Chemistry & Biology, Vol. 9, 1059–1072, October, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00247-8

The Chemical Biology of Apoptosis: Exploring Protein-Protein Interactions and the Life and Death of Cells with Small Molecules

Review

Ziwei Huang[1]
Departments of Biochemistry and Chemistry
University of Illinois at Urbana-Champaign
Urbana, Illinois 61801

Apoptosis, a fundamental process for both human health and disease, is initiated and regulated by protein-protein interactions, notable examples of which are the interactions involving Bcl-2 and IAP protein families. This article discusses recent advances in the use of chemical approaches in discovering and studying small molecules targeted to proteins of the Bcl-2 and IAP families. These small molecules and their complexes with receptors provide the tools and model systems to probe the basic mechanism of molecule recognition underling the life and death of cells and develop novel strategies for therapeutic intervention of the dysregulated apoptotic process. The review of these studies highlights the opportunity and challenge in this emerging area of chemical and chemical biological research.

Introduction

Apoptosis or programmed cell death is the prevalent mechanism complementary to proliferation that is critical for the normal development and function of multicellular organisms [1]. As approximately 10^{11}–10^{12} cells are produced every day in healthy adult humans, this rapid proliferation needs to be balanced by apoptosis to maintain a constant cell number. Changing this balance in either direction has pathological consequences. Abnormally high rate of cell death is found in neurodegenerative diseases including Alzheimer's and Parkinson's diseases, AIDS and cardiovascular diseases, whereas retarded cell death contributes to a wide variety of human cancers [2].

There are two major pathways for apoptosis that have been elucidated so far, both of which involve a family of cysteine proteases with aspartate specificity, called caspases, as the executioners of apoptosis [3, 4]. The mitochondria provide one of the apoptotic pathways from which cytochrome c is released upon the stimulation by a variety of cell-death triggers [5]. This leads to activation of Apaf-1 (apoptosis protease activating factor-1), caspase-9, and caspase-3. Another apoptotic pathway involves the ligation of death receptors such as Fas and activation of caspase-8 and subsequently caspase-3 [6] (Figure 1).

Protein-protein interactions are important for a wide variety of physiological as well as pathological processes. The significance of protein-protein interactions has never been more apparent than in the post-genome era, in which the functional characterization of every protein encoded by the human genome will require understanding of its molecular interactions with others. The inhibition or promotion of these interactions either by natural molecules or by unnatural synthetic ones is of great interest for understanding the mechanism of biological recognition and translating the information of gene sequence to the discovery of new therapeutic agents. Much progress has been made recently in developing various strategies to manipulate protein-protein interactions with small molecules [7, 8].

In apoptosis, protein-protein interactions are the underlying theme in both mitochondria and death receptor pathways (Figure 1). Obviously, life and death are matters that cannot be taken lightly. Thus, nature has evolved a sophisticated and tightly controlled network of protein-protein interactions to ensure the accuracy and check-and-balance of the cell-death machinery. The activation of caspases, which are central for apoptosis, depends on various protein-protein interactions. For example, caspase-8 is activated through a cascade of sequential oligomerization of death receptors, the adaptor molecules FADD (Fas-associated protein with death domain), and procaspase-8, whereas caspase-9 is activated through the association of at least cytochrome c, Apaf-1, and procaspase-9 to form apoptosome. The action of these "killer" enzymes before or after activation is controlled or safeguarded through protein-protein interactions involving multiple families of proteins, including the Bcl-2 (B cell lymphoma-2) and IAP (inhibitor of apoptosis proteins) families, which have recently received intensive attention.

The Bcl-2 family is a large group of apoptosis regulators which, through the diverse interactions among themselves and with other proteins, control the release of apoptogenic factors, such as cytochrome c and Smac (second mitochondria-derived activator of caspases) or its murine homolog DIABLO (direct IAP binding protein with low pI), needed for caspase activation [9, 10]. While the Bcl-2 family regulates the integrity of mitochondria and normally has little impact on signals from death receptors, a crosstalk is found in some cells where caspase-8 activated by death receptors cleaves inert Bid to release tBid, which then triggers the mitochondiral apoptotic pathway (Figure 1). The IAP family is another family of proteins that reprieve caspases' execution of apoptosis through physically interacting with caspases and thereby directly inhibiting their function. In turn, the function of the IAP family is inhibited by Smac/DIABLO, which, upon its release from the mitochondria together with cytochrome c, binds IAPs and relieves them from the complexes with caspases.

Given the fundamental role of apoptosis in both normal physiology and a wide variety of human diseases, there has been an explosion of research in the past decade which has made apoptosis one of the central areas in biomedical sciences. There are many recent reviews in the biological [11–15], clinical [16–19], and structural [20, 21] aspects of apoptosis. This article focuses on the chemical and chemical biological perspectives of apoptosis in terms of how chemical approaches are used to explore and control the function of proteins

[1]Correspondence: z-huang@life.uiuc.edu

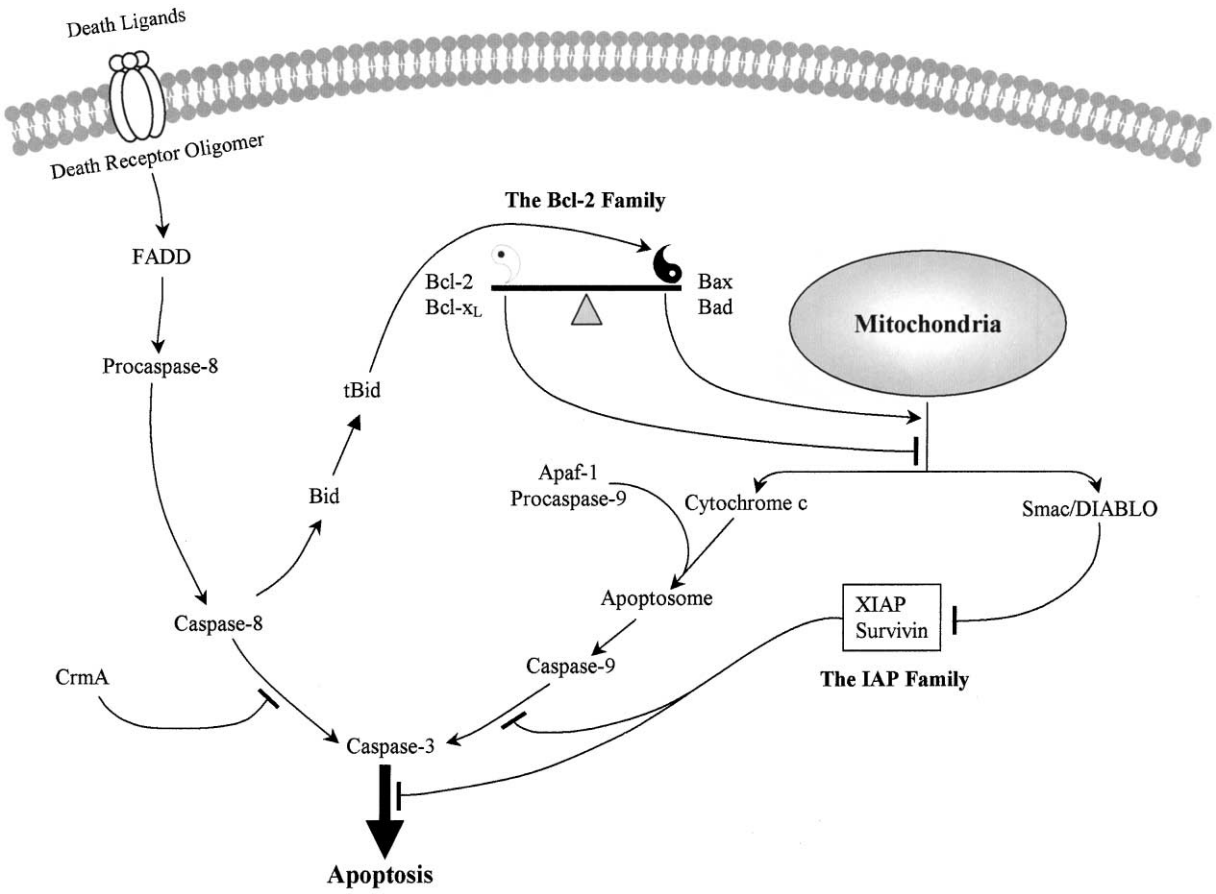

Figure 1. Protein-Protein Interactions in Programmed Cell Death

A network of protein-protein interactions along the two apoptotic pathways involving the mitochondria and death receptor.

involved in the regulation of cell death and survival. Chemists have long favored the dissection and mimicry of large and complicated biological systems by smaller and simplified molecular models that are amenable to chemical synthesis and modification. Such strategies have powerful application in the study of apoptosis in conjunction with genetic, biological, and structural techniques. The chemical research of apoptosis often involves the use of small molecules, including synthetic peptides, natural products, and designed compounds, that mimic or bind specific protein surface sites and thus modulate their interactions with other proteins. Cell-permeable synthetic peptides and nonpeptidic molecules targeted to a specific apoptosis-related protein can be used to perturb its function instantaneously for the dissection and determination of the kinetic time course (which is not possible with genetic techniques) of the complex network of many molecular interactions during apoptotic processes.

In this article, I will discuss the use of chemical approaches in discovering and studying small molecules targeted to the protein-protein interactions involving the two apoptosis-regulating protein families of Bcl-2 and IAP. The Bcl-2 family will be the main focus for which recent advances in the discovery and synthesis of many different classes of peptide and nonpeptidic inhibitors are described. After that, I will briefly describe some recent work on peptide ligands of IAPs. In addition to serving as model systems to probe and understand the basic mechanism of molecule recognition in programmed cell death, small molecule inhibitors of Bcl-2 and IAP family proteins have tremendous potential for clinical application, as some of them have recently been shown to be potent anticancer agents.

