Review

Physiological Functions of Caspases Beyond Cell Death

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The principal focus of interest in caspase enzymes has been on their role in apoptotic cell death. This focus began with the discovery that *ced-3*, the critical executioner gene in the apoptotic pathway of *Caenorhabditis elegans*, is homologous to interleukin-1 β converting enzyme (ICE), a proinflammatory caspase seen in mammals. Since that discovery, caspases have been implicated in a broader range of functions outside of the death pathway. In this review, we propose that a unifying aspect of these functions is the processing of proteins critical to major changes in cell state, including not only cell death but terminal differentiation to *formes frustes* of cell death, including keratinocyte formation, differentiation of lens cells, erythrocyte differentiation, and formation of platelets. Caspase activation has also been implicated in cell fusion, differentiation of monocytes to macrophages and dendritic cells, and the clonal expansion of T and B lymphocytes. Specific substrates activated by caspases and responsible for nonapoptotic change in function are discussed, as well as the importance of distinguishing apoptosis from other caspase-mediated processes.

Caspases are a family of cysteinyl proteases. The first caspase identified was ICE. ICE proteolytically activates the proform of the cytokine interleukin (IL)-1 β to its active form. ICE was later found to also activate IL-18.¹⁻³ This restricted view of caspase function in inflammatory cytokine processing, however, changed greatly when a caspase homolog in *C. elegans*, CED-3, was shown to mediate programmed cell death.^{4,5} Identification of similar executioner caspases required for cytokine-initiated death of mammalian cells, together with the similarity of the morphological hallmarks of programmed cell death in *C. elegans* to the shrinkage, blebbing, and absence of inflammation observed in

certain forms of mammalian cell death, led to the general belief that the presence of activated caspases and/or cleaved caspase substrates was diagnostic of apoptotic or programmed cell death.⁶

The interactions among the protein products of the four genes implicated by genetic complementation in *C. elegans* are shown in Figure 1A. This figure makes two important points for this review: the mammalian system of caspases is much more complex than the single pathway system of *C. elegans*, and the final event of caspase-mediated death, a process we call "catastrophic proteolysis," is believed to involve proteolysis of a large number of proteins critical for cell survival.

At least 15 members of the caspase family have been identified in mammals. In addition to human caspase-1, caspase-5 and its mouse ortholog, caspase-11, are used by living cells to process and activate proinflammatory cytokines.² These inflammatory caspases might be viewed as a variant specialization of the caspase family. As discussed later, this is not a strict dichotomy. The larger set of caspases identified with apoptosis (ie, apoptotic caspases) now appears to occur as part of normal cellular processes that do not lead to death (Table 1), and inflammatory caspases may contribute to apoptosis.² Apoptosis may be thought of as a signaling cascade. In this view, caspases should be considered as signaling proteolytic molecules akin in function to kinases, phosphatases, lipases, and other components of the cell's signaling machinery.

The Initiator and Effector Paradigm of Proteolytic Activation of Caspases

In this review, we will stress three points: 1) this signaling cascade is only one way cells die; 2) the caspase cascade initiates many processes other than cell death and cannot, therefore, be equated with apoptosis; and 3) the collection of known functions of the

