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Cell death disguised: The mitochondrial permeability transition pore as the c-subunit of the F_1F_O ATP synthase

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ABSTRACT

Ion transport across the mitochondrial inner and outer membranes is central to mitochondrial function, including regulation of oxidative phosphorylation and cell death. Although essential for ATP production by mitochondria, recent findings have confirmed that the c-subunit of the ATP synthase also houses a large conductance uncoupling channel, the mitochondrial permeability transition pore (mPTP), the persistent opening of which produces osmotic dysregulation of the inner mitochondrial membrane and cell death. This review will discuss recent advances in understanding the molecular components of mPTP, its regulatory mechanisms and how these contribute directly to its physiological as well as pathological roles.

1. Mitochondria at the center of cell metabolism and cell death

Mitochondria are complex organelles responsible for producing energy in the form of ATP for most eukaryotic cells. They regulate several other essential processes including calcium (Ca^{2+}) homeostasis, heme and steroid biosynthesis. In addition, the mitochondrion lies at the center of the cellular response to stress and the control of cell death.

To produce energy in the form of ATP, mitochondria utilize substrates produced in the cytosol by carbohydrate, lipid and protein metabolic pathways. These products, particularly acetyl co-enzyme A, enter the tricarboxylic acid cycle. Turns of the TCA cycle synthesize NADH and $FADH_2$ that donate their electrons to the electron transport chain. The energy of the bonds of NADH and $FADH_2$ is used to pump H^+ ions out of the matrix by the NADH dehydrogenase and other electron transport complexes, creating a proton motive force that in turn drives the F_1F_O ATP synthase [1]. Upon kinetic repositioning of the ATP synthase rotor, ATP is synthesized from ADP and P_i [2]. The machinery required for ADP/ATP exchange between the cytoplasm and the matrix including the outer membrane voltage dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT) at the inner membrane are intimately linked to that of the ATP synthase [3].

2. Mitochondrial inner membrane leak: regulator of metabolic rate and uncoupling

There are two currents that complete the current loop of the proton pumping activity of the electron transport complexes. First, ATP is formed by hydrogen ion (H^+) translocation through the ATP synthase in the opposite direction to that of the electron transport complexes. Second, an apparently wasteful leak in the inner mitochondrial membrane provides a pathway for uncoupling of oxidation from phosphorylation as H^+ ions enter the matrix through channels independent of ATP production. Classically, uncoupling proteins carry out this role. Known physiological functions of uncoupling proteins are to generate heat for organisms with large surface to volume ratio, to depolarize mitochondria in order to temper oxidative damage and to regulate metabolic rate during hibernation and at other times [4–7]. In addition to uncoupling proteins, however, intrinsic uncoupling exists within other inner mitochondrial membrane channels and transporters and within the F_1F_O ATP synthase [8,9].

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3. Mitochondrial inner membrane Ca^{2+} cycling regulates cellular Ca^{2+} dynamics: the example of neuronal short term synaptic plasticity

Mitochondrial inner membrane depolarization occurs not only through proton movement but also via the flux of other ions including Ca^{2+} across mitochondrial membranes. Ca^{2+} movement into the mitochondrial matrix is a physiological event that takes place in response to increased cytosolic Ca^{2+} levels. Ca^{2+} buffering is frequently employed by mitochondria in cells that experience rapidly changing cytosolic Ca^{2+} levels.

Mitochondria regulate cytosolic levels of Ca^{2+} and the release of Ca^{2+} and metabolites through an intricate system involving several ion channels. The discovery of the molecular structure for the Ca^{2+} uniporter ion channel (MCU) at the mitochondrial inner membrane has generated increasing interest in mechanisms of Ca^{2+} management within the cell body of many types of cells and also in the presynaptic terminals of neurons [10–13]. Additional isoforms of MCU and its helper MICU that confer tissue specificity and other behaviors have added to our understanding of the mechanisms of activity dependent energy production by mitochondria [14,15]. Mitochondrial Ca^{2+} release also appears to be highly regulated, involving both exchangers and channels, but, unlike the MCU, the molecular components of a Ca^{2+} release channel were only recently discovered and form the main focus of this review.

Ca^{2+} re-release from mitochondria determines short term synaptic plasticity in certain neuronal synapses [16]. During neuronal activity, Ca^{2+} influx across the plasma membrane occurs through glutamate receptors and voltage gated Ca^{2+} channels. After Ca^{2+} enters the cytosol, Ca^{2+} clearance is performed by the actions of Ca^{2+} ATPases at the plasma membrane and by buffering through uptake by intracellular stores including the endoplasmic reticulum (ER) and mitochondria [12,17]; these processes reset the normally low Ca^{2+} levels present in resting neurons or neuronal synapses. The Ca^{2+} that is buffered by intracellular stores is eventually re-released, providing, for example, for residual Ca^{2+} in presynaptic endings. Residual Ca^{2+} increases the amount of Ca^{2+} available for synaptic vesicle fusion, enhancing the amount of neurotransmitter released for a given stimulus [18,19].

