Cell Death and Differentiation (2008) 15, 1139–1146

www.nature.com/cdd

Review

Living with death: the evolution of the mitochondrial pathway of apoptosis in animals

A Oberst[1], C Bender[2,3] and DR Green*[,1]

The mitochondrial pathway of cell death, in which apoptosis proceeds following mitochondrial outer membrane permeabilization, release of cytochrome *c*, and APAF-1 apoptosome-mediated caspase activation, represents the major pathway of physiological apoptosis in vertebrates. However, the well-characterized apoptotic pathways of the invertebrates *C. elegans* and *D. melanogaster* indicate that this apoptotic pathway is not universally conserved among animals. This review will compare the role of the mitochondria in the apoptotic programs of mammals, nematodes, and flies, and will survey our knowledge of the apoptotic pathways of other, less familiar model organisms in an effort to explore the evolutionary origins of the mitochondrial pathway of apoptosis.
Cell Death and Differentiation (2008) **15,** 1139–1146; doi:10.1038/cdd.2008.65; published online 2 May 2008

The events that define apoptosis in all animal cells are mediated by the actions of the caspase proteases. The mechanisms, however, by which apoptotic stimuli are integrated and translated into caspase activation differ markedly among the species in which the apoptotic pathways have been elucidated. In most mammalian cells, caspase activation and apoptosis proceed after mitochondrial outer membrane permeabilization (MOMP). A wide range of signals converge on the mitochondria, and MOMP is observed in response to apoptotic stimuli as diverse as DNA damage, nutrient deficiency, ER stress, growth factor withdrawal, heat shock, and developmental cues. Although a central role for the mitochondria is well recognized in mammalian apoptosis, it is worthwhile to take an unclouded look at what remains a fundamentally surprising and intriguing finding: that our cells have evolved a coordinated program to rupture an indispensable organelle, and that once this occurs an apparently innocuous piece of the respiratory machinery—cytochrome *c*—assembles a group of proteins lurking in the cytosol to bring about the rapid and total destruction of the cell. When examining the minutiae of this process, we often fail to step back and contemplate why these events take place as they do. When did the centrality of the mitochondria arise? How conserved is the role of the mitochondria in general, and the phenomenon of MOMP in particular, among metazoans? The well-characterized death programs of *C. elegans* and *D. melanogaster* provide fundamental insight, but the experienced researcher will regard trends based on two data points with a jaundiced eye. Much-needed additional information is beginning to become available from non-canonical invertebrate model organisms; herein we shall present a comparison between the role of the mitochondria in the apoptotic pathways of mammals, nematodes and flies, and begin working to fill in the sizable gaps between these phylogenically disparate animals with a summary of the information gleaned thus far from more obscure creatures (at least from the point of view of apoptosis research). We will take a self-centered approach and use the pathways and processes of mammalian apoptosis as a referential baseline, so let us begin by reviewing these pathways with an eye to molecules and mechanisms that bear comparison to those of other species.

In mammals, the intrinsic pathway of apoptosis is initiated by MOMP, which leads to diffusion of the contents of the mitochondrial intermembrane space into the cytosol. MOMP is controlled by the Bcl-2 family of proteins, which is divided between proapoptotic (MOMP-promoting) and antiapoptotic (MOMP-inhibiting) members. Members of the Bcl-2 family share up to four Bcl-2 homology (BH) domains; chief among the proapoptotic Bcl-2 proteins are the multidomain proteins Bax and Bak. These contain BH-1, -2, and -3 domains, and knockout studies have identified them as being indispensable for MOMP; their activation is believed to lead to MOMP through a conformational change, inducing their oligomerization and insertion into the outer mitochondrial membrane, where they form large pores through which proteins from the intermembrane space can access the cytosol. Bax and Bak activation is controlled, in turn, but the complex interplay between the other members of the Bcl-2 family, including the antiapoptotic multidomain proteins Bcl-2, Bcl-xL, and MCL-1, and the numerous BH-3-only proteins. Although the specifics of this process remain somewhat controversial, it is thought that the antiapoptotic multidomain proteins inhibit Bax and

[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA; [2]Apoptosis and Cell Death Program, Burnham Institute for Medical Research, La Jolla, CA 92037, USA and [3]Biomedical Sciences Graduate Program, University of California San Diego, La Jolla, CA 92093, USA
*Corresponding author: D Green, Department of Immunology, St. Jude Childern's Research Hospital, 332 N. Lauderdale Street, Memphis, TN 38105, USA.
Tel: 1 901 495 3470; Fax: 1 901 495 5766; E-mail: douglas.green@stjude.org
Keywords: mitochondria; *drosophila*; *C.elegans*; apoptosis; MOMP
Abbreviations: MOMP, mitochondrial outer membrane permeabilization; CARD, caspase activation and recruitment domain; IAP, inhibitor of apoptosis proteins
Received 26.3.08; accepted 31.3.08; Edited by G Melino; published online 02.5.08

Bak by binding to and sequestering the BH-3-only proteins capable of inducing the activating conformational change in Bax and Bak, as well as active Bax and Bak themselves, whereas another subset of BH-3-only proteins act by releasing this inhibition.[1]

Once MOMP has taken place, proteins heretofore sequestered within the mitochondria gain access to the cytosol. Among these is cytochrome *c*, which binds to the WD domain repeats of the cytosolic protein APAF-1. This binding, along with that of dATP, causes a conformational change in APAF-1, which in turn leads to its oligomerization into a septameric structure called the apoptosome.[2] The conformational change induced in APAF-1 upon binding to cytochrome *c* also exposes the former's caspase activation and recruitment domain (CARD), which then recruits and dimerizes caspase-9 through interaction with the CARD in that molecule's prodomain. Caspase-9 is an apical caspase, and as such exists as an inactive monomer in the cytosol of non-apoptotic cells. This caspase, like the apical caspases-2, -8 and -10, has a long N-terminal prodomain, which contains a protein interaction motif. Apical caspases are activated by dimerization;[3] when two caspase-9 monomers are pulled into proximity by the oligomerized CARD domains of the apoptosome, they dimerize and undergo interchain autocatlytic cleavage, yielding a mature, active caspase. This protease then activates the executioner caspases, caspase-3 and -7. Executioner caspases are present as inactive dimers in the cytosol, and lack a long prodomain; they are activated by interchain processing by an apical caspase, and once active lead to the destruction of the cell that we observe as apoptosis (Figure 1).

