CELL DEATH AND AUTOPHAGY

Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy

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Abstract | The BCL-2 protein family determines the commitment of cells to apoptosis, an ancient cell suicide programme that is essential for development, tissue homeostasis and immunity. Too little apoptosis can promote cancer and autoimmune diseases; too much apoptosis can augment ischaemic conditions and drive neurodegeneration. We discuss the biochemical, structural and genetic studies that have clarified how the interplay between members of the BCL-2 family on mitochondria sets the apoptotic threshold. These mechanistic insights into the functions of the BCL-2 family are illuminating the physiological control of apoptosis, the pathological consequences of its dysregulation and the promising search for novel cancer therapies that target the BCL-2 family.

Sertoli cells
Cells that nourish developing sperm cells through the stages of spermatogenesis.

'Eat me' signals
Surface markers on apoptotic cells, such as phosphatidylserine, that facilitate their recognition and phagocytosis by healthy cells.

Necroptosis
A programmed form of necrosis regulated by receptor-interacting Ser/Thr protein (RIP) kinases.

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In multicellular organisms, altruistic cell suicide has many essential roles[1]. In the vertebrate epiblast, cell death eliminates the less fit cells[2]. Later, it shapes the embryo by deleting superfluous cells, for example by removing interdigital cells during limb formation and by hollowing out ducts. Also, excess neurons are deleted to match target cell numbers, and excess male germ cells must be eliminated in early spermatogenesis to avoid overwhelming their supporting Sertoli cells. Throughout life, cell death must balance cell proliferation, particularly in tissues with high turnover, such as the haematopoietic system and intestinal epithelium. In the immune system, cells that are useless or dangerous because their antigen receptors recognize self tissues must be eliminated. Cell death also drives duct formation in the mammary gland and involution at weaning, as well as thymus atrophy with ageing. Finally, the programmed suicide of infected cells limits pathogen spread[3].

The major mode of programmed cell death, apoptosis, was first recognized morphologically[4]. The nuclei and cytoplasm, including the mitochondria, shrink in cells undergoing apoptosis, and the cell contents typically become encased in 'apoptotic bodies' surrounded by plasma membrane. In response to 'eat me' signals on their surface, these apoptotic bodies are rapidly engulfed by nearby phagocytic cells and digested in their lysosomes. The clearance system is so effective that little cell death is apparent, even in tissues that normally undergo high levels of apoptosis, such as the thymus[1]. By contrast, necrosis (passive cell death), which involves swelling of the mitochondria and rupture of the plasma membrane, releases inflammatory cellular contents that might lead to the presentation of self antigens in an immunogenic form and culminate in autoimmune tissue damage[5]. Apoptosis has been well studied in vertebrates, the flatworm *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*. Recently, other forms of programmed cell death, such as necroptosis, have been recognized; however, they have been expertly reviewed elsewhere[6,7] and are not addressed in this Review.

Molecular insights into apoptosis first emerged during the 1980s and 1990s from a remarkable convergence of mammalian cancer cytogenetics and biochemistry with pioneering genetic studies on developmentally programmed cell death in *C. elegans*. The previously unknown gene *BCL2* was identified from the breakpoint region of a recurrent chromosomal translocation in human follicular lymphoma[8]. In seminal studies, enforced expression of BCL-2 rendered haematopoietic cells refractory to death induced by cytokine deprivation[9] and promoted lymphocyte accumulation in mice[10-12], often culminating in autoimmune disease or cancer (see below). Notably, the function of CED-9, which prevents developmental cell death in *C. elegans*[13], could be replaced by human BCL-2 (REFS 14,15). Similarly, the worm killer protein CED-3 (REF. 16) was shown to resemble the mammalian Asp-specific Cys proteases, later to be known as caspases. Subsequent biochemical

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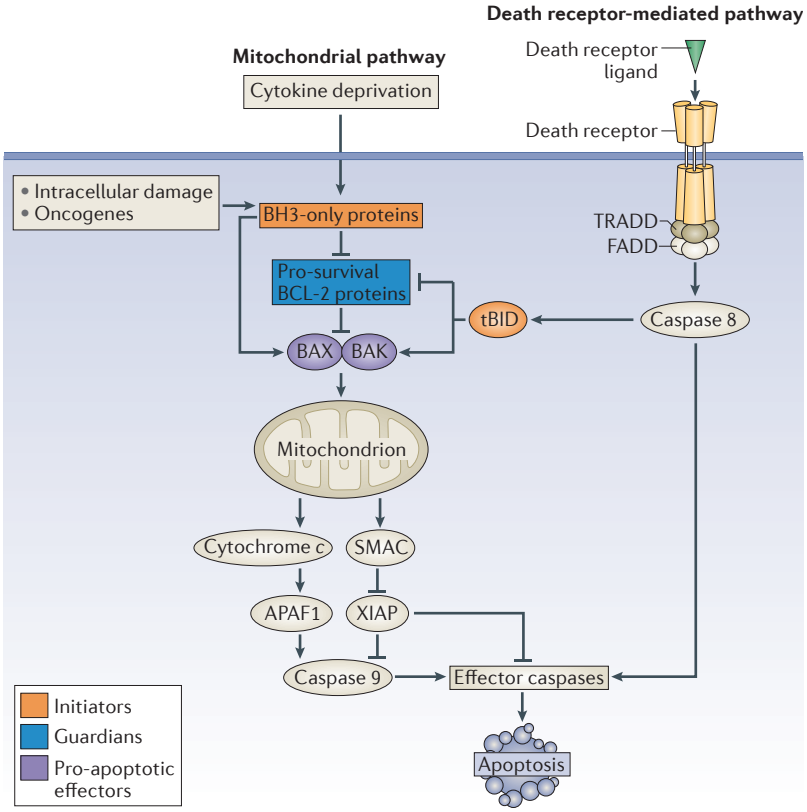

Figure 1 | **The mitochondrial and death receptor-mediated pathways to apoptosis.** Diverse cytotoxic stimuli, including oncogenic stress and chemotherapeutic agents, as well as developmental cues, engage the mitochondrial pathway, which is regulated by BCL-2 family members. These stimuli activate BH3-only family members (initiators), which inhibit the pro-survival BCL-2-like proteins (guardians), thereby enabling activation of the pro-apoptotic effectors BAX and BAK, which then disrupt the mitochondrial outer membrane. The cytochrome *c* released from the mitochondria promotes caspase 9 activation on the scaffold protein APAF1 (apoptotic protease-activating factor 1), whereas the released protein SMAC (second mitochondria-derived activator of caspases)[214,215] blocks the caspase inhibitor XIAP (X-linked inhibitor of apoptosis protein). The death receptor-mediated (or extrinsic) pathway of apoptosis is activated when certain death receptor ligands of the tumour necrosis factor (TNF) family (such as FAS ligand and TNF) engage their cognate death receptors (FAS and TNFR1, respectively) on the plasma membrane, leading to caspase 8 activation via FAS-associated death domain protein (FADD) and TNFR-associated death domain protein (TRADD). TRADD is required for the induction of apoptosis by some death receptors (such as TNFR1) but not others (such as FAS). The two pathways converge at activation of the effector caspases (caspase 3, caspase 7 and caspase 6). In addition, the truncated form of BID (tBID), which is generated by caspase 8-mediated proteolysis of BID in the death receptor-mediated pathway, can engage the mitochondrial pathway to amplify the apoptotic response. This amplification mechanism is required for effective apoptosis in certain cells (denoted 'type 2' cells), such as hepatocytes, but not in 'type 1' cells, such as thymocytes[33,34]. The level of XIAP distinguishes the two cell types, as it is higher in type 2 cells[35].

studies showed that the mammalian proteolytic cascade that is induced by the BCL-2-regulated pathway starts with the scaffold protein APAF1 (apoptotic protease-activating factor 1)[17], which is the homologue of worm CED-4 (REF. 18), in response to cytochr0ome *c* released from mitochondria[17,19]. These discoveries outlined a dedicated, evolutionarily conserved genetic programme to ensure the 'tidy' (non-inflammatory) removal of unwanted cells.

This Review focuses on the commitment to apoptosis in vertebrates, as this pivotal step differs substantially in mechanism and complexity from that in invertebrates, and is highly relevant to human pathology. We concentrate on the mitochondrial (also known as intrinsic, BCL-2-regulated or stress) pathway to cell death, in which the apoptotic threshold is set by interactions on the mitochondrial outer membrane between three functionally and structurally distinct subgroups of the BCL-2 protein family: BH3 (the BCL-2 homology 3)-only proteins (which convey signals to initiate apoptosis), the pro-survival cell guardians such as BCL-2 itself, and the pro-apoptotic effector proteins BAX (BCL-2-associated X protein) and BAK (BCL-2 antagonist/killer) (FIG. 1). Thus, this family can be regarded as a tripartite apoptotic switch. When enough BH3-only proteins have been stimulated in response to various cytotoxic stresses to exceed the apoptotic threshold, BAX and BAK begin to form the oligomers that permeabilize the mitochondrial outer membrane. This releases apoptogenic factors into the cytosol, particularly cytochrome *c*[17,19], which promotes the activation of apical caspase 9 on APAF1 (REF. 20); activated caspase 9 in turn processes and activates the effector caspases. We only briefly mention the independent but convergent death receptor-mediated (also known as extrinsic) pathway, as this pathway has recently been reviewed elsewhere[21]. The death receptor-mediated pathway is triggered when certain members of the tumour necrosis factor receptor (TNFR) family are ligated on the plasma membrane, which leads to the activation of caspase 8 and subsequently of the effector caspases (FIG. 1). We do not address the structural aspects of caspase activation[22] or the downstream events that they induce[23].

