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Review

New insights into mitochondrial structure during cell death

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ABSTRACT

Mitochondria play a pivotal role in the cascade of events associated with cell death pathways that are involved with several forms of neurodegeneration. Recent findings show that in the Bax/Bak-dependent pathway of apoptosis, the release of cytochrome *c* from mitochondria is a consequence of two carefully coordinated events: opening of crista junctions triggered by OPA1 oligomer disassembly and formation of outer membrane pores. Both steps are necessary for the complete release of pro-apoptotic proteins. The remodeling of mitochondrial structure accompanies this pathway, including mitochondrial fission, and cristae and crista junction alterations. Yet, there is controversy surrounding the timing of certain remodeling events and whether they are necessary early events required for the release of pro-apoptotic factors or are simply a downstream after-effect. Here, we analyze the current knowledge of mitochondrial remodeling during cell death and discuss what structural alterations occur to this organelle during neurodegeneration, focusing on the higher resolution structural correlates obtained by electron microscopy and electron tomography.

Contents

Mitochondrial structure and apoptosis

Recent research is building a consensus that mitochondrial structure and dynamics are actively and tightly controlled by cellular stimuli, signaling events, and perturbations inside this organelle (reviewed by Chan, 2006; Mannella, 2008; Zick et al., 2009), including programmed cell death and certain forms of neurodegeneration. Developmentally regulated programmed cell death in the brain uses the Bak/Bax-dependent pathway with tBid acting as a pro-apoptotic effector protein (Jemmerson et al., 2005). Apoptosis-like cell death is also involved in neurodegenerative diseases and stroke. A key event in many forms of neuronal degeneration and cell death is the release from mitochondria of pro-apoptotic effectors, such as cytochrome *c*, HtrA2/Omi, smac/DIABLO, and AIF, that initiate the caspases involved in downstream proteolytic processes for cellular digestion (Munoz-Pinedo et al., 2006). The release mechanism has not yet been elucidated completely but it is known to be regulated by the Bcl-2 protein family (Chipuk and Green, 2008).

A governing principle of mitochondrial architecture is that structure determines function. The three distinct mitochondrial compartments that separate functionality are involved in the major cell death pathways; these are the intermembrane space, intracristal

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space, and matrix, defined by three membrane systems: outer, inner boundary, and cristae. Recent interest in mitochondrial structure/function correlates has centered on cristae and crista junction shape, size, and abundance (Mannella, 2006b; Zick et al., 2009). Because electron transport chain molecules reside on the cristae membranes (Gilkerson et al., 2003; Vogel et al., 2006), the ratio of cristae/mitochondrion surface area can be viewed as the ATP synthesizing capacity of the mitochondrion. When central nervous system (CNS) neurons are compared, this ratio does not vary much (mean between 1.6 and 1.7; Table 1), and is significantly greater than the ratios for peripheral nervous system (PNS) mitochondria. The greatest ratio is found for the rod photoreceptor terminal (spherule) consistent with the high metabolic rate of retina (Johnson et al., 2007). Crista junctions are hypothesized to limit movement of signaling molecules (Fig. 1), enzymatic substrates, products, and metabolites into or out of the intracristal space or between the inner boundary and cristae membranes (Frey et al., 2002; 2006; Mannella, 2006a; Mannella et al., 2001; Perkins et al., 1997, 2001). Whereas the crista junction diameter is similar in neuronal mitochondria, the crista junction density varies substantially (Table 1). The role that the crista junction diameter plays in cell death is discussed below. However, the significance of the variation in crista junction density has not been explored yet and may require further investigations, but one can speculate that it may affect the heterogeneity of mitochondrial components (Perkins and Ellisman, 2007).

In recent years scientists have unraveled the structural alterations to mitochondria linked to the major apoptotic pathways. One such pathway is calcium-induced cytochrome *c* release, prevalent in neurons during stroke and ischemia (Jemmerson et al., 2005). Distinctive to this pathway is rupture of the mitochondrial outer membrane that can be blocked by inhibitors of the mitochondrial permeability transition. Another pathway uses pro-apoptotic proteins, such as Bax, Bak and tBid to initiate cytochrome *c* release independently of the permeability transition and notably without rupturing the mitochondrial outer membrane and may have no large-scale changes in mitochondrial ultrastructure (von Ahsen et al., 2000). Instead, it has been proposed that in order to release cytochrome *c* and other proteins during tBid-mediated apoptosis, pores must form on the mitochondrial outer membrane (Reed and Green, 2002). Pore formation is likely triggered by tBid-induced oligomerization of Bak or Bax (Lovell et al., 2008) but VDAC channels are not involved (Galluzzi and Kroemer, 2007). Supporting this model are the observations that in the absence of Bak or Bax oligomerization, there is no cytochrome *c* release (Kuwana et al., 2002; Scorrano et al., 2003; Wei et al., 2001; Yamaguchi et al., 2008). Once cytochrome *c* is released from mitochondria during apoptosis and activates apoptosomes in the cytosol, death is the certain outcome for the cell (Green and Reed, 1998; Li et al., 1997, 2000; Liu et al., 1996). What happens to the cell's mitochondria, on the other hand, is turning out to be a complicated story. Adding to the complexity is the timing of structural perturbations to this organelle that may differ between apoptotic stimuli and the death pathway taken. Publications in the last few years

