The genome sequence of Caenorhabditis elegans is due to be completed around the end of this year. C. elegans will therefore be the first animal to have its genome completely sequenced.

To mark this outstanding achievement, we are publishing a series of articles celebrating worm genetics. Articles will review the contributions that worm genetics has made to fundamental aspects of biology, such as cell death, signal transduction, sex determination and neurobiology.

**Reviews**

The elimination of unwanted cells by programmed cell death is an important developmental and homeostatic process in multicellular organisms, including the nematode Caenorhabditis elegans. During the development of the C. elegans hermaphrodite, 1090 cells are generated, of which 131 undergo programmed cell death1–4 (Fig. 1). Genetic analyses of C. elegans have identified genes that function in programmed cell death, ordered these genes in a genetic pathway and led to the finding that similar genes control programmed cell death in other organisms, including mammals. In this review, we concentrate on the genetic and developmental studies of C. elegans that have resulted in these discoveries. We mention only briefly molecular biological and biochemical studies of programmed cell death, which have been described in a number of recent reviews5–8. We focus primarily on mechanisms of cell killing and, for this reason, do not discuss in detail the engulfment or degradation of cell corpses.

*egl-1, ced-4, ced-3 and ced-9 are global regulators of programmed cell death in C. elegans*

Three C. elegans genes, *egl-1, ced-4* and *ced-3*, seem to be required for all somatic programmed cell death to occur. Loss-of-function (lf) mutations in *egl-1, ced-4* or *ced-3* lead to the survival of essentially all cells that undergo programmed cell death during wild-type development9–11. The *egl-1* (egl, egg laying defective) gene was defined originally by gain-of-function (gf) mutations that cause a dominant egg-laying defect attributable to the loss of functional HSN neurons in hermaphrodites12, a defect found to be caused by the ectopic programmed deaths of these neurons9. The isolation of a cis-dominant suppressor of the egl-1(gf) egg-laying defect revealed that the loss of *egl-1* function causes an absence of programmed cell death13. The first allele of *ced-4* (ced, cell death abnormal) was identified as an extragenic suppressor of the egg-laying defect of egl-1(gf) mutants14. Mutations in cis-3 were identified as suppressing the presence of persistent cell corpses seen in mutant animals defective in cell-corpse engulfment15. Subsequently, many more alleles of *ced-4* and *ced-3* have been obtained by screening for either the absence of cell corpses (e.g. Ref. 12), or the presence of extra living cells that would have died in wild-type animals (undead cells, e.g. Ref. 13), or by suppressing the lethality caused by loss-of-function mutations in the cell-death protection gene *ced-9* (see below, e.g. Ref. 14). The activity of the gene *ced-9* protects most, if not all, cells from undergoing programmed cell death during C. elegans development. Loss-of-function mutations in C. elegans lead to sterility and maternal-effect lethality, as a consequence of the ectopic activation of programmed cell death in cells that normally live15. In C. elegans, animals carry many cell types, including neurons, ectodermal...
The discovery that programmed cell death in all animals.

biochemical mechanisms of pro-
cised death (see below). Loss-of-function alleles were obtained by identifying cis-dominant suppressors.

The molecular characterization of C. elegans cell-death genes has been key in revealing some of the biochemical mechanisms of programmed cell death in all animals.

The discovery that ced-3 encodes a member of the caspase (cysteine aspartate-specific protease) family of proteases was instrumental in identifying a role for mammalian caspases in the regulation of programmed cell death5. A protein related to the CED-4 protein, APAF-1, appears to be involved in effecting programmed cell death in mammalian cells, and both of these proteins might require nucleotide binding to function19,20. The cloning of the ced-9 gene revealed that its protein product is similar to the human Bcl-2 protein22, which had been implicated in programmed cell death in mammalian cell culture experiments21. This discovery, along with the findings that human Bcl-2 could block programmed cell death in C. elegans and could substitute for the C. elegans ced-9 gene in ced-9-deficient mutants22, strongly suggests that the molecular mechanism of programmed cell death is evolutionarily conserved. Finally, EGL-1 has functional and molecular similarity to the BHS-only (BH, Bcl-2 homology domain) subfamily of Bcl-2-like proteins: BHS-only proteins are capable of inducing programmed cell death when overexpressed in mammalian cell systems (Ref. 10 and references therein). The EGL-1 gene is the first member of the BHS-only family for which a loss-of-function phenotype has been described, and this phenotype indicates not only that a gene of this class can cause programmed cell death when overexpressed, but also that such a gene normally functions in this way.

