A n explosion of information has recently emerged highlighting the role of mitochondria in the life and death of cells. The function of mitochondria as the arbiters of a complex signaling cascade urging the cell to its own orchestrated demise is one of a number of new findings fueling a renewed interest in this essential organelle. Perhaps in no other tissue is it more important to maintain mitochondrial function and prevent cell dropout than in cardiac muscle. Pump function quickly fails when the constant high-energy demand of contraction is not met by the efficient generation of ATP through mitochondrial oxidative phosphorylation, and if cell death is triggered in the adult heart, regardless of whether it is through necrotic or apoptotic pathways, there is no way to regenerate lost myocytes.

**Slow Cardiac Death**

It is perhaps not surprising then to learn that the genetic program during the later stages of development tips the balance of proteins in favor of preventing apoptosis. In this issue of Circulation Research, Cook et al.1 examine the distribution of Bcl-2 family proteins in the rat heart and probe the mechanism of apoptotic cell death by studying H$_2$O$_2$-induced cell death in cultured neonatal myocytes. Interestingly, expression of the antiapoptotic proteins Bcl-2 and Bcl-xL was maintained at high levels throughout development to adulthood whereas the proapoptotic proteins Bad and Bax dropped to undetectable levels in the adult heart. In a similar vein, it has been previously shown that Bcl-2 mRNA is high in fetal rat, drops significantly postnatally, and then increases through day 21 whereas the rate of programmed cell death is inversely related to Bcl-2 expression.2 This suggests that a postnatal apoptotic window is opened to allow for a period of cardiac remodeling during maturation of the heart (reviewed in Reference 3). After this developmental period, however, cardiomyocyte apoptosis has generally been tied to pathology, with increased apoptotic cell death noted in the failing heart, after ischemia/reperfusion injury, and in association with conduction disorders.3,4

The link to mitochondrial function is made by Cook et al1 by observing that Bad is rapidly translocated from the cytosol to the mitochondria of cultured neonatal rat myocytes exposed to H$_2$O$_2$, followed soon after by release of cytochrome c into the cytosol and depolarization of the mitochondrial membrane potential ($\Delta$$\psi$). Subsequently, both Bad and Bcl-2 appear to be degraded. $\Delta$$\psi$, as measured by fluorescence-activated cell sorter (FACS) analysis of populations of myocytes, showed a triphasic response to the apoptotic stimulus, dropping rapidly, recovering at 1 hour, and then continuously declining.

These new findings fit in with the general architecture of the sequence of apoptotic cell death, but they also illustrate that there are a number of foundation bricks missing from this construction. Although the signaling pathways leading from the mitochondria have been explored in elegant detail (eg, AIF and cytochrome c release, APAF-1 and caspase activation), there is usually a question mark placed over the step that matters most: the mitochondrial events taking place between the noxious stimulus (eg, H$_2$O$_2$, UV irradiation, staurosporine, growth factor withdrawal, metabolic inhibition) and the slippery slope of apoptosis. Only by understanding the earliest trigger that provokes the primordial endosymbiont to rebellion can we begin to rationally prevent (eg, in cardiomyopathy) or promote (eg, in cancer treatment) programmed cell death.

**Mitochondria as Harbingers of Death**

A large volume of evidence indicates that mitochondria play a central role in triggering apoptosis (reviewed in Reference 5). Although the earliest chain of events after a noxious stimulus has not been delineated with fine time resolution, cytochrome c release appears to occur within minutes, presumably resulting from a loss of stabilization by Bcl-2 and Bcl-xL in the membrane space between the mitochondrial inner and outer membranes. Notably in the Cook et al1 study, by 5 minutes after H$_2$O$_2$ exposure, Bad already had translocated to the mitochondrial fraction, but it cannot be determined whether this movement trails or precedes cytochrome c release. Furthermore, as in other studies,6,7 depolarization of $\Delta$$\psi$ lags behind these initial changes. With the caveat in mind that FACS analysis cannot distinguish between apoptotic and nonapoptotic cell death, this present result is consistent with earlier findings that cytochrome c release does not necessarily require mitochondrial membrane depolarization. This finding, along with an insensitivity of apoptosis to cyclosporin A inhibition in some models,6 would suggest that the opening of an inner membrane permeability transition pore (PTP) is not the only mechanism mediating cytochrome c release. Additionally, oxidative phosphorylation itself is not a prerequisite...
for the presence of apoptosis, because cells devoid of mitochondrial DNA also exhibit programmed cell death and can be rescued by Bcl-2 overexpression. ATP depletion also fails to explain the early apoptotic signal. Direct assays of ATP content of cells undergoing apoptosis usually show no degradation of ATP until very late in the process, and several studies have shown that ATP is probably required for steering the cell toward apoptotic versus necrotic cell death.

