Mitochondria can Power Cells to Life and Death

Role of Mitochondria in Apoptosis

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Mitochondria (mitos=thread, chondrion=granular body) serve as the life-sustaining power houses of living eukaryotic cells. Mitochondria were discovered by R Altman in 1890 and the word was coined by C Benda in 1897. As we all know, mitochondria are the energy factories of the cell: these double membraned organelles take glucose and combine it with oxygen to produce ATP (chemical energy, usable by the cell), CO_2 and H_2O (see *Box* 1). Recent biological discoveries also strongly link mitochondria to what is commonly known as programmed cell death, or cell suicide (apoptosis).

Very early in the evolutionary history of multicellular organisms, programmed cell death (cell suicide as signaled by the nuclei in normally functioning cells when age or when the state of the cell health and condition dictates) got integrated into the vital genetic machinery. But it shot into limelight only about two decades ago. Apoptosis (literally meaning falling off of leaves from trees) is now known to occur as a universal phenomenon in all the living multicellular organisms in the course of development, tissue homeostasis, parasitic infection and several other pathogenic conditions. Apoptosis is an organized self destruction of redundant and incorrigibly damaged living cells and is different from the usual or necrotic cell death (*Table* 1). The targeted cells, carrying opsonic receptors (receptors promoting ingestion of particulate material by a specific antibody in combination with complement), undergo membrane blebbing (protrusion in the cell membrane which pinches out in the form of a vesicle), nuclear condensation and DNA fragmentation, ultimately leading to packaging of the cellular contents, including DNA, into membrane vesicles known as apoptotic bodies (see *Box* 2 for details). Recent advances in life sciences research

	NECROSIS	APOPTOSIS
Origin	Starvation, physical/chemical injury	growth factor withdrawal, hormonal influence, viral infection
First manifestation	swelling	shrinking, convolution
Membrane integrity	early loss	initially intact
Cytoskeletal changes	leakage of cellular contents	formation of apoptotic bodies
Protein synthesis	not affected by inhibitors	process inhibited by the inhibitors of protein synthesis
DNA degradation	DNA smear formation	DNA laddering
Cells affected	group of contiguous cells	individual/scattered cells
Cell elimination	inflammatory response in neighboring tissues	engulfment by macrophages

Table 1. Morphological and biochemical differences between the accidental (necrosis) and programmed (apoptotic) cell death.

indicate that apoptosis may be the origin of many human ailments. These disorders may be due to failed or excessive apoptosis. Failed apoptosis may give rise to cancer, whereas excessive cell death may result in Alzheimer's disease, Parkinson's disease, atherosclerosis, ischemic heart disease and many others (*Table* 2). A number of agents, including those of viral origin, have been found to inhibit the process of apoptosis (*Table* 3).

Mitochondria and Cell Death in the Evolutionary History

Some two billion years ago, the ancestors of modern eukaryotic

Table 2. Some human diseases associated with inappropriate apoptosis.

Neurological Disorders	Cardiovascular Disorders	Immune System Disorders
Alzheimer's disease	Ischemic heart damage	Multiple sclerosis
Parkinson's disease	Hypertension	Diabetes mellitus
Huntington's disease	Cardiac hypertrophy	Hashimoto's thyroiditis
Epilepsy		AIDS
Muscular atrophy		Other Autoimmune syndromes
Retinitis pigmentosa		
Amytrophic lateral sclerosis		
Others		
Ageing		
Alopecia		
Viral infections		
Cancer: Follicular lymphoma		

Box 1. What are Mitochondria?

Mitochondria, at times referred to as chondriosomes, are rod like or granular/spherical structures in the cytoplasm of every eukaryotic cell and provide the cells with energy in the form of ATP for vital functions. Measuring about 0.5 μm in diameter and 4–7 μm in length, mitochondria vary in shape and number (1-2×10^3 per cell) depending upon where and in which tissue they exist. They can be observed under light microscope after Janus green staining. Animal cells possess more mitochondria than plant cells. Repeated administration of thyroxin (a hormone) in the muscle enhances the number of mitochondria in the muscle cells. On the other hand, tumour cells contain a reduced number of mitochondria.

