Of course, the structure of a protein is simply a means for getting at how the protein works. Two "big" questions keep researchers busy. First, how might ATP hydrolysis by the NBDs be coupled to substrate transport? There is strong biochemical evidence that the two NBDs of ABC transporters work in an alternating catalytic cycle, yet because only part of the NBD is resolved in the MsbA structure, it is not yet possible to ascertain whether the two NBDs interact directly with each other and, if they do, which amino acid residues are involved. Even more important is how the binding and hydrolysis of ATP by the NBDs is coupled to the conformational changes in the TMDs that mediate substrate translocation. The MsbA structure identifies amino acids in the intracellular loops of the TMDs that form a bridge that is likely to transduce conformational changes from the NBDs to the TMDs. However, the snapshot MsbA structure alone (in the absence of ATP and substrate) cannot answer these mechanistic questions.

The second "big" question is the nature of the transmembrane pathway, the substrate binding sites, and the conformational changes induced during the transport cycle. Chang and Roth propose an elegant and plausible model for lipid A transport by MsbA: Lipid A enters the chamber from the inner leaflet of the bilayer and, after an ATP-induced conformational change, is exposed to charged residues that create an unfavorable environment such that lipid A is "flipped" into the upper part of the chamber and ultimately into the outer leaflet of the bilayer (see the figure). However, as the authors themselves point out, this model cannot explain how many other ABC transporters operate.

Our two-dimensional crystallographic and biochemical studies of P-glycoprotein trapped at different stages in the transport cycle add another twist to the story (*10*). Unexpectedly, the membrane-spanning α-helices of the TMDs undergo a remarkable reorganization during the transport cycle. The most dramatic structural changes accompany binding of ATP to the NBDs, an event that results in loss of drug-binding affinity. Thus, the energy of ATP binding (rather than ATP hydrolysis) may provide the initial energy for translocation of substrate. Subsequent ATP hydrolysis and ADP/Pi release returns the transporter to its original configuration through at least one additional conformational intermediate. This reorganization appears to be due to lateral repacking of the α-helices within the plane of the membrane. One model for reorientating the substrate binding site is rotation of the transmembrane α-helices within the membrane (see the figure). An alternative model is helix "tilting"; of course, reorientation may depend on a combination of both. Crucially, the exceptional flexibility of the TMDs of ABC transporters may reconcile otherwise apparently incompatible data.

Although the structure of an ABC transporter is a necessary prerequisite, it is only one step in our efforts to unravel and understand the complex dynamic and vectorial processes that enable these fascinating proteins to translocate solutes across cellular membranes.

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PERSPECTIVES: APOPTOSIS

Till Death Us Do Part

Abigail Hunt and Gerard Evan

The wonder of multicellular organisms is that each individual cell seems to know what to do, where to be, and how to behave. Such remarkable self-organization relies in part on the surprising alacrity of cells to commit suicide, a process termed apoptosis, should they stray or be misplaced from their normal somatic compartment and so become deprived of the requisite social signals needed for their survival. Nowhere is this phenomenon more evident than in epithelial cells, which derive much of their positional information from their association with their neighbors and with the extracellular matrix. Deprived of such associations, epithelial cells typically undergo detachment-induced apoptosis, or anoikis. Such spontaneous suicide effectively confines epithelial tissues to their correct somatic compartments, ensuring the expeditious deletion of cells misplaced during development or through injury, and potently restraining the emergence of invasive malignancies. Accordingly, inactivation of anoikis is a critical step in the progression of epithelial cancers to an invasive and metastatic form. On page 1829 of this issue, Puthalakath *et al.* (*1*) show that detachment of epithelial cells from their extracellular matrix detonates an apoptotic bomb by triggering the release of the pro-apoptotic protein Bmf from the myosin V motor complex of the actin cytoskeleton.

Key players in the determination of cell survival and death are members of the Bcl-2/Bax protein family (*2*). Bcl-2/Bax family members fall into three general classes. Some, like Bcl-2 and its close homolog Bcl-x_L, suppress apoptosis and render a cell (at least temporarily) more resilient to a wide variety of lethal assaults including radiation, metabolic poisoning, growth factor deprivation, and even heat. They accomplish this not by mitigating the damage incurred by such insults, but rather by curbing the cell's suicidal response to the damage.