In addition to cytochrome *c*, several additional factors released upon MOMP have been implicated in apoptosis. Notably, Smac/DIABLO and Omi/HtrA2 bind to and inhibit the inhibitor of apoptosis (IAP) proteins. The IAPs were first identified in baculoviruses,[4] and feature 1–3 copies of a zinc-binding domain, the BIR domain. These domains allow some IAPs to bind to and inhibit caspases; XIAP is a strong *in vitro* inhibitor of caspases-3, -7 and-9, whereas cIAP1 and -2 are weaker caspase inhibitors.[5] XIAP overexpression prevents apoptosis in cultured cells,[6,7]and IAPs are often upregulated in human cancers, which initially led researchers to posit a model in which IAPs block basal caspase activity in healthy cells, thereby preventing 'accidental' cell death until MOMP occurs, at which point Smac and Omi bind to the IAPs, releasing the caspases and allowing apoptosis to proceed. However, this model was not supported by *in vivo* mouse studies, which found that genetic ablation of *smac, omi,* or *XIAP* failed to produce a clear apoptotic phenotype.[8–10] Nonetheless, IAP inhibition through small-molecule Smac mimetics has shown remarkable promise as a selective anticancer therapy. Recent reports[11–13] have indicated that apoptosis in response to IAP inhibition occurs not due to release of caspases-9 and -3 by XIAP, but rather in a caspase-8- and TNF-dependent manner following autoubiquitination and degradation of cIAP1 and -2. Despite these encouraging findings, the relevance of the Smac/Omi-IAP pathway in the apoptosis of normal mammalian cells remains unclear.

Nematodes: like Us, only Different

The study of apoptosis in the nematode worm, *C. elegans,* has an illustrious past; it was the observation that precisely 131 of the 1090 cells generated in the development of an adult hermaphrodite worm die in a predictable, repeatable way that helped to inaugurate the field of apoptosis research at the molecular level. A landmark finding was that programmed cell death in *C.elegans* is under genetic control,[14] and subsequent genetic analyses identified several genes responsible for *cell death defective (ced)* phenotypes. Chief among these were *ced-3, ced-4,* and *egg laying defective (egl)-1*, in which loss-of-function mutations led to survival of nearly all the cells programmed to die, and *ced-9*, in which loss of function leads to excessive apoptosis. Sequence comparison revealed that

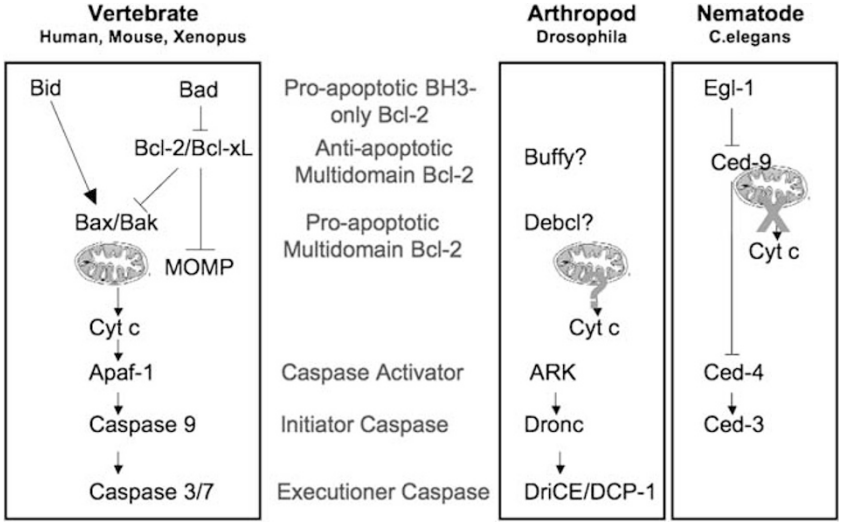

Figure 1 Mammalian cytochrome *c*-dependent apoptotic pathways and their homologs in flies and worms. Leftmost panel depicts the proteins upstream and downstream of cytochrome *c* release in mammals. Panels on the right show homologs of these proteins found in *Drosophila* and *C.elegans*; the degree to which homologs conserve the function of their mammalian counterparts varies, and is summarized in the text. Cytochrome *c* release is controversial in the fly, and is not observed in the worm. Note that this figure depicts only cytochrome *c*-dependent caspase activation; effects of IAPs and their inhibitors, as well as other released mitochondrial factors, are omitted

the protein products of these genes bear striking resemblances to the major players in mammalian apoptosis, and indeed the mechanisms of nematode apoptosis are in many respects similar to those described above for mammals.

The CED-9 protein is an anti-apoptotic protein with four BH domains, homologous to Bcl-2. It is anchored to the mitochondria, and in healthy cells is associated with a dimer of CED-4. CED-4 is homologous to mammalian APAF-1, and like APAF-1 it contains a CARD, though importantly it lacks the WD domain repeats that mediate APAF-1′s interaction with cytochrome *c*. Developmental cues in the worm lead to upregulation of EGL-1, which is a BH-3 only protein; this protein binds to CED-9, disrupting its interaction with CED-4. CED-4 is then free to form a tetrameric apoptosome-like structure, which recruits CED-3. CED-3 is a CARD-containing caspase, most closely homologous to mammalian caspase-2; *C.elegans* lacks the initiatior-executioner caspase hierarchy observed in mammalian cells, so the catalytic activity of CED-3 is directly responsible for cellular disruption (for review see Lettre and Hengartner[15]) (Figure 1).

Although many of the players in nematode apoptosis have mammalian homologs, the way in which they interact differs in several important ways. Most obviously, neither MOMP nor cytochrome *c* plays a role in initiation of apoptosis in the nematode, although the mitochondrially localized proteins CPS-6 and WAH-1 (nematode homologs of mammalian endonuclease-G and AIF, respectively) have been suggested to contribute to DNA degradation and phosphatydilserine externalization later in the apoptotic program.[16–19] As noted, CED-4 lacks the WD-domain repeats present in APAF-1 that are necessary for cytochrome *c* binding, and the release of CED-4 from sequestration by CED-9 appears to be sufficient to cause formation of the nematode apoptosome. Furthermore, despite early reports to the contrary[20,21] it is now accepted that mammalian Bcl-2 homologs do not functionally interact with APAF-1.[22–24]

Given these significant differences, it is intriguing that the antiapoptotic functions of both Bcl-2 and CED-9 involve the mitochondria. Indeed, the functions of the mammalian Bcl-2 family members and those of CED-9/EGL-1 overlap in a number of interesting ways: despite sharing only around 22% homology with CED-9, expression of Bcl-2 inhibits apoptosis in *C.elegans*,[25] and Bcl-2 expression was found to rescue the excess of apoptosis found in CED-9 loss-of-function worms.[26] This is particularly surprising in light of the inability of Bcl-2 to interact with APAF-1. How, then, is Bcl-2, which acts by preventing MOMP, able to substitute for CED-9, whose antiapoptotic effect is exerted through sequestration of the nematode APAF-1 homolog CED-4? A more recent study[27] found that Bcl-2 does not interact with CED-4 in a yeast two hybrid screen; it does, however, interact with EGL-1. Furthermore, when the basic nematode apoptotic program is recapitulated in yeast, CED-9, but not Bcl-2, was able to prevent lethality caused by CED-3/CED-4 co-expression. The authors proposed that Bcl-2 prevents cell death in wild-type nematodes by binding EGL-1 through that protein's BH-3 domain, and that the ability of Bcl-2 to rescue the apoptotic phenotype of CED-9-deficient worms could be due to Bcl-2 preventing EGL-1 from neutralizing the small amount of maternally derived CED-9 present at the developmental stage at which the original experiments were performed.[26–28]

