Journal of
Medicinal
Chemistry

Volume 43, Number 18 September 7, 2000

Perspective

Caspases as Targets for Anti-Inflammatory and Anti-Apoptotic Drug Discovery

Robert V. Talanian,* Kenneth D. Brady, and Vincent L. Cryns†

BASF Bioresearch Corporation, 100 Research Drive, Worcester, Massachusetts 01605, and Endocrinology Division, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Illinois 60611

Received February 15, 2000

Introduction

The biochemical mechanisms underlying proinflammatory cytokine maturation and cellular apoptosis are linked by the family of cysteine proteases known as caspases. These enzymes have required roles in both processes and are widely considered promising targets for drug discovery. Both inflammation and apoptosis are at the core of common medical conditions for which available drug therapies are poor. Cytokines have central roles in inflammatory diseases such as rheumatoid arthritis and septic shock, and inhibition of their action or activation is a proven or promising approach to modulation of these diseases.[1] Apoptosis can play a role in disease when it is either excessive or insufficient. Tissue damage following stroke or myocardial infarction is largely apoptotic, and there is growing evidence that inhibition of that apoptosis can lessen tissue damage and improve a patient's prospects. Conversely, there is mounting evidence that apoptosis mechanisms are defective in cancer, and it is reasonable to speculate that a selective apoptosis-stimulating therapeutic would have anticancer activity, either alone or as a chemotherapeutic sensitizer.

The caspases are a family of cysteine proteases with at least 12 human members and strong phylogenetic conservation. Because of their roles in inflammation and apoptosis, the caspases have received enormous research interest and there has been an explosion in understanding of their properties and biological roles. The caspases share structural properties. Each in its active form is a homodimer of heterodimers derived from proteolytic processing and association of inactive precursor polypeptide chains. Caspase specificity dictates cleavage C-terminal to aspartic acid residues, a property shared only with Granzyme B, a serine protease that also participates in apoptosis. The various biological roles of the caspases derive from differences in their specificity as well as the effects of their distinct regulatory pro-domains. In cytokine maturation, at least one caspase cleaves and activates precursor molecules including those of interleukin-1β (IL-1β) and interleukin-18 (IL-18). In apoptosis, caspase cleavage of a limited number of substrates results in activation of some enzymes (including, prominently, caspases), inactivation of others, and disassembly of cytoskeletal proteins. How these changes add up to apoptosis is not well understood, but it is clear that the process is precise and orderly. Evidence from gene knockout animals and from relatively nonspecific peptidic caspase inhibitors suggests that inhibition of caspases would be sufficient to block apoptosis or caspase-mediated cytokine maturation. These results encourage further efforts to delineate the roles of each of these enzymes and to discover inhibitory drugs that might be effective in a variety of important diseases.

The promise of caspases as drug targets has encouraged a great deal of effort to find potent and selective inhibitors with pharmaceutically acceptable properties. Studies of caspase structure, specificity, and catalytic mechanism have yielded insight into the design of compounds selective for this new class of cysteine protease inhibitors. Important strategies for inhibitor design include use of appropriate electrophiles for interaction with the catalytic cysteine and design of

* To whom correspondence should be addressed. Tel: 508-849-2581. Fax: 508-754-7784. E-mail: talanir@BASF.com.
† Northwestern University Medical School.

10.1021/jm000060f CCC: $19.00
Published on Web 08/19/2000

Table 1. Known Caspase Family Members and Properties[a]

caspase	P_4 peptide specificity[b]	proposed physiologic roles	pro-domain function[c]
1	W, Y	cytokine maturation	(proximity-induced activation?)
4	W, L	(cytokine maturation?)[d]	unknown
5	W	(cytokine maturation?)[d]	unknown
13	unknown	(cytokine maturation?)[d]	unknown
2	D	death receptor signaling	proximity-induced activation
8	I, L	death receptor signaling	proximity-induced activation
9	L	mitochondrial signaling	proximity-induced activation
10	I	death receptor signaling	proximity-induced activation
14[e]	unknown	(death receptor signaling?)[d]	unknown; short
3	D	effector; many substrates	unknown; short
6	T, V	effector; cytoskeleton[f]	unknown; short
7	D	effector; many substrates	unknown; short

[a] Cloning of caspase-1 through -10 and -13 is reviewed in ref 2; a human caspase-14 sequence is reported in ref 3. [b] Single amino acid codes are given for preferred residues at the P_4 substrate position and represent consensus between combinatorial and defined peptide specificity studies.[43,42] For some, two similarly preferred amino acids are listed. [c] Caspase-3, -6, -7, and -13 have pro-domains of 40 amino acids or less; in contrast, the pro-domains of the other caspases are >100 residues. [d] Proposed role based on homologies to better-understood caspases. [e] Catalytic activity for caspase-14 has not been demonstrated. [f] Caspase-6 can also directly activate caspase-3 and possibly -7 and so can be considered to have both effector and signaling roles.[288]

peptidomimetic moieties to interact with critical substrate-recognition binding sites.

The caspase field has seen rapid advance and has been the topic of many excellent reviews, especially regarding the biochemical mechanisms of apoptosis (see, for example, ref 2 and references therein). Identification of the key molecular participants in inflammation and apoptosis, as well as their functions and interrelationships, is continually supporting particular caspases and associated molecules as potential new targets for drug discovery. Many approaches to the design of inhibitors of caspase-1 have been described in the literature, but there have been few reports on inhibitors of other caspases. Success in clinical use of caspase-directed drugs has not been reported. Accordingly, in this Perspective, we will focus on recent developments in caspase-mediated apoptosis and cytokine maturation that direct our selection of drug targets, as well as strategies and progress in drug lead discovery, primarily toward caspase-1.

The Caspase Gene Family

A Conserved Family of Cysteine Proteases. There are 12 described human caspases[3,2] (Table 1). Orthologs are known for most in the mouse[3−8] as well as in several other organisms. Most of the human caspases were cloned or recognized in sequence databases based on homology with known caspases. Therefore, homology in this family is to some degree a self-fulfilling prophecy. Granzyme B, a serine protease unrelated in sequence to the caspases, shares both substrate specificity and a biological role in promoting apoptosis with the caspases and so can be considered functionally a caspase family member. There may be many other such proapoptotic Asp-specific proteases not yet described.

The role of caspases in apoptosis is also highly conserved. This was first proposed based on homology between caspase-1 and CED-3, a protein that is required for developmental apoptosis in the nematode *C. elegans*.[9] CED-3 is a cysteine protease,[10,11] and its proapoptotic role in this model organism has been studied in detail.[12]

Caspase Roles in Cytokine Maturation and Apoptosis. Caspases have well-established biochemical roles in both cytokine maturation and apoptosis. As described in detail below, caspases activate proinflammatory cytokines and cleave numerous substrates associated with apoptosis, all of which can be blocked by caspase inhibitors. Further, caspase-deficient animals are defective in cytokine maturation or apoptosis, depending on which caspase is absent. Homologies and functional studies suggest categorization of most of the caspases in cytokine maturation, apoptosis signaling, and apoptosis effector roles, as summarized in Table 1.

At a molecular level, cytokine maturation and apoptosis seem unrelated. However, the two are clearly cross-regulatory at least at a higher functional level. Many cytokines have strong proliferative and anti-apoptotic effects on cells. Conversely, elimination of autoreactive T lymphocytes or those in "immune-privileged" tissues, as well as resolution of immune responses, is effected by caspase-mediated apoptosis.[13−18] Additionally, cytotoxic lymphocytes destroy target cells (for example, damaged or virally infected) by mechanisms including induction of target cell apoptosis.[19] It is not obvious, however, why caspases are involved in both processes. For example, there is no evidence for opposite regulation of cytokine-maturing and apoptosis-inducing caspases during lymphocyte proliferation and down-regulation. Nor is there evidence for combined pro- and anti-apoptotic roles of single caspases, such as that of TNF signaling through the TNF receptor subtype 1 (TNFR1).[20] Such mechanism(s) might provide evolutionary pressure to keep caspases involved in both processes.

Caspase Structure and Function

The caspases are transcribed as inactive zymogens and are activated by proteolytic removal of a pro-domain and separation of the large (∼p20) and small (∼p10) subunits of the active enzyme. Some caspases are cleaved at two closely spaced sites between the large and small subunits, releasing a short linker peptide.[2]

Caspase Pro-Domains. Many of the differences in function between the caspases are due to their pro-domains. They range from 23 to 219 amino acids, and all are proteolytically removed during caspase activation. At least for the longer pro-domains, a major function is zymogen subcellular localization. The caspase-2 pro-domain causes nuclear localization,[21] and indeed fusion of this domain to another caspase or to an unrelated protein can also drive nuclear localization.[21] Some caspases change subcellular localization on acti-

vation.[22] Caspase-3 and -7, which have short (23-residue) pro-domains, migrate to different compartments upon activation during apoptosis,[23] suggesting either that these short domains are fully competent to direct localization and/or that localization information resides in other parts of the proteins.

Interaction with Adaptor Proteins. As described below, caspase interaction with adaptor proteins can result in proximity-induced cross-activation of the caspase zymogens. The caspase zymogens possess minimal catalytic activity, which is sufficient in protein complexes for cross-activation.[24−26] These interactions are mediated by homologous 'adaptor domains' (for a review, see ref 27) present in the long pro-domains of most caspases as well as in many death receptors and adaptor proteins. Those present in caspases are known as death effector domains (DEDs) and caspase recruitment domains (CARDs).[28] Solution or crystal structures of the DEDs of the receptor Fas[29] and the adaptor FADD,[30] as well as the CARDs of the adaptor RAIDD/CRADD[31] and Apaf-1,[32,33] have been reported. These form α-helical bundles of similar topology. The cocrystal structure of a complex of the CARDs of Apaf-1 and caspase-9 revealed the first caspase pro-domain structural information.[34] CARD/CARD interactions are stabilized by complementary basic and acidic amino acids on the adaptors and caspases, respectively, as well as by hydrophobic amino acids.[34]

Crystal Structures. Crystal structures have been reported for the activated forms of caspases representing all three biological roles: caspase-1 (cytokine-maturing),[35,36] caspase-3 (apoptosis effectors),[37,38] and caspase-8 (apoptosis signaling).[39,40] The structures reveal important similarities and differences between the enzymes. The topologies of the three are similar. Three-dimensional alignment between the Cα atoms of caspase-1 and -3 shows root-mean-square deviations of only 1.2 Å;[37,38] for the caspase-1/8 and caspase-3/8 pairs the values were 0.8 and 0.9 Å, respectively.[39] The active species of each is a homodimer of (p20)(p10) heterodimers. The caspase-1 homodimer interface is formed by three pairs of antiparallel β-strands: one at the core of the complex (contributed by the two p10 subunits) and two symmetric pairs (contributed by the p20 and p10 subunits of opposite halves of the homodimer) extending away from the core. The homodimer forms 16 interface backbone hydrogen bonds[35,36] and buries approximately 5200 Å of surface area,[36] presumably stabilizing the complex. Similar domain interactions and topologies are observed for caspase-3 and -8. For all three enzymes, the two active sites of the homodimer are distant, and in agreement with catalytic and inhibitor studies, no allosteric interactions between active sites are evident.

Caspase Specificity. Caspase specificity toward peptidic substrates has been studied in detail as a means of both developing specific substrate and inhibitor probes of each enzyme and predicting preferences toward natural substrates and thereby their biological roles. Starting with literature precedents for peptide derivatives known as substrates or inhibitors of other cysteine proteases, the cleavage site within IL-1β rapidly led to a tetrapeptidic aminomethylcoumarin (amc) fluorogenic substrate (Ac-YVAD-amc, $K_m = 14\ \mu M$) and aldehyde (cho) inhibitor (Ac-YVAD-cho, $K_i = 0.76$ nM) for caspase-1.[41] Combinatorial[42] and defined peptide[43] specificity approaches have been applied to the caspase family. Most caspases recognize tetrapeptidic probes efficiently, following the original observations for caspase-1,[41] with the exceptions that caspase-2 requires a pentapeptide substrate and that caspase-5 requires much longer substrates, spanning four "nonprime" as well as five or more "prime-side" residues (R. V. Talanian, unpublished observations). These approaches have led rapidly and predictably to similarly efficient substrates and potent inhibitors for each of the caspases so far studied.[41,43] Caspases display an apparently absolute requirement for Asp in the P_1 position of substrates, a preference for Glu in P_3, and one of several preferences in P_4 (principally Asp, aliphatic (Val, Leu, and the like) and aromatic (mainly Trp and Tyr)) that differentiate the caspases somewhat (Table 1). These properties rank the caspases among the most specific proteases known. While they do vary in specificity, especially at P_4, these differences are not sufficient to dictate distinct roles for the enzymes. Tissue distribution studies show that most caspases are expressed broadly, giving few clues to the possible individual functions of the enzymes. The function of pro-domains and associated adaptor proteins may contribute to whatever nonredundant functions the caspases may posess.

It is difficult to predict biological roles from peptide substrate specificity. Efficiently cleaved protein substrates often contain cleavage site sequences that do not match optimal peptide substrate sequences. For example, the P_4-P_1 caspase-1 cleavage sites in IL-1β and IL-18 are YVHD and LESD, respectively, whereas peptide studies suggest a consensus caspase-1 preference for the sequence WEVD. One might not have confidently predicted either cytokine as a caspase-1 substrate from its peptide specificity. Surveys of protein sequence databases to find caspase substrates are therefore impractical. Peptidic probes are typically recognized by several of the caspases, limiting their value as probes of their individual in vivo functions. In some cases substrates can be ruled out on the basis of selectivity. For example, caspase-3 has a nearly absolute requirement for Asp in P_4, and as predicted, it cleaves neither IL-1β nor IL-18 efficiently. Currently, commercially available monoclonal antibodies remain the best specific probes of individual caspases. A panel of specific small-molecule inhibitors for each caspase would be extremely useful.

Structural Basis of Substrate Specificity. The caspase structures shed some light on their substrate recognition properties. Each was determined with a bound tetrapeptidic inhibitor: either a potent (<1 nM) reversible aldehyde[36,37,40] or an irreversible alkyl-enzyme adduct.[35,38,39] In each case, the peptide bound in an extended, β-like conformation, with side chains interactions in the S_1 through S_4 subsites. The caspase-1 structure confirmed the expected role of Cys285 as the catalytic nucleophile based on biochemical studies[41] and revealed roles for His237 and the backbone carbonyl of Pro177 in a H-bonding network. The analogous carbonyl−His Nε interaction is seen most clearly in the 1.2 Å structure of caspase-8.[40] Caspases require Asp in substrate P_1 positions. This strict conservation of function corresponds with a strict conservation of the amino

acids constituting the S_1 pocket, for which the three enzymes are identical except for a Ser-to-Thr substitution in caspase-8. The S_1 pockets contain two Arg residues that complement the P_1 Asp side chain charge. The S_2 pockets are shallow, consistent with relatively broad substrate tolerance. The caspases share a preference for Glu in P_3. This is due to ionic interaction with the highly conserved Arg341, which also forms part of the S_1 pocket. P_4 is the major site for specificity variation between the caspases, and again the structures offer some explanation. The caspase-1 S_4 pocket is a large hydrophobic channel, consistent with its aromatic amino acid preference. S_4 of caspase-3 is substantially smaller, being at least partially filled by a 10-residue insert with respect to caspase-1. The site offers hydrogen bond donors complementary to the Asp side chain that is preferred by caspase-3 in this position, as well as a steric match consistent with relatively poor cleavage of peptides containing P_4 Glu.[43,42] Similar interactions with P_4 Asp may be possible for caspase-8. The caspases vary not only in their specificity in P_4 but also in their degree of specificity. For example, caspase-1 prefers aromatic residues in P_4 but recognizes many other residues efficiently, including those containing Asp. In contrast, caspase-3 is highly selective for Asp in P_4. Flexibility, a property not easily measured by crystallography, may be a major contributor to the observed variation in the degree of caspase specificity. Dynamics simulations or cocrystallization with a variety of ligands might explain the variations in the degree of substrate specificity between the caspases and the implications of that variability for drug discovery.

