DEVELOPMENTAL CELL BIOLOGY

Developmental apoptosis in *C. elegans*: a complex CEDnario

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Abstract | Apoptosis, an evolutionarily conserved programme of cellular self-destruction, is essential for the development and survival of most multicellular animals. It is required to ensure functional organ architecture and to maintain tissue homeostasis. During development of the simple nematode *Caenorhabditis elegans*, apoptosis claims over 10% of the somatic cells that are generated — these cells were healthy but unnecessary. Exciting insights into the regulation and execution of apoptosis in *C. elegans* have recently been made. These new findings will undoubtedly influence our perception of developmental apoptosis in more complex species, including humans.

Autophagy
A pathway for the recycling of cellular contents, in which materials inside the cell are packaged into vesicles and are then targeted to the vacuole or lysosome for bulk turnover.

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A tight balance between cell division and cell death is a fundamental principal of metazoan development: morphogenetic programmes are often characterized by high rates of cell proliferation, which are soon followed by waves of cell death (reviewed in REF. 1). Elimination of the webbing between digits in humans and mice, and the deletion of mammary tissue in males are good examples of this pattern. Developmental cell death can also have homeostatic functions, or can be used to eliminate aberrant, damaged or harmful cells (reviewed in REFS 2–5). Although several forms of cell death have been described in multicellular organisms[3], for the purpose of this review we will focus exclusively on apoptosis, a gene-directed cellular self-destruction programme that generally serves biologically meaningful functions. Apoptotic cells usually present characteristic morphological changes, including chromatin condensation and DNA laddering, loss of mitochondrial-membrane potential and of plasma-membrane phospholipid asymmetry, and detachment from the cellular matrix[6–8]. Apoptosis is an important, but not the sole, form of 'programmed' cell death that occurs during metazoan development. For example, recent studies have shown that autophagy can also contribute to developmental cell death (reviewed in REF. 9).

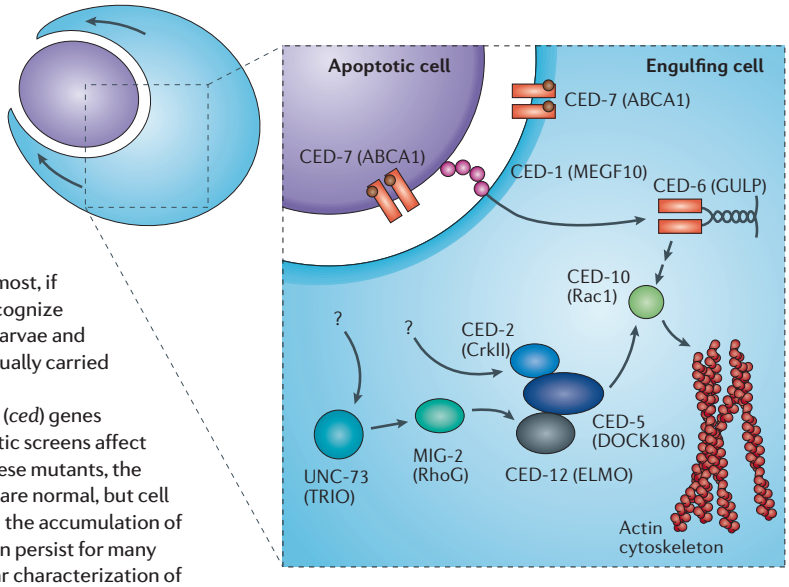
Developmental apoptosis has been studied in all the main model systems of developmental biology, including the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse *Mus musculus*. Knowledge that was gained from these model organisms greatly contributed to our knowledge of apoptosis. For example, pioneering work in *C. elegans* established that apoptotic cell death is under genetic control and that this molecular programme is conserved throughout evolution[10,11]. Here, we focus our attention once again on this small invertebrate, and review some of the most exciting recent findings concerning the regulation and execution of developmental apoptosis in *C. elegans*. We hope that lessons drawn from *C. elegans* can guide, or at least inspire, apoptosis research in other systems. As dysregulation of apoptosis is associated with several human pathologies, such as cancer and neurodegenerative disorders, a better understanding of developmental apoptosis in *C. elegans* could also have interesting prognostic and therapeutic implications.

In the following sections, we will discuss in more detail the various molecules that control apoptosis during *C. elegans* development. We begin with a description of the *C. elegans* core apoptotic machinery, and present a molecular model of how apoptosis is triggered in *C. elegans*. We then discuss the complexity of the regulation of developmental apoptosis in *C. elegans*, using three cell types as examples. Finally, we highlight some pertinent, but still unanswered, questions concerning apoptosis in this organism. Engulfment of apoptotic cells and apoptotic DNA degradation, topics that each constitute a field of their own, will not be covered extensively here (see BOX 1 and REFS 12–14).

Apoptosis during development

The development of *C. elegans* is invariant: reproducible patterns of cell division, cell death and cell migration give rise to animals with exactly the same number of cells and an invariant anatomy. Apoptotic cell death is a common cell fate in *C. elegans*[15–17]: out of the 1,090 cells that are generated to form an adult hermaphrodite, exactly

Box 1 | **Apoptotic cell-corpse clearance**



Dying cells in *Caenorhabditis elegans* are rapidly removed from the scene by neighbouring cells, who engulf (eat) them and subsequently degrade them within phagolysosomes (FIG. 2). Unlike mammals, which have macrophages, there are no specialized 'death eaters' in *C. elegans*: during development, most, if not all, cells have the ability to recognize and engulf apoptotic corpses; in larvae and adults, cell-corpse clearance is usually carried out by larger epithelial cells.

Many of the *cell death abnormal* (*ced*) genes that have been identified in genetic screens affect the engulfment step (FIG. 2). In these mutants, the pattern and kinetics of cell death are normal, but cell clearance is impaired, resulting in the accumulation of 'long-lived cell corpses', which can persist for many hours, or even days. The molecular characterization of these engulfment genes identified two partially redundant signal-transduction pathways (see figure). The first pathway includes the guanine nucleotide-exchange factor (GEF) heterodimer CED-5–CED-12 and its upstream regulators CED-2, abnormal cell migration (MIG)-2 and uncoordinated (UNC)-73. The second pathway leads from the cell surface to its interior via the putative receptor protein CED-1 and its adaptor protein CED-6. The latter pathway also contains an ABC transporter protein, CED-7. The substrate, or substrates, that are transported by CED-7 and their role in cell cleareance are unknown at present. The two pathways cooperate to activate the small GTPase CED-10 within the engulfing cell[103] (for a recent review of the engulfment pathway in *C. elegans*, see REF. 104). CED-10 activation, in turn, promotes the cytoskeletal rearrangements that are required for the successful ingestion of such a large 'prey'. Still missing in the nematode corpse-clearance pathway are the signal, or signals, that mark a cell for removal. It is also surprising that not a single negative regulator of engulfment has been described so far. More sophisticated genetic screens might allow identification of these missing links in the future.

Importantly, the engulfment machinery that has been identified in *C. elegans* is highly conserved throughout evolution (TABLE 1). All *C. elegans* engulfment genes that have been identified so far have homologues in humans and, in most cases, there is clear evidence that these human homologues also promote the clearance of apoptotic cells. So, the engulfment pathway that has been characterized in *C. elegans* must be of ancient origin, and can be used to better understand how our own dying cells are removed. The mammalian homologues of the *C. elegans* proteins are indicated in parentheses in the diagram. The figure is adapted, with permission, from REF. 105 © (2001) Elsevier. ABCA1, ATP-binding cassette A1; ELMO, engulfment and cell-motility protein; GULP, engulfment adapter protein; MEGF10, multiple epidermal-growth-factor-like motifs-10.

131 cells — and always the same ones — undergo apoptosis. Most of the cells that die during early development in *C. elegans* are neurons, although several hypodermal, muscle and pharyngeal cells also suffer the same fate.

Apoptotic cells in *C. elegans*, much like their counterparts in mammals, follow a series of morphological changes that clearly distinguish them from healthy cells[7,15]. When observed by light microscopy, the transition from life to death in *C. elegans* culminates in the appearance of highly refractile button-like disks, which are rapidly recognized and removed by neighbouring cells (FIG. 1a)[18]. Three waves of death characterize developmental apoptosis in *C. elegans* (FIG. 1b). The first one occurs between 250 and 450 minutes after fertilization and removes almost a fifth (113/628) of the cells that are generated during embryonic development[17]. The second wave of death is observed in the larval stage L2 and removes some of the newly generated neurons. No apoptotic cell death occurs in the soma after the L2 stage. The third wave of death is observed in the adult hermaphrodite germ line, where about half of all potential oocytes are removed by apoptosis (BOX 2)[19].

The apoptotic pathway in *C. elegans*

Over the past 25 years, the genetic dissection of programmed cell death in *C. elegans* has led to the identification of >20 cell-death genes (TABLE 1). These genes have been placed into a genetic pathway, which includes four distinct steps. First, a cell decides to die. In a second step, it kills itself in a cell-autonomous fashion. The dead cell is subsequently recognized and engulfed by a neighbouring cell. Finally, the engulfed cell is degraded[10,18,20–22] (FIG. 2). Surprisingly, genes that are involved in engulfment and apoptotic DNA degradation have been shown to also contribute to the killing process. So, the cell-death pathway in *C. elegans* might not be as strictly linear as is indicated in FIG. 2 (see below)[23–26].

Phagolysosome
A cellular body that is formed by the union of a phagosome or ingested particle with a lysosome that contains hydrolytic enzymes.

1.5-fold stage
A *C. elegans* developmental stage that occurs 420–460 minutes after the first cleavage at 20 °C. The cell number remains at ~560 cells, with some new cells generated and some cells going through programmed cell death. The shape of the embryo is elongated and folds back on itself by ~50%.