We first describe the recent molecular insights, shown by structural studies, into how the BCL-2 family sets the apoptotic threshold and regulates commitment to apoptosis. We briefly consider how regulatory signals impinge on the family and then address its physiological functions in cell death control, its roles in the development of pathology[24,25] and the effect on cancer therapy. We briefly note the proposed involvement of the BCL-2 family in several non-apoptotic processes, which are discussed further in other reviews[26–29]. Finally, we describe how the insights into BCL-2 family structure and function are guiding the development of therapeutics that target BCL-2 family members to improve the treatment of cancer and potentially also certain autoimmune and infectious diseases.

The tripartite BCL-2 apoptotic switch

Proteins of the BCL-2 family have blocks of sequence homology known as BH domains (FIG. 2a). Taking into account both structural and sequence homology, it is now clear that the six human pro-survival family members — BCL-2 itself, BCL-X_L, BCL-W (also known as BCL2L2), MCL1 (myeloid cell leukaemia sequence 1), A1 (also known as BFL1 in humans) and BCL-B (also known as BCL2L10) — as well as the pro-apoptotic effector proteins BAX, BAK and BOK (BCL-2-related ovarian killer protein) share four BH domains[30] and adopt

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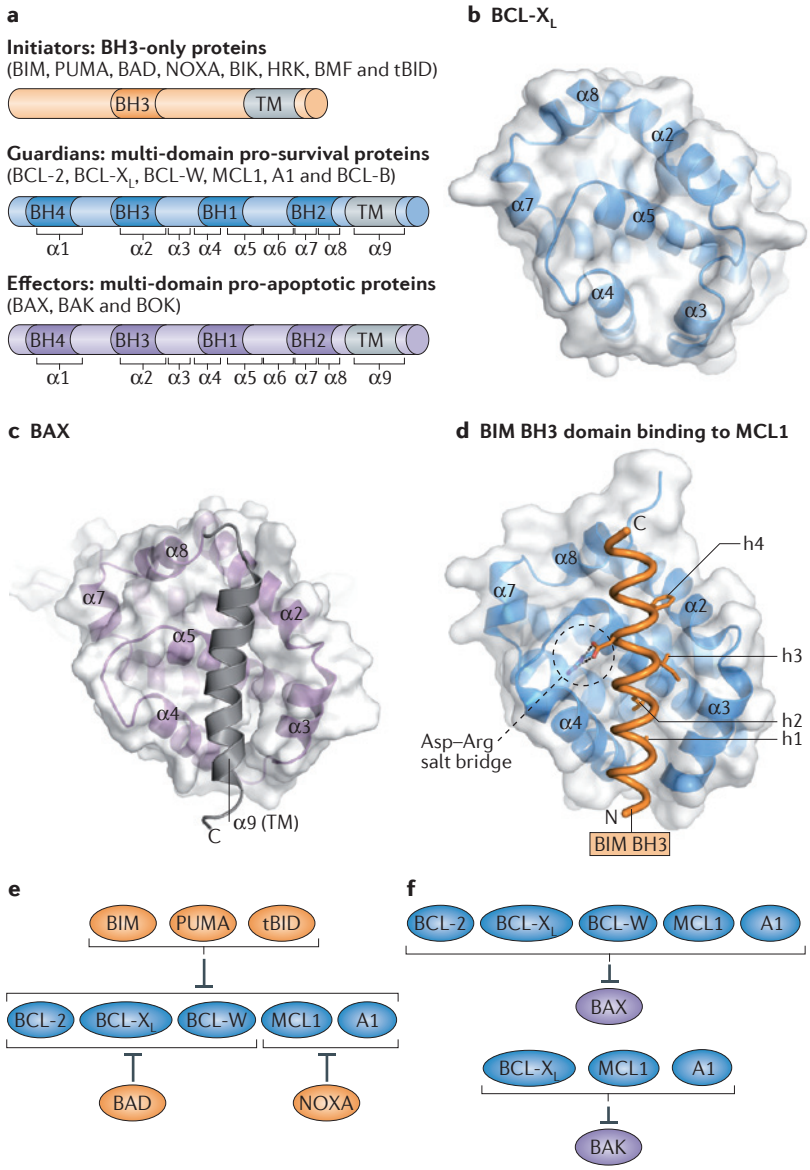

Figure 2 | **The structure of BCL-2 family proteins and the selectivity in their interactions.** BCL-2 family members are related by regions of sequence and structural homology (**a**). When structural as well as sequence homology is considered[30] the multi-domain members of the family (the pro-survival proteins and the effector proteins BAX, BAK and perhaps BOK) share four such BCL-2 homology regions (BH1–BH4), whereas the BH3-only proteins contain only the BH3 amphipathic helix, which mediates their interaction with the groove of multi-domain BCL-2 family members. Most family members also have a transmembrane (TM) domain for anchoring to organelles, most notably the mitochondria. The mouse homologue of human BCL-B, BOO, bears mutations that are thought to render it non-functional[123]. The BCL-2 family structural fold, of seven amphipathic α-helices bundled around a central hydrophobic helix (α5), is shared by the pro-survival members of the family, illustrated here by BCL-X_L (**b**), and by the pre-activated forms of BAX and BAK, represented here by cytosolic BAX, which has its transmembrane domain (α9) sequestered within its surface groove[64] (**c**). The surface groove of multi-domain BCL-2 family proteins mediates interactions with the BH3 domain of pro-apoptotic family members, shown here by the structure of a BIM BH3 peptide bound to MCL1 (**d**)[51]. All BH3 domains insert four hydrophobic residues (h1–h4) into hydrophobic pockets in the surface groove and extend an Asp to a conserved Arg through a salt bridge. Some BH3-only proteins can bind to and neutralize all pro-survival proteins (and vice versa), whereas others (such as BAD or NOXA) bind only a limited subset[39,42,45] (**e**). BAK is inhibited predominantly by BCL-X_L, MCL1 and A1 (REFS 44,59), although BCL-2 can contribute in some contexts[95,216], whereas BAX probably can be inhibited by all of the pro-survival proteins[43] (**f**).

similar globular structures: a helical bundle surrounding a central hydrophobic core helix (α5)[31] (FIG. 2b,c). This fold generates a hydrophobic surface groove delineated by α-helices 2, 3, 4 and 5. This groove constitutes a crucial interface for interactions with the BH3 domain of pro-apoptotic members of the BCL-2 family, such as BIM (BCL-2-interacting mediator of cell death; also known as BCL2L11) (FIG. 2d). These interactions primarily occur on intracellular membranes, particularly the mitochondrial outer membrane, to which many family members are directed by a carboxy-terminal hydrophobic transmembrane domain (FIG. 2a).

Unlike the globular multi-BH domain family members, most BH3-only proteins (FIG. 2a) are intrinsically disordered[32], although their BH3 region becomes an amphipathic helix on interaction with protein partners (FIG. 2d). BID (BH3-interacting domain death agonist), which links the death receptor-mediated apoptotic pathway to the mitochondrial apoptotic pathway to amplify caspase activation[33–35] (FIG. 1), is the structural exception. Its fold resembles that of the multi-BH domain members, with key BH3 residues being buried and thus inactive[36,37]. Cleavage of BID — for example by caspase 8 — generates the active truncated form (tBID), in which the BH3 domain is exposed. It is possible that under certain circumstances intact BID might function in a manner similar to BAX or BAK.

Interactions between BCL-2 family members. BH3-only proteins, which are induced transcriptionally or post-translationally (see below) by cytotoxic stress signals (reviewed in REF. 38), carry out their pro-apoptotic function by two mechanisms[39–41]: neutralization of the pro-survival BCL-2 family proteins[42–44] and direct activation of the pro-apoptotic effectors BAX and BAK[45–48] (BOX 1). Neutralization of the pro-survival proteins has been well characterized, both structurally and functionally, and this information has informed the development of novel therapeutics that target these proteins. The BH3 amphipathic helix of BH3-only proteins binds the hydrophobic groove of pro-survival proteins predominantly by the insertion of four hydrophobic residues (h1–h4) along one face into hydrophobic pockets in the groove, and by the formation of a salt bridge between a conserved BH3 Asp residue and a conserved Arg residue in the BH1 domain of the pro-survival proteins[49–51] (FIG. 2d). However, mutational studies indicate that interactions across the entire interface contribute to binding[52,53]. Owing to subtle differences in their BH3 domains, and in the grooves of the pro-survival proteins, some BH3-only proteins (such as BAD (BCL-2 antagonist of cell death) and NOXA (also known as PMAIP1)) are selective for subsets of their pro-survival relatives, whereas other BH3-only proteins, particularly BIM, tBID and PUMA (p53 upregulated modulator of apoptosis; also known as BBC3), probably neutralize all of the pro-survival proteins[39,42,45] (FIG. 2e). Interestingly, structural studies have shown that the BH3-binding groove of the pro-survival BCL-2 family members has considerable plasticity[54,55], which probably contributes to their ability to associate with multiple distinct BH3 domains (FIG. 2e,f).