Despite their frequent depiction as isolated, globular organelles, in healthy cells the mitochondria form a complex and interconnected network, subject to frequent fusion, fission, and remodeling events (reviewed in Hoppins *et al*.[29]). Imaging of mammalian cells undergoing apoptosis has revealed that mitochondria fragment during MOMP; subsequent studies have lent credence to the idea that mitochondrial dynamics and MOMP are intimately linked with increased mitochondrial fission leading to sensitivity to MOMP and increased fusion blocking or delaying it.[30–32] While both pro- and antiapoptotic proteins of the Bcl-2 family have been implicated in these processes, causal links have been difficult to draw, due in part to the challenge of distinguishing between their roles in mitochondrial dynamics and their roles in apoptosis. Intriguingly, given the lack of MOMP in the apoptotic program of *C. elegans*, it appears that mitochondrial dynamics do play a role in nematode cell death. Jagasia *et al*.[33] found that EGL-1 causes mitochondrial fragmentation in apoptotic nematode cells, and that this function is antagonized by CED-9; furthermore, upregulation of the mitochondrial fission protein Drp-1 was necessary and partially sufficient to induce apoptosis. Surprisingly, Delivani *et al*.[34] found that the roles of these *C. elegans* proteins in mitochondrial dynamics extend to mammalian cells. Expression of CED-9 in mammalian cells causes mitochondrial fusion, wheras EGL-1 co-expression antagonized this function, leading to mitochondrial fragmentation. Perhaps most interestingly, neither CED-9-mediated fusion nor EGL-1-mediated fission affected the dynamics or timing of cytochrome *c* release. These findings indicate that CED-9 and EGL-1 play a role in mitochondrial dynamics independent of their apoptotic function in regulating the release of CED-4, as well as implying that changes in mitochondrial morphology *per se* do not affect sensitivity to MOMP in mammalian cells. They also raise questions about the idea that mitochondrial remodeling is required for efficient release of intermembrane space proteins, as nematode cells remodel their mitochondria in similar ways in the absence of MOMP. Finally, it is unclear what role mitochondrial fragmentation plays in *C. elegans* apoptosis, as the only known role for mitochondria in the process is as localization platforms for sequestration of CED-4 by CED-9.

Conservation of a Mitochondrial Role in Cell Death: a Fly in the Ointment?

Let us now turn our attention to *Drosophila melanogaster,* the third model organism in which the particulars of the apoptotic pathway have been worked out in some detail. The *Drosophila* caspase family comprises seven members, and like mammalian caspases (but unlike the nematode), these can be divided into initiator and executioner caspases based upon the presence or absence of long N-terminal prodomains. DRONC has emerged as the essential apoptotic initiator caspase in *Drosophila*; like mammalian caspases 2 and 9, as well as CED-3, DRONC contains an N-terminal CARD, through which it interacts with ARK, the fly homolog of APAF-1 and CED-4. Once activated, DRONC cleaves DRICE and DCP-1,

Drosophila executioner caspases homologous to mammalian caspase-3 (Figure 1). Unlike APAF-1, however, ARK does not appear to require cytochrome *c* to activate DRONC. Instead, the *Drosophila* apoptotic program is controlled by DIAP1, a fly homolog of the mammalian IAP proteins. DIAP1 contains two Zinc-coordinating BIR domains, which allow it to bind to DRONC, keeping the activity of that caspase in check. Apoptosis is initiated by the DIAP1 antagonists HID, Grim, and Reaper, which act by binding to DIAP1 and inducing its autoubiquitination and proeasome-mediated degradation (reviewed in Vaux and Silke[35], Kumar and Doumanis[36], Kumar[37]). Cells in which DIAP1 is ablated show spontaneous apoptosis, indicating that removal of the inhibitory function of DIAP1 is sufficient to initiate the apoptotic program in flies. It is worth noting that this stands in contrast to the situation in mammals, where genetic ablation of the IAPs does not produce an overt apoptotic phenotype. Although IAPs and their antagonists are present in mammals, they do not appear to play the central role in the control of apoptosis found in the fly. Smac/DIABLO and Omi/HtrA2, the mammalian IAP antagonists, do not show sequence homology to HID, Grim, or Reaper; however, fly and mammalian IAP antagonists do share an unmodified N-terminal alanine, generated by proteolytic cleavage in the case of Smac and Omi, and removal of the initiator methionine in Grim, Reaper, and HID.[38] This functional group is also present in small-molecule Smac mimetics, and is believed to be required for neutralization of BIR domain-mediated inhibition. Smac and Omi also differ from fly IAP antagonists in the mechanism by which they are unleashed. In the fly, HID, Grim, and Reaper are transcriptionally upregulated in response to developmental cues in most cell types; transcriptional upregulation of ARK and DRONC may also be involved in evasion of DIAP1-mediated caspase inhibition.[39] By contrast, in mammalian apoptosis Smac and Omi are released from the mitochondria upon MOMP, along with cytochrome *c*.

This observation highlights a thorny question: what role, if any, do the mitochondria play in *Drosophila* apoptosis? ARK is structurally more similar to APAF-1 than to CED-4, and it contains several of the WD-domain repeats, absent in CED-4, through which APAF-1 binds cytochrome *c* during formation of the mammalian apoptosome; however, the role of cytochrome *c* in *Drosophila* apoptosis remains controversial. The finding that DIAP1 ablation is sufficient to cause caspase activation indicates that ARK is constitutively able to activate DRONC, independent of mitochondrial factors.[40] The structure of a putative ARK apoptosome has been solved using electron cryo-microscopy to a resolution of 18.8 Å, and this structure does not include cytochrome *c*, although it does conserve the requirement for dATP found in the mammalian apoptosome.[41]

Additional biochemical and genetic data has also indicated that apoptosome formation and caspase activation may be cytochrome *c* independent in *Drosophila*, although this has been the topic of some debate. In one study, silencing expression of either or both *Drosophila* cytochrome *c* proteins was found not to affect apoptosis in the fly, and addition of either recombinant forms of these proteins or whole mitochondrial extracts to cell-free systems failed to significantly activate caspases in *Drosophila* cell lysates, although addition of recombinant *Drosophila* cytochrome *c* does yield caspase activation in mammalian lysates.[42–44] However, studies on flies lacking *d-cyt-c*, one of the *Drosophila* cytochrome *c* genes, found severely delayed apoptosis in the developing retina,[45] and a genetic screen found that mutations in *d-cyt-c* reduced caspase activation during spermatid individualization,[46] an effect phenocopied by ARK or DRONC loss-of-function mutations. An earlier study found that an otherwise hidden epitope of cytochrome *c* is presented early in the *Drosophila* apoptotic program, although this putative conformational change is apparently caspase dependent and proceeds without release of cytochrome *c* from the mitochondria.[47] These findings have led to the supposition that alterations in mitochondrial morphology might lead to formation of a cytochrome *c* dependent apoptosome at the mitochondrial surface as part of a feed-forward signal in caspase activation.[46,48] While intriguing, such a scenario requires further validation; much of the discord in this area may be due to the differences in cell type and apoptotic stimulus used in the various studies.