The Bcl-2 Family: The Molecular "Yin" and "Yang" in Apoptosis

Structure, Function, and Molecular Interactions

The Bcl-2 family proteins are key regulators of the apoptotic pathway involving the mitochondria [9, 10]. Members of the Bcl-2 family include both antiapoptotic proteins, exemplified by Bcl-2, and proapoptotic proteins, exemplified by Bax. Some features of these proteins are reminiscent of the ancient Chinese theory of "yin" and "yang," which, in an over-simplified interpretation, symbolize two basic opposing elements such as death versus life, dark versus light, negative versus positive, etc. (Figure 2). The healthy state relies on the interaction of yin and yang in a unity of opposites, whereas loss of this balance results in the abnormal diseased state. In some sense, prodeath proteins such as Bax and Bad might be thought to act like yin, whereas the opposite

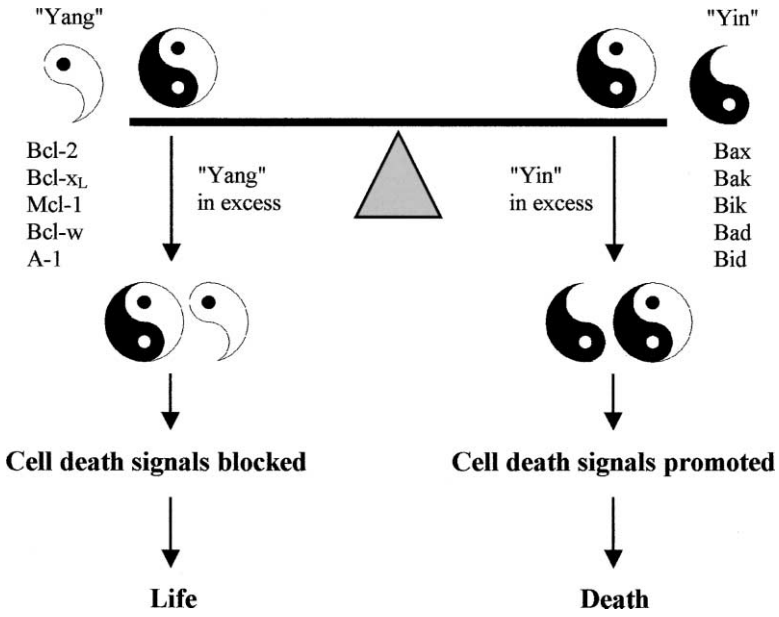

Figure 2. The Molecular "Yin" and "Yang" in Apoptosis

The interaction and relative ratio of Bcl-2 and Bax (or their functional homologs), like that of "yang" and "yin," is essential for determining the fate of cells. According to ancient Chinese theory, yin and yang symbolize two basic opposing elements, such as death versus life. The healthy state relies on the interaction of yin and yang in a unity of opposites, whereas loss of this balance results in the abnormal diseased state. In many ways, prodeath proteins, such as Bax, act like yin, whereas the opposite prolife proteins, such as Bcl-2, act like yang. Capable of heterodimerization, they neutralize each other's function and retain a delicate balance. However, the excess of either one can tilt the balance toward cell death or survival.

prolife proteins such as Bcl-2 and Bcl-x_L act like yang. Capable of heterodimerization, they neutralize each other's function and retain a delicate balance. However, the excess of either one can tilt the balance toward cell death or survival. In terms of sequences, Bcl-2 family proteins share at least one of four homologous regions termed Bcl homology (BH) domains (BH1 to BH4) (Figure 3A). Based on sequence homology, a subclass of proapoptotic proteins termed "BH3-only" can be classified that share sequence homology only in the BH3 domain. While all of proapoptotic members use the BH3 domain to interact with antiapoptotic proteins, BH3-only proteins, including Bad and Bid, appear to act mainly as antagonists of antiapoptotic members such as Bcl-2 and Bcl-x_L. In contrast to the opposing biological functions and wide differences in amino-acid sequences, experimentally determined structures of Bcl-2 [22], Bcl-x_L [23, 24], Bax [25], and Bid [26, 27] are surprisingly similar (Figure 3B).

The mechanism by which Bcl-2 family proteins regulate apoptosis has been a subject of intensive research. Currently it remains controversial and several models have been proposed. An attractive mode of action is the heterodimerization between antiapoptotic and proapoptotic Bcl-2 family members (Figure 2) [17, 28, 29]. Some information about the structural basis of these interactions is provided by the three-dimensional structure of Bcl-x_L in complex with a peptide derived from the BH3 domain of Bak (Figure 4A) [30]. The structure reveals a hydrophobic surface pocket on Bcl-x_L formed by the BH1-3 domains bound by the Bak BH3 domain peptide in helical conformation. Since the BH3 domain is buried in the structures of proapoptotic proteins Bid [26, 27] and Bax [25], this raises the speculation that conformational changes are necessary for the exposure of the BH3 domain of a proapoptotic protein and its inhibition of the functional pocket on antiapoptotic partners. In the cell environment, proapoptotic Bcl-2 family members are suggested to undergo such conformational changes [31] triggered by dephosphorylation [32] or proteolytic cleavage by caspases (such as the cleavage of Bid to generate tBid; Figure 1) [33–35].

The BH3 Domain: Analysis and Mimicry by Peptides

BH3 Peptide Binding and Mechanistic Studies In Vitro or in Cell-free Systems

Among the four BH domains of the Bcl-2 family, the BH3 domains in proapoptotic Bcl-2 family members (Figure 4A) play a key role in cell death by interacting with the surface pocket of Bcl-2 or Bcl-x_L and neutralizing its antiapoptotic activity [36]. The three-dimensional structures of Bcl-x_L and Bcl-2 as determined by X-ray crystallography and/or NMR spectroscopy reveal a hydrophobic surface pocket formed by the BH1-3 domains (Figure 4B) [22, 23]. Residues at the BH1 and BH2 domains are essential for the antiapoptotic function of Bcl-2 or Bcl-x_L, as studies have shown that mutations at these sites abolished their biological function [37]. Death agonists, such as Bax, Bak and Bad, use their BH3 domains to bind to the surface pocket and promote apoptosis [38–40]. How these BH3 domains recognize their antiapoptotic partners and the mechanism by which they trigger apoptosis have been the subject of much investigation.

Sattler et al. synthesized peptides containing the BH3 domain of several Bcl-2 family proteins, including Bcl-2, Bcl-x_L, Bax, Bik, and Bak [30]. These synthetic BH3 peptides displayed widely different binding affinities to Bcl-x_L, while the peptide derived from Bak BH3 domain (residues 72–87, see Figure 4A) showed relatively high binding affinity ($K_D = 0.34\ \mu M$). The difference in binding affinity of these BH3 peptides derived from different Bcl-2 family members and Ala-substituted analogs of Bak BH3 peptide was explained by the NMR structure of Bcl-x_L bound by Bak BH3 peptide [30].

Compared with other BH3 peptides described above, the BH3 peptide derived from mouse Bad protein (residues 140–165 of mBad, Figure 4A) showed higher affinity

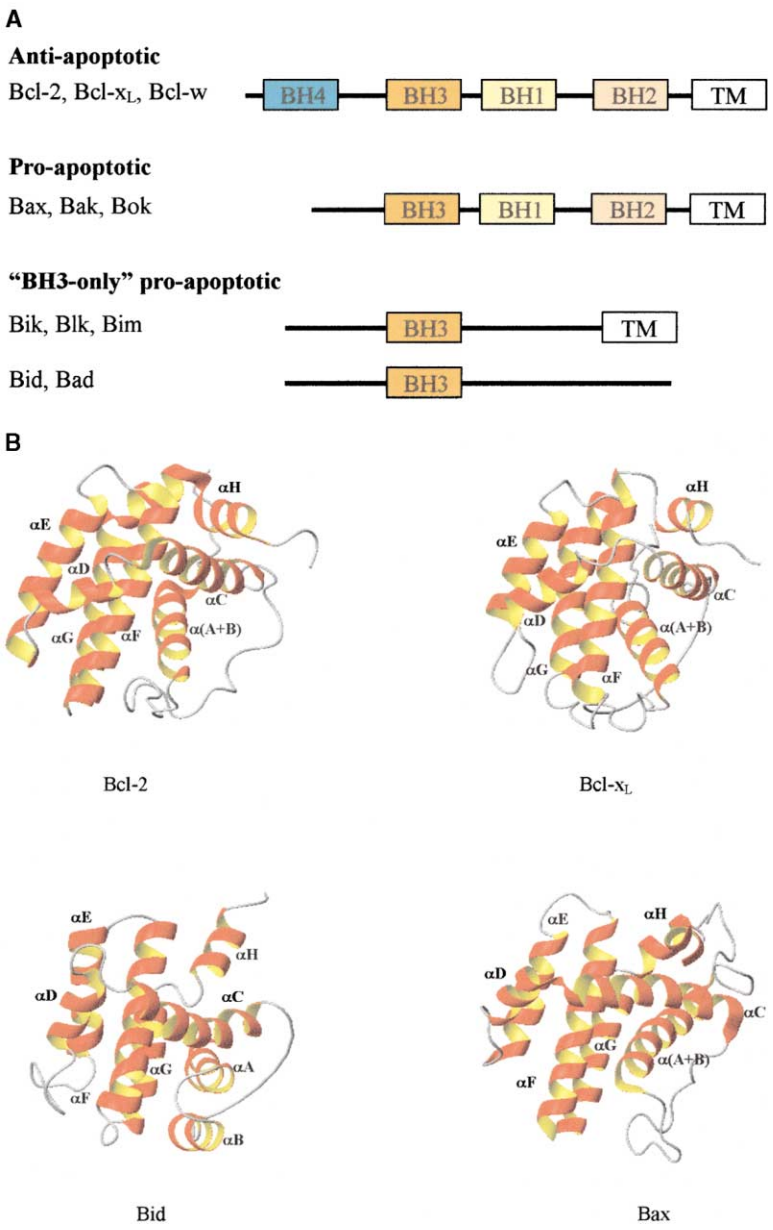

Figure 3. The Sequence and Structural Homology of the Bcl-2 Family

(A) One or more of the four homologous regions termed BH domains (BH1 to BH4) shared by the Bcl-2 family. A subclass of pro-apoptotic proteins termed "BH3-only" share sequence homology only in the BH3 domain. (B) The structures of Bcl-2 [22], Bcl-x_L [23], Bax [25], and Bid [26, 27] displaying the similarity in their overall structure and folding pattern, despite their opposing biological functions and wide differences in amino-acid sequences. Note that the C-terminal helix of Bax, which is a putative transmembrane domain and is packed against the surface pocket on Bax, is not shown in the figure. Also note that Bid possesses two helical segments (αA and αB) in BH4 region, whereas Bcl-2 and Bcl-x_L display a continuous helix combining these two segments, as denoted by α(A+B).

for Bcl-x_L, with a K_D value of 6 nM [41]. The structure of Bcl-x_L protein complexed with a 25-residue peptide derived from human Bad BH3 domain (NLWAAQRY GRELRRMSDEFVDSFKK) was determined by using NMR spectroscopy [42]. The overall structure is similar to the complex of Bcl-x_L bound by a 16-residue Bak BH3 peptide. The N and C termini of Bad BH3 peptide form additional interactions with Bcl-x_L protein. However, such contacts were suggested to play no major role in the increase of receptor binding, based on results from mutant peptides containing Ala substitutions at N and C termini. Rather, it was argued that the high Bcl-x_L binding affinity was contributed by the enhanced helical stability of the longer Bad BH3 peptide sequences as compared with other shorter 16-amino-acid BH3 peptides. Using an in vitro protein-protein binding assay, Ottilie, Diaz et al. evaluated the effects of peptides (either 16 amino acids or longer) derived from the BH3 domains of Bak, Bax, and Bad in blocking the heterodimerization of Bcl-x_L with death agonists [43, 44]. These BH3 peptides were shown to inhibit Bcl-x_L-Bax and Bcl-x_L-Bad interactions in a dose-dependent manner. Consistent with its high binding affinity to Bcl-x_L [41], peptides derived from Bad BH3 (26 amino acids) were more potent than other BH3-derived peptides in blocking protein-protein interactions involving Bcl-x_L.