Caspase Regulation

Transcription and Translation. Both the cytokine-maturing and pro-apoptotic functions of caspases can occur in the absence of transcription or translation. Quantitative experiments using human PBMC preparations or the monocytic cell line THP.1 showed that, when unstimulated, each contains a large pool of caspase-1 precursor and that LPS stimulation activated only a small fraction of the caspase-1 zymogen without depleting the precursor pool.[44] During apoptosis, substantial fractions of caspase zymogens can be activated, but protein synthesis inhibitors are often ineffective in blocking caspase activation. Little is known regarding transcriptional regulation of most caspases. The limited available data suggests that caspase transcription or translation is not a promising target for anticaspase drug discovery.

Proteolysis. Because caspase activation is primarily autoproteolytic, the obvious targets for inhibition of caspase activation are the caspases themselves. As described above, death receptor-mediated activation of caspase-8 and -9 occurs by proximity-induced cross-cleavage of the zymogens. The effector caspase-3 and -7 contain at their p10/p20 maturation sites the sequences IETD(175)-S and IQAD(198)-S, respectively.[45,46] The sites match the specificity of other caspases well (but not of caspase-3 and -7 themselves), suggesting that the maturation of these enzymes is catalyzed by others such as caspase-6, -8, and -9, for which the cleavage sequences are far more suitable.[43,42]

Phosphorylation. There is some evidence that phosphoryation or other covalent posttranslational modification (other than proteolytic activation) participates in caspase regulation. The serine/threonine kinase Akt/PKB is activated by phosphoinositide 3-phosphate-dependent kinases and opposes apoptosis by several mechanisms including catalytic inactivation of caspase-9.[47] This suggests a particularly important regulatory role for caspase-9 in apoptosis generally, since there is currently no evidence for similar Akt-mediated inactivation of other caspases. Other caspases may also be negatively regulated by phosphorylation,[48] but the evidence is indirect and the phosphorylating kinase(s) unknown. Thus it is at least premature to propose kinases or phosphatases as drug targets for modulation of caspase activation or activity.

Inhibitory Proteins. The first caspase-inhibitory molecules described were viral serpins, which, by inhibiting caspases, block inflammatory and apoptotic mechanisms that would otherwise oppose their proliferation.[49−52] Another class of viral caspase inhibitors unrelated in sequence to serpins is the IAPs (for *I*nhibitor of *AP*optosis), for which the human homologues NAIP, c-IAP-1/HIAP-2/hMIHB, c-IAP-2/HIAP-1/hMIHC, XIAP/hILP/MIHA, and survivin[53−60] are known. A constitutive role for NAIP in inhibition of neuronal cell death is suggested by the observation of deletions in this gene associated with the neurodegenerative disease spinal muscular atrophy (SMA).[54] The global role of the IAP proteins in regulation of apoptosis remains unclear.

Nitric Oxide. NO has roles in both promoting and opposing apoptosis.[61,62] One important mechanism of NO-induced cytotoxicity[63,64] may be peroxynitrite formation (from reaction of NO and superoxide anions)[65] and subsequent DNA adduct formation,[66,67] resulting in p53 induction.[68] NO can have anti-apoptotic effects as well, as initially demonstrated for B lymphocytes.[69] NO is a thiol-modifying agent[70] and can inhibit all caspases in vitro in a redox-reversible manner[71] by adduct formation with the active site cysteine and possibly others.[72,73] The anti-apoptotic effects of shear stress[74] or a cytokine mixture[75] can be blocked by NOS inhibitors, suggesting that endogenously produced NO has anti-apoptotic activity. Similarly, NOS inhibitor studies suggest that cytokine maturation by caspases is negatively and constitutively regulated by NO.[76] Fas-induced apoptosis is stimulated by NOS inhibitors,[77] and Fas stimulation results in denitrosylation of constitutively NO-inhibited caspases,[78] revealing a second mechanism (in addition to proteolysis) by which Fas stimulation activates caspases. Pharmacologic manipulation of caspase-mediated apoptosis has been demonstrated by inhibition of TNF-α/galactosamine-induced apoptosis and hepatotoxicity in rats by the NO donor V-PYRRO/NO, which derives tissue specificity from its liver metabolism.[79] NO modulation may therefore be a viable means of altering caspase activity in disease, but achieving tissue specificity by this approach may frequently be difficult.

Signal Transduction Pathways

Cytokine Maturation. Caspase-1 cleaves and thereby activates the pro-forms of the inflammatory cytokines IL-1β and IL-18.[41,80−82] Gene knockout[83,84] and inhibitor studies[41,85−87] support a critical role for caspase-1 in

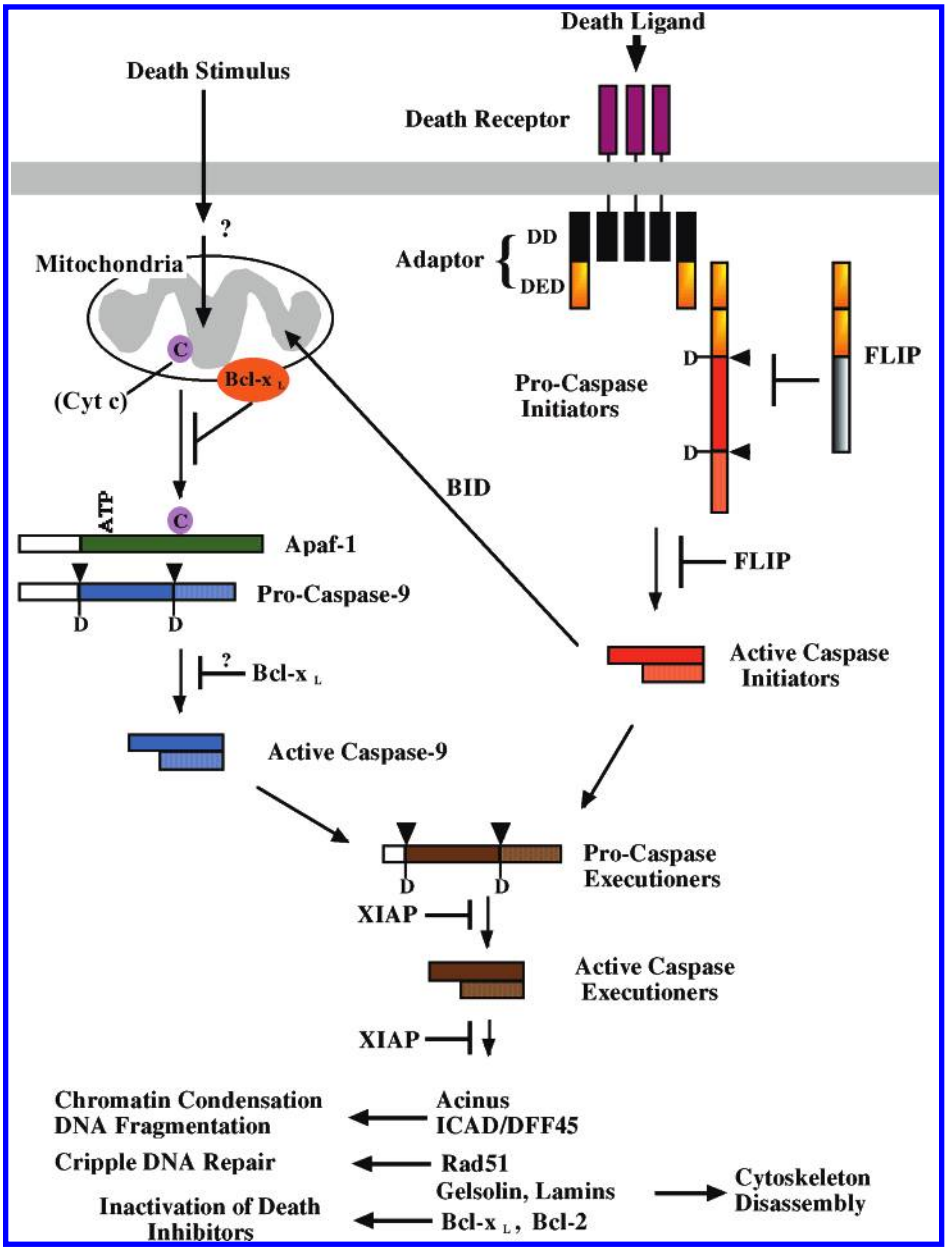


Figure 1. A cellular dead end: caspase activation and proteolytic signaling in apoptosis. (Reprinted by permission with minor modification.[12])

both processes. Although several of the caspases are highly homologous to caspase-1 (especially caspase-4, -5, and -13), it remains unclear whether any participate in cytokine maturation. Caspase-1 and -11 knockout mice have similar phenotypes.[8] The cross-species homologies of the caspase-1 subfamily, in contrast to other caspases, are insufficient to predict with confidence the human ortholog of murine caspase-11 (this is why no human enzyme has been named caspase-11 or -12). Still, these studies suggest that, at least in mice, one or more additional caspases are required for IL-1β maturation, perhaps in a cascade. Any such enzyme might be a viable target for anti-inflammatory inhibitor design.

Protein−protein interactions involved in caspase activation may also be viable drug targets. The caspase-1 pro-domain interacts with the kinase CARDIAK, through the CARD domains on each.[88] CARDIAK also interacts with the TNF receptor-associated adaptors TRAF1 and TRAF2.[88] The Toll-like receptor TLR4 recognizes LPS, and related receptors recognize other bacterial cell wall components.[89] These events result in release of cytokines including IL-1β, but the steps in signal transduction between such membrane events and caspase activation are unknown. One hypothesis is that CARDIAK acts as an adaptor linking Toll-like receptor(s) to caspase-1 activation, analogous to the role that FADD plays in Fas-induced caspase-8 activation. The role, if any, of the kinase activity of CARDIAK is unknown.

Caspase Cross-Activation. Caspase zymogen activation may occur in a loosely ordered fashion, in which individual caspases have primarily "signaling" or "effector" roles.[90] There are at least two mutually interacting/amplifying routes: a mitochondrial pathway and a death receptor pathway (see Figure 1). Although apoptotic cell death may be mediated by one pathway or the other depending on the stimulus, some mechanisms of cell death require both for efficient execution.[91−93] For instance, activation of upstream caspases by recruitment to death receptors can directly trigger mitochon-

Table 2. Representative Caspase Substrates in Apoptosis

category	protein(s)	consequence of cleavage	reference
apoptosis-modulating	Bcl-2, Bcl-x_L	change from anti- to pro-apoptotic activity	124−127
	BID	stimulates mitochondrial cytochrome *c* release	94, 95
	XIAP	eliminates anti-apoptotic activity	128
	RIP	impairs TNF-α-induced NF-κB activation; sensitizes cell to TNF-α-induced apoptosis	130
	IκBα	blocks NF-κB activation; sensitizes cell to apoptosis	131
DNA metabolism/repair	Rad51	cleavage product binds but does not repair DNA lesions	145
	PARP, DNA-PKcs	products bind DNA lesions but fails to signal for repair	148−152
	acinus	product induces chromatin condensation	153
	DFF-45	activation of nuclease DFF-40; DNA fragmentation	154−156
structural	gelsolin	dysregulation of actin-depolymerizing activity	164
	actin, lamin, Gas2, others	direct morphological effects on cell and nuclear membranes	157−161, 166
	FAK, β-catenin	disruption of cell−cell contacts	162, 168, 170

drial injury and cytochrome *c* release by the proteolysis of key targets such as BID.[94,95] Both pathways rely on protein interaction-induced proximity of procaspases for activation.

Signal Amplification via Mitochondria. In the mitochondrial pathway, the molecular event initiating caspase activation is the cytoplasmic release of cytochrome *c* from the mitochondria (Figure 1).[96] This incendiary event is modulated by Bcl-2 family members that are anti-apoptotic (e.g., Bcl-2 and Bcl-x_L) and pro-apoptotic (e.g., Bax and Bak), and it does not require caspase activity.[96−102] How these regulate cytochrome *c* release is unclear. In one model, Bcl-2 and Bcl-x_L prevent the early apoptotic mitochondrial influx of ions and subsequent swelling that leads to outer membrane rupture and cytochrome *c* release into the cytoplasm.[103] In contrast, Bax promotes these mitochondrial events, perhaps by interacting with components of the mitochondrial permeability transition pore complex.[104,102] In another (not mutually exclusive) model, Bax and Bak bind to and open the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, allowing cytochrome *c* to pass through this channel; conversely, Bcl-x_L binds to and closes the VDAC, preventing cytochrome *c* release into the cytoplasm.[105]

Cytochrome *c* initiates the assembly of an oligomeric complex of itself, ATP/dATP, and the adaptor protein Apaf-1, which recruits pro-caspase-9, resulting in its proximity-induced cross-activation.[106−112] Apaf-1 contains an N-terminal CARD and a C-terminal domain with multiple WD-40 repeats. These repeats interact with the CED-4-like region of APAF-1, blocking oligomerization. This interaction is reversed by binding of cytochrome *c* and ATP/dATP.[106] The multimeric Apaf-1/cytochrome *c* complex recruits procaspase-9 by CARD−CARD interactions between Apaf-1 and procaspase-9.

Death Receptor Signaling. Binding of death-inducing ligands of the TNF/NGF family such as Fas, TNF-α, and TRAIL to the extracellular domains of their cognate "death receptors" (Fas, TNFR1, and DR-4/DR-5, respectively) results in a series of protein−protein interactions culminating in the recruitment of procaspase-8 and -10 to the death-inducing signaling complex (DISC) and their subsequent cross-activation (Figure 1; reviewed in refs 12, 113−115). Death ligand binding to its receptor leads to the recruitment of several "death domain"-containing proteins including FADD/MORT1.[116,117] This adaptor protein has an N-terminal DED also present in the prodomains of caspase-8 and -10.[118,119] FADD/MORT1 recruits these procaspases to the DISC via DED interactions. The caspase-induced proximity of the caspase zymogens promotes their cross-activation. The recruitment and activation of these DED-containing procaspases can be blocked by FLIP, an endogenous death receptor antagonist that resembles procaspase-8 but is catalytically inactive. Recruitment of FLIP to the DISC via its DEDs displaces procaspase-8 and -10 from the DISC, preventing activation.[120−123]

Caspase Proteolytic Signaling in Apoptosis: A Dead End

Despite the wealth of data supporting the critical role of caspases in apoptosis execution, the essential mechanisms by which caspases kill the cell are just beginning to be understood. Caspases are highly specific proteases and effect death by cleaving a limited number of cellular proteins. Presumably, it is the collective action of multiple caspases on multiple downstream targets that effects the biochemical and morphological characteristics of apoptosis. Although a wide array of proteins have been proposed as caspase substrates (see refs 2, 12 for comprehensive lists), relatively few have been clearly linked to induction of cell death. Some of those that are relatively well-understood are summarized in Table 2 and described below.

Inactivation of Anti-Apoptotic Proteins. Several apoptotic cell death antagonists are inactivated by caspases. For instance, caspases cleave the anti-apoptotic molecules Bcl-2 and Bcl-x_L in their N-terminal domains to generate C-terminal products that trigger mitochondrial release of cytochrome *c* and subsequent cell death.[124−127] Cleavage-resistant Bcl-2 and Bcl-x_L mutants are more potent inhibitors of apoptosis than the corresponding wild-type proteins.[124−127] Similarly, caspase cleavage of XIAP disrupts its ability to inhibit death receptor-induced cell death.[128] By at least two mechanisms, caspases also disrupt activation of the transcription factor NF-κB, which normally activates anti-apoptotic gene expression, including members of the IAP family.[129] First, caspases inactivate RIP, which normally signals downstream from TNFR1.[130] Caspase-cleaved RIP is severely impaired in NF-κB activation and sensitizes cells to TNF-α-induced cell death. A caspase cleavage-resistant RIP mutant antagonizes death receptor-induced apoptosis.[130] Second, caspases cleave the inhibitor of NF-κB, IκBα, producing a C-

terminal fragment that is resistant to proteasomal degradation and stably inhibits NF-κB, sensitizing cells to apoptosis.[131]

Activation of Pro-Apoptotic Proteins. Cleavage of the pro-apoptotic Bcl-2 family member BID by caspase-8 produces a C-terminal product that moves from the cytoplasm to the mitochondria, where it induces cytochrome *c* release.[94,95] In this way, BID links the death receptor pathway to the mitochondrial pathway and amplifies the death signal initiated by death receptor activation. The importance of this mitochondrial amplification mechanism in vivo is illustrated by the resistance of BID-deficient mice to Fas-induced liver destruction and subsequent death.[132] Caspases also cleave and activate a number of protein kinases, such as MEKK-1, PKCδ, -θ, and -μ, and PAK2, by removing autoinhibitory domains.[133−139] With the exception of PKCμ,[139] overexpression of each of these caspase-cleaved kinases (but not their full-length counterparts) induces apoptosis. The downstream targets of these kinases relevant to cell death are unknown.