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Figure 1. A: *C. elegans* caspase pathway. Cell death is the genetically determined final event in differentiation for specific cells in the classical studies of *C. elegans*, as determined using genetic complementation. Four genes control this pathway: *egl-1*, *ced-9*, *ced-4*, and *ced-3*. Transcription-regulated synthesis of EGL-1 inhibits CED-9. Release of inhibition on CED-9 allows for oligomerization of CED-3 by CED-4. Activation of CED-3 leads to cell death by proteolysis and subsequent uptake by neighboring cells. The function of this pathway in *C. elegans* is limited to cell death based on studies of mutants with deficiency in these four genes. **B:** Mammalian caspase pathways. The mammalian caspase pathways are more complex than the canonical cell death pathway in *C. elegans* (A). The extrinsic pathway is a receptor-mediated activation of initiator caspases that requires adaptors. The exact stoichiometry of activating (FADD) and inhibitory (FLIP) adaptors is not known. Active initiator caspases subsequently cleave and activate effector caspases. As discussed in the text, the set of critical caspase substrate to mediate cell death has not been identified. The second, or intrinsic, pathway is initiated by damage to mitochondria or other organelles leading to activation of caspases. The homologs of *C. elegans* CED-9 and EGL-1 are the BH4- and BH3-containing proteins of the Bcl-2 family members in mammals. The function of these Bcl-2 members is to regulate mitochondrial integrity and disruption of mitochondrial membranes leading to release of the CED-4 homolog, APAF-1, and cytochrome *c* for assembly of apoptosome to activate caspase-9. Activated caspase-9, in turn, cleaves and activates effector caspases. Other mitochondrial proteins can be released to amplify caspase activation or for caspase-independent cell death as discussed in the text.

Table 1. Cell Types with Active Caspases in Physiological Functions Other Than Apoptosis

Cell type	Evidence of active caspases	Description of caspase functions
Lens cells	Use of pan-caspase inhibitor to disrupt caspase activity	PARP cleavage, DNA fragmentation precedes lens fiber formation
Keratinocytes	Use of pan-caspase inhibitor to disrupt caspase activity	Normal loss of nucleus
Erythrocytes	Activation of caspase-3 via mitochondrial pathway; siRNA against caspase-3	Cleavage of lamin B and activation of chromatin condensation (acinus); reduction of enucleation with caspase-3 siRNA
Megakaryocytes	Detection of cleaved caspase-3 and caspase-9; use of pan- and specific caspase inhibitors; overexpression of Bcl-2	PARP cleavage; Bcl-2 inhibited proplatelet formation in megakaryocytes
B and T cells	Use of pan- and specific caspase inhibitors; detection of cleaved caspases	Restricted proteolysis of caspase substrates to permit cell cycle progression
Monocytes/macrophages	Use of pan- and specific caspase inhibitors; detection of caspase substrates	Alternate proteolysis of caspase substrates; differentiation of monocyte leads to activation of acinus; attachment of macrophages leads to PARP cleavage
Myocytes	Detection of caspase-3 activity	MST-like protein activated by caspase-3 leads to myotube formation
Osteoblasts	Use of specific caspase-8, -3, and -2 inhibitors	Caspase inhibition blocked G ₀ /G ₁ cell-cycle arrest
Trophoblasts	Detection of active caspase-8 and use of caspase-8 siRNA	Requirement for caspase-8 in syncytial formation of cytotrophoblasts
Spermatids	Use of caspase inhibitors and cytochrome c ^{-/-} <i>Drosophila</i>	Caspase activation is required for removal of bulk cytoplasm and for individualization of spermatids

caspase substrates suggests that the unique role of the caspases may be to induce rapid and irreversible changes in cell function, of which death is only one example. The mammalian apoptotic cascade begins with activation of initiator caspases (a subset of the caspase family), which initiates subsequent proteolytic activation of effector caspases (Figure 1B).

Initiator and effector caspases have distinct structural domains. Initiator caspases contain prodomains that interact with other molecules to aggregate caspases into homo- or heterophilic polymers required for caspase activation. Two such prodomains are caspase activation and recruitment domain (CARD) and the death effector domain (DED).⁷ DEDs consist of six or seven anti-parallel α -helices that provide homotypic interaction required to recruit procaspases to receptor-adaptor protein complexes (eg, the death-inducing signaling complex, DISC, with Fas).⁸ CARD has been observed to have a structure similar to DED, thereby allowing interaction with and subsequent activation of caspase proforms.