Cosulich et al. studied the biological activity of synthetic BH3 peptides in triggering apoptosis in a cell-free system based on extracts of *Xenopus* eggs [45]. Peptides of 16 amino acids derived from the BH3 domains of Bak, Bax, or Bid were found to induce apoptosis by causing rapid activation of caspases, whereas a Bak BH3 mutant peptide containing Ala substitution at Leu-78, which is critical for Bcl-x_L binding [30], did not

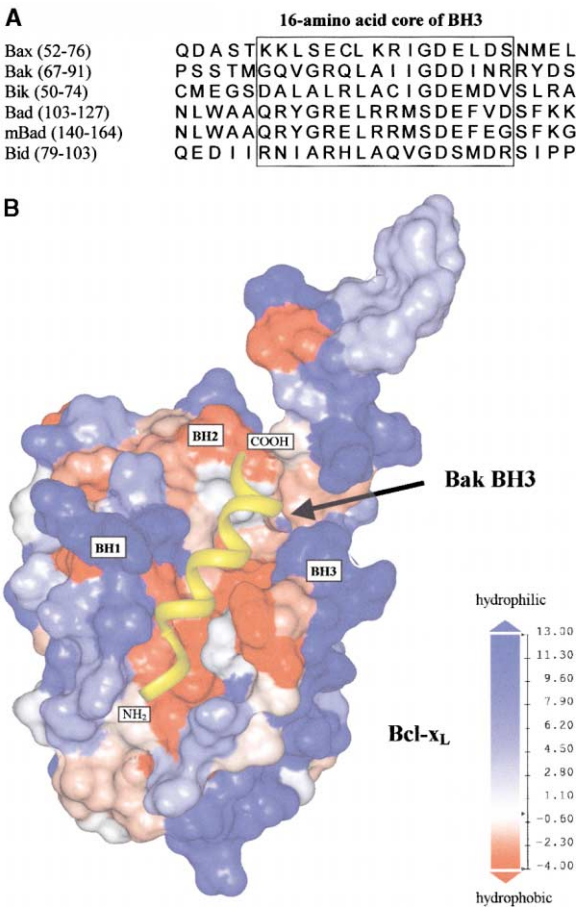

Figure 4. The BH3 Domain: Sequences and Complex Structure with Bcl-x_L

(A) The aligned amino-acid sequences of the BH3 domain in representative members of the proapoptotic and BH3-only subfamilies. The amino-acid sequence of the BH3 domain of mouse Bad (mBad) is shown because it has been used for peptide design. All other BH3 sequences are from human proteins. The numbering of residues corresponds to that in the native protein.

(B) The three-dimensional structure of Bcl-x_L in complex with a peptide derived from the BH3 domain of Bak [30]. This surface pocket is conserved in other antiapoptotic proteins, such as Bcl-2 [22], and proapoptotic proteins, such as Bax [25]. The molecular recognition of this surface pocket and the BH3 domain determines the affinity and specificity of protein-protein interactions of the Bcl-2 family. Thus, peptides or nonpeptidic molecules mimicking the binding of the BH3 domain can modulate the function of the Bcl-2 family and be used as novel therapeutic agents.

show any effect. Since cytochrome c is involved in the activation of apoptosis, the activity of synthetic BH3 peptides in affecting cytochrome c release in isolated rat mitochondria was evaluated. Whereas a 16-amino-acid peptide derived from Bax BH3 domain was shown to lack the activity in inducing cytochrome c release [46], another study observed significant cytochrome c release after treatment with a 20-amino-acid Bax BH3 peptide (residues 55–74) or a 15-amino-acid Bak BH3 peptide (residues 73–87) [47]. Morgan et al. described in a conference report how synthetic peptides derived from the BH3 domain of Bak or Bax diminished the association of Bcl-2 with Bak in PC-3 cells [48]. When introduced into prostate carcinoma PC-3 and DU145 cells by electroporation, these peptides caused apoptosis in these cells, which could be blocked by a broad-spectrum caspase inhibitor zVAD-fmk.

Biological Activity of BH3 Peptides in Intact Cells

In addition to structure and function studies in vitro and in cell-free systems as described above, synthetic peptides derived from the Bcl-2 family can be used to probe and modulate Bcl-2-regulated apoptotic pathways in living cells. To do this, one needs to modify these peptides to make them cell permeable, because normally peptides have little ability to cross the cell membrane and arrive at their intracellular targets, such as Bcl-2 family proteins. Several strategies have been reported in which synthetic peptides derived from Bcl-2 family proteins are linked with transporter peptides or nonpeptidic molecules that can deliver functional peptides into the cell.

The internalization domain of the Antennapedia (Ant) protein, RQIKIWFQNRRMKWKK, has been used in a number of studies as the transporter to deliver functional peptides into cells [49–54]. Holinger et al. synthesized a fusion Ant-BH3 peptide combining the 16-amino-acid Ant internalization sequence and the Bak BH3 sequence (residues 71–89, Figure 4A) and found that this fusion peptide caused the activation of caspases and triggered apoptosis in intact HeLa cells [55]. While microinjection of recombinant Bcl-x_L into these cells suppressed programmed cell death induced by Fas, Ant-BH3 peptide was shown to antagonize the function of Bcl-x_L by inhibiting its ability to suppress Fas-induced apoptosis. It was suggested that the cell-killing effect of Ant-BH3 peptide was due to its binding to Bcl-2-related death antagonists, because a mutant Ant-BH3 peptide containing a single Ala substitution at Val-78 lost the Bcl-x_L binding activity and was incapable of inducing apoptosis. Interestingly, Ant-BH3 appeared to trigger apoptosis in a cytochrome c-independent manner. It did not cause the early loss of mitochondrial membrane potential or cytochrome c release from the mitochondria.

Fatty acids are a class of nonpeptidic molecules that can help peptides cross the cell membrane. For example, myristic acid and stearic acid have been used for the intracellular delivery of peptide inhibitors of protein kinase C [56] and protein-tyrosine phosphatase [57], respectively. Using a smaller fatty acid, the decanoic acid, as the cell-permeable moiety (CPM), Wang et al. synthesized cell-permeable Bcl-2 binding peptides by attaching this CPM to the N terminus of synthetic BH3 peptides [58]. One peptide, termed CPM-1285, that contained the BH3 domain of mouse Bad protein (residues 140–165 of mBad, Figure 4A) was shown to enter human myeloid leukemia HL-60 cells by confocal microscopy. The in vitro Bcl-2 binding assay suggested that CPM-1285 strongly competed with the binding of a fluorescein-labeled Bak BH3 peptide with an IC_{50} of 130 nM. In intact HL-60 cells, CPM-1285 induced the activation of caspase-3 and triggered apoptosis characterized by DNA fragmentation and cleavage of PARP. The apoptosis induced by CPM-1285 was dependent on caspase activation, since the addition of a caspase inhibitor, zVAD-fmk, completely blocked the effect of CPM-1285. Furthermore, CPM-1285 was shown to slow human my-

eloid leukemia growth in severe combined immunodeficient (SCID) mice.

In addition to apoptosis induction, the cell-permeable peptide approach was applied to inhibit apoptosis. Shimizu et al. showed that the BH4 domain of antiapoptotic Bcl-2 or Bcl-x_L was required for inhibiting VDAC activity and apoptotic release of cytochrome c [59]. Using the protein transduction domain of HIV TAT protein that is known to facilitate the delivery of proteins into cells [60–62], they synthesized a fusion TAT-BH4 peptide that combined the HIV TAT domain (RKKRRQRRR) and Bcl-x_L BH4 sequence [59]. This TAT-BH4 peptide prevented apoptosis in HeLa cells, whereas the TAT-only sequence did not show any effect. These results suggest that peptides mimicking the BH4 domain of antiapoptotic Bcl-2 family proteins, when transferred into cells, act as inhibitors of apoptosis by suppressing VDAC activity and subsequently the release of cytochrome c through VDAC.

Nonpeptidic Molecules that Inhibit BH3 Domain Binding to Bcl-2 or Bcl-x_L

Methods to Detect Small Molecule Binding to Bcl-2 or Bcl-x_L

In addition to synthetic BH3 peptides, nonpeptidic organic molecules that specifically target the BH3 binding pocket on Bcl-2 or Bcl-x_L have been highly sought after by many groups in both academia and industry. Such nonpeptidic compounds can be used as probes for the research of the molecular recognition of Bcl-2 or Bcl-x_L surface pocket by small molecules and leads for the development of new anticancer agents. Prerequisite to these efforts is the development of accurate and sensitive protein-ligand binding assays for screening small molecules and measuring their affinity with the surface pocket. Below, a summary is provided for several Bcl-2 or Bcl-x_L ligand binding assays which can be divided into two basic types: direct ligand binding detection and measurement of competition with a known labeled probe for receptor binding.

The earliest method used to detect ligand binding to the Bcl-2 or Bcl-x_L surface pocket was reported by Sattler et al., who analyzed the Bcl-x_L binding affinity of a series of synthetic BH3 peptides based on changes in the fluorescence emission of the Trp residues of Bcl-x_L in the presence of increasing concentrations of peptides [30]. Rather than measuring the fluorescence emission of receptor Trp residues, Tzung et al. studied the receptor binding of fluorescent compounds such as antimycin A_3 by measuring the increase in fluorescence upon protein binding [63]. Yet another method for direct ligand binding measurement was based on NMR titrations of Bcl-x_L with inhibitors, as reported by Lugovskoy, Degterev et al. [64, 65]. This NMR-based method has the advantage in providing not only the binding affinity of a ligand but also its contact residues on the receptor. Alternative to these methods for direct ligand binding measurement, Wang et al. reported a competitive binding assay based on fluorescence polarization (FP) [58, 66]. By labeling a Bak BH3 peptide with 5-carboxyfluorescein at the peptide's N terminus and measuring the decrease of fluorescence polarization of the labeled peptide from its value of the receptor-bound state, they applied this FP competitive binding assay to analyze the binding affinity of synthetic peptides and nonpeptidic compounds. The FP-based assay is relatively easy to conduct and highly amenable for high-throughput screening. This assay and its subsequently modified protocols using different labeled BH3 peptide probes have been commonly used for screening compound libraries and determining ligand affinity by a number of groups [22, 42, 65, 67, 68].

Discovery of Nonpeptidic Inhibitors of Bcl-2 or Bcl-x_L

In general, it has been a difficult challenge to find small molecule inhibitors of protein-protein interactions. Thus, even though several assays were available for compound screening, the discovery of small molecule inhibitors of Bcl-2 or Bcl-x_L remained elusive until the last two years, when multiple, different classes of nonpeptidic inhibitors were reported. The first inhibitor of Bcl-2 was reported by Wang et al. in 2000 and was discovered by using a computer screening strategy [66]. This method relies on the high-resolution three-dimensional structure of a targeted receptor protein and computer-aided techniques to search a large number of organic compounds for potential ligand molecules. Combining both the rational design by new computational methods and the diversity of existing compound databases, this strategy has become a powerful tool for nonpeptidic ligand discovery [69]. Using this approach, Wang et al. conducted a virtual screening on a computer of a large collection of more than 190,000 organic molecules and identified HA14-1 (Figure 5A) [66]. In vitro binding studies demonstrated the interaction of HA14-1 with the surface pocket of Bcl-2 (IC_{50} = 9 μM in competing with the Bcl-2 binding of a fluorecein-labeled Bak BH3 peptide). HA14-1 induced apoptosis in HL-60 cells that overexpress Bcl-2. In conjunction with the discovery of HA14-1, Yu et al. developed an efficient synthetic method for the preparation of HA14-1 derivatives, which can facilitate efforts in further lead optimization to increase the binding potency and specificity of HA14-1 analogs for Bcl-2 protein [70].

