The Journal of Experimental Biology 211, 1829-1840
Published by The Company of Biologists 2008
doi:10.1242/jeb.000299

Commentary

Mitochondria in energy-limited states: mechanisms that blunt the signaling of cell death

Steven C. Hand* and Michael A. Menze

Division of Cellular, Developmental and Integrative Biology, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

*Author for correspondence (e-mail: shand@lsu.edu)

Accepted 20 March 2008

Summary

Cellular conditions experienced during energy-limited states – elevated calcium, shifts in cellular adenylate status, compromised mitochondrial membrane potential – are precisely those that trigger, at least in mammals, the mitochondrion to initiate opening of the permeability transition pore, to assemble additional protein release channels, and to release pro-apoptotic factors. These pro-apoptotic factors in turn activate initiator and executer caspases. How is activation of mitochondria-based pathways for the signaling of apoptotic and necrotic cell death avoided under conditions of hypoxia, anoxia, diapause, estivation and anhydrobiosis? Functional trade-offs in environmental tolerance may have occurred in parallel with the evolution of diversified pathways for the signaling of cell death in eukaryotic organisms. Embryos of the brine shrimp, *Artemia franciscana*, survive extended periods of anoxia and diapause, and evidence indicates that opening of the mitochondrial permeability transition pore and release of cytochrome *c* (cyt-*c*) do not occur. Further, caspase activation in this crustacean is not dependent on cyt-*c*. Its caspases display regulation by nucleotides that is consistent with 'applying the brakes' to cell death during energy limitation. Unraveling the mechanisms by which organisms in extreme environments avoid cell death may suggest possible interventions during disease states and biostabilization of mammalian cells.

Key words: apoptosis, necrosis, permeability transition pore, metabolic depression, anoxia, diapause, caspase activation.

Introduction: survival during energy-limited states

Bioenergetic constraints, as a consequence of metabolic depression, occur when animals are challenged with stressful physical environments (Guppy and Withers, 1999; Hand and Hardewig, 1996; Hochachka and Guppy, 1987; Lutz and Milton, 2004; Storey and Storey, 2007), or when hypometabolism is ontogenetically programmed, as during entry into diapause. Diapausing organisms become hypometabolic even when environmental conditions exist that normally would promote active metabolism and development (Denlinger, 2002; Hand et al., 2001; Lees, 1955; Reynolds and Hand, 2004; Tauber and Tauber, 1976). It is clear that the degree of metabolic depression is tightly correlated with survivorship during energy-limited states like anoxia (e.g. Hand, 1998). When the primary metabolic pathway for ATP production (oxidative phosphorylation) is blocked, for example by the absence of an electron acceptor, energy utilization must be curtailed simultaneously. Otherwise, survival would be brief. Some animals like embryos of the brine shrimp, *Artemia franciscana*, exhibit profound metabolic depression and survive anoxia at room temperature for years (Clegg, 1997) with no evidence of apoptotic or necrotic cell death.

Many requirements for prolonged metabolic depression have been documented. Gene expression is restricted because the manufacture of new macromolecules is simply too expensive to maintain. Thus one observes the arrest of DNA transcription (Eads and Hand, 2003b; Hardewig et al., 1996; van Breukelen and Hand, 2000) and protein synthesis (Fraser et al., 2001; Hofmann and Hand, 1990; Hofmann and Hand, 1994; Kwast and Hand, 1996a; Kwast and Hand, 1996b; Land et al., 1993; Land and Hochachka, 1994; Pakay et al., 2003; Podrabsky and Hand, 2000; Wieser and Krumschnabel, 2001), and the extension of protein and mRNA half-lives (Anchordoguy and Hand, 1995; Anchordoguy and Hand, 1994; Anchordoguy et al., 1993; Eads and Hand, 2003a; van Breukelen et al., 2000). Channel arrest and other mechanisms to depress passive ion leak have been observed at both the plasma and mitochondrial membranes (Boutilier and St-Pierre, 2002; Buck and Hochachka, 1993; Gnaiger et al., 2000; St-Pierre et al., 2000). But unless depression of ion leakage is essentially total (highly improbable), ionic disturbances like calcium overload in the intracellular compartment will eventually occur and compromise of the mitochondrial membrane potential is likely. Dissipation of ion gradients (Covi and Hand, 2005; Covi and Hand, 2007; Covi et al., 2005) may occur when energy flow is restricted to the point that ion transport across membranes cannot keep up with passive ion leak. Such conditions are precisely the ones that unavoidably trigger the initiation of apoptosis in mammalian species. So how do some animals survive energy-limited states for days, weeks and years?

Survival presumably requires that unwanted initiation of cell death, in any of its various forms, is blunted or precluded. The focus of this commentary is to consider the role of the mitochondrion in cell death processes, to highlight fundamental similarities and differences in the regulation of cell death that exist across phylogenetically diverse groups, and to evaluate recent information

that indicates 'putting the brakes' on apoptosis is a critical event for cell survival during energy-limited states. Current data do not permit a comprehensive evolutionary analysis of the regulation of cell death (cf. Zmasek et al., 2007). However, an emerging picture is that some regulatory systems for controlling cell death are more sensitive to energy disruption than others. For example, a relatively modest compromise in calcium homeostasis may trigger apoptosis in mammals, whereas severe energy limitation may not initiate apoptosis in certain non-mammalian species. A character trait, like prolonged tolerance to anoxia, may be a consequence, in part, of the specific characteristics of apoptosis that are operative across species. Functional trade-offs in environmental tolerance may have occurred in parallel with the evolution of diversified pathways for the signaling of cell death in eukaryotic organisms.

Categories of cell death

Some confusion in nomenclature results from the fact that types of cell death historically are defined by morphological criteria without a precise reference to a biochemical mechanism (Kroemer et al., 2005). With an increased understanding of the multiple biochemical subroutines leading to cell death, especially in mammals, more mechanism-based definitions have appeared, sometimes leading to, in our opinion, less appealing constructs such as 'programmed necrosis' (Edinger and Thompson, 2004; Melino et al., 2005). Regardless of the specific category, cells eventually pass a point of no return (when rescue is no longer possible), which may or may not coincide with bioenergetic catastrophe depending on the specific cell death pathway executed. Biochemical reactions that occur after the point of no return may be either completely unregulated or highly orchestrated for some interval of time.

Apoptosis, necrosis, autophagy

Up to 11 different types of cell death have been defined, and the classification of cell death subroutines is an ongoing subject (Melino et al., 2005). For our purposes here, we will distinguish two major forms of cell death: programmed cell death (PCD) and necrosis. As the name denotes, PCD involves the execution of a genetic program, requires at least some minimal level of ATP to be executed in an orderly fashion and is characterized by cell shrinkage (Table 1). The cell is destroyed without the release of breakdown products into the extracellular space. Necrosis is characterized by a more severe disruption of bioenergetics, cellular swelling and eventual rupture. In contrast to PCD, cellular contents are released during necrosis and can inflame/activate other neighboring cells, thereby spreading the necrotic region. While autophagy is considered by some as a distinct type of PCD, it is in our view more appropriately viewed as a rescue mechanism in times of starvation, when the cell recycles internal components within double-membrane vesicles for the purpose of nutritional gain. Under severe nutrient limitation, cell death may occur simultaneously with autophagy, rather than as a result of autophagy (Lum et al., 2005).

Extrinsic *versus* intrinsic pathway to apoptosis in mammals

Two initiation pathways are triggered by different events to promote apoptosis in mammals. The extrinsic pathway is triggered through ligation of death receptors resident in the plasma membrane that transmit the death signal into the interior of the cell (Tran et al., 2004). This pathway frequently serves as a mechanism to remove cells during development, differentiation and tissue remodeling. The intrinsic pathway occurs as a response to moderate perturbation of intracellular homeostasis by various cellular stresses [e.g. increased reactive oxygen species (ROS) formation, xenobiotics, hypoxia/anoxia, viral or bacterial proteins, and accumulation of misfolded proteins] (Ferri and Kroemer, 2001). The mitochondrion is an important integrator of both pathways (although not essential for all modes of extrinsic cell death), and plays a leading role in the amplification of the death signal.

Mitochondrial involvement in cell death and comparison of pathways

Correlations that implicated mitochondrial involvement in apoptosis have accumulated for some time (for a review, see Kroemer et al., 1995), but it was in 1996–1997 that a major expansion of our understanding occurred, when it became clear mitochondria were not just bioenergetic organelles but also controlled life and death decisions in the cell (Liu et al., 1996; Yang et al., 1997). The fundamental step of cytochrome *c* (cyt-*c*) release by mitochondria in the progression of mammalian apoptosis sparked the realization that mitochondria play a critical gatekeeper role.

In order to appreciate evolutionary differences that may contribute to the sensitivity of cell death activation to energy limitation, we will briefly describe the apoptotic machinery for the three most well-studied systems, those of *Caenorhabditis elegans*, *Drosophila melanogaster* and mammalian cells. The intuitive notion that apoptotic networks increase in a linear way from simple to complex, as one moves from cnidarian–bilaterian ancestors to nematodes, flies and vertebrates, is apparently incorrect (Zmasek et al., 2007). Rather, there have been numerous losses of apoptotic paralogs among the apoptosome-forming proteins, and both losses and expansions in Bcl-2 and caspase families. Different members of these families led to the extant proteins represented across these groups and may explain some of the functional differences in proteins that once were thought to be orthologous (Zmasek et al., 2007). Thus, the homologies drawn below are for the most part meant to imply functional similarity.

The mitochondrial contribution to the death program in *C. elegans* is only recently becoming appreciated (Rolland and Conradt, 2006). In response to a death stimulus, the pro-apoptotic Bcl-2 family protein EGL-1 interacts with CED-9, which is a mitochondria-bound, anti-apoptotic homolog of mammalian Bcl-2 (Fig. 1A). The interaction causes the displacement and release of a CED-4 dimer. CED-4 is a mammalian homolog of APAF-1 and in turn activates the caspase 3 homolog CED-3 (Yan et al., 2005). During this process, fragmentation of the mitochondrial network can be observed (Jagasia et al., 2005), and two pro-apoptotic factors are released. Release of the AIF homolog WAH-1 (Wang et al., 2007; Wang et al., 2002) and the presence of CPS-6 [an endonuclease G

Table 1. Features of major death subroutines in metazoans

	Apoptosis (type I cell death)	Autophagy (type II cell death)	Necrosis
Genetic program	Yes	Yes	None
Cell membrane	Intact/blebbing	Intact/blebbing	Lysed
Cytoplasm	Condensation/apoptotic bodies	Autophagic vesicles	Swollen
Mitochondria	Intact/swollen	Intact	Ruptured
Chromatin	Condensed	Condensed	Flocculent
ATP (energy status)	Dependent	Dependent	Independent

