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Review

Cell death specification in *C. elegans*

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Years of research have identified a highly conserved mechanism required for apoptotic cell killing. How certain cells are specified to die is not well understood. With a rich history in programmed cell death research, the nematode *C. elegans* offers an excellent animal model with which to study cell death specification events. Developing hermaphrodites have 131 invariant cell death events that can be studied with single cell resolution. Recent genetic studies have begun to identify diverse sets of factors required for the proper specification of individual cell death events. The limited findings thus far suggest that cell death specification is controlled through transcriptional regulation of at least two members of the core cell death pathway, *egl-1* and *ced-3*. However, it remains unclear if additional modes of cell death specification exist. Here we briefly summarize current findings in the field of *C. elegans* cell death specification and consider those questions that remain to be answered.

Programmed Cell Death in *C. elegans*

The process of developmental cell death can be divided into three distinct phases. First, individual cells are selected for death during the specification step. Second, in the killing phase, cell-specific activation of caspases leads to the destruction of the cell. Finally, the execution phase is defined by chromosome degradation, cytoplasmic shrinkage and clearance of the dead cells by engulfment (reviewed in ref. 1). In this review, we focus on the current understanding of how the killing phase is regulated in specific cells to achieve cell death specification.

Genetic screens for mutants defective in cell death have identified four members of the core cell-killing pathway in *C. elegans*. These include the cell death promoting genes *egl-1*, *ced-4* and *ced-3,* as well as a single cell death inhibitor *ced-9* (Fig. 1A).[2-4] This genetic pathway is well conserved and has been studied extensively in a broad range of organisms.[5]

During programmed cell death, cells are killed by active caspases, aspartate-specific cysteine proteases, which cleave an unknown number of protein targets. Caspase cleavage can yield one of two results: inactivation of cell death protective factors and/or activation of cell death promoting factors. The cumulative effect of these cleavage events is the systematic disassembly of the cell.[6-8] The *C. elegans* gene *ced-3* encodes a caspase required for programmed cell death.[3,9,10] CED-3 is expressed as a latent proenzyme that requires autoproteolytic activation to acquire caspase activity.[9,11] In vivo CED-3 activation requires the pro-apoptotic protein CED-4, although there is experimental evidence for weak CED-3 autoactivation under overexpression conditions.[12,13] CED-4, the *C. elegans* Apaf-1 homolog, forms an oligomer during apoptosis that serves as a scaffold for CED-3 self-activation.[14,15] In living cells, CED-4 is sequestered at the mitochondria as a dimer by CED-9, the *C. elegans* Bcl-2 homolog.[12,15,16] CED-9 activity is absolutely required for cell viability as animals homozygous for a loss-of-function (lf) allele of *ced-9* exhibit widespread cell death, resulting in embryonic lethality.[4] The small BH3-only (Bcl-2 homology region 3) protein EGL-1 antagonizes the anti-apoptotic activity of CED-9 by binding to CED-9, resulting in a conformational change that leads to the release of the CED-4 dimer from the CED-4/CED-9 complex.[2,17-19] A gain-of-function (gf) mutation in CED-9 that impairs EGL-1 binding results in a significant reduction in cell death during development.[4,18] The expression of *egl-1* is thought to be restricted to those cells that are going to die.[20-22] Overexpression of *egl-1* using a heat shock promoter results in embryonic lethality due to widespread programmed cell death.[2] Thus transcriptional regulation of *egl-1* is hypothesized to be the major mode of cell death specification in *C. elegans*. However there is recent evidence for alternative modes of cell death specification, which leaves this a rich area of study.