In both *C. elegans* and mammalian cells, proteins of the Bcl-2 family play a pivotal role in the mitochondrial pathway of cell death. *Drosophila* also expresses two proteins with homology to Bcl-2, termed Buffy and Debcl.[49–52] These proteins share significant sequence homology, and both contain three BH domains. Sequence comparison and overexpression studies have suggested that Buffy is an antiapoptotic multidomain Bcl-2 protein similar to mammalian Bcl-2, where Debcl is proapoptotic, with greatest observed homology to mammalian Bok. Knockdown of these proteins was consistent with these roles; RNAi against Buffy led to increased apoptosis, whereas injection of Debcl dsRNA caused an accumulation of posterior glial cells. However, despite these findings, a recent study found that flies with loss-of-function mutations in *buffy, debcl*, or both display normal developmental cell death and are viable and fertile, although a modest effect on apoptosis following ionizing radiation was observed.[53] As in the case of cytochrome *c* involvement in fly apoptosis, these conflicting results may reflect differences in cell type and stimulus.

As noted, significant evidence has been arrayed that mitochondrial factors such as cytochrome *c* are not required for caspase activation in *Drosophila*; additionally, Bcl-2 proteins do not appear to play a major role in the core apoptotic pathway of the fly. This stands in stark contrast to the situation observed in the nematode and mammal, and has led to supposition that *Drosophila* might be an evolutionary outlier, or indeed that the mitochondrial pathway of apoptosis might not be as conserved as once thought. However, a recent study by Abdelwahid *et al*.[54] indicates a significant, if somewhat puzzling, role for the mitochondria in programmed cell death in the fly. Abdelwahid *et al*. found that, upon apoptosis, Reaper and HID proteins cause mitochondrial fragmentation and release of cytochrome *c* in both cultured S2 cells and in the developing fly embryo. This effect was observed to depend both on HID and Reaper and on caspase activation; death induced by DIAP1 knockdown or genotoxic stress (in which HID and Reaper are not upregulated) did not induce mitochondrial changes, despite significant caspase activation. Furthermore, fragmentation of the mitochondria upon HID or Reaper upregulation was blocked by the pancaspase inhibitor zVAD-fmk. As in mammals and

nematodes, Drp1 was found to mediate mitochondrial fragmentation in apoptotic cells, and although the authors confirmed that cytochrome *c* is not required for apoptosis in the fly, they found that Drp1 knockdown led to a reduction in cell death. It may be that, while the observed mitochondrial disruption does not contribute to caspase activation *per se* (given that caspase activity is required for the disruption itself), it is nonetheless an important fail-safe mechanism to ensure cell death by rupturing the mitochondria, as well as contributing to efficient packaging of the dying cell for phagocytosis. The role of HID and Reaper in this process is provocative; in nematodes, mammals, and even yeast, the Bcl-2 family proteins are implicated in Drp-1-mediated fragmentation during apoptosis.[33,55,56] In flies, where the Bcl-2 family evidently plays a less pivotal role in cell death, the essential apoptotic mediators HID and Rea*per* seem to have assumed this role, and Abdelwahid *et al*. found that a mutant of Reaper that does not block DIAP-1 function was still able to cause mitochondrial fragmentation, indicating that apoptosis induction and mitochondrial disruption represent two distinct functions of Reaper. This duality of function mirrors the effect found for CED-9/EGL-1 in the nematode,[33] and may indicate that mitochondrial fragmentation plays an essential, if incompletely understood, role in apoptosis *in vivo*, leading different organisms to link this process to essential apoptotic factors.

Out of the Mainstream, into the Ocean

Having surveyed the well-characterized model organisms without emergence of a clear consensus on the role of the mitochondrial pathway in apoptosis, we are forced to turn our attention farther afield. A small group of brave souls has begun working to characterize the pathways of apoptosis in non-canonical invertebrate model organisms; a survey of these findings might prove informative in breaking the stalemate presented by the distinct pathways of *C.elegans* and *Drosophila.*

The deuterostome clade consists of three major phyla: (1) the chordates which includes vertebrates (mammals, birds, amphibians, fish, reptiles) and the invertebrate chordates, cephalochordates (amphioxus) and urochordates (sea squirts), (2) the hemichordates (acorn worms), and (3) the echinoderms (sea urchins, starfish).[57] Significant, if fragmentary, information is available regarding the features of apoptosis in non-vertebrate deuterostomes. Caspase activation has been observed in organisms of all phyla; however, the mechanisms leading to caspase activation are generally poorly understood. Figure 2 depicts an animal phylogeny showing proposed evolutionary relationships between these phyla, as Table 1 summarizes the apoptotic molecules found to date in these animals.

Cephalochordates are small eel-like animals that live buried in the sand. Apoptosis has been detected in the embryonic and larval stages of amphioxus (*Branchiostoma floridae*) development.[73] In addition, AmphiCASP-3/7, a capsase homologous to human caspases 3 and 7, has been cloned. AmphiCASP-3/7 is expressed throughout amphioxus development, from the gastrula to larval stage. When transfected into 293T cells, wild-type AmphiCASP-3/7 promotes apoptosis, and a mutant with the prodomain deleted\had an enhanced ability to promote apoptosis, indicating that AmphiCASP-3/7 is activated by cleavage. Recombinant AmphiCASP-3/7 preferentially cleaves synthetic caspase substrates containing a DEVD sequence, but not substrates containing either VEID or IETD sequences. However, transfection of AmphiCASP-3/7 into MCF7 cells (deficient in caspase-3) did not rescue the apoptotic phenotype.[73]

The urochordate ascidian *Ciona intestinalis* (sea squirt) develops through a larval stage, which is characterized by a typical chordate body plan including a notochord. The adult is a sessile filter feeder and metamorphosis requires massive