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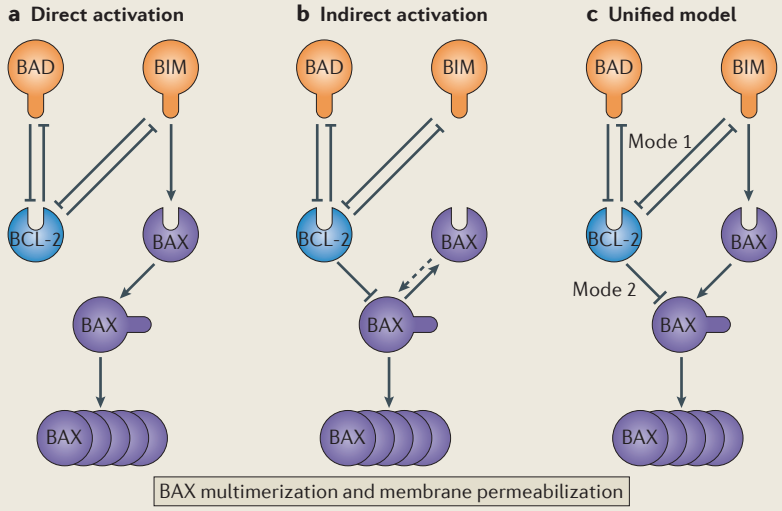
Box 1 | **Models for control of the apoptotic switch**



Three models addressing how interactions between members of the BCL-2 family control the apoptotic switch are described (see the figure). In the direct activation model certain BH3-only proteins, particularly the truncated form of BID (tBID), BIM and perhaps PUMA, directly engage and activate the pro-apoptotic effectors BAX and BAK[39,45–48,62] (see the figure, part **a**). Detection of such complexes has been problematic, probably because the interactions are transient, but the associations were inferred on the basis that BAX could permeabilize liposomes only together with certain BH3 peptides, such as those of BIM and BID[39,45,46,62]. BH3-only proteins that lack this direct 'activator' function, such as BAD, are designated 'sensitizers' to indicate that they exclusively engage pro-survival BCL-2 family members, thereby releasing bound BH3-only activator proteins[47]. Precisely which BH3-only proteins are activators remains debatable.

Whereas the pro-survival proteins, such as BCL-2, primarily sequester BH3-only proteins in the direct activation model, in the indirect activation model[42–44] (see the figure, part **b**), pro-survival proteins must also sequester any BAX or BAK molecule that becomes activated and exposes its BH3 domain[57,58]. In this model, BAX and BAK might be activated in multiple ways, such as by an unknown modification or spontaneously at a low rate. This model emphasizes that BAX and BAK become free to permeabilize the mitochondrial outer membrane only if all of the pro-survival proteins are neutralized by BH3-only proteins or their mimics[42–44].

The current consensus is that both the direct and indirect models apply in many circumstances. Thus, the unified model[41] (see the figure, part **c**) requires that the pro-survival proteins sequester not only BH3-only proteins ('mode 1') but also activated BAX and BAK ('mode 2'). The effect on cell turnover *in vivo* of BIM with altered binding specificities is consistent with this model[40]. The similar 'embedded together' model[84], which is supported by an elegant analysis of the interactions between tBID, BAX and BCL-X_L on a synthetic membrane[209], emphasizes that all of the crucial interactions are influenced by the membrane in which the molecules are embedded[210].

Activator BH3-only proteins
BH3-only proteins, such as BIM and the truncated form of BID (tBID) that can directly bind and activate BAX or BAK.

Sensitizer BH3-only proteins
BH3-only proteins, such as BAD, that can activate BAX or BAK only indirectly by neutralizing pro-survival BCL-2 family members, thereby preventing them from restraining BAX or BAK.

Pro-survival proteins can also bind the BH3 domains of activated BAX and BAK (see below), and thereby restrain their pro-apoptotic activity[56–58]. All of the pro-survival proteins seem to bind BAX, but BAK seems to be restrained mainly by MCL1, A1 and BCL-X_L (REFS 43,44,59) (FIG. 2f). The interfaces in complexes of pro-survival proteins with BAX or BAK BH3 peptides closely resemble those in their complexes with the BH3 peptides of BH3-only proteins[49,60,61]. This suggests a simple competition model for apoptotic regulation, whereby increased BH3-only protein levels (resulting in increased binding of pro-survival proteins) prevent or overcome the restraint of activated BAX or BAK by pro-survival proteins[42–44]. An alternative, but not mutually exclusive, model (BOX 1) suggests that the pro-survival proteins prevent apoptosis by neutralizing the BH3-only proteins that can directly activate BAX and BAK[39,45–47,62]. Indeed, genetic and biochemical evidence supports the idea that both models operate within a tripartite network of interactions between the three subgroups of the BCL-2 family[40,41]. The dominant pathway may vary with biological context, such as non-transformed cells versus tumour cells, or according to the cell death stimulus.

Activation of BAX and BAK. In healthy cells, BAK resides on the mitochondria, with its transmembrane domain (α9) spanning the outer membrane[63]. By contrast, BAX is primarily cytosolic in healthy cells, with its transmembrane domain tucked within its canonical hydrophobic groove[64] (FIG. 2c), but cytotoxic signals promote the accumulation of BAX on the mitochondria[64]. Recent evidence indicates that BAX regularly cycles to the outer mitochondrial membrane in healthy cells but is then translocated back, possibly by interactions with pro-survival proteins such as BCL-X_L (REFS 65,66). Hence, the mitochondrial enrichment of BAX during apoptosis may reflect either the activation of BAX in the cytosol, probably by a BH3-only protein (see below), or simply a shift in this equilibrium, such as less retro-translocation of BAX from the mitochondria.

During apoptosis, BAX and BAK change their structure from inert monomers with folds resembling the pro-survival proteins (FIG. 2b,c) into homo-oligomers of unknown structure that can permeabilize the mitochondrial outer membrane. This transition can be driven by activator BH3-only proteins (BOX 1), such as BIM or tBID, and, experimentally, even by their BH3 peptides. For BAX, two distinct activation sites have been proposed: the canonical hydrophobic groove, which is homologous to that of the pro-survival proteins[67] (FIG. 3a), and an alternative site on the opposite side of BAX[48] (FIG. 3b).

Insights into the activation mechanism recently emerged from studies of a form of BAX truncated to remove its transmembrane domain, BAX (ΔTM)[67]; this form mimics full-length BAX when its tail has inserted into the mitochondrial membrane. Notably, BAX (ΔTM) treated with a BID BH3 peptide (but not with BH3 peptides from sensitizer BH3-only proteins, such as BAD (BOX 1)), together with an ionic detergent, yielded crystals with the BID peptide bound in the groove of BAX (FIG. 3a). Mutagenesis studies based on this structure have defined the characteristics that distinguish 'activator' from 'sensitizer' BH3 domains, and have enabled the design of novel BH3 sequences with activator function[67]. For example, activation by the BID BH3 domain requires two Ile residues near its amino terminus at 'h0', a few residues before the canonical BH3 region h1–h4 (FIG. 3a).

The BAX 'rear' activation site, which involves helices α1 and α6 (FIG. 3b), was proposed on the basis of NMR studies using activator BH3 peptides modified with a hydrocarbon staple and full-length BAX, which has its transmembrane domain located within its canonical hydrophobic groove[48,68]. The rear activation site might be

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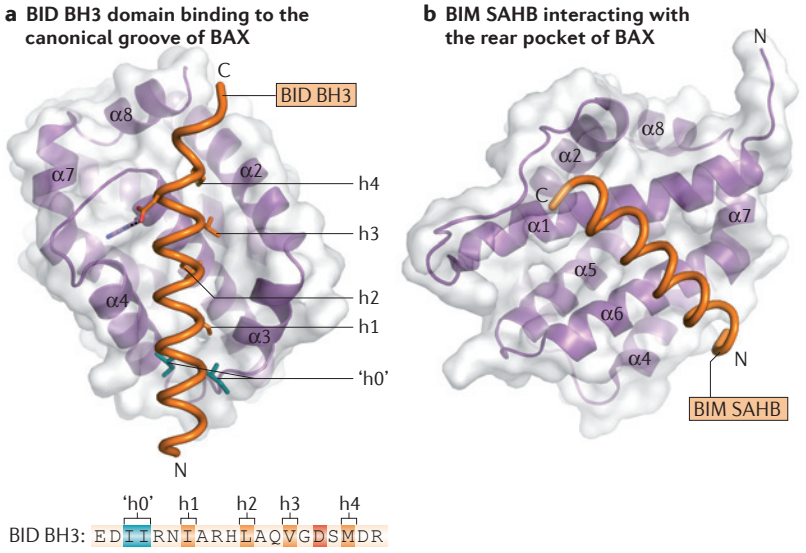