As with the opening of the PTP, there are other possible triggers that fall into the category of exceptions to the fourth postulate of Mitchell’s chemiosmotic hypothesis, i.e., that the mitochondrial inner membrane is generally impermeable to anions and cations, except for the protons pumped by the electron transport chain and dissipated by the ATP synthase. With some homology to bacterial toxins, Bcl family proteins may form ion conductive pores, as demonstrated by incorporation of these proteins into lipid bilayers. This has not been demonstrated, however, in intact cells, and it is not clear how such pores would contribute to cytochrome c release, especially considering that Bcl-2 and Bcl-xl are presumably anchored on the highly permeable outer membrane of the mitochondrion. This does not necessarily preclude a connection with the inner membrane though, because Bcl family proteins are located at the contact sites between inner and outer membranes. These sites are the meeting point of numerous proteins, including the outer membrane voltage-dependent anion channel (VDAC), peripheral benzodiazepine receptors (PBRs), hexokinase, creatine kinase, adenine nucleotide translocase (ANT), cyclophilin, and proteins involved in the mitochondrial protein import pathway. Combinations of some of these proteins are believed to constitute the PTP (VDAC, cyclophilin, ANT) and other multiconductance inner membrane pores.

This leads to a wider discussion of the role of other known and yet-to-be-discovered ion channels of the mitochondrial inner membrane (reviewed in Reference 17). In addition to the multiconductance PTP, both anion conductive (eg, the outwardly rectifying ~100-pS channel and other low-conductance anion channels) and cation conductive-channels (eg, the Ca^2+ uniporter, the mitochondrial ATP-sensitive K+ channel [mitoKATP], and the Ca^2+-activated K+ channel) exist on the mitochondrial inner membrane. It is interesting to note that cyclosporine-insensitive inner membrane anion channels are potently inhibited by the benzodiazepine ligand PK11195, which interferes with the antiapoptotic effects of Bcl-2. However, this must be interpreted cautiously because benzodiazepines, as well as many other cardiotoxic drugs, also interact with the mitochondrial PTP. One wonders whether part of the effectiveness of clinical treatment is due to mitochondria as a drug target. By the same token, recent evidence suggests that although mitoKATP channels may be the primary effector of cardioprotection in the heart, the mitoKATP opener diazoxide may trigger the opening of the PTP and thus initiate cytochrome c release. Because the aforementioned ion channels have been implicated in matrix volume regulation, there is a direct connection with the idea that mitochondrial swelling may play a role in apoptosis. Indeed, the biophysical characterization and cloning of ion channels on the mitochondrial inner membrane may represent a new frontier of investigation, hopefully leading to a growth in this area akin to that achieved for surface membrane ion channels. The possible contribution of these channels to apoptotic and nonapoptotic cell death could then be directly addressed, leading to new questions about the physiological triggers of ion flux across the inner membrane.