Genetic evidence suggests that EGL-1, CED-4 and CED-3 function exclusively in programmed cell death. Programmed cell death is not essential for the viability of C. elegans, and animals homozygous for loss-of-function mutations in egl-1, ced-4 or ced-3 appear superficially normal in size and movement. Undead cells in C. elegans can differentiate13,23, and can even function23. Loss-of-function alleles of ced-4 include some that are null by genetic and molecular criteria12. More than 90 alleles of ced-3 have been obtained, of which more than 60 have been analyzed genetically and molecularly (Ref. 16; H.R. Horvitz et al., unpublished), and some are altered in residues essential for CED-3 enzymatic activity (Refs 16, 27; S. Shaham, B. Davies, P. Reddien and H.R. Horvitz, unpublished). However, no allele is known that is unambiguously predicted to eliminate expression of the entire CED-3 protein. Based on this consideration, it is conceivable that ced-3 has a role during the development of C. elegans that is unrelated to its enzymatic activity and that null alleles of ced-3 lead to additional (possibly lethal) defects. There is only one known loss-of-function allele of egl-1. This allele behaves as a null by genetic criteria, but molecular analysis indicates that at least a portion of the EGL-1 protein could still be produced, so it is not clear whether this mutation completely eliminates egl-1 activity20.

Because ced-9(22) causes early lethality it is difficult to know whether ced-9 is required for any process other than the regulation of programmed cell death. However, double mutants between a null allele of ced-9 and either ced-3 or ced-4 do not appear to have any defects beyond those seen in ced-3 or ced-4 single mutants14, arguing that ced-9 function is as cell-death specific as are the functions of ced-3 and ced-4.

Pathway to death

Genetic experiments have ordered the functions of egl-1, ced-9, ced-4 and ced-3. First, as described above, loss-of-function mutations in ced-4 or ced-3, indicating that ced-9 normally functions to negatively regulate ced-4 and ced-3 (Ref. 15). A simple pathway consistent with the observations places ced-9 genetically upstream of ced-4 and ced-3 (Fig. 2a). However, these observations do not exclude the possibility that ced-9 might function biochemically to inhibit the activities rather than the expression or activation of ced-4 and ced-3 (e.g. Ref. 28). By contrast, loss of egl-1 function, while preventing somatic cell death just as does loss of ced-4 or ced-3 function, does not suppress the lethality resulting from a loss of ced-9 function23. This finding suggests

FIGURE 1. Distribution of programmed cell deaths within the cell lineage of the Caenorhabditis elegans hermaphrodite. Horizontal lines indicate early embryonic cell divisions, colors represent cell fates and areas are proportional to the total numbers of embryonic and postembryonic descendants from the primary blastomeres AB, MS, E, C and D. The germline, generated from blast cell P4, unlike other tissues in C. elegans, continuously proliferates and gives rise to variable numbers of cells and cell deaths24,25. Programmed cell deaths occur in the AB, MS and C cell lineages, but not in the E cell lineage, which produces only intestine, or the D cell lineage, which produces only muscle21,26. Cell death is a particularly common fate in the AB cell lineage, which produces most of the nervous system and in which 116 of the 722 cells generated proceed to die. Adapted from Ref. 4.
that egl-1 acts genetically upstream of and functions as a negative regulator of ced-9 (Fig. 2a).