Figure 3 | **Direct activation of BAX by BH3-only proteins.** Two distinct sites on BAX have been proposed to allow certain BH3-only proteins to engage and activate: its canonical hydrophobic surface groove, based on the crystal structure of a BID BH3 peptide bound to BAX[67] (**a**); or an alternative rear site, based on a molecular model calculated from nuclear magnetic resonance data of full-length BAX with a bound BIM stapled peptide[48] (**b**). The BID BH3 peptide contacts the BAX groove (**a**) not only through the h1–h4 residues and the conserved Asp residue that are used to engage pro-survival BCL-2 family members (compare with FIG. 2d) but also, uniquely, through two Ile residues near its amino terminus (denoted 'h0' on the structure), which are required for effective BAX activation by the BID BH3 domain[67]. Mutagenesis of BH3 peptides indicates that the other highlighted residues shown in the BID BH3 sequence might also contribute to BID activator function[67]. The BIM SAHB (stabilized α helix of BCL-2 domains) lies across the α1 and α6 helices of BAX (**b**). Binding to this site is proposed to shift the α1–α2 loop and allosterically displace the BAX transmembrane domain (α9), which lies on the other side of cytosolic BAX[48,62,68].

a trigger for extruding the BAX transmembrane domain from the groove and thereby accelerating the basal shuttling of BAX between the cytosol (inert BAX) and the outer mitochondrial membrane (activated BAX). The groove activation site may then drive subsequent transitions, as some biochemical evidence indicates[62,68,69]. However, the failure of mutations of the rear activation site to abolish apoptosis induction[70,71] indicates that it might not be essential for the activation of BAX.

In the case of BAK, several groups have identified its canonical surface groove as the sole interaction site for activator BH3 domains[69,72–74]. The complex of BAK with a bound stapled BID BH3 domain[74] closely resembles that of BAX with a bound BID BH3 peptide[67].

Conversion of BAX and BAK into killers. After cells receive a cytotoxic stimulus, early structural transitions in BAX and BAK, presumably driven by activator BH3-only proteins (BOX 1), include the exposure of cryptic N-terminal residues[63,75] and transient exposure of their BH3 domain before its insertion into the groove of a neighbouring BAX or BAK protein[74,76–78]. The structure of BAX bound to an activator BH3 domain[67] unexpectedly revealed that this interaction produces a destabilizing cavity inside BAX, near the BAX BH3 domain. This might promote the extrusion of the BH3 domain.

Staple
A hydrocarbon bridge introduced into a peptide that links amino acids four or seven residues apart to maintain the peptide in a helical conformation, which is thought to convey higher affinity for its target, as well as greater stability and perhaps cell penetration.

The structure also showed that BAX rearranges into a 'core' domain consisting of α2–α5 (and possibly α1) and a 'latch' domain comprising α6–α8 (FIG. 4, step 3). These observations are consistent with the transitory exposure of the BAX and BAK BH3 domains during commitment to apoptosis[76,77], and the importance of BAX helices α2–α5 in oligomerization[79]. BH3 peptide binding to BAK also produces N-terminal exposure and oligomerization[73,74,78], but it remains to be determined whether BAK undergoes an equivalent core–latch disengagement.

Crosslinking and biophysical studies have suggested several models of the interfaces within BAX and BAK oligomers[76,77,80–83]. In the best-supported model[76], two activated BAK (or BAX) molecules each insert their BH3 domain into the canonical groove of the other molecule to form a novel 'symmetric' dimer[76,77,81,82]. Crystallographic studies have shown the structure of the BAX core domain in such a BH3-in-groove dimer[67] (FIG. 4, step 4). The presence of symmetric dimers within the BAX and BAK homo-oligomers argues against proposed head-to-tail ('daisy-chain') models of oligomerization[84,85]. Instead, the dimers must join by a second interface to build the oligomers[76,80,86]. Crosslinking studies have identified parallel α6 helices at or near a second interface[77,80], but the second interface and the structure of larger oligomers remain to be defined.

How BAX and BAK oligomers engage and perforate the outer mitochondrial membrane also remains unresolved. Cys-labelling studies led to the proposal that the BAX α5 and α6 helices insert as a hairpin into the mitochondrial outer membrane early in apoptosis[87], but this seems to be incompatible with the BH3-in-groove symmetric dimer structure (FIG. 4), particularly because BAX with α5 pinned to α6 lost apoptotic function[67]. It seems pertinent that the BAX core BH3-in-groove symmetric dimer has a planar surface of α4 and α5 chains exposing multiple aromatic residues[67] (FIG. 4, step 5). If this surface sits on the membrane in the BAX oligomers and inserts these side chains between lipid head groups in the outer membrane leaflet, the resulting excess bulk in that leaflet might increase membrane tension and curvature. This could promote stable bilayer breaks[88], leading to proteolipidic pores (ones not entirely bound by protein)[67]. Recent reports indicate that substantial oligomerization of BAK might precede pore formation[78], and that oligomer mass might determine pore size[89].

Thus, the cardinal interaction in the BCL-2 family is that of the BH3 domain of a pro-apoptotic family member, for example a BH3-only protein, with the surface groove of a multi-domain relative. Whereas the high-affinity binding of a BH3-only protein to a pro-survival protein produces a stable complex, the lower affinity, transient binding of certain BH3-only proteins to the groove of BAX or BAK instead leads to major structural transitions within BAX or BAK (FIG. 4). Importantly, these conformational changes in BAX and BAK include the 'release' of their own BH3 domain, which can then engage the groove of another activated BAX or BAK molecule to form the symmetric dimers that constitute the central element of the oligomers that perforate the outer mitochondrial membrane. This understanding

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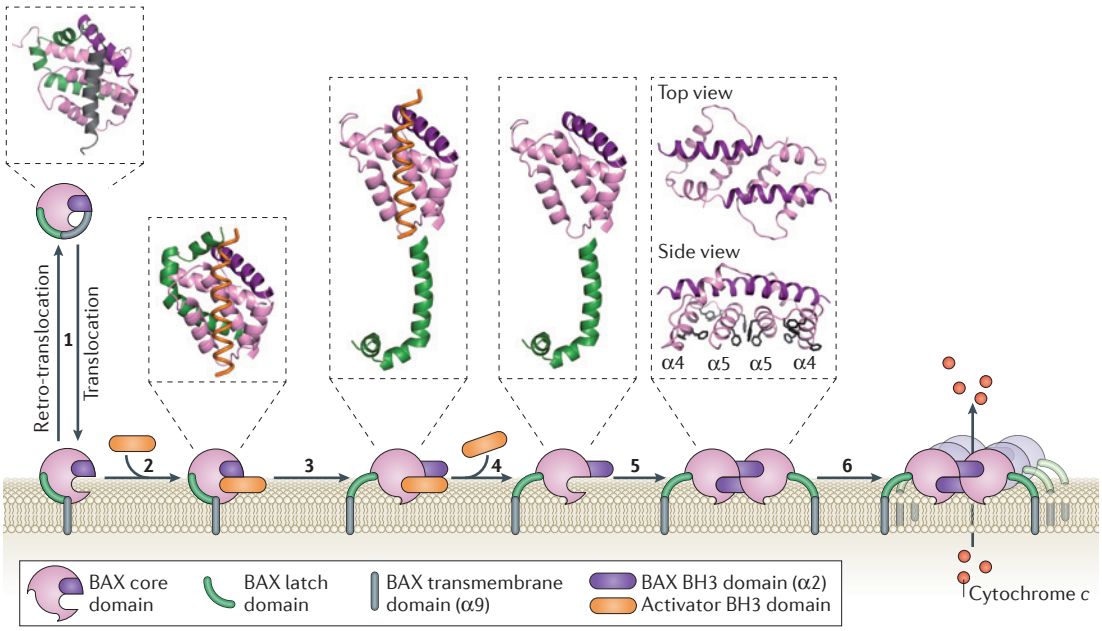

Figure 4 | **Model for the activation and oligomerization of BAX and BAK.** The figure illustrates structural transitions in BAX and BAK proposed from crosslinking studies[76,77,80,82], biophysical measurements[81,83], association of BH3 peptides[67,73,74] and, where indicated, by the BAX three-dimensional structures shown[67]. Step 1 illustrates the proposed shuttling of BAX to the mitochondrial outer membrane and its retro-translocation to the cytosol[65,66]. The movement of BAX to the mitochondrial membrane may be triggered or increased by engagement of the rear BAX site by a BH3-only protein (see FIG. 3b). The transmembrane (TM) domain released from the BAX surface groove can insert across the mitochondrial outer membrane, generating a membrane-bound form of BAX (which resembles native BAK). In step 2, an activator BH3 domain binds to the BAX groove. In step 3, this binding promotes release of the 'latch' domain from the 'core' domain of BAX and destabilizes the BAX BH3 domain, α2 (REF. 67); this step has not yet been documented for BAK. The initiating activator BH3 domain then disengages (step 4). In step 5, protrusion of the BAX BH3 domain allows two such molecules to form the BAX BH3-in-groove dimer[76,77]. The structure of the BAX core (α2–α5) as a symmetrical dimer[67] reveals a hydrophobic layer of two α4 and two α5 chains with 12 protruding aromatic residues (side view). Although it is unclear how the larger oligomers form (step 6), the core α4–α5 surface might engage the outer leaflet of the outer mitochondrial membrane, thereby inducing tension and contributing to membrane permeabilization[67].

of the BH3–groove interaction, together with genetic insights into the physiological roles of BCL-2 family members, has galvanized the search for drugs that target this family, as we discuss later.