The classical effector caspases have short prodomains (ie, they lack CARD and DED). Initiator caspases are usually of low abundance, but their function is amplified by the proteolytic activation of more abundant effector caspases that do not possess the long CARD or multiple DEDs. Upstream initiator caspases, such as caspase-8 and caspase-9, in turn proteolytically activate caspase-3, -6, and -7 (Figure 1B).⁹

Initiator caspases may themselves function as effector caspases in the death process. For example, caspase-8 acts on members of the Bcl-2 family. The Bcl-2 proteins, including the *C. elegans* gene *ced-9*, regulate integrity of the mitochondrial membrane. Bcl-2-like proteins with four BH (Bcl-2 homology) domains protect mitochondria from injury, whereas members with a truncated terminal domain are homologous to the proapoptotic gene product

of nematode *egl-1* and function to initiate mitochondrial leakage. Activated caspase-8 can cleave the EGL-1 mammalian homolog of the Bcl-2 family, Bid, to become tBid. Truncated Bid interacts with other truncated family members, Bax and/or Bak, to damage mitochondria. The mitochondria release cytochrome c to associate with apoptosis protease-activating factor (APAF-1; a homolog of the nematode death gene *ced-4*) for activation of caspase-9, and concomitantly, Smac/DIABLO is also released to disrupt inhibition of caspase-9 by IAP (Figure 1).¹⁰

Proteolytic cleavage is not required for activation of all caspases. Procaspase-9 activity is dependent on bending the protein into an appropriate structure similar to the one that forms when the proforms of other caspases are cut.¹¹ Active caspase-9 requires apoptosome formation. The apoptosome is a protein complex comprised of cytochrome c released from mitochondria, APAF-1, and procaspase-9 (Figure 1B).¹¹ Active caspase-9 initiates a proteolytic caspase cascade to activate effector caspases.

Cell Death without Caspase Activation

A long list of cell death pathways not dependent on caspase activation has been described. This list includes calcium-dependent proteolysis, proteolysis by serine and aspartyl proteases, halogenation, and free radical forma-

Table 2. Caspase Substrates

Caspases
Nuclear proteins
Transcription factors and cell-cycle regulators
Caspase-sensitive kinases
Cytoskeletal proteins
Proinflammatory cytokines

Figure 2. Summary of caspase functions and substrates. Functions of mammalian caspases include activation of inflammatory cytokines, cell death, proliferation, differentiation, fission, and fusion. In addition to sharing caspases, these processes may share common caspase substrates.

tion via reactive oxygen species.^{12–14} A well-established example of apoptosis without caspase activation (ie, death following the morphological pattern of apoptosis) is death attributable to the release of apoptosis-inducing factor (AIF). AIF, a free-radical scavenger oxidoreductase, is normally confined to the mitochondria.¹⁵ After mitochondrial injury, AIF translocates into the nucleus to initiate DNase activity. This mechanism is now known to be conserved between *C. elegans* and mammals.¹⁶ The *C. elegans* homolog of AIF, WAH-1, is released from mitochondria by EGL-1 in a CED-3-dependent manner and interacts with CPS-6 (an endonuclease-G homolog) to promote DNA degradation. Thus, mitochondrial injury may lie downstream of caspase-mediated death or may initiate cell death without caspase involvement.

It is important to mention that the specific steps leading to death in most of these examples of noncaspase-mediated cell death are unknown. Similarly, even in caspase-mediated death, the respective roles played by different caspase substrates are not known, even though they have been organized into different classes based on functions or locations (Table 2). Whether caspase-dependent or not, there is still a need to identify specific targets critical to committing the cell to die.

Caspase Activation without Cell Death

Although inflammatory caspases have been implicated in certain forms of cell death, their most well-described function is processing of proinflammatory cytokines to their active forms (Figure 2). Caspase-1^{−/−} and caspase-11^{−/−} mice fail to activate and release IL-1 β , and caspase-11^{−/−} mice fail to activate caspase-1 in response to lipopolysaccharide stimulation.^{17,18} *In vitro* studies, however, did not confirm direct interaction of caspase-11 and caspase-1. In addition to activation of IL-1 β by proteolytic cleavage, caspase-1^{−/−} mice also failed to release IL-1 α . Caspase-1, therefore, can directly

cleave IL-1 β and, potentially, other substrates to mediate release of both IL-1 β and IL-1 α from living cells.

