Mitochondrial IF$_1$ preserves cristae structure to limit apoptotic cell death signaling

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The functional integrity of mitochondria is strictly dependent on the molecular dynamics governing their shape, size, and structure. A sequence of fusion and fission events serve to tailor the mitochondrial reticulum and preserve its morphological and functional homeostasis.[1] The mitochondrial ultrastructure, defined by the alignment and density of cristae, plays a central role in mitochondrial function, dictating both the efficiency of oxidative phosphorylation, through production of adenosine 5-triphosphate (ATP), and the execution of programmed cell death (or apoptosis) by tuning the release of pro-apoptotic molecules stored within the intermembrane space, such as cytochrome c (Cyt c). Concomitantly with the dismantling of the inner mitochondrial structure, during activation of the intrinsic pathway of apoptosis, a signaling platform assembles on mitochondria, driving the permeabilization of the outer mitochondrial membrane (OMM) and sustaining the release of pro-apoptotic proteins.

Alterations in the structural dynamics of mitochondria therefore influence the rate and efficiency of apoptosis. We have recently found that the protein IF$_1$ (the inhibitor of the F$_1$ complex of the ATP synthase) contributes to the regulation of Cyt c release and the signaling cascade that culminates in apoptosis.[2]

The biochemistry of this small mitochondrial protein (~10 kDa) has been studied in exquisite detail as an inhibitor of the hydrolysis of adenosine triphosphate (ATP) by the F$_1$Fo-ATPsynthase (see ref. 3 and references therein). This event is likely to be important during conditions of oxygen deprivation, making IF$_1$ protective in ischemia by slowing the rate of ATP depletion. Its effect on the activity of the F$_1$Fo-ATPsynthase during physiological oxidative phosphorylation remains controversial.[4]

Quite distinct from its role as an inhibitor of the F$_1$Fo-ATPase, we found that overexpression of IF$_1$ increased cristae density and number, while knockdown of the protein could leave mitochondria with only minimal cristae structures, although they could maintain a considerable potential.[5] It seems likely that this results from a promotion of dimerization of the F$_1$Fo-ATPsynthase, which, in turn, increases membrane curvature and hence cristae density. Strikingly, we also found that increased IF$_1$ expression levels limit the progression of apoptotic cell death by greatly delaying the release of Cyt c. This is consistent with the rise in cristae density, which limits cristae remodeling, an event that is required for complete cytochrome c release. This closely resembles the mechanism by which the pro-fusion protein optic atrophy type 1 (Opa1) reduces the mobilization of Cyt c and delays apoptosis by retaining cristae shape during the activation of apoptosis,[6] while the pro-fission dynamin-related protein 1 (Drp1) mediates an opposite effect on apoptosis, supporting oligomerization of the pro-apoptotic protein Bax and hence augmenting release of the Cyt c.

We found that the overexpression of IF$_1$ preserved mitochondrial shape during pharmacological activation of apoptosis, delaying the mobilization of Cyt c and the downstream signaling apoptotic cascade.

The anti-apoptotic role of IF$_1$ involves other elements in the regulation of apoptosis, namely the multidomain pro-apoptotic proteins Bax and Drp1. During apoptosis, Bax behaves similarly to Drp1, being recruited to the outer mitochondrial membrane (OMM), where it inserts and oligomerizes to form foci that are functionally linked to the permeabilization of the OMM.[7]

Under apoptotic conditions, we found that the overexpression of IF$_1$ limited the formation of these foci, reducing the accumulation of Bax as well as that of Drp1 on mitochondria; whereby, in cells in which IF$_1$ expression was knocked down, increased mitochondrial translocation of Drp1 was accompanied by increased oligomerization of Bax.[2] Suppression of the apoptotic foci and limitation of the formation of constriction sites, is therefore consistent with the idea that IF$_1$ plays an anti-apoptotic role by limiting mitochondrial fragmentation.

In the scheme illustrated in **Figure 1**, we propose that the ratio of expression of IF$_1$ relative to that of the F$_1$Fo-ATPsynthase controls the release of Cyt c from mitochondria and hence regulates the completion of the apoptotic program. The progression of apoptosis leading to cell death engages an amplification pathway mediated by (1) Cyt c-dependent release of ER Ca^{2+},[8] (2) Ca^{2+}-dependent recruitment of the GTPase Dynamin-related protein 1 (Drp1), (3) Bax insertion into the outer mitochondrial membrane, and (4) further release of Cyt c. This cascade, which amplifies and accelerates Cyt c release, was significantly slowed down by

the overexpression of IF_1, and apoptotic cell death was suppressed.

