Calcium orchestrates apoptosis

Mark P. Mattson and Sic L. Chan

Apoptosis is a feature of many diseases and is critical for the sculpting and maintenance of tissues. New work demonstrates that calcium released from the endoplasmic reticulum synchronizes the mass exodus of cytochrome *c* from the mitochondria, a phenomenon that coordinates apoptosis.

One unresolved problem in cell biology has been to explain how a cell dies rapidly while maintaining its energy levels, preserving the structure of its mitochondria and endoplasmic reticulum, and not adversely affecting its neighbours. Research over the past decade has filled in some pieces of this puzzle by establishing the functions of several proteins — including Bcl-2 family members, cytochrome *c* and caspases — in an evolutionarily conserved form of programmed cell death called apoptosis[1]. During development, apoptosis is necessary for tissues and organs to acquire their unique structures and functions. By eliminating 'worn out' cells, apoptosis also serves adaptive functions in self-renewing tissues of the adult, with bone marrow or blood and epithelia being well-studied examples[2]. Beyond its importance for understanding development and tissue homeostasis, the molecular regulation of apoptosis has moved to the forefront of biomedical research: it is an important factor in prominent diseases, including cancers (in which tumour cells are resistant to apoptosis) and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (where unwanted apoptosis of neurons occurs)[3].

Apoptosis can be triggered by many different stimuli, including engagement of 'death receptors' by cytokines (such as tumour necrosis factor and Fas ligand), growth factor insufficiencies, toxins, oxidative stress, and calcium influx through plasma membrane channels or release from the endoplasmic reticulum. Early research into apoptosis focused on the nucleus because of the marked morphological changes it undergoes. However, it was quickly realised that the nuclear changes are far removed from the stimulus that triggers apoptosis, as they only occur after the cell death decision has been made. Early and pivotal events in apoptosis are now known to occur in mitochondria and the endoplasmic reticulum (Fig. 1), and the release of cytochrome *c* (from mitochondria) and calcium (from the endoplasmic reticulum) into the cytosol are requisites for apoptosis in many cases[4]. The function of calcium in apoptosis is particularly fascinating, particularly when we consider the prominence of calcium in regulating a multitude of physiological processes and the involvement of perturbed cellular calcium homeostasis in the pathogenesis of disorders of the cardiovascular, immune and nervous systems[5]. Nevertheless, until now the specific mechanisms through which calcium dynamics are controlled and by which calcium participates in apoptotic cascades have been elusive. In this issue, Boehning *et al.*[6] identify calcium as a messenger that coordinates mitochondrial–endoplasmic reticulum interactions that drive apoptotis. In an elegant and comprehensive set of experiments, they establish that a small amount of cytochrome *c* released from mitochondria can bind to and promote calcium conductance through inositol-1,4,5-trisphosphate ($InsP_3$) receptors in the endoplasmic reticulum membrane. The released calcium then triggers a mass exodus of cytochrome *c* from all mitochondria in the cell, thus activating the caspase and nuclease enzymes that finalise the apoptotic process.

Using yeast two-hybrid technology, Boehning *et al.*[6] show that cytochrome *c* binds to a carboxy-terminal domain of the $InsP_3$ receptor. They demonstrated the translocation of cytochrome *c* from mitochondria to the endoplasmic reticulum in intact cells in which apoptosis was triggered by several different stimuli, including the bacterial alkaloid staurosporine and the endogenous lipid ceramide. The authors further showed that binding of cytochrome *c* to the $InsP_3$ receptor occurs early in the cell death cascade and enhances calcium release from the endoplasmic reticulum, resulting in a large global increase in the cytoplasmic free calcium concentration (Fig. 1). Expression of the C-terminal domain of the $InsP_3$ receptor reduced the ability of staurosporine to induce calcium release and apoptosis, consistent with a requirement for cytochrome *c* binding to this region of the $InsP_3$ receptor. Cytochrome *c* translocation to the endoplasmic reticulum and calcium release were necessary for the subsequent massive exodus of cytochrome *c* from all mitochondria in the cell because these events did not occur in cells devoid of $InsP_3$ receptors. Apparently, calcium uptake into mitochondria secondary to elevation of cytoplasmic calcium concentration is the stimulus for massive release of cytochrome *c* from the mitochondria. Caspases are not involved in cyctochrome *c* translocation to the endoplasmic reticulum and subsequent calcium release because these processes were unaffected by caspase inhibitors.