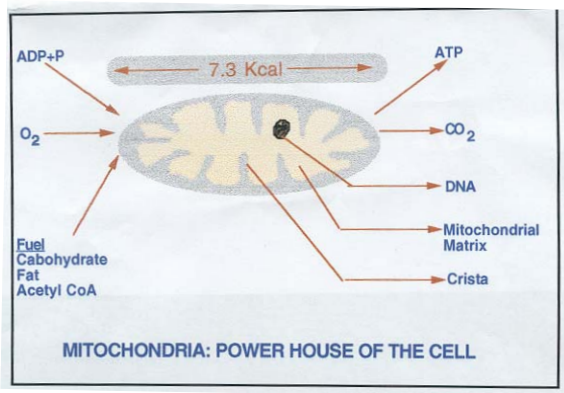

Figure 1. Structure and function of mitochondria.

Mitchondria are double (outer and inner) membrane structures with the inner membrane making finger-like projections (cristae) into the mitochondrial matrix, which hosts, besides other things, a closed, covalent and circular DNA. Cristae give rise to stalked particles of 8.5 nm at regular intervals of 10 nm. These are called inner membrane spheres or F1 particles, and are involved in ATP synthesis. Mitochondria may accumulate iron containing pigments or protein moieties to form yolk bodies. Injuries to mitochondria may cause their degeneration into cytolysosomes or chondriospheres (fusion of mitochondria during degeneration to form large bodies).

Box 2. Apoptosis

Apoptosis is a genetically driven universal biological phenomenon in eukaryotes to control cell number and to eliminate cells which jeopardise the survival of the organism. *Caenorhabditis elegans*, a nematode, has proved an excellent system for dissecting the genetic anatomy of apoptosis. All together 14 genes, including ced-3, ced-4 and ced-9 associated with apoptosis have been investigated with satisfactory details. Ced-3 encodes a caspase protein, the chief destroyer of vital macromolecules, whereas ced-9 is an antiapoptotic protein which inhibits apoptosis. Ced-4 acts as a regulating bridge between the two functionally opposite genes. Normally, ced-9 remains complexed with ced-4 and ced-3, keeping ced-3 inactive. On receiving an appropriate apoptotic stimulus ced-9 is dissociated from the complex allowing ced-4 to activate ced-3. Vertebrates have evolved genes that resemble *C.elegans* cell death genes. Mammalian caspases are functional homologues of ced-3. Apaf-1 has been recognized in mammals as a ced-4 homologue. Bcl-2 gene family is related to ced-9 but is composed of two sub-groups, one inhibiting and the other promoting apoptosis.

Gene Product	Source
BCL-2	Eukaryotic
CrmA	Cow pox virus
E1A	Adenovirus (19 kDa, 55 kDa)
HSV-1	Gene g134.5
IAPs	Baculovirus homologues found in eukaryotes as well
LMW5-HL	African swine fever virus, homologue of bc1-2
LMP-1	EBV, upregulates host bc1-2 expression
P35	Baculovirus

Table 3. Inhibitors of apoptosis.

cells picked up an ancestor of the present day purple bacteria as a symbiont (relationship in which both the partners benefit from each other and live in close association). There are reports which suggest that both mitochondria and chloroplasts are the descendants of symbiotic prokaryotes living within the eukaryotic cell. Prospects of huge energy benefits for both, accruing from the emerging oxygen atmosphere, which was toxic to other life forms, presumably kept them together. The result of this endosymbiosis was the emergence of protoeukaryotic cells hosting intracellular bodies, which were to become mitochondria. This alliance was frequently threatened because of (i) the conflicts in selection between their genomes, and (ii) the fully aerobic atmosphere. The protomitochondria, however, played the lead role in oxygen metabolism providing oxidative phosphorylation (ATP generation). However, conditions that favored the protomitochondria over the host cell would kill the cell and release the endosymbiont. The symbiosis, therefore, remained perilously fragile until essential genes for mitochondrial metabolism and biogenesis were acquired by the nuclear genome culminating in the modern 'obligate symbiosis'.