Inactivation of DNA Repair Factors. Caspases also promote apoptosis by disrupting repair processes that are essential for cell survival. Several enzymes that recognize and/or repair DNA damage, such as PARP, DNA-PKcs, RF-C140, Rad51, and ATM, are cleaved and inactivated by caspases.[140−146] Cleavage of Rad51 has been most directly linked to the execution of cell death.[145] Rad51 is essential in genetic recombination and in the repair of DNA double-strand breaks.[147] Ionizing radiation induces caspase cleavage of Rad51 into two fragments that bind DNA but have no recombinase activity. Expression of a caspase cleavage-resistant Rad51 mutant provides partial protection against ionizing radiation-induced (but not TNF-α-induced) cell death. Poly(ADP-ribose) polymerase (PARP) and the catalytic subunit of DNA-dependent proten kinase (DNA-PKcs) each recognize DNA lesions and signal for other factors to effect their repair. Caspase cleavage of each leaves a fragment that recognizes lesions but fails to signal for repair, and may actually block such repair.[148−152]

Activation of DNA Fragmentation. Caspases mediate chromatin condensation and DNA fragmentation by proteolytic activation of two distinct proteins. Caspases cleave the nuclear factor Acinus to produce a C-terminal fragment that, unlike the full-length protein, induces chromatin condensation but not DNA fragmentation in vitro and in vivo.[153] DNA fragmentation is dependent on caspase activation of the endonuclease DFF-40 (CAD in mice) that is normally present in the cytoplasm complexed to the inhibitor DFF-45 (ICAD in mice).[154−156] Caspases inactivate DFF-45, allowing DFF-40 to move to the nucleus where it degrades DNA.

Disassembly of the Cytoskeleton. Caspases also effect characteristic morphological changes directly as well as severing intercellular contacts by cleaving several structural proteins. Caspases cleave several key components of the microfilament system (actin in some systems, Gas2, and gelsolin), the intermediate filament network (nuclear lamins, cytokeratins, and vimentin), and adhesion complexes (FAK and β-catenin).[157−170] Again, the functional significance of these individual proteolytic events is largely unknown. Gelsolin cleavage gives an N-terminal product that stimulates actin filament depolymerization in a calcium-independent manner and directly induces apoptosis.[164] Conversely, overexpression of a caspase cleavage-resistant mutant lamin A delays the dismantling of the nuclear envelope and subsequent chromatin condensation/DNA fragmentation that normally occurs during apoptosis.[160] Although the details are unclear, these findings clearly indicate that caspase cleavage of cytoskeletal targets leads to the dismantling of the actin and intermediate filament networks and the severing of intercellular contacts.

Caspases in Disease

In addition to their role in developmental and homeostatic programmed cell death, caspases mediate pathological cell death in many diseases.[12,171,172] These can be classified into (i) disorders of *deficient* caspase activity (inactivating caspase mutations or up-regulation of endogenous caspase inhibitors) resulting in enhanced cell survival (e.g., cancer and autoimmune diseases) and (ii) disorders of *excessive/inappropriate* caspase activity (increased caspase activation or loss of endogenous caspase inhibitors) leading to accelerated cell death (e.g., neurodegenerative diseases and ischemic tissue injury). In this section, we will review the evidence implicating caspases in these diverse disease processes and examine caspases as potential therapeutic targets.

Disorders of Deficient Caspase Activity. Perhaps the strongest evidence supporting participation of caspases in disease comes from the observation that autoimmune lymphoproliferative syndrome (ALPS) type II, an inherited disease characterized by lymphocyte and dendritic cell resistance to apoptosis, is caused by missense mutations in the caspase-10 gene.[173] These mutations impair the catalytic activity of caspase-10 and inhibit its ability to induce apoptosis when transfected into mammalian cells. Some of these mutations act in a dominant negative fashion. Introduction of one patient's mutant caspase-10 into dendritic cells containing wild-type caspase-10 rendered them partially resistant to death receptor-induced apoptosis. The impaired clearance of antigen-presenting dendritic cells in lymph nodes in this disorder may lead to excessive stimulation of immune cells and subsequent breakdown of immune tolerance.[173]

Neoplasms apply similar genetic strategies to acquire resistance to apoptosis and to some anticancer drugs.[174] Loss of caspase-8 expression by genetic deletion or by hypermethylation has been described in a subset of aggressive neuroblastomas that are resistant to death receptor-mediated apoptosis.[175] These findings suggest that caspase-8 and others may function as tumor suppressors whose inactivation in neoplasms promotes cancer cell survival by reducing their sensitivity to cell death. Similarly, caspase-3 expression levels correlate with prognosis and survival in neuroblastomas[176] and non-small-cell lung carcinomas.[177] Some tumors have acquired diminished caspase activity by genetic alterations that lead to the overexpression of endogenous caspase inhibitors. For instance, up-regulation of the caspase-inhibitory protein FLIP has been observed in metastatic melanomas and Burkitt's lymphoma cell lines.[122,178] Similarly, survivin is commonly expressed in a broad spectrum of malignancies but not in normal

adult tissue.[179] This protein binds and inactivates caspase-3 and -7 and confers resistance to apoptosis.[180] These observations suggest that inhibition of caspase activity is a common theme in tumorigenesis, providing neoplasms with a selective survival advantage.

Disorders of Excessive/Inappropriate Caspase Activity. Several lines of evidence indicate that caspases are inappropriately activated in neurodegenerative disorders and contribute to the death of neurons. Activated caspase-8 has been identified in degenerating neurons from Huntington's disease patients,[181] and caspase-3 expression is up-regulated in apoptotic hippocampal neurons from Alzheimer's disease patients.[182] Moreover, murine caspase-12, a family member confined to the endoplasmic reticulum,[183] is an essential mediator of neurotoxicity by amyloid-β: mice deficient in caspase-12 are selectively resistant to endoplasmic reticulum stressors such as amyloid-β.[183] Also, actin and amyloid-β precursor protein (APP) are specifically cleaved by caspases in senile plaque-associated neurons from Alzheimer's patients.[182,184] Many of the gene products implicated in neurodegenerative diseases, including the presenilins, APP, tau, and the variable-length polyglutamine tract-containing proteins huntingtin, the androgen receptor, ataxin-3, and atrophin-1, are caspase substrates.[182,185−191] Mutations in these genes may lower the threshold for caspase activation in neurons, leading to caspase cleavage and the generation of pro-apoptotic products. For example, caspase cleavage of ataxin-3 containing an expanded polyglutamine tract generates a truncated polyglutamine peptide that directly recruits pro-caspase-8 to polyglutamine inclusions; these events lead to caspase-8 proteolytic activation and neuronal apoptosis.[181] Conversely, mutants of huntingtin resistant to cleavage by caspase-3 or -6 display reduced cytotoxicity.[192] Loss of endogenous caspase inhibitors in neurons may also lead to inappropriate caspase activation. One such candidate is the neuronal apoptosis-inhibitory protein (NAIP) gene, which is deleted in a subset of patients with spinal muscular atrophy.[54] NAIP is a member of the mammalian IAP family that contains active-site directed inhibitors of the caspases, although the precise mechanism of action of NAIP is not known.[59,60,180]

Caspases have also been implicated in tissue injury in response to ischemia,[193−201] spinal cord/head trauma,[202,203] and burns.[202,203] In both the brain and heart, ischemia and reperfusion injury lead to mitochondrial release of cytochrome *c*, proteolytic activation of multiple caspases, cleavage of caspase substrates such as PARP and PKCδ, and subsequent neuronal or myocardial cell death.[193,195,196,199,200,204] At least in the brain, ischemic injury results in the release of caspase-9 from mitochondria and its eventual translocation into the nucleus where it may cleave substrates necessary for apoptosis.[200] In the heart, caspases are also activated in a variety of human cardiomyopathies that lead to progressive loss of cardiac myocytes and congestive heart failure.[205] Finally, the delayed inflammatory cell response that is observed in many types of ischemic tissue injury is dependent on caspase activation (most likely the caspase-1 subfamily) and exacerbates tissue destruction.[206,207] These findings implicate caspases in the tissue destruction triggered by a variety of insults, and they suggest that caspase inhibition might be therapeutically beneficial by attenuating cell death in these settings.

Caspase-Directed Therapies. Given the growing evidence that caspases are important mediators of diverse diseases, it is not surprising that they have become the focus of intense scrutiny as therapeutic targets. In diseases of deficient caspase activity such as cancer, the delivery of caspases to tumor cells by gene therapy might directly induce apoptosis or at least restore tumor sensitivity to apoptotic induction. One strategy being examined is the use of caspase constructs that are proteolytically activated by chemically induced dimerization.[24,208,209] Specifically, caspase constructs containing FK506 binding motifs are activated in vivo by addition of a cell-permeable dimerizer (which facilitates autoproteolytic activation). In theory, this allows regulation of caspase activity (and subsequent cell death) by titrating the ligand. The major shortcoming of this approach (and other gene therapies) is the inability to target selectively the gene to a tumor. Inhibition of the caspase inhibitor survivin expression by expression of antisense cDNA activates programmed cell death and inhibits cell proliferation.[210] This approach offers some selectivity because it is absent in terminally differentiated normal adult tissue but expressed in a broad spectrum of malignancies.[58] Another novel caspase-targeting strategy is the use of an HIV protease-activated caspase-3 to selectively kill HIV-infected cells.[211] In this approach, the pro-domain of caspase-3 is replaced with a portion of the HIV TAT domain containing the HIV protease cleavage site, which allows efficient uptake of the denatured protein into mammalian cells.[211,212] Introduction of the TAT-caspase-3 protein (but not a catalytically inactive mutant) into HIV-infected Jurkat cells potently induced apoptosis; cell death was completely blocked by treatment with an HIV protease inhibitor.[211] These findings suggest that the selective activation of caspases in diseased cells and their subsequent unobtrusive elimination by apoptosis may be an attainable therapeutic goal.

In contrast, diseases of excessive caspase activation may be treatable by caspase inhibition. In neurodegenerative disorders, strong evidence supporting the potential efficacy of caspase inhibition comes from the observation that neuronal expression of a dominant negative caspase-1 transgene delays the progression of neurological symptoms and death in murine models of amyotrophic lateral sclerosis and Huntington's disease.[213,214] Similarly, in stroke models, neuronal expression of this same dominant negative caspase-1, XIAP/hILP/MIHA, or NAIP reduces brain infarct size and motor/cognitive deficits.[215−217] Selective peptide inhibitors of caspases reduce ischemic neuronal,[193,194,196,218] myocardial,[197,199] and small-bowel[198] tissue damage. Caspase inhibition also improves neurologic outcome following focal cerebral ischemia and prolongs life in a mouse model of amyotrophic lateral sclerosis.[193,219] These studies provide evidence that caspase inhibition in these disorders may be therapeutically beneficial.

Many critical issues surrounding the potential therapeutic value of caspase inhibitors have yet to be resolved. For one, we do not know which caspase(s) to

inhibit in a given disease; targeting the right caspase-(s) might avoid the toxicity of global caspase inhibition. Ideally, such inhibitors would be cell-permeable, small molecules with high affinity for the relevant caspases, rather than the short-lived, broadly reactive peptidic inhibitors currently known. In addition, the therapeutic window for caspase inhibition has yet to be established. Delayed administration of peptide caspase inhibitors was neuroprotective in some models[196,218] but not in others.[194] Finally, it is unknown whether cells treated with caspase inhibitors will remain viable for prolonged periods. Some manifestations of apoptotic cell death, such as membrane blebbing, the formation of apoptotic bodies, and mitochondrial injury/cytochrome *c* release, may be caspase-independent, suggesting that cells "rescued" by caspase inhibitors may still die.[220−222] If so, inhibition of these caspase-independent pathways will also be required of effective therapeutic strategies. It seems likely that disorders characterized by acute tissue injury, such as strokes and myocardial infarction, would lend themselves most readily to therapeutic intervention with caspase inhibitors. In these settings, caspase inhibition may be sufficient to protect tissue from ischemic damage for a period of hours to days, and the possible untoward consequences of systemic caspase inhibition would be minimized.

Caspase Inhibitor Discovery

Medicinal chemists have succeeded during the past two decades in developing clinically useful inhibitors of proteases from the serine, aspartic, and metalloprotease classes. The fourth class, the cysteine proteases, includes promising targets for therapeutic intervention in widespread conditions such as as malaria,[223,224] gingivitis,[225,226] osteoporosis,[227,228] and rheumatoid arthritis.[229,230] Building on years of academic investigations into the mechanistic properties of papain, cathepsin B, and a handful of other model enzymes, pharmaceutical labs continue to develop strategies for inhibiting the cysteine proteases. A decade after the first report of the activity of caspase-1,[231,232] there remain few published mechanistic studies of cysteine proteases. This is unfortunate, since these enzymes are structurally and mechanistically distinct from the papain superfamily enzymes, and basic studies of their catalytic properties could reveal new principles.

Development of caspase-1 inhibitors remains highly competitive, and the list of peer-reviewed publications describing quantitative and detailed SAR for caspase-1 inhibitors is short relative to the amount of chemistry described in the patent literature. For this reason, and since the authors' interests are primarily enzymological, discussion of the properties of caspase-1 that have made it a uniquely interesting and challenging target will provide a framework for examination of the chemical entities described in the literature. Examples of many of the compounds mentioned are provided in Table 3.

Nearly all published literature on caspase inhibitors described compounds directed at caspase-1. The early discovery and unique biological roles of this enzyme prompted vigorous drug discovery efforts at several pharmaceutical companies, and a correspondingly rich scientific and patent literature has developed. Followers of the field are aware of strong interest by several pharmaceutical companies in understanding and inhibiting the other caspases, but in contrast to that of caspase-1 the list of publications describing specific inhibitors is extremely short. Differences in the crystal structures and substrate specificities of the caspases, described above, essentially represent the (published) state of the art in the design of inhibitors of most caspases. Therefore, this review focuses on caspase-1 inhibitors, describing other caspase inhibitors when possible.

Caspase-1 Is a Mechanistically Unique Cysteine Protease. Caspase-1 was first identified as a cysteine protease by its reactivity with iodoacetamide.[41] Subsequent evaluation of the enzyme's reactivity with aspartic aldehydes[233] and ketones[234] furthered analogies with the papain family of cysteine proteases. Crystal structures demonstrated that the active-site geometry is quite distinct from that of papain (see Figure 2).[35,36] Reactivity of caspase-1 toward acyloxymethyl ketones showed an unusual lack of correlation with the pK_a of the leaving group,[235] and the enzyme can even be alkylated by sulfonylaminomethyl ketones in a structure-specific manner.[236] Figure 2 compares the geometry of the caspase-1 catalytic machinery with that of papain, where both enzymes are complexed with aldehyde inhibitors. While the imidazolium ion of papain protonates the leaving amino group[237] upon collapse of the transition state, that of caspase-1 is better positioned to provide a salt bridge with the oxyanion of the developing transition state and may fortuitously donate a proton to the leaving group of some activated aspartic ketones. While the behaviors of caspase-1 are largely dominated by the soft thiolate anion, careful mechanistic studies of this enzyme may yield additional surprises due to the enzyme's uniquely stabilized transition state.