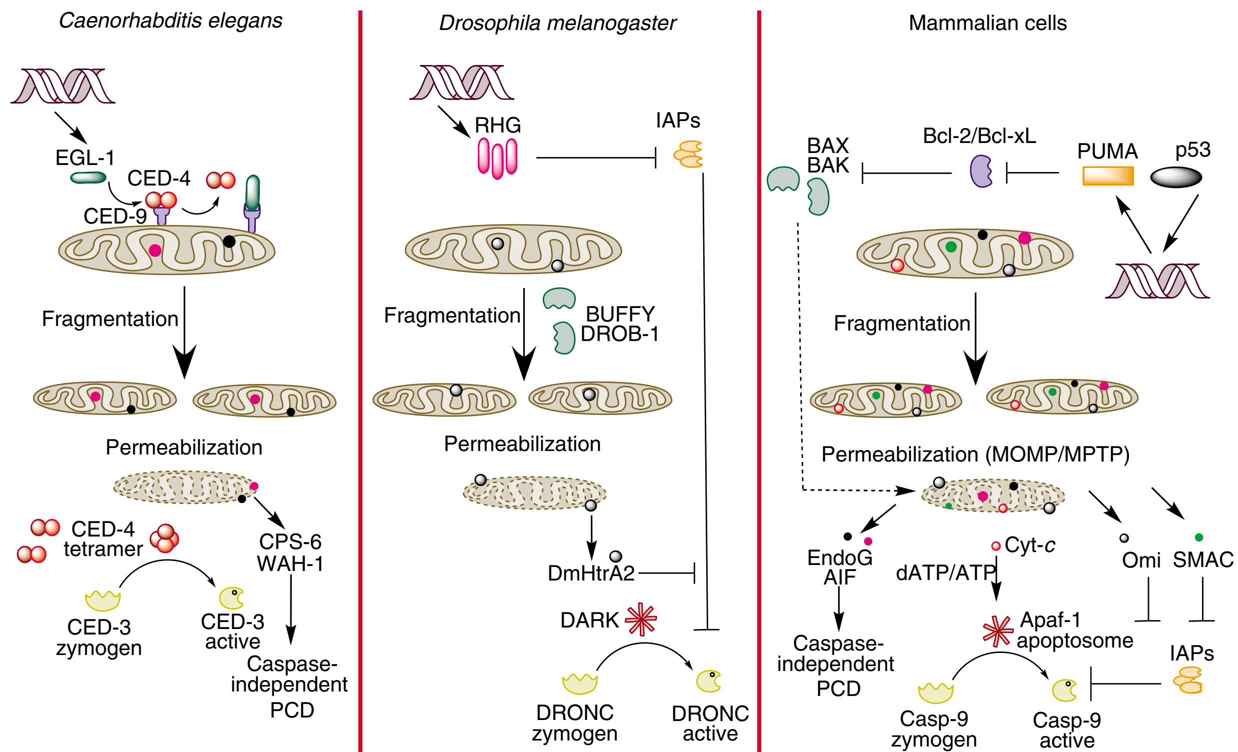

Fig. 1. Comparison of pathways for programmed cell death (PCD) in *Caenorhabditis elegans*, *Drosophila melanogaster* and mammalian cells. For details see text.

(EndoG) homolog (Parrish et al., 2001)] underscores the importance of mitochondrial involvement in apoptosis.

In *D. melanogaster* (Fig. 1B) cell death is largely regulated by the ***reaper***, ***hid*** and ***grim*** gene products (RHG killer proteins) and their inactivation of IAPs, a class of proteins not found in *C. elegans* that inhibit caspase activity (Hay et al., 2004; Kornbluth and White, 2005). Furthermore, two Bcl-2 proteins with modest pro- and anti-apoptotic features are found (BUFFY, DROB-1) that localize to the mitochondria (Igaki and Miura, 2004) and possibly impact mitochondrial functions and participate in stress-induced PCD (Sevrioukov et al., 2007). Highly conserved homologs of AIF and EndoG that contain mitochondrial target sequences are found in *Drosophila* (accession number NP_722765 and NP_610737, respectively), but thus far functional roles in PCD are undefined. However, recent reports underscore the importance of the mitochondrial involvement in apoptosis by demonstrating fragmentation (Abdelwahid et al., 2007) as well as permeabilization of the mitochondrial network and release of the pro-apoptotic protein DmHtrA2, which inactivates IAPs (Challa et al., 2007; Igaki et al., 2007). A pro-apoptotic action of cyt-*c* is controversial (Arama et al., 2006; Means et al., 2005) and may be restricted to certain cell types or tissues in *Drosophila*. As in *C. elegans*, activation of caspases can be mediated by an adaptor platform, the CED-4 homolog DARK (Yu et al., 2006). DARK interacts with the initiator caspase DRONC, an ortholog of the mammalian caspase 9 (Mills et al., 2006), and several downstream executor caspases have been described (Hay and Guo, 2006).

At least five factors that reside in the mammalian mitochondrion are involved in caspase-dependent and -independent PCD (Saelens et al., 2004) (Fig. 1C). Depending on the specific nature of the death signal, these factors are released through permeabilization of the outer, or inner and outer, mitochondrial membrane (Kroemer et al., 2007). This process is accompanied by an extensive fragmentation of the mitochondrial network that happens prior to caspase activation (Youle and Karbowski, 2005). After release from the mitochondrion, the NADH-oxidase AIF and the endonuclease EndoG translocate to the nucleus where they are involved in chromatin condensation and DNA degradation (Low, 2003; Modjtahedi et al., 2006). Other effectors impact the PCD machinery in a caspase-specific fashion. The RHG analog SMAC/DIABLO releases caspases from inhibition by IAPs. IAPs are intrinsic regulators of the caspase cascade and are the only known endogenous proteins that regulate the activity of both initiator (caspase 9) and effector (caspase 3, caspase 7) caspases (Liston et al., 2003). The serine protease Omi contributes to PCD in two ways. Omi neutralizes inhibition of caspases by IAPs, and also contributes to caspase-independent apoptosis through its protease activity (Saelens et al., 2004). Discovery of the Janus-faced nature of cyt-*c* reshaped our picture of the mitochondrion's role in life and death more than any other factor (Liu et al., 1996). Inside the mitochondrion, cyt-*c* is essential for oxidative phosphorylation, but after release into the cytoplasm, it initiates the assembly of the apoptosome, i.e. the molecular machinery that activates caspase 9.

Energy status and cell death

As documented for a number of mammalian cell lines, a drop in ATP is commonly associated with apoptosis [(Bossy-Wetzel et al., 1998; de Graaf et al., 2002; Izyumov et al., 2004; Marton et al., 1997; Vander Heiden et al., 1999); exceptions exist (e.g. Atlante et al., 2005)]. This relationship with ATP level exists despite the fact that there are a number of steps in the apoptotic pathway that

actually require ATP (see below, 'Specific requirements for ATP in cell death'). However, it is appropriate to point out that a major drop in cellular ATP can occur without limiting the activities of many of these steps, due to the micromolar K_m values for the enzymes involved (cf. Skulachev, 2006) and the rather high ATP binding affinities for non-catalytic proteins that participate in the process (Riedl et al., 2005). Further, the amount of ATP needed to permit the progression of apoptosis is actually quite small, and the source of ATP is not critical, e.g. it can be produced from either glycolysis or oxidative phosphorylation (Nicotera et al., 2000; Skulachev, 2006). Thus, modest compromise in mitochondrial function (and a concomitant drop in ATP) does not necessarily dictate that necrosis will be favored and apoptosis disfavored. Nevertheless it is true that necrotic cell death is typically observed under conditions of severe ATP depletion in mammalian systems (Atlante et al., 2005; Eguchi et al., 1997; Leist et al., 1997; Nicotera et al., 2000; Nicotera et al., 1998; Nicotera and Melino, 2004).

Skulachev and colleagues have studied the interplay in HeLa cells between cellular ATP levels and the occurrence of apoptosis *versus* necrosis (Izyumov et al., 2004). These authors report that not only is the magnitude of the ATP drop important for favoring one form of cell death over the other, but also the length of time HeLa cells experience compromised ATP levels is a determining factor, with longer exposures being more conducive to necrosis. Such linkages to both the duration and degree of ATP depression were first shown for Jurkat cells (Leist et al., 1997), an immortalized line of human T lymphocytes. When interpreting cell death studies with immortalized cell lines, a cautionary note it is that such cells typically display an altered metabolic poise (more glycolytic based), and the features of cell death are apt to differ from primary cells or tissues.

If placed in the perspective of the overall energy budget for a cell, the ATP requirements for operating the cell death pathways are likely to be small. The main consumers of cellular energy in the basal state are three processes. (1) The metabolic cost of active transport by the Na^+/K^+-ATPase for the maintenance of ion gradients averages 36% [range 15–58% (Covi and Hand, 2007)] of the basal metabolic rate across many cell types (Hand and Hardewig, 1996; Rolfe and Brown, 1997). Similarly, the cost of maintaining the proton gradient across the mitochondrial inner membrane, i.e. offsetting the proton leak, is also estimated to be quite substantial, accounting for 20–40% of the respiration rate of hepatocytes isolated from a rat and a lizard (Brand et al., 1994). Thus, over half the cell's energy can be devoted to processes of ion transport alone. (2) Under resting conditions, the metabolic cost of protein synthesis ranges between 18 and 26% in various tissues and cell types (Hawkins, 1991), and even higher values are observed for tissues during growth or increased biosynthetic activity (Land et al., 1993). The cost of ubiquitin-dependent protein degradation is sizable as well (Land and Hochachka, 1994). (3) Finally, DNA transcription and replication are responsible for up to 10% of basal cellular metabolism (Rolfe and Brown, 1997). Consequently, initiation and execution of apoptosis are unlikely to represent even a noticeable fraction of the energy budget. In the latter phases of cell death, when various physiological processes have been disrupted, the relative cost of cell death processes within the cellular energy budget undoubtedly increases. Unfortunately, a quantitative inventory of how much ATP is utilized during apoptosis is not available (Chiarugi, 2005).

Physiological stress and mitochondrial permeabilization

During metabolic states like diapause or during environmental stress (e.g. hypoxia, anoxia) when cellular ATP is at a premium, the ion transport capabilities directed at maintaining steady-state calcium distributions inside the cell can become restricted. Because the extracellular free Ca^{2+} concentration is about 10 000 times higher than the intracellular free Ca^{2+} concentration, the electrochemical gradient favors calcium entry in the cell. Cells utilize two primary mechanisms for removing excess calcium from the cytoplasm, both of which are energetically expensive: active export across the plasma membrane, and import into the ER and mitochondria. At steady state, free calcium in the ER is in the several hundred micromolar range (Alvarez and Montero, 2002). Thus, if the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) loses its ability to scavenge calcium from the cytoplasm, free calcium in the cytoplasm will rise precipitously (Walter and Hajnoczky, 2005). High cytoplasmic calcium has stimulatory effects at multiple steps in cell death pathways (Fig. 2; see 'Mitochondrial permeability transition pore (MPTP)' and 'Specific requirements for ATP in cell death' below).

Calcium distribution in mammalian mitochondria is a steady state, where electrophoretic calcium uptake *via* the calcium uniporter and/or rapid-uptake mode is opposed by calcium efflux through two separate pathways (Gunter et al., 2000; Gunter et al., 2004). Because the maximal rate of calcium efflux is much lower than calcium uptake, mitochondria exposed to high calcium are susceptible to the hazard of calcium overload (Bernardi, 1999). Calcium overload in the matrix can lead to opening of the mitochondrial permeability transition pore (MPTP) in mammalian cells (Fig. 2; see 'Mitochondrial permeability transition pore (MPTP)' below).

Oxidative stress as a result of the generation of ROS is another important initiator/facilitator of cell death pathways, and a sizable fraction of cellular ROS is generated by mitochondria. Yet, in the metabolic states being considered in the present commentary, mitochondrial electron transport is greatly downregulated (e.g. Clegg et al., 1996; Gnaiger et al., 2000; Hand, 1998; Reynolds and Hand, 2004). ROS generation by the mitochondrion cannot occur under anoxia due to the absence of the substrate for ROS and a terminal electron acceptor to support electron transport. Likewise, during diapause mitochondrial respiration can be depressed by as much as 97% by unidentified mechanisms (Clegg et al., 1996; Reynolds and Hand, 2004). Thus, in neither case is the generation of ROS an issue. For further information on the interplay between oxidative stress and cell death, a number of useful reviews are available (Brookes et al., 2004; Kakkar and Singh, 2007; Orrenius et al., 2007; Ryter et al., 2007).