Because in a ced-9 loss-of-function mutant background the allelic states of ced-4 and ced-3 affect the fates of cells that would normally live, either the ced-4 and ced-3 gene products are normally present in most cells before the time at which cells differentiate or the loss of ced-9 activity leads to ectopic expression of ced-4 and ced-3. Although the expression patterns of ced-4 and ced-3 at the RNA and protein levels have not been described, other molecular genetic evidence suggests that the former model is more likely to be correct. Specifically, transcriptional overexpression of ced-4 and/or ced-3 from transgenes, at least in certain types of developing C. elegans neurons, causes these cells to undergo programmed cell death29. The efficiency of this killing is dependent on the genotype of the animal carrying the transgene29. For instance, killing by ced-3 overexpression is more efficient when the transgenic animal is wild-type for ced-4 and/or mutant for loss-of-function of ced-9 (as described in more detail below). Because changes in the ced-4 or ced-9 genotype influence killing, ced-4 and ced-9 products are likely to be normally present in these cells29. Similar arguments suggest that ced-3 is also likely to be present.

Transcriptional overexpression has also been used to order the functions of cell-death genes. Specifically, in the absence of ced-4 activity, ced-3 overexpression is able to kill cells, although at a reduced level in comparison with killing in a wild-type ced-4 background (Fig. 2b). By contrast, when ced-3 activity is no longer inhibited by ced-4, overexpressed ced-3 can still kill, albeit at a slightly reduced efficiency. By contrast, in the absence of ced-3 activity, ced-4 is almost incapable of killing cells. These results suggest ced-4 acts upstream of ced-3 to activate programmed cell death. ced-3 endogenous ced-4 activity protects cells from programmed cell death induced by overexpression of ced-3, because killing is more efficient in a ced-9(e1) background than in a ced-9(+) background. While elimination of ced-4 function reduces the killing activity of ced-3, this residual ced-3 activity is no longer inhibited by ced-5, suggesting that ced-5 functions to protect against programmed cell death, at least in part, by negatively regulating ced-4.

(b) The overexpression of egl-4 can also kill cells. This killing is blocked by loss-of-function mutations in ced-4 or ced-3 or by a gain-of-function mutation in ced-9, suggesting that egl-3 acts upstream of these genes.

Figure 2. Interactions among egl-1, ced-9, ced-3 and, ced-5. Genetic epistasis experiments and studies in which ced-4, ced-9, ced-4 and ced-3 have been overexpressed to kill cells in different genetic backgrounds have suggested an order of action of these genes10,15,29. The relative efficiency of cell killing is represented by the number of plus (+) signs, as interpreted from data in Refs 10, 20. (a) Loss-of-function mutations in ced-4 or ced-9 suppress the lethality caused by loss of ced-9 function, suggesting that ced-9 functions upstream of ced-4 and ced-3. By contrast, a loss of function mutation in egl-1 does not suppress ced-9 lethality, suggesting that egl-3 functions upstream of ced-5. (b) The overexpression of ced-4 or ced-9 can kill cell in wild-type C. elegans embryos. In the absence of ced-4 activity, overexpressed ced-3 can still kill, albeit at a slightly reduced efficiency. By contrast, in the absence of ced-3 activity, ced-4 is almost incapable of killing cells. These results suggest ced-4 acts upstream of ced-3 to activate programmed cell death. (c) Endogenous ced-9 activity protects cells from programmed cell death induced by overexpression of ced-3, because killing is more efficient in a ced-9(e1) background than in a ced-9(+) background. While elimination of ced-4 function reduces the killing activity of ced-3, this residual ced-3 activity is no longer inhibited by ced-9, suggesting that ced-5 functions to protect against programmed cell death, at least in part, by negatively regulating ced-4. (d) The overexpression of egl-4 can also kill cells. This killing is blocked by loss-of-function mutations in ced-4 or ced-3 or by a gain-of-function mutation in ced-9, suggesting that egl-1 acts upstream of these genes.
ced-3 either through another caspase or independently of caspase activity. At least two additional genes with similarity to ced-3 are present in C. elegans genomic sequences (P. Reddien and H.R. Horvitz, unpublished; M. Hengartner, pers. commun.).

Biochemical data have supported the hypothesis that ced-4 is an upstream activator of ced-3 and have provided a possible physical basis for their interaction. The CED-4 protein might bind directly to the proenzyme form of the CED-3 protein to facilitate its proteolytic conversion to the active enzyme\(^9,10,33\). In mammals APAF-1 (a CED-4-related protein) binds to and stimulates the processing of caspase-9 (a CED-3-like protein)\(^9,33\). High levels of CED-4 might relieve the need for CED-4 to facilitate conversion from the proenzyme form, explaining why ced-4 overexpression can kill in the absence of ced-4 activity.