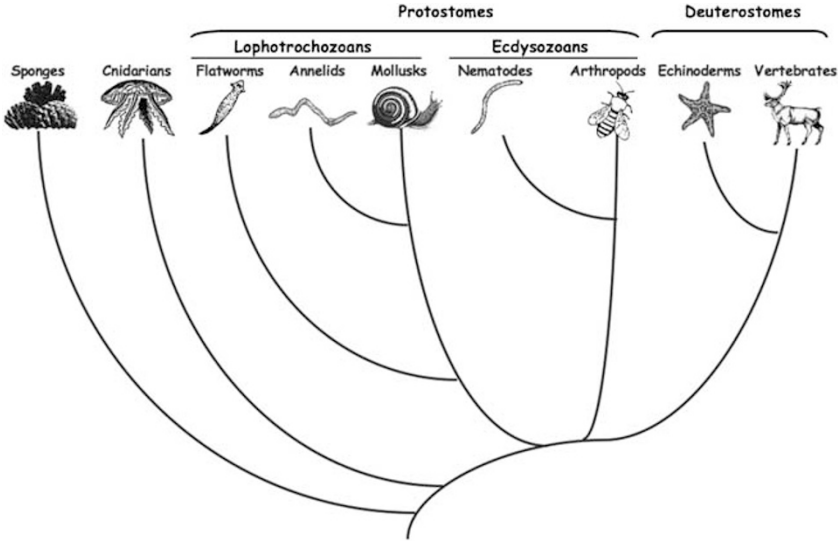

Figure 2 Animal phylogeny. MOMP is well characterized only in vertebrates. The apoptotic programs of the nematode ecdysozoan *C.elegans* and the arthropod ecdysozoan *D.melanogaster* have been studied extensively, and show varying degrees of mitochondrial involvement but lack direct evidence of MOMP. This leaves open the question of whether MOMP arose in vertebrates, or arose earlier but was lost in ecdysozoans. Much-need additional information from more basal phyla is emerging (surveyed in the text)

Table 1 Summary of apoptotic molecules and pathways identified thus far in the invertebrate phyla depicted in Figure 2

Phylum	Apoptotic molecules identified
Echinodermata (Purple sea urchin)	Apical and initiator caspases, three APAF-1 homologs including one with WD repeats, pro- and antiapopotic Bcl-2 family homologs, seven possible IAPs[58,59]
Platyhelminthe (Planaria)	Eleven caspases, one APAF-1 homolog, nine Bcl-2 homologs[60,61]
Cnidaria (Starlet sea anemone)	p53/p63 homolog, five caspase homologs, APAF-1 homolog containing WD repeats, seven Bcl-2 homologs, four IAP homologs[62–66]
Porifera (sponges)	Apical and initiator caspase homologs, pro- and antiapoptotic Bcl-2 family members. Caspase-dependent cell death observed in allograft rejection[67–72]

reorganization of the body plan including regression of the tail. The regression of the tail has been demonstrated to be caspase dependent, and caspase inhibitors blocked metamorphosis. Fifteen independent caspase-like sequences have been identified in Ciona and while the mechanism of caspase activation is unknown, it appears that ERK activation may be involved in developmental apoptosis in Ciona.[74] Two Bcl-2 family protein homologs have also been identified in Ciona. ciBAX is a homolog of the proapoptotic multidomain protein BAX, whereas ciBcl-xL is a homolog of the antiapoptotic Bcl-xL. Ectopic expression of ciBAX, but not a BH3-deleted mutant, in Ciona embryos resulted in cell dissociation and apoptosis during the gastrula stage. The ciBAX-induced dissociation and apoptosis was inhibited by ciBcl-xL,[75] suggesting that the mitochondrial pathway may be present in Ciona.

The genome of an echinoderm, the purple sea urchin *Strongylocentrotus purpuratus*, has recently been sequenced[58] and homologs of the components of the vertebrate apoptotic pathways have been identified.[59] The *S. purpuratus* genome contains five classes of caspases, including both apical and executioner caspases. Caspase-8 and -9 homologs have been detected, containing tandem DED repeats or a CARD in their prodomains, respectively.[59] Three APAF-1 gene homologs were also identified in the *S. purpuratus* genome, one of which contained a CARD and seven WD repeats.[59] Ten genes coding for putative Bcl-2 family proteins were also identified, including one homolog each of the proapoptotic multidomain proteins, BAX and BAK, and one homolog of the antiapoptotic proteins Bcl-2/BclxL; however, to date no homologs of the BH3-only proteins BAD, BID, or BIM have been identified.[59] In addition, the *S. purpuratus* genome contains seven sequences that encode at least one BIR (baculoviral IAP repeat) domain characteristic of the IAPs, seven potential TNF receptor genes, four potential TNF ligand genes, and homologs of adaptor proteins involved in the extrinsic pathway.[59]

The Platyhelminthes includes both parasitic and free-living species of flatworms.[60] Freshwater planarians, a free-living flatworm, have long been studied for their remarkable regenerative abilities. For example, in decapitated planaria both the head and trunk fragments each regenerate the missing body parts and integrate them into the original fragments resulting in two complete, proportional, and fully functional animals. Cell loss (and presumably death) has been observed during both morphollaxis (proportional remodeling without cell proliferation) and epimorphosis (regeneration with cell proliferation). Although cell death has been observed in planaria for more than a century, the molecular mechanisms regulating death remain unknown. The genome of one species of planaria, *Schmidtea mediterranea* has been sequenced and has recently been made available on SmedGD.[61] A number of genes with homology to mammalian genes encoding proteins known to be involved in apoptosis, including 11 caspases, one APAF-1 and nine Bcl-2 proteins, have been identified (J Pelletieri, personal communication).

It has recently been observed that germ cells in the starlet sea anemone Nematostella, a member of the phylum cnidaria, undergo apoptosis in response to ultraviolet radiation in a dose-dependent manner. Although the molecular mechanism of UV-induced death in the anemone is unknown, it appears to be dependent on nvp63,[62] a protein with homology to mammalian p53, a proapoptotic protein that (1) functions as a transcription factor and (2) binds the antiapoptotic proteins Bcl-2 and BclxL to activate the proapoptotic proteins BAX and BAK to initiate MOMP.[63] Sequencing of the genome of *Nematostella vectensis* has been completed,[64] and analysis has revealed a number of apoptotic genes, including five caspase homologs, four IAP homologs, seven Bcl-2 family protein homologs, proteins containing homologs to death domains, death effector domains, CARD domains, and nucleotide-binding domains[59] and homologs of iCAD and CAD, proteins responsible for the characteristic DNA fragmentation observed in apoptosis.[65] In addition, an APAF-1 homolog containing two CARDs, a nucleotide-binding domain and WD repeats was recently identified.[66]