In addition to activated caspases for cytokine processing, activation of apoptotic caspases has been shown in survival of cardiomyocytes, in reverse remodeling, and in abortive apoptosis of neurons. Activated initiator caspases (8 and 9) were detected in neurons of Alzheimer's disease lesions. These proteases, however, failed to propagate the apoptotic signal to activate downstream effector caspases (3, 6, and 7).¹⁹ Aborted or interrupted apoptosis has also been demonstrated in cardiocytes of reverse modeling.^{20,21} Endomyocardial biopsy specimens from patients with left ventricular assisted device implantation had activated caspase cascade, but mitochondria and nuclei remained intact. These phenomena illustrate that activation of the caspase cascade does not necessarily lead to catastrophic proteolysis resulting in cell death, and each successive step can be interrupted resulting in abortive apoptosis.

The existence of a broader set of nondeath caspase functions is suggested by targeted gene disruption (knockout). *Ced-3*^{−/−} nematodes have an increase in the number of egg-laying cells as expected. In the worm, cell death is, therefore, tightly coupled to a caspase. Similarly, deletion of caspase-3 might be expected to produce overgrowth of tissues showing activation of this enzyme during cell death. This expectation, however, fails in the mouse. Caspase-3^{−/−} mice on the 129 background are embryonic lethal; however, caspase-3^{−/−} mice on the C57BL/6 background are phenotypically normal.²² Caspase-2^{−/−} mice have two divergent phenotypes. Lack of caspase-2 leads to excess germ cells in ovaries, but death of motor neurons during development was accelerated when deprived of neuronal growth factor.²³ The absence of effect on growth suggests that mammalian caspase functions may not be limited to cell death. The next sections review caspase function in cell division, cell differentiation, and cell fusion (Table 1).

Caspase Activation in Cellular Proliferation

Fas (CD95) is the best-known member of the death receptor family shown in Figure 1B. Targeted genetic disruption of the Fas-associated initiator caspase-8, its dominant-negative homolog (c-FLIP), or its adaptor molecule (FADD) has provided striking evidence of effects that would not be expected if its primary function were to form the death-inducing signaling complex (DISC) for cell death. Instead of overgrowth, mice with deficiency in any of the DISC proteins exhibit prominent abdominal hemorrhage and impaired heart muscle development in trabeculae and ventricular musculature.^{24,25} In addition, physiological cardiac hypertrophy after pressure overload is diminished in the MRL strain of *lpr/lpr* mice lacking functional Fas.²⁶ In the immune system, hematopoietic precursor cells from *lpr/lpr* mice reveal strongly impaired colony-forming activity and a defect in maintaining sufficient numbers of T-cell progenitors entering thymic development.²⁷ Additional evidence from bone marrow

adoptive transfer studies of Fas/FasL-deficient cells into Rag-2^{-/-} mice indicate that Fas/FasL signaling is also critical for B-cell development.²⁷

Activation-induced proliferation of T cells is impaired in FADD^{-/-} mice or mice expressing a dominant-negative FADD protein.^{28–30} Chimeric mice transgenic for dominant-negative FADD and deficient in RAG-1 further support a role for FADD in proliferation.^{28–30} Additionally, T cells lacking functional FADD arrest at the G₀/G₁ transition of cell cycle. Precursors of T cells from these animals fail to proliferate in response to CD3 ϵ ligation. Concomitant signaling through the pre-T-cell receptor (pre-TCR) and death receptors appears to trigger cell survival, proliferation, and differentiation; whereas, death-receptor signaling in thymocytes lacking pre-TCR induced apoptosis.³¹ Interestingly, serine protein kinase phosphorylates FADD during G₂/M phase transition, but not in cells arrested in G₁/S of the cell cycle.³² These events could link FADD and, possibly, caspase-8 and FLIP to the cell cycle. Indeed, caspase-8 is cleaved in nonapoptotic cells after TCR stimulation.^{33,34} Other supportive, yet indirect, evidence for a role of FADD/FLIP/caspase-8 in cell growth is the observation that proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors, such as zVAD-fmk.^{33–35}