Specification of Cell Death Fates

During the development of a *C. elegans* hermaphrodite, 1090 cells are born and 131 of them undergo programmed cell death.[23,24] The precise way in which these cell death events are controlled is largely unknown. To date the specification of cell death has been studied for only five cell types. These include the pharyngeal NSM (Neurosecretory motor neuron) sister cells,[22,25,26] a ventral cord cell P11.aaap,[21] the tail-spike cells,[27] and finally two sets of sex-specific neurons: the HSNs (Hermaphrodite Specific Neurons)[2,20,28] and the male specific sensory neurons CEMs (Cephalic Male).[29,30] The findings from these studies suggest that a diverse set of cell-specific factors are required for the proper specification of programmed cell deaths.

NSM sister cell. The NSMs are a set of two serotonergic motor neurons in the anterior pharynx of the worm.[31,32] These cells appear to be responsible for signaling the presence of food to the rest of the worm, which stimulates pharyngeal pumping, egg laying, and

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a depression of locomotion.[33] During embryonic development, the mother of the NSM cell gives rise to two daughter cells, the NSM that lives and the NSM sister cell that is destined to die.[24]

The specification of NSM sister cell death was analyzed by introducing an *egl-1* rescuing transgene back to the *egl-1(n3082 n1084)* mutant.[22] In this *egl-1* loss-of-function background virtually all programmed cell death events are inhibited including the death of the NSM sister cells.[2] This *egl-1* genomic fragment included 1 kb of 5' promoter as well as ~5.6 kb of 3' sequence and efficiently rescued NSM sister cell death. By analyzing subclones of the *egl-1* genomic fragment, Thellmann et al., identified a 352 bp region downstream of the *egl-1* open reading frame (ORF) that is required for rescue of NSM sister cell death. This region is conserved between two different nematode species, *C. elegans* and *C. briggsae,* and contains four conserved E-box motifs, the binding sites for bHLH (basic helix-loop-helix) DNA-binding proteins (Fig. 1B).[34] Analysis of available genetic alleles of the *C. elegans* bHLH genes or animals treated with RNA*i* of candidate genes reveals a requirement for *hlh-2* and *hlh-3* in the death of NSM sister cells. Recombinant HLH-2 on its own or with HLH-3 can bind the *egl-1* E-boxes in vitro. Together these results suggest that HLH-2/HLH-3 likely act as *egl-1* activators in NSM sister cells to properly specify their programmed cell deaths.