In 2001, three independent papers describing discoveries of small molecule inhibitors of Bcl-2 or Bcl-x_L were published. Using the FP competitive binding assay to screen a chemical library of 16,320 compounds, Degterey et al. identified two classes of small molecule ligands of Bcl-x_L, termed BH3I-1 and BH3I-2 because they inhibit BH3 peptide binding to Bcl-x_L [65] (Figure 5B). These compounds were also shown to inhibit BH3 peptide binding to Bcl-2. The affinity of these compounds is in the low micromolar range (Ki of 2.4–15.6 μM) as determined by FP and NMR titration assay. The binding site of the compounds in the surface pocket of Bcl-x_L was estimated by NMR experiments based on chemical-shift perturbations exhibited by residues on Bcl-x_L upon compound binding. These compounds were shown to induce apoptosis in Jurkat cells overexpressing Bcl-x_L. In a subsequent study, Lugovskoy et al. analyzed the structure-activity relationships of these compounds based on NMR and molecular modeling studies. In addition, they used computer screening to identify an analog of BH3I-1 that showed similar binding affinity to Bcl-x_L [64].

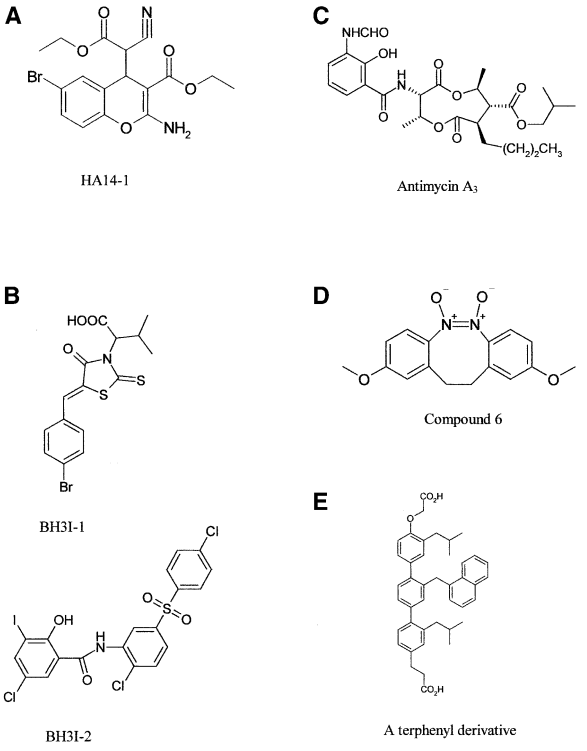

Figure 5. Nonpeptidic Inhibitors of Bcl-2 and Bcl-x_L

The chemical structures of recently discovered nonpeptidic ligands of Bcl-2 and Bcl-x_L, reported by several groups independently, including (A) HA14-1 [66], (B) BH3I-1 and BH3I-2 [65], (C) antimycin A_3 [63], (D) compound 6 [67], and (E) a terphenyl derivative [68].

A natural product, antimycin A (Figure 5C), which is a known antibiotic and binds cytochrome b [71, 72], was reported by Tzung et al. to have biological activity in mimicking the death-inducing BH3 domain [63]. Based on fluorescence emission spectra, antimycin A was shown to compete with Bak BH3 peptide binding to the surface pocket of Bcl-2 and Bcl-x_L. Antimycin A inhibited the pore-forming activity of Bcl-x_L in synthetic liposomes and induced apoptosis in murine hepatocyte cell lines transfected with Bcl-x_L. To address the issue of selectivity of antimycin A for the Bcl-2 or Bcl-x_L targets, an analog, 2-methoxy-antimycin A_3, which was previously known to be inactive as an inhibitor of cytochrome b-c_1 [72, 73], was studied and shown to retain binding to Bcl-2. This suggested the feasibility of using antimycin A as a template to develop more specific inhibitors of Bcl-2 or Bcl-x_L devoid of general mitochondrial toxicity.

Enyedy et al. used the structure-based computer screening approach to search a collection of 206,876 compounds and identified seven compounds that had Bcl-2 binding with IC_{50} values by the FP assay ranging from 1.6 to 14.0 μM [67]. Compound 6 was the most potent compound and had an IC_{50} value of 4 μM in reducing the viability of HL-60 cells expressing a high level of Bcl-2 (Figure 5D). It was further shown that compound 6 was active in inducing apoptosis in HL-60 and MDA-231 cells that express Bcl-2 but had much less or no activity in T47D and MDA453 cells that express low or undetectable levels of Bcl-2. Finally, changes in chemical shifts as determined by NMR experiments showed those residues around the BH3 binding pocket of Bcl-x_L to be involved in the binding of compound 6.

The most recent addition to the reported list of nonpeptidic ligands of Bcl-2/Bcl-x_L surface pocket was a series of terphenyl derivatives published by Kutzki et al. [68]. Previously, this group showed that the terphenyl scaffold, in a staggered conformation, closely reproduces the projection of functionality on the surface of an α helix [74, 75]. Extending this scaffold for the mimicry of the BH3 helix that binds the Bcl-2/Bcl-x_L surface pocket, they synthesized terphenyl molecules containing alkyl or aryl substituents on the three ortho positions which were designed to mimic the key hydrophobic side chains (i.e., Val-74, Leu-78, Ile-81, and Ile-85) of Bak BH3 peptide. Such compounds were indeed found to bind to Bcl-x_L as determined by the FP competitive binding assay, with a K_D of 114 nM for the most potent derivative (Figure 5E). Further NMR experiments and computational docking studies showed that this compound binds to the same hydrophobic surface pocket on Bcl-x_L as the Bak BH3 peptide.

Clinical Application of Inhibitors of Bcl-2 or Bcl-x_L

The Bcl-2 family is implicated in many human diseases. In particular, cancer is linked to both the original discoveries of many genes belonging to this family and much of subsequent biological research to understand their function. The antiapoptotic members of the Bcl-2 family are known to contribute to neoplastic cell expansion by preventing normal cell turnover caused by physiological cell-death mechanisms. For example, high levels and aberrant patterns of Bcl-2 gene expression are found in a wide variety of human cancers, including ~70% of breast cancers, ~30%–60% of prostate cancers, ~90% of colorectal cancers, ~60% of gastric cancers, 100% of small-cell lung carcinomas, ~20% of non-small-cell lung cancers, ~30% of neuroblastomas, ~80% of B cell lymphomas, and variable percentages of melanomas, renal cell, and thyroid cancers, as well as acute and chronic lymphocytic and nonlymphocytic leukemias [17, 76]. The expression levels of Bcl-2 proteins also correlate with relative resistance to a wide spectrum of current chemotherapeutic drugs and γ-irradiation. Since Bcl-2 can protect against such a wide variety of drugs that have very different mechanisms of action, it appears that all of these drugs use a common final pathway for the eventual induction of cell death which is regulated by Bcl-2.

The revealed role of the Bcl-2 family in cancer and resistance to cytotoxic therapies has opened new avenues in the development of novel anticancer strategies [77, 78]. An attractive strategy for inducing apoptosis in cancer cells or overcoming their chemoresistance is to inhibit the protective function of Bcl-2 or related antiapoptotic proteins such as Bcl-x_L that are often overexpressed or upregulated in cancer cells. Apoptosis-inducing activity in cancer cell lines was observed in the above-described small molecule inhibitors of Bcl-2 or Bcl-x_L. HA14-1 was shown to induce apoptosis in several types of cancer cell lines, including human myeloid leukemia (HL-60) [66], breast cancer (MDA-MB-

468) [79], and prostate cancer (PC3, LNCaP) (unpublished results from laboratory). The apoptotic effect of HA14-1 in breast and prostate cancer cells was found to be dependent on Bcl-2 expression, as the compound was not active in breast and prostate cancer cells that have very low or undetectable levels of Bcl-2. Compound 6 also displayed a selective killing effect on Bcl-2-positive cancer cells [67]. Most recently, HA14-1 was shown to induce the apoptosis of primary acute myelogenous leukemia (AML) and to be synergistic with PD184352, a MEK inhibitor, in reducing the number of CFU-blast of primary AML patients 50% more than those treated with either alone [80]. The potential benefits of combining HA14-1 with other cytotoxic agents in cancer treatment were also suggested by another study in which the use of HA14-1 together with an epothilone B analog was significantly more effective in killing human breast cancer cells than the use of either alone [79]. As to in vivo animal models, although the activity of this and other nonpeptidic inhibitors of Bcl-2 or Bcl-x_L have not been reported and await further studies, CPM-1285, a cell-permeable peptide inhibitor of Bcl-2, has been shown to slow human myeloid leukemia growth in SCID mice [58].

An important question to be considered in the clinical application of Bcl-2 or Bcl-x_L inhibitors is whether these compounds are toxic to normal cells, which is a common limitation of current chemotherapeutic drugs. Because the function of Bcl-2 is not absolutely necessary in many normal cell types [81], a systemic inhibition of Bcl-2 may not affect the normal cellular function. Furthermore, oncogenic changes in certain cancer cells render them more susceptible than normal cells to apoptosis [82]. This seems to be consistent with the observation that a higher level of Bcl-2 or Bcl-x_L is found in many cancers, which may serve to protect the vulnerability of these cancers. Therefore, it can be expected that small molecule inhibitors targeting cancer cells highly expressing Bcl-2 or Bcl-x_L are more selective than conventional cytotoxic drugs. In this regard, it was encouraging to note that peptide inhibitor CPM-1285 had little effect on the viability of normal human peripheral blood lymphocytes or the proliferative response of activated lymphocytes stimulated with PHA mitogen for 48 hr at concentrations where the peptide strongly induced apoptosis in cancer cells [58]. Also, a nonpeptidic inhibitor HA14-1 had no noticeable effect on normal human hematopoietic $CD34^+$ cells (determined by the average number of colony-forming cells with or without HA14-1 treatment), even though at the same treatment condition HA14-1 strongly decreased the number of CFU-blast of primary AML (personal communication, Dr. Michael Andreeff's group at M.D. Anderson Cancer Center). Finally, recent encouraging data from the clinical trial indicating that antisense oligonucleotides targeted against Bcl-2 gene can specifically inhibit non-Hodgkin's lymphoma in humans provided an important validation of Bcl-2 as a therapeutic target [83]. Since the clinical value of antisense oligonucleotides is often limited by their lack of enzymatic stability, cell permeability, and oral activity, small molecule inhibitors devoid of such limitations are more desired agents for clinical application.

