

# Caspase activation – stepping on the gas or releasing the brakes? Lessons from humans and flies

Guy S Salvesen<sup>\*.1</sup> and John M Abrams<sup>\*.2</sup>

<sup>1</sup>Program in Apoptosis and Cell Death Research, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92122, USA; and <sup>2</sup>Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA

**The central components of the execution phase of apoptosis in worms, flies, and humans are members of the caspase protease family. Work in *Drosophila* and mammalian systems has revealed a web of interactions that govern the activity of these proteases, and two fundamental control points have been identified. These are zymogen activation – the process that converts a latent caspase into its active form, and inhibition of the resulting active protease. In humans, the driving force for caspase activity is activation of the zymogens, but in *Drosophila*, a major thrust is derepression of caspase inhibitors. In this review, we consider evidence for these two distinct events in terms of the regulation of caspase activity. This sets the scene for therapy to reinstate the normal death mechanisms that have been overcome in a cancer cell's quest for immortality.**

*Oncogene* (2004) 23, 2774–2784. doi:10.1038/sj.onc.1207522

**Keywords:** caspase; IAP; apoptosis; zymogen activation; *drosophila*

## Introduction

Conditions that drive entry into the cell cycle also prime the apoptotic machinery (Green and Evan, 2002). This ensures that cell fate is largely determined by the presence of local survival signals that hold the apoptotic response in check (Raff, 1992). In the absence of such signals cells enter apoptosis unless they have down-regulated the ability to respond to the death cues. It is now clear that many cancers arise because of defects in the normal apoptosis control. The principal molecular machines that execute apoptosis, the packaging of a cell for phagocytic removal that is coincident with its demise, consist of proteolytic enzymes from the caspase family. Consequently, delineating the controls placed on the generation of caspase activity is required if we are to grasp the full view of the oncogenic process.

Caspases are highly selective proteases that have an exquisite preference for cleaving proteins after Asp

residues. This fastidious specificity ensures that apoptosis is primarily a set of limited proteolytic cleavages, and not the degradative process that is often assumed to accompany proteolysis (Salvesen and Dixit, 1997). The execution phase of apoptosis is thus thought to be a result of the limited caspase-dependent cleavage of hundreds of cellular proteins (Fischer *et al.*, 2003), the sum of which results in the morphology characterizing this form of programmed cell death (PCD).

A majority of studies on apoptosis are based on the assumption that caspase precursors are activated by cleavage, a common mechanism for most protease zymogen activations. Whereas this appears to be true for the executioner caspases, a paradigm shift is underway that points to a completely distinct activation mechanism for the initiator caspases that trigger the apoptotic pathways in humans. In contrast to the prevailing view that human caspase activation is driven mainly by caspase activation (stepping on the gas), research in *drosophila* suggests that apoptosis is initiated by transcriptional controls that liberate constitutively active caspases from their complex with natural inhibitors (releasing the brakes). This review considers these two fundamentally distinct mechanisms of caspase activation with implications for caspase biology.

## Proteolytic cycles

Whether in coagulation, inflammation or gastrulation, protease activity is regulated by an activation/activity/inhibition cycle depicted in Figure 1. Indeed, several of these cycles can act sequentially to cause a cascade. The reason for protease cascades is still debated, but one attractive hypothesis suggests that it converts a binary event (activate/don't activate) into an analog signal where levels of downstream protease – executioner protease – can be controlled in a graded response by inhibitors.

Almost independent of protease type, there exist three fundamental steps at which protease activity is naturally regulated at the post-translational level *in vivo*: (i) zymogen activation, (ii) access to substrates, (iii) inhibition. These principal control points are beautifully

\*Correspondence: G Salvesen; E-mail: gsalvesen@burnham.org or J Abrams; john.abrams@utsouthwestern.edu

exemplified by the caspases. Almost all proteases are stored as zymogens to protect sensitive cellular machinery during biosynthesis, and to allow timing and localization of the ultimate proteolytic events. Second, because most proteases show only limited degrees of stringency in substrate recognition *in vitro*, an important way to deliver specificity *in vivo* is to direct the protease to or from the cellular location of its target substrates. Third, the extent of substrate cleavage *in vivo* is dictated by a dynamic competition between substrate and natural inhibitors for the active site of the protease, and so the concentration of the inhibitors demonstrates the final regulation point in the cycle.

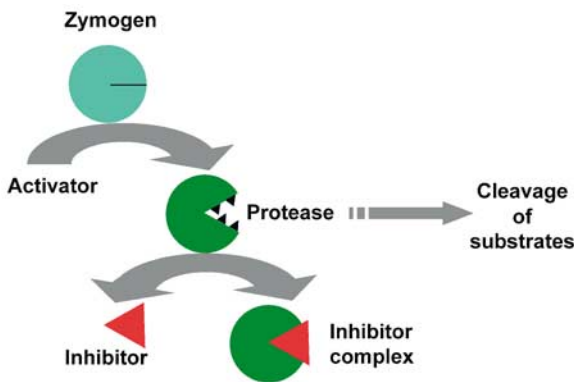
### Caspase types

The human genome encodes 11 caspases, and these can be divided into subgroups depending on inherent substrate specificity (Thornberry *et al.*, 1997), domain composition (Denault and Salvesen, 2002) or presumed roles *in vivo* (Nicholson, 1999). Table 1 relates them on the basis of domain composition, as this is currently the only way to compare human and fly caspases. Long

prodomain apoptotic initiator types or long prodomain cytokine activator types contain Caspase Recruitment Domains (CARD) or Death Effector Domains (DED) preceding the catalytic domain. These domains are 6–7 helix bundles that direct the caspases to their activation platforms (Fesik, 2000). The short prodomain, apoptotic effector type caspases contain short prodomains of unknown structure. The fly genome encodes seven members of the caspase gene family (Kumar and Doumanis, 2000). Based on prodomain structure, two of these correspond to initiator caspases and four resemble effector-type or executioner caspases. The seventh member, Strica, bears a long and unusual amino-terminal prodomain with no homologies to other proteins (Kumar and Doumanis, 2000). Their functional order has been largely inferred through shared phylogenetic relationships and, hence, critical aspects of the currently predicted pathways await validation through direct biochemical analyses. Likewise, much remains to be learned regarding the developmental functions of these genes, since most of the current genetic evidence relies on multigenic deficiencies, dominant negatives (Meier *et al.*, 2000) or injected dsRNAs (Quinn *et al.*, 2000).

### The human cytokine activators

The first caspase, caspase 1, was discovered as an aspartic-specific cysteine protease participating in the proteolytic maturation of pro-IL-1 $\beta$  (Cerretti *et al.*, 1992; Thornberry *et al.*, 1992). In agreement with this proposed role, ablation of the caspase 1 gene results in mice that cannot process pro-IL-1 $\beta$ , or the related proinflammatory cytokine pro-IL-18 (Kuida *et al.*, 1995). Interestingly, a similar defect is seen in mice ablated in caspase 11, and the suggestion has been made that mouse caspases 1 and 11 collaborate in proinflammatory cytokine activation (Wang *et al.*, 1998). These mice are highly resistant to septic shock and have lower production of a variety of other cytokines in response to experimental LPS infusion. Significantly, no overt apoptotic phenotype or developmental defect has been observed demonstrating that caspases 1 and 11 are not involved in apoptosis, except possibly in a paracrine manner by sensitizing cells to apoptosis via cytokine activation (Friedlander *et al.*, 1997). Human caspases 4 and 5 are less studied enzymes, and they are almost certainly cytokine activators because of the high



**Figure 1** A proteolytic cycle. The fundamental mechanisms governing activity of proteases are conserved in caspase activation. Latent proteases await an activation signal. The activator may be an oligomeric activation platform, or it may be another protease. Once active, substrate and inhibitor compete for protease binding, and the outcome is defined by the local concentration of inhibitor. Significantly, active protease may be released from its inhibitory complex by factors that bind the inhibitor. This is signified by the double-headed arrow, since many cognate protease inhibitors form reversible complexes with their target proteases (Turk *et al.*, 2002). A cascade is defined when the substrate of one protease (caspase 8 for example) is another protease zymogen (caspase 3 for example)