Caspase family
A family of Cys proteases that cleave after Asp residues. Initiator caspases are typically activated in response to particular stimuli, whereas effector caspases are particularly important for the ordered dismantling of vital cellular structures.

Apoptosome
In mammalian cells, the apoptosome is a complex that forms when cytochrome *c* is released from mitochondria and interacts with the cytosolic protein APAF1, which, in turn, recruits pro-caspase-9. In the presence of ATP, this interaction results in the allosteric activation of caspase-9 and in the formation of a caspase-3-activation complex.

Bcl-2 family
Anti- and pro-apoptotic proteins that, in mammals, control mitochondrial-membrane permeabilization, a key event in apoptosis. The members of this family share characteristic domains of homology known as Bcl-2 homology (BH) domains.

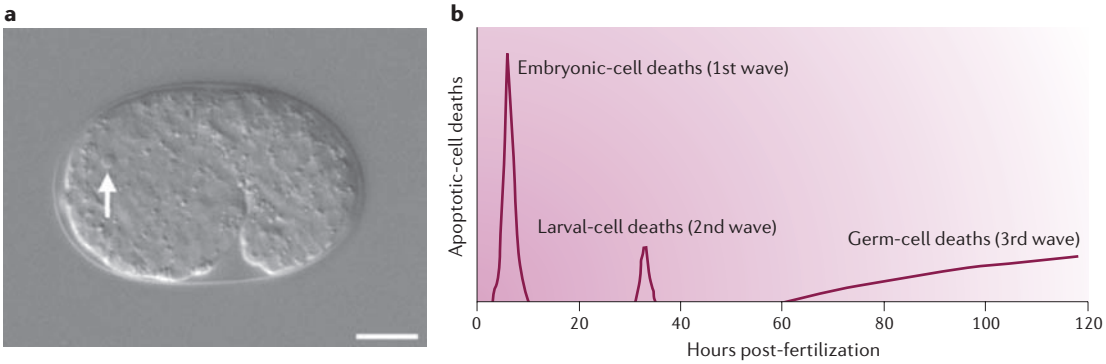


Figure 1 | **Developmental apoptosis in *C. elegans*. a** | Somatic cell death in *Caenorhabditis elegans*. The image shows a wild-type embryo at an early 1.5-fold stage, as visualized using Nomarski optics. The white arrow indicates a highly refractile apoptotic cell. Scale bar, 10 µm. **b** | Waves of apoptotic cell death during *C. elegans* development. Apoptosis is first observed in the embryo 250–450 minutes after fertilization, when it claims 113 cells. At the larval stage L2, 18 somatic cells undergo apoptosis. Finally, in the adult hermaphrodite, roughly half the developing germ cells die by apoptosis. The graph illustrates the number of apoptotic cells that can be observed by light microscopy at any given time during the development of a *C. elegans* hermaphrodite.

***The essential four*: ced-3, ced-4, ced-9 *and* egl-1.** Genetic screens for mutants with abnormal cell-death patterns identified four genes that regulate all somatic cell deaths in *C. elegans*: *cell death abnormal (ced)-3*, *ced-4*, *ced-9* and *egg-laying defective (egl)-1*. Loss-of-function (lf) mutations in *ced-3*, *ced-4* and *egl-1* result in the survival of almost all (~131) doomed cells, which indicates that these three genes have pro-apoptotic functions[10,20]. By contrast, *ced-9* has anti-apoptotic activity because a *ced-9* gain-of-function (gf) mutation blocks apoptosis, whereas *ced-9*(lf) mutants die during early development due to excessive cell death[22]. Epistasis studies have allowed the ordering of these genes in a pathway, with *egl-1* acting as a negative regulator of *ced-9*, and *ced-9*, in turn, negatively regulating *ced-4* and *ced-3* (REF. 27) (FIG. 2).

The molecular identification of *ced-3*, *ced-4*, *ced-9* and *egl-1* in the 1990s provided important clues to understand how the apoptotic machinery is engaged, not only in *C. elegans*, but also in other species. Indeed, all four genes code for conserved apoptotic regulators. *ced-3* encodes a protease of the caspase family — a group of enzymes pivotal for their role in the execution of apoptosis[28–31]. Whereas several-hundred caspase substrates have already been described in mammals[32], CED-3 substrates are still largely elusive. CED-4 is an adaptor protein that is similar to mammalian apoptotic protease-activating factor-1 (APAF1)[33]. Like their mammalian homologues, CED-3 and CED-4 oligomerize to form an apoptosome-like complex, and this interaction is required for CED-3 activation and apoptosis[34,35].

ced-9 and *egl-1* encode members of the Bcl-2 family: CED-9 is an anti-apoptotic protein with four Bcl-2 homology (BH) domains, whereas EGL-1 is a pro-apoptotic BH3-only-domain protein[36–38]. In mammals, the Bcl-2 family includes pro- and anti-apoptotic proteins, which all share at least one BH domain; these proteins have key roles in the control of apoptosis at the level of mitochondria (see below, and reviewed in REFS 39,40). The *C. elegans* genome encodes only three Bcl-2-family members: CED-9, EGL-1 and the BH3-only-domain protein CED-13. In contrast to EGL-1, CED-13 does not have a role in developmental apoptosis in *C. elegans*; its only known function so far is a modest role in promoting germ-cell death after DNA damage[41] (BOX 2).

Molecular model of apoptosis activation

The execution of apoptosis in *C. elegans* is regulated through a series of direct protein–protein interactions between the four main components described above (CED-3, CED-4, CED-9 and EGL-1). The immediate cause of death in *C. elegans* is the proteolytic cleavage of CED-3 from its precursor form (proCED-3) into the processed, active enzyme. For this activation to occur, several proCED-3 molecules must associate with a CED-4 tetramer to form a large oligomeric complex known as the *C. elegans* apoptosome. In the absence of apoptotic stimuli, however, CED-4 does not exist as a tetramer, but, rather, as a dimer that

Box 2 | **Germ-cell apoptosis**

In the mammalian female ovaries, apoptosis claims more than 99.9% of the potential germ cells. Although most germ-cell deaths take place before birth, apoptosis continues to decimate the number of oocytes during juvenile and adult life. Once the supply of oocytes has been exhausted through ovulation and apoptosis, the ovaries senesce, leading to infertility and, in humans, to the menopause (reviewed in REFS 106,107).

Physiological (developmental) germ-cell death also occurs during oogenesis in *Caenorhabditis elegans* hermaphrodites (but not in male gonads); it increases with the age of the animals — claiming on average ~50% of the developing oocytes — and, contrary to somatic apoptosis in *C. elegans*, is not cell-lineage dependent[19]. Physiological germline apoptosis requires cell death abnormal (CED)-4 and CED-3, and is blocked by CED-9. However, it is not dependent on egg-laying defective (EGL)-1, nor on any other Bcl-2 homology (BH)3-domain protein that has been tested so far. As in mammals, the reason why so many apparently healthy germ cells succumb to apoptosis is unknown. One possibility is that germline apoptosis has a homeostatic function, whereby it eliminates excess germ cells that function as nurse cells to provide nutrients to maturing oocytes.

Finally, germ-cell apoptosis can also be induced ectopically in *C. elegans* hermaphrodites by agents that cause DNA damage, or by pathogen infections as part of the *C. elegans* innate immune response[86,102,108,109].

is sequestered on the outer surface of mitochondria through a direct protein–protein interaction with mitochondria-bound CED-9 (FIG. 3)[42–45]. This sequestration (and possibly other limitations) prevents CED-4 from assembling with, and activating, CED-3. In the 131 cells that are destined to die, developmental cues lead to the expression of the pro-apoptotic EGL-1 protein. EGL-1 binds through its BH3 domain to CED-9 and induces a conformational change that disrupts the CED-4–CED-9 interaction. Once released, CED-4 translocates from mitochondria to the outer surface of the nuclear membrane. Released CED-4 is now also free to interact with CED-3 and activate apoptosis (FIG. 3)[34,38,46–49].

Table 1 | **Proteins with a function in apoptosis in *C. elegans***

***C. elegans* protein**	**Description**	***D. melanogaster* homologue***	**Mammalian homologue***
Decision			
CES-1	C2H2-type zinc-finger transcription factor (Snail family)	Scratch	Slug
CES-2	bZIP transcription factor	PAR-domain protein-1	D-site-binding protein
HLH-2	bHLH transcription factor (Daughterless-like)	Daughterless	Transcription factor E2-α
HLH-3	bHLH transcription factor (Achaete-scute-like)	Asense	Achaete-scute homologue-1
TRA-1	Zinc-finger protein	Cubitus interruptus	GLI3
EOR-1	BTB/zinc-finger transcription factor	Meiotic central spindle	PLZF
EOR-2	New	CG17233	KIAA1205
Execution			
CED-3	Caspase	Drice	Caspase-3/Caspase-9
CED-4	Adaptor protein	Ark	APAF1
CED-9	Anti-apoptotic Bcl-2-like protein	Buffy	BCL2
EGL-1 (CED-13)	Pro-apoptotic BH3-only-domain protein	?	BH3-domain protein
Engulfment			
CED-1	Transmembrane protein	Draper	MEGF10
CED-2	SH2- and SH3-containing adaptor protein	Crk	CrkII
CED-5	Scaffolding protein	Myoblast city	DOCK180
CED-6	Adaptor protein	Ced-6	GULP
CED-7	ABC transporter	CG1718	ABCA1
CED-10	Small GTPase, Rac type	Rac1	Rac1
CED-12	PH-domain protein	Ced-12	ELMO
MIG-2	Small GTPase, Rho type	Mig-2-like	RhoG
UNC-73	Guanine nucleotide-exchange factor	Trio	TRIO
NEX-1	Calcium-dependent phospholipid-binding protein	Annexin B11	Annexin A13
PSR-1	Phosphatidylserine receptor	Psr	PSR
DNA degradation			
NUC-1	Deoxyribonuclease	DNaseII	DNaseII
CPS-6	Mitochondrial endonuclease	CG8862	EndoG
WAH-1	Oxidoreductase	CG7263	AIF
CRN-1	5′ → 3′ exonuclease	Fen1	FEN1
Others			
CED-8	Membrane transporter	CG32579	XK
ICD-1	Mitochondrial protein	Enhancer of Bicaudal	βNAC