Future Research in Molecule Recognition and Drug Design of the Bcl-2 Family

From chemical and structural perspectives, a central question that needs to be further studied is the mechanism by which the BH3 domain recognizes the surface pocket commonly seen in the Bcl-2 family. The BH3 domain seems to be responsible for the affinity of different protein-protein interactions within the Bcl-2 family, since synthetic peptides derived from the BH3 domain of different Bcl-2 family members showed interactions with Bcl-x_L corresponding to their native proteins [30]. It remains to be further elucidated as to the amino-acid sequence and structural bases that dictate the selectivity and hierarchy of associations between BH3 peptides (or native BH3 domains in proapoptotic proteins) with the surface pocket of antiapoptotic Bcl-2 or Bcl-x_L. The experimentally determined structures of proapoptotic Bax and Bid reveal that their BH3 domain is buried in the core of the protein, implying that conformational changes are necessary for the BH3 domain to interact with its antiapoptotic partners [25–27]. More needs to be learned about the mechanism and kinetics of the conformational switch and refolding of BH3 domains during their binding to Bcl-2 or Bcl-x_L surface pocket. To address these questions, synthetic BH3 peptides may provide a useful model for further investigation employing synthetic and combinatorial chemistry and biophysical techniques.

Significant progress in the field was made with the recent discovery by several groups independently of multiple classes of nonpeptidic inhibitors capable of mimicking the binding of BH3 domain to the surface pocket of Bcl-2 and/or Bcl-x_L. Whereas the structures of Bcl-2 or Bcl-x_L bound by these inhibitors were predicted by using molecular modeling alone or in combination with NMR experimental data, high-resolution cocrystal or NMR structure is needed for the delineation of detailed binding modes of these molecules with Bcl-2 or Bcl-x_L surface pocket. This will be important for the design of new analogs with higher affinity for the surface pocket. Another important question is the mechanism underlying the specificity of small molecules recognizing the surface pocket of different Bcl-2 family members. All of the nonpeptidic inhibitors described above bind both Bcl-2 and Bcl-x_L, which may not be surprising given that presumably they all recognize the surface pocket highly conserved in these two proteins. This may be acceptable or even desirable for clinical application in certain cancers, as suggested by the study of antisense oligonucletides that are bispecific for Bcl-2 and Bcl-x_L [84, 85]. Still, inhibitors with monospecificity for either Bcl-2 or Bcl-x_L are needed if they are to be used to probe the function of a specific protein or treat cancer cells where targeting of only one protein is desired. Toward the development of such specific inhibitors, the existing compounds can provide starting templates to generate new derivatives based on structural design and combinatorial chemistry.

So far, all of the reported small molecule (both peptidic and nonpeptidic) inhibitors target the surface pocket of antiapoptotic Bcl-2 or Bcl-x_L for triggering apoptosis in cancer cells. It may also be worthwhile to target proapoptotic Bcl-2 family members with small molecules to

prevent unwanted cell death; for example, to prevent death of neuronal cells which has been observed in neurodegerative diseases in which proapoptotic proteins such as Bax and Bad are known to play an important role [18]. This is a highly promising and yet currently unexplored new area of research where the concepts and methods used for the recent development of small molecule inhibitors of Bcl-2 and Bcl-x_L described above can be readily applied. Structural biology has revealed a surprising conservation of both the overall structure and the surface pocket similar to those of Bcl-2 and Bcl-x_L in Bax, whose surface pocket can also accommodate the binding of BH3 domains. Of course, there are differences in shape and amino-acid composition in the surface pocket of these Bcl-2 family proteins that presumably account for differences in affinity and selectivity in molecular interactions. It is thought that proapoptotic members of the Bcl-2 family possessing a BH3 domain "hidden" inside the surface pocket are activated by cleavage by caspases or posttranslational modification events to expose the BH3 recognition motif and manifest death-promoting activity. It is thus intriguing to speculate that small molecules bound to the surface pocket of Bax or other proapoptotic proteins might stabilize their conformation and prevent the projection of the deadly BH3 motif. Therefore, the conformational changes and molecular recognition involving Bcl-2 family proteins appear to be central for both understanding and controlling their biological function.

Finally, understanding the general mechanism by which natural as well as designed, synthetic ligands recognize Bcl-2 family proteins will allow chemistry to play a pioneering role in research of apoptosis in the post-genome era. Currently, the Bcl-2 family already includes a large number of proteins, many of which await further structure-function characterization. The list keeps expanding as new members are discovered rapidly. Most recently, 24 Bcl-2 homology-containing sequences were predicted by using bioinformatic techniques from the available human genome databases [86]. The discoveries of specific ligands for these and other yet to be identified new members, based on understanding of molecular recognition and application of computational and combinatorial chemistry, will provide a panel of powerful tools for characterizing their functions and molecular interactions.

The IAPs: A Deadly Trio with Caspases and Smac/DIABLO

Structure, Function, and Molecular Interactions

Continuing on the topic of protein-protein interactions in apoptosis and their inhibition by small molecules, I describe another class of molecular interactions involving proteins of the IAP family, which have been the subjects of intensive biomedical research in recent years, and present new targets for inhibitor design and discovery. The Bcl-2 and IAP families are regulators of caspases at two different levels: the Bcl-2 family controls signaling events upstream of capases, while the IAP family directly binds and inhibits caspases. Caspases, like other enzymes that are traditionally favored by the pharmaceutical industry, have been the target of much effort to develop synthetic inhibitors [4, 87]. Here, I focus on the natural inhibitors of caspases, IAPs.

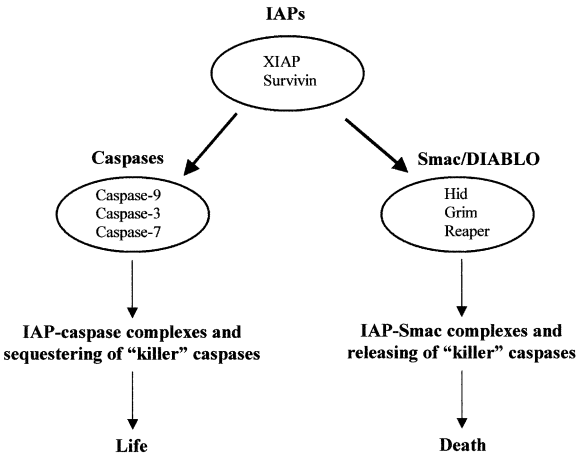

Figure 6. A Trio that Dictates the Life and Death of Cells

IAPs, such as XIAP, selectively interact with and inhibit caspase-3, -7, and -9. IAPs also interact with Smac/DIABLO (or its homologs Hid, Grim, or Reaper in *Drosophila* cells), which is released from the mitochrondria together with cytochrome c upon death stimuli. The binding of Smac/DIABLO removes IAPs from their association with caspases and thus relieves their caspase-inhibiting function. This competing binding among the trio of IAPs, caspases, and Smac/DIABLO leads to either cell survival or death.

The IAP family is conserved from viruses to yeast to humans and presently contains eight distinct human cellular members that were just discovered in the past 5–6 years, including XIAP (X-linked IAP), c-IAP1, c-IAP2, and survivin [88]. In humans, IAPs such as XIAP, c-IAP1, and c-IAP2 selectively inhibit caspase-3, -7, and -9 through direct molecular interactions but not caspase-1, -6, -8, and -10. In addition, IAPs can interact with Smac/DIABLO, which is released from the mitochrondria together with cytochrome c upon death stimuli. The binding of Smac/DIABLO removes IAPs from their association with caspases and thus relieves their caspase-inhibiting function. A similar picture is found in flies: IAPs such as DIAP1 and DIAP2 bind and inhibit several *Drosophila* caspases, whereas the caspase-inhibiting action of DIAP1 is removed by the binding of Hid, Grim, or Reaper, which are homologs of Smac/DIABLO in *Drosophila* cells [89]. Thus, from flies to mammals, the competing binding among the trio of IAPs, caspases, and Smac/DIABLO or its functional homologs leads to only one outcome: life or death (Figure 6).

Like the BH domains for the Bcl-2 family, the defining characteristic of the IAP family is that its members all share one or more BIR (baculovirus IAP repeat) domains (Figure 7A). Among members of the IAP family, XIAP and survivin have recently received enormous attention in the biological and clinical communities [88, 90]. Correspondingly, there has been an explosion in structural studies of these proteins and their interactions with caspases and Smac/DIABLO which, in just the past three years, has resulted in the publication (often simultaneous publication by two or three independent groups) of the structures of XIAP [91, 92], survivin [93–95], and the complex structures of XIAP with caspase-3, -7 [96–98],

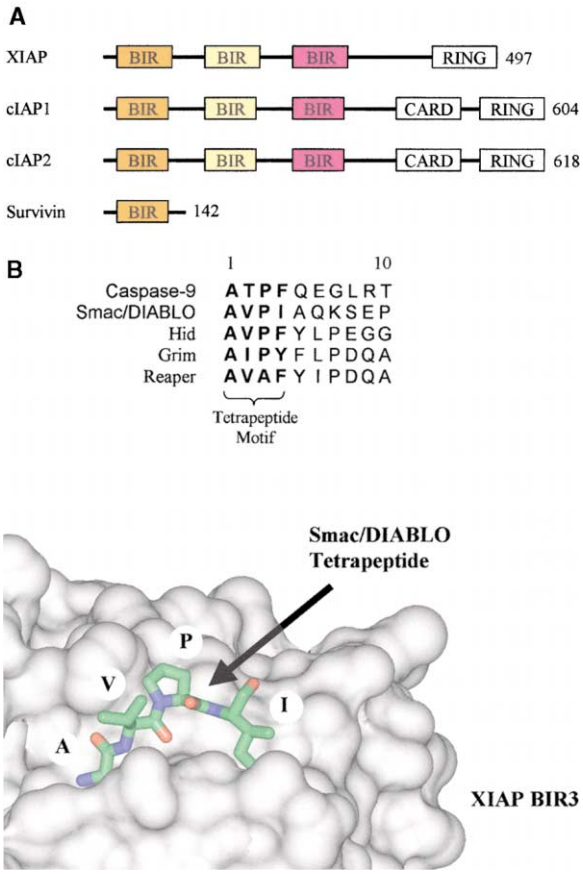

Figure 7. The BIR Domains of the IAP Family and Their Recognition by Natural and Synthetic Ligands

(A) One or more BIR domains (BIR1-3) shared by the IAP family.
(B) The surface pocket on XIAP bound by the tetrapeptide motif [99, 100], which is conserved in both caspase-9 and Smac/DIABLO or its *Drosophila* homologs Hid, Grim, and Reaper. This surface pocket offers an attractive site for discovering small molecule inhibitors.

or Smac/DIABLO [99, 100]. From these studies, a picture of how XIAP works with caspase-3, -7, and Smac/DIABLO has emerged [101]. It has been shown that a long peptide segment preceding the BIR2 domain of XIAP occupies the active site of caspase-3 or -7 (thus blocking the access of substrates) and adjacent surfaces that are specific to these two caspases (thus achieving the specificity over other caspases) [96–98]. However, the mechanism by which XIAP inhibits caspase-9 is quite different. Rather than directly restricting the enzyme active site for substrate cleavage, the BIR3 domain of XIAP interacts with the N-terminal tetrapeptide (ATPF) of caspase-9, which becomes available after the cleavage of procaspase-9, thus sequestering caspase-9 and preventing it from its deadly action [102]. Surprisingly, a similar tetrapeptide motif (AVPI) exists in the N terminus of Smac/DIABLO that has been shown in X-ray and NMR structures to bind to a surface pocket on the BIR3 domain of XIAP [99, 100]. Thus, Smac/DIABLO competes with the same tetrapeptide binding pocket on the XIAP BIR3 domain and relieves XIAP from its complex with caspase-9. In *Drosophila*, a similar binding pocket is found on the BIR2 domain of the DIAP1 protein, a member of the IAP family which is recognized by the conserved N-terminal tetrapeptide motif in Hid and Grim [103].