**Table 1** Categories of human and *Drosophila* caspases

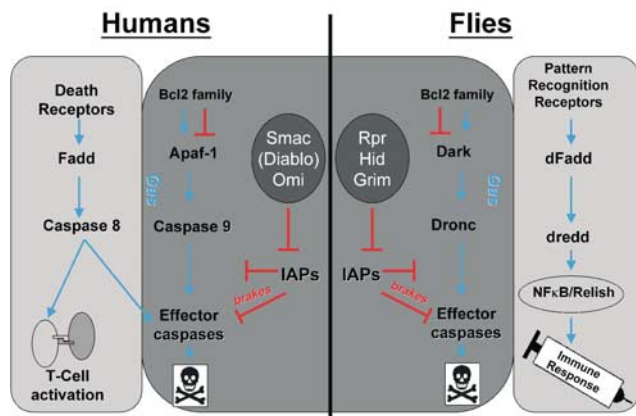
Domain organization type	Human	<i>Drosophila</i>
Long prodomain (DED, CARD), apoptotic initiator type	Caspases 2, 8, 9, 10	Dronc, Dredd <sup>a</sup>
Short prodomain, apoptotic effector type	Caspases 3, 6, 7	Dcp1, Drice, Decay, Damm
Long prodomain (CARD), cytokine activator type	Caspases 1, 4, 5	
Others	Caspase 14 <sup>a</sup>	Strica

<sup>a</sup>Dredd, though it contains a DED recruitment unit, is predominantly involved in innate immunity. Caspase 14 is a short prodomain caspase involved in terminal keratinocyte differentiation (Lippens *et al.*, 2000). Although Strica bears a long prodomain, phylogenetic analysis based on the catalytic domain (p10, p20), shows clustering with the executioner caspases (see Lamkanfi *et al.*, 2002)

sequence similarity, domain organization and comparable substrate specificity they share with caspase 1. Presumably, caspases 4 and 5, which seem to be orthologs of mouse caspase 11, process cytokines in response to different inflammatory stimuli or pathological situations than caspase 1, or cooperate with caspase 1 to produce an adequate inflammatory response. With respect to this, caspase 5 has been found to be associated with caspase 1 under specific conditions *in vitro* (Martinon *et al.*, 2002).

### The human apoptotic initiators

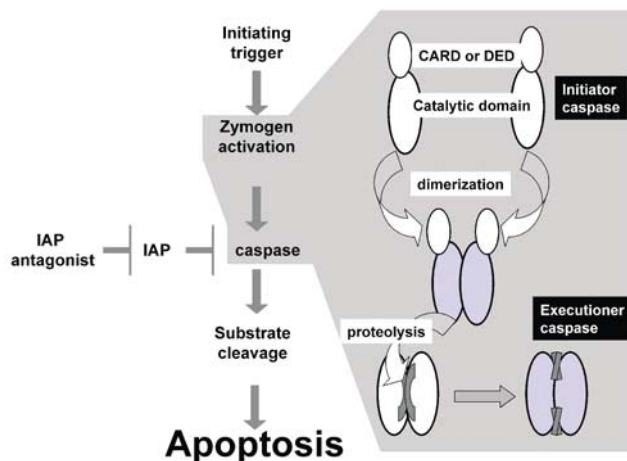
Initiator caspases constitute the point at which cell signals are converted to proteolytic activity, probably one of the most important decisions a cell can make during its life. The need for initiator caspases may be threefold. First, they permit sensing and integration of different inputs, transmitting to a common execution phase. Second, they enforce amplification of the apoptotic system by generating substantial amounts of active executioner caspases. Third, they allow for a point of regulation before the final commitment to death. In humans, we recognize two distinct initiation points: the extrinsic pathway and the intrinsic pathway (Figures 2 and 3). Recruitment and activation of the initiator caspases is achieved by adapter molecules that bridge to death receptors via homophilic DEDs – for



**Figure 2** Apoptotic caspase pathways in humans and flies – conservation. The centrally conserved intrinsic pathway in humans and flies is shown by the dark shading, and involves caspase 9 or Dronc as the initiating caspase. Upstream decisions are integrated by members of the Bcl-2 family, and signals are transduced to the activation platforms of Apaf-1 or Dark. All intrinsic caspase components are under the control of IAPs, which in turn can be derepressed – to release caspase activity – by the species-specific antagonists in the ovals. In the lighter shading are the extrinsic activation pathways. In mammals, the caspase 8 pathway plays a profound role in initiating apoptosis, but may also paradoxically provide cell activation stimuli. In contrast, the extrinsic route in flies plays a minor role in apoptosis, being more involved in innate immunity. The figure highlights the forward drive by zymogen activation (the Gas) and control by IAPs (the Brakes) that dominate the intrinsic pathway

caspases 8 and 10, or via CARDs – between caspase 9 and the cofactor Apaf 1. In many cultured cellular models in which caspases 8 and 10 are expressed, they seem redundant probably owing to the high identity (48%), similar substrate specificity and domain organization.

Humans with mutant caspase 10 display an autoimmune lymphoproliferative syndrome caused by defective lymphocyte apoptosis (Wang *et al.*, 1999a). Humans with mutant caspase 8, while also exhibiting defects in lymphocyte apoptosis, have pronounced defects in their ability to activate lymphocytes, with resulting immunodeficiency (Chun *et al.*, 2002). Importantly, the latter study revealed that caspase 8 deficiency is compatible with development in humans, although it is embryonic lethal in mice (Varfolomeev *et al.*, 1998), probably because mice lack caspase 10 (Reed *et al.*, 2003). Moreover, the results of analysis of disease suffered by humans with defects in caspase 8 reveal a role for this caspase in T-cell activation, clearly distinct from its associated proapoptotic role.



**Figure 3** The basis of IAP antagonists as proapoptotic proteins – variation between humans and flies. In flies there is evidence for a continuous low-level production of caspases, which are neutralized by *Drosophila* IAP-1 (Rodriguez *et al.*, 2002). In this scenario, simple upregulation of one or more fly IAP antagonists could ignite the system into apoptosis, and to this extent specification of apoptosis in transcriptionally regulated (White *et al.*, 1994). In contrast to flies, the currently known mammalian IAP antagonists are mitochondrial proteins and require translocation before they can influence the inhibitory activity of IAPs. This implies a distinct point of impact for the regulation of IAP antagonists in mammals since both positive initiator caspase signaling and mitochondrial fluxes would presumably be required, and the system may be less reliant upon transcriptional regulation. Input from the left of the diagram may be the most important event in flies, whereas in mammals, input from the top of the diagram could be more important. The shaded area expands the zymogen activation phase, in which apical (initiator) caspases acquire the ability to cleave downstream targets following dimerization at activator platforms, mediated by DED or CARD recruitment domains. The prime targets of the apoptotic initiator caspases are the executioner caspases, which pre-exist as dimers requiring simply limited proteolysis for activation

### The human apoptotic executioners

In contrast to the initiators, caspases 3, 6, and 7 each possess a short distinct N-terminal peptide (23–28 residues). The reason for having three executioner caspases is not clear, and indeed the evidence for the importance of caspase 6 is lacking. Whereas caspase 3 is essential for normal embryonic development, the phenotype of mice ablated in caspase 6 appears to be normal (Zheng *et al.*, 2000). The phenotype of mice ablated in caspase 7 has yet to be reported, yet it is evident that caspases 3 and 7 are almost synonymous in their substrate and inhibitor specificity. Nevertheless, biochemical experiments place the activation of caspases 6 (Orth *et al.*, 1996) and 7 (Yang *et al.*, 1998; Denault and Salvesen, 2003) downstream of caspase 3 and so we will consider them as executioner caspases.

