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The insect caspases

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Abstract Developmental and tissue homeostasis is a delicate balance between cell proliferation and cell death. The activation of caspases, a conserved family of cysteine proteases, is a main event in the initiation and execution of programmed cell death. While caspases have been characterized from many organisms, comparatively little is known about insect caspases. In *Drosophila melanogaster*, seven caspases have been characterized; three initiators and four effectors. In mosquitoes, several putative caspases have been identified in the genomes of *Aedes aegypti* and *Anopheles gambiae.* A small number of caspases have been identified in the Lepidoptera, the flour beetle, *Tribolium castaneum*, and the pea aphid, *Acyrthosiphon pisum*. The availability of new insect genome sequences will provide a unique opportunity to examine the caspase family across an evolutionarily diverse phylum and will provide valuable insights into their function and regulation.

Keywords Insects · Caspase function · *Drosophila* · *Aedes* mosquito · Lepidoptera

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Introduction

Apoptosis is a process that is widely observed in all eukaryotic cells [1–4]. In multi-cellular organisms, apoptosis is an essential mechanism required to sculpt tissues and eliminate unwanted cells during development, and also is involved in regulating tissue-size homeostasis and immunity. Apoptosis also plays a key role in eliminating cells containing intracellular pathogens, cells with damaged DNA, and cells that are proliferating inappropriately [5, 6]. The apoptotic process is highly organized involving DNA fragmentation, membrane blebbing, cell shrinkage and fragmentation into membrane-enclosed vesicles called apoptotic bodies [1, 3]. The highly regulated series of events that lead to the apoptotic destruction of cells is a concerted and fine tuned effort by a family of conserved proteases, the caspases.

Caspases—the beginning and the end

Caspases (cysteine aspartate-specific proteinases) are a family of cysteine proteases that serve as both the initiators and the executioners of apoptosis. Most caspases possess an active site cysteine and have a strict requirement for cleaving protein substrates containing Asp. As is true of many proteases, caspases are present in cells as inactive zymogens and are activated by proteolytic processing. Caspase zymogens comprise three domains: an N-terminal prodomain, and the large (p20) and small (p10) catalytic domains [1, 3]. The active enzyme is a heterotetramer composed of two active large/small heterodimers and two active sites [3, 7, 8]. Conversion of the caspase zymogen to the active enzyme requires two cleavages, one separating the prodomain from the large subunit and another

separating the large and small subunits. Each cleavage involves Asp-X bonds and occurs in a progressive fashion with cleavage between the large and small subunits preceding the removal of the prodomain. Most caspases function within auto- and trans-activation cascades in which caspases containing long prodomains (initiators) activate the cascades and those with short prodomains (effectors) are involved in the downstream processing of the substrates required to dismantle the cell [9, 10].

The apoptotic caspases are considered to be initiators or effectors depending on their point of entry into the cell death pathway. The long N-terminal prodomains of initiator caspases commonly contain protein–protein interaction domains, typically caspase recruitment domains (CARD) or death effector domains (DED) [7, 8, 11–14]. These domains are believed to mediate the recruitment of caspase zymogens into complexes, which promotes the oligomerization and activation of the bound caspases [12, 15–17]. In mammals, the initiator caspases-8, -9, -10 are activated following their recruitment by the adaptor proteins FADD (caspases-8 and -10) and Apaf-1 (caspase-9) [3, 12, 15, 16, 18]. Once they are activated, the initiators, in turn, activate the short prodomain effector caspases, caspases-3, -6, -7 by proteolytic processing [3, 12, 15, 16, 18]. The effector caspases then go on to cleave key cellular substrates including protein kinases, signal transduction proteins, chromatin modifying enzymes PARP (poly (ADP ribose) polymerase), DNA repair proteins, and the inhibitory subunits of certain endonucleases [7, 19, 20].