Requirement for an Inhibitory Electrophile. In the late 1970s, captopril became the first effective and safe therapeutic small-molecule protease inhibitor, targeting the zinc metalloprotease angiotensin-I-converting enzyme (ACE). Captopril is a small drug molecule (217 Da), and much of its binding potency derives from the strong interaction of its thiol group with the essential electrophilic zinc atom of ACE. More recently, improvements have been made in the binding affinity and specificity, the reactive thiol has been replaced with carboxyl or phosphinyl groups, and the bioavailability and safety of the resulting compounds have also been improved.[238] However, a strong interaction between a functional group of the inhibitor and the active-site zinc cation remains essential.

An analogous requirement applies to caspase inhibitors.[239] All known small-molecule inhibitors of cysteine proteases with reasonable potency include an electrophilic moiety that interacts strongly with the catalytic cysteine. The peptide aldehyde Ac-YVAD-cho (**1**) is a potent inhibitor of caspase-1.[41] Replacement of the aldehyde functionality with hydrogen results in a compound with 280 000-fold less potency (**2**).[240]

To fulfill this requirement and to satisfy the P_1 specificity, much of the emphasis in caspase-1 chemistry has been on development of novel electrophilic aspartic acid derivatives. Many of the electrophilic moieties used in caspase-1 inhibitors are listed in Table 4. After initial application of Ac-YVAD-cho[233] to inhibit and affinity-

Table 3. Exemplary Caspase Inhibitors[a]

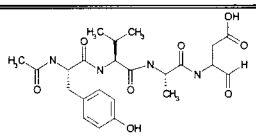
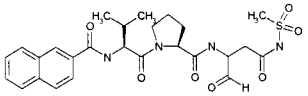
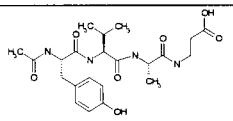
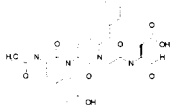
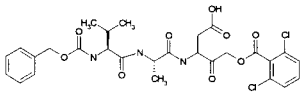
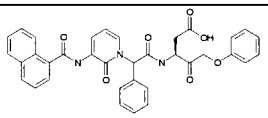
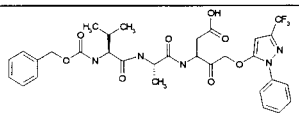
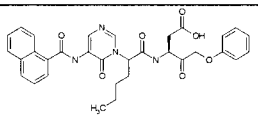
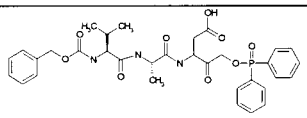
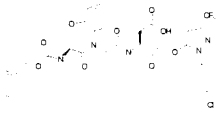
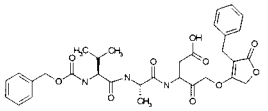
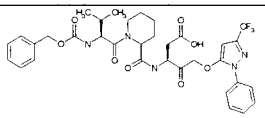
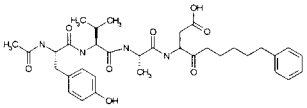
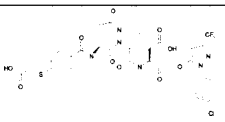
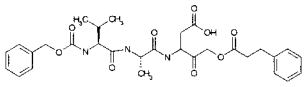
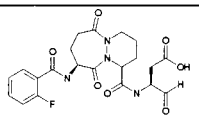
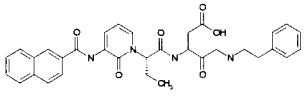
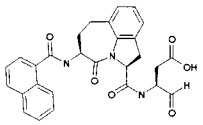
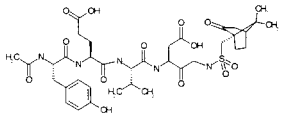
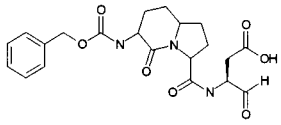
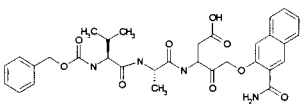
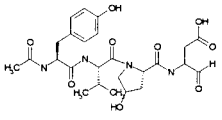
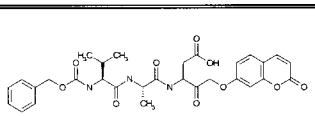
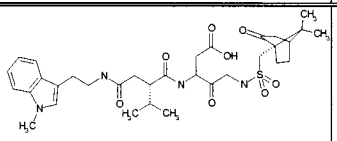
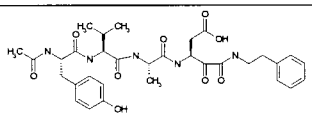
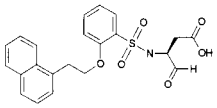
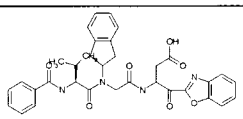
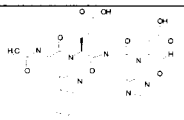
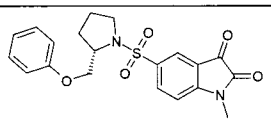
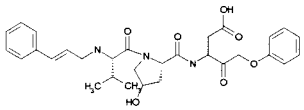
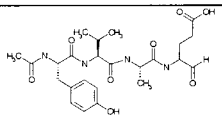
	Structure	IC_{50}[b] (μM)	k_{on}[b] ($M^{-1}s^{-1}$)		Structure	IC_{50}[b] (μM)	k_{on}[b] ($M^{-1}s^{-1}$)
1		0.00076	380000	**17**		0.038	
2		200		**18**		0.054	
3			407000	**19**		0.01	
4			280000	**20**		0.046	
5			117000	**21**			114000
6			252000	**22**			271000
7		0.0185		**23**			1220000
8		0.0019	1600000	**24**		0.001	
9		0.00037		**25**		0.01	
10		0.00011		**26**		0.036	
11		0.0017	430000	**27**		0.002	

Table 3 (Continued)

	Structure	IC_{50}[b] (μM)	k_{on}[b] ($M^{-1}s^{-1}$)		Structure	IC_{50}[b] (μM)	k_{on}[b] ($M^{-1}s^{-1}$)
12		0.017	160000	**28**		0.0004	
13		0.009		**29**		0.062	
14		0.002		**30**		0.000056	
15		0.015[c] 0.047[d]		**31**		0.013	
16		0.009					

[a] Literature references are given in the text. [b] IC_{50} and k_{on} values are derived from a wide variety of assay protocols and may not be directly comparable. All values refer to inhibition of caspase-1, unless specified. [c] $K_{i,app}$ for inhibition of caspase-3. [d] $K_{i,app}$ for inhibition of caspase-7.

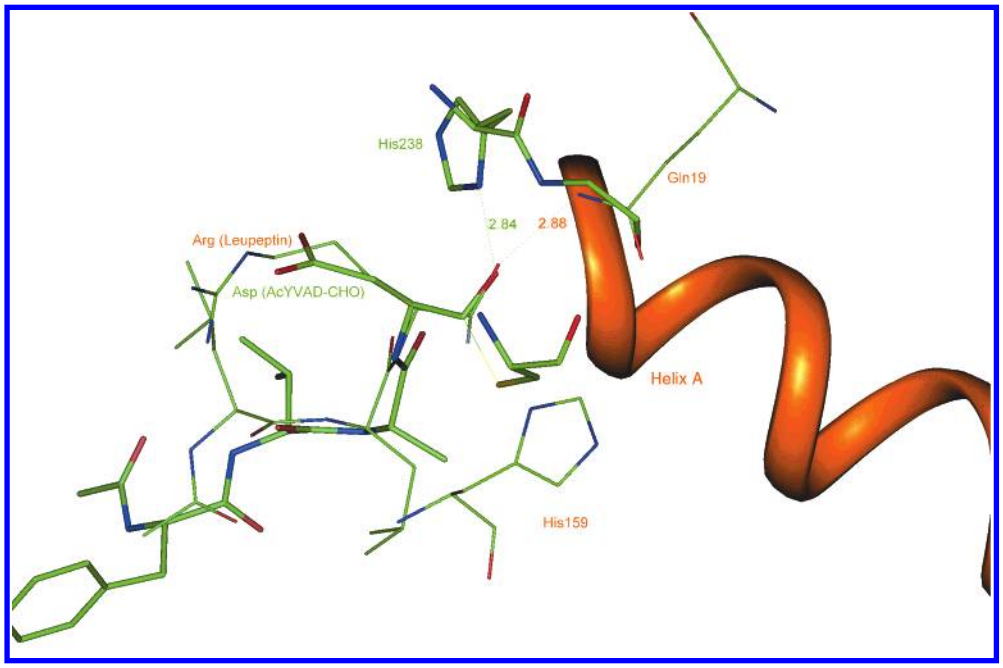
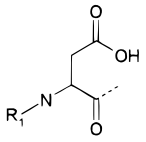
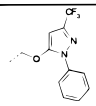
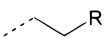
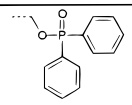
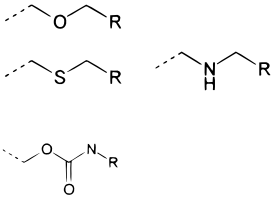
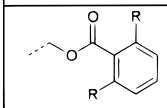
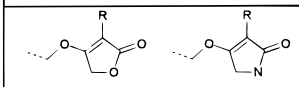
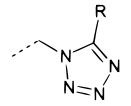
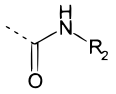
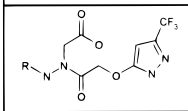
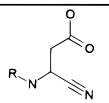
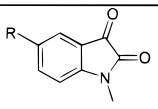
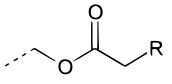
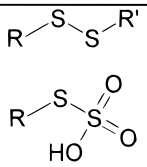
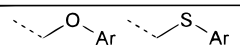
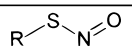
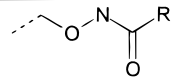
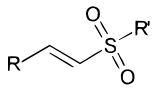
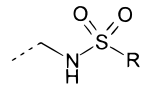
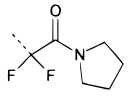


Figure 2. Caspase-1 complex with Ac-YVAD-cho (thick bonds) superimposed on the structure[298] of papain with leupeptin (thin bonds) such that the aldehyde ligands are aligned. Hydrogen bonds between the aldehyde oxygen and His238 of caspase-1 (green) or Gln19 of papain (orange) are shown, as well as the long helix of papain, which may play a role in stabilizing the oxyanion in that enzyme.[299]

purify caspase-1,[41] the benzoyloxymethyl ketones (**3**) were found to be potent inactivators of caspase-1.[235,241,242] On the basis of the ideas of Krantz,[243] chemists at Sterling-Winthrop expanded the repertoire of leaving groups (**4**−**6**). While this approach yielded irreversible enzyme inactivators, Merck chemists in-

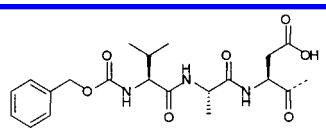
Table 4. Alternative Electrophilic Functions with Caspase-Inhibitory Activity

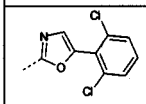
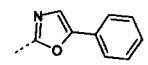
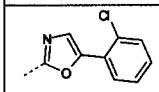
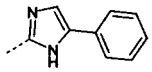
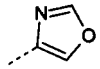
Template: 

Inactivators	Reference	Reversible Inhibitors	Reference
	242		233,274,278
	277		234
	289		245
	241 235		
	290		249,251
	291		292
	293		262
			253
Bimodal Inhibitors		**Other**	
			258
	248,240		71
	294		295
	296,236		297

vestigated alternative aspartic ketones.[244] Phenylalkyl ketones[234] became very effective inhibitors after optimization of the alkyl chain length (**7**). Several "activated" ketones[245] inhibited caspase-1, particularly the acyloxymethyl (**8**),[240,246] aminomethyl (**9**), and sulfonylaminomethyl (**10**) ketones. In a series of potent aminomethyl ketones with varying substituents from the amine nitrogen, a phenylethyl substitution was optimal.[247] The aryloxymethyl ketones (**11**, **12**)[248] also showed great promise. Many of the acyloxymethyl and aryloxymethyl ketones are mechanistically bimodal[240] and demonstrate clear structure−activity relationships in the P′ regions of the enzyme. Many α-ketoamides are effective caspase-1 inhibitors. While some acyl tripep-

Table 5. Aryl Ketone Inhibitors of Caspase-1 (from ref 249)



Aryl Group	K_i (μM)
	0.16
	0.24
	0.26
	6.3
	8.8
	10
	13
	14

tidic ketoamides achieve potencies less than 10 nM (**13**; J. Powers, unpublished observations), some ketoamides are surprisingly inactive, possibly due to poor adaptability of the rigid, planar amide functionality.

The aryl ketone caspase-1 inhibitors (**14** and Table 5) have been described only in the patent literature. Table 5 shows the reported potencies of a series of isoxazolyl, thiazolyl, and imidazolyl ketones.[249] The high potency of benzoxazolyl and isoxazolinyl ketones against neutrophil elastase is due in part to favorable hydrogen bonding between the ring nitrogen of the inhibitor and the protonated imidazolium of the transition state of the serine protease.[250] Given the hydrogen bond that His238 of caspase-1 forms with the ester or ether oxygen of several very potent acyloxy and aryloxymethyl ketones,[236] one might hope for similar interactions in these aryl ketones. Unfortunately, the potencies shown in Table 5 do not seem to reflect especially strong interactions. Similarly, tetrapeptide oxadiazolyl ketones[251] are reported with IC_{50} values above 100 nM. Perhaps the large planar group of these ketones is poorly accommodated. One benzoxazolyl ketone has been described as a very potent caspase-1 inhibitor (**14**).[252]

Very recently, isatin derivatives have been described as potent inhibitors of caspase-3 and -7 (**15**).[253] These are reported to be completely reversible in action, and crystallographic analysis of the caspase-3/inhibitor complex revealed formation of a tetrahedral adduct with the ketone carbonyl of the isatin heterocycle.

Many other electrophilic substances react nonspecifically with caspase-1.[254] Cephalosporins and related β-lactams inhibit caspase-1,[255] though the activity, mechanism, or SAR of these compounds is not reported. Similarly, reactive electrophilic heterocyles including 3,3-dichloro-2-oxindoles[256] and indole-2,3-diones[257] are incompletely described caspase-1 inhibitors. Among other types of electrophiles, caspase-1 reacts readily with oxidized glutathione, a property that can greatly simplify purification and storage of the enzyme.[258] ICE also reacts reversibly with other disulfides[259] and with nitroso donors.[71] Some nitriles are highly potent against serine and cysteine proteases,[260,261] but one nitrile has been shown as a very weak caspase inhibitor.[262] The relatively nonspecific halomethyl ketones are broadly used in research applications and are of possible therapeutic utility for treatment of acute indications.[263]

A number of potentially useful electrophiles have not been described as caspase-1 inhibitors, due either to lack of effort or to lack of success. 1,5-Diacyl hydrazides[264] and 1,3-diamino ketones[265] are effective inhibitors of the cysteine protease cathepsin-K. The epoxide E64 is a prototypical cysteine protease inhibitor but is inactive against caspase-1, possibly due to its inability to meet the caspase-1 requirement for aspartic acid. Vinyl sulfones are being tested as cruzain[266] and falcipain[224] inhibitors but have not yet been described as caspase inhibitors.

Ketones are usually more chemically stable and less reactive than aldehydes, and they necessarily add additional mass to a prospective drug. Still, aldehyde inhibitors of caspase-1 continue to be highly exemplified in the patent literature. Fortuitously, the essential carboxylate side chain readily condenses with the aldehyde (or ketone) carbonyl, forming the stable cyclic hemiacyl.