Other family members, such as Bax and Bak, closely resemble Bcl-2/Bcl-x_L in structure and share three of their four signature Bcl-2 homology (BH) regions. However, instead of suppressing apoptosis, they promote it, dimerizing with Bcl-2 and Bcl-x_L and antagonizing the protective activity of these proteins. More distant relatives are the "BH3-only" proteins whose apparent exclusive role is to promote apoptosis. The emerging consensus is that many of the diverse pathways that regulate cell survival and cell death have, as their terminal effector, one or more BH3-only proteins (see the figure). For example, the BH3-only proteins Noxa and PUMA are transcriptional targets of the p53 tumor suppressor protein, which is induced in response to DNA damage and promotes apoptosis (*3, 4*). The BH3-only protein Bid is cleaved in response to signaling through the Fas, tumor necrosis factor (TNF), or TRAIL death receptors; cleavage converts Bid to its active, proapoptotic form tBid (*5*). The lethal action of the BH3-only protein Bad is forestalled only as long as survival factor signaling through the serine-threonine kinase Akt/PKB pathway keeps it phosphorylated and sequestered by the cytosolic 14-3-3 proteins (*6*). The abiding image is one in which our cells are peppered with unexploded BH3 bombs, their fuses primed and, in some cases, lit, only to be quenched by survival signals from neighboring cells.

In their new work, Puthalakath *et al.* identify a BH3-only protein, Bmf, which appears to act as a detachment sentinel during anoikis. Identified initially through its interaction with the anti-apoptotic Bcl-2 family member Mcl-1, Bmf is, like its

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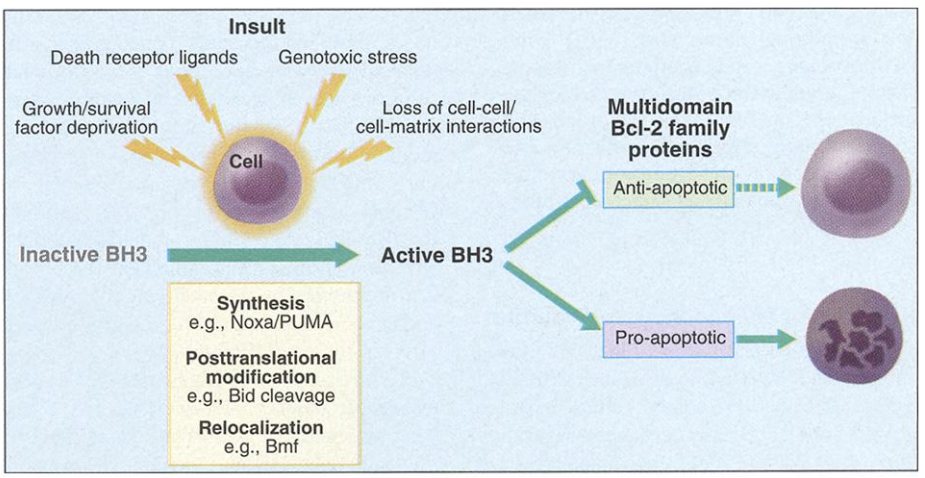
BH3-only kindred, a potent killer. However, its lethal proclivities must be tightly held in check until required because it is constitutively expressed in many healthy tissues. Indeed, careful analysis shows that Bmf, like the similar BH3-only protein Bim recently identified by the same group (*7*), is held in abeyance through its attachment to the cytoskeleton, and is thus denied access to its apoptotic effector targets. Specifically, Bmf associates with the dynein light chain 2 (DLC2) component of the myosin V motor and is thereby sequestered by the cell's actin cytoskeleton. Disruption of the actin cytoskeleton, either by drugs and toxins that depolymerize actin filaments or more physiologically by detachment of cells from the extracellular matrix, triggers release and consequent activation of Bmf, initiating the downstream apoptotic program. Until now, anoikis was thought to occur because detachment deprives a cell of survival signals generated by integrin-dependent interactions between cells and the extracellular matrix and catenin/cadherin-dependent interactions between cells (*8*). Puthalakath *et al.* now show that anoikis involves not only a lack of survival signals, but also the active liberation of the pro-apoptotic effector Bmf. As added spice, the gene encoding Bmf lies on chromosome 15q14, a region that is lost in many metastatic carcinomas.