Ca^{2+}-sensitive ligand gated mitochondrial channels, which are widely conserved and found in species from invertebrates to mammals, open in response to elevated Ca^{2+} within the mitochondrial matrix. In the squid presynaptic terminal, opening of a Ca^{2+}-activated mitochondrial channel is correlated with enhanced neurotransmitter release [20]. Electrophysiological recordings [21] demonstrate that within the resting presynaptic terminal, the conductance of mitochondrial membranes is low [20]. In contrast, during high frequency electrical stimulation of the presynaptic nerve, a large increase in mitochondrial membrane ion channel activity takes place [20]. The delay in onset of the mitochondrial activity and the persistence of the mitochondrial activity after stimulation are in keeping with the role of a channel and/or exchanger in re-releasing Ca^{2+} from mitochondria for short term plasticity [20,22,23]. Furthermore, mitochondrial activity and short term increases in post stimulation synaptic transmitter release are both abrogated by applying the uncoupler FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), which depolarizes mitochondria, preventing Ca^{2+} handling [20].

4. Bcl-2 family proteins: regulators of mitochondrial outer membrane permeability and cell death

In contrast to the normal physiological role for Ca^{2+} release channels and exchangers in mitochondrial membranes, one of the main regulators of pathological mitochondrial permeability or leakiness are the proteins of the Bcl-2 family. Programmed cell death (apoptosis) in vertebrate cells may be initiated by signaling at the plasma membrane or by intracellular pathways that lead to changes in mitochondria [24]. The final common pathway for programmed cell death in many systems is mitochondrial outer membrane permeabilization (MOMP) [25–28]. Pro-apoptotic Bcl-2 family members such as Bax regulate MOMP by inducing the formation of large outer membrane pores comprised of activated oligomerized proteins, aided by other pro-apoptotic moieties [25,27,29]. In their canonical role, the anti-apoptotic Bcl-2 family proteins such as Bcl-x_L protect cells against MOMP by interacting with, and inhibiting the pore forming properties of, the pro-apoptotic family members [28,30].

MOMP leads to the release of several inter-membrane space proteins such as cytochrome *c* [31,32]. The resultant decrease in cytochrome *c* levels compromises the ability of mitochondria to maintain the mitochondrial inner membrane potential and to produce ATP [33]. In addition, cytochrome *c* released into the cytoplasm activates downstream cytosolic enzyme pathways including effector caspases that execute cell death [34].

5. The mitochondrial permeability transition and pathophysiology

In some cases, an increase in mitochondrial outer membrane permeability may also be triggered by an acute inner membrane depolarization [35], particularly after cytosolic and mitochondrial Ca^{2+} overload. Although Ca^{2+} uptake and re-release from mitochondria is a normal physiological event in cells, accumulation of Ca^{2+} in the matrix can have detrimental effects, including diminution in energy production by the ATP synthase [36]. Ca^{2+} overload can produce an uncoupling process described historically as a rapid increase in permeability of the mitochondrial inner membrane to solutes and the halting of ATP production [37–39]. This phenomenon is termed permeability transition (PT).

PT can be reversible or irreversible [35,37,40–45]. If not reversed, PT leads to an even more extreme form of catastrophic PT associated with structural breakdown of the mitochondrial matrix accompanied by outer mitochondrial membrane rupture and cell death [35]. The interaction of this kind of mitochondrial cell death with apoptotic death produced by MOMP has been debated. Although the two types of cell death seem to be overlapping, it is safe to say that pathological PT is associated with necrotic cell death such as is found in ischemia or injury whereas MOMP occurring in the presence of sufficient amounts of ATP may have a more important role in developmental and genetically predetermined death [46,47]. Inter-membrane space pro-apoptotic factors such as cytochrome *c* and Smac/DIABLO are released during both forms of cell death. In MOMP, outer membrane permeabilization alone leads to release of these factors, whereas in prolonged PT, rupture of the outer membrane after inner membrane swelling releases pro-apoptotic factors into the cytosol [35].

6. Physiological functions of the permeability transition pore

PT has been extensively studied for its role in ischemic injury in brain, heart and other organs as well as in neurodegenerative conditions [48]. In the heart, data suggest that opening of the mPTP during early reperfusion after ischemia is a harmful event that precipitates further damage to the myocardium [49]. However additional data also suggest that transient mPTP opening during preconditioning can be protective, thus serving a physiological role even during injury [43].