The Shallow, Wet Pocket. In the early 1980s, nafamostat was applied as a therapeutically useful serine protease inhibitor.[267] This relatively nonspecific inhibitor mimics the arginine residues that characterize the natural substrates of thrombin, kallikreins, and most serine proteases of the complement-activation cascades. Crystallography of these enzymes has shown that the pocket accommodating the arginine side chain is largely hydrophobic, except for a carboxylate side chain at the bottom of the pocket which can form a salt bridge with the substrate guanidinium group.[268,269] Expulsion of water from the hydrophobic channel, plus formation of a buried salt bridge, contributes greatly to the high potency achievable by noncovalent inhibitors of the arginine-specific serine proteases. Additionally, thrombin has another hydrophobic pocket near to P_3, enabling the specific, potent (subnanomolar) noncovalent inhibitors of this important enzyme.[270]

Caspases also cleave peptides immediately following a charged residue, though this is not particularly favorable for inhibitor design. The carboxymethyl side chain of Asp is much shorter than the guanidinopropyl side chain of Arg, necessitating a shallow binding

pocket. In contrast to the hydrophobic channel at P_1 of the Arg/Lys-specific serine proteases, the P_1 pocket of caspase-1 presents a uniformly polar, positively charged environment to the Asp side chain of the substrate.[35,36] The guanidinium group of Arg179 lies at the bottom of this pocket, and the guanidinium group of Arg341 is partly solvent-exposed near the top of the pocket. In the deeper parts of the caspase-1 pocket are the carboxamide group of Gln238 and the backbone carbonyl of Ser236. The Nδ1 of His237, which is probably positively charged, also points into the pocket. In the absence of substrate, this pocket is likely to attract anions such as chloride, acetate, phosphate, and sulfate, along with associated water. To bind a substrate, these must first be expelled, reducing the net energy gain on binding. Since the pocket is shallow, the newly formed salt bridge is poorly shielded from solvent. The dielectric constant in the region of this salt bridge is therefore likely to be high, reducing the energy of the Coulombic interaction. While this pocket clearly exerts a strong effect on caspase selectivity, it offers minimal favorable binding enthalpy.

Little is published on P_1 replacements in caspase inhibitors or substrates. Ketone and aldehyde inhibitors bearing a glutamate side chain inhibit caspase-1 with 10−100-fold lower potency than the corresponding aspartic inhibitor (**16**).[271] Phosphonate and sulfonate isosteres are very poor ICE substrates (K. D. Brady, unpublished observations) and probably offer no advantage for drug development. In the context of acyl tripeptide aldehyde inhibitors, the relatively conservative −COOH to −CONHOH substitution was deleterious to the inhibitory activity (100-fold loss of activity), while substitution with the relatively bulky group $-SO_2NHCH_3$ (**17**) caused only a 3-fold reduction in activity relative to carboxylate.[272] Crystallographic analysis indicated that distortion of the hydrogen-bonding network and a major rotation of the His237 imidazole ring (which forms one wall of the P_1 pocket) accommodated binding of this bulky group. Isatin-derived caspase inhibitors (**14**)[253] demonstrate that P_1-interacting moieties are dispensable. These compounds lack any P_1 side chain, and crystallographic analysis of a complex with caspase-3 shows that the pocket is occupied by a water molecule.

P_2 and P_3 Replacements. Cleavage of tripeptidic (or shorter) peptides by caspase-1 is barely detectable;[273] efficient catalysis (e.g., $k_{cat}/K_M > 10^4\ M^{-1}\ s^{-1}$) minimally requires a peptide extending to P_4. Inhibitors that bind like the substrates have similar requirements, and truncation of reversible (e.g., aldehyde or ketone) peptidic inhibitors reduces potency to levels impractical for therapeutic applications.[248,274] The tetrapeptide chain interacts with the enzyme as an extended β-strand, a situation analogous to that of peptidomimetic inhibitor development for renin[275] and neutrophil elastase.[276] Replacement of the peptide backbone with structures that reduce metabolic lability, reduce entropy losses upon binding, and optimize favorable contacts with the enzyme is the chemist's challenge.

In its peptide substrates, caspase-1 shows a nearly absolute requirement for Asp at P_1 and a strong preference for aromatic amino acids at P_4, but its requirements at P_3 and especially P_2 are less strict.[43,42] Crystal structures of tetrapeptide (Ac-YVAD) inhibitors bound to caspase-1 show that the S_2 and S_3 pockets are shallow and that the P_2 and P_3 side chains are solvent-exposed. *N*-Methylated peptide inhibitors demonstrated that only the P_1 and P_3 amide nitrogens were essential for inhibitory action,[274,277] and crystallography showed that the P_3 carbonyl oxygen participates in a hydrogen bond with the enzyme. Glycylaminopyridones (**18**)[278,279] that accommodate this pattern bind adequately. A benzyl substituent from the 6-position of the pyridone ring (**18**) optimally accesses the S_2 subsite, but a variety of alkyl or aromatic substitutions from the P_2 glycine yielded more effective compounds (**19**). Pyrimidone mimetics (**20**)[280] were slightly less active than the equivalent pyridones.[279]

Suspecting that the planar geometry of the pyrimidone ring led to suboptimal extension toward P_4, Dolle et al. prepared a variety of nonaromatic, cyclic mimetics.[281] Like the substituted pyridones described above, benzoxazepines (**21**) performed poorly relative to peptide-based inhibitors, and so subsequent designs included formal P_2 side chains (**22**), leading finally to the very effective pyridazinodiazepine core (**23**, **24**). In a series of patents, Vertex Pharmaceuticals extended the chemistry around these bicyclic mimetics.[249,282] A thiazoloazepine mimetic that is an effective caspase-3 inhibitor was independently discovered.[283]

Other mimetic approaches are described in the patent literature. Tricyclic dihydroindoloazepine mimetics (**25**) are potent caspase-3 inhibitors.[284] Since proline and pipecolic acid are very suitable P_2 substitutions, a diversity of cyclic and acyclic substitutions from C_α and N of P_2 are possible (**14**, **26**, **27**).[249,252,272,283,285] Since the P_2 nitrogen is dispensable, succinamide derivatives have been described as very active caspase inhibitors (**28**).[262] Arylsulfonamide-substituted aspartic aldehydes (**29**) are also reported as modestly potent inhibitors.[271] In a short series of isatin (**15**) derivatives, interactions with the S_2 subsite conferred selectivity for caspase-3 and -7 compared to caspase-1, -2, -4, -6, and -8 and, to a lesser degree, caspase-9.[253]

P_4 Replacements. The S_4 subsite of caspase-1 provides an important opportunity for enhancement of inhibitory potencies. The tetrapeptide Ac-WEHD-cho (**30**),[286] prepared as an analogue of the best known tetrapeptide caspase-1 substrate, is the most potent (K_i = 56 pM) inhibitor reported to date. Relatively little systematic exploration of P_4 is reported in the literature. Okamoto et al. explored a short list of cyclic and aliphatic extensions from P_4 and found 1-naphthoic and cinnamic (**31**) terminations equally optimal,[272] though neither group was as effective as Ac-Tyr. Differential effects of a variety of substituted 2-indolyl derivatives on human caspase-3, -6, and -8 have been described.[287] The examples in Table 3 reflect other terminal acyl groups in common use at P_4.

Future Directions

A wealth of "proof of concept" experiments in animal models of a wide variety of diseases supports optimism that caspase inhibition would be effective in a wide variety of clinial indications. Target selection remains a major challenge. Biochemical studies clearly differentiate the caspases that have critical roles in in-

flammtory versus apoptotic disease, but the latter seems more complex and the target(s) less obvious. The potential adverse consequences of chronically inhibiting apoptosis without effective means of achieving tissue selectivity is another concern. Chronic inhibition of apoptosis will be challenging without the discovery of highly tissue-specific caspase function or drug delivery.

The requirement for electrophilic groups in caspase inhibitors is an obstacle in the development of clinically safe drugs, since many (such as aldehydes) are susceptible to reactions in vivo such as Schiff base formation or nonspecific reaction with cysteine. A great deal of creativity has been applied to this problem, and its successful solution may dictate the outcome of clinical trials. There have been few reports of inhibitors selective for caspases other than caspase-1; these reports however suggest that the problem of achieving selectivity between caspases is surmountable but may be most difficult between the most highly homologous caspases.

Description in the literature of caspase-1-directed compounds is by far the most advanced. Such compounds provide ample demonstration of the effectiveness of caspase-1 inhibition in inflammatory disease. Since the role of this enzyme seems to be limited to cytokine maturation, chronic inhibition of caspase-1 may not directly cause unacceptable side effects. Sequence homology suggests that up to three related caspases may also participate in cytokine maturation and therefore be analogously promising drug targets, but little evidence supports this idea. Distinct roles of these enzymes in cytokine maturation might suggest opportunities for the design of drugs with useful biological specificity. Although more than a decade has passed since the promise of caspase-1 as an anti-inflammatory target was widely appreciated, progress in the field suggests that clinically effective drugs are achievable and will be available for the treatment of diseases such as rheumatoid arthritis in coming years.

In principle, inhibition of apoptosis might be effective in a variety of chronic indications such as neurodegenerative diseases. The major challenge in this approach will be to achieve sufficient selectivity to avoid blocking normal apoptosis which could, for example, cause autoimmune disorders, cancer, or infections. It is still possible that one or more caspases function only in certain tissues and so would offer opportunities for tissue-specific inhibition. However, all of the best-understood pro-apoptotic caspases are widely distributed and probably serve central roles in apoptosis in many, if not all, tissues. Tissue-specific drug delivery might provide an alternative means of achieving the necessary selectivity but may be achievable only in limited cases. We therefore expect that the treatment of chronic diseases of excessive apoptosis by caspase inhibition will be difficult.

In contrast, inhibition of apoptosis in acute indications such as stroke and myocardial infarct seems more promising. Growing evidence supports the central role of caspases in these processes. Much of this evidence comes from studies with nonspecific caspase inhibitors that have unacceptable pharmaceutical properties (for example, peptide derivatives) but which are sufficient for proof of concept experiments. The side effects due to global inhibition of apoptosis in acute settings may be easily acceptable, and cross-reactivity of such caspase-directed drugs against noncaspase targets may also be acceptable to some degree. These relatively relaxed constraints on drug leads increase the chance of success. Despite the lack of reported clinical success so far by this approach, we anticipate that the design of clinically effective inhibitors of acute apoptosis may be the most easily achieved of the three classes of targets described here and are likely to become available during this decade.

Biographies

Robert V. Talanian received a B.S. in chemistry from the Rensselaer Polytechnic Institute in 1984 and a Ph.D. in pharmacology from the University of Massachusetts Medical School in 1989 under the direction of George Wright. He then performed NIH-supported postdoctoral research at the Whitehead Institute for Biomedical Research under the direction of Peter Kim. In 1992 he joined BASF Bioresearch Corp., where he is a Group Leader in the Department of Biochemistry.

Kenneth D. Brady received a S. B. in biology in 1980 from the Massachusetts Institute of Technology and spent several years as a technician in electrochemical research and then as a computer operator in business settings. He earned his Ph.D. in biology from Brandeis University in 1989 and was supported by the American Cancer Society for a postdoctoral fellowship at the Harvard School of Public Health under Armen Tashjian. He joined BASF Bioresearch Corp. as an enzymologist in 1993.

Vincent L. Cryns received an A.B. *summa cum laude* in biochemistry from Harvard College in 1983 and an M.D. from Harvard Medical School in 1987. He completed a postdoctoral fellowship with Junying Yuan in the Department of Cell Biology at Harvard Medical School. In 1997 he became an Assistant Professor of Medicine at Northwestern University Medical School.

References

(1) Schreiber, R. D.; Chaplin, D. D. In *Samter's Immunologic Diseases;* Frank, M. M., Austen, K. F., Claman, H. N., Unanue, E. R., Eds.; Little, Brown and Co.: Boston, MA, 1994; pp 279−310.
(2) Earnshaw, W. C.; Martins, L. M.; Kaufman, S. H. Mammalian caspases: Structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem*. **1999**, *68*, 383−424.
(3) Van de Craen, M.; et al. Identification of a new caspase homologue: Caspase-14. *Cell Death Differ*. **1998**, *5*, 838−846.
(4) Juan, T. S.-C.; et al. Identification and mapping Casp7, a cysteine protease resembling CPP32β, interleukin-1β converting enzymes, and CED-3. *Genomics* **1997**, *40*, 86−93.
(5) Van de Craen, M.; et al. Characterization of seven murine caspase family members. *FEBS Lett*. **1997**, *403*, 61−69.
(6) Ahmad, M.; et al. Identification and characterization of murine caspase-14, a new member of the caspase family. *Cancer Res.* **1998**, *58*, 5201−5205.
(7) Hu, S.; Snipas, S. J.; Vincenz, C.; Salvesen, G.; Dixit, V. M. Caspase-14 is a novel developmentally regulated protease. *J. Biol. Chem*. **1998**, *273*, 29648−29653.
(8) Wang, S.; et al. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* **1998**, *92*, 501−509.
(9) Yuan, J.; Shaham, S.; Ledoux, S.; Ellis, H. M.; Horvitz, H. R. The C. elegans cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* **1993**, *75*, 641−652.
(10) Hugunin, M.; Quintal, L. J.; Mankovich, J. A.; Ghayur, T. Protease activity of in vitro transcribed and translated *Caenorhabditis elegans* cell death gene (*ced-3*) product. *J. Biol. Chem*. **1996**, *271*, 3517−3522.
(11) Xue, D.; Shaham, S.; Horvitz, H. R. The *Caenorhabditis elegans* cell-death protein CED-3 is a cysteine protease with substrate specificities similar to those of the human CPP32 protease. *Genes Dev*. **1996**, *10*, 1073−1083.
(12) Cryns, V.; Yuan, J. Proteases to die for. *Genes Dev*. **1998**, *12*, 1551−1570.
(13) Singer, G. G.; Abbas, A. K. The Fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* **1994**, *1*, 365−371.