### The fly apical caspases

Dronc is the sole 'CARD-carrying' caspase in the fly genome (Kumar and Doumanis, 2000) and, in this respect, the enzyme is most homologous to caspase 9. Expression of this locus is ubiquitous but acutely responsive to the steroid hormone ecdysone (Dorstyn *et al.*, 1999a). When overexpressed, this gene provokes apoptosis in both cultured cells and in the animal (Dorstyn *et al.*, 1999a; Meier *et al.*, 2000; Quinn *et al.*, 2000), which requires the participation of 'downstream' caspases (Dorstyn *et al.*, 1999a). Although single gene mutations at this locus have not been characterized, studies with multigenic deficiencies, dominant negatives (Meier *et al.*, 2000), and injected dsRNAs (Quinn *et al.*, 2000) suggest that this caspase is a central player in the apoptotic programmed cell death pathway. Interestingly, in addition to Asp, Dronc may cleave following Glu residues in some proteins (including its own precursor) implying a more tolerant specificity (Hawkins *et al.*, 2000). Notably, bacterially produced Dronc was found to be a particularly poor catalyst with kinetics ~40–180-fold less than caspase 9, itself a rather inefficient enzyme when expressed in bacteria. Hence, like caspase 9, efficient Dronc activity might require the formation of a holoenzyme complex involving intimate associations with Dark, the *Drosophila* ortholog of Apaf-1 (Kanuka *et al.*, 1999; Rodriguez *et al.*, 1999; Zhou *et al.*, 1999).

Another apical caspase in flies, Dredd (Chen *et al.*, 1998), most closely resembles mammalian caspases 8 and 10. The long prodomain found in Dredd includes significant sequence similarities spanning the DEDS of these mammalian counterparts. Hu and Yang (2000) noted that a region of *Drosophila* Fadd (dFadd) binds to and shares substantial similarity with this same portion of the Dredd prodomain, and referred to this shared motif as the 'death-inducing domain'. Single gene mutations at Dredd are viable and recessive (Leulier *et al.*, 2000). Although *dredd*<sup>-</sup> animals are cell death defective when examined in sensitizing backgrounds, the

absence of Dredd did not cause global defects in PCD. Instead, genetic analyses established that Dredd plays a fundamental role in the innate immune response toward bacterial pathogens (Leulier *et al.*, 2000). When Dredd mutants were challenged by microbial pathogens, induction of several antimicrobial genes failed. Consistent with these observations, Dredd activation processing was responsive not only to apoptotic signals (Chen *et al.*, 1998), but to LPS (Georgel *et al.*, 2001; Stoven *et al.*, 2003a, b) and coexpression of dFadd (Hu and Yang, 2000) as well. Collectively, the findings established a limited role for this caspase in apoptosis but uncovered an obligatory role for this apical caspase in transducing responses to microbial pathogens. In flies, as in mammals, innate immunity is governed by Toll receptors that propagate signals through members of the NF $\kappa$ B family to induce specific sets of antimicrobial peptides (reviewed in Hoffmann and Reichhart, 2002). Since the function of Dredd maps upstream of the *Drosophila* NF $\kappa$ B protein, RELISH (Stoven *et al.*, 2003a, b), which itself requires proteolytic cleavage for activation, the findings illuminate a compelling new link between signal-dependent activation of NF $\kappa$ B proteins and the action of a caspase. As Dredd and Relish can physically interact, a likely scenario proposes that Dredd directly cleaves Relish, perhaps in a phosphorylation-dependent manner (Stoven *et al.*, 2000, 2003a, b).

The third long prodomain caspase encoded in the fly genome, Strica, bears an unusual N-terminal prodomain with no homologies to other proteins (Kumar and Doumanis, 2000). Likewise, the putative prodomain exhibits no matches to previously characterized motifs (e.g. CARD, DED) but it is distinctly rich in serines and threonines. Little functional data on this enzyme currently exists, but the few studies that have been performed on this protein failed to detect activation processing of, nor proteolytic activity against, commercial substrates. However, if overexpressed, Strica can trigger apoptosis and was able to associate with DIAP1 and DIAP2 (Doumanis *et al.*, 2001). Mutations in Strica have not been isolated and so the physiologic function of this enzyme is currently not known.

### The fly executioner caspases

Dcp1, Drice, Damm, and Decay lack extensive prodomains and hence qualify as effector caspases. Single gene mutations do not yet exist in any of these genes and so the requirements for these proteins in development are not yet known (previously described DCP1 alleles are now known to be compound mutations that also affect a flanking gene (McCall *et al.*, 2003)). The substrate specificities determined for Dcp1 and Drice are notably similar to human caspase 3, as well as nematode Ced-3, and forced expression of truncated variants can trigger apoptosis (Fraser and Evan, 1997; Song *et al.*, 2000). In addition, although both enzymes are able to cleave *Drosophila* lamin their substrate specificities are not identical, as evidenced by distinct activities of these

enzymes for human lamins (Song *et al.*, 2000). Evidence from immunodepletion studies on cultured *Drosophila* cells implicates Drice as the predominant (if not the sole) effector caspase responsible for apoptosis at least in S2 cells (Fraser *et al.*, 1997).

Ectopic expression of the two less well-characterized enzymes, Decay and Damm, also triggers apoptosis (Dorstyn *et al.*, 1999b; Harvey *et al.*, 2001). These proteins are most closely aligned with human caspases 3 and 7 (Decay) and caspase 6 (Damm), respectively. In the former case (Dorstyn *et al.*, 1999b), this resemblance extended to enzymatic activity (Decay was active against a caspase 3 substrate) while, in contrast, Damm was not active against a synthetic substrate preferred by caspase 6 (Harvey *et al.*, 2001).

As mentioned earlier, while the functional order of these caspases programmed cell death is largely inferred, there is broad consensus that Dronc is an important initiator caspase (Dorstyn *et al.*, 1999a, 2002; Meier *et al.*, 2000; Muro *et al.*, 2002; Yu *et al.*, 2002). The most rigorous biochemical demonstration that Dronc activates Drice comes from *in vitro* studies where Dronc was shown to process Drice (Hawkins *et al.*, 2000). From other *in vitro* studies, there is also evidence that reciprocal 'cross-cleavage' between Dcpl and Drice may occur (Fraser *et al.*, 1997; Song *et al.*, 2000) but whether this cleavage relationship occurs *in vivo* is not yet known.

### Caspase zymogen activation: stepping on the gas

Most biochemical and structural work on caspase activation has been performed with human caspases 3, 7, 8, and 9, and a reasonably clear picture has emerged to demonstrate variation and conservation in their activation mechanisms. The executioner caspases 3 and 7 are activated by direct proteolysis at internal sites that generate the large and small subunits of their catalytic domains. In contrast, the initiator caspases 8 and 9 do not require cleavage for activation, but are activated within polymeric activation platforms.

The zymogens of the initiator caspases exist within the cell as inactive monomers. These monomeric zymogens require dimerization in order to assume an active conformation, and this activation is independent of cleavage (Stennicke *et al.*, 1999; Boatright *et al.*, 2003; Donepudi *et al.*, 2003). The dimerization event occurs at multiprotein activating complexes to which the caspase zymogens are recruited to by virtue of their N-terminal recruitment domain. The activating complex involved depends on the origin of the death stimulus: in the intrinsic pathway it occurs within the Apaf-1 containing apoptosome and in the extrinsic pathway it occurs within the polymeric DISC.

### Extrinsic pathway activation: caspase 8 and Dredd

In mammals, the DISC activates the extrinsic cell death pathway and is recruited to the cytoplasmic portion of

death receptors. Within this complex, the adaptor protein FADD forms the essential link to apical caspases 8 and 10 via homotypic interactions involving DED domains. The fly genome clearly encodes an ortholog of Fadd, designated Dfadd, and, like its mammalian counterpart, this protein binds to and regulates an apical caspase, in this case Dredd (Hu and Yang, 2000; Naitza *et al.*, 2002). However, while flies do express a TNF-like axis, current evidence argues that it probably does not engage the Dfadd/Dredd module to launch an apoptotic caspase cascade (Igaki *et al.*, 2002; Kanda *et al.*, 2002; Moreno *et al.*, 2002; Kauppila *et al.*, 2003). Instead, the Dfadd/Dredd module predominantly (and perhaps exclusively) regulates innate immune responses triggered by proteins known as peptidoglycan-recognition proteins, some of which encode transmembrane bacterial sensors (Hoffman, 2003). Transduction of signals by these nonself sensors is propagated through Dredd to the NF $\kappa$ B protein, Relish (orthologous to the mammalian NF $\kappa$ B proteins, p100, and p105) and it has been proposed that Relish is in fact a substrate for the Dredd caspase. Moreover, because Relish activation also requires I $\kappa$ B kinases together with cleavage at an aspartate (Silverman *et al.*, 2000; Stoven *et al.*, 2000, 2003a, b; Lu *et al.*, 2001), the findings raise the possibility that Dredd might directly cleave RELISH in a phosphorylation-dependent manner. This scenario contrasts sharply with mechanisms proposed for mammalian NF $\kappa$ B counterparts (p100 and p105), where proteolytic cleavage is thought to occur via the proteasome (Silverman and Maniatis, 2001). However, evidence arguing for orthologous physiologic relationships between caspase 8 and NF $\kappa$ B proteins also exists (Chaudhary *et al.*, 2000) and it therefore seems probable that this ancient innate immune response pathway is well conserved, at least in some mammalian cell types.