Similar overexpression experiments suggest that ced-9 acts by inhibiting ced-4 function\(^9\) (Fig. 2c). The presence of an endogenous wild-type ced-9 allele partially inhibits the ability of overexpressed ced-3 to kill cells that normally live; killing by ectopically expressed ced-3 is less efficient in a ced-9(wild-type) animal than in a ced-9 loss-of-function animal. However, in a ced-4 mutant background, killing by ced-3 is no less efficient in animals carrying a wild-type ced-9 allele than in animals carrying a ced-9(lf) allele. These results indicate that, in the absence of ced-4 activity, ced-9 fails to protect against killing by overexpressed ced-3. Thus, ced-9 protection against ced-3 killing is mediated at least in part by ced-4 (Fig. 2c). Results consistent with this hypothesis were also obtained by examining the effects of expression of combinations of ced-3, ced-4 and ced-3 in insect and mammalian cells\(^34,35\).

A physical basis for the results of these genetic studies is suggested by the observation that CED-9 and CED-4 can interact directly (reviewed in Ref. 33). This interaction has been proposed to block the activity of CED-4, although the mechanism of such an inhibition is unknown. An interaction between CED-9 and CED-4 has been demonstrated in vitro and in yeast, insect and mammalian cells but not yet directly in C. elegans, although some ced-9 and ced-4 allele-specific interactions have been observed\(^6\). The human CED-9-like protein APAF-1 and a Bcl-2 family member, Bcl-x\(_L\), might also interact directly\(^19,35\).

Overexpression of egl-1 can kill cells that normally live\(^19\). This killing is dependent on ced-4 and ced-3, placing egl-1 genetically upstream of these genes (Fig. 2d). As described above, egl-1 also seems to function upstream of and as a negative regulator of ced-9. The EGL-1 protein binds to the CED-9 protein, and it has been proposed that EGL-1 acts by releasing CED-3 from CED-9 (Ref. 10). Similarly, in mammals Bcl-2-family proteins bind Bcl-2-family proteins (various references cited in Ref. 10).

Thus, current genetic and molecular data suggest the following model for the activation of programmed cell death (Fig. 3). After activation by upstream signals, the EGL-1 protein interacts with CED-9 and releases CED-3 protein from membrane-associated CED-9 protein (CED-9, like other Bcl-2 family members, has a hydrophobic C-terminus that probably causes it to be membrane-associated\(^19\)). Free CED-4 then interacts with CED-9/Bcl-2-family proteins\(^40,41\), one possibility is that CED-9 might bind directly to CED-3 protein to facilitate its proteolytic function\(^18\), like protein)\(^18,21\). High levels of CED-3 might relieve the need for CED-4 to facilitate conversion from the proenzyme form, explaining why ced-4 overexpression can kill in the absence of ced-4 activity.

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function, indicating that ectopic programmed cell deaths caused by upstream of CED-4 encoded by alternatively spliced transcripts34. The disappearance appears to result from two protein isoforms of CED-4, both cell-death inhibitor. These opposing activities of CED-4 seem to be dependent upon its splice sites: for example, CED-4L could interact with the killing form, CED-4S (S, short). When overexpressed from a transgene between residues 212 and 213 of the 549-amino acid sequence (although not in position)Δ4. The ced-4 gene appeared to be an essential gene, because within the third intron there is a 72-nucleotide region of very high (90%) identity among all three species. This region proved to correspond to an alternatively spliced exon that is present in a second, less abundant, ced-4 tran- script and that is predicted to produce a protein, CED-4L (L, long), with 24 additional amino acids inserted between residues 212 and 213 of the 549-amino acid killing form, CED-4S (S, short). When overexpressed from a transgene CED-4L can protect against programmed cell death, while when overexpressed from a transgene CED-4L can protect against ectopic programmed cell deaths caused by ced-9. Studies, indicating that ced-9 functions genetically upstream of ced-4L, just as it functions genetically upstream of ced-4S. It is not known how the splicing choice is controlled or whether this control is differentially regulated in different cells (e.g. those that normally live and those that normally die) to alter their susceptibility to death-inducing signals or perhaps to initiate cell death program. Furthermore, how the CED-4L protein inhibits programmed cell death is unknown. The simplest model is that CED-4L functions as an interfering dominant-negative form of the CED-4 protein. For example, CED-4L could interact with the same targets as does CED-4S but not activate these targets, while blocking CED-4S from doing so. Consistent with this hypothesis, recent biochemical evidence suggests that CED-4L can bind to, but not promote the processing of, the proenzyme form of CED-3 (Ref. 43).

