

Drosophila Apoptosis and the Regulation of the Caspase Cascade

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Abstract

The caspase cascade in *Drosophila* is controlled primarily by DIAP1 (*Drosophila* inhibitor of apoptosis), which exerts its regulatory effect by ubiquitinating the caspase DRONC, thus targeting it for proteasomal degradation. Pro-apoptotic signaling stimulates auto-ubiquitination by DIAP1 and frees DRONC to activate downstream caspases.

Introduction

The apoptotic pathway in *Drosophila* is critically dependent on the class of cysteine proteases known as caspases, of which seven have been identified in the *Drosophila* genome (Kumar and Doumanis, 2000; see Table 1). Caspases are synthesized as zymogens and are activated by proteolysis upon pro-apoptotic signaling. Apical or “initiator” caspases primarily activate the downstream “effector” caspases whose protease activity is directed toward the deconstruction of cellular machinery.

Table 1: The seven known *Drosophila* caspases and their functions. Adapted from Richardson and Kumar, 2002; Kumar and Doumanis, 2000.

| <i>Caspase</i> | <i>Function</i> | <i>Prodomain</i> | <i>Caspase</i> | <i>Function</i> | <i>Prodomain</i> |
|----------------|-----------------|------------------|----------------|-----------------|------------------|
| DRONC | Initiator | Long | DECAY | Unknown | Short |
| DREDD | Initiator? | Long | DAMM | Unknown | Short |
| DRICE | Effector | Short | STRICA | Unknown | Long |
| DCP-1 | Effector | Short | | | |

This classification scheme is correlated with the size of structural elements called prodomains which lie N-terminal to the caspase active sites and frequently contain protein interaction motifs. Initiator prodomains are typically long and effector prodomains generally short (Kumar and Dumanis, 2000).

While intensive research has constructed a skeleton outline of the caspase-activating apoptosis pathway, significant questions remain unanswered. The roles of three of the caspases are as yet uncharacterized, and the details of the feedback mechanisms within the cascade have yet to be worked out in full. Nevertheless, significant inroads have been made in determining the critical elements in the regulation of the *Drosophila* caspase cascade.

The Caspase Activation Pathway

DIAP1 is currently understood to be the single most important regulatory element in the caspase cascade (See Figure 1). Its primary mode of regulation is the ubiquitination of the initiator caspase DRONC, which is thus targeted for proteasomal degradation (Palaga and Osborne, 2002). DIAP1 has also been reported to bind to and inhibit the effector caspase DRICE without ubiquitinating it (Yan et al., 2004).

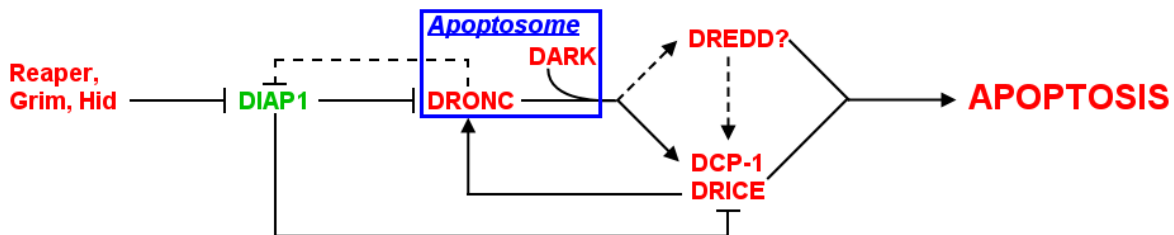


Figure 1: The *Drosophila* apoptosis pathway. Red proteins are pro-apoptotic and green are anti-apoptotic. Dashed lines represent less well-established interactions. Adapted from Richardson and Kumar, 2002.

In apoptotic cells, DIAP1 is itself inhibited by upstream regulatory proteins known as Reaper, Hid, and Grim (RHG); when these proteins are active, DIAP1 ubiquitinates itself and is degraded by the proteasome. A partial negative feedback loop exists in this interaction – DIAP1 is capable of ubiquitinating Reaper as well (Olson et al., 2003). After removal of DIAP1 inhibition, DRONC recruits the activator protein DARK to form a structure known as the apoptosome, which is specialized for further caspase activation (Martin,

2002). Alternatively, DARK may be required for early processing of DRONC into its active form (Muro et al., 2002). Once activated, DRONC is capable of activating DRICE, which in turn cleaves DRONC and renders it invulnerable to DIAP1 inhibition (Yan et al., 2004). Finally, DCP-1's position as a general-purpose downstream protease is relatively uncontroversial, although it has not been reported to participate in any feedback mechanisms (Richardson and Kumar, 2002).