An interesting addition to the mechanism of human caspase 8 activation is the involvement of FLIP (FLICE-like inhibitory protein – FLICE being one of the original names for caspase 8). FLIP is a caspase 8 homolog with crucial differences, notably its lack of a catalytic cysteine that renders it incapable of proteolytic activity. At low levels of expression (close to those occurring in a normal cell), FLIP enhances Fas-induced caspase 8 activation at the DISC. However, at higher levels (such as that found in certain tumors) FLIP inhibits caspase 8 activation (Chang *et al.*, 2002), presumably by saturating available recruitment sites on the DISC and preventing caspase 8 recruitment. This study was complimented by studies revealing that FLIP was capable of forming heterodimers with caspase 8 that possessed catalytic activity (Micheau *et al.*, 2002), incidentally confirming the dimerization activation mechanism of caspase 8.

### Intrinsic pathway activation: caspase 9 and Dronc

The objective of the intrinsic pathway is to integrate developmental and stress cues into activation of the

apical caspase 9 or Dronc. In humans, a key component of the integrator is the mitochondrion. Importantly, oligomerization of Apaf-1 into a functional apoptosome requires release of cytochrome *c* from mitochondria, and is therefore clearly a post-translational event (Liu *et al.*, 1996; Kluck *et al.*, 1997; Li *et al.*, 1997). In stark contrast, a parallel apoptotic role for cytochrome *c* in flies is controversial. Prior to overt signs of apoptosis, an otherwise hidden epitope on Dark, the fly counterpart of Apaf-1, is exposed (Varkey *et al.*, 1999; Arama *et al.*, 2003) and exogenous cytochrome *c* weakly enhanced apoptosome-like activities in lysates from fly cells (Kanuka *et al.*, 1999; Dorstyn *et al.*, 2002). However, most studies in fly systems find no evidence for release of cytochrome *c* from mitochondria during apoptosis (Varkey *et al.*, 1999; Dorstyn *et al.*, 2002) and, in gene silencing experiments, dsRNAs targeting DARK suppressed apoptosis but dsRNAs silencing cytochrome *c* RNAs showed no effect (Zimmermann *et al.*, 2002). Hence, while a vital role for Dark in PCD is firmly established, important questions regarding how its activity is regulated and whether cytochrome *c* plays a role remain to be answered. This introduces the possibility of a fundamental distinction in the caspase pathway of flies and humans, since it suggests the probability that human caspase activation is regulated by activation, but that fly caspase activation is constitutive (Rodriguez *et al.*, 2002).

Although caspase 9 is the common initiator of the intrinsic pathway, recent work demonstrates that caspase 2 is required for an apoptotic response to neurotrophic deprivation (Troy *et al.*, 2001) and DNA damage (Lassus *et al.*, 2002), a subset of intrinsic stimuli, in certain primary cells or cell lines. Caspase 2 appears to be activated by interaction with a high molecular weight complex that requires the CARD of caspase 2 (Read *et al.*, 2002). The components of this complex have yet to be identified, but they are independent of Apaf-1. Similar to the other initiator caspases, the zymogen of caspase 2 is a latent monomer and cleavage is not required for its activation (Read *et al.*, 2002). Rather, the active form of caspase 2 exists as both cleaved and uncleaved, in complex with a high molecular weight activator of currently unknown composition. Significantly, a unified model for apical (initiator) caspase activation suggests that all long prodomain caspases may be recruited as monomers to activation platforms that function to dimerize them to the active form (Boatright *et al.*, 2003). Once the active form is achieved, downstream caspase activation takes place strictly by limited proteolysis.

### Execution phase activation: caspases 3 and 7

In apparent incongruity to the initiators, the executioner caspase 3 and 7 zymogens pre-exist within the cytosol as inactive dimers (Boatright *et al.*, 2003). They are activated by limited proteolysis within their interdomain linker carried out by an initiator caspase, and occasion-

ally by other proteases under specific circumstances. The crystal structures of zymogen caspase 7, active caspase 7, and inhibitor-bound caspase 7 serve as a model with which to rationalize the apparent conflict between the cleavage mechanism for executioner caspase activation, and the dimerization mechanism for apical caspase activation (Wei *et al.*, 2000; Chai *et al.*, 2001b; Riedl *et al.*, 2001a).

At cytosolic concentration in human cells, the caspase 3 (Bose and Clark, 2001; Pop *et al.*, 2001) and 7 zymogens are already dimers (Boatright *et al.*, 2003), but cleavage within their respective linker segments is required for activation (Chai *et al.*, 2001b; Riedl *et al.*, 2001a). The same reordering of catalytic and substrate binding residues occurs in caspase 7 as seen in caspase 9, so the fundamental mechanism of zymogen activation is equivalent. Only the driving forces are distinct since the inter-subunit linker segment of procaspase 7 blocks ordering of the active site, and upon cleavage the new N- and C-terminal sequences so generated aid in active site stabilization. The property that allows the distinct driving forces (dimerization of monomers or cleavage of pre-existing dimers) to converge on the same activation mechanism seems to be the unusual plasticity of the residues constituting the caspase active site, which rather unusually for proteases are predominantly placed on flexible loops and not ordered secondary structure.

### Caspase-inhibiting IAPs: the brakes

Although the first level of regulating proteolytic pathways is by zymogen activation, an equally important level is achieved by specific inhibitors that can govern the activity of the active components. The endogenous inhibitors of caspases, those present in mammals and flies, are members of the inhibitor of apoptosis (IAP) family. IAPs contain one, two, or three baculovirus IAP repeat (BIR) domains, which represent the defining characteristic of the family. The best-characterized endogenous human caspase inhibitor is the X-linked IAP (XIAP), a member of the IAP family. The IAPs are broadly distributed and, as their name indicates, the founding members are capable of selectively blocking apoptosis, having initially been identified in baculoviruses (reviewed in Verhagen *et al.*, 2001a). Eight distinct IAPs have been identified in humans. XIAP (which is the human family paradigm) has been found by multiple research groups to be a potent but restricted inhibitor targeting caspase 3, 7, and 9 (reviewed in Deveraux and Reed, 1999). Similarly, evidence implicates human cIAPs 1 and 2, ML-IAP, ILP-2, and Drosophila DIAP-1 as caspase inhibitors (reviewed in Salvesen and Duckett, 2002).

The second BIR domain (BIR2) of XIAP specifically target caspases 3 and 7 ( $K_i \approx 0.1-1$  nM), and regions closely related to the third BIR domain (BIR3) specifically target caspase 9 ( $K_i \approx 10$  nM). This led to the general assumption that the BIR domain itself was important for caspase inhibition. Surprisingly, the

recent structures of BIR2 in complex with caspases 3 and 7 have revealed the BIR domain to have almost no direct role in the inhibitory mechanism. All the important inhibitory contacts are made by the flexible region preceding the BIR domain (Chai *et al.*, 2001a; Huang *et al.*, 2001; Riedl *et al.*, 2001b). Interestingly, the mechanism of inhibition of caspase 9 by the BIR3 domain requires cleavage in the intersubunit linker to generate the new sequence NH<sub>2</sub>-ATPF (Srinivasula *et al.*, 2001). In part, this explains the cleavage of caspase 9 during apoptosis, which as described above is not required for its activation. Paradoxically, it seems required for its inactivation by XIAP, which then inhibits caspase 9 by reversing the dimeric activation process (Shiozaki *et al.*, 2003). The importance of IAPs in cancer progression is underscored by observations of tumor-associated elevations in their levels. For example, upregulation of IAP family members is common in prostate cancers of both humans and mice (Krajewska *et al.*, 2003). Moreover, IAP expression shows a positive correlation with chemoresistance and poor treatment outcome (Tamm *et al.*, 2000).