Activating and regulating the caspases: insects and mammals do it differently

The last 10 years has seen a staggering increase in our knowledge of apoptosis in general and the role of the caspases that execute the process. Since the identification of caspase-1 (interleukin-1-β-converting enzyme, or ICE) [21, 22], caspases have been identified in many different organisms ranging from mammals (11 caspases in human and 10 in mice), to nematodes (3 caspases) [23]. While the role of human caspases has been the focus of much research, their function, regulation, and interactions are complex. As is the case with any complex biological system, crucial insights on the role of these molecules can be gained from the study of similar molecules in other model systems, including the insects. *Drosophila melanogaster* (*Drosophila*) has emerged as a model organism to study many forms of cell death and has made major contributions to the understanding of cell death regulation and its role in development. Importantly, comparative studies between *Drosophila* and mammals have highlighted a number of differences that have provided insights into the evolution of apoptosis in general.

Although several basic strategies are used to activate and regulate cell caspase-dependent cell death, mammals and insects emphasize distinct points of control. In mammals, the decision to activate caspase-dependent cell death is usually made at the level of positive death signals that result in the activation of initiator caspases (caspase-8, -9, -10), and the subsequent activation of downstream effector caspases (caspase-3, -6, -7). The life and death decisions that promote caspase activation are calculated largely by pro- and anti-apoptotic members of the Bcl-2 family of proteins [24, 25]. Once activated, caspase activity is dampened by several mechanisms, namely the inhibitor of apoptosis (IAP) family of proteins [26–29].

In contrast, caspase activity in *Drosophila* is regulated primarily after activation. The primary apoptotic caspase in *Drosophila* is the caspase-9 homologue Dronc, and many cells experience chronic Dronc activation. Cells survive because they express the IAP protein 1, DIAP1 [30, 31], reviewed in Hay and Gao [32] and Kumar [33]. DIAP1 suppresses Dronc activity, as well as that of the downstream caspases activated by Dronc [30, 31, 34]. In addition, the expression of the pro-death RGH proteins (Reaper, Grim, Hid, Sickle, Jafrac2) disrupts DIAP1-caspase interactions, resulting in the activation of the caspase cascade [30, 31, 35–41]. Thus, cell death in *Drosophila* is the result of a series of complex interactions between RHG proteins, DIAP1 and Dronc. Interestingly, no specific apoptotic roles have been defined for the caspase-8 homologue, Dredd.

Significantly less is known about insect caspases other than those described in *Drosophila*, however, there are many reasons to explore this diversity. Firstly, insects comprise ancient and diverse phyla that have the potential to offer important insights into the evolution of apoptotic machinery and pathways. In addition, medical considerations can justify the study of apoptosis in those insects that are exposed to and transmit human pathogens. In this review, we provide an overview of the *Drosophila* caspases and summarize what is known about the caspases in non-drosophilid insects. The recent availability of several insect genomes has provided a glimpse of the caspases that exist outside of *Drosophila*, highlighting the conservation of molecules predicted to function within apoptotic pathways as well as what appears to be expansions of the caspase gene family (see Table 1).

The initiator caspases

The execution of apoptosis depends on conserved molecules that transmit and regulate the death signals. Most

Table 1 A summary of caspase function and mutant phenotypes for published insect caspases

Caspase	Function in vivo	Mutant phenotype	References
Drosophila Dredd	NF-κB activation, JNK activation, innate immune signaling; involved in spermatid individualization	Developmental normal, viable and fertile adults, immune compromised	[42–46]
Aedes Dredd	NF-κB activation and innate immune signaling	Not available	[47]
Drosophila Dronc	Essential for most developmental and stress-induced apoptosis; ecdysone inducible; involved in spermatid individualization	Pupal lethal, multiple cell and tissue-specific deaths in embryos, larvae and pupae	[37, 46, 48–69]
Aedes Dronc	Developmental apoptosis; ecdysone inducible	Not available	[70]
Drosophila Strica	Developmental apoptosis in restricted tissues including eye, salivary glands; egg chamber development during mid-oogenesis	Development normal with delayed removal of salivary glands and defective egg chambers during oogenesis	[46, 47, 65, 71–73]
Drosophila Drice	Essential for most developmental apoptosis; ecdysone inducible	Pre-pupal lethal, reduced cell death in the pupal retina, embryonic nervous system and adult wing; reduced response to stress and irradiation-induced apoptosis	[46, 48, 74–79]
Drosophila Dcp-1	Minor role and tissue-specific function in developmental apoptosis	Development normal, viable and fertile adults	[46, 48, 80–82]
Drosophila Decay	No clear function in apoptosis; suggested redundant function	Development normal, viable and fertile adults; *decay* RNAi shows weak suppression of rough eye phenotype	[46, 83]
Drosophila Damm	No physiological role established	No specific mutants available; RNAi shows no contribution to canonical cell death pathway	[46, 84]
Spopdoptera frugiperda caspase-1	Role in development and UV-induced apoptosis; induced by baculovirus infection	RNAi repressed stable cells showed a significant increase in resistance to UV- and baculovirus-induced apoptosis.	[85–87]
Spodoptera littoralis caspase-1	Role in development, in vitro role established for UV-induced and virus-induced apoptosis	Not available	[88, 89]
Helicoverpa armigera (Hearm caspase-1)	Role in development and larval-pupal metamorphosis	Not available	[90]