Whether the proliferative effect of FADD requires caspase-8 activation or unrelated signaling events has not been fully established. Expression of CrmA, a caspase-8 inhibitor, in T cells completely blocked CD95-mediated apoptosis without affecting T-cell proliferation.²⁹ Budd³⁶ has proposed that FLIP, the dominant-negative caspase-8 homolog, although lacking functional enzymatic activity of caspase-8, may interact with TRAF1, TRAF2, and Raf-1 leading to activation of nuclear factor (NF)- κ B with subsequent production of survival signals/functions for proliferating T cells. Ironically, this hypothesis implies that Fas can function as either a pro- or anti-apoptotic receptor dependent on context of activation. Because FLIP itself is a nonenzymatic homolog of caspase-8, activation of the TRAF would comprise an intriguing example of caspase activation of a signaling pathway independent of the proteolytic properties of this enzyme family.

Similarly, initiator caspases (6 and 8) are cleaved and activated in B cells stimulated for entry into G₁.³⁷ Activation of upstream initiator caspases, however, did not lead to activation of downstream effector caspase-3. Moreover, selective inhibition of caspase-3 did not affect B-cell transition from G₀ to G₁, whereas inhibition of caspase-8 or caspase-6 blocked B-cell proliferation. As described in abortive apoptosis of neurons in Alzheimer's disease specimens, the activation of upstream initiator caspases does not automatically lead to activation of downstream effector caspases, as observed in caspase-dependent apoptosis. Although there is no empirical evidence for direct effects of active caspases on cytokinesis during mitosis, the morphological and biochemical changes that occur in the nucleus during apoptosis can be compared to those associated with nuclear envelope breakdown and chromatin condensation during mitosis.

As discussed later, similar parallels can be drawn between cellular fragmentation during cell death and caspase-mediated cell fission/fusion.

As already noted above, targeted disruption of caspase-3 in mice on the 129 background causes embryonic death *in utero*. There is no evidence that lethality results from tissue overgrowth. Instead, lethality is attributable to failure of the bone marrow to differentiate, and stromal cells from caspase-3^{-/-} animals are growth-arrested. The mechanism for this arrest is not known. As expected, these cells show up-regulated p21/p53 and down-regulated Cdk2/Cdc2.³⁸ Moreover, although wild-type marrow stem cells are inhibited from growth when treated with transforming growth factor- β , caspase-3^{-/-} cells undergo *in vitro* senescence, or even cell death. In contrast, treatment of wild-type stromal cells with bone morphogenic protein 4 (BMP-4) induces a potent activation of caspase-8, caspase-2, and caspase-3 without cell death but resulting in cell-cycle G₀/G₁ arrest.³⁹ Because p53-mediated cell death is associated with cell-cycle arrest,⁴⁰ these results raise the intriguing possibility that the primary function of caspase-3 during marrow development might be the regulation of developmental changes in cell-cycle regulation.⁴¹

Caspase Activation in Terminal Differentiation

A number of cell types undergo terminal differentiation by entering a postmitotic state without a full complement of organelles. This form of terminal differentiation might be thought of as a *forme fruste* state that resembles an incomplete apoptotic process. The best two examples of this process are the enucleation of keratinocytes and the enucleation of lens fiber cells.^{42–44} Activation of caspase-3 and caspase-14 (a cornified epithelium-specific caspase) during keratinocyte formation may be necessary for removal of organelles in the final stages of differentiation. The molecular mechanism for organelle removal in lens cells is unknown. However, the equatorial epithelium has been shown to have high levels of caspase-3-like activity during the initial differentiation of lens cells.⁴⁵ Moreover, staurosporine (a broad spectrum kinase inhibitor that induces caspase-dependent cell death) stimulates *in vitro* differentiation of lens cells in the presence of a proapoptotic Bcl-2 member and requires release of cytochrome *c*, implying activation of caspase-9.⁴⁵

Another example of a *forme fruste* state is erythrocyte differentiation.⁴⁶ Two transcription factors activated during erythropoiesis, GATA-1 and Tal-1, are caspase substrates. In the absence of sufficient erythropoietin, caspase cleavage of either GATA-1 or Tal-1 leads to cell death.⁴⁷ However, low-level caspase activity is required for activation of DNaseII for enucleation of erythroblasts via macrophages.⁴⁸ It is not known whether caspases activate substrates directly involved in enucleation, or act indirectly through cleavage of transcription factors or disruption of cellular signaling pathways.