Mitochondrial permeability transition pore (MPTP)

Basic features of the regulated MPTP in mammals

If mammalian mitochondria are exposed to high calcium concentrations in the presence of the co-activator P_i (Fig. 2), especially when accompanied by adenine nucleotide depletion and a reduced inner membrane potential (Petronilli et al., 1993a), a large swelling can be observed that is associated with the uncoupling of respiration and release of cyt-*c* (Gunter and Pfeiffer, 1990; Halestrap et al., 2000; Haworth and Hunter, 1979). These phenomena are due to a sudden increase in permeability of the inner mitochondrial membrane to solutes with a molecular mass of up to 1500 Da, a phenomenon known as the mammalian mitochondrial permeability transition (Bernardi, 1996; Hunter et al., 1976). The swelling of the matrix compartment causes rupture of the outer mitochondrial membrane and release of multiple pro-apoptotic factors from the intermembrane space. The mitochondrial permeability transition is mediated by a multi-protein complex,

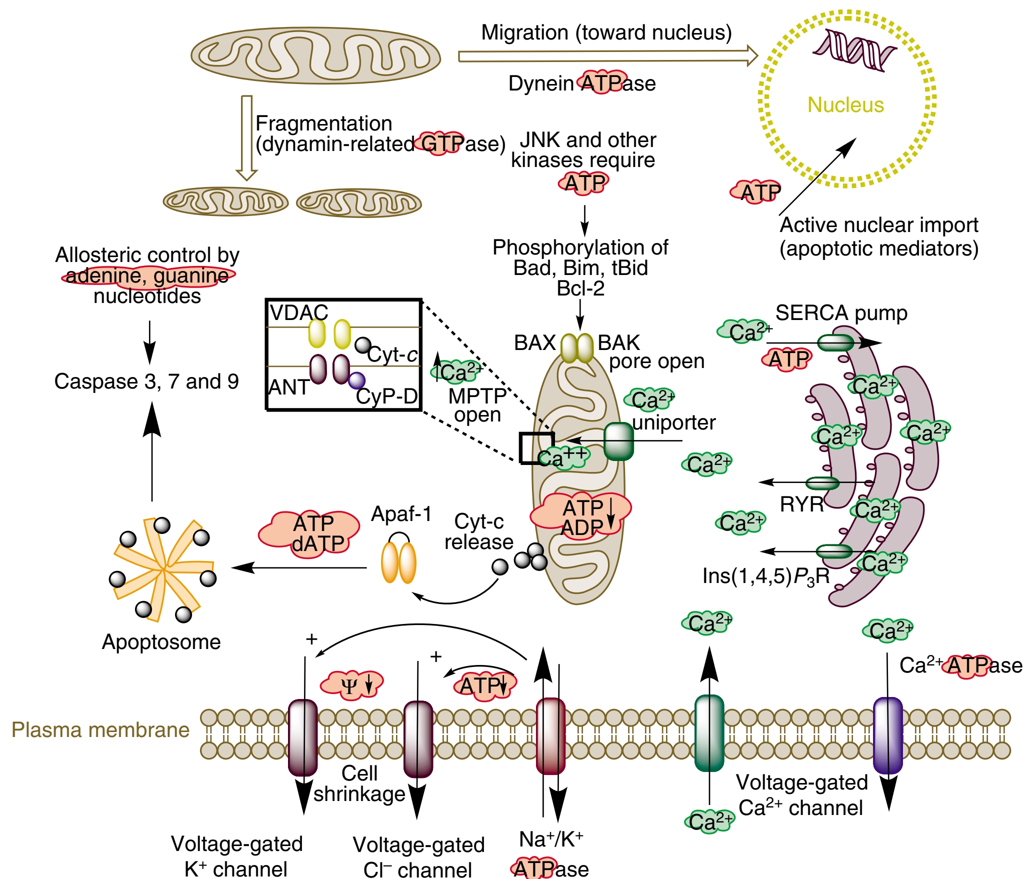

Fig. 2. Steps at which energy availability (shaded red) and calcium (shaded green) may impact PCD. For further explanation see text.

which can be defined as a voltage-dependent, cyclosporin A-sensitive and calcium-activated inner membrane channel (Bernardi et al., 1999).

The molecular composition of the MPTP is still not established. Historically, the minimum constituents of the regulated MPTP were proposed to be the voltage-dependent anion channel (VDAC), the adenine nucleotide translocators (ANTs), and the peptidyl-prolyl cistrans isomerase (PPI) cyclophilin D (Crompton, 1999; Halestrap and Brennerb, 2003; Pliyev and Gurvits, 1999). Although the sensitivities to inducers are altered, recent evidence has shown that the MPTP can form a functional complex in the absence of ANTs (Kokoszka et al., 2004), without VDAC (Baines et al., 2007; Krauskopf et al., 2006), and without cyclophilin D (Baines et al., 2005; Basso et al., 2005). Not surprisingly, as a result alternative compositional models have been proposed (Bernardi et al., 2006; He and Lemasters, 2002; Rasola and Bernardi, 2007).

Non-specific (unregulated) pore

He and Lemasters (He and Lemasters, 2002) proposed a two-step mode of mercury-induced mitochondrial permeabilization: activation of the regulated MPTP by low mercury and unspecific ‘damage’ of membrane proteins at higher mercury, leading to an unregulated pore. More than 30 mitochondrial carriers have been described (Passarella et al., 2003), and mercury is known to interact with several of these and transform their properties to be more channel like and non-specific (Dierks et al., 1990a; Dierks et al., 1990b). In the absence of a defined macromolecular composition for the regulated MPTP, it is possible that oxidation/degradation of variable combinations of mitochondrial proteins that then associate and in some manner permeabilize the inner membrane might represent a plausible explanation for even the regulated pore. However, for such a scenario to hold, it must account for specific pore opening by the physiological inducers calcium plus phosphate, the specific inhibition by cyclosporin A, and voltage dependency.

Non-mammalian MPTPs

Despite an enormous amount of literature on the mammalian MPTP, little is known about MPTPs in non-mammalian species. *In vitro* studies have shown that in lipid vesicles, reconstituted ANTs from *Neurospora crassa* can form mega-channels that exhibit characteristics similar to the MPTP (response to calcium, ADP and bongkrekic acid) (Brustovetsky et al., 2002). However, the occurrence of an *in vivo* MPTP in *N. crassa* mitochondria was not examined. Mitochondria from the yeast *Saccharomyces cerevisiae* exhibit a permeability transition pore that is inhibited by ADP and has a size exclusion comparable to mammalian MPTPs, but the yeast pore is not induced by calcium and is not cyclosporin A sensitive (Jung et al., 1997). In addition, a calcium-induced transition could not be found in mitochondria from the yeast *Endomyces magnusii* (Deryabina et al., 2004). Investigations of purified potato and wheat mitochondria show a calcium-sensitive permeability transition that is inhibited by cyclosporin A in the presence of dithiothreitol (Arpagaus et al., 2002; Virolainen et al., 2002). Isolated liver mitochondria from the great green goby (*Zosterisessor ophiocephallas*) show calcium-induced swelling if

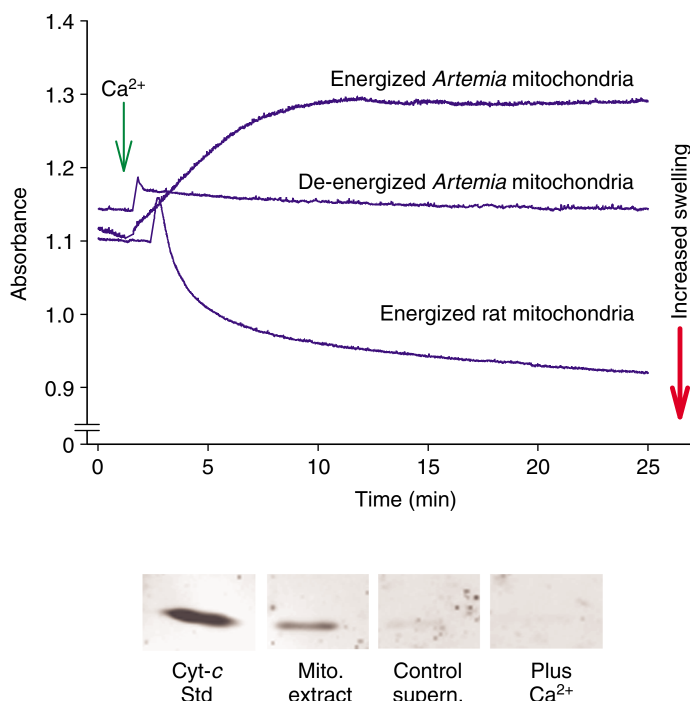

Fig. 3. Calcium does not open the MPTP in *A. franciscana*. Upper frame, presence or absence of swelling induced by calcium plus phosphate in isolated mitochondria from post-diapause embryos of *A. franciscana* and rat liver. Mitochondrial absorbance (a.u.) at 540 nm (inversely related to swelling) was measured after addition of 1 mmol l^{-1} calcium to *A. franciscana* mitochondria and 0.1 mmol l^{-1} to rat liver mitochondria. Mitochondria were energized with 5 mmol l^{-1} succinate. Lower frame, Western blot analysis showing lack of cytochrome *c* (cyt-*c*) release from *A. franciscana* mitochondria in response to calcium. Blots were probed with cyt-*c* antibody. Cyt-*c* Std, positive control, purified horse heart cyt-*c*; Mito. extract, positive control, supernatant from homogenized mitochondria; Control supern., supernatant from energized mitochondria without Ca^{2+} addition; Plus Ca^{2+}, supernatant from energized mitochondria plus 1 mmol l^{-1} Ca^{2+} (modified from Menze et al., 2005b).

incubated in the presence of P_i, but the amount of calcium required to induce the mitochondrial permeability transition was substantially higher than that needed for mitochondria from rat liver (Toninello et al., 2000). While data are not fully conclusive, a MPTP may exist in liver mitochondria from the Baltic lamprey *Lampetra fluviatilis* (Savina et al., 2006).

Recalcitrant animal MPTPs

Although uncoupled proton flux across the inner membrane is reduced under severe hypoxia in *A. franciscana* embryos (Gnaiger et al., 2000), it is nevertheless inconceivable that the mitochondrial $\Delta\Psi$ is not eventually compromised during prolonged anoxia, which in this animal can be tolerated for several years at room temperature (Clegg, 1997), or during months of diapause. A decline in membrane potential increases the probability of MPTP opening by calcium (Bernardi, 1992; Petronilli et al., 1993a; Petronilli et al., 1993b). A large increase in intracellular calcium is a hallmark change observed in many cells exposed to anoxia (Hochachka, 1986). Thus a question arose as to how *A. franciscana* avoided apoptotic and necrotic cell death during long-term anoxia, an environmental challenge that should foster the release of cyt-*c* and other pro-apoptotic factors from mitochondria due to MPTP activation.

We showed that mitochondria from *A. franciscana* possessed VDAC, ANT and cyclophilin D, yet the traditional assay used to measure opening of the MPTP (mitochondrial swelling) clearly showed that the pore did not respond to the addition of calcium and phosphate in de-energized mitochondria (Fig. 3) (Menze et al., 2005b). In contrast, calcium addition to energized mitochondria from *A. franciscana* actually promoted an increase in absorbance, which was apparently due to their extensive capacity for calcium loading (Fig. 3). The formation of calcium phosphate complexes in the matrix causes an increase in the refractive index (Nicholls and Chalmers, 2004). In comparison, rat liver mitochondria showed the typical pattern of rapid swelling in response to calcium plus phosphate (Fig. 3), which could be blocked by cyclosporin A. Thus, by this measure, a calcium-regulated opening of the MPTP was not detectable in *A. franciscana* mitochondria. Not surprisingly, based on this lack of swelling, incubation of energized mitochondria from *A. franciscana* with 1 mmol l^{-1} calcium for 30 min did not induce the release of cyt-*c* (Fig. 3, lower panel). Use of positive controls (alamethicin, an artificial pore-forming agent; treatment with high mercury concentrations) confirmed that mitochondria from *A. franciscana* intrinsically possessed the capacity for swelling (Menze et al., 2005b). In other words, swelling could be artificially stimulated and measured, but known inducers of the regulated MPTP were without effect.