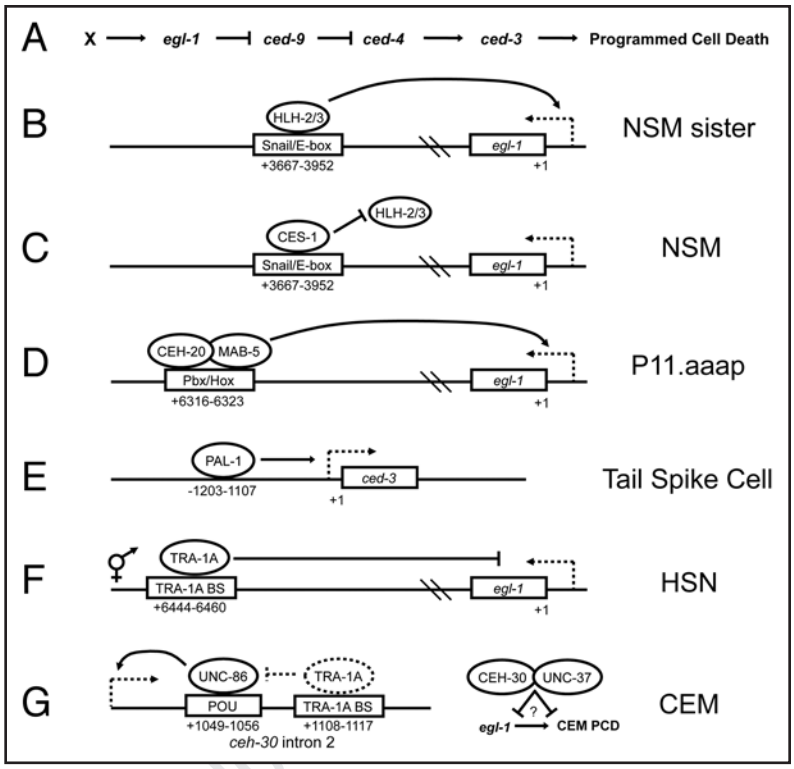

Figure 1. *C. elegans* programmed cell death specification. (A) The core cell death pathway in *C. elegans* where X activates *egl-1*. X may be any number of known and unknown cell-specific factors that regulate cell death fate. (B) The NSM sister cell is specified for death by HLH-2/HLH-3 activation of *egl-1*. Four separate E-boxes, bHLH binding sites, were identified in *egl-1*; Box: I 3947-3952, Box II: 3788-3793, Box III: 3713-3718, Box IV: 3667-3672. (C) In the NSMs, CES-1 inhibits cell death by competing with HLH-2/HLH-3 in binding to the Snail elements that coincide with the E-boxes in *egl-1*. (D) The P11.aaap cell is specified for cell death by CEH-20/MAB-5 activation of *egl-1*. (E) The tail-spike cell is specified for cell death by PAL-1 transcriptional activation of *ced-3* via binding to 3 regions of the *ced-3* promoter (Region A: -1203-1178, Region B: -1162-1137 and Region C: -1131-1107). (F) The HSN is protected from cell death in hermaphrodites by TRA-1A binding to *egl-1* and repressing *egl-1* transcription. (G) The male CEM is protected from cell death due to transcriptional upregulation of *ceh-30* by UNC-86 in the absence of TRA-1A (denoted by dashed lines), which antagonizes the activity of UNC-86. The direct target(s) of the CEH-30/UNC-37 repressosome remain to be identified.

Specification of the NSM sister cell cell death appears to be a rather complex process. Previous work showed that *ces-1(gf)* and *ces-2(lf)* alleles are capable of inhibiting NSM sister cell death.[25,26] In addition, *ces-1(gf)* but not *ces-2(lf)* mutations can inhibit the death of another pharyngeal neuronal cell type, the I2 sister cells.[35] Interestingly, CES-1, a C2H2-type Zinc finger protein,[35] was found to bind Snail-binding sites that coincide with the E-boxes in the *egl-1* gene required for NSM sister cell death.[22] A model was proposed, in which high levels of CES-1 in *ces-1(gf)* or *ces-2(lf)* backgrounds lead to competition for binding to the Snail/E-box DNA elements with HLH-2/HLH-3 and thus block NSM sister cell death (Fig. 1C). It is possible that in the I2 sister cells CES-1 binds the same and/or additional DNA elements in competition with I2 sister cell-specific factors.[35] A recent study further characterized the roles of *ces-1*, *ces-2* and a new factor *dnj-11* in the specification of NSM sister cell death.[36] *ces-1* transcription is inhibited by CES-2, a basic Leucine-zipper transcription factor, and DNJ-11, a previously uncharacterized DnaJ domain-containing protein. This transcriptional repression serves to keep CES-1 levels low in the NSM lineage so that CES-1 is only present in the NSMs. In addition to their roles in NSM cell fate specification, these three genes are required for the proper control of the asymmetric division of the NSM mother cell. This relationship between asymmetric cell division and the specification of programmed cell death is interesting and deserves further study.

P11.aaap. The ventral cord precursor cells (referred to as P0 through P12) give rise to motor neurons, hypodermal cells, and cells that undergo programmed cell death.[23] The pattern of cell divisions within each sub-lineage takes place in a stereotypical, reiterative way to give rise to progenitor cells. These cell fate specifications are controlled in part by the *C. elegans* homeobox (Hox) genes.[37,38] Two closely related cells, P11.aaap and P12.aaap (the posterior daughter of the anterior daughter of the anterior daughter of the anterior daughter of the P cell) normally undergo programmed cell death.[23] These cell deaths depend on the activity of *C. elegans Antennapedia* ortholog *mab-5*.[38] Recent work by Liu et al., determined the mode of cell death specification in P11.aaap.[21]