The roles of the remaining caspases in the activation sequence are less well-studied. DREDD is reported to be an initiator caspase, although no clear position in the pathway has been assigned to it. One review suggests that it may also play a role far upstream in the pro-apoptotic signaling of the innate immunity pathway (Richardson and Kumar, 2002). It has even been suggested that DREDD concentration is a rate-limiting factor in apoptosis induced via RHG activation (Kumar and Doumanis, 2000). DECAY has been reported to be an effector caspase similar to DCP-1 (Danial and Korsmeyer, 2004), but this assignment is tenuous; the roles of DECAY, DAMM, and STRICA in the caspase cascade at this point remain ambiguous.

A critical element of the caspase activation pathway is the substrate specificity of each caspase. The canonical caspase substrate is the peptide sequence DxxD, where x represents any amino acid; however, individual caspases have more specific substrate requirements. Effector caspases like DCP-1, DRICE, and DECAY typically recognize the sequence DEVD (Kumar and Doumanis, 2000). DRONC, the only known *Drosophila* caspase capable of cleaving a peptide ending in either glutamate or aspartate, can cleave TQTE, TETD, and IETD as well as DEVD (Hawkins et al., 2000). Because DRONC must proteolytically activate itself as well as other caspases, it must be capable of recognizing a site toward which proteases (including the other caspases) have no activity. Were this not the case, DRONC would be susceptible to activation even in the absence of pro-apoptotic signaling.

Structure-Function Relationships in Caspase Regulators

Comparatively few crystal structures exist for members of the caspase cascade; however, the structure of DIAP1 has been solved in complex with several of its binding partners. DIAP1 possesses three main

structural subdomains, including a C-terminal RING motif responsible for the molecule’s ubiquitin ligase activity (Wilson et al., 2002) and two N-terminal zinc-containing BIR domains (BIR1 and BIR2), which are important for protein-protein binding and are found in mammalian as well as *Drosophila* IAP proteins (Wu et al., 2001).

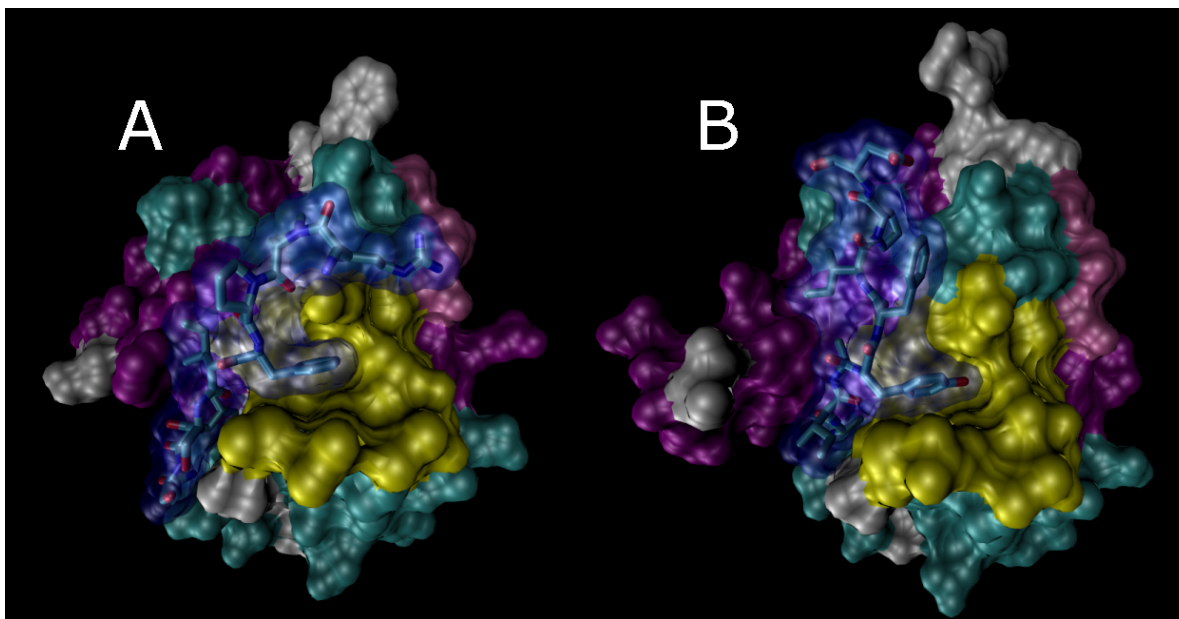


Figure 2: Crystal structures of DIAP1 bound to a fragment of DRONC (A) and Grim (B). (A) The DIAP1 BIR2 domain bound to a short fragment of DRONC (outlined in blue). The deep binding pocket contains a phenylalanine residue required for the binding interaction (Chai et al., 2003). (B) The same domain bound to a Grim fragment in a slightly different orientation. Both Grim and DRONC bind to the same site on the DIAP1 domain (Wu et al., 2001).