To confirm the apparent absence of MPTP opening in *A. franciscana*, we measured the kinetics of calcium-induced calcium release in mitochondria isolated from both *A. francsicana* and rat liver by using a fluorescent probe that reports external free calcium (Fig. 4) (Menze et al., 2005b). Mitochondria from the two species differed dramatically in their responses to exogenously added calcium. Calcium additions of 0.1 mmol l^{-1} and higher to energized rat mitochondria promoted an increase in fluorescence well above that seen in the absence of mitochondria (control value), a result that indicates the opening of the MPTP and release of calcium stores from the matrix (Fig. 4B). In contrast, energized mitochondria from *A. franciscana* (Fig. 4A) were able to reduce the external calcium concentration, compared with controls, across the entire range of experimental calcium. Thus the capacity for calcium uptake by mitochondria from *A. franciscana* was far greater than that seen for rat liver mitochondria. Second, although a clear calcium-dependent opening of the MPTP occurred at 0.1 mmol l^{-1} calcium in rat liver mitochondria, no calcium-dependent MPTP opening was indicated for *A. franciscana* mitochondria, because the calcium level in the presence of energized mitochondria never reached, much less exceeded, the controls (no mitochondria). Thus calcium-induced calcium release, a hallmark of MPTP opening, did not occur at any level of exogenously added calcium.

Although the absence of a regulated MPTP in *A. franciscana* mitochondria could contribute to the extreme anoxia tolerance in this species, we speculate that the absence of a regulated MPTP may be a feature of many invertebrates. First, a recalcitrant MPTP, which was seen in the anoxia-tolerant embryonic stage of *A. franciscana*, is also present in nauplius larvae that do not show the anoxia tolerance of embryos (Menze et al., 2005b). Second, a similar recalcitrant MPTP has been documented in a ghost shrimp, *Lepidophthalmus louisianensis* (J. Holman and S.C.H., unpublished observations). Finally, indirect evidence suggests that a regulated MPTP may be absent in mitochondria from the oyster *Crassostrea virginica* (Sokolova et al., 2004). Specifically, cadmium up to 1000 µmol l^{-1} [sufficent to open the mammalian MPTP in a cyclosporin A-sensitive manner (Belyaeva et al., 2002)] in the presence of phosphate did not cause swelling of isolated

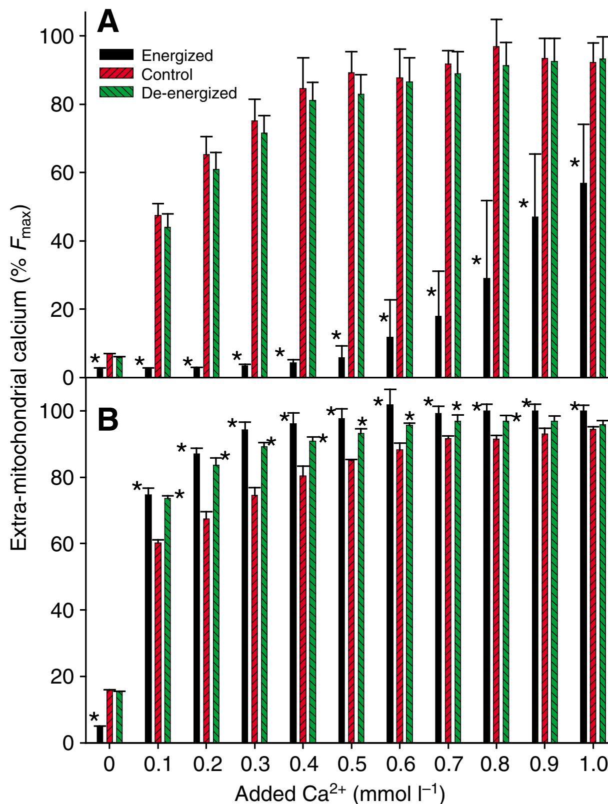

Fig. 4. Calcium uptake and release by isolated mitochondria. Extra-mitochondrial free calcium concentration was measured with the calcium probe fluo-5N. (A) Energized mitochondria from *A. franciscana* lowered the concentration of exogenously added Ca^{2+} at all concentrations investigated compared with controls (no mitochondria present). Calcium-induced calcium release was not observed, consistent with the lack of MPTP opening. (B) Rat liver mitochondria reduced the free Ca^{2+} concentration only in the case where no exogenous Ca^{2+} was added. Upon addition of 0.1 mmol l^{-1} Ca^{2+}, release of calcium was observed, indicating MPTP opening. For further explanation, refer to text. Each bar represents the mean ± s.d. of N=3 experiments. *Significantly different from control (P<0.05) (modified from Menze et al., 2005b).

mitochondria, but physiological inducers were not evaluated. The molecular explanation for the absence of a regulated MPTP in *A. franciscana* is currently under investigation and could inform us of new mechanisms for regulating the pore.

Mitochondrial outer membrane permeabilization (MOMP)

A complex interplay between the pro- and anti-apoptotic proteins belonging to the Bcl-2 family controls the permeability of the outer mitochondrial membrane in mammals. Proteins in this family are characterized by the presence of up to four Bcl-2 homology regions (BH1–4). Based on the presence of these regions and the cellular function of the protein, Bcl-2 family proteins are divided into three subfamilies: multi-domain anti-apoptotic (e.g. Bcl-2, Bcl-xL), multi-domain pro-apoptotic (e.g. Bax, Bak) and pro-apoptotic BH3-only proteins (e.g. Bid, Bad) (Gross et al., 1999). The mechanism of MOMP is mediated by the pore-forming proteins Bak and Bax (Fig. 1), whose activation is promoted by BH3-only proteins. This activation could occur either by direct binding to Bax/Bak, or indirectly by displacement of the anti-apoptotic Bcl-2 subfamily members from Bax/Bak (Willis and Adams, 2005). In non-apoptotic cells Bak is tail-anchored to the outer mitochondrial membrane, whereas Bax is mostly cytosolic (Lucken-Ardjomande and Martinou, 2005; Youle and Strasser, 2008). During apoptosis Bax translocates to the mitochondrion where it changes conformation and inserts into the outer mitochondrial membrane (Hsu et al., 1997). The precise mechanism by which MOMP is mediated is still unclear. The predominant view is that Bax and Bak undergo conformational changes, oligomerize and form pores in the outer mitochondrial membrane (Antignani and Youle, 2006). To complicate matters further, the tumor suppressor protein p53 can impact MOMP by promoting in the nucleus expression of PUMA (a BH3-only protein) (Fig. 1), which then releases p53 from Bcl-xL, thereby freeing p53 to activate Bax in the cytoplasm (Chipuk et al., 2005).

Loss of anti-apoptotic Bcl-2 family members under anoxia

Mammalian cell death during hypoxia/anoxia occurs *via* the intrinsic pathway of apoptosis (Brunelle et al., 2007; McClintock et al., 2002; Shimizu et al., 1995; Shroff et al., 2007). Key regulators of apoptosis during anoxia are the Bcl-2 family proteins (Shimizu et al., 1995). The pathway is initiated by the loss of function of the pro-survival Bcl-2 family members Mcl-1 and Bcl-2/Bcl-xL, resulting in Bax- or Bak-dependent release of cyt-*c* and subsequent caspase 9-dependent cell death. A key member of the Bcl-2 family of pro-survival proteins, Mcl-1, undergoes ubiquitin-dependent degradation by the proteasome. While the mechanisms are currently ill-defined, it is suggested that inhibition of the electron transport chain due to oxygen deprivation is in some way linked to the negation of the pro-survival function of Bcl-2 family proteins.

One would predict that the above mechanism, if operative in *A. franciscana* embryos, must be short-circuited under anoxia. At present nothing is known about homologs of Bcl-2 family proteins in *A. franciscana*. Whether or not pro-survival proteins similar to CED-9 in *C. elegans* exist, and whether such proteins are involved in anoxia-induced cell death is unclear. However, it is well established that ubiquitin-dependent (proteosomal) degradation of proteins is acutely arrested under anoxia in *A. franciscana* embryos (Anchordoguy and Hand, 1995; Anchordoguy and Hand, 1994). If Bcl-2 family homologues are degraded in *A. franciscana* in a similar way to that in mammals, then this inhibition of ubiquitin-dependent degradation may be a potential mechanism by which initiation of Bax–Bak-dependent MOMP is avoided under anoxia.

Specific requirements for ATP in cell death

As mentioned above, a number of steps in the process of apoptosis require ATP (Fig. 2) (Atlante et al., 2005; Chiarugi, 2005; Eguchi et al., 1997; Kass et al., 1996; Leist et al., 1997; Nicotera et al., 2000; Nicotera et al., 1998; Skulachev, 2006), although we have argued that the absolute quantities and fluxes are modest and have been over emphasized in recent literature. The inactivation of anti-apoptotic Bcl-2 by phosphorylation by JNK (c-Jun NH_2 terminal kinase) (Fan et al., 2000; Yamamoto et al., 1999), activation of pro-apoptotic factors Bad, Bim and Bmf (Donovan et al., 2002; Lei and Davis, 2003), and other steps relying on covalent modification with ATP as a phosphate donor use tiny amounts of ATP. The movement and fragmentation of mitochondria, which are routinely associated with apoptosis, require the activities of ATPases and GTPases, but estimates of energy consumption for such processes are unavailable to our knowledge. For the extrinsic pathway of apoptosis, ATP-driven nuclear import of apoptogenic factors from the cytoplasm is also an energy-dependent step (Chiarugi, 2005; Yasuhara et al., 1997). For assembly of the mammalian apoptosome, Apaf-1-

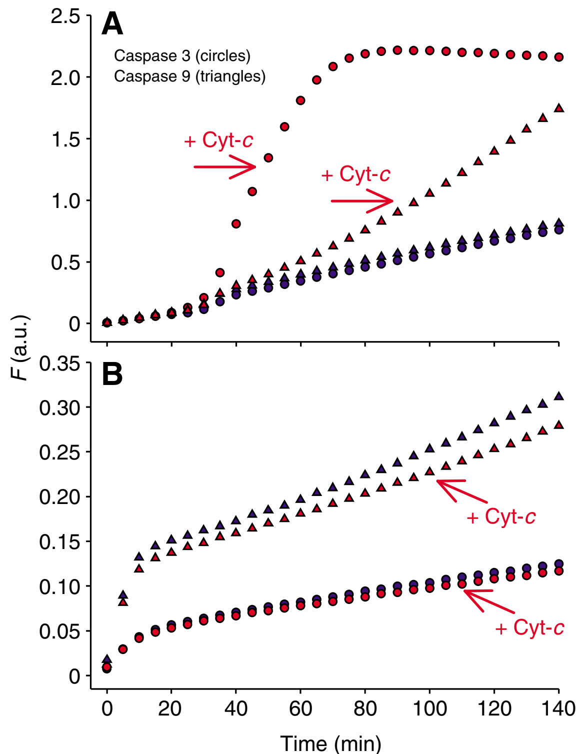

Fig. 5. Impact of cytochrome *c* (cyt-*c*) addition on the activation of caspase 3 and caspase 9 in cytosolic extracts from (A) human hepatoma cells (C3A) and (B) diapause embryos of *Artemia franciscana*. Caspase activities were measured following the increase in fluorescence (*F*; in arbitrary units, a.u.) due to cleavage of Z-LEHD-R110 (caspase 9) or Z-DEVD-R110 (caspase 3). Cyt-*c* additions were saturating for C3A cells. No activation by cyt-*c* was observed for *A. franciscana* extracts (modified from Menze and Hand, 2007).