signals initiating cell death converge first on the long prodomain, or initiator, caspases. In mammals these initiator caspases are caspase-8, -9, -10 and in *Drosophila,* Dredd, Dronc and Strica.

Drosophila dredd

Dredd is a death domain containing caspase that shares modest sequence similarity with mammalian caspase-8 [42]. The long prodomain in Dredd contains two death-inducing domains (DID) that interact with the caspase adaptor, dFADD (*Drosophila Fas associated death domain containing protein*) [43]. Dredd was isolated initially as a potential inducer of apoptosis functioning downstream of the cell death activator reaper (Rpr). Although apoptotic roles for Dredd have not been excluded, data from the analysis of *dredd* mutants suggests that the primary function of Dredd is immune related [44, 45]. *Dredd* mutants produce viable adults but display a significantly reduced ability to activate the immune deficient pathway (IMD)-induced expression of antimicrobial peptides in response to challenge with Gram-negative bacteria, and also display a greatly enhanced lethality upon septic infection [45]. During IMD signaling, Dredd is predicted to be involved in the cleavage of Relish, the *Drosophila* homologue of mammalian NF-κB [91]. Dredd forms complexes with Relish in cell culture and Relish cleavage occurs at a caspase consensus cleavage site, but the lack of available Dredd antibodies has made it difficult to identify these interactions in vivo. More recent data has implicated Dredd in the activation of the JNK pathway, although the mechanism of this activation remains unclear [92].

Dredd and dFADD have additional non-apoptotic functions including a well-established role in spermatid individualization [48].

Drosophila dronc

Dronc is the primary apoptotic caspase in *Drosophila* and the only CARD-carrying caspase in the *Drosophila* genome. As such, it is often regarded as the true caspase-9 orthologue, although by sequence similarity, Dronc is most similar to human caspase-2 [49, 93]. The N-terminal CARD domain found in Dronc interacts with the adaptor protein

DARK to form high molecular weight complexes required for auto-processing and Dronc activation [50, 51, 94].

Dronc has a unique substrate specificity among caspases, cleaving after an aspartate, similar to other caspases but also after glutamate residues. The unusual substrate profile may be due to the unique sequence, PFCRG, in the catalytic site, which is different from the QAC(R/Q/G)(G/E) sequence found in all other caspases [7, 49, 52]. Dronc efficiently cleaves substrates containing VDVAD, as well as those containing VEID, IETD, and DEVD [49, 52]. The only known cellular targets for Dronc are effector caspases Drice and Dcp-1 (both of which are cleaved at a sequence containing TETD), and the IAP protein, DIAP1 [37, 38, 52, 95].

There are several lines of evidence that suggest Dronc is the primary effector of caspase-dependent cell death in *Drosophila*. Both in vivo and in vitro studies show that Dronc is essential for developmental apoptosis in most larval tissues and the apoptotic response to toxic agents and x- and γ-irradiation [37, 51–57]. *Dronc* null animals show suppressed Hid-induced apoptosis, indicating that Dronc acts downstream of the RHG proteins [53–57]. Loss of *dronc* function also blocks apoptosis induced by decreasing levels of the IAP protein, DIAP1, suggesting that Dronc mediates cell death through signals that lead to DIAP1 degradation [38, 46, 58–61].

