

For debate: defective mitochondria, free radicals, cell death, aging-reality or myth-ochondria?

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Abstract

As both experimental evidence and theoretical considerations may suggest that free radicals and mitochondria might be associated as key factors in aging, these organelles have been implicated in various versions of the free radical theory of aging. However, except for a few cases, no evidence for a death process specifically activated in respiratory defective cells could be found in patients with a mitochondrial disorder, including those harboring high levels of mutant mtDNA associated with profound respiratory chain deficiencies. This and more recent evidence suggest that damages produced by free-radicals endogenously generated in the mitochondria result in a distinctive biochemical profile, only occur under exceptional conditions and that a dysfunction of the respiratory chain does not cause opening of the permeability transition pore and is not sufficient per se to trigger massive entrance of cells into death processes, neither apoptosis nor necrosis. Therefore, defective mitochondria and their particular genome, should not be considered as a major and primary source of free radicals either leading cells into a death cascade or resulting in an accelerated aging process. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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Life-span of aerobic organisms mostly depends upon their ability to simultaneously use oxygen and neutralize highly toxic oxygen radical derivatives they concurrently produce. The ability to do so might depend on both metabolic potential and/or genetic factors (Guarente, 1997). In this regard, mitochondria occupy a unique position, being at once:

1. the cell's major oxygen consumers through the activity of the respiratory chain (RC);
2. a privileged site for free radical generation through unstable flavin and ubiquinone (CoQ) species of the RC; while
3. the fully reduced form of this latter compound can behave as a potent antioxidant (Emster and Dallner, 1995).

The proposal of a direct involvement of free radicals in aging has been substantiated by the observation of an extended life-span brought about by the overexpression of cytosolic Cu-Zn superoxide dismutase and catalase in *Drosophila melanogaster* (Orr and Sohal, 1994). Similarly, the increased life-span of *Caenorhabditis elegans* due to mutations in the Clk-1 gene, homologous to the yeast and rat CAT5/CoQ7 gene, which encodes a protein necessary for CoQ synthesis (Jonassen et al., 1996; Marbois and Clarke, 1996), suggests the involvement of CoQ as a control partner in aging (Wong et al., 1995; Lakowski and Hekimi, 1996). However, it is unclear whether the putative CoQ potential to control free radicals in aging is specifically associated with its mitochondrial function, since CoQ distribution in the cell is far from being restricted to the mitochondrial membranes (Emster and Dallner, 1995). Nevertheless, as both experimental evidence and theoretical considerations give credit to the assumption that free radicals and mitochondria might be associated as key factors in aging, it is not surprising that these organelles have been implicated in various versions of the free radical theory of aging (Linnane et al., 1989; Sohal and Dubey, 1994; Shigenaga et al., 1994; Kowald and Kirkwood, 1996; Skulachev et al., 1996; de Grey, 1997).

To make the picture even more puzzling, it has also been shown that opening of the mitochondrial permeability transition pore and the subsequent release of intermembrane cytochrome c and AIF (apoptosis-inducing factor) constitute critical events in cells' decision to die or survive (Vayssiere et al., 1994; Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997), and as to whether die by apoptosis or by necrosis (Richter et al., 1996). Associated loss of mitochondrial functions, such as the ability to maintain a trans-membrane potential or to readily produce ATP, has also been described (Kroemer et al., 1995). The idea that the regulation of energy metabolism has a major control effect on cell death led to the subsequent confusing suggestion that mitochondrial respiratory chain defects might also constitute causal events in cell death, disease and/or aging. Here we would like to stress that studying pathological situations encountered in patients with RC disorders actually rather conflicts than supports a unifying hypothesis (as tentatively depicted in Fig. 1) on the general implication of mitochondrial dysfunction in these different events, which do not necessarily bear relationship.

The peculiarities of the human mitochondrial genome (mtDNA) have been often put forward in this debate. Its proximity to the RC, potential source of free radicals

and the relative inefficiency of mtDNA repair systems have long been advocated to account for the high mutation rate observed in mtDNA (Wallace 1992). All this contributes to make mutant mtDNA an attractive candidate as a potent actor in the free radical-triggered aging cascade. Accordingly, using the methods now available to detect very low amounts of variant DNA, mutant mtDNA species have been shown to slightly accumulate with age in humans (Cortopassi and Arnheim 1990). A profusion of studies followed that initial report, with conflicting results on the nature, amount, or even occurrence of these mutations (Cortopassi et al., 1992; Munscher et al., 1993; Zhang et al., 1993; Pallotti et al., 1996). In any case, the quantities detected (less than 1% of total mtDNA), cast doubt on the significance of such observations. Involvement of a putative defective mitochondria-triggered cell death (Fig. 1) might, however lend weight to these observations. If a RC dysfunction is able per se to initiate cell death process, then we might only see a transient step of the process, cells harboring high level of mutant mtDNA being eliminated. Studying pathological situations encountered in patients with mitochondrial disorders could answer some of these questions.