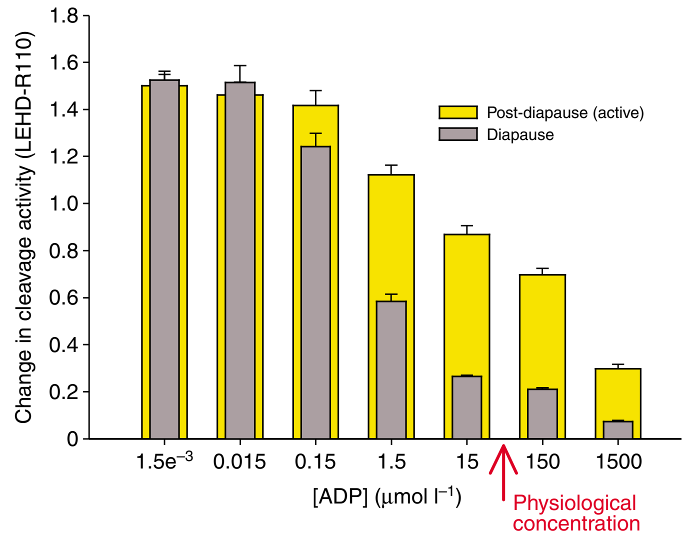

Fig. 6. Influence of Mg-ADP on caspase 9 (Z-LEHD-R110) activity in cytosolic extracts from diapause and post-diapause embryos of *A. franciscana*. ADP inhibition was about 70% greater in diapause extracts at the approximate physiological concentration of ADP (15–150 μmol l^{-1}) than in post-diapause extracts. Each bar is the mean ± s.d. of *N*=4–7 experiments (modified from Menze and Hand, 2007).

dependent activation of caspase 9 is maximal at 1 μmol l^{-1} ATP (Riedl et al., 2005), which may be compared with cellular levels of one to several millimolar ATP.

The underlying issue is that even if cellular ATP were to drop by 50%, then engaging the apoptotic program would do little to further compromise (drain) the ATP pool. Apoptotic processes have precise affinities for ATP, and cellular concentrations of ATP must be adequate to satisfy the affinities of the proteins involved. Once ATP in the cell falls to a level that limits binding by relevant proteins, execution of apoptosis will undoubtedly be impacted, but this drop in ATP would need to be approximately 99% to restrict steps with high affinities.

Where depression in ATP production rate may have significant physiological impacts is on processes like ion transport that consume large quantities of ATP. Restriction of active calcium uptake by SERCA into the ER may render the pump unable to balance the loss of Ca^{2+} through release channels (inositol-1,4,5-trisphosphate receptor–Ca^{2+} channel, ryanodine receptor/Ca^{2+} channel) or leak pathways. A parallel scenario can be developed for ATP limitation of the plasma membrane calcium pump. The resultant rise in free cytoplasmic calcium has far-reaching effects on cell death (Fig. 2). Perturbation of the Na^+/K^+-ATPase by reduced ATP availability is apparently key. Activation of apoptosis by exposing lymphocytes or thymocytes to anti-Fas (which stimulates a plasma membrane death receptor) promotes inactivation of the Na^+/K^+-ATPase, thereby significantly decreasing K^+ uptake and irreversibly depolarizing the cell membrane (Remillard and Yuan, 2004). This depolarization activates voltage-dependent K^+ and Cl^- channels, promoting cell shrinkage due to the accentuated loss of KCl (Fig. 2). This drop in intracellular KCl is also known to directly stimulate caspase activity and thus apoptosis (see ‘Impact of mitochondrial poration on downstream events’ below). Finally, proteins whose functions are sensitive to adenylate ratios (ATP/ADP in the case of ANT; AMP/ATP for the AMP-activated protein kinase), proteins with low affinities for ATP, or proteins where the allosteric impact of nucleotides (e.g. certain caspases) may fall in the millimolar range, could all serve as ATP-sensing steps. Decreasing the ATP/ADP ratio can alter the conformation of ANT and increase the probability of MPTP opening by calcium (Bernardi et al., 2006).

Impact of mitochondrial poration on downstream events

Cyt-*c* and apoptosome assembly

A classic downstream effect of the mitochondrial release of pro-apoptotic factors is the cyt-*c* stimulation of apoptosome assembly and subsequent activation of the initiation and executer caspases. Even though mitochondria from *A. franciscana* are resistant to calcium-induced opening of the mitochondrial permeability transition pore, cyt-*c* release could also occur by MOMP through interaction with pro-apoptotic Bcl-2 family members, a process about which little is known in *A. franciscana*. Therefore, we investigated whether cyt-*c* is able to elevate caspase 9 and caspase 3 activity *in vitro* (Menze and Hand, 2007). In clear contrast to mammalian cells, caspase activation by cyt-*c* is absent in *A. franciscana* embryos (Fig. 5). As expected, extracts obtained from human C3A cells responded with a sharp increase in caspase 3 and a less pronounced increase in caspase 9 activity after addition of cyt-*c*. Work on the assembly and function of DARK, the apoptosome of *D. melanogaster*, has indicated that cyt-*c* is not required for its *in vitro* assembly, nor is cyt-*c* incorporated into the final structure (Yu et al., 2006). Thus, our data are consistent with a view that the apoptosome in arthropods can assemble and activate

caspases in the absence of cyt-*c*. Cyt-*c* participation is also absent in the activation of meta-caspases in yeast cells, which do not even contain an Apaf-1 homolog in their genome (Madeo et al., 2002). Fully establishing the evolutionary pattern for the involvement of cyt-*c* in the apoptotic cascade of eukaryotic organisms would be instructive from multiple perspectives. Integration of cyt-*c* signaling into the carefully balanced process of caspase activation requires many steps beyond the acquisition of intracellular organelles like mitochondria.

Caspase regulation

Despite the absence of a cyt-*c* effect, many basic features of caspase regulation in mammals are observed in *A. franciscana*. High intracellular nucleotide concentrations represent a critical pro-survival condition that prevents caspase activation due to the interaction with both cyt-*c* (Chandra et al., 2006) and caspase 9 (Chereau et al., 2005), whereas high intracellular potassium inhibits apoptosome formation by binding to Apaf-1 (Cain et al., 2001). Both regulatory effects are observed in *A. franciscana*, although the mechanisms of action are likely to differ.

Multiple species of adenylates and guanylates impact the caspase activities in *A. franciscana* (Menze and Hand, 2007) and, moreover, there are differences in response between diapause and non-diapause embryos to Mg^{2+}-ADP (Fig. 6). Analysis of the IC_{50} value showed that inhibition by Mg-ADP is far greater in diapause embryos (IC_{50}=0.66 μmol l^{-1}) than in post-diapause embryos (IC_{50}=44.4 μmol l^{-1}). This observation is apt to be of physiological relevance and may represent a new mechanism to prevent caspase activation during cell stasis. In *A. franciscana* embryos, ADP levels remain constant during diapause (J. A. Covi, J. Reynolds and S.C.H., unpublished observations). Consequently, this pattern could prevent caspase activation under energy limitation when ATP levels fall. In mammalian systems a physiological role for ADP in caspase 9 regulation has not been reported (Chandra et al., 2006; Chereau et al., 2005), and ADP does not stimulate Apaf-1 activation of caspases (Riedl et al., 2005).

Another novel feature of *A. francsicana* caspases is that GTP inhibits caspase 9 activity, and the regulatory pattern for GTP on caspase 9 in *A. franciscana* embryos is very similar to that for ATP (Menze and Hand, 2007). Because intracellular GTP remains high (>2 mmol l^{-1}) in embryos exposed to anoxia (Stocco et al., 1972), it could serve as a means to prevent maladaptive apoptosis during oxygen deprivation that otherwise might occur during the precipitous drop in ATP that occurs under anoxia (Carpenter and Hand, 1986; Stocco et al., 1972). The remarkably high levels of soluble guanylates present in *A. franciscana* embryos are a consequence of the huge stores of P^1,P^4-diguanosine 5′-tetraphosphate (Gp_4G) and its complex metabolic interconversions (Finamore and Warner, 1963; Stocco et al., 1972). The suggestion that in mammals GTP/dGTP may bind to cyt-*c*, prevent apoptosome formation, and thereby indirectly inhibit caspase activation (Chereau et al., 2005) cannot explain the GTP effect in *A. franciscana*. Rather, GTP apparently promotes a more direct inhibition by binding to caspase 9 or perhaps to the adaptor platform Apaf-1/Ced-4. Multiple mechanisms are in place that could serve to ‘apply the brakes’ to apoptosis during energy-limited states in *A. franciscana*.

Concluding comments and future directions

We have highlighted emerging issues that provide insight into how animals exposed to energy-limited states for extended periods do not experience cell death in any of its forms. While a mammalian cell’s fate to die from necrosis *versus* apoptosis is dictated largely by the abundance of cellular ATP (Kroemer et al., 2007), compromised adenylate status does not activate either death routine in animals capable of severe metabolic arrest. The extended and simultaneous arrest of oxidative phosphorylation and glycolytic flux experienced by *A. franciscana* would be viewed as a ‘bioenergetic catastrophe’ (Kroemer et al., 2007) for mammalian cells. Yet the 4000 gastrula-stage cells of *A. franciscana* survive the condition under diapause and anoxia for months to years at room temperature. To be sure, *A. franciscana* is not a ‘freak of nature’. Numerous species from 34 of 35 major animal phyla display the capacity for severe metabolic arrest (dormancy) at various points in their life cycles (Crowe and Clegg, 1973). Biological interpretations that place the findings from *A. franciscana* in an evolutionary context are hampered by the paucity of data on other tolerant species. Still, our current view is that characteristics discussed here for *A. franciscana* (e.g. lack of a functional MPTP) are apt to be features of many animal phyla. In contrast, the regulatory mechanisms for cell death that have evolved in mammals may have been linked to substantial trade-offs in tolerance to energy-limited states.

Unresolved questions are what is the signaling/sensing process by which a transient, moderate drop in ATP can stimulate initiation of apoptosis, or by which a more severe, prolonged drop favors necrosis? Is the ATP effect direct or indirect, i.e. mediated through an impact on another cellular process like ion homeostasis? Understanding these mechanisms might eliminate much controversy as to what governs progression along one *versus* another divergent pathway to cell death (Newmeyer and Ferguson-Miller, 2003). As we learn more about energy requirements for various forms of cell death, distinctions among subtypes are becoming blurred (Chiarugi, 2005). Evidence is building that the mitochondrial apoptotic pathway may bifurcate at the post-mitochondrial level, with one branch being caspase and ATP dependent, and another independent of both (Chiarugi, 2005).

There are marked differences in *A. franciscana* mitochondria, relative to those of mammals, in the MPTP and capacities for calcium uptake. Further, caspase activation in *A. franciscana* does not rely on cytrochrome *c* release from mitochondria, and its caspases show novel and interesting responses to nucleotides and calcium that may blunt activation that would readily occur under energy-limited conditions in mammalian cells. Yet there are areas where more data are required in order to piece together an integrated understanding of cell death pathways and their regulation in *A. franciscana*. In addition to the MPTP, information is needed on the regulation of MOMP *via* mechanisms comparable to Bax/Bak poration in mammals. What other pro-apoptotic factors (aside from cyt-*c*) may be potentially released by MOMP? How reliant is *A. franciscana* on IAPs for controlling caspase activity? How well developed is crosstalk between the ER and mitochondrion in *A. franciscana*, and are there features of mitochondrial fragmentation that place constraints on mitochondrial-based pathways for apoptosis? Such information will help explain how processes of cell death are blunted during states of metabolic arrest, and it may lead to new therapies for intervention to prevent cell death in disease states and during biostabilization of mammalian cells (Buchanan et al., 2005; Crowe et al., 2005; Elliott et al., 2006; Hand and Hagedorn, 2008; Liu et al., 2005; Menze et al., 2005a).

