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News and Commentary

Divide et impera: Ca^{2+} signals, mitochondrial fission and sensitization to apoptosis

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Ancient Romans knew it: the best way to take control over an enemy during a battle was to divide its army. Recent data suggest that during apoptosis mitochondria may be condemned to the role of Romans' enemies, being divided to become defeated. Mitochondrial defeat is represented by the release of several proteins, needed in the cytosol to activate the effector caspases.[1] Among the released proteins, cytochrome *c*, the only soluble component of the respiratory chain, is an essential cofactor for the formation of the so-called apoptosome with the subsequent activation of caspase-9. The mechanism by which cytochrome *c* is released is heavily investigated and debated. Molecules of the BCL-2 family have a prominent role in the control of this process: proapoptotic BCL-2 members promote the release of cytochrome *c*, whereas the antiapoptotic ones prevent it.[2] BCL-2 family proteins are characterized by homology in short amphipathic stretches of amino acids, called BCL-2 homology (BH) domains. Antiapoptotic members display sequence conservation throughout all four BH domains. Proapoptotic BCL-2 members can be further subdivided into 'multidomain' members possessing homology in BH1-3 domains (such as BAX and BAK) and 'BH3-only' members that display only ~ 9 amino acids of homology within this domain.[3] In a widely accepted model, the BH3-only molecules connect upstream signals to the mitochondrial pathway: for example, BID recruits the mitochondria during death receptor-induced apoptosis[4] and BIM is essential for T cell receptor-induced apoptosis.[5] Once activated, BH3-only proteins function as ligands for the multidomain proapoptotics BAX and BAK, induce their homo/hetero-oligomerization and ultimately the release of cytochrome *c* from mitochondria.[6] Several different and sometimes mutually exclusive mechanisms have been proposed to explain the mitochondrial events that lead to the efflux of cytochrome *c* (see Martinou and Green[7] for a review), often generating more confusion than knowledge.

Mitochondrial structural organization further complicates this already bamboozled scenario. The classic textbook depiction of mitochondria as individual, isolated organelles with an outer membrane that separates them from the cytosol and an inner membrane invaginated in large baffles, the *cristae*, that freely communicate with the intermembrane space, is probably oversimplified. High-voltage electron tomography coupled with three-dimension image reconstruction of mitochondria depicted a more complex internal structure. The *cristae* constitute a separate compartment, shaped like bags and connected by narrow tubular junctions to a thin intermembrane space.[8] *In situ*, mitochondria appear to be organized in a net, where individual organelles dynamically fuse and divide.[9] Moreover, this mitochondrial reticulum is in close proximity with the endoplasmic reticulum (ER), often closer than the discrimination capabilities of high-resolution confocal microscopy.[10] This structural organization influences multiple parameters of mitochondrial physiology during life and death of the cell. The narrow tubular junctions generate gradients of ions and small molecules along the *cristae* and are responsible for the segregation of cytochrome *c* in the *cristae* compartment,[11] where the majority of the respiratory chain complexes also localize.[12–14] Mitochondrial fusion *in situ* is efficient[15] and fused mitochondria can behave as a functional unit, where a stimulus or a signal hitting one end of the mitochondrial wire is readily transmitted to other distal components of the net.[16] This property can be extremely useful to convey signals across the cytoplasm rapidly, especially in large cells such as cardiomyocytes.[17] The proximity between ER and mitochondria reflects a close functional communication between the two organelles, in particular in the context of Ca^{2+} signalling. The activation of the low-affinity mitochondrial Ca^{2+} uniporter, responsible for mitochondrial Ca^{2+} uptake,[18] requires that the mitochondria are exposed to high Ca^{2+} microdomains originated at the mouth of inositol trisphosphate receptors at the surface of the ER.[19] Mitochondrial Ca^{2+} uptake is crucial to shape cytosolic Ca^{2+} oscillations[20] and in turn to modulate mitochondrial function, including activation of mitochondrial metabolism[21] and control of the permeability transition (PT) pore, a Ca^{2+} and voltage-dependent channel of the inner mitochondrial membrane that participates in certain models of apoptosis.[18]

Recent works show that these structural features can play a role in the control of apoptosis. On one side, Ca^{2+} signalling between ER and mitochondria is crucial in the regulation of certain apoptotic pathways. Intriguingly, BCL-2 family proteins can modulate steady-state ER Ca^{2+} content and hence the amount of Ca^{2+} that is taken up by mitochondria.[22,23] Overexpression of BCL-2 as well as ablation of BAX and BAK results in reduced steady-state ER Ca^{2+} concentration, reduced mitochondrial Ca^{2+} uptake and ultimately resistance to apoptosis induced by Ca^{2+}-dependent stimuli.[24] On the other hand, changes in the shape of the mitochondrial reticulum and clustering of mitochondria in the perinuclear region occur in response to several apoptotic stimuli.[25] These changes of the mitochondrial network are accompanied by a remodelling of the internal mitochondrial structure and result in enhanced cytochrome *c* release.[26,27] The shape of the