Signals regulating the BCL-2 family

Whereas the regulation of BCL-2 family proteins through interactions with other members of the family is becoming well defined (BOX 1), their control by signals from proteins outside the family remains poorly understood. For example, a great many unrelated proteins have been reported to bind to BCL-2 family members, but few such putative interactions have survived more detailed scrutiny. The reported interaction of a cytosolic form of the tumour suppressor protein p53 with several pro-survival BCL-2 family proteins and BAX still draws attention[90]; however, its physiological importance is questionable because genetic data indicate that the ability of p53 to induce *PUMA* and *NOXA* transcription can account for all of its pro-apoptotic function[91]. The intriguing interaction on the endoplasmic reticulum between pro-survival BCL-2 family members and Beclin 1 (BOX 2) seems to inhibit autophagy rather than drive apoptosis, as the Beclin 1 BH3 domain only weakly binds the pro-survival proteins. It remains to be defined how nutrient-deprivation autophagy factor 1 (NAF1) reportedly associates with BCL-2 and augments this interaction[92] (BOX 2).

BCL-2 family proteins are subject to many post-translational modifications, particularly phosphorylation and ubiquitylation, but the biological consequences of these modifications are often controversial, as is the identity of the relevant kinases, phosphatases and ubiquitin ligases[93]. For example, the pro-survival BCL-2 family members are predominantly phosphorylated at several Ser or Thr residues within their flexible α1–α2 loop, but these modifications (often of the same residues) have been variously linked to decreased or increased pro-survival activity, increased or decreased protein stability, a block in mitosis (for example, on taxane treatment), ability to associate with other proteins and subcellular localization[93,94]. Some clarity is provided by recent evidence that the phosphorylation of BCL-2 at Ser70, or at several other loop residues, alters the loop conformation, which greatly increases binding of BCL-2 to BAK and BIM and renders cells more refractory to chemotherapeutic agents[95]. Presumably, different loop conformations affect the association of BCL-2 with other proteins.

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Box 2 | **Proposed non-apoptotic roles for BCL-2 proteins**

Mitochondrial fission and fusion

The mitochondrial network fragments early in apoptosis, potentially linking apoptosis to mitochondrial fission and fusion (reviewed in REF. 26). However, the link remains controversial; for example, ablation of BAX and BAK only modestly decreases mitochondrial fusion, and fission is unaffected[211]. One proposed link is dynamin-related protein 1 (DRP1), a soluble factor that is recruited to mitochondria to promote fission, but DRP1 does not seem to be required for mitochondrial membrane permeabilization; and permeabilization is not required for fragmentation[26]. Although no compelling molecular link has emerged, BAX and BAK may preferentially permeabilize on the outer mitochondrial membrane where fission and/or fusion initiate[26].

Autophagy

During autophagy, which is an ancient process to mobilize metabolites and energy for cell survival under adverse conditions, cellular components are packaged within membranes and digested within lysosomes. Beclin 1, an essential inducer of autophagy, has a region that is thought to resemble a BH3 domain, through which it was reported to engage pro-survival BCL-2 proteins, although apparently only on the endoplasmic reticulum (ER), and these complexes seem to inhibit autophagy (reviewed in REF. 28). BCL-2 reportedly may be recruited to the ER by NAF1 (nutrient-deprivation autophagy factor 1), which augments its sequestration of Beclin 1 (REF. 92). However, although NAF1 itself has no BH3 domain, the binding of BH3-only proteins to BCL-2 can release NAF1 and free Beclin 1 to induce autophagy. Beclin 1 can also be freed by phosphorylation of the Beclin 1 BH3 domain, or of three sites in the BCL-2 flexible α1–α2 loop. The loop phosphorylation might be crucial[94] because BCL-2 with those sites mutated *in situ* prevented autophagy induction in response to nutrient starvation[212].

Mitochondrial function

Although one MCL1 isoform resides as expected on the mitochondrial outer membrane, a truncated isoform resides inside the mitochondrial matrix. Notably, cells engineered to express only the apoptosis-regulating outer membrane isoform of MCL1 were reported to have abnormal mitochondrial morphology, less mitochondrial DNA and decreased respiration and ATP production[29,99]. Conditional MCL1 deletion in cardiomyocytes induced similar mitochondrial defects[119], but their basis remains unknown. BCL-X_L has also been implicated in entering mitochondria and affecting their function[213].

MCL1 is subject to tight regulation in terms of its transcription, translation and protein turnover (it has a half-life of <30 minutes)[29,96]. MCL1 can be degraded by a ubiquitin-independent pathway or targeted to the proteasome by several ubiquitin ligases (such as MULE (also known as HUWEI), β-transducin repeat-containing protein (βTrCP) and F-box and WD40 domain-containing 7 (FBW7; also known as FBXW7)) and conversely is stabilized by the deubiquitylase USP9X[29,96–98]. The N-terminal region of MCL1 also affects its stability; excision of an N-terminal segment generates an isoform of MCL1 that is found in the mitochondrial matrix and has been implicated in mitochondrial structure and function[29,99] (BOX 2).

The BH3-only proteins are regulated in diverse ways[93,100]. The transcription of *PUMA* and *NOXA* is regulated by p53, but the transcription of both can also be upregulated in a p53-independent manner. The forkhead box O transcription factor FOXO3A is implicated in the induction of *BIM* and *PUMA* transcription, but recent *in vivo* studies question its physiological relevance for *BIM* transcription[101]. Post-translational modifications of the BH3-only proteins are common. Several proteases, most notably caspase 8, process BID into the active tBID (FIG. 1). BAD is phosphorylated and sequestered by 14-3-3 proteins in the cytosol, but the biological effects of the phosphorylation of most BH3-only proteins are controversial[93,100]. For example, the most abundant BIM isoform, extra-long BIM (BIM_{EL}), is thought to be phosphorylated by ERK1 and ERK2 (at Ser65 in mouse BIM) and thereby targeted for proteasomal degradation; however, removal of the ERK docking site by *in situ* modification of the *Bim* locus in mice did not have any effect on cell populations in which BIM has crucial roles[102].

Collectively, these results indicate that a definitive understanding of how upstream signals impinge on the BCL-2 family may require physiological studies in normal cell populations, after *in situ* gene modification in mice.

The BCL-2 family in normal physiology

Studies using transgenic and gene-targeted mice have clarified the functions of many BCL-2 family members in both normal physiology and diverse pathological settings. Collectively, they leave little doubt that these proteins control the survival of all mammalian cells. Some biochemical findings have led to proposals that family members also participate in non-apoptotic processes, including mitochondrial fission and fusion, autophagy and mitochondrial function (BOX 2) (as discussed further in other reviews[26–29]), but physiological evidence for these links remains limited.

The pro-survival proteins. When they are overexpressed in transgenic mice, all of the pro-survival BCL-2 family members render many cell types resistant to diverse apoptotic stimuli[10,12,103]. Conversely, constitutive or conditional gene deletion has identified crucial roles for specific family members in distinct cell populations, presumably those in which the remaining expressed pro-survival BCL-2 family members cannot maintain the apoptotic threshold. BCL-2 ablation causes fatal polycystic kidney disease owing to the death of renal epithelial progenitor cells, and it depletes mature lymphoid cells and melanocyte progenitors[104]. Remarkably, concomitant loss of the BH3-only protein BIM rescues all of these defects[105]. BCL-X_L-deficient mice die near day 14 of embryogenesis owing to defective fetal erythropoiesis and neuronal attrition[106]. Moreover, loss of even one *Bcl2l1* allele (which encodes BCL-X_L) impairs spermatogenesis[107] and reduces platelet numbers[108], whereas conditional loss of both alleles kills hepatocytes, causing liver fibrosis[109]. Concomitant BIM loss rescues the testicular and erythroid defects[110], but not the excess neuronal death, which might be driven by other BH3-only proteins. BCL-W-deficient mice have only a spermatogenic defect[111,112], which probably reflects the death of supporting Sertoli cells[113].

MCL1 ablation has the greatest physiological effect. Its constitutive loss precludes implantation, although it is unknown whether this results from excess apoptosis[114]. Conditional MCL1 deletion has revealed its crucial role in the survival of diverse cell types, including haematopoietic stem and progenitor cells[115,116], as well as effector lymphocytes[117,118]. Remarkably, conditional deletion of MCL1 in cardiomyocytes rapidly provokes cardiomyopathy and heart failure[119,120]. These defects can be attributed to cardiomyocyte apoptosis (and secondary necrosis) because concomitant deletion of the pro-apoptotic effectors BAX and BAK precluded them;

Mitochondrial fission and fusion
Mitochondria divide by fission but also continually fuse into tubular networks; thus, their structure is dynamic.

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however, certain mitochondrial defects remained[119], which indicates that MCL1 might have an additional, non-cell death-related mitochondrial role (BOX 2).

