α/TNFR1 models. Binding of FasL to its cell surface receptor Fas results in the recruitment of adapter protein FADD at the cytoplasmic end. FADD in turn, via its death effector domain (DED), mediates the recruitment of procaspase-8. This complex is called death inducing signaling complex (DISC) and it finally results in the auto-catalytic activation of procaspase-8 (Kischkel et al., 1995). Similarly, engagement of TNFR1 triggers the recruitment of the adaptor molecule TRADD and receptor-interacting protein (RIP), which is followed by formation of complex-I and complex-II. The initial plasma membrane bound complex, which promotes cell survival (complex-I), consists of TNFR1, TRADD, RIP1, and TRAF2. In a second step, TRADD and RIP1 associate with FADD and caspase-8, forming the cytoplasmic complex-II that finally initiates cell death (Micheau and Tschopp, 2003).

In the intrinsic pathway of apoptosis, death signal is generated inside the cell following chemical treatment, irradiation, viral infection or growth factor deprivation leading to release of mitochondrial factors such as cytochrome-*c*, AIF, Smac/Diablo into the cytosol. Once released into cytosol, cytochrome-*c* interacts with Apaf-1, which then recruits caspase-9. This whole complex is called apoptosome and it acts as a holoenzyme resulting in caspase-9 activation in a manner that does not require proteolytic cleavage, finally leading to caspase-3 activation (Kim et al., 2005). Out of all these steps, the release of cytochrome-*c* into cytosol is considered the key regulatory step irreversibly committing cells to apoptosis in most cases of intrinsic apoptosis. Though the mechanism of cytochrome-*c* release has been widely studied, it has been the subject of extensive debate with different contrasting models. While some models suggest that cytochrome-*c* release can take place in the absence of permeability transition and is mediated by proapoptotic Bcl_2 family proteins mainly Bax, some suggest involvement of various components of permeability transition pore with or without cooperative action of Bax.

Role of cytochrome-*c* in apoptosis was first suggested by Liu et al. (1996). Authors initially found that addition of dATP results in caspase-3 activation in HeLa cell cytosolic extract, and further analysis has led to purification of a protein required for dATP-triggered caspase-3 activation, which was found to be cytochrome-*c*.

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Soon after this discovery, the role of cytochrome-*c* in apoptosis induction was well established and numerous studies have since been carried out to understand the nature of its release. As mitochondria release all their cytochrome-*c* within minutes following apoptotic stimulus in a temperature-independent fashion over a range of 24–37 ℃ (Goldstein et al., 2000), it is thought that an enzymatic transport may not be involved in this process. A two-step mechanism has also been proposed to explain cytochrome-*c* release. Bernardi and Azzone (1981) showed that only 15–20% of cytochrome-*c* is available in mitochondrial intermembrane space while the rest is compartmentalized in cristae. Outer membrane permeabilization leads to the release of cytochrome-*c* present in intermembrane space. Reorganization of cristae causes release of cytochrome-*c* stored in cristae in a CsA sensitive manner (Scorrano et al., 2002a,b). Very recently, Bax has been implicated in the remodeling and opening of cristae (Yamaguchi et al., 2008; Zhang et al., 2008).

Several competing models have been proposed to explain the mechanism of cytochrome-*c* release from mitochondria. One model suggests opening of permeability transition pore (PTP) resulting in the leakage of cytochrome-*c* into cytosol. PTP spans through both the inner and the outer mitochondrial membranes. The adenine-nucleotide translocator (ANT, located in the inner mitochondrial membrane), a voltage-dependent anion channel (VDAC, located in the outer mitochondrial membrane) and cyclophilin D, a small soluble protein found inside mitochondria are considered to be major components of the PTP, though some studies also suggest presence of several other proteins including hexokinase-II (HK-II) (Fig. 1). Outer mitochondrial membrane has semi-permeable nature due to VDAC which allows passage of solutes of up to 5 kDa, thereby allowing the free exchange of respiratory-chain substrates such as NADH, FADH and ATP/ADP between the mitochondrial intermembrane space and the cytosol. In contrast, the inner mitochondrial membrane (IMM) is almost impermeable – a feature that is essential for generation of the electrochemical proton gradient ($\Delta\psi_m$) used for oxidative phosphorylation (Bernardi, 1999). In response to any apoptotic stimulus, mitochondrial calcium levels increase, and high concentration of Ca^{2+} in the mitochondrial matrix and oxidative stress activate voltage operated PT pore causing persistent opening of pore, allowing not only Ca^{2+} but also low-molecular-mass matrix components (M_r <1500) to easily pass through the mitochondrial membranes (Green and Kroemer, 2004). Apoptosis through Ca^{2+}-mediated mitochondrial permeability transition has been observed in various cell types in several studies involving a variety of treatments including Ca^{2+} ionophores, thapsigargin, neurotoxins, chemotherapeutic agents and pro-oxidants and cell death can often be prevented by inhibitors of mitochondrial Ca^{2+} uptake or PTP formation, such as ruthenium red and cyclosporin A (Walter et al., 1998; Pritchard et al., 2000; Lee et al., 2005; Bradham et al., 1998).

