Connected to Death: The (Unexpurgated) Mitochondrial Pathway of Apoptosis

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The mitochondrial pathway of apoptosis in vertebrates is dependent on the process of mitochondrial outer membrane permeabilization (MOMP), which leads to the release of proteins from the mitochondrial intermembrane space into the cytosol. "Upstairs" of this event are the Bcl-2 family proteins that regulate and mediate MOMP; "downstairs" is the activation of caspases that orchestrate the dismantling of the cell. In the Connections Map database at *Science*'s Signal Transduction Knowledge Environment (STKE), the pathways that define the mitochondrial pathway of apotosis are illustrated, with the bulk of control occurring "upstairs" of MOMP.

Cell death is a fundamental fact of life, and in multicellular organisms it can be critically important for the organization of cells and tissues in the body. In animals, the preeminent mechanism of most (but not all) physiological cell deaths is by apoptosis, and in the vertebrates, most (but not all) apoptosis proceeds through the mitochondrial pathway, illustrated in the Connections Map database (*1*–*4*) at *Science*'s STKE. These pathways present current information on the key elements of the mitochondrial pathway of apoptosis as they are understood in vertebrate systems, along with many of the molecules that may regulate elements of the pathway. The extent to which this pathway, as we define it below, is represented in other animals is controversial. The pathway is ''unexpurgated" in that most of the protein functions and protein-protein interactions that have been described as regulating this process are included, regardless of whether they have been confirmed, challenged, or viewed skeptically (*1*–*4*). In this overview, only the best documented elements are discussed, although these too are not without controversy.

The mitochondrial pathway of apoptosis in vertebrate cells centers on and is defined by a pivotal event in the cell death process: mitochondrial outer membrane permeabilization (MOMP) (Fig. 1). Proteins sequestered in the mitochondrial intermembrane space, between the inner and outer mitochondrial membranes (IMM and OMM, respectively), gain access to other proteins in the cytosol, and this results in apoptosis. The mitochondrial pathway concerns the causes and consequences of MOMP. In describing this pathway in simplified form, we have an "upstairs/downstairs" situation where, at first pass, most of the aristocratic decisions are made before MOMP (upstairs) and the workmanlike consequences occur thereafter (downstairs). As in entitled households, of course, this is only an approximation.

Upstairs of MOMP are the members of the Bcl-2 family, divided between those that are antiapoptotic (prevent MOMP) and those that are proapoptotic (promote MOMP). These proteins share up to four Bcl-2 homology domains (BH1 to BH4). The proapoptotic "multidomain" proteins (sharing BH1, 2, and 3), Bax and Bak, appear to be prerequisite for MOMP and are likely to directly mediate it by forming size-indeterminate openings in the OMM (*1*, *5*). They are present in most cells in inactive form, and their activation to cause MOMP is triggered by other proteins, including a subset of the "BH3-only" proteins (Bcl-2 family members sharing only the BH3 domain), but perhaps other non–Bcl-2 proteins as well (*2*). This poorly understood activation event is inhibited by the antiapoptotic Bcl-2 proteins that sequester the activating proteins and probably also the active multidomain proteins (*3*). This inhibition can be reversed by several other BH3-only proteins ("derepressors" or "sensitizers"), as well as by protein modifications (such as phosphorylation or deamidation) of the antiapoptotic proteins to control MOMP. The BH3-only proteins, both "activators" and "derepressors," are regulated in various ways—only some of which are fully understood—and their regulation is likely to represent many of the major portals through which different signaling events converge on the mitochondrial pathway of apoptosis (*3*).

Once MOMP occurs, proteins of the intermembrane space are released to the cytosol (*4*). One is holocytochrome c, which binds to cytosolic, monomeric apoptotic protease activating factor–1 (APAF-1). The interaction with cytochrome c permits a 2′-deoxyadenosine 5′-triphosphate (dATP)–mediated conformational change in APAF-1, promoting APAF-1 oligomerization to form an "apoptosome." The apoptosome then binds the proform of a protease, caspase-9. The oligomerization of caspase-9 on the apoptosome activates the protease; initiator caspases such as caspase-9 can only be activated by dimerization (*6*). The active caspase-9 cleaves two other caspases, caspase-3 and caspase-7. These are ''executioner" caspases, and, unlike initiator caspases, these can only be activated by cleavage at specific sites, which are targeted by initiator caspases. Once activated, the executioner caspases orchestrate apoptosis through the cleavage of key substrates within the cell, resulting in demolition and packaging of the dying cell for removal by phagocytic cells.