Given that null mutations of *mab-5* result specifically in the survival of P(11, 12).aaap but not other ventral cord cells and that *mab-5* overexpression does not lead to widespread cell death,[39] Liu et al., examined candidate cofactors that might work with *mab-5* to promote cell death. The *C. elegans Pbx* homolog *ceh-20* is required for proper P(11,12).aaap cell death. Worms homozygous for *ceh-20(ay42)*, a strong loss-of-function allele, have surviving P(11,12).aaap cells. Analysis of a 7.6 kb *egl-1* genomic rescuing fragment identified a single conserved Pbx/Hox binding site 6 kb downstream of the *egl-1* start codon that is required for proper P11.aaap but not P12.aaap cell death. When this site is mutated in an *egl-1* GFP

reporter, GFP is no longer expressed in P11.aaap. Furthermore, GFP expression in P11.aaap from the wild-type reporter requires *ceh-20* and *mab-5*. In vitro a CEH-20/MAB-5 protein complex is capable of binding a wild-type DNA probe containing the Pbx/Hox binding site but binding to a probe containing the mutatated Pbx/Hox site is greatly reduced. Finally Liu et al., showed that both *ceh-20* and *mab-5* are expressed in P11.aaap, suggesting a model by which *egl-1* is directly activated by a CEH-20/MAB-5 complex in the P11.aaap cell to specify its death fate (Fig. 1D).

Questions remain regarding the specification of these cell deaths in the ventral cord. First of all, it is interesting that the closely related P11 and P12 lineages have overlapping but not identical requirements for cell death specification. The death of P12.aaap appears to be distinct in its requirement for *mab-5*. In the P11 lineage, *mab-5* expression is detected in all five descendent cells including P11.aaap. However in the P12 lineage *mab-5* expression seems to be limited to the first cell division and cannot be detected after that.[39] In addition, the Pbx/Hox site required for P11.aaap death is not required for P12.aaap death, indicating that a distinct mechanism is required for proper P12.aaap cell death specification. Interestingly, expression of *mab-5* and *ceh-20* is also seen in the P11.aaap sister cell (P11.aaaa), although this cell does not undergo programmed cell death.[23] This result suggests that proper cell death specification in the P11 lineage also requires unidentified cell death inhibitors.

Tail-spike cells (a delayed death event). The tail-spike cells are a pair of cells in the tail tip that produce a bundle of microtubule filaments over which the cuticle of the tail forms.[24] The tail-spike cells are unique among cells that undergo programmed cell death in *C. elegans* because they are highly differentiated and long lived prior to their death.[23,24] Thus far, findings related to cell death specification in the worm have been limited to regulators of *egl-1* transcription; however, the specification of tail-spike cell death takes a step away from this trend in cell death specification.

Genetic analysis of tail-spike cell death revealed that *ced-3* and *ced-4* are absolutely required for killing, while *egl-1* appears to be only partially required.[27] For example, in *ced-3* or *ced-4* loss-of-function backgrounds, one hundred percent of tail-spike cells survive, compared to only thirty percent tail-spike cell survival in an *egl-1* loss-of-function background. In addition to a partial genetic requirement of *egl-1*, an *egl-1* GFP reporter does not express in the tail-spike cell. The *egl-1*-dependent tail-spike death does require wild-type *ced-9*. These data suggest that the majority of tail-spike cell killing activity is *egl-1* independent, which is in stark contrast to previously known cell death specification events.