Encoded within the fly genome are four BIR containing genes, DIAP1, DIAP2, dBruce, and Deterin. Among these, DIAP1 is the best studied and transgenic analyses, as well as gain-of-function and loss-of-function mutations, clearly demonstrate a central, apoptotic role for this protein (reviewed in Hay, 2000). When overexpressed, DIAP2, dBruce, and Deterin can also suppress death in certain contexts and, notably, deletion alleles at dBruce enhanced killing by Rpr and Grim (Vernooy *et al.*, 2002). DIAP1 binds and inhibits the action of Drice, Dcp1, and Dronc (Kaiser *et al.*, 1998; Wang *et al.*, 1999b; Hay, 2000; Meier *et al.*, 2000) but similar activities for other BIR containing proteins have not been reported.

IAPs clearly have functions in addition to caspase inhibition because they have been found in organisms such as yeast, which neither contain caspases nor undergo apoptosis (Uren *et al.*, 1998). Moreover, though most of the mechanistic studies on IAPs relate to their binding and direct inhibition of caspase catalytic activity, it is likely that some of the IAPs function to downregulate caspases not by inhibiting them, but by acting as E3 ligases for their rapid removal via the proteasomal route (Huang *et al.*, 2000). Irrespective of the actual mechanism for terminating caspase activity, the IAPs set the scent for the final phase of caspase control – derepression as a method of activation.

### IAP antagonists: releasing the brakes

Reaper proteins act by derepressing IAPs. The four known activators of apoptosis in the Reaper region (RPR, GRIM, HID, and SKL) are all transcribed in the same orientation, encode partially redundant functions and share an N-terminal amino-acid motif referred to as either the RHG motif (Wing *et al.*, 2001; Christich *et al.*, 2002; Srinivasula *et al.*, 2002; Wilson *et al.*, 2002), or the

IAP-binding motif (IBM) (Salvesen and Duckett, 2002; Tenev *et al.*, 2002). How do these proteins elicit the death of a cell? One likely explanation focuses on derepression functions that liberate caspases from IAPs, which themselves act as constitutive caspase inhibitors. Among the fly IAP proteins, DIAP1 is thought to act as a central, rate-limiting brake upon caspases through direct physical contact with these enzymes (Meier and Evan, 1998; Goyal *et al.*, 2000; Hay, 2000; Lisi *et al.*, 2000; Wu *et al.*, 2001). While the precise mechanisms are not completely understood, Reaper proteins and a recently identified RHG containing protein, Jafrac2, (Tenev *et al.*, 2002) are thought to antagonize DIAP1 through direct interactions (Wilson *et al.*, 2002) involving contacts formed by residues in the RHG motif and the BIR domains of DIAP1 (Shi, 2001). Elegant structural studies, recently reported by Shi and colleagues, have further elucidated the precise binding surfaces for this association and mapped these relative to the site on DIAP1 that recognizes Dronc (Chai *et al.*, 2003). Their studies nicely explain how associations between DIAP1/Dronc or DIAP1/RHG motif of Reaper proteins are mutually exclusive since the same binding pocket with the second BIR repeat of DIAP1 can accommodate only one but not both proteins (Chai *et al.*, 2003). The ultimate outcome of these interactions promotes displacement of activate caspases via mechanisms linked to: (1) autoubiquitination of DIAP1 (Ryoo *et al.*, 2002), (2) suppression of DIAP1-mediated ubiquitination of Dronc (Meier *et al.*, 2000; Chai *et al.*, 2003) and (3) N-end rule degradation of DIAP1 (Ditzel *et al.*, 2003).

Numerous observations are consistent with a caspase/DIAP1 liberation model for cell killing by Reaper-like proteins. First, the apoptotic action of these proteins is effectively suppressed by caspase inhibitors and by DIAP1 in both cultured cells and in the animal (Abrams, 1999; Hay, 2000). Second, removal of DIAP1 from cells (Igaki *et al.*, 2002; Zimmermann *et al.*, 2002) or embryos (Wang *et al.*, 1999b; Rodriguez *et al.*, 2002) promotes rapid apoptotic death. Third, DIAP1 binds and inhibits at least two of the fly caspases, Drice and Dronc (Kaiser *et al.*, 1998; Wang *et al.*, 1999b; Meier *et al.*, 2000). Fourth, RPR, GRIM, and HID can bind to IAPs through their common N-terminal RHG motif and also antagonize IAP function in heterologous, yeast-based assays (Vucic *et al.*, 1997; Kaiser *et al.*, 1998; Vucic *et al.*, 1998; Wang *et al.*, 1999b; Hay, 2000; Wu *et al.*, 2001). Fifth, the RHG motif is sufficient to elicit apoptosis (Vucic *et al.*, 1998; Claveria *et al.*, 2002) and flies carrying DIAP1 mutations that selectively impair binding to RPR and HID exhibit resistance to apoptosis by these proteins (Goyal *et al.*, 2000; Lisi *et al.*, 2000). Collectively, these observations strongly favor models whereby Reaper proteins function, at least in part, to release active caspases from inhibitory IAP complexes. Additional support for the liberation model comes from structural studies that highlighted shared features among the RHG motif and the N-termini of two mammalian proteins, Smac (Diablo in mice) and Omi/HtrA2, which also bind and antagonize IAP

function (Hegde *et al.*, 2001; Martins *et al.*, 2001; Verhagen *et al.*, 2001b; van Loo *et al.*, 2002). Comparative analyses of BIR domains that were complexed with N-terminal peptides from HID, GRIM (Wu *et al.*, 2001), and Smac (Chai *et al.*, 2000; Wu *et al.*, 2000), described minimal surfaces required for binding to BIRs with common contacts shared between the fly and mammalian peptides.

Although attractive and compelling, other lines of evidence are inconsistent with strict 'liberation' models that presume DIAP1 is the sole effector of RPR proteins. First, while the RHG motif is sufficient to evoke apoptosis, RPR and GRIM also exert potent killing activities distinct from this IAP binding domain (Chen *et al.*, 1996; Vucic *et al.*, 1998; Wing *et al.*, 1998, 2001). Intriguing parallels to recent studies on SMAC are worth noting here, as this mammalian IAP antagonist also provokes apoptosis without its IAP binding domain (Roberts *et al.*, 2001). Likewise, *in vitro* studies have uncovered significant biochemical activities for RPR proteins that do not require the RHG domain and appear to be independent of DIAP1 activity. For instance, in heterologous systems, apoptogenic activity of RPR required a ubiquitin-like domain containing protein, SCYTHE, which binds to RPR outside of the N terminal IBM (Thress *et al.*, 1998, 1999). More recently, both RPR and GRIM were found to repress translation of proteins *in vitro* and, where tested, this activity was observed in the absence of the N terminal IBM (Holley *et al.*, 2002; Yoo *et al.*, 2002). Finally, epistasis studies in cultured cells (Zimmermann *et al.*, 2002) and in the fly (Rodriguez *et al.*, 2002) indicated that cells are not 'preloaded' with sufficient levels of IAP-inhibited processed caspases to achieve cell killing. Instead, the results favored a 'gas and brake' model whereby concurrent input from Dark, together with removal of IAP inhibition, drives caspase activation to levels that exceed a threshold necessary for apoptosis. Taken together, the collective body of research shows that RPR proteins cause cell death by relieving caspases from the antagonistic action of IAPs and might, concurrently, also engage other death-associated signals, perhaps through unknown effectors.

### Gas, brakes, and the control of cancer

The above discussion has been used to focus readers on the apparent differences in caspase control in humans and flies. Current evidence suggests that human caspase activation is a post-translational pathway that must be triggered by specific initiators. Of course, transcription sets the scene – the level of the pro- and antiapoptotic proteins, but the stringent controls on caspase zymogen activation restrain a constant low level activation, or 'tick-over', of caspase activity. In contrast, flies may possess this tick-over, much as has been suggested for complement activation – another proteolytic cascade (Manderson *et al.*, 2001). Are these different modes of regulation minor peculiarities, or do they provide more

profound variations in the design of the intrinsic cell death machinery? Are we all blindly feeling different parts of the same elephant?