Like Bcl-2 or Bcl-x_L, elevated levels of IAPs are found in cancer cells. XIAP is abnormally overexpressed in many cancer cells [88]. Survivin is expressed in a variety of cancers but lacks expression in differentiated adult tissues [90]. Inhibition of these protein targets by small molecules may allow for selective elimination of cancer cells that overexpress IAPs or sensitization of these cells to chemotherapies. Consistent with this notion, an antisense oligonucleotide that reduces survivin expression was shown to induce apoptosis and sensitize lung cancer cells to a chemotherapeutic drug, etoposide, but had no effect on the viability of normal blood leukocytes [104].

The Tetrapeptide Binding Pocket on IAPs: Target for Ligand Design

The surface pocket on XIAP or other IAPs for the binding of the tetrapeptide motif conserved in both caspase-9 and Smac/DIABLO or its *Drosophila* homologs Hid, Grim, and Reaper presents an attractive target for discovering small molecule inhibitors (Figure 7B) [105]. Synthetic peptides or nonpeptidic mimics bound to this pocket should disrupt the interaction between IAPs and caspases, thus releasing active caspases-3, -7, and -9 and facilitating apoptosis. In 2000, in vitro studies of synthetic peptides containing the tetrapeptide motif of Smac/DIABLO were reported by several groups independently. Chai et al. showed that a peptide consisting of the N-terminal seven residues (i.e., the tetrapeptide motif plus three additional residues) could promote procaspase-3 activation at 10–300 μM, whereas a mutant peptide containing the Met substitution at the Ala-1 position or a reversed peptide sequence was not active [106]. Srinivasula et al. synthesized peptides containing the first 7 or 35 residues of Smac/DIABLO and found that both were effective in promoting caspase-3 activation in the XIAP-containing extracts at 100–500 μM concentrations and that the longer peptide was noticeably more effective than the short peptide [107].

A detailed analysis of the structure-activity relationship of Smac/DIABLO N-terminal peptides was reported by Liu et al., who determined the NMR structure of the XIAP BIR3 domain complexed with a nine-residue peptide derived from Smac/DIABLO and measured the binding affinity of a series of Smac/DIABLO N-terminal peptides of various lengths and mutants using a FP competitive binding assay [100]. This nine-residue peptide adopts an extended conformation with a kink at Pro3 when bound to the surface pocket on XIAP BIR3 domain (Figure 7B). The main chain of Ala-1, Val-2, and Ile-4 forms extensive hydrogen bonds with the pocket, while the side chains of Val-2, Pro3, and Ile-4 engage in hydrophobic interactions. By contrast, residues beyond this tetrapeptide motif do not show any interaction with the pocket. On the pocket, mutational analysis has suggested the important role in ligand binding of residues Leu-307, Trp-310, Trp-323, E314, and D296. Consistent with the NMR and mutational analyses, synthetic peptides containing modifications at the N-terminal amine (Ala-1) or Ala replacement at Val-2, Pro3, or Ile-4 resulted

in loss or decrease in XIAP binding. Interestingly, a peptide containing only the first five residues is almost as potent as the nine-residue peptide.

More recently, combinatorial chemistry has been applied to the development of novel ligands for the tetrapeptide binding pocket on the BIR3 domain of IAPs. Kipp et al. synthesized libraries of tetrapeptides using the Smac/DIABLO N-terminal sequence as a starting point [108]. With a competitive binding assay based on a solvent-sensitive fluorogenic dye molecule, badan, the binding affinities of these tetrapeptides, which contain variations at different residues, were determined in order to dissect the contribution of each residue of the tetrapeptide to the total binding energy with the surface pocket on the BIR3 domain of XIAP. From the analysis of a total of six libraries of related tetrapeptides, it was found that Arg and Phe substitutions at positions 2 and 4, respectively, led to significant increase in binding affinity. Combining these two substitutions resulted in a tetrapeptide, ARPF, that had the highest affinity, with a K_D of 20 nM.

Future Research in Molecule Recognition and Drug Design of the IAP Family

The interactions of the IAP family with caspases and Smac/DIABLO present new targets for inhibitor design. As highlighted by the tetrapeptide binding pocket on the IAPs, small synthetic peptides derived from Smac/DIABLO have been shown to be active in inhibiting the function of IAPs and promoting caspase activation. Despite their small sizes (the shortest being four residues), these peptides display high affinity for the IAP BIR3 domain, down to the low nM concentrations. These results strongly suggest the feasibility of discovering small molecules capable of mimicking the effect of Smac/DIABLO. While some of these Smac/DIABLO peptides were shown to cause the activation of caspase-3 in vitro, further studies are needed to demonstrate whether they can promote caspase activation and apoptosis in living cells and animals. Interestingly, short peptides containing the N-terminal four or seven residues of Smac/DIABLO were able to enter Jurkat cells and potentiate apoptosis triggered by other agents [109]. To improve the cell permeability of IAP targeting peptides, the strategies described above in the studies of cell-permeable peptide inhibitors of Bcl-2 may be readily applied. Indeed, a recent paper by Fulda et al. showed that a Smac N-terminal seven-residue peptide linked to the protein transduction domain of HIV Tat protein sensitized tumor cells to the effect of apoptosis-inducing agents in vitro and in vivo [110].

Perhaps a more important goal will be the transformation of the high-affinity tetrapeptide into nonpeptidic molecules through peptidomimetic modifications and/or discovery of novel organic compounds that bind to the same surface pocket. Such nonpeptidic molecules will be more suitable agents for probing the function of IAPs and inhibiting their function in vivo. So far, nonpeptidic inhibitors have not been reported for the IAPs, whose biology and structure are starting to be understood only very recently. Thus, this is a largely unexplored field that offers great opportunity to chemists. The rapid progress made recently in the discovery of nonpeptidic inhibitors of Bcl-2 and Bcl-x_L seems to suggest that similar advances in the study of IAPs may not be far off, given the enormous interest in these proteins in both academia and pharmaceutical companies.

Conclusion

A highly regulated network of protein-protein interactions is the central theme underlying the machinery of programmed cell death. Understanding the chemical basis of these interactions is a key to unlock the mystery of life and death of cells at the atomic level, while controlling the outcome of these interactions offers tremendous benefits in the treatment of many human diseases. Focusing on the Bcl-2 family as a model system, I summarize the progress, especially during the last two years, in the discovery of small molecule ligands targeting Bcl-2 or Bcl-x_L and use of these ligands as both probes and inhibitors of relevant molecular interactions and biological function. These studies have provided an exciting prospect in further developing and using these novel small molecules for chemical biological research of molecular interactions involving the Bcl-2 family and in applying similar strategies to explore and control other molecular interactions that are also important for apoptosis. The interactions of IAPs with caspases and Smac/DIABLO is one additional example described in this article for the application of small molecule approaches to study intracellular protein-protein interactions mediating apoptotic signaling. Besides Bcl-2 and IAP families, there are many other protein-protein interactions that can be targeted by structure-based and chemical biological strategies, such as those involving FADD adaptor motifs, various procaspases, Apaf-1, and apoptosome, just to name a few. With the frenetic pace of biomedical research in apoptosis, the list of known and yet to be known protein-protein interactions of both biological and therapeutic significance goes on and so does the tremendous challenge and opportunity for chemists and biochemists in years to come.

Acknowledgments

I apologize to my colleagues if I have missed or failed to cite their works and papers here due to the specific focus and space constraint of this review and the rapid development of this field. The work conducted in our laboratory was supported by grants from the National Institutes of Health, the American Cancer Society, and the Sidney Kimmel Foundation for Cancer Research. I would like to thank Professors Robert Gennis, Paul Hergenrother, and John Katzenellenbogen (University of Illinois at Urbana-Champaign) for helpful comments and suggestions and Dr. Dongxiang Liu for help in preparing the figures.

References

1. Wyllie, A.H., Kerr, J.F., and Currie, A.R. (1980). Cell death: the significance of apoptosis. Int. Rev. Cytol. *68*, 251–306.
2. Thompson, C.B. (1995). Apoptosis in the pathogenesis and treatment of disease. Science *267*, 1456–1462.
3. Alnemri, E.S., Livingston, D.J., Nicholson, D.W., Salvesen, G., Thornberry, N.A., Wong, W.W., and Yuan, J. (1996). Human ICE/CED-3 protease nomenclature. Cell *87*, 171.
4. Thornberry, N., and Lazebnik, Y. (1998). Caspases: enemies within. Science *281*, 1312–1316.