Dronc is ubiquitously expressed in all fly cells and its transcription is acutely responsive to the steroid hormone ecdysone, which mediates the histolysis of larval tissues during metamorphosis [49, 62–67]. Maternal/zygotic *dronc* mutants are embryonically lethal and loss of function *dronc* mutants exhibit greatly reduced apoptosis, including the delayed removal of tissues such as salivary glands and are generally pupal lethal [51, 54, 56]. Interestingly, the histolysis of the larval midgut during molting occurs normally in *dronc* mutants suggesting alternative mechanisms for activating the downstream effector caspases [57].

Dronc also has been implicated in a number of non-apoptotic functions including the compensatory proliferation of cells, spermatid individualization, and cell migration [48, 68, 69, 96, 97].

Drosophila strica/dream

Strica is a long prodomain containing caspase with no homology to other previously characterized motifs (e.g., CARD or DID) but instead contains a novel serine- and threonine-rich prodomain. Although comparatively less is known about Strica, in vitro expression studies have established that Strica will induce cell death in cell lines, and ectopic expression in the *Drosophila* eye results in a rough eye phenotype [71, 72]. In addition, Strica is required for Hid-mediated killing, although the mechanism for this activation remains unknown [46].

No defects have been reported in *strica* mutants during embryogenesis, however, several tissue-specific phenotypes have been observed. In the pupal retina, depletion of *strica* was found to affect the elimination of interommatidial cells during the early but not the late stages of cell death suggesting that *strica* is required for the correct timing, but not specifically the execution, of interommatidial cell death [46]. Strica also is required for the timely removal of salivary glands, consistent with the observation that *strica* transcripts are up-regulated in the salivary glands prior to histolysis [46, 65]. Strica also plays a role in *Drosophila* oogenesis as small deletions in *strica* result in moderate egg chamber abnormalities during mid-oogenesis [73]. Interestingly, a deletion of the entire gene resulted in only a few egg chamber abnormalities suggesting that another caspase may compensate for the loss of *strica*. In agreement with these findings, *strica*:*dronc* double mutants demonstrated a high frequency of egg chambers defective in both mid- and late-stage oogenesis demonstrating that Strica and Dronc play redundant roles in programmed cell death observed in *Drosophila* ovaries [73]. Finally, while nothing is known about specific cellular targets for Strica, the available data suggest that Strica may target and activate Dcp-1 during mid-oogenesis [73].

The other insect initiators

Few initiator caspases have been identified in non-drosophilid insects; homologues of both *Drosophila* Dredd and *Drosophila* Dronc have been identified in genomes of *Aedes aegypti*, *Anopheles gambiae*, and homologues of *Drosophila* Dredd have been identified in *Tribolium castaneum* (GI:189237119) and *Bombyx mori* (AB2982816). To date, only those in *Ae. aegypti* have been characterized.

Aedes dredd

Aedes Dredd (AeDredd) shares the most sequence similarity with *Drosophila* Dredd and with human caspase-8 [47]. It contains two putative N-terminal death inducing domains (DIDs) and shares a similar substrate specificity with *Drosophila* Dredd with a preference for substrates containing IETD but also will use substrates containing VDVAD, YVAD, LEHD and VEID [47]. AeDredd was shown to interact with a mosquito FADD adaptor, named *Aedes* FADD and is required for IMD signaling and antibacterial immunity in *Ae. aegypti* [98]. No specific apoptotic role has been established for AeDredd and no *Ae. aegypti* mutants are currently available.

Aedes dronc

A homologue of the *Drosophila* Dronc, named AeDronc, also has been characterized in *Ae. aegypti* [70]. AeDronc

shares significant sequence similarity with *Drosophila* Dronc, and modest similarity with mammalian/human caspases-2 and -9. As is the case with Dronc, the sequence surrounding the catalytic cysteine, SICRG, is unique. AeDronc preferentially cleaves substrates containing VDVAD but unlike *Drosophila* Dronc will also cleave substrates containing LEHD, AEVD and WEHD [70]. Although no in vitro studies have been conducted with regards to the AeDronc activation, a homologue of the adaptor protein DARK has recently been identified in the *Ae. aegypti* genome [99] and there is conservation of the specific cleavage sites predicted to be required for AeDronc activation suggesting that auto-processing and activation occur through a similar mechanism [70]. Whether AeDronc has the ability to process substrates containing glutamate is currently unknown. Lastly there is limited conservation of the predicted DIAP1 binding site suggesting that regulation of AeDronc activation may occur through similar mechanisms [70].