*Homologues are defined as the best reciprocal BLAST hit and do not imply conservation of functions. ABC, ATP-binding cassette; AIF, apoptosis-inducing factor; APAF1, apoptotic protease-activating factor-1; Bcl-2, B-cell lymphoma-2; BH3, Bcl-2-homology domain-3; bHLH, basic helix–loop–helix; BTB, 'Broad complex, Tramtrack, Bric-a-brac' domain; bZIP, basic-region leucine zipper; *C. elegans*, *Caenorhabditis elegans*; CES, cell-death selection abnormal; CED, cell death abnormal; CPS-6, CED-3 protease suppressor-6; CRN, cell-death-related nuclease; *D. melanogaster*, *Drosophila melanogaster*; EGL-1, egg-laying defective-1; ELMO, engulfment and cell-motility protein; EndoG, endonuclease G; EOR, egl-1 suppressor/DiO uptake defective/raf enhancer; Fen1, Flap endonuclease-1; GULP, engulfment adapter protein; HLH2/3, helix–loop–helix protein-2/3; ICD-1, inhibitor of cell death-1; MEGF10, multiple epidermal-growth-factor-like motifs-10; MIG-2, abnormal cell migration-2; βNAC, β-subunit of the nascent-polypeptide-associated complex; NEX-1, annexin-family member-1; NUC-1, abnormal nuclease-1; PH domain, pleckstrin-homology domain; PLZF, promyelocytic leukaemia zinc-finger protein; PSR-1, phosphatidylserine receptor-1; SH2/3, Src-homology-2/3 domain; TRA-1, transformer-1; UNC-73, uncoordinated-73; WAH-1, worm AIF homologue-1; XK, Kell blood-group precursor.

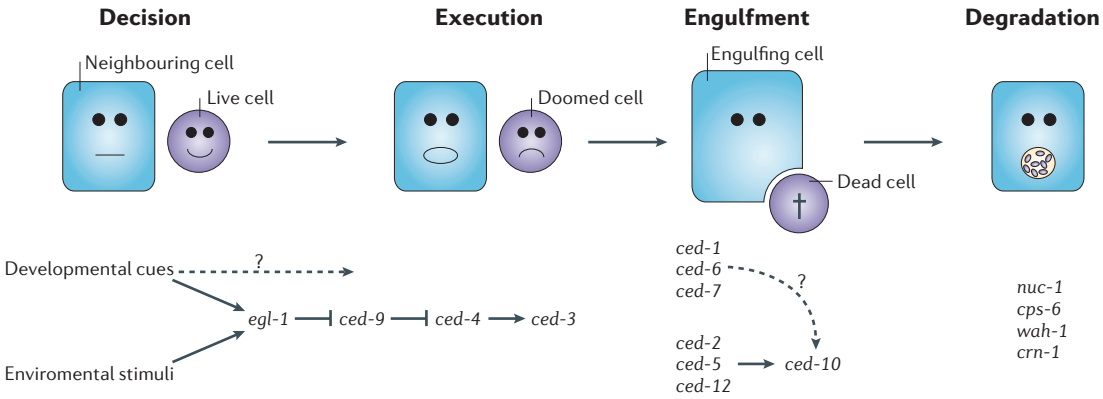


Figure 2 | **The genetic pathway for programmed cell death in *C. elegans*.** The induction of *egg-laying defective* (*egl*)*-1* transcription, either by developmental cues or external signals, is key in the decision of a cell to undergo apoptosis in *C. elegans*. The *cell death abnormal* (*ced*)*-9* gene normally blocks apoptosis by inhibiting *ced-4* activity. However, once activated, *egl-1* inhibits the anti-apoptotic gene *ced-9*, thereby allowing the pro-apoptotic genes *ced-4* and *ced-3* to interact and execute cell suicide. The dead cell is then recognized and engulfed by a neighbouring cell: apoptotic-corpse clearance requires the participation of two partially redundant genetic pathways (*ced-1–ced-6–ced-7 and ced-2–ced-5–ced-12*) that converge at the level of *ced-10*. Finally, several genes (*abnormal nuclease-1* (*nuc-1*), *CED-3 protease suppressor-6* (*cps-6*), *worm AIF homologue-1* (*wah-1*) and *cell-death-related nuclease-1* (*crn-1*)) encode proteins with nuclease activity that participate in the degradation of the dead cell within the engulfing cell (see TABLE 1 for a molecular description of the genes involved in apoptosis in *C. elegans*). Because it is still unknown how genes that activate or repress the physiological germ-cell-death pathway interact with the death machinery, we did not include this pathway in the diagram. Environmental stimuli represent all external signals (for example, γ-irradiation) that can induce *egl-1*-dependent germ-cell death. Positive (→) and negative (⟞) genetic interactions are shown. Dashed lines represent indirect interactions.

***A structural view of apoptosis activation in* C. elegans.** Almost all of the protein complexes that are postulated to be involved in the activation of CED-3 have now been crystallized by Shi and colleagues, which allows a detailed view of the mechanism of apoptosis activation in *C. elegans*. For example, the structure of the CED-9–EGL-1 complex revealed how EGL-1 disrupts the CED-4–CED-9 interaction[50]. The authors found that CED-9 uses two different non-overlapping surface areas to bind to EGL-1 and CED-4. In the presence of EGL-1, CED-9 undergoes a series of conformational changes to create a hydrophobic surface cleft that can accommodate the extended BH3 α-helix of EGL-1. At the same time, these conformational changes induce a rearrangement of the CED-4-binding surface on CED-9, which is likely to destabilize the CED-4–CED-9 interaction and allow the release of CED-4. Finally, the crystal structure also explains why the original *ced-9*(gf) mutation blocks apoptosis[22]. This mutation changes a small Gly residue to a bulkier Glu residue in the EGL-1-binding pocket of CED-9. This substitution interferes with EGL-1 binding to CED-9, but does not affect the CED-4–CED-9-interaction surface. As a consequence, the mutated CED-9(G169E) protein constitutively binds to CED-4 with high affinity, and CED-3 is never activated, irrespective of whether EGL-1 is present or not[36,50,51].

In a second study[52], the same group recently reported the crystal structure of the CED-9–CED-4 complex. Unexpectedly, the authors found that CED-4 binds to CED-9 as an asymmetric dimer, with CED-9 interacting with only one of the two CED-4 molecules. Gel-filtration experiments and electron micrographs also showed that after their release from CED-9, two CED-4 dimers come together to form a tetramer. This tetramer seems to be the mature form, which can now interact with, and facilitate, CED-3 autoactivation (FIG. 3). The CED-4 tetramer provides an interesting counterpoint to the proposed structure of the mammalian APAF1 apoptosome, which has been suggested to be a heptamer. It is, of course, possible that several conformations of these protein complexes are allowed. For example, biochemical data reported in a recent publication by Colman and co-workers[53] are more consistent with a 2:2 stoichiometry for the CED-9–CED-4 complex than with the 2:1 stoichiometry found by Shi and colleagues.

***Mitochondria and apoptosis in* C. elegans.** Mitochondria have an important role in the regulation of apoptosis in mammals. In the mitochondrial pathway (also known as the intrinsic pathway), active Bax or Bak proteins bind to the outer mitochondrial membrane and induce its permeabilization, through a process that is still controversial. As a result of this permeabilization, a battery of pro-apoptotic proteins, which are normally corralled inside the mitochondrial intermembrane space, are now given free access to the cytosol. The most well-known of this group of escapees is the electron carrier cytochrome *c*, which interacts with cytosolic APAF1 to form, together with caspase-9, the mammalian apoptosome[54].

In *C. elegans*, two lines of evidence argue against cytochrome *c* having a role in apoptosome formation.

Bcl-2 homology (BH) domains
Sequence analysis of the proteins in the Bcl-2 family has identified four BH domains (BH1–BH4). These domains contribute at several levels to the function of the members of the Bcl-2 family in cell death and survival.

BH3-only-domain protein
Sequence alignment among the Bcl-2-family proteins has identified four Bcl-2 homology (BH) domains, BH1–BH4. The BH3-only members contain a single BH3 domain and are pro-apoptotic.