This work was supported by NIH (Institute of General Medical Sciences) grant 1-RO1-GM071345-01 and Defense Advanced Research Projects Agency (DARPA) contract number N00173-01-1-G011.

References

Abdelwahid, E., Yokokura, T., Krieser, R. J., Balasundaram, S., Fowle, W. H. and White, K. (2007). Mitochondrial disruption in *Drosophila* apoptosis. *Dev. Cell* **12**, 793-806.
Alvarez, J. and Montero, M. (2002). Measuring [Ca2+] in the endoplasmic reticulum with aequorin. *Cell Calcium* **32**, 251-260.
Anchordoguy, T. and Hand, S. (1995). Reactivation of ubiquitination in *Artemia franciscana* embryos during recovery from anoxia-induced quiescence. *J. Exp. Biol.* **198**, 1299-1305.
Anchordoguy, T. J. and Hand, S. C. (1994). Acute blockage of the ubiquitin-mediated proteolytic pathway during invertebrate quiescence. *Am. J. Physiol.* **267**, R895-R900.
Anchordoguy, T. J., Hofmann, G. E. and Hand, S. C. (1993). Extension of enzyme half-life during quiescence in *Artemia* embryos. *Am J. Physiol.* **264**, R85-R89.
Antignani, A. and Youle, R. J. (2006). How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Curr. Opin. Cell Biol.* **18**, 685-689.
Arama, E., Bader, M., Srivastava, M., Bergmann, A. and Steller, H. (2006). The two *Drosophila* cytochrome c proteins can function in both respiration and caspase activation. *EMBO J.* **25**, 232-243.
Arpagaus, S., Rawyler, A. and Braendle, R. (2002). Occurrence and characteristics of the mitochondrial permeability transition in plants. *J. Biol. Chem.* **277**, 1780-1787.
Atlante, A., Giannattasio, S., Bobba, A., Gagliardi, S., Petragallo, V., Calissano, P., Marra, E. and Passarella, S. (2005). An increase in the ATP levels occurs in cerebellar granule cells en route to apoptosis in which ATP derives from both oxidative phosphorylation and anaerobic glycolysis. *Biochim. Biophys. Acta* **1708**, 50-62.
Baines, C. P., Kaiser, R. A., Purcell, N. H., Blair, N. S., Osinska, H., Hambleton, M. A., Brunskill, E. W., Sayen, M. R., Gottlieb, R. A., Dorn, G. W. et al. (2005). Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**, 658-662.
Baines, C. P., Kaiser, R. A., Sheiko, T., Craigen, W. J. and Molkentin, J. D. (2007). Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat. Cell Biol.* **9**, 550-555.
Basso, E., Fante, L., Fowlkes, J., Petronilli, V., Forte, M. A. and Bernardi, P. (2005). Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J. Biol. Chem.* **280**, 18558-18561.
Belyaeva, E. A., Glazunov, V. V. and Korotkov, S. M. (2002). Cyclosporin A-sensitive permeability transition pore is involved in Cd(2+)-induced dysfunction of isolated rat liver mitochondria: doubts no more. *Arch. Biochem. Biophys.* **405**, 252-264.
Bernardi, P. (1992). Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *J. Biol. Chem.* **267**, 8834-8839.
Bernardi, P. (1996). The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochim. Biophys. Acta* **1275**, 5-9.
Bernardi, P. (1999). Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol. Rev.* **79**, 1127-1155.
Bernardi, P., Scorrano, L., Colonna, R., Petronilli, V. and Di Lisa, F. (1999). Mitochondria and cell death. Mechanistic aspects and methodological issues. *Eur. J. Biochem.* **264**, 687-701.
Bernardi, P., Krauskopf, A., Basso, E., Petronilli, V., Blalchy-Dyson, E., Di Lisa, F. and Forte, M. A. (2006). The mitochondrial permeability transition from *in vitro* artifact to disease target. *FEBS J.* **273**, 2077-2099.
Bossy-Wetzel, E., Newmeyer, D. D. and Green, D. R. (1998). Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. *EMBO J.* **17**, 37-49.
Boutilier, R. G. and St-Pierre, J. (2002). Adaptive plasticity of skeletal muscle energetics in hibernating frogs: mitochondrial proton leak during metabolic depression. *J. Exp. Biol.* **205**, 2287-2296.
Brand, M. D., Chien, L. F., Ainscow, E. K., Rolfe, D. F. and Porter, R. K. (1994). The causes and functions of mitochondrial proton leak. *Biochim. Biophys. Acta* **1187**, 132-139.
Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W. and Sheu, S. S. (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol.* **287**, C817-C833.
Brunelle, J. K., Shroff, E. H., Perlman, H., Strasser, A., Moraes, C. T., Flavell, R. A., Danial, N. N., Keith, B., Thompson, C. B. and Chandel, N. S. (2007). Loss of Mcl-1 protein and inhibition of electron transport chain together induce anoxic cell death. *Mol. Cell. Biol.* **27**, 1222-1235.
Brustovetsky, N., Tropschug, M., Heimpel, S., Heidkamper, D. and Klingenberg, M. (2002). A large Ca2+-dependent channel formed by recombinant ADP/ATP carrier from *Neurospora crassa* resembles the mitochondrial permeability transition pore. *Biochemistry* **41**, 11804-11811.
Buchanan, S. S., Menze, M. A., Hand, S. C., Pyatt, D. W. and Carpenter, J. F. (2005). Cryopreservation of human hematopoetic stem and progenitor cells loaded with trehalose: transient permeabilization via the adenosine triphosphate-dependent P2Z receptor channel. *Cell Preserv. Technol.* **3**, 212-222.
Buck, L. T. and Hochachka, P. W. (1993). Anoxic suppression of Na(+)-K(+)-ATPase and constant membrane potential in hepatocytes: support for channel arrest. *Am. J. Physiol.* **265**, R1020-R1025.
Cain, K., Langlais, C., Sun, X. M., Brown, D. G. and Cohen, G. M. (2001). Physiological concentrations of K+ inhibit cytochrome c-dependent formation of the apoptosome. *J. Biol. Chem.* **276**, 41985-41990.
Carpenter, J. F. and Hand, S. C. (1986). Arrestment of carbohydrate-metabolism during anaerobic dormancy and aerobic acidosis in *Artemia* embryos-determination of Ph-sensitive control points. *J. Comp. Physiol. B* **156**, 451-459.
Challa, M., Malladi, S., Pellock, B. J., Dresnek, D., Varadarajan, S., Yin, Y. W., White, K. and Bratton, S. B. (2007). Drosophila Omi, a mitochondrial-localized IAP antagonist and proapoptotic serine protease. *EMBO J.* **26**, 3144-3156.
Chandra, D., Bratton, S. B., Person, M. D., Tian, Y., Martin, A. G., Ayres, M., Fearnhead, H. O., Gandhi, V. and Tang, D. G. (2006). Intracellular nucleotides act as critical prosurvival factors by binding to cytochrome c and inhibiting apoptosome. *Cell* **125**, 1333-1346.
Chereau, D., Zou, H., Spada, A. P. and Wu, J. C. (2005). A nucleotide binding site in caspase-9 regulates apoptosome activation. *Biochemistry* **44**, 4971-4976.
Chiarugi, A. (2005). "Simple but not simpler": toward a unified picture of energy requirements in cell death. *FASEB J.* **19**, 1783-1788.
Chipuk, J. E., Bouchier-Hayes, L., Kuwana, T., Newmeyer, D. D. and Green, D. R. (2005). PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. *Science* **309**, 1732-1735.
Clegg, J. (1997). Embryos of *Artemia franciscana* survive four years of continuous anoxia: the case for complete metabolic rate depression. *J. Exp. Biol.* **200**, 467-475.
Clegg, J. S., Drinkwater, L. E. and Sorgeloos, P. (1996). The metabolic status of diapause embryos of *Artemia franciscana* (SFB). *Physiol. Zool.* **69**, 49-66.
Covi, J. A. and Hand, S. C. (2005). V-ATPase expression during development of *Artemia franciscana* embryos: potential role for proton gradients in anoxia signaling. *J. Exp. Biol.* **208**, 2783-2798.
Covi, J. A. and Hand, S. C. (2007). Energizing an invertebrate embryo: bafilomycin-dependent respiration and the metabolic cost of proton pumping by the V-ATPase. *Physiol. Biochem. Zool.* **80**, 422-432.
Covi, J. A., Treleaven, W. D. and Hand, S. C. (2005). V-ATPase inhibition prevents recovery from anoxia in *Artemia franciscana* embryos: quiescence signaling through dissipation of proton gradients. *J. Exp. Biol.* **208**, 2799-2808.
Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochem. J.* **341**, 233-249.
Crowe, J. H. and Clegg, J. S. (1973). *Anhydrobiosis*. Stroudsburg: Dowden, Hutchinson and Ross.
Crowe, J. H., Crowe, L. M., Wolkers, W. F., Oliver, A. E., Ma, X., Auh, J.-H., Tang, M., Zhu, S., Norris, J. and Tablin, F. (2005). Stabilization of dry mammalian cells: lessons from nature. *Integr. Comp. Biol.* **45**, 810-820.
de Graaf, A. O., Meijerink, J. P., van den Heuvel, L. P., DeAbreu, R. A., de Witte, T., Jansen, J. H. and Smeitink, J. A. (2002). Bcl-2 protects against apoptosis induced by antimycin A and bongkrekic acid without restoring cellular ATP levels. *Biochim. Biophys. Acta* **1554**, 57-65.
Denlinger, D. L. (2002). Regulation of diapause. *Annu. Rev. Entomol.* **47**, 93-122.
Deryabina, Y. I., Isakova, E. P., Shurubor, E. I. and Zvyagilskaya, R. A. (2004). Calcium-dependent nonspecific permeability of the inner mitochondrial membrane is not induced in mitochondria of the yeast *Endomyces magnusii*. *Biochemistry Mosc.* **69**, 1025-1033.
Dierks, T., Salentin, A., Heberger, C. and Kramer, R. (1990a). The mitochondrial aspartate glutamate and Adp/Atp carrier switch from obligate counterexchange to unidirectional transport after modification by Sh-reagents. *Biochim. Biophys. Acta* **1028**, 268-280.
Dierks, T., Salentin, A. and Kramer, R. (1990b). Pore-like and carrier-like properties of the mitochondrial aspartate glutamate carrier after modification by Sh-reagents – evidence for a preformed channel as a structural requirement of carrier-mediated transport. *Biochim. Biophys. Acta* **1028**, 281-288.
Donovan, N., Becker, E. B., Konishi, Y. and Bonni, A. (2002). JNK phosphorylation and activation of BAD couples the stress-activated signaling pathway to the cell death machinery. *J. Biol. Chem.* **277**, 40944-40949.
Eads, B. D. and Hand, S. C. (2003a). Mitochondrial mRNA stability and polyadenylation during anoxia-induced quiescence in the brine shrimp *Artemia franciscana*. *J. Exp. Biol.* **206**, 3681-3692.
Eads, B. D. and Hand, S. C. (2003b). Transcriptional initiation under conditions of anoxia-induced quiescence in mitochondria from *Artemia franciscana* embryos. *J. Exp. Biol.* **206**, 577-589.
Edinger, A. L. and Thompson, C. B. (2004). Death by design: apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.* **16**, 663-669.
Eguchi, Y., Shimizu, S. and Tsujimoto, Y. (1997). Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res.* **57**, 1835-1840.
Elliott, G. D., Liu, X. H., Cusick, J. L., Menze, M., Vincent, J., Witt, T., Hand, S. and Toner, M. (2006). Trehalose uptake through P2X(7) purinergic channels provides dehydration protection. *Cryobiology* **52**, 114-127.
Fan, M., Goodwin, M., Vu, T., Brantley-Finley, C., Gaarde, W. A. and Chambers, T. C. (2000). Vinblastine-induced phosphorylation of Bcl-2 and Bcl-XL is mediated by JNK and occurs in parallel with inactivation of the Raf-1/MEK/ERK cascade. *J. Biol. Chem.* **275**, 29980-29985.
Ferri, K. F. and Kroemer, G. (2001). Organelle-specific initiation of cell death pathways. *Nat. Cell Biol.* **3**, E255-E263.
Finamore, F. J. and Warner, A. H. (1963). The occurrence of P1, P4-diguanosine 5′-tetraphosphate in brine shrimp eggs. *J. Biol. Chem.* **238**, 344-348.
Fraser, K. P., Houlihan, D. F., Lutz, P. L., Leone-Kabler, S., Manuel, L. and Brechin, J. G. (2001). Complete suppression of protein synthesis during anoxia with no post-anoxia protein synthesis debt in the red-eared slider turtle *Trachemys scripta* elegans. *J. Exp. Biol.* **204**, 4353-4360.
Gnaiger, E., Mendez, G. and Hand, S. C. (2000). High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. *Proc. Natl. Acad. Sci. USA* **97**, 11080-11085.
Gross, A., McDonnell, J. M. and Korsmeyer, S. J. (1999). BCL-2 family members and the mitochondria in apoptosis. *Genes Dev.* **13**, 1899-1911.
Gunter, T. E. and Pfeiffer, D. R. (1990). Mechanisms by which mitochondria transport calcium. *Am. J. Physiol.* **258**, C755-C786.