Alternatively, another model suggests initial permeabilization of the inner mitochondrial membrane exerts osmotic pressure on the mitochondrial matrix, thereby leading to swelling and rupture of the outer mitochondrial membrane and subsequent cytochrome-*c* release (Feldmann et al., 2000). Though equal osmotic pressure is exerted on inner mitochondrial membrane as well, it does not lead to rupturing owing to its larger surface area because of folded cristae.

In these two models of cytochrome-*c* release, outer mitochondrial permeabilization is achieved either by opening of mitochondrial permeability transition pore (PTP) or by rupturing of OMM. In either case mitochondria loose their structural and functional integrity. This type of mitochondrial membrane permeabilization is relevant during instances like ischemia–reperfusion injury. However, relevance of this type of OMM permeabilization during physiological apoptosis was questioned by several later studies. von Ahsen et al. (2000) have shown that mitochondrial structure and function are preserved after cytochrome-*c* release, favoring involvement of selective channels over non-specific pores like PTP for cytochrome-*c* release. Bax, which is a pro-apoptotic member of Bcl_2 family, is known to form selective channels for cytochrome-*c* release. Again, different models of Bax mediated cytochrome-*c* have been proposed. Some suggest that Bax alone is sufficient for OMM permeabilization while others suggest that Bax and components of PTP cooperatively function to induce OMM permeabilization. The main purpose of this review is to briefly present a glimpse of various models of Bax mediated cytochrome-*c* release without going into overly details of each mechanism.

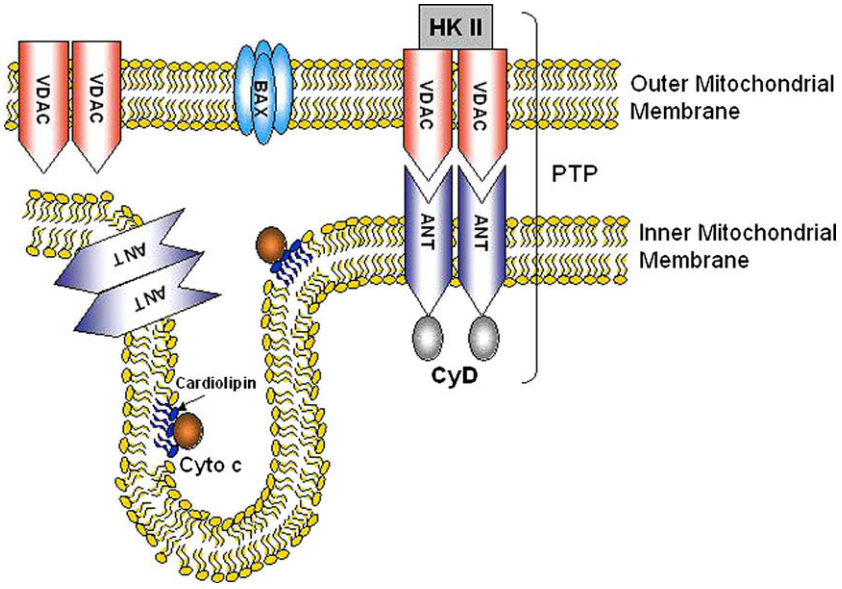

Fig. 1. Various components of permeability transition pore. VDACs are located on the outer mitochondrial membrane and ANTs are located on inner mitochondrial membrane. Cyclophilin D is attached to ANT on the matrix side. When VDAC and ANT come to juxtaposing position, it is called permeability transition pore.