5. Green, D.R., and Reed, J.C. (1998). Mitochondria and apoptosis. Science *281*, 1309–1312.
6. Ashkenazi, A., and Dixit, V.M. (1998). Death receptors: signaling and modulation. Science *281*, 1305–1308.
7. Peczuh, M.W., and Hamilton, A.D. (2000). Peptide and protein recognition by designed molecules. Chem. Rev. *100*, 2479–2494.
8. Cochran, A.G. (2001). Protein-protein interfaces: mimics and inhibitors. Curr. Opin. Chem. Biol. *5*, 654–659.
9. Adams, J., and Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. Science *281*, 1322–1326.
10. Chao, D., and Korsmeyer, S. (1998). Bcl-2 family: regulators of cell death. Annu. Rev. Immunol. *16*, 395–419.
11. Vaux, D.L., and Korsmeyer, S.J. (1999). Cell death in development. Cell *96*, 245–254.
12. Adams, J.M., and Cory, S. (2001). Life-or-death decisions by the Bcl-2 protein family. Trends Biochem. Sci. *25*, 61–66.
13. Hengartner, M.O. (2000). The biochemistry of apoptosis. Nature *407*, 770–776.
14. Zimmermann, K.C., Bonzon, C., and Green, D.R. (2001). The machinery of programmed cell death. Pharmacol. Ther. *92*, 57–70.
15. Budihardjo, I., Oliver, H., Lutter, M., Luo, X., and Wang, X. (1999). Biochemical pathways of caspase activation during apoptosis. Annu. Rev. Cell Dev. Biol. *15*, 269–290.
16. Wyllie, A.H. (1997). Apoptosis and carcinogenesis. Eur. J. Cell Biol. *73*, 189–197.
17. Reed, J.C., Miyashita, T., Takayama, S., Wang, H.G., Sato, T., Krajewski, S., Aime-Sempe, C., Bodrug, S., Kitada, S., and Hanada, M. (1996). BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. J. Cell. Biochem. *60*, 23–32.
18. Mattson, M.P. (2000). Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell Biol. *1*, 120–129.
19. Andreeff, M., Goodrich, D.W., and Pardee, A.B. (2000). Chapter 2: Cell Proliferation, Differentiation and Apoptosis. (Hamilton, Ontario, Canada: B.C. Decker, Inc.).
20. Fesik, S.W. (2000). Insights into programmed cell death through structural biology. Cell *103*, 273–282.
21. Shi, Y. (2001). A structural view of mitochondria-mediated apoptosis. Nat. Struct. Biol. *8*, 394–401.
22. Petros, A.M., Medek, A., Nettesheim, D.G., Kim, D.H., Yoon, H.S., Swift, K., Matayoshi, E.D., Oltersdorf, T., and Fesik, S.W. (2001). Solution structure of the antiapoptotic protein bcl-2. Proc. Natl. Acad. Sci. USA *98*, 3012–3017.
23. Muchmore, S.W., Sattler, M., Liang, H., Meadows, R.P., Harlan, J.E., Yoon, H.S., Nettesheim, D., Chang, B.S., Thompson, C.B., Wong, S.L., et al. (1996). X-ray and NMR structure of human Bcl-x_L, an inhibitor of programmed cell death. Nature *381*, 335–341.
24. Aritomi, M., Kunishima, N., Inohara, N., Ishibashi, Y., Ohta, S., and Morikawa, K. (1997). Crystal structure of rat Bcl-x_L. Implications for the function of the Bcl-2 protein family. J. Biol. Chem. *272*, 27886–27892.
25. Suzuki, M., Youle, R.J., and Tjandra, N. (2000). Structure of Bax: coregulation of dimer formation and intracellular localization. Cell *103*, 645–654.
26. McDonnell, J.M., Fushman, D., Milliman, C.L., Korsmeyer, S.J., and Cowburn, D. (1999). Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. Cell *96*, 625–634.
27. Chou, J.J., Li, H., Salvesen, G.S., Yuan, J., and Wagner, G. (1999). Solution structure of BID, an intracellular amplifier of apoptotic signaling. Cell *96*, 615–624.
28. Yang, E., Zha, J., Jockel, J., Boise, L., Thompson, C., and Korsmeyer, S. (1995). Bad, a heterodimeric partner for Bcl-x_L and Bcl-2, displaces Bax and promotes cell death. Cell *80*, 285–291.
29. Oltvai, Z.N., Milliman, C.L., and Korsmeyer, S.J. (1993). Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell *74*, 609–619.
30. Sattler, M., Liang, H., Nettesheim, D., Meadows, R.P., Harlan, J.E., Eberstadt, M., Yoon, H.S., Shuker, S.B., Chang, B.S., Minn, A.J., et al. (1997). Structure of Bcl-x_L-Bak peptide complex: recognition between regulators of apoptosis. Science *275*, 983–986.
31. Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J.C. (1999). Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J. Cell Biol. *144*, 891–901.
32. Zha, J., Harada, H., Yang, E., Jockel, J., and Korsmeyer, S.J. (1996). Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not Bcl-x_L. Cell *87*, 619–628.
33. Slee, E.A., Keogh, S.A., and Martin, S.J. (2000). Cleavage of BID during cytotoxic drug and UV radiation-induced apoptosis occurs downstream of the point of Bcl-2 action and is catalysed by caspase-3: a potential feedback loop for amplification of apoptosis-associated mitochondrial cytochrome c release. Cell Death Differ. *95*, 556–565.
34. Li, H., Zhu, H., Xu, C.J., and Yuan, J. (1998). Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell *94*, 491–501.
35. Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell *94*, 481–490.
36. Kelekar, A., and Thompson, C.B. (1998). Bcl-2-family proteins: the role of the BH3 domain in apoptosis. Trends Cell Biol. *8*, 324–330.
37. Yin, X.M., Oltvai, Z.N., and Korsmeyer, S.J. (1994). BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature *369*, 321–323.
38. Chittenden, T., Flemington, C., Houghton, A.B., Ebb, R.G., Gallo, G.J., Elangovan, B., Chinnadurai, G., and Lutz, R.J. (1995). A conserved domain in Bak, distinct from BH1 and BH2, mediates cell death and protein binding functions. EMBO J. *14*, 5589–5596.
39. Boyd, J.M., Gallo, G.J., Elangovan, B., Houghton, A.B., Malstrom, S., Avery, B.J., Ebb, R.G., Subramanian, T., Chittenden, T., Lutz, R.J., et al. (1995). Bik, a novel death-inducing protein shares a distinct sequence motif with Bcl-2 family proteins and interacts with viral and cellular survival-promoting proteins. Oncogene *11*, 1921–1928.
40. Zha, H., Aime-Sempe, C., Sato, T., and Reed, J.C. (1996). Proapoptotic protein Bax heterodimerizes with Bcl-2 and homodimerizes with Bax via a novel domain (BH3) distinct from BH1 and BH2. J. Biol. Chem. *271*, 7440–7444.
41. Kelekar, A., Chang, B., Harlan, J., Fesik, S., and Thompson, C. (1997). Bad is a BH3 domain-containing protein that forms an inactivating dimer with Bcl-x_L. Mol. Cell. Biol. *17*, 7040–7046.
42. Petros, A.M., Nettesheim, D.G., Wang, Y., Olejniczak, E.T., Meadows, R.P., Mack, J., Swift, K., Matayoshi, E.D., Zhang, H., Thompson, C.B., et al. (2000). Rationale for Bcl-x_L/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. Protein Sci. *9*, 2528–2534.
43. Ottilie, S., Diaz, J.L., Horne, W., Chang, J., Wang, Y., Wilson, G., Chang, S., Weeks, S., Fritz, L.C., and Oltersdorf, T. (1997). Dimerization properties of human BAD. Identification of a BH-3 domain and analysis of its binding to mutant BCL-2 and BCL-x_L proteins. J. Biol. Chem. *272*, 30866–30872.
44. Diaz, J.L., Oltersdorf, T., Horne, W., McConnell, M., Wilson, G., Weeks, S., Garcia, T., and Fritz, L.C. (1997). A common binding site mediates heterodimerization and homodimerization of Bcl-2 family members. J. Biol. Chem. *272*, 11350–11355.
45. Cosulich, S., Worrall, V., Hedge, P., Green, S., and Clarke, P. (1997). Regulation of apoptosis by BH3 domains in a cell-free system. Curr. Biol. *7*, 913–920.
46. Jurgensmeier, J.M., Xie, Z., Deveraux, Q., Ellerby, L., Bredesen, D., and Reed, J.C. (1998). Bax directly induces release of cytochrome c from isolated mitochondria. Proc. Natl. Acad. Sci. USA *95*, 4997–5002.
47. Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R.J., Matsuda, H., and Tsujimoto, Y. (1998). Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. Proc. Natl. Acad. Sci. USA *95*, 14681–14686.
48. Morgan, B.A., Finnegan, N.M., Prevost, G., and Cotter, T.G.

(2000). 91st Annual Meeting of the American Association for Cancer Research. *42*, 4693.