Gunter, T. E., Buntinas, L., Sparagna, G., Eliseev, R. and Gunter, K. (2000). Mitochondrial calcium transport: mechanisms and functions. *Cell Calcium* **28**, 285-296.

Gunter, T. E., Yule, D. I., Gunter, K. K., Eliseev, R. A. and Salter, J. D. (2004). Calcium and mitochondria. *FEBS Lett.* **567**, 96-102.

Guppy, M. and Withers, P. (1999). Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol. Rev. Camb. Philos. Soc.* **74**, 1-40.

Halestrap, A. P. and Brennerb, C. (2003). The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death. *Curr. Med. Chem.* **10**, 1507-1525.

Halestrap, A. P., Doran, E., Gillespie, J. P. and O'Toole, A. (2000). Mitochondria and cell death. *Biochem. Soc. Trans.* **28**, 170-177.

Hand, S. C. (1998). Quiescence in *Artemia franciscana* embryos: reversible arrest of metabolism and gene expression at low oxygen levels. *J. Exp. Biol.* **201**, 1233-1242.

Hand, S. C. and Hagedorn, M. (2008). New approaches for cell and animal preservation: lessons from aquatic organisms. In *Oceans and Human Health: Risks and Remedies from the Seas* (ed. P. J. Walsh, L. E. Smith, L. E. Fleming, H. Solo-Gabriele and W. H. Gerwick), pp. 611-629. New York: Academic Press.

Hand, S. C. and Hardewig, I. (1996). Downregulation of cellular metabolism during environmental stress: mechanisms and implications. *Annu. Rev. Physiol.* **58**, 539-563.

Hand, S. C., Podrabsky, J. E., Eads, B. D. and Van Breukelen, F. (2001). Interrupted development in aquatic organism: ecological context and physiological mechanism. In *Environment and Animal Development. Genes, Life Histories and Plasticity* (ed. D. Atkinson and M. Thorndyke), pp. 219-234. Oxford: BIOS Scientific Publishers.

Hardewig, I., Anchordoguy, T. J., Crawford, D. L. and Hand, S. C. (1996). Profiles of nuclear and mitochondrial encoded mRNAs in developing and quiescent embryos of *Artemia franciscana. Mol. Cell. Biochem.* **158**, 139-147.

Hawkins, A. J. S. (1991). Protein turnover: a functional appraisal. *Funct. Ecol.* **5**, 222-233.

Haworth, R. A. and Hunter, D. R. (1979). The Ca2+-induced membrane transition in mitochondria. II. Nature of the Ca2+ trigger site. *Arch. Biochem. Biophys.* **195**, 460-467.

Hay, B. A. and Guo, M. (2006). Caspase-dependent cell death in *Drosophila*. *Annu. Rev. Cell Dev. Biol.* **22**, 623-650.

Hay, B. A., Huh, J. R. and Guo, M. (2004). The genetics of cell death: approaches, insights and opportunities in *Drosophila*. *Nat. Rev. Genet.* **5**, 911-922.

He, L. and Lemasters, J. J. (2002). Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? *FEBS Lett.* **512**, 1-7.

Hochachka, P. W. (1986). Defense strategies against hypoxia and hypothermia. *Science* **231**, 234-241.

Hochachka, P. W. and Guppy, M. (1987). *Metabolic Arrest and the Control of Biological Time*. Cambridge, MA: Harvard University Press.

Hofmann, G. E. and Hand, S. C. (1990). Arrest of cytochrome-c oxidase synthesis coordinated with catabolic arrest in dormant *Artemia* embryos. *Am. J. Physiol.* **258**, R1184-R1191.

Hofmann, G. E. and Hand, S. C. (1994). Global arrest of translation during invertebrate quiescence. *Proc. Natl. Acad. Sci. USA* **91**, 8492-8496.

Hsu, Y. T., Wolter, K. G. and Youle, R. J. (1997). Cytosol-to-membrane redistribution of Bax and Bcl-X(L) during apoptosis. *Proc. Natl. Acad. Sci. USA* **94**, 3668-3672.

Hunter, D. R., Haworth, R. A. and Southard, J. H. (1976). Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J. Biol. Chem.* **251**, 5069-5077.

Igaki, T. and Miura, M. (2004). Role of Bcl-2 family members in invertebrates. *Biochim. Biophys. Acta* **1644**, 73-81.

Igaki, T., Suzuki, Y., Tokushige, N., Aonuma, H., Takahashi, R. and Miura, M. (2007). Evolution of mitochondrial cell death pathway: proapoptotic role of HtrA2/Omi in *Drosophila*. *Biochem. Biophys. Res. Commun.* **356**, 993-997.

Izyumov, D. S., Avetisyan, A. V., Pletjushkina, O. Y., Sakharov, D. V., Wirtz, K. W., Chernyak, B. V. and Skulachev, V. P. (2004). "Wages of fear": transient threefold decrease in intracellular ATP level imposes apoptosis. *Biochim. Biophys. Acta* **1658**, 141-147.

Jagasia, R., Grote, P., Westermann, B. and Conradt, B. (2005). DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* **433**, 754-760.

Jung, D. W., Bradshaw, P. C. and Pfeiffer, D. R. (1997). Properties of a cyclosporin-insensitive permeability transition pore in yeast mitochondria. *J. Biol. Chem.* **272**, 21104-21112.

Kakkar, P. and Singh, B. K. (2007). Mitochondria: a hub of redox activities and cellular distress control. *Mol. Cell. Biochem.* **305**, 235-253.

Kass, G. E., Eriksson, J. E., Weis, M., Orrenius, S. and Chow, S. C. (1996). Chromatin condensation during apoptosis requires ATP. *Biochem. J.* **318**, 749-752.

Kokoszka, J. E., Waymire, K. G., Levy, S. E., Sligh, J. E., Cai, J., Jones, D. P., MacGregor, G. R. and Wallace, D. C. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**, 461-465.

Kornbluth, S. and White, K. (2005). Apoptosis in *Drosophila*: neither fish nor fowl (nor man, nor worm). *J. Cell Sci.* **118**, 1779-1787.

Krauskopf, A., Eriksson, O., Craigen, W. J., Forte, M. A. and Bernardi, P. (2006). Properties of the permeability transition in VDAC1(–/–) mitochondria. *Biochim. Biophys. Acta* **1757**, 590-595.

Kroemer, G., Petit, P., Zamzami, N., Vayssiere, J. L. and Mignotte, B. (1995). The biochemistry of programmed cell death. *FASEB J.* **9**, 1277-1287.

Kroemer, G., El-Deiry, W. S., Golstein, P., Peter, M. E., Vaux, D., Vandenabeele, P., Zhivotovsky, B., Blagosklonny, M. V., Malorni, W., Knight, R. A. et al. (2005). Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. *Cell Death Differ.* **12** Suppl. 2, 1463-1467.

Kroemer, G., Galluzzi, L. and Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* **87**, 99-163.

Kwast, K. E. and Hand, S. C. (1996a). Acute depression of mitochondrial protein synthesis during anoxia: contributions of oxygen sensing, matrix acidification, and redox state. *J. Biol. Chem.* **271**, 7313-7319.

Kwast, K. E. and Hand, S. C. (1996b). Oxygen and pH regulation of protein synthesis in mitochondria from *Artemia franciscana* embryos. *Biochem. J.* **313**, 207-213.

Land, S. C. and Hochachka, P. W. (1994). Protein turnover during metabolic arrest in turtle hepatocytes: role and energy dependence of proteolysis. *Am. J. Physiol.* **266**, C1028-C1036.

Land, S. C., Buck, L. T. and Hochachka, P. W. (1993). Response of protein synthesis to anoxia and recovery in anoxia-tolerant hepatocytes. *Am. J. Physiol.* **265**, R41-R48.

Lees, A. D. (1955). *Physiology of Diapause in Arthropods.* Cambridge: Cambridge University Press.

Lei, K. and Davis, R. J. (2003). JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc. Natl. Acad. Sci. USA* **100**, 2432-2437.

Leist, M., Single, B., Castoldi, A. F., Kuhnle, S. and Nicotera, P. (1997). Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J. Exp. Med.* **185**, 1481-1486.

Liston, P., Fong, W. G. and Korneluk, R. G. (2003). The inhibitors of apoptosis: there is more to life than Bcl2. *Oncogene* **22**, 8568-8580.

Liu, X., Kim, C. N., Yang, J., Jemmerson, R. and Wang, X. (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* **86**, 147-157.

Liu, X. H., Aksan, A., Menze, M. A., Hand, S. C. and Toner, M. (2005). Trehalose loading through the mitochondrial permeability transition pore enhances desiccation tolerance in rat liver mitochondria. *Biochim. Biophys. Acta* **1717**, 21-26.

Low, R. L. (2003). Mitochondrial Endonuclease G function in apoptosis and mtDNA metabolism: a historical perspective. *Mitochondrion* **2**, 225-236.

Lucken-Ardjomande, S. and Martinou, J. C. (2005). Newcomers in the process of mitochondrial permeabilization. *J. Cell Sci.* **118**, 473-483.

Lum, J. J., DeBerardinis, R. J. and Thompson, C. B. (2005). Autophagy in metazoans: cell survival in the land of plenty. *Nat. Rev. Mol. Cell Biol.* **6**, 439-448.

Lutz, P. L. and Milton, S. L. (2004). Negotiating brain anoxia survival in the turtle. *J. Exp. Biol.* **207**, 3141-3147.

Madeo, F., Herker, E., Maldener, C., Wissing, S., Lachelt, S., Herlan, M., Fehr, M., Lauber, K., Sigrist, S. J., Wesselborg, S. et al. (2002). A caspase-related protease regulates apoptosis in yeast. *Mol. Cell* **9**, 911-917.