Table 3. Caspase Substrates That Are Activated by Proteolytic Caspase Cascade

Cytokines		
Pro-IL-1 β	Interleukin-1 β precursor: essential inflammatory mediator	Activated
Pro-IL-16	Interleukin-16 precursor: induces T-cell chemotaxis	Activated
Pro-IL-18	IFN- γ -inducing factor: induced IFN- γ production	Activated
Pro-EMAP-II	Endothelial monocyte-activating polypeptide-II: p43 component of aminoacyl-tRNA synthetase complex	Activated
Tyr protein kinases		
Fyn	T-cell Src kinase: removal of N-terminal myristoylation sites leads to relocalization and increased activity	Activated
Lyn	B-cell Src kinase: removal of N-terminal myristoylation sites leads to relocalization and increased activity	Activated
Ser/Thr protein kinases		
MEKK1	MEK kinase-1: cleavage product is constitutively active, redistributed intracellularly and proapoptotic	Activated
Mst1, Mst2, Mst3	Mammalian STE20-related kinases: removal of c-terminal regulatory domain results in constitutive activity, relocalization and activation of stress kinases and caspases	Activated
PKC δ , ϵ , η , μ , θ , ζ	Protein kinase C	Activated
PRC1, 2	PKC-related kinases: constitutively active kinase	Activated
ROCK-1	Rho-associated kinase-1: activation of MLCK and membrane blebbing	Activated
SLK	STE20-related kinase, JNK-pathway: N-terminal kinase promotes apoptosis and cytoskeletal rearrangement, C-terminal fragment disassembles actin fibers	Activated
Protein phosphatases		
Calcineurin	Calmodulin-dependent phosphatase involved in NFAT activation and cytokine synthesis, IL-2 release	Activated
PP2A	Protein phosphatase 2A	Activated
G-proteins		
Ras-GAP	Ras GTPase-activating protein: limited cleavage activate PI3K, further into two inactive fragments	Activated

Caspase Activation in Cellular Fission and Fusion

Caspases are responsible for activating Rho-kinase-1, which produces vesiculation of cell fragmentation during apoptosis (Tables 2 and 3).^{49,50} It is reasonable to speculate that cellular fission may use similar pathways in living cells. As shown in the final stage of spermatid individualization in *Drosophila*, cytochrome c-dependent caspase activation is necessary for the removal of bulk cytoplasm.⁵¹ Similarly, caspase activation has been detected during fragmentation of proplatelets from megakaryocytes, without concomitant induction of cell death. Incubation with peptide-based caspase inhibitors or overexpression of Bcl-2 has been shown to block proplatelet formation. Platelet formation is also reduced in transgenic mice overexpressing Bcl-2, while the number of megakaryocytes remains unchanged.⁵²

At the other end of the morphogenic spectrum, cellular fusion of myoblasts into myotubes and syncytiotrophoblasts into placental trophoblast also requires active caspases. Caspase cleavage of mammalian sterile twenty-like (*Mstl*) kinase is required for formation of myotubes and expression of muscle-specific proteins.^{53,54} Similarly, fusion of cytotrophoblasts into syncytiotrophoblasts can be inhibited by down-regulation of caspase-8 protein expression or inhibition of caspase-8 protein activity.⁵⁵ Whether myotubes, syncytiotrophoblast formation, and morphogenic changes during apoptosis involve common targets is unknown. *Mstl* is an interesting candidate for mediating fusion because overexpression of truncated *Mstl* has been demonstrated to induce apoptotic features such as cell rounding and shrinkage, detachment from substratum, chromatin condensation,

and DNA fragmentation in several cell lines.⁵⁶ Moreover, activated *Mstl* has been implicated in other nondeath functions, including megakaryocyte differentiation.^{57–60}