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Evidence of Mitochondrial Involvement in Cell Death

There are several lines of biochemical and cellular evidence that directly link mitochondria to cell death. Bax, a mammalian cell death protein targets mitochondrial membranes to induce mitochondrial damage and cell death. Bcl-2, an anti-apoptotic pro-

tein, also exists abundantly in the mitochondrial membrane. In a cell-free system, mitochondrial presence is necessary to induce the nuclear condensation and DNA fragmentation, which is considered as a signature of apoptosis. Induction of caspase, that actually executes the cell death in presence of ATP, also requires cytochrome-c (cyto-c) released from mitochondria. These observations provide unequivocal evidence of an important involvement of mitochondria in apoptosis.

How do Mitochondria Orchestrate Apoptosis?

Three inter-related pathways are regulated in mitochondria to bring about cell death: (1) Disruption of electron transport (electron transport system is organised within the membrane and the electrons are passed from one carrier to another on the respiratory chain whereas the protons (H^+) are ejected towards the cytoplasmic side while the OH^- remains on the matrix side, thereby creating a difference in the pH resulting in the electric potential) and oxidative phosphorylation (ATP synthesis by phosphorylation of ADP using the energy provided by the electron transfer during aerobic respiration), leading to non-generation or less generation of ATP. (ii) Release of factors that trigger activation of key enzyme(s) of apoptosis i.e. Caspase family of proteases. (iii) Change in the cellular redox potential.

(i) Disruption of Electron Transport Chain: Several agents, such as gamma-radiation, ceramide, and Fas (receptor) ligation have been demonstrated to cause disruption of the electron transport chain at various levels (reduction in ATP generation) leading ultimately to apoptosis. However, it is important to understand that a drop in ATP production occurs late in the process, hence this affects downstream events in the apoptotic pathway. Loss of mitochondrial ATP, as such, can thus kill a cell by necrosis (*Table* 1) but it may not necessarily induce apoptosis. In other words, loss of ATP could be an effect of apoptosis, but not necessarily a cause.

(ii) Caspase Activating Factors: Caspase is the ultimate death

executioner in most of the apoptotic programmes. Mitochondria release some protein factors, now recognized as cyto-c upon activation, which in turn activate caspases in the presence of ATP in the cytoplasm. Involvement of cyto-c in the death programme was demonstrated by studies on cell-free systems, in which spontaneous bcl-2 mediated inhibition of nuclear condensation and DNA fragmentation were observed to depend on the presence of mitochondria. Cytochrome-c was earlier reported by T Ramasarma and his group to be released from the mitochondria under conditions of starvation – a condition similar to growth factor withdrawal – which signals apoptosis. Experiments on vertebrates demonstrate that cytosolic cyto-c exists as 'apoptosome', which comprises cyto-c, Apaf 1 and procaspase-9 (*Figure 2*). From the apoptosome, active caspase-9 is released, which then gears up other types of caspases to orchestrate the biochemical execution of the cell. The precise pathway, however, remains unknown.

Surprisingly, most of the caspase-inhibitors failed to prevent cyto-c release induced by apoptotic agents including UV, staurosporine (inhibitor of protein kinase C) and over expression of bax (a gene product that induces apoptosis). Release of cyto-c from mitochondria may thus kill cells in two ways: (i) release of active caspase via an express pathway involving Apaf 1,

Box 3. Processing of the Apoptotic Signals.

The cellular apoptotic machinery is kept in check by two factors: (i) survival signals from the cells' environment and (ii) internal sensors for cellular integrity. The apoptotic machinery is, therefore, geared up as and when cellular contact with its surroundings is snapped or there is an irreparable damage and conflicting signals for cell division reach the cell simultaneously. In the mammalian immune system, an 'instructive' mechanism of cell destruction is followed. The lead role in this mechanism of apoptosis is played by the 'death receptors' present on the cell surface, which transmit death signals initiated by specific 'death ligands'. These receptors are capable of activating caspases within seconds of ligand-binding, destroying the cells within hours. Death receptors are encoded by TNF (tumor necrosis factor)–receptors gene superfamily, comprising cellular 'death domain'. The latter enables death receptors to gear up the apoptotic machinery. Some of the best known of these death receptors are CD 95 (also called fas or Apo1) and TNFR1 (also known as p55 or CD 120a).