The problem in interpretation may be inherent in the relative ease of studying pathways in flies by epistatic genetics, and can be confounded in humans (or mice) by redundancy in IAPs. Nevertheless, fundamental differences in IAP antagonists suggest basic differences in caspase regulation. Paramount among these is that in *Drosophila* the death-inducing proteins Hid, Grim, Reaper, and Sickie all contain homologous IAP binding motifs at their N-terminus (sometimes called the RHG or IBM motif). In distinction to the known mammalian IAP antagonists, the *Drosophila* ones do not have transit peptides, and are therefore fully functional translation synthesis and removal of their initiator methionine. In contrast, the known human IAP antagonists Smac and Omi/HtrA2 are not active following translation since they are housed in mitochondria awaiting a signal for their release. This supports the transcription/translation driven control of apoptosis in flies, and the post-translational driven activation in humans.

Given that transformed cells invariably must lose their ability to die when out of context, it is now fairly certain that part of this oncogenic transformation results from inactivation of key proapoptotic components or upregulation of antiapoptotic ones (Reed, 1999). Among these are mutations in death receptors and adaptors, mutations in caspases 8 and 10, and upregulation of IAPs (reviewed in Kaufmann and Vaux, 2003). Paradoxically, or maybe essentially (Green and Evan, 2002), cancer cells become sensitive to apoptosis-inducing chemotherapy. In the very nice review in *Oncogene* that should be read in conjunction with this one, Kaufmann and Vaux (2003) pointed out that most chemotherapeutic agents act by triggering the intrinsic (caspase 9) pathway at a level upstream on mitochondria.

But toxicity raises its head with chemotherapy, and so adjuvant cancer therapy is clearly a current theme. With respect to this, we may learn a lot by studying the mechanisms of caspase control. If the objective is to activate caspases in cells that have switched off parts of the pathway, then attacking two or more points in the apoptotic pathways to rekindle death is a valuable strategy. Success in combined TRAIL/Apo2L (caspase 8 activation) and IAP antagonist experimental therapy demonstrates the lowering of the threshold of TRAIL induced death by pretreatment with IAP antagonists (Fulda *et al.*, 2002). Small molecules that lower the kinetic barrier for apical caspase activation can now be considered, and a possible example of this is seen in the discovery of a small molecule that influences apoptosome function (Jiang *et al.*, 2003). Exploration of nonpeptidic IAP antagonists is producing results, at least in experimental cell culture paradigms (Wu *et al.*, 2003), and more significantly in mouse models of tumor regression (Schimmer *et al.*, 2003).

Flies are indispensable for understanding basic mechanisms and it is unlikely that the importance of

IAP antagonists would have been appreciated without the fly paradigm. We also note that in development and in cancers, pressures driving the cell cycle may be universally coupled to those which sensitize cells toward apoptosis (Abrams, 2002; Nahle *et al.*, 2002). In other words, early staged neoplastic cells probably exist in a constitutively idling state, with elevated flux operating through pathways that provide 'the gas'. Accordingly, early staged neoplastic cells must also exist under intense selective favoring repression of caspase activity through genetic and/or epigenetic

means. Hence, while flies may be an imperfect model for understanding the late stages of human cancers, they could represent an ideal model for the early phases of oncogenesis, where fundamental properties of caspase repression by IAPs can be easily accessed.

#### Acknowledgements

GSS was supported by NIH Grants CA69381 and AG15402; JMA was financially supported by NIH Grant AG12466 and the American Cancer Society.

#### References

- Abrams JM. (1999). *Trends Cell Biol.*, **9**, 435–440.
- Abrams JM. (2002). *Cell*, **110**, 403–406.
- Arama E, Agapite J and Steller H. (2003). *Dev. Cell*, **4**, 687–697.
- Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen I, Ricci J-E, Edris WA, Sutherlin DP, Green DR and Salvesen GS. (2003). *Mol. Cell*, **11**, 529–541.
- Bose K and Clark AC. (2001). *Biochemistry*, **40**, 14236–14242.
- Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, March CJ, Kronheim SR, Druck T, Cannizzaro LA, Huebner K and Black RA. (1992). *Science*, **256**, 97–100.
- Chai J, Du C, Wu JW, Kyin S, Wang X and Shi Y. (2000). *Nature*, **406**, 855–862.
- Chai J, Shiozaki E, Srinivasula SM, Wu Q, Dataa P, Alnemri ES and Yigong Shi Y. (2001a). *Cell*, **104**, 769–780.
- Chai J, Wu Q, Shiozaki E, Srinivasula SM, Alnemri ES and Shi Y. (2001b). *Cell*, **107**, 399–407.
- Chai J, Yan N, Huh JR, Wu JW, Li W, Hay BA and Shi Y. (2003). *Nat. Struct. Biol.*, **10**, 892–898.
- Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME and Yang X. (2002). *EMBO J.*, **21**, 3704–3714.
- Chaudhary PM, Eby MT, Jasmin A, Kumar A, Liu L and Hood L. (2000). *Oncogene*, **19**, 4451–4460.
- Chen P, Lee P, Otto L and Abrams J. (1996). *J. Biol. Chem.*, **271**, 25735–25737.
- Chen P, Rodriguez A, Erskine R, Thach T and Abrams JM. (1998). *Dev. Biol.*, **201**, 202–216.
- Christich A, Kauppila S, Chen P, Sogame N, Ho SI and Abrams JM. (2002). *Curr. Biol.*, **12**, 137–140.
- Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, Dale JK, Puck J, Davis J, Hall CG, Skoda-Smith S, Atkinson TP, Straus SE and Lenardo MJ. (2002). *Nature*, **419**, 395–399.
- Claveria C, Caminero E, Martinez AC, Campuzano S and Torres M. (2002). *EMBO J.*, **21**, 3327–3336.
- Denault JB and Salvesen GS. (2002). *Chem. Rev.*, **102**, 4489–4500.
- Denault JB and Salvesen GS. (2003). *J. Biol. Chem.*, **278**, 34042–34050.
- Deveraux QL and Reed JC. (1999). *Genes Dev.*, **13**, 239–252.
- Ditzel M, Wilson R, Tenev T, Zachariou A, Paul A, Deas E and Meier P. (2003). *Nat. Cell Biol.*, **5**, 467–473.
- Donepudi M, Mac Sweeney A, Briand C and Gruetter MG. (2003). *Mol. Cell*, **11**, 543–549.
- Dorstyn L, Colussi PA, Quinn LM, Richardson H and Kumar S. (1999a). *Proc. Natl. Acad. Sci. USA*, **96**, 4307–4312.
- Dorstyn L, Read S, Cakouros D, Huh JR, Hay BA and Kumar S. (2002). *J. Cell Biol.*, **156**, 1089–1098.
- Dorstyn L, Read SH, Quinn LM, Richardson H and Kumar S. (1999b). *J. Biol. Chem.*, **274**, 30778–30783.
- Doumanis J, Quinn L, Richardson H and Kumar S. (2001). *Cell Death Differ.*, **8**, 387–394.
- Fesik SW. (2000). *Cell*, **103**, 273–282.
- Fischer U, Janicke RU and Schulze-Osthoff K. (2003). *Cell Death Differ.*, **10**, 76–100.
- Fraser AG and Evan GI. (1997). *EMBO J.*, **16**, 2805–2813.
- Fraser AG, Mccarthy NJ and Evan GI. (1997). *EMBO J.*, **16**, 6192–6199.
- Friedlander RM, Brown RH, Gagliardini V, Wang J and Yuan J. (1997). *Nature*, **388**, 31.
- Fulda S, Wick W, Weller M and Debatin KM. (2002). *Nat. Med.*, **8**, 808–815.
- Georgel P, Naitza S, Kappler C, Ferrandon D, Zachary D, Swimmer C, Kopczynski C, Duyk G, Reichhart JM and Hoffmann JA. (2001). *Dev. Cell*, **1**, 503–514.
- Goyal L, McCall K, Agapite J, Hartweg E and Steller H. (2000). *EMBO J.*, **19**, 589–597.
- Green DR and Evan GI. (2002). *Cancer Cell*, **1**, 19–30.
- Harvey NL, Daish T, Mills K, Dorstyn L, Quinn LM, Read SH, Richardson H and Kumar S. (2001). *J. Biol. Chem.*, **276**, 25342–25350.
- Hawkins CJ, Yoo SJ, Peterson EP, Wang SL, Vernoooy SY and Hay BA. (2000). *J. Biol. Chem.*, **275**, 27084–27093.
- Hay BA. (2000). *Cell Death Diff.*, **7**, 1045–1056.
- Hegde R, Srinivasula SM, Zhang Z, Wassell R, Mukattash R, Cilenti L, DuBois G, Lazebnik Y, Zervos AS, Fernandes-Alnemri T and Alnemri ES. (2001). *J. Biol. Chem.*
- Hoffman JA. (2003). *Nature*, **426**, 33–38.
- Hoffmann JA and Reichhart JM. (2002). *Nat. Immunol.*, **3**, 121–126.
- Holley CL, Olson MR, Colon-Ramos DA and Kornbluth S. (2002). *Nat. Cell Biol.*, **4**, 439–444.
- Hu SM and Yang XL. (2000). *J. Biol. Chem.*, **275**, 30761–30764.
- Huang H, Joazeiro CA, Bonfoco E, Kamada S, Levenson JD and Hunter T. (2000). *J. Biol. Chem.*, **275**, 26661–26664.
- Huang Y, Park YC, Rich RL, Segal D, Myszkowski DG and Wu H. (2001). *Cell*, **104**, 781–790.
- Igaki T, Kanda H, Yamamoto-Goto Y, Kanuka H, Kuranaga E, Aigaki T and Miura M. (2002). *EMBO J.*, **21**, 3009–3018.
- Jiang X, Kim HE, Shu H, Zhao Y, Zhang H, Kofron J, Donnelly J, Burns D, Ng SC, Rosenberg S and Wang X. (2003). *Science*, **299**, 223–226.
- Kaiser WJ, Vucic D and Miller LK. (1998). *FEBS Lett.*, **440**, 243–248.
- Kanda H, Igaki T, Kanuka H, Yagi T and Miura M. (2002). *J. Biol. Chem.*, **277**, 28372–28375.
- Kanuka H, Sawamoto K, Inohara N, Matsuno K, Okano H and Miura M. (1999). *Mol. Cell*, **4**, 757–769.
- Kaufmann SH and Vaux DL. (2003). *Oncogene*, **22**, 7414–7430.