Marton, A., Mihalik, R., Bratincsak, A., Adleff, V., Petak, I., Vegh, M., Bauer, P. I. and Krajcsi, P. (1997). Apoptotic cell death induced by inhibitors of energy conservation-Bcl-2 inhibits apoptosis downstream of a fall of ATP level. *Eur. J. Biochem.* **250**, 467-475.

McClintock, D. S., Santore, M. T., Lee, V. Y., Brunelle, J., Budinger, G. R., Zong, W. X., Thompson, C. B., Hay, N. and Chandel, N. S. (2002). Bcl-2 family members and functional electron transport chain regulate oxygen deprivation-induced cell death. *Mol. Cell. Biol.* **22**, 94-104.

Means, J. C., Muro, I. and Clem, R. J. (2005). Lack of involvement of mitochondrial factors in caspase activation in a *Drosophila* cell-free system. *Cell Death Differ.* **13**, 1222-1234.

Melino, G., Knight, R. A. and Nicotera, P. (2005). How many ways to die? How many different models of cell death? *Cell Death Differ.* **12** Suppl. 2, 1457-1462.

Menze, M. A. and Hand, S. C. (2007). Caspase activity during cell stasis: avoidance of apoptosis in an invertebrate extremophile, *Artemia franciscana*. *Am. J. Physiol.* **292**, R2039-R2047.

Menze, M. A., Clavenna, M. J. and Hand, S. C. (2005a). Depression of cell metabolism and proliferation by membrane-permeable and -impermeable modulators: role for AMP-to-ATP ratio. *Am. J. Physiol.* **288**, R501-R510.

Menze, M. A., Hutchinson, K., Laborde, S. M. and Hand, S. C. (2005b). Mitochondrial permeability transition in the crustacean *Artemia franciscana*: absence of a calcium-regulated pore in the face of profound calcium storage. *Am. J. Physiol.* **289**, R68-R76.

Mills, K., Daish, T., Harvey, K. F., Pfleger, C. M., Hariharan, I. K. and Kumar, S. (2006). The *Drosophila melanogaster* Apaf-1 homologue ARK is required for most, but not all, programmed cell death. *J. Cell Biol.* **172**, 809-815.

Modjtahedi, N., Giordanetto, F., Madeo, F. and Kroemer, G. (2006). Apoptosis-inducing factor: vital and lethal. *Trends Cell Biol.* **16**, 264-272.

Newmeyer, D. D. and Ferguson-Miller, S. (2003). Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* **112**, 481-490.

Nicholls, D. G. and Chalmers, S. (2004). The integration of mitochondrial calcium transport and storage. *J. Bioenerg. Biomembr.* **36**, 277-281.

Nicotera, P. and Melino, G. (2004). Regulation of the apoptosis-necrosis switch. *Oncogene* **23**, 2757-2765.

Nicotera, P., Leist, M. and Ferrando-May, E. (1998). Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol. Lett.* **102-103**, 139-142.

Nicotera, P., Leist, M., Fava, E., Berliocchi, L. and Volbracht, C. (2000). Energy requirement for caspase activation and neuronal cell death. *Brain Pathol.* **10**, 276-282.

Orrenius, S., Gogvadze, V. and Zhivotovsky, B. (2007). Mitochondrial oxidative stress: implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* **47**, 143-183.

Pakay, J. L., Hobbs, A. A., Kimball, S. R. and Guppy, M. (2003). The role of eukaryotic initiation factor 2alpha during the metabolic depression associated with estivation. *J. Exp. Biol.* **206**, 2363-2371.

Parrish, J., Li, L., Klotz, K., Ledwich, D., Wang, X. and Xue, D. (2001). Mitochondrial endonuclease G is important for apoptosis in *C. elegans*. *Nature* **412**, 90-94.

Passarella, S., Atlante, A., Valenti, D. and de Bari, L. (2003). The role of mitochondrial transport in energy metabolism. *Mitochondrion* **2**, 319-343.

Petronilli, V., Cola, C. and Bernardi, P. (1993a). Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore. II. The minimal requirements for

pore induction underscore a key role for transmembrane electrical potential, matrix pH, and matrix Ca2+. *J. Biol. Chem.* **268**, 1011-1016.
Petronilli, V., Cola, C., Massari, S., Colonna, R. and Bernardi, P. (1993b). Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria. *J. Biol. Chem.* **268**, 21939-21945.
Pliyev, B. K. and Gurvits, B. Y. (1999). Peptidyl-prolyl cis-trans isomerases: structure and functions. *Biochemistry Mosc.* **64**, 738-751.
Podrabsky, J. E. and Hand, S. C. (2000). Depression of protein synthesis during diapause in embryos of the annual killifish *Austrofundulus limnaeus*. *Physiol. Biochem. Zool.* **73**, 799-808.
Rasola, A. and Bernardi, P. (2007). The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. *Apoptosis* **12**, 815-833.
Remillard, C. V. and Yuan, J. X. (2004). Activation of K+ channels: an essential pathway in programmed cell death. *Am. J. Physiol.* **286**, L49-L67.
Reynolds, J. A. and Hand, S. C. (2004). Differences in isolated mitochondria are insufficient to account for respiratory depression during diapause in artemia franciscana embryos. *Physiol. Biochem. Zool.* **77**, 366-377.
Riedl, S. J., Li, W., Chao, Y., Schwarzenbacher, R. and Shi, Y. (2005). Structure of the apoptotic protease-activating factor 1 bound to ADP. *Nature* **434**, 926-933.
Rolfe, D. F. and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731-758.
Rolland, S. and Conradt, B. (2006). The role of mitochondria in apoptosis induction in *Caenorhabditis elegans*: more than just innocent bystanders? *Cell Death Differ.* **13**, 1281-1286.
Ryter, S. W., Kim, H. P., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X. and Choi, A. M. (2007). Mechanisms of cell death in oxidative stress. *Antioxid. Redox Signal.* **9**, 49-89.
Saelens, X., Festjens, N., Vande Walle, L., van Gurp, M., van Loo, G. and Vandenabeele, P. (2004). Toxic proteins released from mitochondria in cell death. *Oncogene* **23**, 2861-2874.
Savina, M. V., Emelyanova, L. V. and Belyaeva, E. A. (2006). Bioenergetic parameters of lamprey and frog liver mitochondria during metabolic depression and activity. *Comp. Biochem. Physiol.* **145B**, 296-305.
Sevrioukov, E. A., Burr, J., Huang, E. W., Assi, H. H., Monserrate, J. P., Purves, D. C., Wu, J. N., Song, E. J. and Brachmann, C. B. (2007). *Drosophila* Bcl-2 proteins participate in stress-induced apoptosis, but are not required for normal development. *Genesis* **45**, 184-193.
Shimizu, S., Eguchi, Y., Kosaka, H., Kamiike, W., Matsuda, H. and Tsujimoto, Y. (1995). Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-xL. *Nature* **374**, 811-813.
Shroff, E. H., Snyder, C. and Chandel, N. S. (2007). Bcl-2 family members regulate anoxia-induced cell death. *Antioxid. Redox Signal.* **9**, 1405-1409.
Skulachev, V. P. (2006). Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis* **11**, 473-485.
Sokolova, I. M., Evans, S. and Hughes, F. M. (2004). Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. *J. Exp. Biol.* **207**, 3369-3380.
Stocco, D. M., Beers, P. C. and Warner, A. H. (1972). Effect of anoxia on nucleotide metabolism in encysted embryos of the brine shrimp. *Dev. Biol.* **27**, 479-493.
Storey, K. B. and Storey, J. M. (2007). Tribute to P. L. Lutz: putting life on 'pause' – molecular regulation of hypometabolism. *J. Exp. Biol.* **210**, 1700-1714.
St-Pierre, J., Brand, M. D. and Boutilier, R. G. (2000). The effect of metabolic depression on proton leak rate in mitochondria from hibernating frogs. *J. Exp. Biol.* **203**, 1469-1476.
Tauber, S. E. and Tauber, C. A. (1976). Insect seasonality: diapause maintenance, termination, and post-diapause development. *Annu. Rev. Entomol.* **21**, 81-107.
Toninello, A., Salvi, M. and Colombo, L. (2000). The membrane permeability transition in liver mitochondria of the great green goby *Zosterisessor ophiocephalus* (Pallas). *J. Exp. Biol.* **203**, 3425-3434.
Tran, S. E., Meinander, A. and Eriksson, J. E. (2004). Instant decisions: transcription-independent control of death-receptor-mediated apoptosis. *Trends Biochem. Sci.* **29**, 601-608.
van Breukelen, F. and Hand, S. C. (2000). Characterization of ATP-dependent proteolysis in embryos of the brine shrimp, *Artemia franciscana*. *J. Comp. Physiol. B* **170**, 125-133.
van Breukelen, F., Maier, R. and Hand, S. C. (2000). Depression of nuclear transcription and extension of mRNA half-life under anoxia in *Artemia franciscana* embryos. *J. Exp. Biol.* **203**, 1123-1130.
Vander Heiden, M. G., Chandel, N. S., Schumacker, P. T. and Thompson, C. B. (1999). Bcl-xL prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. *Mol. Cell* **3**, 159-167.
Virolainen, E., Blokhina, O. and Fagerstedt, K. (2002). Ca(2+)-induced high amplitude swelling and cytochrome c release from wheat (*Triticum aestivum* L.) mitochondria under anoxic stress. *Ann. Bot.* **90**, 509-516.
Walter, L. and Hajnoczky, G. (2005). Mitochondria and endoplasmic reticulum: the lethal interorganelle cross-talk. *J. Bioenerg. Biomembr.* **37**, 191-206.
Wang, X., Yang, C., Chai, J., Shi, Y. and Xue, D. (2002). Mechanisms of AIF-mediated apoptotic DNA degradation in *Caenorhabditis elegans*. *Science* **298**, 1587-1592.
Wang, X., Wang, J., Gengyo-Ando, K., Gu, L., Sun, C. L., Yang, C., Shi, Y., Kobayashi, T., Mitani, S., Xie, X. S. et al. (2007). C. elegans mitochondrial factor WAH-1 promotes phosphatidylserine externalization in apoptotic cells through phospholipid scramblase SCRM-1. *Nat. Cell Biol.* **9**, 541-549.
Wieser, W. and Krumschnabel, G. (2001). Hierarchies of ATP-consuming processes: direct compared with indirect measurements, and comparative aspects. *Biochem. J.* **355**, 389-395.
Willis, S. N. and Adams, J. M. (2005). Life in the balance: how BH3-only proteins induce apoptosis. *Curr. Opin. Cell Biol.* **17**, 617-625.
Yamamoto, K., Ichijo, H. and Korsmeyer, S. J. (1999). BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. *Mol. Cell. Biol.* **19**, 8469-8478.
Yan, N., Chai, J., Lee, E. S., Gu, L., Liu, Q., He, J., Wu, J. W., Kokel, D., Li, H., Hao, Q. et al. (2005). Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* **437**, 831-837.
Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P. and Wang, X. (1997). Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* **275**, 1129-1132.
Yasuhara, N., Eguchi, Y., Tachibana, T., Imamoto, N., Yoneda, Y. and Tsujimoto, Y. (1997). Essential role of active nuclear transport in apoptosis. *Genes Cells* **2**, 55-64.
Youle, R. J. and Karbowski, M. (2005). Mitochondrial fission in apoptosis. *Nat. Rev. Mol. Cell Biol.* **6**, 657-663.
Youle, R. J. and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* **9**, 47-59.
Yu, X., Wang, L., Acehan, D., Wang, X. and Akey, C. W. (2006). Three-dimensional structure of a double apoptosome formed by the *Drosophila* Apaf-1 related killer. *J. Mol. Biol.* **355**, 577-589.
Zmasek, C. M., Zhang, Q., Ye, Y. and Godzik, A. (2007). Surprising complexity of the ancestral apoptosis network. *Genome Biol.* **8**, R226.