(14) Dhein, J.; Walczak, H.; Debatin, K.-M.; Krammer, P. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* **1995**, *373*, 438−441.
(15) Griffith, T. S.; Brunner, T.; Fletcher, S. M.; Green, D. R.; Ferguson, T. A. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* **1995**, *270*, 1189−1192.
(16) Ju, S.-T.; et al. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* **1995**, *373*, 444−448.
(17) Abbas, A. K. Die and let live: Eliminating dangerous lymphocytes. *Cell* **1996**, *84*, 655−657.
(18) Jiang, D.; Zheng, L. X.; Lenardo, M. J. Caspases in T-cell receptor-induced thymocyte apoptosis. *Cell Death Differ.* **1999**, *6*, 402−411.
(19) Atkinson, E. A.; Bleackley, R. C. Mechanisms of lysis by cytotoxic T cells. *Crit. Rev. Immunol.* **1995**, *15*, 359−384.
(20) Baichwal, V. R.; Baeuerle, P. A. Kinases in pro-inflammatory signal transduction pathways: New opportunities for drug discovery. *Annu. Rep. Med. Chem.* **1998**, *33*, 233−242.
(21) Colussi, P. A.; Harvey, N. L.; Kumar, S. Prodomain-dependent nuclear localization of the caspase-2 (Nedd2) precursor. A novel function for a caspase prodomain. *J. Biol. Chem.* **1998**, *273*, 24535−24542.
(22) Zhivotovsky, B.; Samali, A.; Gahm, A.; Orrenius, S. Caspases: Their intracellular localization and translocation during apoptosis. *Cell Death Differ.* **1999**, *6*, 644−651.
(23) Chandler, J. M.; Cohen, G. M.; MacFarlane, M. Different subcellular distribution of caspase-3 and caspase-7 following Fas-induced apoptosis in mouse liver. *J. Biol. Chem.* **1998**, *273*, 10815−10818.
(24) Muzio, M.; Stockwell, B. R.; Stennicke, H. R.; Salvesen, G. S.; Dixit, V. M. An induced proximity model for caspase-8 activation. *J. Biol. Chem.* **1998**, *273*, 2926−2930.
(25) Yang, X.; Chang, H. Y.; Baltimore, D. Autoproteolytic activation of pro-caspases by oligomerization. *Mol. Cell* **1998**, *1*, 319−325.
(26) Stennicke, H. R.; et al. Caspase-9 can be activated without proteolytic processing. *J. Biol. Chem.* **1999**, *274*, 8359−8362.
(27) Aravind, L.; Dixit, V. M.; Koonin, E. V. The domains of death: Evolution of the apoptosis machinery. *Trends Biochem. Sci.* **1999**, *24*, 47−53.
(28) Hofmann, K.; Bucher, P.; Tschopp, J. The CARD domain: A new apoptotic signaling motif. *Trends Biochem. Sci.* **1997**, *22*, 155−156.
(29) Huang, B.; Eberstadt, M.; Olejniczak, E. T.; Meadows, R. P.; Fesik, S. W. NMR structure and mutagenesis of the Fas (APO-1/CD95) death domain. *Nature* **1996**, *384*, 638−641.
(30) Eberstadt, M.; et al. NMR structure and mutagenesis of the FADD (Mort1) death-effector domain. *Nature* **1998**, *392*, 941−945.
(31) Chou, J. J.; Matsuo, H.; Duan, H.; Wagner, G. Solution structure of the RAIDD CARD and model for CARD/CARD interaction in caspase-2 and caspase-9 recruitment. *Cell* **1998**, *94*, 171−180.
(32) Vaughn, D. E.; Rodriguez, J.; Lazebnik, Y.; Joshua-Tor, L. Crystal structure of Apaf-1 caspase recruitment domain: An α-helical Greek key fold for apoptotic signaling. *J. Mol. Biol.* **1999**, *293*, 439−447.
(33) Zhou, P.; Chou, J.; Olea, R. S.; Yuan, J.; Wagner, G. Solution structure of Apaf-1 CARD and its interaction with caspase-9 CARD: A structural basis for specific adaptor/caspase interaction. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11265−11270.
(34) Qin, H.; et al. Structural basis of procaspase-9 recruitment by the apoptotic protease-activating factor 1. *Nature* **1999**, *399*, 549−557.
(35) Walker, N. P. C.; et al. Crystal structure of the cysteine protease interleukin-1β-converting enzyme: A $(p20/p10)_2$ homodimer. *Cell* **1994**, *78*, 343−352.
(36) Wilson, K. P.; et al. Structure and mechanism of interleukin-1β converting enzyme. *Nature* **1994**, *370*, 270−275.
(37) Rotonda, J.; et al. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat. Struct. Biol.* **1996**, *3*, 619−625.
(38) Mittl, P. R. E.; et al. Structure of recombinant human CPP32 in complex with the tetrapeptide acetyl-Asp-Val-Ala-Asp fluoromethyl ketone. *J. Biol. Chem.* **1997**, *272*, 6539−6547.
(39) Blanchard, H.; et al. The three-dimensional structure of caspase-8: An initiator enzyme in apoptosis. *Structure* **1999**, *7*, 1125−1133.
(40) Watt, W.; et al. The atomic-resolution structure of human caspase-8, a key activator of apoptosis. *Structure* **1999**, *7*, 1135−1143.
(41) Thornberry, N. A.; et al. A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. *Nature* **1992**, *356*, 768−774.
(42) Thornberry, N. A.; et al. A combinatorial approach defines specificities of members of the caspase family and granzyme B. *J. Biol. Chem.* **1997**, *272*, 17907−17911.
(43) Talanian, R. V.; et al. Substrate specificities of caspase family proteases. *J. Biol. Chem.* **1997**, *272*, 9677−9682.
(44) Miossec, C.; Decoen, M.-C.; Durand, L.; Fassy, F.; Diu- Hercend, A. Use of monoclonal antibodies to study interleukin-1β-converting enzyme expression: Only precursor forms are detected in interleukin-1β-secreting cells. *Eur. J. Immunol.* **1996**, *26*, 1032−1042.
(45) Fernandes-Alnemri, T.; Litwack, G.; Alnemri, E. S. CPP32, a novel human apoptotic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1β-converting enzyme. *J. Biol. Chem.* **1994**, *269*, 30761−30764.
(46) Fernandes-Alnemri, T.; et al. Mch3, a novel human apoptotic cysteine protease highly related to CPP32. *Cancer Res.* **1995**, *55*, 6045−6052.
(47) Cardone, M. H.; et al. Regulation of cell death protease caspase-9 by phosphorylation. *Science* **1998**, *282*, 1318−1321.
(48) Martins, L. M.; Kottke, T. J.; Kaufmann, S. H.; Earnshaw, W. C. Phosphorylated forms of activated caspases are present in cytosol from HL-60 cells during etoposide-induced apoptosis. *Blood* **1998**, *92*, 3042−3049.
(49) Clem, R. J.; Fechheimer, M.; Miller, L. K. Prevention of apoptosis by a baculovirus gene during infection of insect cells. *Science* **1991**, *254*, 1388−1390.
(50) Ray, C. A.; et al. Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1β converting enzyme. *Cell* **1992**, *69*, 597−604.
(51) Bump, N. J.; et al. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. *Science* **1995**, *269*, 1885−1888.
(52) Chen, P.; Tian, J.; Kovesdi, I.; Bruder, J. T. Interaction of the adenovirus 14.7-kDa protein with FLICE inhibits Fas ligand-induced apoptosis. *J. Biol. Chem.* **1998**, *273*, 5815−5820.
(53) Rothe, M.; Pan, M.-G.; Henzel, W. J.; Ayres, T. M.; Goeddel, D. V. The TNFR2-TRAF signaling complex contains two novel proteins related to Baculoviral inhibitor of apoptosis proteins. *Cell* **1995**, *83*, 1243−1252.
(54) Roy, N.; et al. The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* **1995**, *80*, 167−178.
(55) Duckett, C. S.; et al. A conserved family of cellular genes related to the baculovirus *iap* gene and encoding apoptosis inhibitors. *EMBO J.* **1996**, *15*, 2685−2694.
(56) Liston, P.; et al. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature* **1996**, *379*, 349−353.
(57) Uren, A. G.; Pakusch, M.; Hawkins, C. J.; Puls, K. L.; Vaux, D. L. Cloning and expression of apoptosis inhibitory protein homologues that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4947−4978.
(58) Ambrosini, G.; Adida, C.; Altieri, D. C. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat. Med.* **1997**, *3*, 917−921.
(59) Deveraux, Q. L.; Takahashi, R.; Salvesen, G. S.; Reed, J. C. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* **1997**, *388*, 300−304.
(60) Roy, N.; Deveraux, Q. L.; Takahashi, R.; Salvesen, G. S.; Reed, J. C. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J.* **1997**, *16*, 6914−6925.
(61) Brüne, B.; von Knethen, A.; Sandau, K. B. Nitric oxide (NO): An effector of apoptosis. *Cell Death Differ.* **1999**, *6*, 969−975.
(62) Li, J. R.; Billiar, T. R. The anti-apoptotic actions of nitric oxide in hepatocytes. *Cell Death Differ.* **1999**, *6*, 952−955.
(63) Hibbs, J. B., Jr.; Taintor, R. R.; Vavrin, Z. Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science* **1987**, *235*, 473−476.
(64) Hibbs, J. B., Jr.; Taintor, R. R.; Vavrin, Z.; Rachlin, E. M. Nitric oxide: A cytotoxic activated macrophage effector molecule. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 87−94.
(65) Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1620−1624.
(66) Wink, D. A.; et al. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* **1991**, *254*, 1001−1003.
(67) Fehsel, K.; et al. Islet cell DNA is a target of inflammatory attack by nitric oxide. *Diabetes* **1993**, *42*, 496−500.
(68) Messmer, U. K.; Ankarcrona, M.; Nicotera, P.; Brüne, B. p53 expression in nitric oxide-induced apoptosis. *FEBS Lett.* **1994**, *355*, 23−26.
(69) Mannick, J. B.; Asano, K.; Izumi, K.; Kieff, E.; Stamler, J. S. Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein−Barr virus reactivation. *Cell* **1994**, *79*, 1137−1146.
(70) Stamler, J. S.; et al. S-Nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 444−448.

(71) Li, J.; Billiar, T. R.; Talanian, R. V.; Kim, Y. M. Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. *Biochem. Biophys. Res. Commun.* **1997**, *240*, 419−424.
(72) Rössig, L.; et al. Nitric oxide inhibits caspase-3 by S-nitrosation in vivo. *J. Biol. Chem.* **1999**, *274*, 6823−6826.
(73) Zech, B.; Wilm, M.; van Eldik, R.; Brüne, B. Mass spectrometric analysis of nitric oxide-modified caspase-3. *J. Biol. Chem.* **1999**, *274*, 20931−20936.
(74) Dimmeler, S.; Haendeler, J.; Nehls, M.; Zeiher, A. M. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1β-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J. Exp. Med.* **1997**, *185*, 601−607.
(75) Kim, Y.-M.; Talanian, R. V.; Billiar, T. R. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. *J. Biol. Chem.* **1997**, *272*, 31138−31148.
(76) Kim, Y.-M.; Talanian, R. V.; Li, J.; Billiar, T. R. Nitric oxide prevents IL-1 and IGIF (IL-18) release from macrophages by inhibiting caspase-1 (ICE). *J. Immunol.* **1998**, *161*, 4122−4128.
(77) Mannick, J. B.; Miao, X. Q.; Stamler, J. S. Nitric oxide inhibits Fas-induced apoptosis. *J. Biol. Chem.* **1997**, *272*, 24125−24128.
(78) Mannick, J. B.; et al. Fas-induced caspase denitrosylation. *Science* **1999**, *284*, 651−654.
(79) Saavedra, J. E.; et al. Targeting nitric oxide (NO) delivery in Vivo. Design of a liver-selective NO donor prodrug that blocks tumor necrosis factor-α-induced apoptosis and toxicity in the liver. *J. Med. Chem.* **1997**, *40*, 1947−1954.
(80) Cerretti, D. P.; et al. Molecular cloning of the interleukin-1β converting enzyme. *Science* **1992**, *256*, 97−100.
(81) Ghayur, T.; et al. Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. *Nature* **1997**, *386*, 619−623.
(82) Gu, Y.; et al. Activation of interferon-γ inducing factor mediated by interleukin-1β converting enzyme. *Science* **1997**, *275*, 206−209.
(83) Kuida, K.; et al. Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. *Science* **1995**, *267*, 2000−2003.
(84) Li, P.; et al. Mice deficient in IL-1β-converting enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. *Cell* **1995**, *80*, 401−411.
(85) Elford, P. R.; Heng, R.; Révészn, L.; MacKenzie, A. R. Reduction of inflammation and pyrexia in the rat by oral administration of SDZ 224-015, an inhibitor of the interleukin-1β converting enzyme. *Br. J. Pharmacol.* **1995**, *115*, 601−606.
(86) Fletcher, D. S.; et al. A synthetic inhibitor of interleukin-1β converting enzyme prevents endotoxin-induced interleukin-1β production in vitro and in vivo. *J. Interferon Cytokine Res.* **1995**, *15*, 243−248.
(87) Miller, B. E.; et al. Inhibition of mature IL-1β production in murine macrophages and a murine model of inflammation by WIN 67694, an inhibitor of IL-1β converting enzyme. *J. Immunol.* **1995**, *154*, 1331−1338.
(88) Thome, M.; et al. Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. *Curr. Biol.* **1998**, *8*, 885−888.
(89) Takeuchi, O.; et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* **1999**, *11*, 443−451.
(90) Slee, E. A.; Adrain, C.; Martin, S. J. Serial killers: Ordering caspase activation events in apoptosis. *Cell Death Differ.* **1999**, *6*, 1067−1074.
(91) Kuwana, T.; et al. Apoptosis induction by caspase-8 is amplified through the mitochondrial release of cytochrome *c*. *J. Biol. Chem.* **1998**, *273*, 16589−16594.
(92) Scaffidi, C.; et al. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.* **1998**, *17*, 1675−1687.
(93) Scaffidi, C.; et al. Differential modulation of apoptosis sensitivity in CD95 type I and type II cells. *J. Biol. Chem.* **1999**, *274*, 22532−22538.
(94) Li, H.; Zhu, H.; Xu, C. J.; Yuan, J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* **1998**, *94*, 491−501.
(95) Luo, X.; Budihardjo, I.; Zou, H.; Slaughter, C.; Wang, X. Bid, a Bcl2 interacting protein, mediates cytochrome *c* release from mitochondria in response to activation of cell surface death receptors. *Cell* **1998**, *94*, 481−90.
(96) Liu, X.; Kim, C. N.; Yang, J.; Jemmerson, R.; Wang, X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. *Cell* **1996**, *86*, 147−157.
(97) Bossy-Wetzel, E.; Bakiri, L.; Yaniv, M. Induction of apoptosis by the transcription factor c-Jun. *EMBO J.* **1997**, *16*, 1695−1709.
(98) Kharbanda, S.; et al. Role for Bcl-x_L as an inhibitor of cytosolic cytochrome *c* accumulation in DNA-damage induced apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6939−6942.
(99) Kluck, R. M.; Bossy-Wetzel, E.; Green, D. R.; Newmeyer, D. D. The release of cytochrome *c* from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science* **1997**, *275*, 1132−1136.
(100) Yang, J.; et al. Prevention of apoptosis by Bcl-2: Release of cytochrome *c* from mitochondria blocked. *Science* **1997**, *275*, 1129−1132.
(101) Jürgenmeister, J.; et al. Bax directly induces release of cytochrome *c* from isolated mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4997−5002.
(102) Narita, M.; et al. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome *c* release in isolated mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14681−14686.
(103) Vander Heiden, M. G.; Chandel, N. S.; Williamson, E. K.; Schumacker, P. T.; Thompson, C. B. Bcl-x_L regulates the membrane potential and volume homeostasis of mitochondria. *Cell* **1997**, *91*, 627−637.
(104) Marzo, I.; et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* **1998**, *281*, 2027−2031.
(105) Shimizu, S.; Narita, M.; Tsujimoto, Y. Bcl-2 famiy proteins regulate the release of apoptogenic cytochrome *c* by the mitochondrial channel VDAC. *Nature* **1999**, *399*, 483−487.
(106) Hu, Y.; Ding, L.; Spencer, D.; Núñez, G. WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation. *J. Biol. Chem.* **1998**, *273*, 33489−33494.
(107) Srinivasula, S. M.; Ahmad, M.; Fernandes-Alnemri, T.; Alnemri, E. S. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell* **1998**, *1*, 949−957.
(108) Yang, X.; Chang, H. Y.; Baltimore, D. Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. *Science* **1998**, *281*, 1355−1357.
(109) Adrain, C.; Slee, E.; Harte, M.; Martin, S. Regulation of apoptotic protease activating factor-1 oligomerization and apoptosis by the WD-40 repeat region. *J. Biol. Chem.* **1999**, *274*, 20855−20860.
(110) Saleh, A.; Srinivasula, S.; Acharya, S.; Fishel, R.; Alnemri, E. Cytochrome *c* and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. *J. Biol. Chem.* **1999**, *274*, 17941−17945.
(111) Zou, H.; Li, Y.; Liu, X.; Wang, X. An APAF-1/cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. *J. Biol. Chem.* **1999**, *274*, 11549−11556.
(112) Cain, K.; et al. Apaf-1 oligomerizes into biologically active ∼700-kDa and inactive ∼1.4-MDa apoptosome complexes. *J. Biol. Chem.* **2000**, *275*, 6067−6070.
(113) Golstein, P. TRAIL and its receptors. *Curr. Biol.* **1997**, *7*, R750−R753.
(114) Ashkenazi, A.; Dixit, V. M. Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.* **1999**, *11*, 255−260.
(115) Salvesen, G.; Dixit, V. Caspase activation: The induced-proximity model. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10964−10967.
(116) Boldin, M. P.; et al. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J. Biol. Chem.* **1995**, *270*, 7795−7798.
(117) Chinnaiyan, A. M.; O'Rourke, K.; Tewari, M.; Dixit, V. M. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* **1995**, *81*, 505−512.
(118) Boldin, M. P.; Goncharov, T. M.; Goltsev, Y. V.; Wallach, D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* **1996**, *85*, 803−815.
(119) Muzio, M.; et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signal complex. *Cell* **1996**, *85*, 817−827.
(120) Goltsev, Y. V.; et al. CASH, a novel caspase homologue with death effector domains. *J. Biol. Chem.* **1997**, *272*, 19641−19644.
(121) Hu, S.; Vincenz, C.; Ni, J.; Gentz, R.; Dixit, V. M. I−FLICE, a novel inhibitor of tumor necrosis factor receptor-1 and CD-95-induced apoptosis. *J. Biol. Chem.* **1997**, *272*, 17255−17257.
(122) Irmler, M.; et al. Inhibition of death receptor signals by cellular FLIP. *Nature* **1997**, *388*, 190−195.
(123) Srinivasula, S. M.; et al. FLAME-1, a novel FADD-like anti-apoptotic molecule that regulates Fas/TNFR1-induced apoptosis. *J. Biol. Chem.* **1997**, *272*, 18542−18545.
(124) Cheng, E. H. Y.; et al. Conversion of Bcl-2 to a Bax-like death effector by caspases. *Science* **1997**, *278*, 1966−1968.
(125) Clem, R.; et al. Modulation of cell death by Bcl-X_L through caspase interaction. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 554−559.
(126) Grandgirard, D.; et al. Alphaviruses induce apoptosis in Bcl-2-overexpressing cells: Evidence for a caspase-mediated, proteolytic inactivation of Bcl-2. *EMBO J.* **1998**, *17*, 1268−1278.
(127) Kirsch, D.; et al. Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome *c*. *J. Biol. Chem.* **1999**, *2115*, 21155−21161.
(128) Deveraux, Q. L.; et al. Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *EMBO J.* **1999**, *18*, 5242−5251.