The present findings establish a calcium-based mechanism for amplification and synchronization of cytochrome *c* release and execution of the cell-death process triggered by staurosporine and ceramide in cultured tumour cell lines[6]. Although it is not known if the same mechanism applies to other death triggers and cell types, this seems probable given the conservation of the apoptotic cascade downstream of the cell death trigger.

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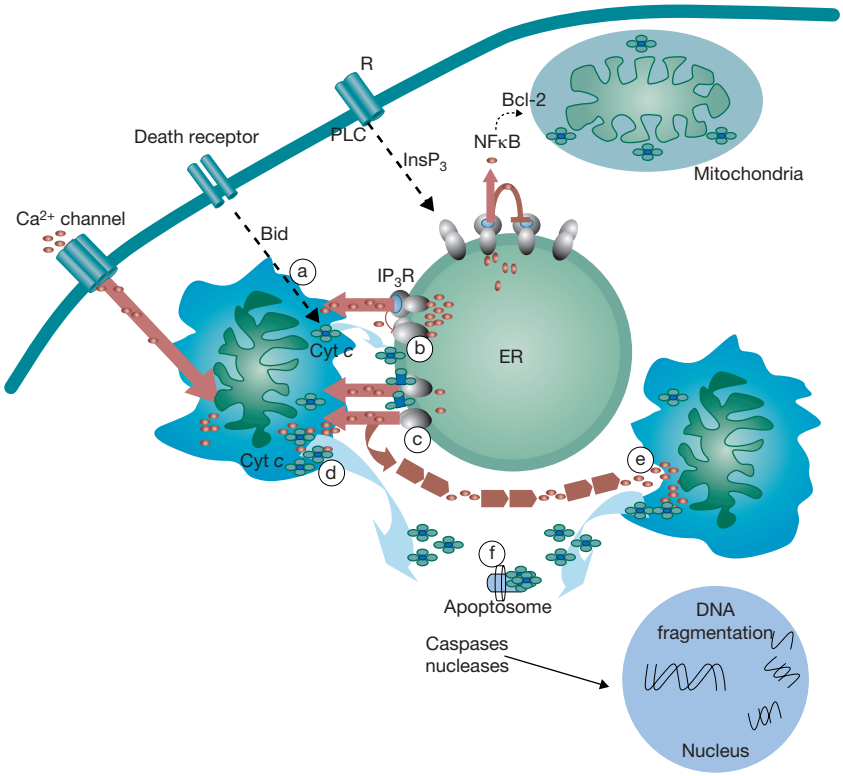

Figure 1 The roles of calcium and cytochrome *c* as inter-organellar messengers in apoptosis. The cell is initially exposed to a localized stimulus that has the potential to trigger apoptosis - two such stimuli shown here are calcium influx through plasma membrane channels and activation of cell surface death receptors. The death stimuli (calcium influx into the mitochondrion or binding of the protein Bid to the mitochondrial membrane) induce a permeability transition in the membrane of an adjacent mitochondrion, resulting in the release of cytochrome *c* from that mitochondrion (**a**). Cytochrome *c* then diffuses to the adjacent endoplasmic reticulum (ER) and binds to $InsP_3$ receptors (**b**), thereby enhancing calcium release from the endoplasmic reticulum (**c**). The calcium released from the endoplasmic reticulum causes a global increase in the cytoplasmic calcium concentration (**d**), resulting in calcium uptake by mitochondria throughout the cell that triggers the simultaneous release of cytochrome *c* from all mitochondria (**e**). The cytochrome *c* in the cytoplasm then induces formation of the apoptosome in which caspases are activated (**f**). Caspases and nucleases then finalize the cell death process by cleaving various protein substrates (caspases) and DNA (nucleases). Although calcium release from the endoplasmic reticulum can be a signal for execution of apoptosis, it can also activate cell survival pathways involving proteins such as the transcription factor NF-κB and the mitochondrial membrane-stabilizing protein Bcl-2.