It is possible that the killing function of ced-4L and the protective function of ced-4 are related. CED-9 might kill the same targets as does CED-4S but not activate these targets, while blocking CED-4S from doing so. Consistent with this hypothesis, recent biochemical evidence suggests that CED-4L can bind to, but not promote the processing of, the proenzyme form of CED-3 (Ref. 43).

Developmental regulation of programmed cell death

How does a cell decide to undergo programmed cell death? In mammals, cell interactions act to trigger at least some programmed cell deaths. In C. elegans, a few cell deaths depend upon gene expression and other cell–cell interactions (reviewed in Ref. 49), much less is known about how the killing occurs during development. Although many living cells in C. elegans probably express ced-4 and ced-3, transcriptional analysis of other genes of these genes, or of egl-1, is sufficient to cause the deaths of at least some cell types. Thus, elevation of egl-1, ced-4 and/or ced-3, is sufficient to cause the deaths of at least some cell types. This is not a result of stochastically occurring defects in cellular physiology. Furthermore, patterns of programmed cell deaths can be altered by mutations in genes known to be involved in the control of other cell deaths (e.g. Refs 46–48), further indicating that programmed cell death is no different from developmental cell fates in general.

How do developmental signals control the activities of the killing machinery, which includes at least egl-1, ced-9, ced-4 and ced-3, to initiate the death process in particular cells? Although some understanding has been reached of how certain cell–cell interactions in the mammalian immune system lead to the activation of the cell-death machinery (reviewed in Ref. 49), much less is known about how the machinery is activated during development in C. elegans or in any other organism. One possibility is that the activity of one or more of these genes is under transcriptional control. Although many living cells in C. elegans probably express ced-4 and ced-3, transcriptional analysis of other genes of these genes, or of egl-1, is sufficient to cause the deaths of at least some cell types. Thus, elevation of egl-1, ced-4 and/or ced-3, is sufficient to cause the deaths of at least some cell types. Genetic analysis has identified two genes, ces-1 and ces-2 (ces, cell death specification) that control a subset of programmed cell deaths in C. elegans. While mutations in ces-1 or ces-2 block the deaths of certain neural cells, no other discernible cell death, cell lineage or cell fate defects have been observed in ces-1 or ces-2 mutants, suggesting that ces-1 and ces-2 are specifically involved in regulating the programmed cell deaths of these cells. ces-2 encodes a member of the basic-leucine zipper (bZIP) family of transcription factors; consistent with the hypothesis that programmed cell death is controlled at the level of differential gene expression. Mammalian members of the bZIP family, which most closely resemble ces-2 (the basic and acidic rich or PAR family), might also have a role in the cell-specific regulation of programmed cell death. It is conceivable that CES-2 is a direct transcriptional regulator of egl-1, ced-4 or ced-3. It is important to note, however, that the Get phenotype of C. elegans cell deaths occurs throughout the cell lineage (Fig. 1), and the majority of cell deaths occur during the developmental period; in most other cells terminally differentiated. Also, like other cell fates in C. elegans, programmed cell death is observed in an essentially identical pattern with the same linearly equivalent cells undergoing programmed cell death from animal to animal. This reproducibility in the cells that die suggests that programmed cell death in C. elegans is not a result of stochastically occurring defects in cellular physiology. Furthermore, patterns of programmed cell deaths can be altered by mutations in genes known to be involved in the control of other cell fates (e.g. Refs 46–48), further indicating that programmed cell death is no different from developmental cell fates in general.
of ced-1 mutant animals is caused by gain-of-function alleles and that of ced-2 mutants is caused by a partial loss-of-function allele. Because these alleles do not result in a complete loss of gene activity, it remains possible that the cell-specific cell-death phenotypes of ces-1 or ces-2 mutants are due to a more general requirement for these genes in programmed cell death or in cell fate determination.