Significant study has been carried out on apoptosis in sponges of the phylum Porifera. Cell death can be readily observed in this organism in transplantation experiments, where an autograft (from the same sponge) or an allograft (from a different sponge) is inserted into a host. Although the autografts fuse within 5 days, approximately half of the cells in the allograft show positive TUNEL staining and characteristic DNA fragmentation during the same period.[67] Extracts made from allografts, but not autografts, cleave a caspase substrate, indicating caspase-like activity.[68] Apoptotic genes have been identified in several species of sponges, and two sponge caspases have been cloned (GEOCYCAS3l and GEOCYCAS3s). GEOCYCAS3l has a long prodomain which appears to be somewhat homologous to a CARD domain, whereas GEOCYCAS3s contains a short prodomain; both are upregulated during allograft rejection.[68,69] Three genes with BH have also been cloned in sponges. GCBHP1 and GCBHP2 both show homology to Bcl-2 in the BH1 and BH2 regions and both regions show closer homology to mammalian Bcl-2 family members than to CED-9.[70] GCBHP2 expression is upregulated in sponge cells exposed to low

(but not high) levels of tributyltin or heat shock, and GCBHP2 appears to have an antiapoptotic role. Mammalian cells transfected with GCBHP2 were somewhat resistant to serum starvation and tributylin-induced apoptosis.[71] In addition, a homolog of BAK (LBBAK2l) with homology in the BH1, BH3, and transmembrane domains and a homolog of Bcl-2 (LBBCL-2a) with homology in all four BH domains have been cloned. Expression of caspases and Bcl-2 family proteins has been analyzed in the developing sponge and it appears that the antiapoptotic proteins are expressed in the proliferative zone, whereas the proapoptotic proteins are expressed in the non-proliferative zone.[72]

A Fairy Tale Ending

Having surveyed our current understanding of the subject, the degree to which the mitochondrial pathway of apoptosis is conserved remains inconclusive. On the basis of well-characterized apoptotic pathways of the ecdysozoans *C.elegans* and *Drosophila*, as well as those present in mammals, we are left with two possibilities: either the apoptotic pathway requiring MOMP upstream of caspase activation arose in the chordates, or it arose earlier but was lost in some or all of the ecdysozoan groups (Figure 2). Recent study on the role of mitochondria in fly apoptosis notwithstanding, *Drosophila* does represent something of a fly in the ointment. Mitochondrial localization of the CED-9/CED-4 complex in *C.elegans*, as well as the conserved role of proteins of the Bcl-2 family in mitochondrial dynamics between mammals and nematodes, could be hypothesized to be vestiges of the mitochondrial pathway of apoptosis. But does *Drosophila* represent the end of an atypical branch of the evolutionary tree in which the mitochondrial pathway has disappeared alltogether, or is the variance in apoptotic pathways among the phyla greater than expected?

In pursuing this question, one generality that emerges is the futility of attempting to draw conclusions based on DNA or protein sequence. As mentioned above, numerous invertebrates express homologs to apoptotic proteins described in mammals; however, one need look no further than *Drosophila* to discern the futility of drawing firm conclusions about apoptotic pathways based on sequence data. On the basis of sequence, *Drosophila* might be expected to require MOMP and cytochrome *c* for caspase activation; after all, ARK contains the WD domains through which APAF-1 binds cytochrome *c*, and the *Drosophila* genome also encodes both pro- and antiapoptotic multidomain proteins of the Bcl-2 family, Debcl and Buffy, respectively. However, a preponderance of data indicates that cytochrome *c* is not required for *Drosophila* apoptosis. To turn the argument on its head, *Drosophila* apoptosis hinges on the action of a BIR-domain containing IAP, DIAP-1; however, despite the presence of mammalian homologs to DIAP-1, in mammals IAPs appear to be dispensable for normal execution of apoptosis. However, we are blessed to live in a time in which whole genome sequences seem to appear on a monthly basis, this is a problem that will yield to biochemistry and cell biology, not to molecular sequencing alone.

Supposition as to the reason that mitochondria play an important role in apoptosis in at least some animals is just that: supposition. However, the similarities between many of the molecules involved in apoptosis and those recruited in the cellular response to infection are difficult to ignore (see review by Munoz and Martin in this issue, and James and Green[76]). APAF-1 shares sequence homology with cytoplasmic mediators of the innate immune response called the NOD-like receptors, and the mitochondria arose as bacterial symbionts living within early eukaryotic cells. The idea that the earliest form of apoptosis may have been in response to pathogen invasion is attractive because it circumvents the problem of altruism in very simple organisms; the purposeful deletion of damaged or developmentally superfluous cells in a complex, multicellular animal confers a clear advantage on the organism as a whole, but the advantageousness of apoptosis is harder to envision in single-celled organisms, where cell death and death of the organism are synonymous. One way apoptosis could arise in unicellular organisms is through infection; apoptosis could be advantageous if a clonally identical group of such organisms acquired a way for group members to commit suicide before invading pathogens could proliferate and infect other members of the group.[76] What if such suicide were mediated by activation of cysteine proteases by simple, innate-immunity-like pattern-recognizing molecules—not unlike the NOD-like receptors—and what if one of the molecules they recognized was an iron-binding protein unique to metabolism in certain bacteria, a relative of what we now call cytochrome *c*? Now suppose that, untold eons later, a bacterium expressing that protein established a highly advantageous symbiotic relationship with an early eukaryotic cell, providing the metabolic efficiency needed for an eventual jump to multicellularity. And suppose that, as evolutionary pressures mandated targeted deletion of specific cells during development of increasingly complex organisms, the pathways of pathogen-mediated cell death were dusted off and reconfigured, but remained arranged around the one pathogen-like element present in each eukaryotic cell, the mitochondrion. Interesting though such speculation may be, it will remain nothing more than a fairy tale until further supporting biochemical information is gathered from phylogenically diverse organisms.

1. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol* 2008; **18**: 157–164.
2. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol Cell* 2002; **9**: 423–432.
3. Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM *et al.* A unified model for apical caspase activation. *Mol Cell* 2003; **11**: 529–541.
4. Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J Virol* 1993; **67**: 2168–2174.
5. Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 1997; **388**: 300–304.
6. Uren AG, Pakusch M, Hawkins CJ, Puls KL, Vaux DL. Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. *Proc Natl Acad Sci USA* 1996; **93**: 4974–4978.
7. Duckett CS, Nava VE, Gedrich RW, Clem RJ, Van Dongen JL, Gilfillan MC *et al.* A conserved family of cellular genes related to the baculovirus iap gene and encoding apoptosis inhibitors. *EMBO J* 1996; **15**: 2685–2694.
8. Harlin H, Reffey SB, Duckett CS, Lindsten T, Thompson CB. Characterization of XIAP-deficient mice. *Mol Cell Biol* 2001; **21**: 3604–3608.
9. Okada H, Suh WK, Jin J, Woo M, Du C, Elia A *et al.* Generation and characterization of Smac/DIABLO-deficient mice. *Mol Cell Biol* 2002; **22**: 3509–3517.
10. Martins LM, Morrison A, Klupsch K, Fedele V, Moisoi N, Teismann P *et al.* Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* 2004; **24**: 9848–9862.
11. Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU *et al.* IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell* 2007; **131**: 682–693.

12. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P *et al.* IAP antagonists induce autoubiquitination of c-IAPs NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell* 2007; **131**: 669–681.
13. Petersen SL, Wang L, Yalcin-Chin A, Li L, Peyton M, Minna J *et al.* Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* 2007; **12**: 445–456.
14. Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 1986; **44**: 817–829.
15. Lettre G, Hengartner MO. Developmental apoptosis in *C. elegans*: a complex CEDnario. *Nat Rev Mol Cell Biol* 2006; **7**: 97–108.
16. Parrish JZ, Xue D. Functional genomic analysis of apoptotic DNA degradation in *C. elegans*. *Mol Cell* 2003; **11**: 987–996.
17. Parrish JZ, Yang C, Shen B, Xue D. CRN-1, a Caenorhabditis elegans FEN-1 homologue, cooperates with CPS-6/EndoG to promote apoptotic DNA degradation. *EMBO J* 2003; **22**: 3451–3460.
18. Wang X, Wang J, Gengyo-Ando K, Gu L, Sun CL, Yang C *et al. C. elegans* mitochondrial factor WAH-1 promotes phosphatidylserine externalization in apoptotic cells through phospholipid scramblase SCRM-1. *Nat Cell Biol* 2007; **9**: 541–549.
19. Wang X, Yang C, Chai J, Shi Y, Xue D. Mechanisms of AIF-mediated apoptotic DNA degradation in *Caenorhabditis elegans*. *Science* 2002; **298**: 1587–1592.
20. Hu Y, Benedict MA, Wu D, Inohara N, Nunez G. Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. *Proc Natl Acad Sci USA* 1998; **95**: 4386–4391.
21. Inohara N, Gourley TS, Carrio R, Muniz M, Merino J, Garcia I *et al.* Diva a Bcl-2 homologue that binds directly to Apaf-1 and induces BH3-independent cell death. *J Biol Chem* 1998; **273**: 32479–32486.
22. Conus S, Rosse T, Borner C. Failure of Bcl-2 family members to interact with Apaf-1 in normal and apoptotic cells. *Cell Death Differ* 2000; **7**: 947–954.
23. Hausmann G, O'Reilly LA, van Driel R, Beaumont JG, Strasser A, Adams JM *et al.* Pro-apoptotic apoptosis protease-activating factor 1 (Apaf-1) has a cytoplasmic localization distinct from Bcl-2 or Bcl-x(L). *J Cell Biol* 2000; **149**: 623–634.
24. Moriishi K, Huang DC, Cory S, Adams JM. Bcl-2 family members do not inhibit apoptosis by binding the caspase activator Apaf-1. *Proc Natl Acad Sci USA* 1999; **96**: 9683–9688.
25. Vaux DL, Weissman IL, Kim SK. Prevention of programmed cell death in *Caenorhabditis elegans* by human bcl-2. *Science* 1992; **258**: 1955–1957.
26. Hengartner MO, Horvitz HR. *C. elegans* cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. *Cell* 1994; **76**: 665–676.
27. Jabbour AM, Puryer MA, Yu JY, Lithgow T, Riffkin CD, Ashley DM *et al.* Human Bcl-2 cannot directly inhibit the *Caenorhabditis elegans* Apaf-1 homologue CED-4, but can interact with EGL-1. *J Cell Sci* 2006; **119**: 2572–2582.
28. Hengartner MO, Ellis RE, Horvitz HR. *Caenorhabditis elegans* gene ced-9 protects cells from programmed cell death. *Nature* 1992; **356**: 494–499.
29. Hoppins S, Lackner L, Nunnari J. The machines that divide and fuse mitochondria. *Annu Rev Biochem* 2007; **76**: 751–780.
30. Neuspiel M, Zunino R, Gangaraju S, Rippstein P, McBride H. Activated mitofusin 2 signals mitochondrial fusion, interferes with Bax activation, and reduces susceptibility to radical induced depolarization. *J Biol Chem* 2005; **280**: 25060–25070.
31. Sugioka R, Shimizu S, Tsujimoto Y. Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J Biol Chem* 2004; **279**: 52726–52734.
32. Lee YJ, Jeong SY, Karbowski M, Smith CL, Youle RJ. Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell* 2004; **15**: 5001–5011.
33. Jagasia R, Grote P, Westermann B, Conradt B. DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* 2005; **433**: 754–760.
34. Delivani P, Adrain C, Taylor RC, Duriez PJ, Martin SJ. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol Cell* 2006; **21**: 761–773.
35. Vaux DL, Silke J. IAPs RINGs and ubiquitylation. *Nat Rev Mol Cell Biol* 2005; **6**: 287–297.
36. Kumar S, Doumanis J. The fly caspases. *Cell Death Differ* 2000; **7**: 1039–1044.
37. Kumar S. Caspase function in programmed cell death. *Cell Death Differ* 2007; **14**: 32–43.
38. Silke J, Verhagen AM, Ekert PG, Vaux DL. Sequence as well as functional similarity for DIABLO/Smac and Grim, Reaper and Hid? *Cell Death Differ* 2000; **7**: 1275.
39. Kumar S, Cakouros D. Transcriptional control of the core cell-death machinery. *Trends Biochem Sci* 2004; **29**: 193–199.
40. Wang SL, Hawkins CJ, Yoo SJ, Muller HA, Hay BA. The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 1999; **98**: 453–463.
41. Yu X, Wang L, Acehan D, Wang X, Akey CW. Three-dimensional structure of a double apoptosome formed by the *Drosophila* Apaf-1 related killer. *J Mol Biol* 2006; **355**: 577–589.
42. Dorstyn L, Mills K, Lazebnik Y, Kumar S. The two cytochrome *c* species DC3 and DC4, are not required for caspase activation and apoptosis in *Drosophila* cells. *J Cell Biol* 2004; **167**: 405–410.
43. Means JC, Muro I, Clem RJ. Lack of involvement of mitochondrial factors in caspase activation in a *Drosophila* cell-free system. *Cell Death Differ* 2006; **13**: 1222–1234.
44. Zimmermann KC, Ricci JE, Droin NM, Green DR. The role of ARK in stress-induced apoptosis in *Drosophila* cells. *J Cell Biol* 2002; **156**: 1077–1087.
45. Mendes CS, Arama E, Brown S, Scherr H, Srivastava M, Bergmann A *et al.* Cytochrome c-d regulates developmental apoptosis in the *Drosophila* retina. *EMBO Rep* 2006; **7**: 933–939.
46. Arama E, Bader M, Srivastava M, Bergmann A, Steller H. The two *Drosophila* cytochrome C proteins can function in both respiration and caspase activation. *EMBO J* 2006; **25**: 232–243.