Indeed, endoplasmic reticulum calcium release and cytochrome *c* release from mitochondria, and subsequent engagement of the apoptosome (a protein complex that also incudes Apaf-1, caspase-9 and caspase-3), are implicated in a broad array of cell deaths including those of neurons during development, of T-lymphocytes in response to activation and of epithelial cells during their normal turnover[7]. Mitochondrial membrane permeabilization and release of calcium from the endoplasmic reticulum are also thought to be pivotal events in pathological apoptosis in disorders ranging from myocardial infarction and stroke to infectious diseases and age-related neurodegenerative disorders. In many of these cases, and in other examples of natural and pathological apoptosis, there is indeed evidence for involvement of perturbed calcium homeostasis in the cell death process. Of particular interest in this regard is emerging evidence that endoplasmic reticulum stress and endoplasmic reticulum calcium release are central to the cell death process in disorders such as Alzheimer's disease and stroke[8], as well as in the killing of tumour cells by cytokines and anti-cancer therapies[9].

Cell death triggers may be highly localized to only a relatively small region of a cell. For example, neuronal apoptosis can be induced by activation of glutamate receptors confined to the postsynaptic membrane of a dendrite. In the latter example, the data of Boehning *et al.*[6] predict that a local influx of calcium through glutamate receptor channels induces cytochrome *c* release from the mitochondria nearest the site of influx, and that the cytochrome *c* then binds $InsP_3$ receptors in adjacent endoplasmic reticulum membranes. This would result in release of calcium that then spreads and triggers cytochrome *c* release from mitochondria throughout the dendrites and cell body. In this way, calcium functions as a temporal synchronizer for cytochrome *c* release. The cytochrome *c*–endoplasmic reticulum calcium interaction identified by Boehning *et al.* might also be an important mechanism for the integration of intracellular signals in biological processes not associated with cell death. The calcium-regulating abilities of the endoplasmic reticulum and mitochondria are increasingly appreciated for their involvement in physiological processes such as synaptic plasticity in neurons, excitation–contraction coupling in skeletal muscle cells, adaptive responses of immune cells to infectious agents, and proliferative responses to mitogens[10]. Mitochondrial calcium dynamics are involved in the regulation of cellular energy metabolism and in processes such as cell motility and neurotransmitter release. Because of its importance for apoptosis and many physiological processes, the regulation of calcium release is under tight control, and indeed an increasing number of proteins are being identified that interact with $InsP_3$ receptors. Other proteins may function downstream of endoplasmic reticulum calcium release to modulate apoptosis or cell functions, with a recent example being a protein(s) released from the endoplasmic reticulum after activation of $InsP_3$ receptors that activates the anti-apoptotic transcription factor NF-κB[11]. The latter kinds of mechanisms would be expected to modify the action of cytochrome *c* in apoptosis.

A better understanding of the molecular mechanisms that regulate apoptosis may lead to the development of novel therapeutic interventions for several diseases. Targeting caspases has not proved as effective as originally hoped because of the fact that these enzymes function at a relatively late stage of apoptosis, when much cell dysfunction and damage has already occurred[12]. In contrast, intervening in apoptosis at an early step in the process would be expected to preserve both the function and survival of the cell. Drugs might be identified that can selectively block binding of cyctochrome *c* to the $InsP_3$ receptor for the treatment of disorders involving pathological apoptosis.

The demonstration by Boehning *et al.* that expression of a peptide corresponding to the C-terminal domain of the $InsP_3$ receptor can block cytochrome *c* binding and prevent apoptosis[6] provides 'proof of principle' for such an approach. In the case of cancer therapies, it may be possible to enhance the cytochrome *c*–calcium release pathways by identifying drugs that mimic the effect of cytochrome *c* on endoplasmic reticulum calcium release. Further investigations of the interplay between mitochondria and the endoplasmic reticulum in the regulation of cell survival and death will undoubtedly reveal additional molecular targets for therapeutics. □

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Kinesin: walking or limping?