What is the *egl-1*-independent mode of cell killing in the tail-spike cells? One clue came from the tail-spike cell reporter used in this work, since $P_{ced\text{-}3}$*::gfp* transgenes, which contain a 1.5 kb region of the *ced-3* promoter, occasionally blocked tail-spike cell death. This observation suggests that extra copies of the *ced-3* promoter sequence may titrate factors required for *ced-3* transcription. Analysis of the expression patterns of the *ced-3* reporter showed a consistent induction of *ced-3* expression in the tail-spike cell approximately 30 minutes prior to the appearance of morphological characteristics of cell death. Analysis of the *ced-3* promoter identified a 349 bp region that is conserved between *C. elegans* and *C. briggsae* and that contains three motifs necessary for *ced-3* expression in the tail-spike cells. In a genetic screen to identify regulators of *ced-3* transcription in the tail-spike cells, two alleles of *pal-1* were identified that are required for proper *ced-3* reporter expression in the tail-spike cells. PAL-1, a homolog of the *Caudal* homeodomain protein, is required for posterior development in *C. elegans* and is expressed in the tail-spike cells.[40] Recombinant PAL-1 binds the required *ced-3* promoter elements in vitro. These data suggest that *pal-1* may promote the expression of *ced-3* in the tail-spike cell just before the onset of cell death (Fig. 1E) and presents a novel mode of cell death specification in *C. elegans*.

Important questions remain regarding how tail-spike cell death is regulated. As overexpression of *ced-3* is not an efficient way to activate cell death,[13] how does *ced-3* upregulation in the tail-spike cell result in efficient cell killing? Genetic analysis indicates that *ced-4* is absolutely required and *egl-1* is partially required for the tail-spike cell death, raising the important question of if and how *ced-4* and *egl-1* are regulated in the tail-spike cells and how *ced-4*, *egl-1* and *ced-3* upregulation cooperate to promote tail-spike cell death. It will be interesting to find out if this mode of cell death specification is also used in other cell types. Finally, PAL-1 is widely expressed during *C. elegans* development and this expression pattern includes many cells that do not die. This observation suggests that additional factors promoting tail-spike cell death or inhibiting PAL-1-induced cell death in other cells remain to be identified.

Sex-specific programmed cell death. *C. elegans* has two sexes, males and self-reproducing hermaphrodites, which exhibit sexually dimorphic cell death events.[23,24] The *C. elegans* sex-determination pathway plays an important role in determining the sexual identity of these cells, leading to appropriate cell death specification.[20,29,30,41,42] Sex determination is an extensively studied pathway that is initiated by X chromosome counting. The X to autosome ratio influences sex determination and dosage compensation through regulation of factors common to both pathways (reviewed in refs. 43 and 44). The sex determination pathway leads to cell non-autonomous regulation of TRA-1, the terminal sex determination transcription factor in *C. elegans*.[45,46] The *tra-1* gene produces two products: TRA-1A that has five zinc fingers and DNA binding activity and TRA-1B with only two zinc fingers and no DNA binding activity.[47,48] TRA-1A is critical for sex-determination and acts to promote hermaphrodite development by repressing genes required for male somatic development.[20,29,30,49]

HSNs (male-specific cell death). The HSNs are two serotonergic motor neurons that innervate vulval muscle and stimulate hermaphrodite egg laying. These neurons are born in both male and hermaphrodite embryos but undergo programmed cell death during male embryonic development since they are not required in males.[24] In mutants where HSNs are absent or non-functional, hermaphrodites adopt the Egl (egg laying defective) phenotype—eggs are not efficiently laid and progeny hatch within the mother.[50-52]

One of the first leads into the regulation of sex-specific cell death events was the identification of *egl-1* through several gain-of-function alleles that result in the Egl phenotype.[2,20] Seven *egl-1* gain-of-function mutations alter and disrupt a conserved TRA-1A binding site just over 6.4 kb downstream of the *egl-1* start codon. Characterization of transgenic animals expressing GFP under the control of the *egl-1* promoter demonstrated male-specific GFP expression in the HSNs. However when the gain-of-function lesion is introduced into the GFP reporter, GFP expression is no longer limited to male HSNs

and is also seen in the HSNs of hermaphrodites. Inappropriate expression of *egl-1* in hermaphrodite HSNs due to *egl-1* gain-of-function mutations or reduced *tra-1* activity leads to their ectopic death. These data along with TRA-1A in vitro binding assays suggest that TRA-1A acts to transcriptionally repress *egl-1* expression in the HSNs of developing hermaphrodites (Fig. 1F).