The preservation of mitochondrial morphology by IF_1 during apoptosis is consistent with its influence on the inner membrane architecture, regulating Cyt c release. Such a role appears to be independent from the protein's canonical role as an inhibitor of the F_1Fo-ATPase. Thus, we found that the dissipation of the $\Delta\Psi_m$ during apoptosis occurred concomitantly with the release of Cyt c and the permeabilization of mitochondrial membranes independently of the IF_1 level of expression.

Interestingly, IF_1 is strongly overexpressed in many human tumors, suggesting that the suppression of apoptotic cell death by this small protein may prove highly significant in the metabolic and structural adaptations associated with tumor development.
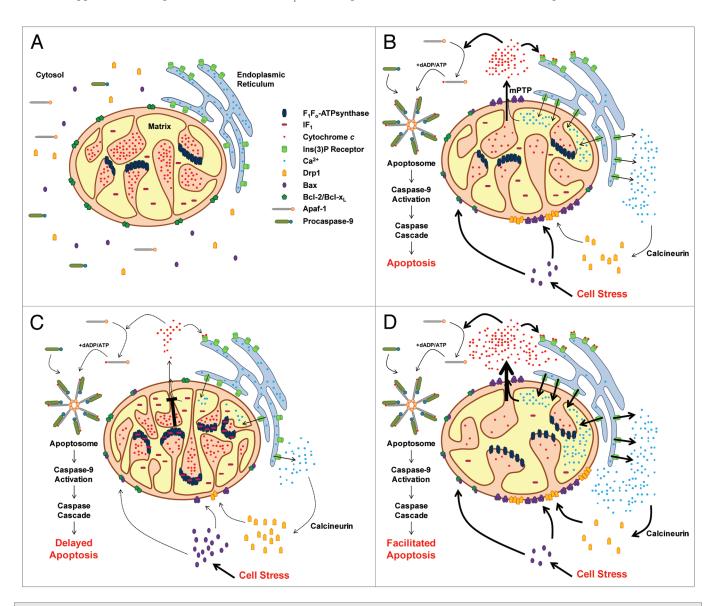

Figure 1. (**A**) In resting conditions, the pro-apoptotic protein Bax resides inactive in the cytosol, while the anti-apoptotic proteins Bcl-2/Bcl-xL are associated with the mitochondrial outer membrane, where they prevent the translocation and the subsequent oligomerization of Bax onto mitochondria. Since this event is crucial for progression into the late stages of apoptosis, the balance between pro- and anti-apoptotic proteins determines the susceptibility of a cell to an apoptotic stimulus. (**B**) When apoptosis is triggered, Bax translocates and oligomerizes onto the mitochondrial outer membrane, inducing its permeabilization and the release of Cyt c into the cytosol. This event allows the oligomerization of Apaf-1 into the apoptosome and the subsequent activation of the caspase cascade, which promotes apoptotic cell death. Cyt c also binds Ins(3)P receptors present on the ER membrane, stimulating the efflux of Ca^{2+} from this intracellular store. In the cytosol, Ca^{2+} activates calcineurin, which, in turn, induces Drp1 recruitment onto the mitochondrial membrane, where it strengthens the membrane association of Bax and stimulates mitochondrial fragmentation. Ca^{2+} is also accumulated by mitochondria, reinforcing the release of Cyt c and the progression of apoptosis. (**C**) When IF_1 is overexpressed, its association with the F_1F_oATPsynthase stabilizes the oligomerisation of the complex and preserves the inner mitochondrial membrane structure by maintaining the cristae, reducing the release of Cyt c and the Cyt c-induced mobilization of Ca^{2+} from the ER. The formation of the apoptosome and the activation of CaN are decreased, and the execution of apoptosis disrupted. (**D**) An opposite scenario characterizes cells with reduced levels of IF_1 expression. The number of mitochondrial cristae is lowered, and cristae junctions are weakened: this facilitates mitochondrial Cyt c release and the efflux of Ca^{2+} from the ER, rendering the cell more susceptible to apoptosis.

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