Aedronc transcripts are expressed ubiquitously and, similar to *Drosophila dronc*, are found at highest levels in developmental stages experiencing pulses of ecdysone [70, 100]. In addition, in vivo studies have confirmed that *Aedronc* transcription is up-regulated in response to ecdysone [70]. These data suggest that AeDronc plays a central role in developmental apoptosis, similar to that observed in *Drosophila*, though a lack of specific *dronc* mutants in mosquitoes has made this difficult to test.

Homologues of strica/dream and damm

Homologues of *Drosophila* Strica/Dream have been identified in the genomes of both *Ae. aegypti* and *An. gambiae* [99]. No specific molecular or biochemical information is available on either putative caspase, however, the *Ae. aegypti* transcript (CASPS16) is predicted to contain a serine- and threonine-rich prodomain and transcripts of CASPS16 can be detected in all developmental stages, and in the adult midgut [99]. The role of these putative initiator caspases and the function of their serine–threonine rich prodomains remain unknown but may represent novel functions, protein–protein interactions or cell death signaling pathways within insects.

Recent phyologenetic analyses suggest that the mosquito homologues of Strica/Dream and the putative effector caspase Damm, have evolved from a gene duplication event that occurred after the Drosophila/mosquito divergence, with one of the duplicated genes either losing (Damm) or gaining (Strica/Dream) a long prodomain sequence [99]. Interestingly, each of these genes has been duplicated in mosquitoes. The significance of these apparent gene duplications or the functions of either caspase in mosquitoes is not known but may highlight a role for caspases in the regulation of an immune system exposed to a wide variety of pathogens.

The current lack of putative initiator caspases in non-drosophilid insects is neither surprising nor representative of what likely exists in the genomes of insects. Predicting initiator caspases can be difficult because sequence similarity across the death domain superfamily is low making it difficult to identify and predict relationships based on primary sequence alone. There is no doubt that a more in-depth analysis of the sequence information emerging from the new insect genome projects will prove fruitful in the future.

The effector caspases

Following the transmission of a cell death signal, the initiator caspases will cleave and activate the downstream effector caspases. These caspases lack long prodomains and the ability to self-activate. Once activated by an initiator caspase, effector caspases then cleave the substrates responsible for the dismantling of the cell. In mammals, the apoptotic effector caspases are caspase-3, -6 and -7, and in *Drosophila* are Drice, Dcp-1, Decay and Damm.

Drosophila drice

Drice is the most abundant and widely expressed *Drosophila* caspase and is a primary target for the initiator caspase Dronc [37, 52, 74, 75, 93], reviewed in Kumar [33]. By sequence analysis, Drice has a short prodomain and is most similar to mammalian caspase-3. Drice has a substrate specificity with optimal activity on the caspase-3 substrate DEVD and many of the known cellular targets for Drice include lamins, DmO, DIAP1, Dronc, the baculovirus caspase inhibitor p35, and *Drosophila* ICAD [74, 75, 93].

Drice also is an ecdysone-inducible caspase that acts downstream of the cell death activators Reaper and Hid, and is required for most cell death that occurs during development. RNAi studies have shown that in most cases, Drice is activated by Dronc, that loss of *dronc* function severely impairs Drice activation, and that depletion of *drice* from *Drosophila* S2 cells inhibits apoptosis in response to a variety of stimuli that are known to be Dronc dependent [46, 50, 53, 57, 76–78]. In vivo studies have established that *drice* mutants, like *dronc* mutants, are mostly pupal lethal and have reduced cell death in the pupal retina, embryonic nervous system and adult wing, and show a reduced response to stress and irradiation or the inhibition of protein synthesis [77, 79]. It is interesting to note that cell death in some *Drosophila* cell types appears to be Dronc- and Drice-independent. Thus, some cell death does occur in the embryos of Dronc and Drice mutants. Studies with *drice/dcp-1* double mutants suggest that some

cells require Drice while others use either Drice or another effector caspase Dcp-1, in a redundant manner [55, 77, 79].