(129) Wang, C. Y.; Mayo, M. W.; Korneluk, R. G.; Goeddel, D. V.; Baldwin, A. S., Jr. NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c- IAP2 to suppress caspase-8 activation. *Science* **1998**, *281*, 1680−1683.
(130) Lin, Y.; Devin, A.; Rodriguez, Y.; Liu, Z. G. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF− induced apoptosis. *Genes Dev.* **1999**, *13*, 2514−2526.
(131) Reuther, J. Y.; Baldwin, A. S. Apoptosis promotes a caspase-induced amino-terminal truncation of IκBα that functions as a stable inhibitor of NF-κB. *J. Biol. Chem.* **1999**, *274*, 20664−20670.
(132) Yin, X.; et al. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* **1999**, *400*, 886−891.
(133) Emoto, Y.; et al. Proteolytic activation of protein kinase Cδ by an ICE-like protease in apoptotic cells. *EMBO J.* **1995**, *14*, 6148−6156.
(134) Cardone, M. H.; Salvesen, G. S.; Widmann, C.; Johnson, G.; Frisch, S. M. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. *Cell* **1997**, *90*, 315−323.
(135) Datta, R.; Kojima, H.; Yoshida, K.; Kufe, D. Caspase-3-mediated cleavage of protein kinase C θ in induction of apoptosis. *J. Biol. Chem.* **1997**, *272*, 20317−20320.
(136) Lee, N.; et al. Activation of hPAK65 by caspase cleavage induces some of the morphological and biochemical changes of apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13642−13647.
(137) Rudel, T.; Bokoch, G. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. *Science* **1997**, *276*, 1571−1574.
(138) Widmann, C.; Gerwins, P.; Johnson, N. L.; Jarpe, M. B.; Johnson, G. L. MEK kinase 1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis. *Mol. Cell Biol.* **1998**, *18*, 2416−2429.
(139) Endo, K.; et al. Proteolytic cleavage and activation of protein kinase Cμ by caspase-3 in the apoptotic response of cells to 1-β-D-Arabinofuranosylcytosine and other genotoxic agents. *J. Biol. Chem.* **2000**, *275*, 18476−18481.
(140) Lazebnik, Y. A.; Kaufmann, S. H.; Desnoyers, S.; Poirier, G. G.; Earnshaw, W. C. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties such as ICE. *Nature* **1994**, *371*, 346−347.
(141) Casciola-Rosen, L.; et al. Apopain/CPP32 cleaves proteins that are essential for cellular repair: A fundamental principle of apoptotic death. *J. Exp. Med.* **1996**, *183*, 1957−1964.
(142) Han, Z.; et al. DNA-dependent protein kinase is a target for a CPP32-like apoptotic protease. *J. Biol. Chem.* **1996**, *271*, 25035−25040.
(143) Rhéaume, E.; et al. The large subunit of replication factor C is a substrate for caspase-3 in vitro and is cleaved by a caspase-3-like protease during Fas-mediated apoptosis. *EMBO J.* **1997**, *16*, 6346−6354.
(144) Ubeda, M.; Habener, J. F. The large subunit of the DNA replication complex C (DSEB/RF-C140) cleaved and inactivated by caspase-3 (CPP32/YAMA) during Fas-induced apoptosis. *J. Biol. Chem.* **1997**, *272*, 19562−19568.
(145) Huang, Y.; et al. Role of caspase-mediated cleavage of rad51 in induction of apoptosis by DNA damage. *Mol. Cell. Biol.* **1999**, *19*, 2986−2997.
(146) Smith, G.; di Fagagna, F.; Lakin, N.; Jackson, S. Cleavage and inactivation of ATM during apoptosis. *Mol. Cell. Biol.* **1999**, *19*, 6076−6084.
(147) Shinohara, A.; Ogawa, H.; Ogawa, T. Rad51 protein involved in repair and recombination in *Saccharomyces cerevisiae* is a RecA-like protein. *Cell* **1992**, *69*, 457−470.
(148) Casciola-Rosen, L. A.; Anhalt, G. J.; Rosen, A. DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J. Exp. Med.* **1995**, *182*, 1625−1634.
(149) Küpper, J.-H.; et al. *trans*-Dominant inhibition of poly(ADP-ribosyl)ation sensitizes cells against γ-irradiation and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine but does not limit DNA replication of a polyomavirus replicon. *Mol. Cell. Biol.* **1995**, *15*, 3154−3163.
(150) Lazebnik, Y. A.; Takahashi, A.; Poirier, G. G.; Kaufmann, S. H.; Earnshaw, W. C. Characterization of the execution phase of apoptosis in vitro using extracts from condemned-phase cells. *J. Cell. Sci. Suppl.* **1995**, *19*, 41−49.
(151) Schreiber, V.; et al. A dominant-negative mutant of human poly-(ADP-ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4753−4757.
(152) Song, Q.; et al. Interleukin-1β-converting enzyme-like protease cleaves DNA-dependent protein kinase in cytotoxic T cell killing. *J. Exp. Med.* **1996**, *184*, 619−626.
(153) Sahara, S.; et al. Acinus is a caspase-3-activated protein required for apoptotic condensation. *Nature* **1999**, *401*, 168−173.
(154) Liu, X.; Zou, H.; Slaughter, C.; Wang, X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* **1997**, *89*, 175−184.
(155) Enari, M.; et al. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* **1998**, *391*, 43−50.
(156) Sakahira, H.; Enari, M.; Nagata, S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* **1998**, *391*, 96−99.
(157) Brancolini, C.; Benedetti, M.; Schneider, C. Microfilament reorganization during apoptosis: The role of Gas2, a possible substrate for ICE-like proteases. *EMBO J.* **1995**, *14*, 5179−5190.
(158) Lazebnik, Y. A.; et al. Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9042−9046.
(159) Kayalar, C.; Örd, T.; Testa, M. P.; Zhong, L.-T.; Bredesen, D. E. Cleavage of actin by interleukin 1β-converting enzyme to reverse DNase I inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2234−2238.
(160) Rao, L.; Perez, D.; White, E. Lamin proteolysis facilitates nuclear events during apoptosis. *J. Cell. Biol.* **1996**, *135*, 1441−1455.
(161) Takahashi, A.; et al. Cleavage of lamin A by Mch2α but not CPP32: Multiple interleukin 1β-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8395−8400.
(162) Brancolini, C.; Lazarevic, D.; Schneider, C. Dismantling cell−cell contacts during apoptosis is coupled to a caspase-dependent proteolytic cleavage of beta-catenin. *J. Cell. Biol.* **1997**, *139*, 759−771.
(163) Caulín, C.; Salvesen, G. S.; Oshima, R. G. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J. Cell. Biol.* **1997**, *138*, 1379−1394.
(164) Kothakota, S.; et al. Caspase-3-generated fragment of gelsolin: Effector of morphological change in apoptosis. *Science* **1997**, *278*, 294−298.
(165) Ku, N.-O.; Liao, J.; Omary, M. B. Apoptosis generates stable fragments of human type I keratins. *J. Biol. Chem.* **1997**, *272*, 33197−33203.
(166) Mashima, T.; et al. Actin cleavage by CPP-32/apopain during the development of apoptosis. *Oncogene* **1997**, *14*, 1007−1012.
(167) van Engeland, M.; Kuijpers, H.; Ramaekers, F.; Reutelingsperger, C.; Schutte, B. Plasma membrane alterations and cytoskeletal changes in apoptosis. *Exp. Cell Res.* **1997**, *235*, 421−430.
(168) Wen, L.-P.; et al. Cleavage of focal adhesion kinase by caspases during apoptosis. *J. Biol. Chem.* **1997**, *272*, 26056−26061.
(169) Hashimoto, M.; et al. Rapid fragmentation of vimentin in human skin fibroblasts exposed to tamoxifen: A possible involvement of caspase-3. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 401−406.
(170) Levkau, B.; Herren, B.; Koyama, H.; Ross, R.; Raines, E. W. Caspase-mediated cleavage of focal adhesion kinase pp125FAK and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.* **1998**, *187*, 579−586.
(171) Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. *Science* **1995**, *267*, 1456−1462.
(172) Nicholson, D. W. ICE/CED-3-like proteases as therapeutic targets for the control of inappropriate apoptosis. *Nat. Biotechnol.* **1996**, *14*, 297−301.
(173) Wang, J.; et al. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell* **1999**, *98*, 47−58.
(174) Debatin, K. Activation of apoptosis pathways by anticancer treatment. *Toxicol. Lett.* **2000**, *112−113*, 41−48.
(175) Teitz, T.; et al. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat. Med.* **2000**, *6*, 529−535.
(176) Nakagawara, A.; et al. High levels of expression and nuclear localization of interleukin-1β converting enzyme (ICE) and CPP32 in favorable human neuroblastomas. *Cancer Res.* **1997**, *57*, 4578−4584.
(177) Koomägi, R.; Volm, M. Relationship between the expression of caspase-3 and the clinical outcome of patients with nonsmall cell lung cancer. *Anticancer Res.* **2000**, *20*, 493−496.
(178) Tepper, C. G.; Seldin, M. F. Modulation of caspase-8 and FLICE-inhibitory protein expression as a potential mechanism of Epstein−Barr virus tumorigenesis in Burkitt's lymphoma. *Blood* **1999**, *94*, 1727−1737.
(179) Adida, C.; et al. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *Am. J. Pathol.* **1998**, *152*, 43−49.
(180) Tamm, I.; et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res.* **1998**, *58*, 5315−5320.
(181) Sánchez, I.; et al. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* **1999**, *22*, 623−633.
(182) Gervais, F.; et al. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-β-precursor protein and amyloidogenic Aβ peptide formation. *Cell* **1999**, *97*, 395−406.