47. Varkey J, Chen P, Jemmerson R, Abrams JM. Altered cytochrome *c* display precedes apoptotic cell death in *Drosophila*. *J Cell Biol* 1999; **144**: 701–710.
48. Walker DW, Benzer S. Mitochondrial 'swirls' induced by oxygen stress and in the *Drosophila* mutant hyperswirl. *Proc Natl Acad Sci USA* 2004; **101**: 10290–10295.
49. Colussi PA, Quinn LM, Huang DC, Coombe M, Read SH, Richardson H *et al.* Debcl a proapoptotic Bcl-2 homologue, is a component of the *Drosophila melanogaster* cell death machinery. *J Cell Biol* 2000; **148**: 703–714.
50. Quinn L, Coombe M, Mills K, Daish T, Colussi P, Kumar S *et al.* Buffy a *Drosophila* Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *EMBO J* 2003; **22**: 3568–3579.
51. Zhang H, Huang Q, Ke N, Matsuyama S, Hammock B, Godzik A *et al. Drosophila* pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanisms. *J Biol Chem* 2000; **275**: 27303–27306.
52. Igaki T, Kanuka H, Inohara N, Sawamoto K, Nunez G, Okano H *et al.* Drob-1, a *Drosophila* member of the Bcl-2/CED-9 family that promotes cell death. *Proc Natl Acad Sci USA* 2000; **97**: 662–667.
53. Sevrioukov EA, Burr J, Huang EW, Assi HH, Monserrate JP, Purves DC *et al. Drosophila* Bcl-2 proteins participate in stress-induced apoptosis, but are not required for normal development. *Genesis* 2007; **45**: 184–193.
54. Abdelwahid E, Yokokura T, Krieser RJ, Balasundaram S, Fowle WH, White K. Mitochondrial disruption in *Drosophila* apoptosis. *Dev Cell* 2007; **12**: 793–806.
55. Fannjiang Y, Cheng WC, Lee SJ, Qi B, Pevsner J, McCaffery JM *et al.* Mitochondrial fission proteins regulate programmed cell death in yeast. *Genes Dev* 2004; **18**: 2785–2797.
56. Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F *et al.* The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* 2001; **1**: 515–525.
57. Bourlat SJ, Juliusdottir T, Lowe CJ, Freeman R, Aronowicz J, Kirschner M *et al.* Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* 2006; **444**: 85–88.
58. Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC *et al.* The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* 2006; **314**: 941–952.
59. Robertson AJ, Croce J, Carbonneau S, Voronina E, Miranda E, McClay DR *et al.* The genomic underpinnings of apoptosis in *Strongylocentrotus purpuratus*. *Dev Biol* 2006; **300**: 321–334.
60. Sanchez Alvarado A. The freshwater planarian *Schmidtea mediterranea*: embryogenesis, stem cells and regeneration. *Curr Opin Genet Dev* 2003; **13**: 438–444.
61. Robb SM, Ross E, Alvarado AS. SmedGD: the *Schmidtea mediterranea* genome database. *Nucleic Acids Res* 2008; **36**: D599–D606.
62. Pankow S, Bamberger C. The p53 tumor suppressor-like protein nvp63 mediates selective germ cell death in the sea anemone *Nematostella vectensis*. *PLoS ONE* 2007; **2**: e782.
63. Chipuk JE, Green DR. Dissecting p53-dependent apoptosis. *Cell Death Differ* 2006; **13**: 994–1002.
64. Sullivan JC, Ryan JF, Watson JA, Webb J, Mullikin JC, Rokhsar D *et al.* StellaBase: the *Nematostella vectensis* genomics database. *Nucleic Acids Res* 2006; **34**: D495–D499.
65. Eckhart L, Fischer H, Tschachler E. Phylogenomics of caspase-activated DNA fragmentation factor. *Biochem Biophys Res Commun* 2007; **356**: 293–299.
66. Zmasek CM, Zhang Q, Ye Y, Godzik A. Surprising complexity of the ancestral apoptosis network. *Genome Biol* 2007; **8**: R226.
67. Wiens M, Krasko A, Blumbach B, Muller IM, Muller WE. Increased expression of the potential proapoptotic molecule DD2 and increased synthesis of leukotriene B4 during allograft rejection in a marine sponge. *Cell Death Differ* 2000; **7**: 461–469.
68. Wiens M, Krasko A, Perovic S, Muller WE. Caspase-mediated apoptosis in sponges: cloning and function of the phylogenetic oldest apoptotic proteases from Metazoa. *Biochim Biophys Acta* 2003; **1593**: 179–189.
69. Tepsuporn S, Kaltenbach JC, Kuhns WJ, Burger MM, Fernandez-Busquets X. Apoptosis in Microciona prolifera Allografts. *Biol Bull* 2003; **205**: 199–201.
70. Wiens M, Krasko A, Muller CI, Muller WE. Molecular evolution of apoptotic pathways: cloning of key domains from sponges (Bcl-2 homology domains and death domains) and their phylogenetic relationships. *J Mol Evol* 2000; **50**: 520–531.
71. Wiens M, Diehl-Seifert B, Muller WE. Sponge Bcl-2 homologous protein (BHP2-GC) confers distinct stress resistance to human HEK-293 cells. *Cell Death Differ* 2001; **8**: 887–898.
72. Wiens M, Belikov SI, Kaluzhnaya OV, Schroder HC, Hamer B, Perovic-Ottstadt S *et al.* Axial (apical-basal) expression of pro-apoptotic and pro-survival genes in the lake baikal demosponge Lubomirskia baicalensis. *DNA Cell Biol* 2006; **25**: 152–164.
73. Bayascas JR, Yuste VJ, Benito E, Garcia-Fernandez J, Comella JX. Isolation of AmphiCASP-3/7, an ancestral caspase from amphioxus (*Branchiostoma floridae*). Evolutionary considerations for vertebrate caspases. *Cell Death Differ* 2002; **9**: 1078–1089.
74. Ciana P, Vegeto E, Beato M, Chambon P, Gustafsson JA, Parker M *et al.* Looking at nuclear receptors from the heights of Erice. Workshop on nuclear receptor structure and function. *EMBO Rep* 2002; **3**: 125–129.
75. Takada N, Yamaguchi H, Shida K, Terajima D, Satou Y, Kasuya A *et al.* The cell death machinery controlled by Bax and Bcl-XL is evolutionarily conserved in *Ciona intestinalis*. *Apoptosis* 2005; **10**: 1211–1220.
76. James ER, Green DR. Infection and the origins of apoptosis. *Cell Death Differ* 2002; **9**: 355–357.