The process of apoptosis "downstairs" of MOMP is regulated at the apoptosome and each of the caspases. The latter are controlled by inhibitor of apoptosis proteins (IAPs) that bind and ubiquitinate the caspases for proteasomal degradation. The function of the IAPs, in turn, is blocked by IAP inhibitors. These include Smac (also known as DIABLO) and Omi (also known as HtrA2), which compete with caspases for IAP binding. Like cytochrome c, Smac and Omi are sequestered in the mitochondrial intermembrane space and are only available to regulate the cytosolic IAPs upon MOMP.

Cell death can also proceed downstairs of MOMP independently of caspase activation. This may occur through the action of lethal factors released upon MOMP [for example, apoptosis-inducing factor (AIF) and endonuclease G have been suggested to have this function] or through irreparable loss of essential mitochondrial functions. Such "caspase-independent" death remains poorly understood (*7*).

What, then, is connected to death? The mitochondrial pathway of apoptosis can be triggered by a bewildering array of conditions, including virtually anything that contributes to cellular stress or loss of housekeeping functions (such as nutrient deprivation, unfolded proteins, cytoskeletal disruption, DNA damage, ion imbalance, and toxins), as well as developmental signals (such as cytokines, steroids, and lipid mediators) and immunologic effector processes (such as cytotoxic effector cells and some cytokines). One important model of tumor suppression holds that all signals to enter the cell cycle simultaneously engage the mitochondrial pathway of apoptosis as a checkpoint on tissue expansion (*8*). With about 100,000 citations to "apoptosis" in the scientific litera-

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ture, one might be tempted to answer the above question, "everything."

Survival versus death of a cell is a binary decision, and as most deaths of vertebrate cells involve in some way the mitochondrial pathway of apoptosis, the Connections Map of this pathway might eventually extend to most of the proteins in the cell. At first this may seem farfetched, but a simple model experiment shows the feasibility of this idea.

The "button experiment" of Kauffman (*9*) illustrates the principle. Start with 500 buttons and at random tie two together with thread. Now pick up a button at random and count how many are tied to it. Repeat. At first the answer will be one or two, but at some point a "phase transition" occurs in which most randomly selected buttons are tied to large numbers of other buttons. In our example, this happens when there are about half as many threads as buttons. By extension to molecules in a cell, we do not need many connections to "tie" all signaling pathways to one another. Many inputs will therefore influence the mitochondrial pathway of apoptosis, and because the outcome is control of cell life and death, the effects can be obvious and potentially overinterpreted.

This, then, is our problem. Simply showing that a molecule (or process, agent, condition, etc.) influences apoptosis does not prove that it directly acts on any of the key players in the pathway. And even if a connection with a protein in the pathway is shown, it need not be pivotal in the outcome for the cell. The challenge for understanding how the mitochondrial pathway of apoptosis is engaged and controlled is to identify the most important interactions dictating cell life or death in a given setting, and to extend the pathway toward those key events. A rigorous investigation of the most relevant relationships is needed. We have tried to provide a map of the many roads to cellular ruin through the mitochondrial pathway; the ones most traveled in apoptotic cell death remain to be determined.