(183) Nakagawa, T.; et al. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β. *Nature* **2000**, *403*, 98−103.
(184) Yang, F.; et al. Antibody to caspase-cleaved actin detects apoptosis in differentiated neuroblastoma and microglia in Alzheimer's disease. *Am. J. Pathol.* **1998**, *152*, 379−389.
(185) Goldberg, Y. P.; et al. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat. Genet.* **1996**, *13*, 442−449.
(186) Kim, T.-W.; Pettingell, W. H.; Jung, Y.-K.; Kovacs, D. M.; Tanzi, R. E. Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. *Science* **1997**, *277*, 373−376.
(187) Loetscher, H.; et al. Presenilins are processed by caspase-type proteases. *J. Biol. Chem.* **1997**, *272*, 20655−20659.
(188) Miyashita, T.; Okamura-Oho, Y.; Mito, Y.; Nagafuchi, S.; Yamada, M. Dentatorubral pallidoluysian atrophy (DRPLA) protein cleaved by caspase-3 during apoptosis. *J. Biol. Chem.* **1997**, *272*, 29238−29242.
(189) Canu, N.; et al. Tau cleavage and dephosphorylation in cerebellar granule neurons undergoing apoptosis. *J. Neurosci.* **1998**, *18*, 7061−7074.
(190) Wellington, C.; et al. Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.* **1998**, *273*, 9158−9167.
(191) Ellerby, L. M.; et al. Cleavage of atrophin-1 at caspase site aspartic acid 109 modulates cytotoxicity. *J. Biol. Chem.* **1999**, *274*, 8730−8736.
(192) Wellington, C. L.; et al. Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J. Biol. Chem.* **2000**, *275*, 19831−19838.
(193) Hara, H.; et al. Attenuation of transient focal cerebral ischemic injury in transgenic mice expressing a mutant ICE inhibitory protein. *J. Cereb. Blood Flow Metab.* **1997**, *17*, 370−375.
(194) Hara, H.; et al. Inhibition of interleukin 1β converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2007−2012.
(195) Black, S. C.; et al. Co-localization of the cysteine protease caspase-3 with apoptotic myocytes after in vivo myocardial ischemia and reperfusion in the rat. *J. Mol. Cell. Cardiol.* **1998**, *30*, 733−742.
(196) Cheng, Y.; et al. Caspase inhibitor affords neuroprotection with delayed administration in a rat model of neonatal hypoxic-ischemic brain injury. *J. Clin. Invest.* **1998**, *101*, 1992−1999.
(197) Yaoita, H.; Ogawa, K.; Maehara, K.; Maruyama, Y. Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. *Circulation* **1998**, *97*, 276−281.
(198) Farber, A.; et al. A specific inhibitor of apoptosis decreases tissue injury after intestinal ischemia-reperfusion in mice. *J. Vasc. Surg.* **1999**, *30*, 752−760.
(199) Holly, T. A.; et al. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. *J. Mol. Cell. Cardiol.* **1999**, *31*, 1709−1715.
(200) Krajewski, S.; et al. Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *11*, 5752−5757.
(201) Velier, J.; et al. Caspase-8 and caspase-3 are expressed by different populations of cortical neurons undergoing delayed cell death after focal stroke in the rat. *J. Neurosci.* **1999**, *19*, 5932−5941.
(202) Clark, R.; et al. Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. *FASEB J.* **1999**, *13*, 813−821.
(203) Springer, J.; Azbill, R.; Knapp, P. Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. *Nat. Med.* **1999**, *5*, 943−946.
(204) Bialik, S.; et al. The mitochondrial apoptotic pathway is activated by serum and glucose deprivation in cardiac myocytes. *Circ. Res.* **1999**, *85*, 403−414.
(205) Narula, J.; et al. Apoptosis in heart failure: Release of cytochrome *c* from mitochondria and activation of caspase-3 in human cardiomyopathy. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8144−8149.
(206) Schielke, G.; Yang, G.; Shivers, B.; Betz, A. Reduced ischemic brain injury in interleukin-1β converting enzyme-deficient mice. *J. Cereb. Blood Flow Metab.* **1998**, *18*, 180−185.
(207) Daemen, M.; et al. Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. *J. Clin. Invest.* **1999**, *104*, 541−549.
(208) MacCorkle, R.; Freeman, K.; Spencer, D. Synthetic activation of caspases: Artificial death switches. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3655−3660.
(209) Fan, L.; Freeman, K.; Khan, T.; Pham, E.; Spencer, D. Improved artificial death switches based on caspases and FADD. *Hum. Gene Ther.* **1999**, *10*, 2273−2285.
(210) Ambrosini, G.; Adida, C.; Sirugo, G.; Altieri, D. C. Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. *J. Biol. Chem.* **1998**, *273*, 11177−11182.
(211) Vocero-Akbani, A.; Vander Heyden, N.; Lissy, N.; Ratner, L.; Dowdy, S. Killing HIV-infected cells by transduction with an HIV protease-activated caspase-3 protein. *Nat. Med.* **1999**, *5*, 29−33.
(212) Schwarze, S. R.; Ho, A.; Vocero-Akbani, A.; Dowdy, S. F. In vivo protein transduction: Delivery of a biologically active protein into the mouse. *Science* **1999**, *285*, 1569−1572.
(213) Friedlander, R. M.; Brown, R. H.; Gagliardini, V.; Wang, J.; Yuan, J. Inhibition of ICE slows ALS in mice. *Nature* **1997**, *388*, 31.
(214) Ona, V.; et al. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* **1999**, *399*, 263−267.
(215) Friedlander, R. M.; et al. Expression of a dominant negative mutant of interleukin-1 β converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. *J. Exp. Med.* **1997**, *185*, 933−940.
(216) Xu, D.; et al. Elevation of neuronal expression of NAIP reduces ischemic damage in the rat hippocampus. *Nat. Med.* **1997**, *3*, 997−1004.
(217) Xu, D.; et al. Attenuation of ischemia-induced cellular and behavioral defecits by X chromosome-linked inhibitor of apoptosis protein overexpression in the rat hippocampus. *J. Neurosci.* **1999**, *19*, 5026−5033.
(218) Fink, K.; et al. Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. *J. Cereb. Blood Flow Metab.* **1998**, *18*, 1071−1076.
(219) Li, M.; et al. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science* **2000**, *288*, 335−339.
(220) Xiang, J.; Chao, D. T.; Korsmeyer, S. J. BAX-induced cell death may not require interleukin 1β-converting enzyme-like proteases. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14559−14563.
(221) McCarthy, N. J.; Whyte, M. K.; Gilbert, C. S.; Evan, G. I. Inhibition of Ced-3/ICE-related proteases does not prevent cell death induced by oncogenes, DNA damage, or the Bcl-2 homologue Bak. *J. Cell Biol.* **1997**, *136*, 215−227.
(222) Zhang, J.; Reedy, M.; Hannun, Y.; Obeid, L. Inhibition of caspases inhibits the release of apoptotic bodies: Bcl-2 inhibits the initiation and formation of apoptotic bodies in chemotherapeutic agent-induced apoptosis. *J. Cell Biol.* **1999**, *145*, 99−108.
(223) Semenov, A.; Olson, J. E.; Rosenthal, P. J. Antimalarial synergy of cysteine and aspartic protease inhibitors. *Antimicrob. Agents Chemother.* **1998**, *42*, 2254−2258.
(224) Olson, J. E.; Lee, G. K.; Semenov, A.; Rosenthal, P. J. Antimalarial effects in mice of orally administered peptidyl cysteine protease inhibitors. *Bioorg. Med. Chem.* **1999**, *7*, 633−638.
(225) Eichinger, A.; et al. Crystal structure of gingipain R: An Arg-specific bacterial cysteine proteinase with a caspase-like fold. *EMBO J.* **1999**, *18*, 5453−5462.
(226) Shinohara, C.; Mori, S.; Ando, T.; Tsuji, T. Arg-gingipain inhibition and anti-bacterial activity selective for Porphyromonas gingivalis by malabaricone C. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 1475−1477.
(227) Gowen, M. Inhibition of cathepsin K − A novel approach to antiresorptive therapy. *Expert Opin. Invest. Drugs* **1997**, *6*, 1199−1202.
(228) Bromme, D. Cysteine proteases as therapeutic targets. *Drug News Perspect.* **1999**, *12*, 73−82.
(229) Esser, R. E.; et al. Cysteine proteinase inhibitors decrease articular cartilage and bone destruction in chronic inflammatory arthritis. *Arthritis Rheum.* **1994**, *37*, 236−247.
(230) Mueller-Ladner, U.; Gay, R. E.; Gay, S. Cysteine proteinases in arthritis and inflammation. *Perspect. Drug Discovery Des.* **1996**, *6*, 87−98.
(231) Black, R. A.; Kronheim, S. A.; Sleath, P. R. Activation of interleukin-1β by a co-induced protease. *FEBS Lett.* **1989**, *247*, 386−390.
(232) Black, R. A.; Kronheim, S. R.; Merriam, J. E.; March, C. J.; Hopp, T. P. A pre-aspartate-specific protease from human leukocytes that cleaves pro-interleukin-1β. *J. Biol. Chem.* **1989**, *264*, 5323−5326.
(233) Chapman, K. T. Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 613−618.
(234) Mjalli, A. M. M.; Chapman, K. T.; MacCoss, M.; Thornberry, N. A. Phenylalkyl ketones as potent reversible inhibitors of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2689−2692.
(235) Thornberry, N. A.; et al. Inactivation of interleukin-1β converting enzyme by peptide (acyloxy)methyl ketones. *Biochemistry* **1994**, *33*, 3934−3940.

(236) Brady, K. D.; et al. A catalytic mechanism for caspase-1 and for bimodal inhibition of caspase-1 by activated aspartic ketones. *Bioorg. Med. Chem.* **1999**, *7*, 621−631.
(237) Brocklehurst, K.; Willenbrock, F.; Salih, E. In *Hydrolytic Enzymes;* Neuberger, A., Brocklehurst, K., Eds.; Elsevier: Amsterdam, 1987; pp 39−158.
(238) White, C. M. Pharmacologic, pharmacokinetic, and therapeutic differences among ACE inhibitors. *Pharmacotherapy* **1998**, *18*, 588−599.
(239) Livingston, D. J. In vitro and in vivo studies of ICE inhibitors. *J. Cell. Biochem.* **1997**, *64*, 19−26.
(240) Brady, K. D. Bimodal inhibition of caspase-1 by aryloxymethyl and acyloxymethyl ketones. *Biochemistry* **1998**, *37*, 8508−8515.
(241) Dolle, R. E.; et al. P1 aspartate-based peptide α-((2,6-dichlorobenzoyl)oxy)methyl ketones as potent time-dependent inhibitors of interleukin-1β-converting enzyme. *J. Med. Chem.* **1994**, *37*, 563−564.
(242) Revesz, L.; et al. Synthesis of P1 aspartate-based peptide acyloxymethyl and fluoromethyl ketones as inhibitors of interleukin-1β-converting enzyme. *Tetrahedron Lett.* **1994**, *35*, 9693−9696.
(243) Krantz, A. T.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. Peptidyl (acyloxy)methyl ketones and the quiescent affinity label concept: The departing group as a variable structural element in the design of inactivators of cysteine proteinases. *Biochemistry* **1991**, *30*, 4678−4687.
(244) Mjalli, A. M. M.; et al. Inhibition of interleukin-1β converting enzyme by N-acyl-aspartic acid ketones. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1405−1408.
(245) Mjalli, A. M. M.; Chapman, K. T.; MacCoss, M.; Thornberry, N. A.; Peterson, E. P. Activated ketones as potent reversible inhibitors of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1965−1968.
(246) Albrecht, H. P.; et al. European Patent Application WO9816502, 1998.
(247) Semple, G.; et al. Peptidomimetic aminomethylene ketone inhibitors of interleukin-1β-converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 959−964.
(248) Mjalli, A. M. M.; et al. Inhibition of interleukin-1β converting enzyme by N-acyl-aspartyl aryloxymethyl ketones. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1409−1414.
(249) Bemis, G. W.; et al. U.S. Patent 5,847,135, 1998.
(250) Edwards, P. D.; Wolanin, D. J.; Andisik, D. W.; Davis, M. W. Peptidyl α-ketoheterocyclic inhibitors of human neutrophil elastase. 2. Effect of varying the hetrocyclic ring on in vitro potency. *J. Med. Chem.* **1995**, *38*, 76−85.
(251) Spruce, L. W.; et al. European Patent Application WO9849190, 1998.
(252) Bemis, G. W.; et al. European Patent Application WO9722618, 1997.
(253) Lee, D.; et al. Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality. *J. Biol. Chem.* **2000**, *275*, 16007−16014.
(254) Graczyk, P. P. Caspase inhibitors − A chemist's perspective. *Restor. Neurol. Neurosci.* **1999**, *14*, 1−23.
(255) Levy, M. A.; Gleason, J. G. European Patent Application WO9707805, 1997.
(256) Lee, D.; Long, S. A. European Patent Application WO9906042, 1999.
(257) Lee, D.; Long, S. A. European Patent Application WO9906367, 1999.
(258) Dang, L. C.; et al. Preparation of an autolysis-resistant interleukin-1β converting enzyme mutant. *Biochemistry* **1996**, *35*, 14910−14916.
(259) Nobel, C. S. I.; Kimland, M.; Nicholson, D. W.; Orrenius, S.; Slater, A. F. G. Disulfiram is a potent inhibitor of proteases of the caspase family. *Chem. Res. Toxicol.* **1997**, *10*, 1319−1324.
(260) Dufour, E.; Storer, A. C.; Menard, R. Peptide aldehydes and nitriles as transition state analogue inhibitors of cysteine proteases. *Biochemistry* **1995**, *34*, 9136−9143.
(261) Li, J.; Wilk, E.; Wilk, S. Aminoacylpyrrolidine-2-nitriles: Potent and stable inhibitors of dipeptidyl-peptidase IV (CD 26). *Arch. Biochem. Biophys.* **1995**, *323*, 148−154.
(262) Caprathe, B. W.; et al. European Patent Application WO9956765, 1999.
(263) Cai, S. X.; Drewe, J.; Wang, Y.; Weber, E. European Patent Application WO9947154, 1999.
(264) Bossard, M. J.; et al. Mechanism of inhibition of cathepsin K by potent, selective 1,5-diacylcarbohydrazides: A new class of mechanism-based inhibitors of thiol proteases. *Biochemistry* **1999**, *38*, 15893−15902.
(265) Marquis, R. W.; et al. Conformationally constrained 1,3-diamino ketones: A series of potent inhibitors of the cysteine protease cathepsin K. *J. Med. Chem.* **1998**, *41*, 3563−3567.
(266) Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H. Cysteine protease inhibitors cure an experimental *Trypanosoma cruzi* infection. *J. Exp. Med.* **1998**, *188*, 725−734.
(267) Okajima, K.; Uchiba, M.; Murakami, K. Nafamostat mesilate. *Cardiovasc. Drug Rev.* **1995**, *13*, 51−65.
(268) Narayana, S. V. L.; et al. Structure of human factor D. A complement system protein at 2.0 Å resolution. *J. Mol. Biol.* **1994**, *235*, 695−708.
(269) Spraggon, G.; et al. The crystal structure of the catalytic domain of human urokinase-type plasminogen activator. *Structure* **1995**, *3*, 681−691.
(270) Wagner, J.; Kallen, J.; Ehrhardt, C.; Evenou, J.-P.; Wagner, D. Rational design, synthesis, and x-ray structure of selective noncovalent thrombin inhibitors. *J. Med. Chem.* **1998**, *41*, 3664−3674.
(271) Allen, H. J.; et al. U.S. Patent 5,744,451, 1998.
(272) Okamoto, Y.; et al. Peptide based interleukin-1β converting enzyme (ICE) inhibitors: Synthesis, structure activity relationships and crystallographic study of the ICE-inhibitor complex. *Chem. Pharm. Bull.* **1999**, *47*, 11−21.
(273) Sleath, P. R.; Hendrickson, R. C.; Kronheim, S. R.; March, C. J.; Black, R. A. Substrate specificity of the protease that processes human interleukin-1β. *J. Biol. Chem.* **1990**, *265*, 14526−14528.
(274) Mullican, M. D.; et al. The synthesis and evaluation of peptidyl aspartyl aldehydes as inhibitors of ICE. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2359−2364.
(275) Lunney, E. A.; Humblet, C. Structure-based design: From renin to HIV-1 protease. *Methods Princ. Med. Chem.* **1998**, *6*, 37−71.
(276) Gubernator, K.; Boehm, H. J. Examples of active areas of structure based-design. *Methods Princ. Med. Chem.* **1998**, *6*, 15−36.
(277) Dolle, R. E.; et al. Aspartyl α-((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy)methyl ketones as interleukin-1β converting enzyme inhibitors. Significance of the P1 and P3 amido nitrogens for enzyme-peptide inhibitor binding. *J. Med. Chem.* **1994**, *37*, 3863−3866.
(278) Golec, J. M. C.; et al. Structure-based design of nonpeptidic pyridone aldehydes as inhibitors of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2181−2186.
(279) Semple, G.; et al. Pyridone-based peptidomimetic inhibitors of interleukin-1β-converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1337−1342.
(280) Dolle, R. E.; et al. First examples of peptidomimetic inhibitors of interleukin-1β converting enzyme. *J. Med. Chem.* **1996**, *39*, 2438−2440.
(281) Dolle, R. E.; et al. Pyridazinodiazepines as a high-affinity, P2−P3 peptidomimetic class of interleukin-1β-converting enzyme inhibitor. *J. Med. Chem.* **1997**, *40*, 1941−1946.
(282) Batchelor, M. J.; et al. U.S. Patent 5,874,424, 1999.
(283) Karanewsky, D. S.; et al. Conformationally constrained inhibitors of caspase-1 (interleukin-1β converting enzyme) and of the human CED-3 homologue caspase-3 (CPP32, apopain). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2757−2762.
(284) Karanewsky, D. S.; Linton, S. D. European Patent Application WO9811109, 1998.
(285) Wannamaker, M. W.; et al. European Patent Application WO9947545, 1999.
(286) Rano, T. A.; et al. A combinatorial approach for determining protease specificities: Application to interleukin-1β converting enzyme (ICE). *Chem. Biol.* **1997**, *4*, 149−155.
(287) Karanewsky, D. S.; Bai, X. European Patent Application WO9811129, 1998.
(288) Xanthoudakis, S.; et al. Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. *EMBO J.* **1999**, *18*, 2049−2056.
(289) Dolle, R. E.; et al. Aspartyl α-((diphenylphosphinyl)oxy)methyl ketones as novel inhibitors of interleukin-1β converting enzyme. Utility of the diphenylphosphinic acid leaving group for the inhibition of cysteine proteases. *J. Med. Chem.* **1995**, *38*, 220−222.
(290) Graybill, T. L.; et al. α-[(Tetronoyl)oxy]- and α-[(tetramoyl)oxy]-methyl ketone inhibitors of the interleukin-1β converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1997**, *7*, 41−46.
(291) Ohmoto, K.; Tanaka, M.; Miyazaki, T.; Ohno, H. European Patent Application WO9724339, 1997.
(292) Powers, J. C.; et al. α-Keto amide inhibitors of interleukin-1β converting enzyme (caspase-1). 2000, in press.
(293) Graybill, T. L.; Dolle, R. E.; Helaszek, C. T.; Ator, M. A.; Strasters, J. Synthesis and evaluation of diacylhydrazines as inhibitors of the interleukin-1β converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1197−1202.
(294) Allen, H. J.; et al. U.S. Patent 5,919,790, 1999.
(295) Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. Vinyl sulfones as mechanism-based cysteine protease inhibitors. *J. Med. Chem.* **1995**, *38*, 3193−3196.
(296) Albrecht, H. P.; et al. European Patent Application WO9816505, 1998.

(297) Robinson, R. P.; Donahue, K. M. Synthesis of a peptidyl difluoro ketone bearing the aspartic acid side chain: An inhhibitor of interleukin-1β converting enzyme. *J. Org. Chem.* **1992**, *57*, 7309−7314.
(298) Schroder, E.; Phillips, C.; Garman, E.; Harlos, K.; Crawford, C. X-ray crystallographic structure of a papain-leupeptin complex. *FEBS Lett.* **1993**, *315*, 38−42.
(299) Doran, J. D.; Carey, P. R. α-Helix dipoles and catalysis: Absorption and Raman spectroscopic studies of acyl cysteine proteases. *Biochemistry* **1996**, *35*, 12495−12502.

JM000060F