Recent regulation of ced-9 activity is unlikely to be the only mechanism controlling the life or death decision. Animals that are doubly mutant for a null allele of both ced-3 and ced-9 are viable, and do not have a large number of ectopic cell deaths. However, in such animals the majority of cells that normally die during wild-type development still do so, indicating that, in this genetic background, cells that normally die are more susceptible to programmed cell death than are cells that normally live. Because ced-9 is inactive in these mutants, this difference in susceptibility cannot arise from differential regulation of ced-9 activity. Hence, some death-promoting signals must feed into components of the cell-death pathway independently of ced-9.

In the throes of death

As cells undergo programmed cell death, their corpses are rapidly engulfed by, and then degraded, within, engulfing cells. In Caenorhabditis elegans, most corpses are engulfed by their closest neighbors (Egl. 52 and J. Sulston and J. White, pers. commun.). Six genes, ced-1, 2, 5, 6, 7 and 10, important for cell corpse engulfment in C. elegans, have been discovered in genetic screens by visually identifying mutants that engulf cell corpses is not altered. Thus, the activities of these genes affect programmed cell death rather than cell corpses persistence indefinitely within engulfing cells. CED-4L and CED-4S controlled? What is the basis for the killing activity of ced-9? What are the steps downstream of CED-3 activation that mediate cell-corpse formation, engulfment and degradation? Further genetic analysis using C. elegans should help reveal the answers to these questions.

While there have been extensive screens to identify mutations that can suppress the lethality conferred by ced-9(0) mutations (H.R. Horvitz et al., unpublished), there has been only limited screening for genes that, like ced-1, function to promote cell death upstream of ced-9. Furthermore, most screens for cell-death mutants have required that animals be viable, so other suppressor genes like ced-9 might not have been identified. Genes with redundant functions in cell killing would also probably have been missed in previous screens. Searching for enhancers or suppressors of cell killing in genetically sensitized backgrounds (e.g. Refs 60, 61) offer two approaches to the identification of such genes.

What are the targets of CED-3 proteolysis? There might be a small number of critical targets that are affected to effect the downstream events of cell death. Alternatively, there might be multiple targets with important cellular functions that are inactivated by CED-3 proteolysis. This latter model assumes that, although cell death initiates as a regulated process, it proceeds by eliminating functions that are required for normal cellular homeostasis. Another possibility is that ced-3 functions to inactivate a cell-death protector. Loss of function of such a gene would presumably confer a lethal phenotype and would not be suppressed by any known cell-death mutant. The identification and characterization of genes involved in the generation of the conserved morphological changes in cell corpses, in cell-corpse degradation and in the generation of engulfment signals might reveal which, if any, of these models is true. A few genetic screens have sought genes involved in corpse formation and breakdown. Perhaps targets of the execution genes could be sought by screening for mutations that synthetically alter cell death patterns, for example by blocking cell death when in combination with mutations that cause other downstream defects.

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While numerous proteolytic targets of mammalian caspases have been identified, the relevance of these targets in vivo to programmed cell death has been mostly untested. The genetic analysis of such genes in C. elegans should determine their functions.

Conclusions

Enormous progress has been made towards understanding the basic molecular mechanisms used by cells to kill themselves. Nonetheless, many questions remain. The remarkable degree of conservation of the cell-death pathway from nematodes to mammals suggests that genetic analysis of programmed cell death in C. elegans will continue to play a major role in revealing the mechanisms responsible for this crucial and fascinating process.

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References

17 Marks, E. et al. (1996) Cell 85, 375–390
22 Hengartner, M.O. and Horvitz, H.R. (1994) Cell 76, 665–676
61 White, K. et al. (1994) Science 264, 577–583

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