Drice also has been implicated in several non-apoptotic functions, including spermatid individualization [48, 77].

Drosophila Dcp-1

Dcp-1 was the first *Drosophila* caspase to be identified and is most similar to Drice and mammalian caspase-3 [80]. As expected, Dcp-1 has a short prodomain and a substrate specificity that closely mimics that of Drice, with a preferred recognition sequence of DEVD and the ability to cleave PARP and p35 in vitro [80, 93]. Like Drice, Dcp-1 acts downstream of the cell death activators, Reaper and Hid, and *dcp-1* mRNA are expressed during the early stages of embryogenesis [46]. In contrast to *drice* mutants, however, *dcp-1* null animals are viable and fertile as homozygotes, with only a lack of germline cell death during mid-oogenesis in response to nutrient deprivation reported as the cell death phenotype [81]. Several studies indicate that Dcp-1 represents a redundancy in the apoptotic pathway substituting for Drice only in specific cell types [55, 79]. Despite the lack of phenotypes observed in *dcp-1* mutants, RNAi studies indicate that the specific depletion of *dcp-1* from cell lines significantly reduces the rate of ecdysone-induced apoptosis suggesting that Dcp-1 may, in fact, contribute to the overall efficiency of cell death [76].

Dcp-1 also has been implicated in spermatid individualization and starvation-induced autophagy during oogenesis [48, 82].

Drosophila decay

Decay is an effector caspase structurally similar to Drice, Dcp-1 and human caspase-3, and cleaves substrates containing DEVD in vitro [83]. When ectopically expressed, Decay induces apoptosis in mammalian cells, though little information is available regarding Decay function in vivo. *Decay* mutants are developmentally viable and fertile, and show no obvious signs of abnormalities [55]. While no defects have been reported in *decay* mutants during embryogenesis, tissue-specific phenotypes have been observed. Decay is involved in Hid-mediated cell death and flies lacking *decay* show weak suppression of the rough eye phenotype [46]. Much of the current data suggests cell death occurs normally in *decay* mutants and that Decay function may be redundant during development.

Drosophila damm

The functions of Damm are not fully understood, as no mutants are available. In vitro, Damm induces cell death and ectopic expression in the *Drosophila* eye results in a rough eye phenotype that sensitizes these cells to apoptosis [84]. In vivo, Damm contributes in a minor fashion to the canonical cell death pathway induced by loss of DIAP1 and no significant rescue of Hid-mediated apoptosis, suggesting that Damm is redundant in this system [46]. It is possible that Damm is required for functions not yet identified or may perhaps function in a tissue- or stage-specific fashion.

The other insect effectors

Mosquito effectors of apoptosis

Several putative effector caspases have been identified in the genomes of mosquitoes and Lepidoptera, however, relatively few have been fully characterized. In the mosquitoes, effector caspase activity has been detected in tissues and cells infected with parasites and viruses [101–103]. Genes homologous to *Drosophila* Drice, Dcp-1, and Decay have been identified in the genomes of *Ae. aegypti* and *An. gambiae,* though it should be noted here that many of the predicted caspases in *An. gambiae* have not been confirmed and many are not full-length [99, 104]. A recent study groups putative mosquito effector caspases into two clades [99]. Clade I caspases share sequence similarity with *Drosophila* Decay and include two *Ae. aegypti* caspases (CASPS18 and CASPS19) and eight *An. gambie* caspases (Ags1, Ags2, Ags3, Ags4, Ags5, Ags6, Ags11, and Ags14). Interestingly, three caspases from *An. gambiae* (Ags1, Ags2, and Ags14) contain a serine or threonine instead of an alanine in the putative active site, while one CASPS18 (*Ae. aegypti*) contains a serine in place of cysteine, making it unlikely that these genes encode functional caspases. These genes may instead encode proteins that regulate caspases in a dominant-negative manner, similar to that shown by the human proteins, Pseudo-ICE and ICEBERG [99, 105].