Unsurprisingly, given its role in ubiquitin ligation, a functional RING domain is required for DIAP1-induced DRONC degradation, but a mutated RING does not disrupt the interaction between DIAP1 and DRONC (Wilson et al., 2002). The BIR2 domain of DIAP1 is required for DRONC binding; interestingly, crystallographic data imply that the DRONC region involved in binding DIAP1 is a short fragment with high homology to the “IAP-binding motif” present at the N-termini of the RHG proteins. This suggests that the RHG proteins disrupt the DIAP1-DRONC interaction by straightforward competitive inhibition (Chai et al., 2003; see Figure 2). Also of interest is the size of the DRONC peptide that mediates DIAP1 binding – a 12-residue region is both necessary and sufficient for DRONC binding to the BIR2 domain of

DIAP1, and a single point mutation is capable of completely destroying the interaction (Chai et al., 2003). A similar competition-based mechanism has been identified for the interaction of the BIR1 domain with DRICE, although this interaction does not result in DRICE ubiquitination (Yan et al., 2004).

The Mechanism of DRONC and DIAP1 Degradation

Ubiquitination and proteasomal degradation play a vital role in the regulation of the caspase cascade, and it is not surprising that a number of secondary regulators influence the ubiquitination of DRONC and DIAP1 (See Figure 3). Although DIAP1's RING domain has E3 ubiquitin ligase activity, it functions in an E2-conjugase-dependent manner (Schreuder et al., 2003). In ubiquitinating either DRONC (in healthy cells)

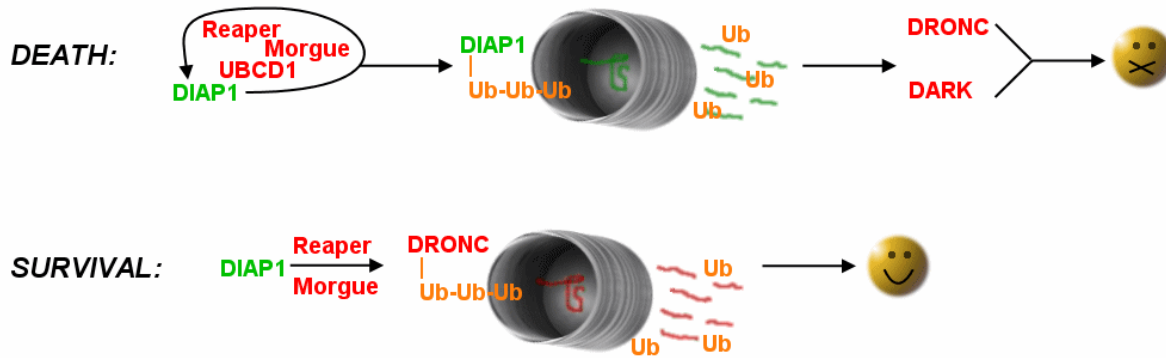


Figure 3: DRONC and DIAP1 degradation. Under apoptotic conditions, DIAP1 ubiquitinates itself and is degraded by the proteasome. Under conditions of cell survival, DIAP1 instead ubiquitinates DRONC and causes its degradation. Adapted from Martin, 2002.

or other DIAP1 molecules (in apoptotic cells), DIAP1's ubiquitin ligase activity emerges only in complex with the ancillary proteins UBCD1 and Morgue (Martin, 2002). UBCD1 is a "legitimate" E2 conjugase; however, while Morgue shares high sequence identity with known conjugases, it lacks a required active site residue and is thus best described as a ubiquitin E2 conjugase variant (UEV). UEV's lack E2 activity on their own but promote the activity of conjugases with which they are bound (Schreuder et al., 2003). It is not known whether either DIAP1 or DRONC retain their biochemical activities after ubiquitination but before proteasomal degradation.

Inactivation of the proteasome by RNA interference targeted toward a single proteasomal subunit pro-

duces varying degrees of apoptotic cell death (Wójcik and DeMartino, 2002). The amount of apoptosis observed is dependent on the particular subunit targeted, although the level of ubiquitinated protein buildup observed in proteasome knockdowns is less subunit-dependent. Known proteasome inhibitors such as lactacystin also increase ubiquitinated protein levels and induce apoptosis. In neither case is it clear whether proteasomal dysfunction actively induces apoptosis, or whether pro-apoptotic proteins such as DRONC that would normally be degraded are running amok in the cell.

Conclusions

Although the basic outline of the caspase activation pathway in *Drosophila* is fairly well characterized, a number of outstanding questions remain. First, the roles of DEWAY, DAMM, STRICA, and to some extent DREDD have yet to be fully explored in the apoptotic pathway of *Drosophila* cells. Second, crystal structures of proteins that participate in the caspase cascade remain rare and would provide important information about structure-function relationships in caspases and their regulators. Finally, the role of the proteasome in regulating levels of apoptotic proteins has yet to be elucidated in specific biochemical detail. Further research in each of these areas will likely yield exciting new discoveries about apoptosis regulation in *Drosophila*.

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