49. Theodore, L., Derossi, D., Chassaing, G., Llirbat, B., Kubes, M., Jordan, P., Chneiweiss, H., Godement, P., and Prochiantz, A. (1995). Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J. Neurosci. *15*, 7158–7167.
50. Ball, K.L., Lain, S., Fahraeus, R., Smythe, C., and Lane, D.P. (1997). Cell-cycle arrest and inhibition of Cdk4 activity by small peptides based on the carboxy-terminal domain of p21WAF1. Curr. Biol. *7*, 71–80.
51. Bonfanti, M., Taverna, S., Salmona, M., D'Incalci, M., and Broggini, M. (1997). p21WAF1-derived peptides linked to an internalization peptide inhibit human cancer cell growth. Cancer Res. *57*, 1442–1446.
52. Selivanova, G., Iotsova, V., Okan, I., Fritsche, M., Strom, M., Groner, B., Grafstrom, R.C., and Wiman, K.G. (1997). Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nat. Med. *3*, 632–638.
53. Williams, E.J., Dunican, D.J., Green, P.J., Howell, F.V., Derossi, D., Walsh, F.S., and Doherty, P. (1997). Selective inhibition of growth factor-stimulated mitogenesis by a cell-permeable Grb2-binding peptide. J. Biol. Chem. *272*, 22349–22354.
54. Hall, H., Williams, E.J., Moore, S.E., Walsh, F.S., Prochiantz, A., and Doherty, P. (1996). Inhibition of FGF-stimulated phosphatidylinositol hydrolysis and neurite outgrowth by a cell-membrane permeable phosphopeptide. Curr. Biol. *6*, 580–587.
55. Holinger, E.P., Chittenden, T., and Lutz, R.J. (1999). Bak BH3 peptides antagonize Bcl-x_L function and induce apoptosis through cytochrome c–independent activation of caspases. J. Biol. Chem. *274*, 13298–13304.
56. Ioannides, C.G., Freedman, R.S., Liskamp, R.M., Ward, N.E., and O'Brian, C.A. (1990). Inhibition of IL-2 receptor induction and IL-2 production in the human leukemic cell line Jurkat by a novel peptide inhibitor of protein kinase C. Cell. Immunol. *131*, 242–252.
57. Kole, H.K., Garant, M.J., Kole, S., and Bernier, M. (1996). A peptide-based protein-tyrosine phosphatase inhibitor specifically enhances insulin receptor function in intact cells. J. Biol. Chem. *271*, 14302–14307.
58. Wang, J.L., Zhang, Z.J., Choksi, S., Shan, S., Lu, Z., Croce, C.M., Alnemri, E.S., Korngold, R., and Huang, Z. (2000). Cell Permeable Bcl-2 Binding Peptides: A Chemical Approach to Apotosis Induction in Tumor Cells. Cancer Res. *60*, 1498–1502.
59. Shimizu, S., Konishi, A., Kodama, T., and Tsujimoto, Y. (2000). BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death. Proc. Natl. Acad. Sci. USA *97*, 3100–3105.
60. Green, M., and Loewenstein, P.M. (1988). Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. Cell *55*, 1179–1188.
61. Derossi, D., Calvet, S., Trembleau, A., Brunissen, A., Chassaing, G., and Prochiantz, A. (1996). Cell internalization of the third helix of the Antennapedia homeodomain is receptor-independent. J. Biol. Chem. *271*, 18188–18193.
62. Schwarze, S.R., Ho, A., Vocero-Akbani, A., and Dowdy, S.F. (1999). In vivo protein transduction: delivery of a biologically active protein into the mouse. Science *285*, 1569–1572.
63. Tzung, S.P., Kim, K.M., Basanez, G., Giedt, C.D., Simon, J., Zimmerberg, J., Zhang, K.Y., and Hockenbery, D.M. (2001). Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat. Cell Biol. *3*, 183–191.
64. Lugovskoy, A.A., Degterev, A.I., Fahmy, A.F., Zhou, P., Gross, J.D., Yuan, J., and Wagner, G. (2002). A novel approach for characterizing protein ligand complexes: molecular basis for specificity of small-molecule Bcl-2 inhibitors. J. Am. Chem. Soc. *124*, 1234–1240.
65. Degterev, A., Lugovskoy, A., Cardone, M., Mulley, B., Wagner, G., Mitchison, T., and Yuan, J. (2001). Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-x_L. Nat. Cell Biol. *3*, 173–182.
66. Wang, J., Liu, D., Zhang, Z., Shan, S., Han, X., Srinivasula, S.M., Croce, C.M., Alnemri, E.S., and Huang, Z. (2000). Structure-based discovery of a novel organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc. Natl. Acad. Sci. USA *97*, 7124–7129.
67. Enyedy, I.J., Ling, Y., Nacro, K., Tomita, Y., Wu, X., Cao, Y., Guo, R., Li, B., Zhu, X., Huang, Y., et al. (2001). Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. J. Med. Chem. *44*, 4313–4324.
68. Kutzki, O., Park, H.S., Ernst, J.T., Orner, B.P., Yin, H., and Hamilton, A.D. Development of a potent Bcl-x_L antagonist based on a-helix mimicry. J. Am. Chem. Soc., in press.
69. Huang, Z. (2000). Structural chemistry and therapeutic intervention of protein-protein interaction in immune response, HIV entry and apoptosis. Pharmacol. Ther. *86*, 201–215.
70. Yu, N., Aramini, J.M., Germann, M.W., and Huang, Z. (2000). Reactions of salicylaldehydes with alkyl cyanoacetates on the surface of solid catalysts: syntheses of 4H-chromene derivatives. Tetrahedron Lett. *41*, 6993–6996.
71. Dunshee, B.R., Leben, C., Keitt, G.W., and Strong, F.M. (1949). The isolation and properties of antimycin A. J. Am. Chem. Soc. *71*, 2436–2437.
72. Tokutake, N., Miyoshi, H., Satoh, T., Hatano, T., and Iwamura, H. (1994). Structural factors of antimycin A molecule required for inhibitory action. Biochim. Biophys. Acta *1185*, 271–278.
73. Miyoshi, H., Tokutake, N., Imaeda, Y., Akagi, T., and Iwamura, H. (1995). A model of antimycin A binding based on structure-activity studies of synthetic antimycin A analogues. Biochim. Biophys. Acta *1229*, 149–154.
74. Orner, B.P., Ernst, J.T., and Hamilton, A.D. (2001). Towards proteomimetics: terphenyl derivatives as structural and functional mimics of extended regions of an a-helix. J. Am. Chem. Soc. *123*, 5382–5383.
75. Ernst, J.T., Kutzki, O., Debnath, A.K., Jiang, S., Lu, H., and Hamilton, A.D. (2002). Design of a protein surface antagonist based on a-helix mimicry: inhibition of gp41 assembly and viral fusion. Angew. Chem. Int. Ed. *41*, 278–281.
76. Reed, J.C. (1994). Bcl-2 and the regulation of programmed cell death. J. Cell Biol. *124*, 1–6.
77. Huang, Z. (2000). The Bcl-2 family of proteins as targets for anticancer drug design. Oncogene *19*, 6627–6631.
78. Liu, D., and Huang, Z. (2001). Synthetic peptides and non-peptidic molecules as probes of structure and function of Bcl-2 family proteins and modulators of apoptosis. Apoptosis *6*, 453–462.
79. Yamaguchi, H., Paranawithana, S.R., Lee, M.W., Huang, Z.W., Bhalla, K.N., and Wang, H.G. (2002). Epothione B analogue (BMS-247550)-mediated cytotoxicity through induction of Bax conformational change in human breast cancer cells. Cancer Res. *62*, 466–471.
80. Milella, M., Kornblau, S.M., Estrov, Z., Konopleva, M., Tari, A., Schober, W.D., Carter, B., Harris, D., Leysath, C.L., Berestein, G.L., et al. (2002). Simultaneous disruption of the Bcl-2 and MEK/MAPK pathways sinergistically induces apoptosis in acute myelogenous leukemia. Blood *62*, 466–471.
81. Veis, D., Sorenson, C., Shutter, J., and Korsmeyer, S. (1993). Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell *75*, 229–240.
82. Evan, G., and Littlewood, T. (1998). A matter of life and cell death. Science *281*, 1317–1322.
83. Webb, A., Cunningham, D., Cotter, F., Clarke, P., di Stefano, F., Ross, P., Corbo, M., and Dziewanowska, Z. (1997). Bcl-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet *349*, 1137–1141.
84. Zangemeister-Wittke, U., Leech, S.H., Olie, R.A., Simoes-Wust, A.P., Gautschi, O., Luedke, G.H., Natt, F., Haner, R., Martin, P., Hall, J., et al. (2000). A novel bispecific antisense oligonucleotide inhibiting both bcl-2 and bcl-x_L expression efficiently induces apoptosis in tumor cells. Clin. Cancer Res. *6*, 2547–2555.
85. Strasberg Rieber, M., Zangemeister-Wittke, U., and Rieber, M. (2001). p53-Independent induction of apoptosis in human melanoma cells by a Bcl-2/Bcl-x_L bispecific antisense oligonucleotide. Clin. Cancer Res. *7*, 1446–1451.

86. Gozani, O., Boyce, M., Yoo, L., Karuman, P., and Yuan, J. (2002). Life and death in paradise. Nat. Cell Biol. *4*, E159–E162.
87. Thornberry, N.A., Rano, T.A., Peterson, E.P., Rasper, D.M., Timkey, T., Garcia-Calvo, M., Houtzager, V.M., Nordstrom, P.A., Roy, S., Vaillancourt, J.P., et al. (1997). A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J. Biol. Chem. *272*, 17907–17911.
88. Deveraux, Q.L., and Reed, J.C. (1999). IAP family proteins–suppressors of apoptosis. Genes Dev. *13*, 239–252.
89. Hay, B.A. (2000). Understanding IAP function and regulation: a view from Drosophila. Cell Death Differ. *7*, 1045–1056.
90. Altieri, D.C. (2001). The molecular basis and potential role of survivin in cancer diagnosis and therapy. Trends Mol. Med. *7*, 542–547.
91. Sun, C., Cai, M., Gunasekera, A.H., Meadows, R.P., Wang, H., Chen, J., Zhang, H., Wu, W., Xu, N., Ng, S.C., et al. (1999). NMR structure and mutagenesis of the inhibitor-of-apoptosis protein XIAP. Nature *401*, 818–822.
92. Sun, C., Cai, M., Meadows, R.P., Xu, N., Gunasekera, A.H., Herrmann, J., Wu, J.C., and Fesik, S.W. (2000). NMR structure and mutagenesis of the third Bir domain of the inhibitor of apoptosis protein XIAP. J. Biol. Chem. *275*, 33777–33781.
93. Chantalat, L., Skoufias, D.A., Kleman, J.P., Jung, B., Dideberg, O., and Margolis, R.L. (2000). Crystal structure of human survivin reveals a bow tie-shaped dimer with two unusual alpha-helical extensions. Mol. Cell *6*, 183–189.
94. Muchmore, S.W., Chen, J., Jakob, C., Zakula, D., Matayoshi, E.D., Wu, W., Zhang, H., Li, F., Ng, S.C., and Altieri, D.C. (2000). Crystal structure and mutagenic analysis of the inhibitor-of-apoptosis protein survivin. Mol. Cell *6*, 173–182.
95. Verdecia, M.A., Huang, H., Dutil, E., Kaiser, D.A., Hunter, T., and Noel, J.P. (2000). Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. Nat. Struct. Biol. *7*, 602–608.
96. Chai, J., Shiozaki, E., Srinivasula, S.M., Wu, Q., Datta, P., Alnemri, E.S., Shi, Y., and Dataa, P. (2001). Structural basis of caspase-7 inhibition by XIAP. Cell *104*, 769–780.
97. Huang, Y., Park, Y.C., Rich, R.L., Segal, D., Myszka, D.G., and Wu, H. (2001). Structural basis of caspase inhibition by XIAP: differential roles of the linker versus the BIR domain. Cell *104*, 781–790.
98. Riedl, S.J., Renatus, M., Schwarzenbacher, R., Zhou, Q., Sun, C., Fesik, S.W., Liddington, R.C., and Salvesen, G.S. (2001). Structural basis for the inhibition of caspase-3 by XIAP. Cell *104*, 791–800.
99. Wu, G., Chai, J., Suber, T.L., Wu, J.W., Du, C., Wang, X., and Shi, Y. (2000). Structural basis of IAP recognition by Smac/DIABLO. Nature *408*, 1008–1012.
100. Liu, Z., Sun, C., Olejniczak, E.T., Meadows, R.P., Betz, S.F., Oost, T., Herrmann, J., Wu, J.C., and Fesik, S.W. (2000). Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. Nature *408*, 1004–1008.
101. Fesik, S.W., and Shi, Y. (2001). Controlling the caspase. Science *294*, 1477–1478.
102. Srinivasula, S.M., Hegde, R., Saleh, A., Datta, P., Shiozaki, E., Chai, J., Lee, R.A., Robbins, P.D., Fernandes-Alnemri, T., Shi, Y., et al. (2001). A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. Nature *410*, 112–116.
103. Wu, J.W., Cocina, A.E., Chai, J., Hay, B.A., and Shi, Y. (2001). Structural analysis of a functional DIAP1 fragment bound to grim and hid peptides. Mol. Cell *8*, 95–104.
104. Olie, R.A., Simoes-Wust, A.P., Baumann, B., Leech, S.H., Fabbro, D., Stahel, R.A., and Zangemeister-Wittke, U. (2001). A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. Cancer Res. *60*, 2805–2809.
105. Shi, Y. (2002). A conserved tetrapeptide motif: potentiating apoptosis through IAP-binding. Cell Death Differ. *9*, 93–95.
106. Chai, J., Du, C., Wu, J.W., Kyin, S., Wang, X., and Shi, Y. (2000). Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature *406*, 855–862.
107. Srinivasula, S.M., Datta, P., Fan, X.J., Fernandes-Alnemri, T., Huang, Z., and Alnemri, E.S. (2000). Molecular determinants of the caspase-promoting activity of Smac/DIABLO and its role in the death receptor pathway. J. Biol. Chem. *275*, 36152–36157.
108. Kipp, R.A., Case, M.A., Wist, A.D., Cresson, C.M., Carrell, M., Griner, E., Wiita, A., Albiniak, P.A., Chai, J., Shi, Y., et al. (2002). Molecular targeting of inhibitor of apoptosis proteins based on small molecule mimics of natural binding partners. Biochemistry *41*, 7344–7349.
109. Guo, F., Nimmanapalli, R., Paranawithana, S., Wittman, S., Griffin, D., Bali, P., O'Bryan, E., Fumero, C., Wang, H.G., and Bhalla, K. (2002). Ectopic overexpression of second mitochondria-derived activator of caspases (Smac/DIABLO) or cotreatment with N-terminus of Smac/DIABLO peptide potentiates epothilone B derivative-(BMS 247550) and Apo-2L/TRAIL-induced apoptosis. Blood *99*, 3419–3426.
110. Fulda, S., Wick, W., Weller, M., and Debatin, K.M. (2002). Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat. Med. *8*, 808–815.