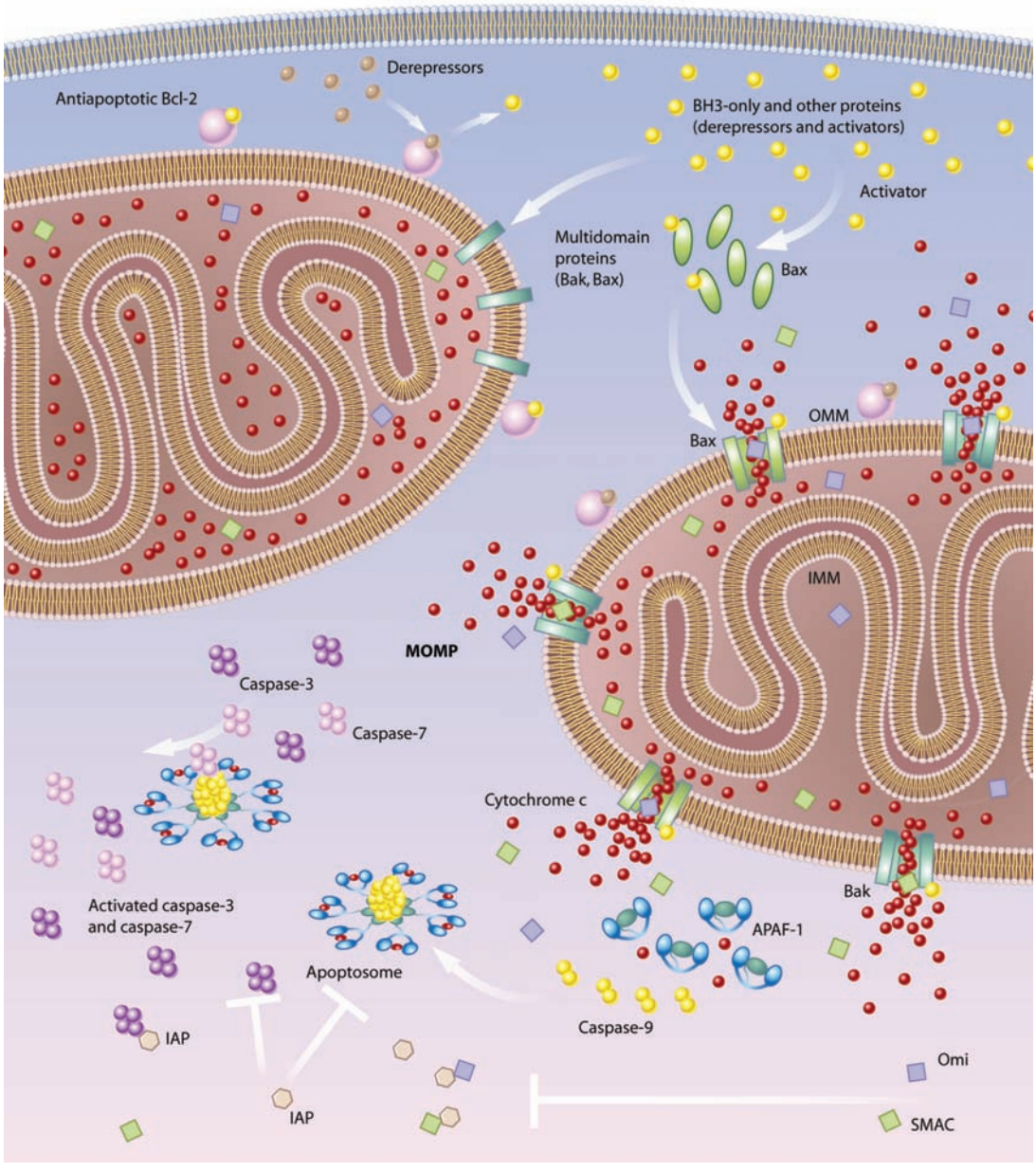

Fig. 1. The vertebrate mitochondrial pathway of apoptosis. At the core of this pathway is the process of mitochondrial outer membrane permeabilization (MOMP). This is mediated predominantly by the proapoptotic Bcl-2 family members, Bax and Bak, and inhibited by the antiapoptotic Bcl-2 family proteins. The BH3-only proteins of this family regulate MOMP either by activating Bax and Bak (activators) or by antagonizing the antiapoptotic Bcl-2 proteins (derepressors). MOMP allows proteins of the mitochondrial intermembrane space to gain access to the cytosol. Cytochrome c triggers the activation of APAF-1, leading to formation of the apoptosome followed by recruitment and activation of caspase-9. Caspase-9, in turn, cleaves and activates the executioner caspases 3 and 7 to orchestrate apoptosis by the cleavage of key substrates. IAP inhibits caspase activation, and this inhibition can be reversed by IAP antagonists (for example, Smac and Omi) released from the mitochondria upon MOMP.

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