- Kauppila S, Maaty WS, Chen P, Tomar RS, Eby MT, Chapo J, Chew S, Rathore N, Zachariah S, Sinha SK, Abrams JM and Chaudhary PM. (2003). *Oncogene*, **22**, 4860–4867.
- Kluck RM, Bossy-Wetzel E, Green DR and Newmeyer DD. (1997). *Science*, **275**, 1132–1136.
- Krajewska M, Krajewski S, Banares S, Huang X, Turner B, Bubendorf L, Kallioniemi OP, Shabaik A, Vitiello A, Peehl D, Gao GJ and Reed JC. (2003). *Clin. Cancer Res.*, **9**, 4914–4925.
- Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MSS and Flavell RA. (1995). *Science*, **267**, 2000–2003.
- Kumar S and Doumanis J. (2000). *Cell Death Differ.*, **7**, 1039–1044.
- Lassus P, Opitz-Araya X and Lazebnik Y. (2002). *Science*, **297**, 1352–1354.
- Leulier F, Rodriguez A, Khush RS, Abrams JM and Lemaitre B. (2000). *EMBO Rep.*, **1**, 353–358.
- Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X. (1997). *Cell*, **91**, 479–489.
- Lippens S, Kockx M, Knaepen M, Mortier L, Polakowska R, Verheyen A, Garzyn M, Zwijzen A, Formstecher P, Huylebroeck D, Vandenabeele P and Declercq W. (2000). *Cell Death Differ.*, **7**, 1218–1224.
- Lisi S, Mazzon I and White K. (2000). *Genetics*, **154**, 669–678.
- Liu X, Kim CN, Yang J, Jemmerson R and Wang X. (1996). *Cell*, **86**, 147–157.
- Lu Y, Wu LP and Anderson KV. (2001). *Genes Dev.*, **15**, 104–110.
- Manderson AP, Pickering MC, Botto M, Walport MJ and Parish CR. (2001). *J. Exp. Med.*, **194**, 747–756.
- Martinon F, Burns K and Tschopp J. (2002). *Mol. Cell*, **10**, 417–426.
- Martins LM, Iaccarino I, Tenev T, Gschmeissner S, Totty NF, Lemoine NR, Savopoulos J, Gray CW, Creasy CL, Dingwall C and Downward J. (2001). *J. Biol. Chem.*
- McCall K, Laurie B, Chang J, Fileppo D, Thompson J, Baum J and Peterson S. (2003). *44th Ann. Drosophila Res. Conf.*, **946A**.
- Meier P and Evan G. (1998). *Cell*, **95**, 295–298.
- Meier P, Silke J, Leever SJ and Evan GI. (2000). *EMBO J.*, **19**, 598–611.
- Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C and Grutter MG. (2002). *J. Biol. Chem.*, **277**, 45162–45171.
- Moreno E, Yan M and Basler K. (2002). *Curr. Biol.*, **12**, 1263–1268.
- Muro I, Hay BA and Clem RJ. (2002). *J. Biol. Chem.*, **277**, 49644–49650.
- Nahle Z, Polakoff J, Davuluri RV, McCurrach ME, Jacobson MD, Narita M, Zhang MQ, Lazebnik Y, Bar-Sagi D and Lowe SW. (2002). *Nat. Cell Biol.*, **4**, 859–864.
- Naitza S, Rosse C, Kappler C, Georgel P, Belvin M, Gubb D, Camonis J, Hoffmann JA and Reichhart JM. (2002). *Immunity*, **17**, 575–581.
- Nicholson DW. (1999). *Cell Death Differ.*, **6**, 1028–1042.
- Orth K, O'Rourke K, Salvesen GS and Dixit VM. (1996). *J. Biol. Chem.*, **271**, 20977–20980.
- Pop C, Chen YR, Smith B, Bose K, Bobay B, Tripathy A, Franzen S and Clark AC. (2001). *Biochemistry*, **40**, 14224–14235.
- Quinn LM, Dorstyn L, Mills K, Colussi PA, Chen P, Coombe M, Abrams J, Kumar S and Richardson H. (2000). *J. Biol. Chem.*, **275**, 40416–40424.
- Raff MC. (1992). *Nature*, **356**, 397–400.
- Read SH, Baliga BC, Ekert PG, Vaux DL and Kumar S. (2002). *J. Cell Biol.*, **159**, 739–745.
- Reed JC. (1999). *Curr. Opin. Oncol.*, **11**, 68–75.
- Reed JC, Doctor K, Rojas A, Zapata JM, Stehlik C, Fiorentino L, Damiano J, Roth W, Matsuzawa S, Newman R, Takayama S, Marusawa H, Xu F, Salvesen G and Godzik A. (2003). *Genome Res.*, **13**, 1376–1388.
- Riedl SJ, Fuentes-Prior P, Ratus M, Kairies N, Krapp R, Huber R, Salvesen GS and Bode W. (2001a). *Proc. Natl. Acad. Sci. USA*, **98**, 14790–14795.
- Riedl SJ, Ratus M, Schwarzenbacher R, Zhou Q, Sun S, Fesik SW, Liddington RC and Salvesen GS. (2001b). *Cell*, **104**, 791–800.
- Roberts DL, Merrison W, MacFarlane M and Cohen GM. (2001). *J. Cell Biol.*, **153**, 221–227.
- Rodriguez A, Chen P, Oliver H and Abrams JM. (2002). *EMBO J.*, **21**, 2189–2197.
- Rodriguez A, Oliver H, Zou H, Chen P, Wang X and Abrams JM. (1999). *Nat. Cell Biol.*, **1**, 272–279.
- Ryoo HD, Bergmann A, Gonen H, Ciechanover A and Steller H. (2002). *Nat. Cell Biol.*, **4**, 432–438.
- Salvesen GS and Dixit VM. (1997). *Cell*, **91**, 443–446.
- Salvesen GS and Duckett CS. (2002). *Nat. Rev. Mol. Cell Biol.*, **3**, 401–410.
- Schimmer AD, Welsh K, Pinilla C, Wang Z, Krajewska M, Bonneau MJ, Pedersen IM, Kitada S, Scott FL, Bailly-Maitre B, Glinsky G, Scudiero D, Sausville E, Salvesen G, Nefzi A, Ostresh JM, Houghten RA and Reed JC. (2004). *Cancer Cell*, **5**, 25–35.
- Shi Y. (2001). *Nat. Struct. Biol.*, **8**, 394–401.
- Shiozaki EN, Chai J, Rigotti DJ, Riedl SJ, Li P, Srinivasula SM, Alnemri ES, Fairman R and Shi Y. (2003). *Mol. Cell*, **11**, 519–527.
- Silverman N and Maniatis T. (2001). *Genes Dev.*, **15**, 2321–2342.
- Silverman N, Zhou R, Stoven S, Pandey N, Hultmark D and Maniatis T. (2000). *Genes Dev.*, **14**, 2461–2471.
- Song ZW, Guan B, Bergman A, Nicholson DW, Thornberry NA, Peterson EP and Steller H. (2000). *Mol. Cell Biol.*, **20**, 2907–2914.
- Srinivasula SM, Datta P, Kobayashi M, Wu JW, Fujioka M, Hegde R, Zhang Z, Mukattash R, Fernandes-Alnemri T, Shi Y, Jaynes JB and Alnemri ES. (2002). *Curr. Biol.*, **12**, 125–130.
- Srinivasula SM, Hegde R, Saleh A, Datta P, Shiozaki E, Chai J, Lee RA, Robbins PD, Fernandes-Alnemri T, Shi Y and Alnemri ES. (2001). *Nature*, **410**, 112–116.
- Stennicke HR, Deveraux QL, Humke EW, Reed JC, Dixit VM and Salvesen GS. (1999). *J. Biol. Chem.*, **274**, 8359–8362.
- Stoven S, Ando I, Kadalayil L, Engstrom Y and Hultmark D. (2000). *EMBO Rep.*, **1**, 347–352.
- Stoven S, Silverman N, Junell A, Hedengren-Olcott M, Erturk D, Engstrom Y, Maniatis T and Hultmark D. (2003a). *Proc. Natl. Acad. Sci. USA*, **100**, 5991–5996.
- Stoven S, Silverman N, Junell A, Hedengren-Olcott M, Erturk D, Engstrom Y, Maniatis T and Hultmark D. (2003b). *Proc. Natl. Acad. Sci. USA*, **100**, 5991–5996.
- Tamm I, Kornblau SM, Segall H, Krajewski S, Welsh K, Kitada S, Scudiero DA, Tudor G, Qui YH, Monks A, Andreeff M and Reed JC. (2000). *Clin. Cancer Res.*, **6**, 1796–1803.
- Tenev T, Zachariou A, Wilson R, Paul A and Meier P. (2002). *EMBO J.*, **21**, 5118–5129.
- Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, Elliston KO, Ayala JM, Casano FJ, Chin J, Ding GJF, Egger LA, Gaffney EP, Limjuco G, Palyha OC, Raju SM, Rolando AM, Salley JP, Yamin TT and Tocci MJ. (1992). *Nature*, **356**, 768–774.

- Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT and Nicholson DW. (1997). *J. Biol. Chem.*, **272**, 17907–17911.
- Thress K, Evans EK and Kornbluth S. (1999). *EMBO J.*, **18**, 5486–5493.
- Thress K, Henzel W, Shillinglaw W and Kornbluth S. (1998). *EMBO J.*, **17**, 6135–6143.
- Troy CM, Rabacchi SA, Hohl JB, Angelastro JM, Greene LA and Shelanski ML. (2001). *J. Neurosci.*, **21**, 5007–5016.
- Turk B, Turk D and Salvesen GS. (2002). *Curr. Pharm. Des.*, **8**, 1623–1637.
- Uren AG, Coulson EJ and Vaux DL. (1998). *Trends Biochem. Sci.*, **23**, 159–162.
- van Loo G, van Gurp M, Depuydt B, Srinivasula SM, Rodriguez I, Alnemri ES, Gevaert K, Vandekerckhove J, Declercq W and Vandennebeele P. (2002). *Cell Death Differ.*, **9**, 20–26.
- Varfolomeev EE, Schuchmann M, Luria V, Chiannikulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P and Wallach D. (1998). *Immunity*, **9**, 267–276.
- Varkey J, Chen P, Jemmerson R and Abrams JM. (1999). *J. Cell Biol.*, **144**, 701–710.
- Verhagen AM, Coulson EJ and Vaux DL. (2001a). *Genome Biol.*, **2**.
- Verhagen AM, Silke J, Ekert PG, Pakusch M, Kaufmann H, Connolly LM, Day CL, Tikoo A, Burke R, Wrobel C, Moritz RL, Simpson RJ and Vaux DL. (2001b). *J. Biol. Chem.*
- Vernooy SY, Chow V, Su J, Verbrugge K, Yang J, Cole S, Olson MR and Hay BA. (2002). *Curr. Biol.*, **12**, 1164–1168.
- Vucic D, Kaiser WJ, Harvey AJ and Miller LK. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 10183–10188.
- Vucic D, Kaiser WJ and Miller LK. (1998). *Mol. Cell Biol.*, **18**, 3300–3309.
- Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE and Lenardo MJ. (1999a). *Cell*, **98**, 47–58.
- Wang S, Miura M, Jung Y-K, Zhu H and Yuan J. (1998). *Cell*, **92**, 501–509.
- Wang SL, Hawkins CJ, Yoo SJ, Muller HAJ and Hay BA. (1999b). *Cell*, **98**, 453–463.
- Wei Y, Fox T, Chambers SP, Sintchak J, Coll JT, Golec JM, Swenson L, Wilson KP and Charifson PS. (2000). *Chem. Biol.*, **7**, 423–432.
- White K, Grether ME, Abrams JM, Young L, Farrell K and Steller H. (1994). *Science*, **264**, 677–683.
- Wilson R, Goyal L, Ditzel M, Zachariou A, Baker DA, Agapite J, Steller H and Meier P. (2002). *Nat. Cell Biol.*, **4**, 445–450.
- Wing JP, Schwartz LM and Nambu JR. (2001). *Mech. Dev.*, **102**, 193–203.
- Wing JP, Zhou L, Schwartz LM and Nambu JR. (1998). *Cell Death Diff.*, **5**, 930–939.
- Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X and Shi Y. (2000). *Nature*, **408**, 1008–1012.
- Wu JW, Cocina AE, Chai JJ, Hay BA and Shi YG. (2001). *Mol. Cell*, **8**, 95–104.
- Wu TY, Wagner KW, Bursulaya B, Schultz PG and Deveraux QL. (2003). *Chem. Biol.*, **10**, 759–767.
- Yang X, Stennicke HR, Wang B, Green DR, Janicke RU, Srinivasan A, Seth P, Salvesen GS and Froelich CJ. (1998). *J. Biol. Chem.*, **273**, 34278–34283.
- Yoo SJ, Huh JR, Muro I, Yu H, Wang L, Wang SL, Feldman RM, Clem RJ, Muller HA and Hay BA. (2002). *Nat. Cell Biol.*, **4**, 416–424.
- Yu SY, Yoo SJ, Yang L, Zapata C, Srinivasan A, Hay BA and Baker NE. (2002). *Development*, **129**, 3269–3278.
- Zheng TS, Hunot S, Kuida K, Momoi T, Srinivasan A, Nicholson DW, Lazebnik Y and Flavell RA. (2000). *Nat. Med.*, **6**, 1241–1247.
- Zhou L, Song Z, Tittel J and Steller H. (1999). *Mol. Cell*, **4**, 745–755.
- Zimmermann KC, Ricci JE, Droin NM and Green DR. (2002). *J. Cell Biol.*, **156**, 1077–1087.