Manfred Schliwa

Some kinesin motors can move along a microtubule for many hundred steps without dissociating. These motors are dimers, but precisely how the two motor domains are coordinated during stepping is still the subject of debate. A novel experimental approach offers new insights.

Molecular motors use polymer tracks to move cellular cargoes around the cell. One such motor — conventional kinesin — is a dimer composed of two heavy chains that travels long distances on microtubules by taking 8-nm steps from one tubulin subunit to the next[1]. Each kinesin monomer possesses a globular motor domain that has a binding site for the track and a binding site for the fuel, ATP. The monomers are linked to one another though coiled-coil regions in the carboxy-terminal stalk. Binding and unbinding to the track and the fuel, respectively, are delicately interwoven so that a single motor can move for long distances without 'falling off' the track, a property referred to as 'processivity'[2]. To accomplish this, the motor has long been thought to use a mechanism where one head remains attached to the track while the other swings forward, and the roles of the two heads are swapped at each step[3]. Much of the biochemical and structural evidence accumulated over the past decade supports this 'hand-over-hand' model. In fact, it made so much intuitive sense that it has found entry into all major cell biology and biochemistry textbooks. Case closed?

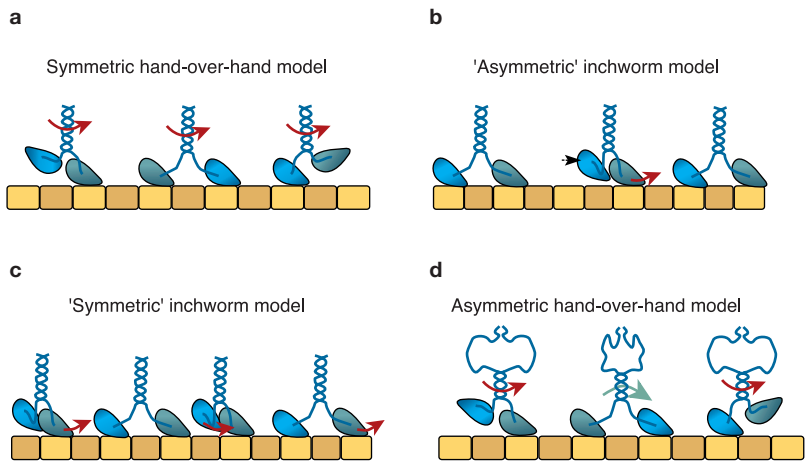

Figure 1 Models for kinesin stepping. (**a**) In the symmetric hand-over-hand model, the trailing head always passes the leading head on the same side (red arrows in front of the coiled-coil neck). (**b**) In the asymmetric inchworm model, only the leading head hydrolyses ATP while the trailing head is pulled up passively. Here, 'asymmetric' refers to ATP hydrolysis occurring only in one head. (**c**) In a symmetric inchworm model, both heads would hydrolyse ATP, and hydrolysis in the trailing head would push the leading head forward. (**d**) In an asymmetric hand-over-hand model, torsion generated during a step would be accommodated by the flexible hinge domain above the neck during one step (red arrow) and relieved by uncoiling in one of the next step(s), as shown by the green arrow behind the neck.

Not yet, said Hua, Chung and Gelles last year[4]. The devil, as usual, may be in the detail. Unlike humans, kinesin does not have a left foot (head) and a right foot (head). The molecule is composed of two identical subunits that should behave exactly the same. This implies that the lagging head should swing by the leading head always in the same fashion, which in turn would rotate the motor by 180° at each step (a 'symmetric hand-over-hand' model; Fig. 1a). We should be able to see that rotation, the authors reasoned, if we attach the motor to a surface with its rear end and watch it move a microtubule. If we allow for sufficient time between steps by slowing down the frequency of stepping to seconds, rather than milliseconds, then we can use the microtubule as a visible indicator of that rotation. That was what they hoped to demonstrate, but in fact what they observed was the opposite: the microtubule does not deviate from a straight path by more than ~30°. Their technically elegant and experimentally sound analysis led the authors to propose an alternative mechanism, referred to as the 'inchworm

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