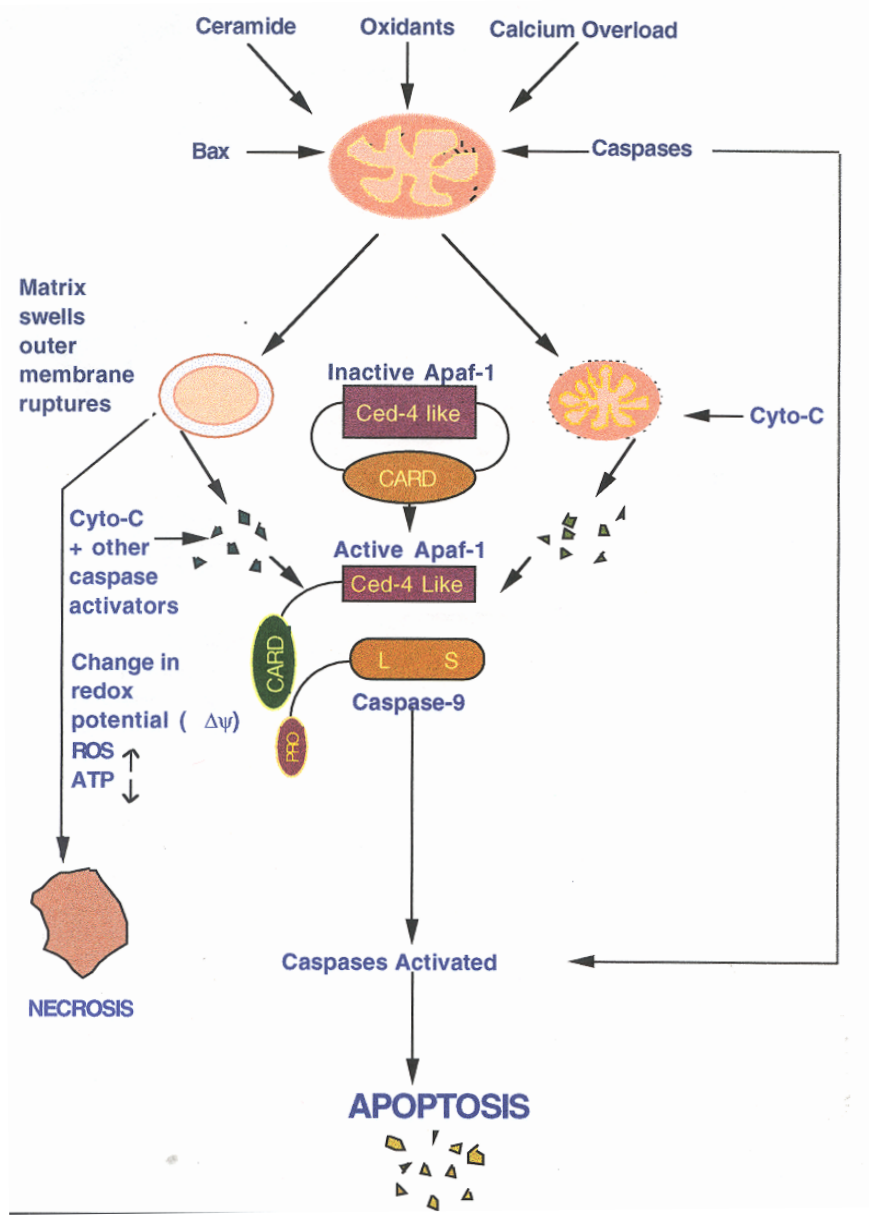

Figure 2. Activation of caspases through mitochondria: Different stimuli (oxidants, bax, ceramide etc.) can trigger mitochondria to release cytochrome-c (cyto-c) and other protein factors including caspases. (i) Cyto-c may directly bind to Apaf1 to enable it to associate with procaspase-9, triggering caspase-9 activation; (ii) Cyto-c release may also lead to loss of membrane potential and ATP and simultaneous increase in ROS causing necrotic death of cells.

or (ii) slow necrotic pathway due to depletion of cyto-c in mitochondria and consequent drop in ATP generation. Mitochondria also release some other apoptosis mediating factors, for example procaspase-3. Whether it assumes an active form before release from mitochondria is poorly understood.