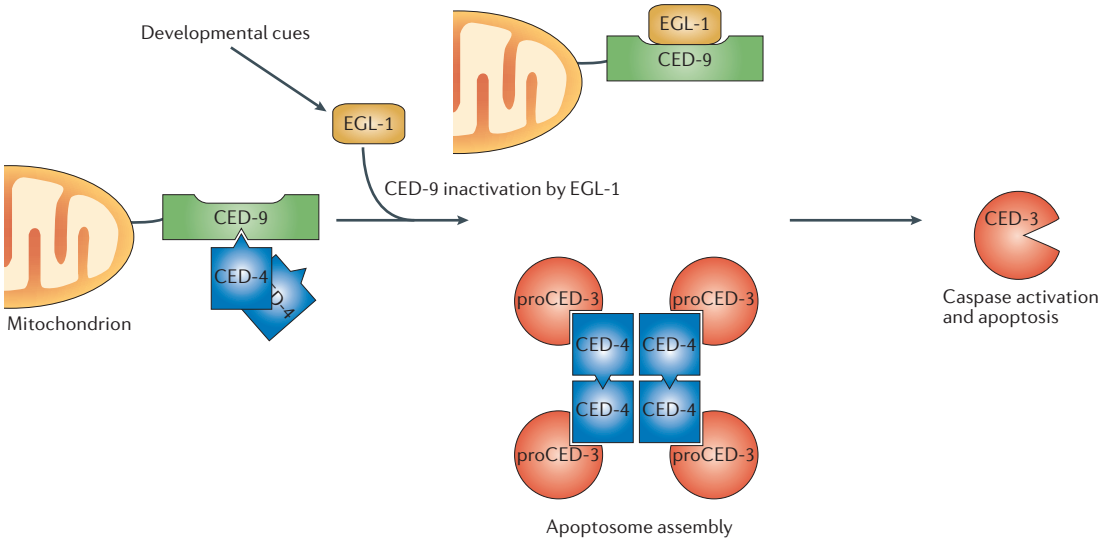


Figure 3 | **Molecular model of apoptosis activation in *C. elegans*.** In living cells, cell death abnormal (CED)-4 dimers are sequestered by CED-9 on the outer surface of mitochondria and are maintained in an inactive conformation. Cells that are destined to die by apoptosis produce the BH3-domain protein egg-laying defective (EGL)-1. Binding of pro-apoptotic EGL-1 to CED-9 induces a conformational change in the latter, resulting in the release of the CED-4 dimer from the CED-9–CED-4 complex. Once freed from the inhibitory interaction with CED-9, two CED-4 dimers associate into a tetramer and recruit proCED-3 molecules to form the so-called apoptosome. CED-3 becomes activated (through conformational change and/or proteolysis), and apoptosis is triggered.

First, unlike APAF1, CED-4 lacks a WD40 domain that binds to cytochrome *c*[55,56]. Second, regulated CED-3 activation can be obtained *in vitro* using only purified CED-3, CED-4, CED-9 and EGL-1 proteins[52]. Two other mitochondrial proteins have, however, been reported to participate in *C. elegans* apoptosis. After apoptosis activation by EGL-1, mitochondrial CPS-6 (CED-3 protease suppressor-6) and WAH-1 (worm AIF homologue-1) — which are the *C. elegans* homologues of mammalian endonuclease G and oxidoreductase apoptosis-inducing factor (AIF), respectively — are released from mitochondria, and cooperate to promote DNA degradation in the dying cell[25,26]. Interestingly, the authors of these studies also presented results that indicate that CPS-6 and WAH-1 synergize to induce cell killing. This observation implies that apoptotic DNA degradation, usually referred to as the final step of the *C. elegans* apoptosis pathway (FIG. 2), can influence cell-death execution. Indeed, decreased apoptosis was also reported by the same group following RNA interference (RNAi) knockdown of an eclectic group of DNases, RNases and other proteins (cell-death-related nucleases, CRNs)[57]. It is possible that DNA damage or aberrant RNA processing can function as pro-apoptotic signals under these conditions, and cooperate with developmental signals to enhance cell killing. It must be pointed out, however, that the effects on apoptosis that have been observed in animals that lacked *cps-6*, *wah-1* or any of the *crn* genes were rather weak. So, whether the changes in cell-death patterns that were observed in these animals are indeed the results of a direct effect on the apoptotic process remains to be confirmed.

Recent work from Conradt and colleagues further supported the idea that mitochondria are involved in apoptosis activation in *C. elegans*[58]. In mammals, mitochondria not only release pro-apoptotic proteins, but also fragment during apoptosis. The significance of this event is still under debate: mitochondrial fragmentation could be either a contributing cause, or a consequence of outer-membrane permeabilization (reviewed in REFS 59,60). Conradt and colleagues used live microscopy to show that mitochondrial fragmentation also occurs in cells that are undergoing apoptosis in *C. elegans*[58]. Mitochondrial fragmentation was dependent on EGL-1, but did not require CED-4 or CED-3, which indicates that it is an early event in the apoptotic programme. Surprisingly, however, both *ced-9*(lf) and *ced-9*(gf) mutations blocked mitochondrial fission. This unexpected finding could be explained if CED-9, in addition to sequestering CED-4 in healthy cells, also promoted mitochondrial fragmentation during cell killing. This hypothesis is indeed consistent with earlier genetic observations that suggested that CED-9 can adopt a pro-apoptotic role in dying cells[36].

To determine whether mitochondrial fragmentation contributes to, or is a consequence of apoptosis activation in *C. elegans*, the authors generated transgenic animals that expressed either a wild-type or a dominant-negative form of *dynamin-related protein-1* (*drp-1*), a gene that is required for mitochondrial fission[61]. Overexpression of wild-type *drp-1* increased mitochondrial fission and resulted in increased cell loss, whereas dominant-negative DRP-1 reduced mitochondrial fragmentation and blocked up to 20% of the normally occurring cell deaths. Although modest, these results hint, for the first time, that mitochondrial fission has a causal role in the activation of apoptosis in *C. elegans*. How fission of this organelle induces apoptosis in *C. elegans* remains

WD40 domain
A 40-amino-acid-long protein motif that contains a WD dipeptide at its carboxyl terminus. This domain is found in many functionally diverse proteins and mediates protein–protein interactions.

Mitochondrial fission
Mitochondrial division is a protein-driven process that regulates, with mitochondrial fusion, the dynamics of the mitochondrial network in mammalian cells. Recent evidence suggests that mitochondrial fragmentation might also have an active role in apoptosis.

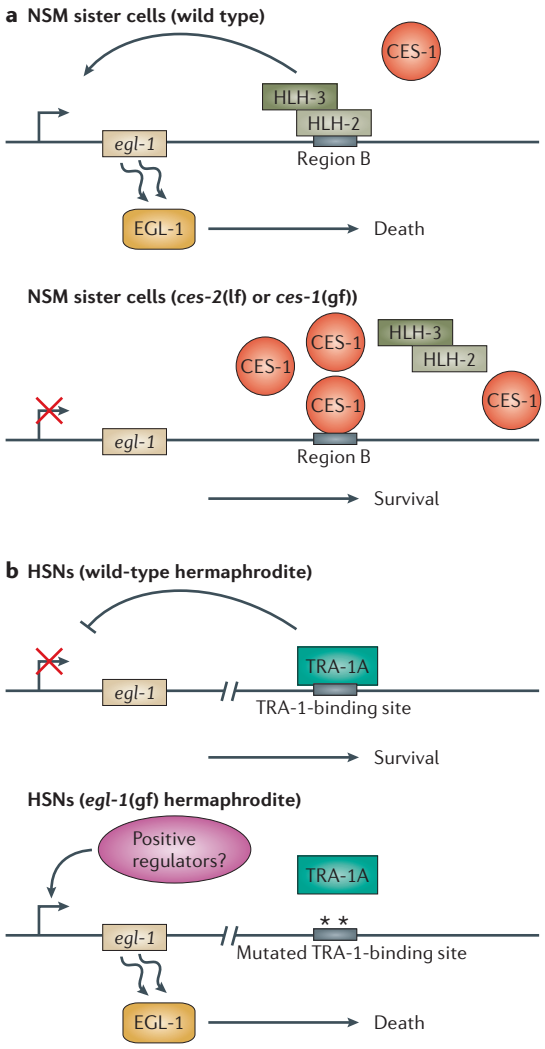


Figure 4 | **Transcriptional regulation of the pro-apoptotic gene *egl-1*. a** | In the two sister cells of the neurosecretory motor (NSM) neurons of wild-type *Caenorhabditis elegans*, heterodimers of the transcription factors helix–loop–helix protein (HLH)-2 and HLH-3 bind to region B of the egg-laying defective (*egl-1*) locus and induce its transcription. EGL-1 can then activate the apoptotic pathway and trigger the death of these cells. *cell-death selection abnormal* (*ces*)*-1* encodes a DNA-binding protein that can also specifically bind region B of the *egl-1* locus. In mutants that have a high level of CES-1 protein — for example, *C. elegans* with *ces-1*(gf) or *ces-2*(lf) mutations (where lf and gf stand for loss of function and gain of function, respectively) — CES-1 can compete with HLH-2–HLH-3 for binding to region B, and therefore block *egl-1* induction. As a result, the NSM sister cells survive. **b** | In wild-type hermaphrodites, the hermaphrodite-specific neurons (HSNs) survive because the induction of pro-apoptotic *egl-1* is blocked by the transcriptional repressor TRA-1A, which binds to a TRA-1-binding site that is downstream of the *egl-1* locus. However, in *egl-1*(gf) mutants, disruption of the TRA-1-binding site prevents TRA-1A binding. Transcription of *egl-1* is induced (by as-yet-unknown positive regulators) and apoptosis is activated — as a result, the HSNs die.

Baculovirus IAP repeat (BIR) domain
Cysteine-based motif of ~65 amino acids. Inhibitors of apoptosis (IAPs) contain several BIR domains.

RING-finger domain
A protein domain that consists of two loops that are held together at their base by Cys and His residues, which form a complex with two zinc ions. Many RING fingers function in protein degradation by facilitating protein ubiquitylation.

unexplained. Does mitochondrial fission induce the release of pro-apoptotic proteins? Does it modulate the activity of the known cell-death proteins? Biochemical approaches are now called for to address this issue[60].