The functions of A1 remain ill-defined owing to the difficulty of targeting the three functional and chromosomally closely linked A1 genes in mice[121]. Nevertheless, loss of the *A1a* gene impaired granulocyte and mast cell survival *ex vivo*[121], and transgenic mice expressing a short hairpin RNA targeting all expressed A1 genes also had a lymphocyte deficit[122]. Mouse knockout studies cannot be used to clarify the roles of human BCL-B, because its mouse homologue (BOO; also known as DIVA and BCL2L10) has inactivating mutations that are not present in BCL-B[123].

The BH3-only proteins. Overexpressed BH3-only proteins, particularly those (BIM, tBID and PUMA) that target all pro-survival BCL-2 family members (FIG. 2e), can trigger apoptosis (for example, see REF. 42). Gene disruption has defined their essential individual roles and some overlapping functions (reviewed in detail in REFS 24,124). In general, loss of the BH3-only proteins that bind all pro-survival proteins and/or directly activate BAX and BAK provokes major abnormalities, whereas ablation of individual more selective binders (such as BAD and BIK (BCL-2-interacting killer)) has only a limited effect. Thus, ablation of BIM, which is activated by cytokine withdrawal, dysregulated calcium flux and certain other apoptotic stimuli, results in excess lymphoid and myeloid cells, showing its role in normal haematopoietic homeostasis[125], as well as in the deletion of autoreactive lymphocytes[126,127] and shutdown of immune responses after clearance of an infection[128–131]. As BID is activated by the death receptor pathway (FIG. 1), BID-deficient mice are spared the lethal hepatitis that is normally induced by activation of FAS (also known as CD95 and TNFRSF6)[33,34] or TNFR1 (REF. 132).

PUMA and *NOXA* are direct transcriptional targets of the tumour suppressor protein p53 (REFS 133,134). Accordingly, PUMA is crucial for the DNA damage-induced apoptosis of diverse cell types, particularly lymphocytes[135,136]; NOXA assists in the lymphocyte attrition[135,137] and has the greater role in the death of fibroblasts and keratinocytes induced by ultraviolet radiation[138]. Notably, PUMA and NOXA seem to account for all of the p53-induced pro-apoptotic activity, because their combined loss renders lymphocytes as resistant to the apoptosis that is induced by γ-irradiation as does p53 loss[91]. PUMA and NOXA also drive the death of primordial follicle oocytes following DNA damage, a process that is mediated by the p53 relative p63 (REF. 139). PUMA also mediates the effects of several p53-independent pro-apoptotic stimuli, including cytokine deprivation and treatment with glucocorticoids or phorbol ester[135,136,140].

Following the loss of BMF (BCL-2-modifying factor), B cells accumulate and are resistant to the effects of glucocorticoids and certain other pro-apoptotic stimuli[141]. Although BIK deficiency leads to no overt abnormalities[142], concomitant loss of BIM renders males infertile[143] because normal spermatogenesis requires the apoptosis of excess primordial progenitors to adjust their numbers to those of their supporting Sertoli cells. BAD loss reportedly rendered fibroblasts partially resistant to the pro-apoptotic effects of glucose or cytokine deprivation, but BAD-deficient mice seem to be generally normal[144], except for a small increase in the number of platelets[145,146]. Activator of apoptosis Harakiri (HRK) seems to be expressed only in neurons, but HRK-deficient neurons have only minor apoptotic defects[147,148].

Consistent with the ability of BIM and PUMA to neutralize multiple pro-survival relatives (FIG. 3a) and/or activate BAX and BAK, mice lacking both BIM and PUMA have exacerbated apoptotic defects[149], and animals also lacking BID may have even more severe abnormalities[150].

The BAX-like proteins. Mice lacking BAK seem normal[151] but have excess platelets as a result of increased platelet lifespan[108]. *Bax*$^{-/-}$ mice also seem largely normal, although the males are sterile owing to the survival of excess primordial germ cells, which impairs subsequent spermatogenesis (see above). Mice that are deficient in both BAX and BAK, however, have serious abnormalities: most die perinatally (for unknown reasons), and the few long-term survivors all develop lymphadenopathy and fatal systemic autoimmune disease[151,152]. All *Bax*$^{-/-}$*Bak*$^{-/-}$ mice have webbed feet and many females have imperforate vaginas, which are classic examples of defects in developmentally programmed cell death[1,6]. Diverse cell types from *Bax*$^{-/-}$*Bak*$^{-/-}$ mice are completely resistant to multiple apoptotic stimuli[151], including enforced expression of BH3-only proteins[153,154]. Thus, the BH3-only proteins function upstream of BAX and BAK (FIG. 1), and cannot cause cell death unless BAX or BAK is present.

Although BAX and BAK have essential and mostly overlapping roles in apoptosis, several tissues that are thought to be shaped by apoptosis (such as luminal structures) seem to be normal in *Bax*$^{-/-}$*Bak*$^{-/-}$ mice. Does their close relative BOK contribute to apoptosis in these tissues? Although mice lacking BOK[155], and even those lacking BOK and either BAX or BAK[156], are largely normal, the increased numbers of primordial follicle oocytes in aged female mice deficient for both BOK and BAX provides the first physiological indication that BOK has a pro-apoptotic function[156]. Analysis of mice lacking BAX, BAK and BOK will be required to determine the overall contribution of the BCL-2-regulated apoptotic pathway to morphogenesis; however, cell removal during development might also involve the death receptor-mediated pathway, non-apoptotic cell death mechanisms (such as necroptosis) or even non-death-related processes[6].

Taken together, the results of genetic manipulation of the BCL-2 family support the concepts that all mammalian cells are poised to undergo cell death; that their survival depends on one or more pro-survival family members, with BCL-X_L or MCL1 often being crucial; that BAX or BAK is an essential effector of apoptosis; that particular BH3-only proteins drive the responses to specific cytotoxic insults and developmental cues, with BIM and PUMA often being essential; and that tissue homeostasis is primarily determined by the balance between the levels of BH3-only proteins and their pro-survival relatives.

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Pathological roles of the BCL-2 family

Defects in the mitochondrial apoptotic pathway are strongly implicated in the development of several diseases, particularly cancer and autoimmunity. Overexpression of pro-survival BCL-2 causes a low incidence of lymphoma in transgenic mice[157,158] and accelerates tumorigenesis driven by the dysregulated expression of *MYC* and certain other oncogenes[9,159]. Loss of some of the pro-apoptotic BCL-2 family members, particularly BIM[160], PUMA[161,162], BAD[163], BMF[163] and BAX[164], also accelerates tumorigenesis that is driven by such oncogenic lesions. Blocks to apoptosis presumably promote tumorigenesis by keeping cells alive long enough to acquire oncogenic mutations that drive their neoplastic progression, as well as by countering the apoptosis that oncogenes such as *MYC* drive under certain conditions of cellular stress.

In humans, BCL-2 is overexpressed as a result of a t(14;18) chromosomal translocation in ~90% of follicular centre B cell lymphomas[165]; *MCL1* or *BCLX* is amplified in diverse tumours[166]; both *BIM* alleles are deleted in 17% of mantle cell B lymphomas[167]; and *BIM* or *PUMA* expression is decreased in diverse malignancies owing to promoter hypermethylation or other causes[161,168].

One important checkpoint for preventing autoimmune disease is the apoptosis of autoreactive B cells and T cells[169]. Accordingly, BCL-2 overexpression in B cells of mice[11], loss of BIM[125] or loss of both BAX and BAK[152,170] can provoke a fatal autoimmune kidney disease that resembles human systemic lupus erythematosus.

Impact on therapy. Defects in the mitochondrial apoptotic pathway also contribute to the resistance of diverse tumours to cytotoxic drugs. For example, the expression of PUMA, NOXA and, surprisingly given that it is not a p53 target gene, BIM determines the response of lymphoma cells to DNA damage-inducing drugs[171]. BIM expression is also required for the killing of tumour cells by glucocorticoids[140,172], paclitaxel[173], inhibitors of oncogenic kinases (such as imatinib for inhibition of breakpoint cluster region protein (BCR)–Abelson (ABL)[174], and erlotinib (Tarceva; Roche) or gefitinib (Iressa; AstraZeneca) for inhibition of the epidermal growth factor receptor (EGFR)[175,176]) or shutdown of the mutant B-RAF signalling pathway[177]. Accordingly, a human *BIM* splice site polymorphism causes resistance to imatinib in chronic myeloid leukaemia and to erlotinib or gefitinib in lung cancers with EGFR mutations[178]. Thus, novel strategies to efficiently activate endogenous BH3-only proteins, or to introduce small molecules mimicking their function (see below), might improve the treatment of diverse types of cancer.

BCL-2 proteins as therapeutic targets

Targeting the regulation or function of pro-survival BCL-2 family members has considerable appeal. Although cancer therapeutics can induce several types of cell death, activation of the BCL-2-regulated apoptotic pathway seems to be crucial for the therapeutic efficacy of most (perhaps all) conventional cytotoxic agents and inhibitors of oncogenic kinases. This process is, however, often blunted in tumours. For example, p53 mutation, which is prevalent in diverse types of tumour, impairs the induction of PUMA and NOXA in response to DNA damage-inducing chemotherapeutics, and many lymphoid malignancies have increased levels of BCL-2 (see above). Somewhat paradoxically, however, most (if not all) tumours seem to be more 'primed to die' than their normal counterparts, probably owing to increased levels of BH3-only proteins[25,45,179]. For example, lymphoid cells expressing high levels of pro-survival BCL-2 accumulate increased levels of pro-apoptotic BIM[179,180], probably because the excess BCL-2 keeps stressed cells that induce BIM alive. Notably, the sensitivity of mitochondria from tumour cells to disruption by a BIM BH3 peptide correlates well with the success of conventional therapies[181,182]. Thus, many types of tumour cell should be vulnerable to novel therapies that directly turn on apoptosis.