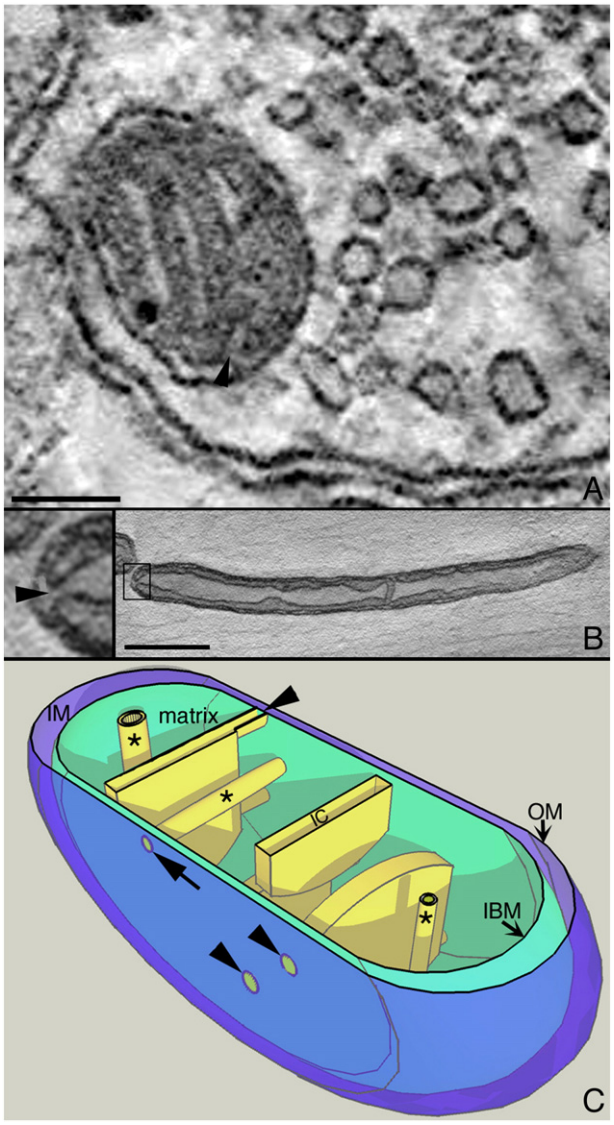

Fig. 1. The crista junction architecture in neurons. (A) A tomographic slice from the volume of a CNS mitochondrion found near a synapse in the striatum. The arrowhead points to a crista junction opening. Synaptic mitochondria in the CNS are typically small with mostly lamellar cristae. Even with lamellar cristae, the narrow, tubular crista junction architecture prevails in brain mitochondria and the opening is at the end and not on the side of the cristae. Scale = 100 nm. (B) A tomographic slice from the volume of a PNS mitochondrion found in the axon of a spinal root. The crista junction is boxed and expanded 4× in the insert at left (arrowhead). PNS axonal mitochondria are typically elongated with longitudinally oriented cristae and often show a condensed matrix as seen here. Even with structural features different from CNS mitochondria, PNS axonal mitochondria nevertheless have the same crista junction architecture and again, the opening is at the end and not on the side of the cristae. Scale = 400 nm. (C) Cut-away model of neuronal mitochondria. Tubular cristae (*) can be oriented in different directions and have different diameters in CNS mitochondria, but are typically longitudinal in PNS mitochondria and the crista junction is at the end of the tube (arrow). The crista junction is relatively uniform in size, even for lamellar cristae (arrowheads). Mitochondria have 3 membrane systems, the outer membrane (OM), inner boundary (IBM), and cristae, and 3 compartments, the matrix, intermembrane space (IM), and intracristal space (IC). The outer membrane was made translucent to better visualize the crista junctions.

Table 1
Cristae surface area and crista junction measurements from tomographic volumes.

Cell type	Cristae/mitochondrion surface area ratio	Crista junction density (per μm²)	Crista junction diameter (nm)
Neurons — CNS			
Rod spherule	5.2 ± .17[a]	54 ± 17 (6)	12 ± 4[b] (102)
Cone pedicle	2.0 ± .78	56 ± 45 (6)	9 ± 2 (32)
Rod inner segment	1.2 ± .47	72 ± 29 (5)	17 ± 4 (148)
Cone inner segment	2.6 ± .50	52 ± 17 (12)	12 ± 3 (215)
Retinal ganglion	1.7 ± .40	25 ± 11 (7)	14 ± 4 (184)
Cerebellum	1.6 ± .28	136 ± 55 (3)	16 ± 5 (85)
Hippocampus	1.7 ± .79	25 ± 15 (3)	14 ± 4 (38)
Striatum	1.6 ± 1.0	65 ± 27 (6)	14 ± 4 (43)
Cortex	1.7 ± .32	37 ± 8 (6)	14 ± 5 (26)
Spinal cord	1.6 ± .41	28 ± 10 (6)	13 ± 3 (99)
Neurons and astrocytes — PNS			
Schwann cell	0.70 ± .18	80 ± 29 (6)	13 ± 4 (40)
Axon (spinal root)	0.41 ± .13	19 ± 10 (6)	13 ± 4 (65)

[a] All values indicated as mean ± sd; number of samples indicated in parentheses. All measurements performed on tomographic reconstructions.
[b] Inner diameter that is only the opening, i.e., the membrane width was excluded.