Ultimately, the crucial experiments will be to determine the triggers and sequence of apoptotic cell death in vivo. There are several key questions about the mechanism of apoptosis in the intact heart. First of all, what is the true incidence of apoptotic cell death in normal and diseased hearts? Although several studies have reported a high rate of apoptosis in heart failure, others have pointed out that at the reported rates of cell dropout, the heart would suffer a rapid catastrophic failure which does not occur. One argument that has been raised is that many of these studies relied on TUNEL staining as an index of apoptosis, and this technique may stain nonapoptotic myocytes, including both necrotic and ultrastructurally sound cells undergoing DNA repair. Still other questions can best be answered by studying adult heart cells, preferably in the intact heart. It is well-known that in neonatal myocytes, energy production depends more on glucose utilization than in the adult heart, where the mitochondrial oxidation of fatty acids takes precedence. This difference, as well as the developmental changes in pro- and antiapoptotic proteins, begs the question of whether or not the results obtained in neonatal cultures are relevant to cardiac disease. Also, how does cardiac workload influence the susceptibility to apoptotic stimuli? How can the earliest mitochondrial perturbations be detected and/or prevented? Are the apoptotic signals confined to individual cells or can they propagate to their neighbors?

**Broader Implications**

Of course, many questions about the triggers of cell death cannot be dissociated from very basic questions about mitochondrial energy metabolism in general. Although it has been more than 45 years since the mechanisms of respiratory control of mitochondria were first investigated, there is still controversy surrounding the issue of what controls respiration in vivo. Recent evidence indicates that Ca^2+ may be the key mitochondrial signal matching the level of energy production to metabolic demand, but control may be distributed among several sites. Little is known about the role of other energy-dissipating pathways (such as inner membrane ion channels and uncoupling proteins) or the cell death signaling pathways on mitochondrial function in situations that fall short of triggering cell death. Furthermore, mitochondria produce and are affected by reactive oxygen species, which have been shown to paradoxically initiate both apoptosis and cardioprotection. How does the opening of mitochondrial K^+ channels act to protect against ischemic damage? Is mild uncoupling good or bad for the heart and over what time scale? What is the physiological role of mitochondrial uncoupling proteins?

There is also much more to learn about the organization and control of the mitochondrial network. Emerging evidence suggests that interconnections between mitochondria, analogous to gap junctions, may permit the mitochondria in a cell...
be interesting to see where this trend will lead us. Who knows? If all the pieces of the puzzle of apoptosis are filled in, then the same authors contributing today may still be a force 100 years hence.

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A New Wave

It is certainly clear that apoptosis has rekindled interest in mitochondrial function, but are we on the verge of renewing the scientific fervor of half a century ago that so elegantly described the components of mitochondrial energy metabolism? A quick check of the PubMed database shows that the number of publications mentioning mitochondria has waxed and waned over the years, with a peak in 1973 roughly coinciding with the 10-year anniversary of Mitchell’s chemiosmotic hypothesis (see Figure, top). Thus, one might speculate that the aftereffects of a new paradigm are to stimulate a burst of testing and inquiry. This is certainly true of apoptosis since the coining of the term in 1972,43 publications mentioning apoptosis have risen dramatically, now making up ≈2% of all Medline entries (Figure 1, top). Will this be true of the more recent connection between mitochondria and apoptosis? A groundswell of interest is apparently underway: the percentage of mitochondrial studies also mentioning apoptosis is rising rapidly (Figure 1, bottom). It will

Top, Number of publications mentioning (in title, abstract, or key words) mitochondria (open symbols) or apoptosis (filled symbols) expressed as a percentage of total Medline entries from 1965 to 1999. Bottom, Percentage of mitochondrial publications also mentioning apoptosis. Arrows refer to seminal publications in the field of mitochondria (Mitchell) or apoptosis (Kerr).

to act as a protonophoric cable.38 Ca^{2+} may also function as a trigger of the excitable mitochondrial network,39 so both internal and external factors are likely to contribute to coordination of mitochondrial function. In some situations of metabolic stress, mitochondrial redox potential and Δψ may become heterogeneous, and clusters of mitochondria may behave independently.40,41 It is unknown what role this plays in cell death, but it leads to speculation that there may be separate functional roles for different clusters of mitochondria in the cell.42 The development of dynamic models of metabolic propagation through cells and tissues will be a crucial aid to the study adaptation to changes in workload, ischemia/reperfusion injury, and cell death.


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Brian O'Rourke

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