Apoptosis inhibition in *C. elegans*

In *D. melanogaster*, but also to a lesser extent in mammals, caspases are maintained in an inactive state through their interaction with inhibitor of apoptosis (IAP) proteins (reviewed in REF. 62). These proteins, which all contain at least one baculovirus IAP repeat (BIR) domain, interact with the catalytic site of caspases and inactivate their protease activity[63]. However, not all BIR-domain-containing proteins can block apoptosis. For example, members of the survivin/BIRC5 subfamily have a BIR fold but do not function in caspase regulation; instead, they are involved in cytokinesis[64]. Many IAP proteins also contain a second motif — the RING-finger domain. Genetic experiments in *D. melanogaster* showed that this domain is important for the anti-apoptotic activity of the protein *in vivo* (reviewed in REF. 62). RING domains are implicated in protein ubiquitylation, a modification that often results in the degradation of the ubiquitin-tagged protein by the proteosome. Accordingly, it has been shown that the RING finger of *D. melanogaster* DIAP1 promotes the ubiquitylation of the caspase Dronc *in vivo*, therefore targeting the caspase for degradation[65]. The anti-apoptotic activity of IAPs is antagonized in *D. melanogaster* by the RHG proteins (Reaper, HID and GRIM, as well as Sickle and JAFRAC2), and in mammals by second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI (SMAC/DIABLO), high-temperature-requirement protein A2 (HtrA2)/OMI and G1-to-S-phase transition-1 (GSPT1)/eRF3 (reviewed in REF. 66).

C. elegans contains only two proteins with BIR motifs, and neither seems to be implicated in the regulation of apoptosis. As is the case in other species, the survivin-family member *bir-1* is required for cytokinesis: the elimination of BIR-1 activity by RNAi leads to embryonic lethality owing to inaccurate chromosome segregation during mitosis, but has no effect on developmental apoptosis[67,68]. Overexpression and RNAi knockdown experiments with the second family member, *bir-2*, also failed to detect any effect on apoptosis[67]. What other function, if any, *bir-2* has in *C. elegans* is still unknown. In conclusion, no caspase inhibitors have so far been identified in *C. elegans*, but comparisons with other protease systems strongly suggest that at least one such inhibitor should exist. It is possible that this putative inhibitor has only a limited role in the regulation of apoptosis, and has therefore avoided detection in the genetic screens that have been carried out so far.

Even after over two decades of genetic screens, new candidate apoptotic proteins are still regularly discovered. One such candidate, *inhibitor of cell death-1* (*icd-1*), was recently found in a screen for genes that are implicated in early embryogenesis in *C. elegans*[69]. The authors discovered that reducing ICD-1 activity by RNAi results in one of two possible phenotypes: either early arrest during embryogenesis or an accumulation of

cell-corpse-like bodies in embryos that developed past the first embryonic stages. Further characterization of *icd-1*(RNAi) animals suggested that various cell types, but especially neurons, undergo apoptosis. Importantly, the authors also showed that *icd-1* overexpression can block apoptosis. The most unexpected finding of this study came when Bloss *et al.* performed epistasis analysis to determine whether the core apoptotic machinery is required for *icd-1*(RNAi)-induced programmed cell death. They found that, in older embryos and in larvae, loss of *ced-4* function, but not loss of *ced-3* function, could suppress the increased number of dying cells in *icd-1*(RNAi) animals. This result would imply that ICD-1 suppresses a CED-4-dependent, CED-3-independent apoptotic programme, therefore indicating a second pro-apoptotic function for CED-4, besides its activation of CED-3. ICD-1 is homologous to the β-subunit of the nascent-polypeptide-associated complex (βNAC), which might regulate protein localization during translation[70]. Like βNAC, ICD-1 localizes to mitochondria.

It remains unclear how ICD-1 blocks apoptosis in *C. elegans*. The protein does not have a BIR fold, but Bloss and colleagues reported that it contains a putative caspase-cleavage site and a caspase-recruitment domain (CARD). Therefore, it might inhibit apoptosis by negatively regulating the caspase CED-3 through titration, or the CARD-containing protein CED-4 through sequestration. However, the fact that *icd-1*(RNAi)-induced apoptosis still occurs in the absence of CED-3 argues against such a model. Another possibility is that the phenotype is nonspecific: ICD-1, like its homologue βNAC, might be important for several aspects of mitochondrial function, and its absence could negatively affect this organelle. For example, ICD-1 elimination could interfere with the anti-apoptotic activity of the mitochondria-bound CED-9. But that would only explain half the story, leaving open the question concerning the CED-3 independence of the phenotype. It is also worth mentioning that an independent group failed to detect any anti-apoptotic activity for *icd-1* using standard cell-death assays in *C. elegans*[58]. Efforts from our group were similarly negative (G.L. and M.O.H., unpublished observations). Clearly, more experiments are needed to determine whether ICD-1 is indeed a direct regulator of apoptosis.

The Müller group recently suggested that the inactivation of *bec-1* — the *C. elegans* homologue of the autophagy gene *beclin* — triggers apoptotic cell death[71]. Interestingly, the authors showed that BEC-1 could interact physically with CED-9 (a similar interaction had previously been reported for mammalian beclin and BCL2). This interaction could provide an exciting, if tentative, molecular link between these two important cell-death pathways. Alternatively, as proposed above for the *crn* genes, it is also possible that the loss of *bec-1* simply interferes with developmental programmes and that the observed increase in apoptosis (or decrease in clearance) is indirect. A detailed analysis of the relevance of the BEC-1–CED-9 interaction might shed light on this matter.

How to kill a *C. elegans* cell: upregulate *egl-1*

To ensure that only the necessary cells are purged during development, the apoptotic machinery must be tightly controlled. The level of biological complexity that is involved in the regulation of developmental apoptosis has hampered its study, and even now we are still ignorant of most of the developmental cues that trigger apoptosis in metazoans. For example, it has been known for several years that the steroid hormone ecdysone activates developmental cell death during metamorphosis in *D. melanogaster* by upregulating the pro-apoptotic genes *reaper* and *hid*, and downregulating the IAP gene *diap1* (REF. 72). But the signals that coordinate these ecdysone pulses in *D. melanogaster* have yet to be identified. The situation is not better in *C. elegans*: we know which cells die, we know when they die, we even know how they die (as a result of the inactivation of CED-9 by EGL-1), but for only 4 of the 131 doomed cells do we know, at least in part, how EGL-1 is activated.

***Transcriptional regulation of* egl-1.** The two sister cells of the neurosecretory motor (NSM) neurons — two serotonergic neurosecretory motor neurons that are located in the animal's pharynx — undergo apoptosis in wild-type *C. elegans*; these deaths depend on the *C. elegans* core apoptotic machinery (*egl-1*, *ced-9*, *ced-4* and *ced-3*), but also on two other genes: *cell-death selection abnormal (ces)-1* and *ces-2*. Mutations that cause a gain of *ces-1* function, or a loss of *ces-2* function, promote survival of the NSM sister cells, without affecting most other cell deaths[73]. *ces-1* and *ces-2* encode a member of the snail/slug family of zinc-finger transcription factors, and a basic-region leucine zipper (bZIP) transcription factor, respectively[74–76]. Epistasis analysis showed that *ces-2* functions upstream of *ces-1*: in *ces-1*(lf) *ces-2*(lf) double mutants, the NSM sister cells die, which implies that *ces-2* causes the death of NSM sister cells by inhibiting the pro-survival activity of *ces-1*.

Recently, elegant genetic and biochemical experiments have established the molecular link between the *ces* genes and *egl-1* transcription[77]. The authors identified two basic helix–loop–helix (bHLH) transcription factors, HLH-2 and HLH-3, which are expressed in the NSM sister cells and are required for their death. In the NSM sister cells of wild-type animals, HLH-2–HLH-3 heterodimers activate *egl-1* transcription by binding to a regulatory sequence, known as region B, in the *egl-1* promoter (FIG. 4a). Interestingly, CES-1 can also bind to region B, which indicates a competition model between HLH-2–HLH-3 and CES-1 for region B of the *egl-1* locus. According to this scenario, the increased levels of CES-1 protein that are found in *ces-1*(gf) or *ces-2*(lf) mutants prevents the death of the NSM sister cells, because CES-1 can successfully compete with HLH-2–HLH-3 for region B, and therefore block *egl-1* transcription (FIG. 4a).

Interestingly, two mammalian proteins that are functionally similar to *C. elegans* CES-2 and CES-1 — the oncogenic E2A-HLF protein and slug (which is encoded by *SNAI2*), respectively — function in an anti-apoptotic transcriptional pathway that is important for the survival

Epistasis analysis
Epistasis is the masking of a phenotype that is caused by a mutation in one gene by a mutation in another gene. Epistasis analysis can therefore be used to dissect the order in which the genes in a genetic pathway act.

Caspase-recruitment domain
(CARD). A conserved domain that is found in c-IAP1 and c-IAP2. The function of the domain in these molecules is unknown at present.

Serotonergic
Describes a nerve that functions by the release of serotonin from the nerve endings.

Zinc-finger transcription factors
Zinc-finger domains are found in numerous nucleic-acid-binding proteins. The zinc-finger motif is an unusually small, self-folding domain in which zinc is a crucial component of its tertiary structure.

Basic-region leucine zipper
(bZIP). A basic leucine-zipper motif that is often associated with transcription factors. Dimerization through the leucine zipper is required for DNA binding. Hetero- or homodimers lend further complexity to the regulation of transcription by members of this family, which include Fos, Jun and the cAMP-responsive-element-binding protein/activating transcription factor (CREB/ATF) family members.