(iii) Change in the Cellular Redox Potential: Superoxide anions are generated largely in mitochondria. During electron transport in the respiratory chain, 1-5% of electrons miss their track to fall prey to O_2 producing O_2^- (superoxide). Agents that cause uncoupling may also enhance production of O_2^-. Reactive oxygen species (ROS) produced in the course of faulty mitochondrial metabolism may induce lipid peroxidation of membrane lipids leading to morphological changes akin to apoptosis. Anaerobic conditions may also gear up cells to undergo apoptosis by certain stimuli. However, ROS can be generated even under conditions of virtual anaerobiosis, and thus their role in apoptosis cannot be contradicted entirely on this basis.

Redox Potential and Permeability Transition (PT)Pore

In normal growing cells, a specific chemical and electrical gradient exists on both sides of the mitochondrial membrane resulting from the asymmetric distribution of protons and ions. Disruption in this transmembrane potential ($\Delta\psi_m$) can be observed in many different cell types, irrespective of the apoptosis-inducing agent. In most of the apoptotic cells, the mitochondrial inner transmembrane potential collapses, which indicates opening of conductance channel known as PT pore. PT pore opening results in cessation of ATP synthesis, matrix Ca^{2+} outflow, depletion of reduced glutathion, etc. Though the exact structure of PT pores is not well understood, there are reports that show the existence of complex formation between the ANT (adenine nucleotide translocator) present in the inner membrane with the outer membrane proteins in the inner-outer membrane contact sites which, in turn interact with hexo and glycerol kinases. However, certain substances (e.g. cyclosporins)

Suggested Reading

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[3] D R Green, *Cell*, 94, 695–698, 1998.

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that inhibit PT pores appear to block apoptosis. Moreover, a specific inhibitor of PT, bongkrekic acid (BA), a ligand of the ANT can inhibit the pre-apoptotic ($\Delta\psi_m$) disruption and subsequent apoptosis in several different experimental systems. This suggests that PT pore inhibitors could also be considered as potential therapeutic targets in future.

What are Caspases?

Caspases are specific cysteine-rich proteases. With the discovery of CED-3, a cell death gene product of a nematode (*Caenorhabditis elegans*) the involvement of caspases in cell death was suggested. CED-3 shares homology with the mammalian interleukin 1β-converting enzyme (ICE). Recently, another mammalian homologue of Ced-3, YAMA, has been identified. Several lines of evidence support its ability to cleave poly (ADP-ribose) polymerase representing YAMA as an effector component of mammalian cell death pathway. Over a dozen caspases have now been discovered, and they all share similarities in amino acid sequence, structure and substrate specificity. Expressed as proenzymes (30-50 kD), all of them consist of three domains: an NH_2 terminal domain, a large subunit (approx. 20 kD), and a small subunit (approx. 10 kD). Activation requires proteolytic processing between the domains, followed by association of the large and small subunits to generate a heterodimer. Crystal structures of some of the active caspases (caspase-1 and 3) show two catalytic sites that apparently function independently. The catalytic domain contains intimately associated large and small subunits with each one contributing residues necessary for substrate binding and catalysis (*Figure* 2).

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Caspases constitute one of most specific cysteine proteases with an absolute requirement for cleavage site after aspartic acid (hence the name *casp*ase). At least four amino acids to the NH_2 terminal side of aspartate serve as the recognition site for cleavage and functional catalysis. The tetrapeptide recognition motif differs significantly among caspases, explaining the diversity of their biological functions. The cleavage of protein by caspases is

Box 4. How can we Study Apoptosis?

Cells undergoing apoptosis can be studied experimentally by light microscopy and/or by biochemical methods:

(i) Light microscopy: Cells treated with trypan blue which stains the dead cells selectively, are observed immediately under light microscope to score for blebbed cells. The apoptotic bodies are taken as a reasonable parameter to study apoptosis.