The molecular modus operandi of the Bcl-2/Bax/BH3-only proteins remains hotly contested (*9*). There are those who argue that the Bcl-2/Bax/BH3-only proteins are critical regulators of the integrity of mitochondria. These energy-generating organelles of cells moonlight as sensitive bellwethers of apoptotic stress and, in response to a wide variety of pro-apoptotic signals, disgorge a plethora of pro-apoptotic effectors from their intermembranous space into the cell cytosol. Such effectors include the flavoprotein AIF, which triggers chromatin condensation and fragmentation (*10*); endonuclease G, which degrades nuclear DNA (*11, 12*); and Smac/DIABLO, which quells the anti-apoptotic action of the IAP proteins (*13, 14*). Also featured in this group is holocytochrome c, which, when not otherwise gainfully employed in mitochondrial electron transport, is a cytosolic activator of the caspase-9–Apaf-1 apoptosome, an apical trigger of the mammalian cascade of proteolytic enzymes called caspases (*15*).



Signaling the executioner. BH3-only proteins are activated in response to diverse apoptotic stimuli. For example, Noxa and PUMA are transcriptionally regulated by p53; Bid is proteolytically cleaved by caspase-8, yielding the pro-apoptotic fragment tBid upon ligation of death receptors; Bmf, which is normally sequestered in the actin cytoskeleton by association with dynein light chain (DLC) 2 of the myosin V motor complex, is released during cell detachment (anoikis). "Active" BH3-only proteins promote cell death through their interactions with either pro- or anti-apoptotic "multidomain" members of the Bcl-2 family of proteins.

Caspases act like molecular chainsaws cleaving critical cellular substrates and implementing much of the apoptosis program. Release of these mitochondrial proteins is suppressed by the Bcl-2/Bcl-x_L protectors and facilitated by the pro-apoptotic Bax/BH3-only killers, suggesting that Bcl-2/Bax proteins directly regulate mitochondrial membrane integrity. An alternative nonmitochondrial Bcl-2/Bax/BH3 modus operandi is suggested by studies of cell death control in the nematode *Caenorhabditis elegans*, an organism whose developmental invariance and genetic tractability have historically made it the model par excellence for cell death studies. The nematode Bcl-2 homolog, Ced-9, sequesters Ced-4, a distant relative of mammalian Apaf-1 that is required to activate the critical nematode apoptotic effector caspase Ced-3. In cells destined to undergo death during nematode development, the BH3 protein EGL-1 severs the connection between Ced-9 and Ced-4, freeing Ced-4, which then activates Ced-3. Of course, *C. elegans* is neither a progenitor nor a simplified version of any mammal but rather is a highly evolved and streamlined product of an alternate evolutionary pathway. Nonetheless, the worm's cell death machinery shares so many of its basic components with mammalian cells that the nematode offers a straightforward and nonmitochondrial molecular cantilever for regulating and implementing mammalian cell death.

This disagreement over the mechanism of action of the Bcl-2/Bax proteins obviously impacts our understanding of the BH3-only proteins. Do they activate the Bax/Bak killers or inhibit the Bcl-2/Bcl-x_L protectors? The existing evidence points both ways. Cells lacking both Bax and Bak, yet retaining functional Bcl-2 and Bcl-x_L, exhibit remarkable resilience to multiple apoptotic insults (*16, 17*). Hence, the mere inhibition of the Bcl-2/Bcl-x_L protectors may be insufficient to trigger cell death in many circumstances, and activation of the Bax/Bak killers may be required. Consistent with this, tBid, the activated form of the BH3-only protein Bid, both binds to and triggers a conformational change in Bax that activates this pro-apoptotic protein (*18*). In contrast, the evidence is unassailable that Bmf binds to anti-apoptotic Bcl-2 family members but not the pro-apoptotic ones, which suggests that its particular pro-apoptotic action is to inhibit the Bcl-2 protectors. Such mechanistic differences may merely indicate that, like the diverse signaling pathways that activate them, the multifarious BH3-only proteins have evolved a variety of different ways to modulate the core Bcl-2/Bax machinery. Exactly what that machinery is, and where in the cell it resides, remains the subject of intense debate.

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