Basic helix–loop–helix (bHLH) transcription factor
A protein that contains two α-helices separated by a loop (the HLH domain), which binds DNA in a sequence-specific manner.

of lymphoid and other haematopoietic cells (reviewed in REFS 76,78). Wu, Look and co-workers recently showed that *SNAI2*, which encodes slug, is upregulated by p53 when haematopoietic progenitor cells are exposed to genotoxic treatments, and that slug protects these cells from undergoing apoptosis by interfering with the expression of another p53 target gene, the pro-apoptotic BH3-only gene *BBC3*, which encodes puma[79]. This model is strikingly similar to the situation in the NSM sister cells in *C. elegans*, where excess CES-1 blocks the expression of BH3-only *egl-1*. These observations indicate that at least some of the pathways that regulate developmental apoptosis in *C. elegans* are also conserved in mammals, and might have a role in some human pathologies (for example, leukaemia).

The life-versus-death decision of the two hermaphrodite-specific neurons (HSNs) has also been studied in great detail. These serotonergic motor neurons are required for egg-laying in the hermaphrodite. On the other hand, they do not have any use in males and therefore normally undergo apoptosis. Genetic screens for mutations that affect egg-laying led to the isolation of several independent dominant gain-of-function mutations in *egl-1*. In these mutants, the HSNs also undergo apoptotic cell death in hermaphrodites, which results in the egg-laying defect (Egl) phenotype, which EGL-1 was named after[20,38]. These mutations were all found to disrupt a binding site in the *egl-1* locus for the transcriptional repressor transformer (TRA)-1A, thereby causing ectopic *egl-1* expression and HSN death[80] (FIG. 4b). Indeed, TRA-1A, the most downstream factor in the *C. elegans* sex-determination pathway, is normally expressed and active only in hermaphrodites. The use of TRA-1A as a direct regulator of *egl-1* transcription allows the nematode to control sexually dimorphic cell death, such as that of the HSN neurons, efficiently.

To identify positive regulators of *egl-1* expression in the HSNs, Hoeppner and colleagues screened for suppressors of the Egl phenotype of *egl-1*(gf) *C. elegans* mutants[81]. They found mutations in two genes, *egl-1 suppressor/DiO uptake defective/raf enhancer (eor)-1* and *eor-2*, that only block apoptosis in the HSNs. *eor-1* encodes a putative transcription factor that is similar to human promyelocytic leukaemia zinc-finger protein (PLZF), whereas *eor-2* encodes a novel, but evolutionarily conserved, protein. Further characterization of the *eor* genes revealed that they not only regulate apoptosis of the HSN neurons, but that they are also required for many cell-differentiation and cell-fate decisions in the *C. elegans* nervous system. How exactly *eor-1* and *eor-2* genetically interact with the core apoptotic machinery to promote cell death in the HSNs is unknown. Given that EOR-1 is probably a transcription factor, an attractive model would be that, in the HSNs, EOR-1 and EOR-2 regulate expression of *egl-1*, or of genes that act in parallel or further downstream. The pleiotropic defects that have been observed in *eor* mutants indicate that these genes presumably also control the expression of many other neuronal target genes.

egl-1-dependent and -independent cell death in the germ line. Apoptosis is also common during germline development: >50% of the differentiating oocytes in *C. elegans* adult hermaphrodites undergo apoptotic suicide. These deaths have been suggested to be required for germline homeostasis[19] (BOX 2). These developmental or 'physiological' cell deaths are mediated by the common core apoptotic machinery: they require *ced-4* and *ced-3*, and are antagonized by *ced-9*. They are, however, *egl-1* independent, which hints at the existence of a distinct physiological germ-cell-death pathway. Loss-of-function mutations in several genes can result in a dramatic increase in the extent of germ-cell apoptosis. Most of these genes are also required for other aspects of germline development; so, it is not clear whether their effect on apoptosis is direct or indirect (E. Kritikou *et al.*, unpublished observations)[82–85].

Unlike somatic cells, germ cells in *C. elegans* can also undergo apoptosis following bacterial infection or in response to genotoxic stress. Interestingly, at least in the latter case, *egl-1* has an important role in activating the death pathway. Therefore, as in mammals, *C. elegans* germ cells possess several upstream pathways that lead to the activation of a common execution programme.

Understanding how *C. elegans* responds to DNA damage has become a very active field of research over the past few years. Forward- and reverse-genetic screens have identified many conserved genes, including CEP-1 and BRC-1, the *C. elegans* homologues of the tumour suppressors p53 and BRCA1 (breast cancer-1), respectively[86–95]. Although many pieces of the puzzle are still missing, the outline of how DNA damage induces germ-cell apoptosis seems simple: depending on the type of damage, specific upstream sensor proteins are triggered. These, in turn, activate CEP-1, which then transcriptionally upregulates *egl-1* expression. If this model holds true, it suggests that the situation in *C. elegans* germ cells is very similar to the (over-simplified) scenario that occurs in mammalian cells after DNA damage, where p53 promotes apoptosis (at least in part) through increased transcription of the genes that encode the pro-apoptotic BH3-only proteins noxa and puma[96,97].

Some outstanding questions

An emerging theme in the field of apoptosis research is that processes that were thought to occur late in the death process, namely engulfment and apoptotic DNA degradation, can contribute to cell killing. For example, Reddien *et al.*[23] and Hoeppner *et al.*[24] showed that mutations that block the clearance of apoptotic cells can also promote cell survival, particularly when pro-apoptotic signals are relatively weak and many cells hover between life and death. In other words, interfering with the removal of dead corpses can convince cells not to die in the first place. A similar effect has been observed in mammalian cells[98], and recent results in *C. elegans* support similar conclusions for genes that are involved in DNA degradation[25,26].

At first glance, this might seem counter-intuitive: why would genes that are involved in 'cleaning up' after completion of the apoptotic programme be required for

its proper execution? One possibility is that engulfment and apoptotic DNA degradation ensure that there is no possibility of turning back once a cell has taken the path to death. Indeed, eating a cell or degrading its genome would seem like good measures to prevent its 'resurrection'. But how does it work? How does the engulfment machinery, or how do the apoptotic nucleases, 'know' when to 'attack' a cell that is not dead yet? In the absence of experimental data, we can only propose that early signals, perhaps released during mitochondrial fission or processed by CED-3, function cell autonomously to initiate DNA degradation and non-autonomously to activate engulfment prior to death. The identification of these signals and the investigation of their mechanisms of action should be amenable to genetic studies in *C. elegans*.

When Sydney Brenner chose *C. elegans* as a model system to study developmental biology, one of his goals was to identify all the developmental cues that are required for cell-fate determination. Looking at how we understand the regulation of developmental apoptosis in *C. elegans*, it is obvious that we are not there yet, and that we might actually never reach that level of understanding. Indeed, we know how *egl-1* is activated in only four of the 131 cells that undergo apoptosis during *C. elegans* development (the NSM sister cells and the HSNs). But, in fact, even in these four cells, our knowledge is only partial. For example, we ignore what specifically activates HLH-2–HLH-3 in the NSM sister cells, and which factors drive expression of *egl-1* in the male HSNs. There are certainly many more genes that influence cell-specific apoptosis in *C. elegans*, but genetic screens to identify them could be burdensome and might be unsuccessful due to unexpected pleiotropic phenotypes (for example, lethality). It is possible that biochemical analysis of the *egl-1* regulatory sequences will be a more fruitful approach to identify candidate apoptotic regulators. The prospect that every cell type uses a different transcriptional cascade to induce *egl-1* hints at how complex the regulation of developmental apoptosis can be, even in a simple animal with a stereotyped lineage such as *C. elegans*.

Apoptosis is important for the proper development of most multicellular species: *D. melanogaster* or *M. musculus* that carry mutations that block cell death usually die during early development[99,100]. But, curiously, *C. elegans* does not seem to require apoptosis to ensure its proper development: mutants with null mutations in *ced-3* or *ced-4* are viable and fertile. So why does *C. elegans* maintain a non-essential process? Of course, non-essential does not mean useless. Indeed, *ced-3* and *ced-4* mutants seem less fit than the wild type: they grow more slowly (possibly due to having more cells to feed), have smaller brood size (possibly because of disrupted germline homeostasis), and fail to eliminate damaged germ cells following genotoxic stress. Another very interesting use for apoptosis in *C. elegans* has emerged from the study of its innate immune system (reviewed in REF. 101). Abbalay and colleagues noticed that *ced-3* and *ced-4* mutants are hyper-susceptible to *Salmonella enterica* serovar Typhimurium infections[102]. *C. elegans* is a soil nematode, and is therefore likely to encounter pathogens in its native environment. It is possible that apoptosis is needed in *C. elegans* to remove cells that have harmful effects during infection — for example, by obstructing organs and tissues that are essential for the *C. elegans* response to pathogen invasion. Alternatively, the activation of CED-3 might promote an innate immune response, as is the case for several caspase-family members in mammals. The investigation of these hypotheses will first require the identification of the responses that are mounted by *C. elegans* to fight infections, and of the different cell types involved — topics of high interest in several research groups around the globe. Only then could we assess how the apoptotic programme contributes to innate immunity in *C. elegans*.