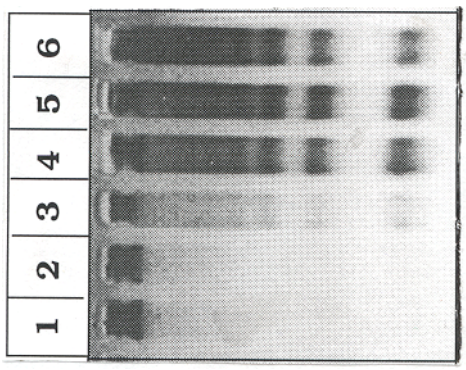

Figure 3. Nucleosomal DNA ladder formation in insect cells as a function of increase in oxidative stress. Lane 1, untreated cells; lanes 2, 3, 4, 5, 6, cells showing increased fragmentation with the increase in oxidative stress.

(ii) Biochemical method: Cells including apoptotic bodies are collected and processed to extract genomic DNA which is then resolved in 2% ethidium bromide agarose gel. Characteristic DNA ladder formation as a result of fragmentation of the genomic DNA on the agarose gel is considered as a key feature of apoptosis often referred as apoptosis signature (*Figure* 3).

Several other biochemical assays are available also to monitor apoptosis. These include TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling) Assay, introduced by Sohan Modak now at Poona University. This assay is based on DNA synthesis using certain fluorescent nucleotides on the chopped 3'OH of fragmented genomic DNA. The fluorescent DNA in cells is then observed under a fluorescent microscope.

Mitochondrial permeability transition (PT): This assay mainly looks at the changes in the permeability of the mitochondrial membrane. PT involves sudden permeability increase of the inner membrane to solutes such as protons, calcium, glutathion, etc. PT can be quantified using radioactive markers or spectrophotometrically. The isolated mitochondria resuspended in protein-free buffer shows colloidosmotic swelling due to PT. This 'large amplitude swelling' causes reduction in the OD_{540} values.

very specific and efficient. This is consistent with the observation that apoptosis is not accompanied by an indiscriminate cleavage of cellular proteins and that cleavage is usually at a single site resulting in loss or change of function.

How do Caspases kill a Cell?

The complete scenario of apoptosis is gradually opening up. Major apoptotic events include genomic DNA fragmentation,

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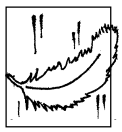
chromatin condensation, membrane blebbing, cell shrinkage and disassembly into membrane-bound vesicles (apoptotic bodies). The process ends with engulfment of apoptotic bodies by the neighboring cells. Involvement of caspases in manifestation of the above mentioned events is not yet fully understood. So far, about 40 substrates of caspases have been recognized. These can be classified into three major categories: (i) those which maintain vital cellular functions, (ii) cytoskeleton, and (iii) those which protect cells against apoptosis and insults. When these and other yet unknown substrates are cleaved by caspases, the cells undergo changes as manifest in apoptosis.

Conclusion

Mitochondria serve as power houses of cells but they also possess 'covered' knives to kill the cells. A number of intrinsic and extrinsic factors exist which regulate cell killing by mitochondria. Caspases are comparable to a surgeon's knife, which can be used to sculpt organisms, get rid of wayward, redundant and incorrigibly damaged cells, but can spell doom if not controlled properly.



In a Lighter Vein...

If Einstein, Rosen, Podolsky and Gödel were engaged in a friendly game of badminton, would they be able to resist talking about some of their profound theories inbetween points? Well, you have to watch the delightful film GENIUS to find out the answer! Walter Mathau plays the role of Einstein to perfection, convincing us in the process that he is to the film world as Einstein is to the physics world. However, the protagonists of this film are actually Einstein's niece and the friendly neighbourhood garage mechanic, the villain being a stuffy psychology professor. Instead of unravelling deep secrets of nature, Einstein is plotting to convince his niece to abandon the idea of marrying the pompous professor and get married to the garage mechanic instead! This movie is an absolute must for physicists/physics students with a sense of humour (assuming of course that there are such people(!)).

Alladi Sitaram