Caspase Activation for Other Physiological Functions

Cell fusion and cell fission may represent intermediate examples of cell death. Table 3 lists a large number of additional substrates implicated in diverse physiological functions expected to be associated with more subtle cellular changes. Interestingly, the initiator caspase caspase-8 acts as an effector for several of these substrates. Caspase cleavage of glutamine receptor (GluR1) by caspase-8 has been shown in neurons stimulated with α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA).⁶¹ Prolonged nicotine exposure of neurons leads to internalization of GluR1; however, prestimulation with AMPA abrogates this process. As previously discussed, caspase-8 is also implicated in several lymphocyte functions other than cell death. Caspase-8 deletion restricted to the T-cell lineage did not alter thymocyte development but resulted in a marked decrease in the number of peripheral T cells.⁶² Mice with T cells that lack caspase-8 cannot mount an effective immune response to viral infection.⁶² Some of these effects may occur because caspase-8 is required for recruitment of IKK α , β -complex, its activation, and subsequent nuclear translocation of NF- κ B. Caspase-8 is required for activation of NF- κ B after stimulation of Fc receptor or TLR-4 in T, B, and NK cells.⁶³

In *Drosophila*, inhibition of caspase activity has been shown to rescue the migration defect in the border

cells.⁶⁴ The development of the *Drosophila* oocyte and its 15 surrounding nurse cells requires that 2 polar follicle cells recruit 6 to 8 neighboring cells to become the border cell cluster.⁶⁵ Expression of dominant-negative Rac can disrupt the directed migration of these neighboring cells to the border between the oocyte and its nurse cells. This defect can be rescued with inhibition of *Drosophila* caspase homolog (DRONC) or overexpression of the endogenous caspase inhibitor IAP (DIAP1).⁶⁴ Active caspase-3 has been demonstrated to cleave and inactivate Rac in human lymphocytes.⁶⁶ DIAP1 inhibition of low-level DRONC activity in border cells might be necessary for normal migration. The link between chemotaxis and cell survival has been observed in neutrophils, in which chemotaxis correlated with reduced spontaneous apoptosis.⁶⁷

The role of nondeath functions of caspases in monocytes is of particular interest.⁶⁸ The circulating monocyte pool provides the source for tissue macrophages and dendritic cells. Together, the monocyte and its progeny are critical for host defense and immune-mediated responses at tissue sites. Differentiation of monocytes to macrophages with macrophage colony-stimulating factor is prevented by pharmacological caspase inhibitors, by overexpression of Bcl-2, or by expression of the caspase inhibitor p35.⁶⁹ Similarly, differentiation of peritoneal macrophages and bone marrow-derived macrophages on attachment requires Fas-dependent activation of caspase-8 and caspase-3 (unpublished data). Caspases are not activated after attachment of macrophages *in vitro* derived from mice lacking functional Fas (*lpr/lpr*) or FasL (*gld/gld*).⁷⁰

Nondeath functions of caspase-3 may play a role in atherosclerosis. Atherosclerotic lesions contain large numbers of macrophages with activated caspase-3 that do not show cleavage of cell death substrates.⁷⁰ Dendritic cell differentiation, or more accurately, the inhibition of dendritic cell maturation, may also depend on activated caspases. Recent studies from the Strominger laboratory^{71,72} have shown that immature dendritic cells exhibit caspase activity. Activated caspases within immature dendritic cells proteolytically cleave subunits of adaptor protein complex 1 (AP-1), consequently compromising the protein sorting function of cleaved AP-1. The level of caspase activities within immature dendritic cells is partially regulated by inducible nitric oxide synthetase.⁷¹