Concluding remarks

Apoptosis, like cell proliferation, is an integral part of the development of most multicellular organisms. Its study in *C. elegans* has led to the identification of several key components of an apoptotic cascade, a cascade that is now known to be conserved throughout evolution. But the story does not end there. Several recent discoveries, as briefly reviewed above, highlight how the study of cell death in *C. elegans* will continue to generate new ideas and hypotheses in the field of apoptosis research. And the future is bright. The ability to combine powerful classic and reverse genetics with state-of-the-art genomics and proteomics approaches to investigate apoptosis in *C. elegans* can only promise even more remarkable discoveries. We have learned a lot by studying developmental apoptosis in *C. elegans*, yet it is obvious that *C. elegans* still has plenty to teach us about why and how it occurs. Not bad at all for a tiny little worm…

1. Hipfner, D. R. & Cohen, S. M. Connecting proliferation and apoptosis in development and disease. *Nature Rev. Mol. Cell Biol.* **5**, 805–815 (2004).
2. Jacobson, M. D., Weil, M. & Raff, M. C. Programmed cell death in animal development. *Cell* **88**, 347–354 (1997).
3. Vaux, D. L. & Korsmeyer, S. J. Cell death in development. *Cell* **96**, 245–254 (1999).
4. Meier, P., Finch, A. & Evan, G. Apoptosis in development. *Nature* **407**, 796–801 (2000).
5. Baehrecke, E. H. How death shapes life during development. *Nature Rev. Mol. Cell Biol.* **3**, 779–787 (2002).
6. Kerr, J. F., Wyllie, A. H. & Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239–257 (1972).
7. Wyllie, A. H., Kerr, J. F. & Currie, A. R. Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68**, 251–306 (1980).
8. Wyllie, A. H. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* **284**, 555–556 (1980).
9. Baehrecke, E. H. Autophagy: dual roles in life and death? *Nature Rev. Mol. Cell Biol.* **6**, 505–510 (2005).
10. Ellis, H. M. & Horvitz, H. R. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817–829 (1986).
This landmark paper describes the genetic regulation of apoptosis in *C. elegans*.
11. Metzstein, M. M., Stanfield, G. M. & Horvitz, H. R. Genetics of programmed cell death in *C. elegans*: past, present and future. *Trends Genet.* **14**, 410–416 (1998).
12. Grimsley, C. & Ravichandran, K. S. Cues for apoptotic cell engulfment: eat-me, don't eat-me and come-get-me signals. *Trends Cell Biol.* **13**, 648–656 (2003).
13. Reddien, P. W. & Horvitz, H. R. The engulfment process of programmed cell death in *Caenorhabditis elegans*. *Annu. Rev. Cell Dev. Biol.* **20**, 193–221 (2004).
14. Nagata, S. DNA degradation in development and programmed cell death. *Annu. Rev. Immunol.* **23**, 853–875 (2005).

15. Sulston, J. E. & Horvitz, H. R. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110–156 (1977).
16. Kimble, J. & Hirsh, D. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **70**, 396–417 (1979).
17. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64–119 (1983).
18. Hedgecock, E. M., Sulston, J. E. & Thomson, J. N. Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* **220**, 1277–1279 (1983).
19. Gumienny, T. L., Lambie, E., Hartwieg, E., Horvitz, H. R. & Hengartner, M. O. Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. *Development* **126**, 1011–1022 (1999).
20. Desai, C., Garriga, G., McIntire, S. L. & Horvitz, H. R. A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. *Nature* **336**, 638–646 (1988).
21. Ellis, R. E., Jacobson, D. M. & Horvitz, H. R. Genes required for the engulfment of cell corpses during programmed cell death in *Caenorhabditis elegans*. *Genetics* **129**, 79–94 (1991).
22. Hengartner, M. O., Ellis, R. E. & Horvitz, H. R. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* **356**, 494–499 (1992).
23. Reddien, P. W., Cameron, S. & Horvitz, H. R. Phagocytosis promotes programmed cell death in *C. elegans*. *Nature* **412**, 198–202 (2001).
24. Hoeppner, D. J., Hengartner, M. O. & Schnabel, R. Engulfment genes cooperate with *ced-3* to promote cell death in *Caenorhabditis elegans*. *Nature* **412**, 202–206 (2001).
25. Parrish, J. *et al*. Mitochondrial endonuclease G is important for apoptosis in *C. elegans*. *Nature* **412**, 90–94 (2001).
26. Wang, X., Yang, C., Chai, J., Shi, Y. & Xue, D. Mechanisms of AIF-mediated apoptotic DNA degradation in *Caenorhabditis elegans*. *Science* **298**, 1587–1592 (2002).
References 23–26 present interesting evidence that engulfment or DNA degradation of apoptotic cells also contribute to cell killing.
27. Shaham, S. & Horvitz, H. R. Developing *Caenorhabditis elegans* neurons may contain both cell-death protective and killer activities. *Genes Dev.* **10**, 578–591 (1996).
28. Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M. & Horvitz, H. R. The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* **75**, 641–652 (1993).
29. Xue, D. & Horvitz, H. R. Inhibition of the *Caenorhabditis elegans* cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. *Nature* **377**, 248–251 (1995).
30. Xue, D., Shaham, S. & Horvitz, H. R. The *Caenorhabditis elegans* cell-death protein CED-3 is a cysteine protease with substrate specificities similar to those of the human CPP32 protease. *Genes Dev.* **10**, 1073–1083 (1996).
31. Riedl, S. J. & Shi, Y. Molecular mechanisms of caspase regulation during apoptosis. *Nature Rev. Mol. Cell Biol.* **5**, 897–907 (2004).
32. Earnshaw, W. C., Martins, L. M. & Kaufmann, S. H. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem.* **68**, 383–424 (1999).
33. Yuan, J. & Horvitz, H. R. The *Caenorhabditis elegans* cell death gene *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* **116**, 309–320 (1992).
34. Yang, X., Chang, H. Y. & Baltimore, D. Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. *Science* **281**, 1355–1357 (1998).
35. Rodriguez, J. & Lazebnik, Y. Caspase-9 and APAF-1 form an active holoenzyme. *Genes Dev.* **13**, 3179–3184 (1999).
36. Hengartner, M. O. & Horvitz, H. R. Activation of *C. elegans* cell death protein CED-9 by an amino-acid substitution in a domain conserved in Bcl-2. *Nature* **369**, 318–320 (1994).
37. Hengartner, M. O. & Horvitz, H. R. *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* **76**, 665–676 (1994).
38. Conradt, B. & Horvitz, H. R. The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* **93**, 519–529 (1998).
39. Cory, S., Huang, D. C. & Adams, J. M. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* **22**, 8590–8607 (2003).
40. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
41. Schumacher, B. *et al*. *C. elegans ced-13* can promote apoptosis and is induced in response to DNA damage. *Cell Death Differ.* **12**, 153–161 (2005).
42. James, C., Gschmeissner, S., Fraser, A. & Evan, G. I. CED-4 induces chromatin condensation in *Schizosaccharomyces pombe* and is inhibited by direct physical association with CED-9. *Curr. Biol.* **7**, 246–252 (1997).
43. Spector, M. S., Desnoyers, S., Hoeppner, D. J. & Hengartner, M. O. Interaction between the *C. elegans* cell-death regulators CED-9 and CED-4. *Nature* **385**, 653–656 (1997).
44. Wu, D., Wallen, H. D. & Nunez, G. Interaction and regulation of subcellular localization of CED-4 by CED-9. *Science* **275**, 1126–1129 (1997).
45. Wu, D., Wallen, H. D., Inohara, N. & Nunez, G. Interaction and regulation of the *Caenorhabditis elegans* death protease CED-3 by CED-4 and CED-9. *J. Biol. Chem.* **272**, 21449–21454 (1997).
46. del Peso, L., Gonzalez, V. M. & Nunez, G. *Caenorhabditis elegans* EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation. *J. Biol. Chem.* **273**, 33495–33500 (1998).
47. del Peso, L., Gonzalez, V. M., Inohara, N., Ellis, R. E. & Nunez, G. Disruption of the CED-9–CED-4 complex by EGL-1 is a critical step for programmed cell death in *Caenorhabditis elegans*. *J. Biol. Chem.* **275**, 27205–27211 (2000).
48. Chen, F. *et al*. Translocation of *C. elegans* CED-4 to nuclear membranes during programmed cell death. *Science* **287**, 1485–1489 (2000).
49. Seshagiri, S. & Miller, L. K. *Caenorhabditis elegans* CED-4 stimulates CED-3 processing and CED-3-induced apoptosis. *Curr. Biol.* **7**, 455–460 (1997).
50. Yan, N. *et al*. Structural, biochemical, and functional analyses of CED-9 recognition by the proapoptotic proteins EGL-1 and CED-4. *Mol. Cell* **15**, 999–1006 (2004).
51. Parrish, J., Metters, H., Chen, L. & Xue, D. Demonstration of the *in vivo* interaction of key cell death regulators by structure-based design of second-site suppressors. *Proc. Natl Acad. Sci. USA* **97**, 11916–11921 (2000).
52. Yan, N. *et al*. Structure of the CED-4–CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* **437**, 831–837 (2005).
References 50 and 52 provide a detailed molecular mechanism of apoptosis activation in *C. elegans*.
53. Fairlie, W. D. *et al*. CED-4 forms a 2:2 heterotetrameric complex with CED-9 until specifically displaced by EGL-1 or CED-13. *Cell Death Differ.* 16 Sept 2005 (doi:10.1038/sj.cdd.4401762).
54. Li, P. *et al*. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91**, 479–489 (1997).
55. Hu, Y., Ding, L., Spencer, D. M. & Nunez, G. WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation. *J. Biol. Chem.* **273**, 33489–33494 (1998).
56. Srinivasula, S. M., Ahmad, M., Fernandes-Alnemri, T. & Alnemri, E. S. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell* **1**, 949–957 (1998).
57. Parrish, J. Z. & Xue, D. Functional genomic analysis of apoptotic DNA degradation in *C. elegans*. *Mol. Cell* **11**, 987–996 (2003).
58. Jagasia, R., Grote, P., Westermann, B. & Conradt, B. DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature* **433**, 754–760 (2005).
Demonstrates, for the first time, a causal role for mitochondria in apoptotic cell death in *C. elegans*.
59. Karbowski, M. & Youle, R. J. Dynamics of mitochondrial morphology in healthy cells and during apoptosis. *Cell Death Differ.* **10**, 870–880 (2003).
60. Youle, R. J. & Karbowski, M. Mitochondrial fission in apoptosis. *Nature Rev. Mol. Cell Biol.* **6**, 657–663 (2005).
61. Labrousse, A. M., Zappaterra, M. D., Rube, D. A. & van der Bliek, A. M. *C. elegans* dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. *Mol. Cell* **4**, 815–826 (1999).
62. Vaux, D. L. & Silke, J. IAPs, RINGs and ubiquitylation. *Nature Rev. Mol. Cell Biol.* **6**, 287–297 (2005).
63. Hinds, M. G., Norton, R. S., Vaux, D. L. & Day, C. L. Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeat. *Nature Struct. Biol.* **6**, 648–651 (1999).
64. Silke, J. & Vaux, D. L. Two kinds of BIR-containing protein — inhibitors of apoptosis, or required for mitosis. *J. Cell Sci.* **114**, 1821–1827 (2001).
65. Wilson, R. *et al*. The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nature Cell Biol.* **4**, 445–450 (2002).
66. Bergmann, A., Yang, A. Y. & Srivastava, M. Regulators of IAP function: coming to grips with the grim reaper. *Curr. Opin. Cell Biol.* **15**, 717–724 (2003).
67. Fraser, A. G., James, C., Evan, G. I. & Hengartner, M. O. *Caenorhabditis elegans* inhibitor of apoptosis protein (IAP) homologue BIR-1 plays a conserved role in cytokinesis. *Curr. Biol.* **9**, 292–301 (1999).
68. Speliotes, E. K., Uren, A., Vaux, D. & Horvitz, H. R. The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Mol. Cell* **6**, 211–223 (2000).
69. Bloss, T. A., Witze, E. S. & Rothman, J. H. Suppression of CED-3-independent apoptosis by mitochondrial βNAC in *Caenorhabditis elegans*. *Nature* **424**, 1066–1071 (2003).
70. Rospert, S., Dubaquie, Y. & Gautschi, M. Nascent-polypeptide-associated complex. *Cell. Mol. Life Sci.* **59**, 1632–1639 (2002).
71. Takacs-Vellai, K. *et al*. Inactivation of the autophagy gene *bec-1* triggers apoptotic cell death in *C. elegans*. *Curr. Biol.* **15**, 1513–1517 (2005).
72. Yin, V. P. & Thummel, C. S. A balance between the *diap1* death inhibitor and *reaper* and *hid* death inducers controls steroid-triggered cell death in *Drosophila*. *Proc. Natl Acad. Sci. USA* **101**, 8022–8027 (2004).
73. Ellis, R. E. & Horvitz, H. R. Two *C. elegans* genes control the programmed deaths of specific cells in the pharynx. *Development* **112**, 591–603 (1991).
74. Metzstein, M. M., Hengartner, M. O., Tsung, N., Ellis, R. E. & Horvitz, H. R. Transcriptional regulator of programmed cell death encoded by *Caenorhabditis elegans* gene *ces-2*. *Nature* **382**, 545–547 (1996).
75. Metzstein, M. M. & Horvitz, H. R. The *C. elegans* cell death specification gene *ces-1* encodes a snail family zinc finger protein. *Mol. Cell* **4**, 309–319 (1999).
76. Nieto, M. A. The snail superfamily of zinc-finger transcription factors. *Nature Rev. Mol. Cell Biol.* **3**, 155–166 (2002).
77. Thellmann, M., Hatzold, J. & Conradt, B. The Snail-like CES-1 protein of *C. elegans* can block the expression of the BH3-only cell-death activator gene *egl-1* by antagonizing the function of bHLH proteins. *Development* **130**, 4057–4071 (2003).
Convincing genetic and biochemical work that describes a transcriptional pathway that regulates apoptosis in the two sister cells of the NSM neurons in *C. elegans*.
78. Seidel, M. G. & Look, A. T. E2A–HLF usurps control of evolutionarily conserved survival pathways. *Oncogene* **20**, 5718–5725 (2001).
79. Wu, W. S. *et al*. Slug antagonizes p53-mediated apoptosis of hematopoietic progenitors by repressing puma. *Cell* **123**, 641–653 (2005).
Recent publication that supports the idea that some of the pathways that regulate apoptosis are conserved from *C. elegans* to mammals.
80. Conradt, B. & Horvitz, H. R. The TRA-1A sex determination protein of *C. elegans* regulates sexually dimorphic cell deaths by repressing the *egl-1* cell death activator gene. *Cell* **98**, 317–327 (1999).
81. Hoeppner, D. J. *et al*. *eor-1* and *eor-2* are required for cell-specific apoptotic death in *C. elegans*. *Dev. Biol.* **274**, 125–138 (2004).
82. Karashima, T., Sugimoto, A. & Yamamoto, M. *Caenorhabditis elegans* homologue of the human azoospermia factor DAZ is required for oogenesis but not for spermatogenesis. *Development* **127**, 1069–1079 (2000).
83. Navarro, R. E., Shim, E. Y., Kohara, Y., Singson, A. & Blackwell, T. K. *cgh-1*, a conserved predicted RNA helicase required for gametogenesis and protection from physiological germline apoptosis in *C. elegans*.