mitochondrial network and of individual mitochondria results from a net balance between fusion and fission processes regulated by proteins belonging to the dynamin family. Dynamins are large, ubiquitous mechanoenzymatic GTPases that control membrane fusion, tubulation, budding and vesicle formation.[28,29] The role of dynamins in controlling mitochondrial shape was first identified in yeast, where deletion of specific dynamin genes results in gross alterations of the mitochondrial network and in functional abnormalities including loss of mitochondrial DNA, growth defects and petite strains.[30] In mammalian cells, the cytosolic dynamin-like protein DRP-1 translocates to the mitochondria to promote fission,[31] whereas the mitochondrial integral proteins MFN-1 and -2 and OPA1 regulate fusion.[32,33] Ablation of *mfn-1* and *-2* in the mouse results in embryonic lethality,[34] loss of function mutations of *OPA1* cause dominant optic atrophy Kjer type 1,[35,36] a human disease characterized by loss of retinal ganglionic cells, and knocking down of *OPA1* using small RNA interference induces cell death.[37] Evidence that favour a role for these proteins during apoptosis starts to accumulate. Youle and colleagues demonstrated that DRP-1 mediates mitochondrial fragmentation associates with cytochrome *c* release and onset of mitochondrial dysfunction and is therefore crucial to ensure the progression of the apoptotic cascade.[27] Overexpression of hFis1, the human ortologue of yeast fis-1p, which in *Saccharomyces cerevisiae* functions as a scaffold for dnm1p to drive its localization on the outer mitochondrial membrane during fission,[30] induces mitochondrial fragmentation, cytochrome *c* release and apoptosis.[38]

A recent paper by Breckenridge *et al*[39] shows that and Ca^{2+} signalling between ER and mitochondria coordinates the mitochondrial fission events of the early stages of cell death. Shore and colleagues previously identified that the ER resident protein BAP31 is a substrate for caspase-8. BAP31 possesses two identical caspases cleavage sites, associates with procaspases-8 and during apoptosis is cleaved into a 20 kDa ER resident fragment that is toxic when ectopically expressed.[40,41] p20 BAP31 triggers mitochondria-dependent death from the ER: it associates with ER membranes from where it cooperates with other proapoptotic factors, such as BID, to induce cytochrome *c* release. Breckenridge *et al*[39] demonstrate that p20 BAP31 causes Ca^{2+} release from the ER with subsequent mitochondrial Ca^{2+} uptake that triggers mitochondrial fission, resulting in enhanced cytochrome *c* release and apoptosis. Interestingly, Shore and colleagues identified a role for this Ca^{2+} connection also in a model in which Ca^{2+} was not suspected to be involved, that is, death induced by BID. BID causes cytochrome *c* release and death only if BAX and BAK are available[42] and apparently independently from mitochondrial Ca^{2+} uptake.[24] The results of Breckenridge *et al* conversely suggest that such a signal elicited by p20 BAP31 renders mitochondria more susceptible to cytochrome *c* release by BID by triggering mitochondrial fission.

It is heavily debated whether the mitochondrial amplification loop is essential to propagate receptor-initiated death signals.[43] Genetic evidences substantiate a model in which certain cell types, such as hepatocytes,[4] require the mitochondrial pathway in response to death receptor activation (the type II cells[44]), whereas in other tissues caspase-8 activation is strong enough to process caspase-3 directly and execute death without a significant mitochondrial intervention.[45] The work of Breckenridge *et al* adds another regulatory organelle to mitochondrial-dependent, receptor-initiated deaths, suggesting that the mitochondrial amplificatory loop can be regulated by the ER, through Ca^{2+} signals that induce DRP-1-dependent mitochondrial fission. Previously, we thought that the ER could only modulate apoptosis induced by Ca^{2+}-dependent, mitochondria utilizing stimuli that depend on adequate mitochondrial Ca^{2+} uptake.[24,25] It is unclear whether only selected cell types will need the ER control to speed up receptor-initiated death. Future investigations are needed to exclude that this role of the ER is not restricted to a subset of type II cells.

Cleavage of BAP31 by caspase-8 elicits a cascade of Ca^{2+} release from the ER and subsequent mitochondrial Ca^{2+} uptake that results in DRP-1 recruitment and activation.[39] This is a first step towards the clarification of the molecular mechanisms of mitochondrial fission induced by Ca^{2+} uptake, a phenomenon known since the early 1980 s.[46] How mitochondrial Ca^{2+} uptake results in recruitment of DRP-1 remains an open question. Is this caused by a diffusible signal, or by conformational changes in mitochondrial proteins that function as adaptors for DRP-1, like fis1p, mdv-1p and -2p in yeast[30]? Since inner and outer membrane fusion and fission are coordinated, it is tempting to speculate that mitochondrial Ca^{2+} uptake might cause an inner mitochondrial membrane remodelling, which in turn recruits the severing machinery to mitochondria. Last but not least, why are fragmented mitochondria more susceptible to the attack of activated proapoptotic BCL-2 family members? Maybe DRP-1, like other dynamins, recruits to the mitochondria proteins that display lipid remodelling activity[47] and change the lipid composition of the mitochondrial membrane. This could in turn render mitochondrial membranes more susceptible to proapoptotic molecules, or generate preferential docking sites for them.[48] Another possibility is that fragmented mitochondria remodel the *cristae* compartment and therefore can release more cytochrome *c* in response to the same proapoptotic stimulus. It will be interesting to investigate whether the same Ca^{2+} signal can coordinately induce inner membrane remodelling and fission of the mitochondrial reticulum. A possibility is that Ca^{2+} induces a sustained transient activity of the PT that is associated with inner mitochondrial membrane remodelling[26,49] and this is used as a signal to recruit severing molecules to the mitochondria. To this end, the study of the role of mitochondrial dynamics in apoptosis might lead to important insights into the processes of mitochondrial fusion and fission.

In conclusion, the recognition of a regulatory role for ER and for Ca^{2+} signals, and of an effector role for mitochondrial fusion and fission in the mitochondrial apoptotic pathways will enhance our understanding of the molecular mechanisms that regulate both apoptosis and the dynamic control of mitochondrial morphology.

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