The T cell antigen receptor (TCR) is another example of a receptor that is coupled to caspase-dependent, nonapoptotic events. TCR triggering leads to activation of caspases and caspase-mediated cleavage of Wee1, a kinase believed to control mitosis as a function of cell size. Although caspases are activated, neither DNA replication factor RFC140 nor DFF45 (the inhibitor of caspase-activated DNase) are cleaved in proliferating T cells.³⁴ Cleavage of RFC140 and DFF45 would lead to inhibition of DNA replication and fragmentation of genomic DNA, events that are not compatible with T-cell proliferation. In contrast, caspase inhibition during stimulation of peripheral blood lymphocytes blocked proliferation, major histocompatibility complex class II expression, and blastic transformation.³⁴

Hypothesis and Counterhypothesis

In setting out to write this brief review, we have attempted to present caspases as components of a signaling pathway invoked when cells must act on a large set of proteins to produce rapid, and possibly irreversible, changes in physiological function and/or cellular morphology. This hypothesis might be called "catastrophic proteolysis" as depicted in Figure 1. Caspase-dependent apoptotic cell death is merely an example of catastrophic proteolysis. In this view, death of the egg-laying cells of *C. elegans*, enucleation of the mammalian erythroblast, erythrocyte differentiation, lens formation, platelet fragmentation, and formation of keratinized epithelium all result from cleavage of a large number of different proteins. The catastrophic proteolysis hypothesis would predict that a large number of substrates would also be proteolytically processed to either active or inactive moieties in lymphocyte differentiation, dendritic cell differentiation, monocyte/macrophage differentiation, and myocyte fusion. All of these physiological processes, in common with cell death, share a need for a fairly rapid and permanent change in cell phenotype (Table 1). If the hypothesis is true, then the set of proteins cleaved in common across these processes may be quite large (Table 2). For example, Table 3 identifies a number of cytoskeletal targets, including kinases that act on the cytoskeleton. Cytoskeletal changes are the unifying process for most caspase activity. As proteomics methods improve, it will be important to learn the spectrum of proteins cleaved during these different processes.

The obvious counterhypothesis is that there are a limited number of specific caspase substrates having a broad functional role in these diverse processes. Two intriguing examples of such substrates are *Mstl* and Rho kinase. Each of these caspase-dependent kinases lies upstream of a broad signaling pathway regulating cell survival and cell shape. *Mstl* and Rho kinase have recently been shown to be upstream of the activation pathway for an entire family of transcription factors involved in expression of cytoskeletal and myogenic proteins.^{49,50,54} Furthermore, these kinases are also able to activate NF- κ B.^{49,50,54} The extent of proteolytic activation of *Mstl* and Rho kinase in different cell processes, other than apoptosis, is not yet known.^{49,50,54}

These two hypotheses, along with other recent reviews of nondeath functions of caspases,^{73,74} raise a common question: how is the activity and specificity of different caspases controlled? To some extent, the answer lies in the substrate specificity of different caspases. A more focused level of control may exist at the level of proteins that inhibit caspases, such as XIAP, or target caspases for ubiquitination or phosphorylation.⁷⁵⁻⁷⁷ We may speculate that these protein-protein interactions and posttranslation modifications will determine the specificity, localization, as well as level of activity responsible for mediation and specific caspase-dependent functional activities.

Finally, we would like to suggest that the large number of viable cell states requiring caspase activation may raise concern regarding our ability to define cell death itself.⁷⁸ Clearly, the presence of activated caspases is not sufficient evidence, by itself, for cell death. The im-

plication of the last statement is that cleavage of specific substrates may not be an adequate definition of cell death either. Activation of caspases and cleavage of their substrates, therefore, cannot be used to determine whether a cell has undergone the point of no return to become a dead cell. The simple dichotomy of whether a cell is dead or alive ignores functions served by cells differentiated into *formes frustes*. Caspase-dependent cell death may be one form of cellular differentiation, and further work is needed to characterize how caspases and their substrates are modulated for physiological functions. The process of differentiation in many cell types extends beyond coordinated transcription and translation programs. As discussed in this review, recent evidence strongly suggests that caspase-dependent signaling events, resulting in comprehensive and coordinated proteolytic posttranslation modifications, represent an important mechanism of regulating fundamental steps involved in cellular differentiation.

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