Development **128**, 3221–3232 (2001).
84. Lettre, G. *et al*. Genome-wide RNAi identifies p53-dependent and -independent regulators of germ cell apoptosis in *C. elegans*. *Cell Death Differ*. **11**, 1198–1203 (2004).
85. Boag, P. R., Nakamura, A. & Blackwell, T. K. A conserved RNA–protein complex component involved in physiological germline apoptosis regulation in *C. elegans*. *Development* **132**, 4975–4986 (2005).
86. Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J. & Hengartner, M. O. A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans*. *Mol. Cell* **5**, 435–443 (2000).
87. Ahmed, S. & Hodgkin, J. MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. *Nature* **403**, 159–164 (2000).
88. Ahmed, S., Alpi, A., Hengartner, M. O. & Gartner, A. *C. elegans* RAD-5/CLK-2 defines a new DNA damage checkpoint protein. *Curr. Biol*. **11**, 1934–1944 (2001).
89. Derry, W. B., Putzke, A. P. & Rothman, J. H. *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. *Science* **294**, 591–595 (2001).
90. Schumacher, B., Hofmann, K., Boulton, S. & Gartner, A. The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. *Curr. Biol*. **11**, 1722–1727 (2001).
91. Hofmann, E. R. *et al*. *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis. *Curr. Biol*. **12**, 1908–1918 (2002).
92. Bergamaschi, D. *et al*. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. *Nature Genet*. **33**, 162–167 (2003).
93. Deng, X. *et al*. *Caenorhabditis elegans* ABL-1 antagonizes p53-mediated germline apoptosis after ionizing irradiation. *Nature Genet*. **36**, 906–912 (2004).
94. Boulton, S. J. *et al*. BRCA1/BARD1 orthologs required for DNA repair in *Caenorhabditis elegans*. *Curr. Biol*. **14**, 33–39 (2004).
95. Schumacher, B. *et al*. Translational repression of *C. elegans* p53 by GLD-1 regulates DNA damage-induced apoptosis. *Cell* **120**, 357–368 (2005).
96. Oda, E. *et al*. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* **288**, 1053–1058 (2000).
97. Nakano, K. & Vousden, K. H. PUMA, a novel proapoptotic gene, is induced by p53. *Mol. Cell* **7**, 683–694 (2001).
98. Marin-Teva, J. L. *et al*. Microglia promote the death of developing Purkinje cells. *Neuron* **41**, 535–547 (2004).
99. White, K. *et al*. Genetic control of programmed cell death in *Drosophila*. *Science* **264**, 677–683 (1994).
100. Lindsten, T. *et al*. The combined functions of proapoptotic Bcl-2 family members *bak* and *bax* are essential for normal development of multiple tissues. *Mol. Cell* **6**, 1389–1399 (2000).
101. Kurz, C. L. & Ewbank, J. J. *Caenorhabditis elegans*: an emerging genetic model for the study of innate immunity. *Nature Rev. Genet*. **4**, 380–390 (2003).
102. Aballay, A. & Ausubel, F. M. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proc. Natl Acad. Sci. USA* **98**, 2735–2739 (2001).
103. Kinchen, J. M. *et al*. Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in *C. elegans*. *Nature* **434**, 93–99 (2005).
104. Kinchen, J. M. & Hengartner, M. O. Tales of cannibalism, suicide, and murder: programmed cell death in *C. elegans*. *Curr. Top. Dev. Biol*. **65**, 1–45 (2005).
105. Hengartner, M. O. Apoptosis: corralling the corpses. Cell **104**, 325–328 (2001).
106. Tilly, J. L. Commuting the death sentence: how oocytes strive to survive. *Nature Rev. Mol. Cell Biol*. **2**, 838–848 (2001).
107. Kim, M. R. & Tilly, J. L. Current concepts in Bcl-2 family member regulation of female germ cell development and survival. *Biochim. Biophys. Acta* **1644**, 205–210 (2004).
108. Aballay, A., Drenkard, E., Hilbun, L. R. & Ausubel, F. M. *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. *Curr. Biol*. **13**, 47–52 (2003).
109. Tenor, J. L., McCormick, B. A., Ausubel, F. M. & Aballay, A. *Caenorhabditis elegans*-based screen identifies *Salmonella* virulence factors required for conserved host–pathogen interactions. *Curr. Biol*. **14**, 1018–1024 (2004).

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