Such considerations have galvanized the search for compounds (BH3 mimetics) that induce apoptosis by mimicking the function of the BH3 domain. However, the long, shallow and mainly hydrophobic groove of the pro-survival BCL-2 proteins — to which the BH3 domain binds (FIG. 2) — is very challenging to target. Although numerous compounds reportedly bind various BCL-2 family members, most have only moderate affinity and nearly all seem to kill cells predominantly by off-target effects that do not require BAX or BAK[183,184].

BH3 mimetic potential and targets. The potential of BH3 mimetics in cancer therapy has been amply demonstrated by ABT-737 (REF. 185) and its orally available clinical derivative ABT-263 (now called navitoclax)[186], which both bind strongly to BCL-2, BCL-X_L and BCL-W, but not markedly to MCL1 or A1. Accordingly, these agents have little activity against tumour cells with high levels of MCL1 or A1 (REF. 183). Surprisingly, their crucial intracellular target in lymphoid cells with high levels of BCL-2 is not unoccupied BCL-2 but BIM–BCL-2 complexes[179,180]. Furthermore, these BH3 mimetics disrupt BIM–BCL-2 complexes much more efficiently than BIM–BCL-X_L or BIM–BCL-W complexes, which explains why high BCL-2 levels promote sensitivity to ABT-737, whereas increased levels of BCL-X_L, similarly to increased levels of MCL1 or A1, instead confer resistance[180]. To kill lymphoid cells, the BIM that is released by binding of ABT-737 to BCL-2 must apparently neutralize pro-survival MCL1 in these cells[180] and might also directly activate BAX or BAK[179]. The identification of BIM–BCL-2 complexes as the crucial target of navitoclax in lymphoid malignancies indicates that effective BH3 mimetics may require affinities for BCL-2 comparable to those of its natural ligands, such as BIM (low nanomolar or even sub-nanomolar affinity), in order to efficiently displace these ligands from BCL-2 and trigger apoptosis.

Early clinical trials of navitoclax as a single agent, which have focused on blood cell cancers or small cell lung cancer, have shown promise, particularly in patients with chronic lymphocytic leukaemia[187]. Moreover, recent preclinical studies indicate that combining navitoclax with other anticancer drugs, either conventional agents[188,189] or targeted therapies[190],

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can increase efficacy, including in the treatment of diverse solid tumours. Many conventional agents probably work in synergy with navitoclax by decreasing the level of MCL1, which has a high rate of turnover at both the mRNA and protein levels[191]. Combination of navitoclax with agents that specifically target tumour cells, such as inhibitors of oncogenic kinases, should have particular promise[190].

The dose-limiting toxicity with navitoclax is a transient acute drop in the number of platelets[187] owing to its inhibition of BCL-X_L, which controls platelet lifespan[108]. A BH3 mimetic that targets only BCL-2 should prevent this problem. Indeed, the recently developed ABT-199 (REF. 192), which is selective for BCL-2, seems, from preclinical studies and the experience of the first few treated patients with chronic lymphocytic leukaemia, to be at least as potent as navitoclax but without decreasing platelet levels[192–194]. These results indicate that BH3 mimetics targeting single pro-survival proteins might be particularly useful. Thus, the recently described WEHI-539 (REF. 195), which is highly specific for BCL-X_L, will help to define the roles of BCL-X_L in normal physiology, as well as in cancer and possibly other diseases. Pertinently, in many solid tumours, BCL-X_L is thought to have a more important pro-survival role than BCL-2.

The development of MCL1 or A1 inhibitors has progressed more slowly, perhaps because their grooves are more rigid than those of BCL-X_L or BCL-2 (REF. 51); so far, only moderately potent MCL1 inhibitors have been reported (for example, see REF. 196). The importance of MCL1 in the development, sustained growth and therapeutic resistance of diverse cancers[166,197], including acute myeloid leukaemia[191,198] and melanoma[166,197,199], makes it an appealing target. Moreover, MCL1 and A1 induce resistance to the BH3 mimetics ABT-263, ABT-737, ABT-199 and WEHI-539 (REFS 179,183,192,195). However, the crucial role of MCL1 in the maintenance of certain non-transformed cells, for example cardiomyocytes (see above), indicates that targeting it might produce severe side effects. Nevertheless, acute myeloid leukaemia cells are more sensitive to the loss of MCL1 than are normal haematopoietic stem or progenitor cells[198], and mice lacking one *Mcl1* allele (mimicking 50% inhibition) seem to be normal[114]; thus, it may be possible to establish an adequate therapeutic window for MCL1-specific BH3 mimetics.

Structural data for compounds targeting BCL-2, BCL-X_L or MCL1 highlight commonalities in their binding mode (FIG. 5). All are anchored in the canonical groove within the P2 hydrophobic pocket, and ABT-737, ABT-263, ABT-199 and WEHI-539 increase binding affinity by extensions into the P4 pocket. Intriguingly, the selectivity of ABT-199 for BCL-2 and of WEHI-539 for BCL-X_L seems to reflect increased polar interactions with their targets (FIG. 5b,c). The P2 pocket seems to be crucial for binding all of these ligands because of its plasticity: to accommodate the BH3 mimetics, the P2 pocket morphs into unique deeper cavities that are not present in the complexes with endogenous ligands. Hence, the plasticity in the pro-survival grooves can be exploited to develop potent and specific ligands.

Alternative therapeutic approaches. The challenges of developing high-affinity small organic BH3 mimetics have prompted initial studies on modified BH3 peptides as potential drugs[200,201]. A stapled BIM BH3 peptide killed cultured leukaemia cells and inhibited the growth of a leukaemia xenograft *in vivo*[201]. In addition, a stapled MCL1 BH3 peptide bound MCL1, preventing it from capturing BAK, and thereby sensitized cancer cells to apoptosis[202]. The therapeutic window for a BIM BH3 peptide, which should eliminate all pro-survival restraints (FIG. 2e) and activate BAX, remains to be determined.

Because MCL1 is regulated in multiple ways and turns over rapidly (see above), targeting its regulation may prove easier than developing an effective BH3 mimetic[191]. Indeed, several conventional chemotherapeutics, including anti-tubulin drugs[98], decrease the level of MCL1, and MCL1 levels are increased in certain tumours by upregulation of the deubiquitylase USP9X, which could be targeted[97]. Drugs that upregulate the MCL1 antagonist NOXA (FIG. 2e) might also have promise.

Applications in autoimmune and infectious diseases. The major role of BCL-2 in the maintenance of lymphocytes indicates that ABT-737, ABT-263 and ABT-199 might be used to treat autoimmune diseases. Indeed, ABT-737 has substantial efficacy in several animal models of autoimmune disease[203,204].

To preclude elimination by host cell apoptosis, many viruses have evolved homologues of pro-survival BCL-2 proteins that often differ markedly in sequence from their host counterparts[3]. Hence, the development of BH3 mimetics specific for viral pro-survival proteins might decrease virus spread within the body. Certain parasites, such as schistosoma, also have distant BCL-2 pro-survival homologues that could be targeted[205].

Figure 5 | **BH3 mimetics binding to the hydrophobic groove of their pro-survival BCL-2 family target proteins.** **a** | ABT-737 bound to BCL-X_L is shown. ABT-737 occupies hydrophobic pockets P2 and P4 and makes a charge interaction with BCL-X_L Glu96 (REF. 217). **b** | A closely related analogue of ABT-199 in complex with BCL-2 (REF. 192). ABT-199 also occupies P2 and P4. The hydrogen bond between the aza-indole moiety and BCL-2 Asp103 confers selectivity for BCL-2 over BCL-X_L. In ABT-199, in which X is nitrogen, contact of Arg107 of BCL-2 with the nitrogen of ABT-199 increases affinity[192]. **c** | BCL-X_L-specific WEHI-539 (REF. 195) bound to BCL-X_L. WEHI-539 also occupies P2 and P4 of BCL-X_L and makes charge interactions with BCL-X_L Glu96 and the conserved Arg139. Two hydrogen bonds may confer selectivity for BCL-X_L over BCL-2 and BCL-W: one between the benzothiazole nitrogen of WEHI-539 and the backbone NH of BCL-X_L Leu108; and one between the hydrazone NH of WEHI-539 and the backbone carbonyl of BCL-X_L Ser106 (REF. 195). **d** | A moderate affinity MCL1 inhibitor[196] bound to MCL1. It occupies only P2 and has a charge interaction with Arg263 of MCL1, which is equivalent to BCL-X_L Arg139. The BCL-2 family proteins are represented as surface (grey, red, blue and yellow represent carbon, oxygen, nitrogen and sulphur atoms, respectively) with their ligands in stick representation (magenta, red, blue and yellow represent carbon, oxygen, nitrogen and sulphur atoms, respectively).