Clade II contains the caspases that share sequence similarity with *Drosophila* Drice and Dcp-1 and includes two caspases from *Ae. aegypti* (CASPS7 and CASPS8) and two caspases from *An. gambiae* (Ags7 and Ags8). One additional caspase, *Ae. aegypti* CASPS20, shares sequence similarity with *Drosophila* Drice but does not fall within either clade. Transcripts for CASPS7, 8 and 20 can be found in all developmental stages and the adult midgut [99].

Lepidoptera effectors of apoptosis

A few effector caspases have been characterized in the Lepidoptera, most of which are homologous to *Drosophila* Drice. The first insect caspase was identified in *Spodoptera frugiperda*, *Sf*-caspase-1, and is similar to mammalian caspase-3 and *Drosophila* Drice [85]. Similar to other

effector caspases, *Sf*-caspase-1 contains a short prodomain and cleaves substrates containing the sequence DEVD, although a complete substrate profile has not been analyzed [86]. Although *Sf*-caspase-1 has not been completely characterized, its activity is suppressed by P35, the caspase inhibitor of AcMNPV, a virus known to known to infect these insects [87]. A similar caspase has been identified in a cell line from *Spodoptera littoralis* [88]. *Sl*-caspase- 1 contains a short prodomain and is involved in apoptosis induced by UV-irradiation and baculovirus-infection. *Sl*-caspase-1 cleaves substrates containing DEVD but not IETD or LEHD substrates, and in contrast to caspase regulation in *Drosophila*, *Sl*- caspase-1 appears to be regulated at the post-transcriptional level [88, 89]. This suggests that the Lepidoptera may have another regulatory check-point in the apoptotic pathway. Lastly, a Drice-homologue has been identified in the cotton bollworm, *Helicoverpa armigera*. This caspase, Hearm caspase-1, contains a short prodomain and is homologous to *Sf*-caspase-1 and *Drosophila* Drice. Hearm-1 is expressed in embryos and the fat body, midgut and hemocytes of feeding and wandering larvae and is inducible by ecdysone [90].

As was the case with the initiator caspases, several effector caspases have been predicted in the genomes of the silkworm, *Bombyx mori* (human caspase1-like molecules; gi:112983103 gi:86371760, gi:86371762), *the flour beetle*, *Tribolium castaneum* (an ecdysone inducible caspase; gi:189241132 and a caspase-1-like molecule, (gi:91079681) and the pea aphid (*Acyrthosiphon pisum*). No biochemical information is available for these caspase homologues.

Insects as cell death models

Apoptosis, and the caspases that determine the fate of a cell, is a conserved process found in all organisms. The idea that cell death might be an evolutionary prerequisite for the development of multicellular organisms is not new; therefore it is not surprising that caspases can be found in all metazoans, including the insects. The conservation of structural and biochemical properties among the caspases underlines the importance of these enzymes and the cell death process itself. In insects, this concept is supported by numerous genetic studies in *Drosophila* where a single caspase, Dronc is essential for most developmental cell death. Importantly, several of these studies suggest that cell death in some cell types occurs in the absence of Dronc and the primary effector caspases Drice, suggesting that, similar to mammals, redundancies have been built into the cell death system of insects. When this redundancy evolved and its significance remains unresolved and the characterization of caspases from the more ancient insect orders may provide valuable insights. Furthermore, studies in both mammals and *Drosophila* have shown that some caspases have no clear role in apoptosis while others appear to play a role in both apoptotic and non-apoptotic processes. Studies with the insects may provide important insights into the function and regulation of caspases in alternative processes.

Finally, insect models such as *Drosophila*, and now the mosquito *Ae. aegypti*, provide a level of intermediate complexity between nematodes and mammals. The mosquito model, in particular, provides us with the opportunity for a comparative study of caspase function in development and the regulation of specific immune processes in insects in which certain intracellular pathogens infect host tissues and multiply before being transmitted to humans. The growing appreciation of the conservation of some apoptotic responses in insects and mammals will produce an exchange of ideas that will continue to invigorate this field.

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