Except for subacute necrotising encephalopathy in Leigh syndrome (Savoiaro et al., 1995) and a few cases of rhabdomyolysis we and others reported (Saunier et al., 1995; Keightley et al., 1996), no evidence for a death process specifically activated in respiratory defective cells could be found in patients with mitochondrial disorders, including those harboring high levels of mutant mtDNA, associated with profound RC deficiencies. Similarly, human skin fibroblasts with a high level of deleted mtDNA and a fully defective RC readily grow in the presence of uridine, which has to be supplied for the maintenance of nucleic acid synthesis in RC deficient cells (Morais et al., 1980). As evidenced by our earlier experiments, these RC deficient fibroblasts do not enter any death process, supporting as many passages (cell divisions) as control cells do (including patient cells grown in the absence of uridine, i.e., rapidly losing RC deficient cell sub-population)

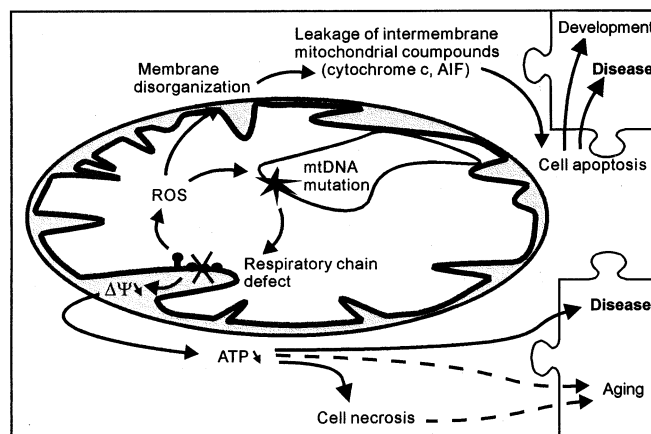


Fig. 1. Defective mitochondria, free radicals, cell death, aging: are all these the pieces of the same puzzle?

(Bourgeron et al., 1993). So far, the hypothesis that a defective RC might per se turn cells into a death cascade has no experimental support. Consequently, the traces of mutant mtDNA occasionally detected in human tissues should be considered as traces, rather than ‘the visible part of the iceberg’, and their potential consequences discussed in accordance. In keeping with this, the largest study carried out in human skeletal muscle aimed at detecting loss of RC activity with age, failed to detect any correlation (Chretien et al., 1998).

One may also consider the biochemical phenotype that should result from endogenous over-production of free radicals by mitochondria. Although its peculiarities make mtDNA a potential target, there are other components, known to be much more sensitive to free radicals, namely iron-sulphur proteins (ISP) (Gardner et al., 1994). Two recent lines of evidence confirmed that mitochondrial ISP are indeed quite sensitive targets for free radicals *in vivo*. It was first shown that the targeted disruption of the mitochondrial Mn-dependent superoxide dismutase gene in mouse results in a characteristic biochemical profile with low succinate dehydrogenase and aconitase activities, which are two mitochondrial ISP (Li et al., 1995). A quite similar biochemical profile was observed at our laboratory in mitochondria from hearts of Friedreich ataxia patients with a free-radical overproduction due to an intra-mitochondrial iron accumulation (Rotig et al., 1997; Rustin et al., 1998; Rotig et al., 1999). Such a quite distinctive biochemical profile hallmarks an endogenously-produced free-radical attack on the mitochondria. Noticeably, it has never been reported in studies tentatively ascribing loss of mitochondrial functions to putative free-radical generation resulting from mtDNA degradation. To date, it rather appears that mitochondrial anti-oxidant systems efficiently adapt to cope with free radicals generated by a defective RC (Ohkoshi et al., 1995).

As a temporary conclusion, based on the available evidence, we suggest that damages produced by free-radicals endogenously generated in the mitochondria (a) result in a quite distinctive biochemical profile; (b) only occur under exceptional conditions; and that (c) a dysfunction of the RC does not cause opening of the permeability transition pore and is not sufficient per se to trigger massive entrance of cells into death processes, neither apoptosis nor necrosis. Therefore, defective mitochondria and their particular genome, should not be considered as major and primary source of free radicals either leading cells into a death cascade or resulting in an accelerated aging process.

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