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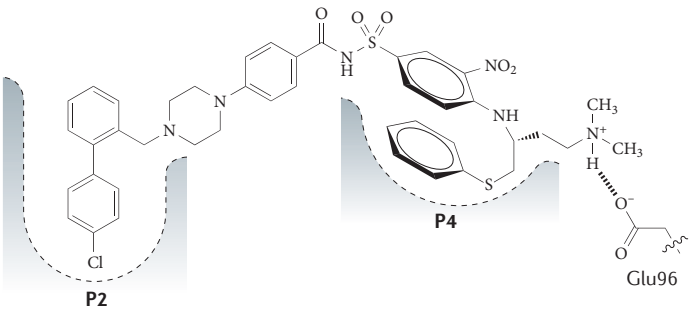
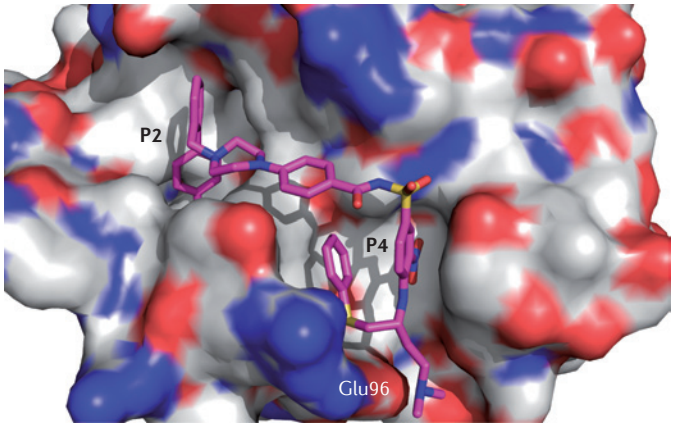
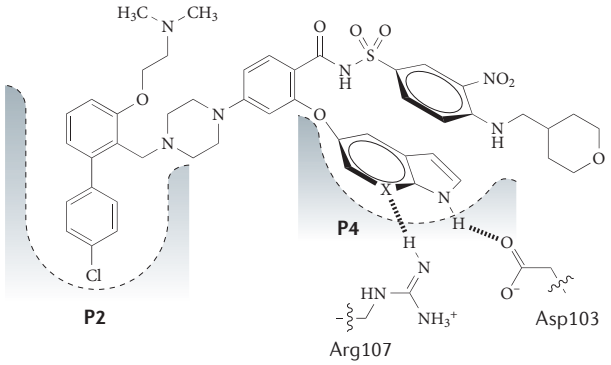
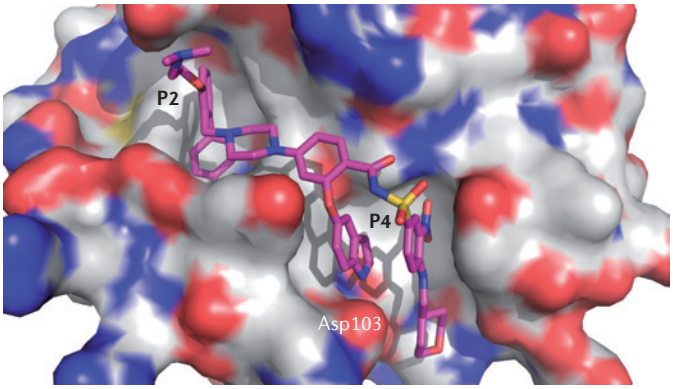
a ABT-737 bound to BCL-X_L



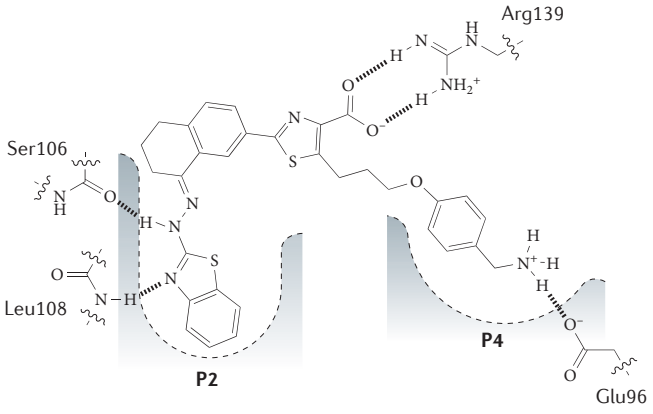


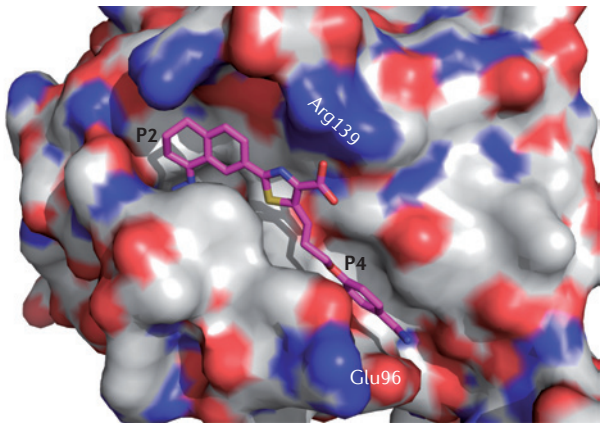
b BCL-2-selective compound bound to BCL-2



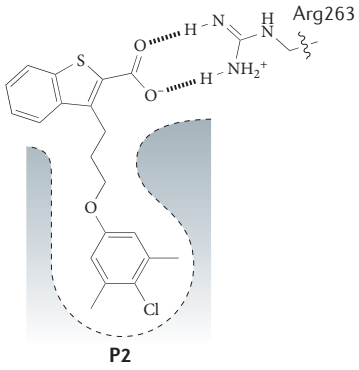


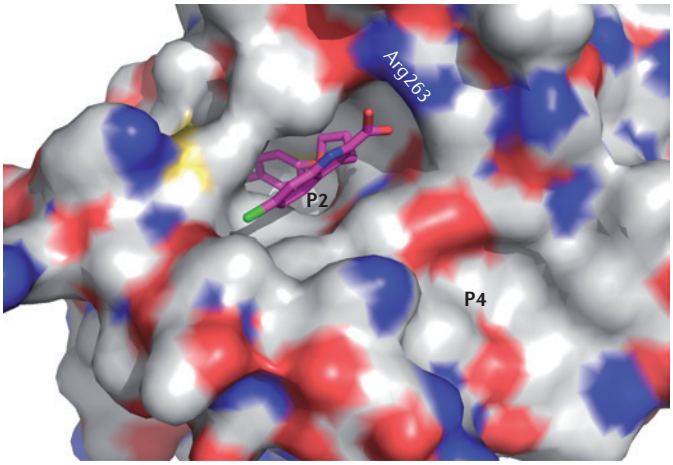
c WEHI-539 bound to BCL-X_L





d MCL1-selective compound bound to MCL1





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BAX and BAK as therapeutic targets. The recent progress in clarifying the structural transitions that drive BAX and BAK activation (FIGS 3,4) should facilitate the development of novel compounds that directly regulate their activity: either promoting their activation for cancer treatment, as reported for BAX[206], or inhibiting it for disorders involving pathologically increased apoptosis. Target sites might include their groove, the 'rear' site in BAX, the region near their BH3 domain to facilitate (or block) its release and the α5–α6 hairpin in BAX to promote or inhibit core–latch disengagement. As increased cell death accompanies ischaemic conditions, such as stroke, cardiac infarction and traumatic brain injury, and inhibition of apoptosis (for example, by BCL-2 overexpression) decreases the pathology[207], drugs that inhibit BAX and/or BAK activation could potentially moderate the devastating consequences. Chronic neurodegenerative disorders, such as Alzheimer's disease, also involve apoptosis, but whether the mitochondrial pathway has a major role is less clear[207].

Perspectives

Our understanding of how the three BCL-2 subfamilies regulate the apoptotic switch has advanced considerably in recent years, but major unresolved issues include the membrane topology of family members during apoptosis, how the membrane affects their association, the structure of BAX and BAK homo-oligomers and the nature and structure of the apoptotic pores that they generate. We need better techniques for determining membrane protein structures and better imaging technologies to monitor associations in living cells, both between family members and with the membrane[208]. To provide more quantitative insights, we need to establish the abundance of family members within certain cells and equilibrium constants for all of their interactions.

Increased understanding of the molecular mechanisms will guide further advances in therapeutically targeting the BCL-2 family. The potential for improved cancer therapy seems to be high, particularly for lymphoid malignancies. Further advances in BH3 mimetic therapy are likely to emerge with agents targeting other pro-survival proteins (such as MCL1 and A1), new ways to assess drug–target engagement inside cells, identification of the most vulnerable types of tumour for each BH3 mimetic and, of course, many tests of combination therapies[190]. The prospect of developing agents that directly activate or inhibit BAX or BAK is also exciting; BAX or BAK inhibitors might open the way to better management of ischaemic conditions and degenerative disorders. We predict that exploiting insights into the regulation of apoptosis for the betterment of health is currently only at its outset.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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