Hoeppner et al., shed additional light on HSN cell death specification when they performed *egl-1(gf)* suppressor screens.[28] In these screens they searched for mutations that suppressed the *egl-1(gf)* phenotype and identified general suppressors of cell death (*ced-3* and *ced-4*) as well as HSN-specific cell death suppressors, *eor-1* and *eor-2*. Mutations in *eor-1* and *eor-2* suppress HSN death in males as well as inappropriate HSN death in *egl-1(gf)* or masculinized hermaphrodites. These genes act upstream of *ced-9* based on the epistasis analysis, however, they do not have any effect on *egl-1* reporter expression in male HSNs. EOR-1 is a zinc finger transcription factor closely related to human PLZF, while EOR-2 is a novel protein. Both genes are widely expressed during early embryonic development and an *eor-2* reporter is expressed in the HSNs during larval development. Since *eor-1* and *eor-2* do not appear to affect *egl-1* transcription in the HSNs, their role in HSN cell death specification remains a topic for future study.

Several important questions remain to be addressed regarding the proper cell death specification of the HSNs. Since TRA-1A is active in all somatic cells of XX animals but only affects HSN cell death, it cannot be a general *egl-1* transcriptional inhibitor. One likely model is that TRA-1A specifically acts to repress the activity of a HSN-specific *egl-1* transcription activator. In a developing XO animal, there is no active TRA-1A in the HSNs and the putative HSN-specific *egl-1* activator is free to bind the *egl-1* promoter, stimulate the transcription of *egl-1,* and trigger programmed cell death. However, in an XX animal, TRA-1A is active and can associate with the TRA-1A binding site 6.4 kb downstream of the *egl-1* start codon. TRA-1A binding to this site could antagonize the activity of the putative HSN-specific *egl-1* activator, leading to *egl-1* transcriptional silencing. Further characterization of factors regulating HSN life and death is required before we fully understand this process.

CEMs (hermaphrodite-specific cell death). The CEMs are a set of four chemosensory cells in the head of male animals. These cells undergo programmed cell death in developing hermaphrodite embryos where they are not required.[24] The CEMs play a role in male chemotaxis towards hermaphrodites during courtship[53] but their absence in males or presence in hermaphrodites causes no obvious morphological phenotypes. Two recent papers, 29 and 30, have identified *ceh-30*, a Bar homeodomain transcription factor, as an important gene that regulates CEM cell death specification.

In a genetic screen to identify factors required for the proper cell death specification of CEMs, a partial loss-of-function allele (*sm130)* of *ceh-30* was isolated that leads to inappropriate CEM cell death in males.[29] In a similar screen, Schwartz and Horvitz identified three *ceh-30* gain-of-function alleles that result in improper CEM survival in hermaphrodites.[30] Interestingly, each of these mutations affect conserved non-coding regions in the second intron of the *ceh-30* gene. The gain-of-function alleles disrupt a conserved TRA-1A binding site, while the loss-of-function allele alters a putative UNC-86 (POU-domain homeoprotein) binding site that is less than one hundred base pairs upstream of the TRA-1A site. Biochemical and genetic analyses indicate that both TRA-1A and UNC-86 bind to the second intron of *ceh-30* and interact to control the expression of the anti-apoptotic *ceh-30* gene in CEMs. In males, TRA-1A is not active and UNC-86, which specifies the CEM cell fate, activates the transcription of *ceh-30* that is essential for CEM survival. In hermaphrodites, TRA-1A is active and its binding to the second intron of *ceh-30* antagonizes the activity of UNC-86 and represses the expression of *ceh-30* in CEMs. Therefore, the regulatory sequences found within the second intron of *ceh-30* define an on/off switch that responds to the sexual signal to regulate sex-specific transcription of the CEM protective gene *ceh-30* (Fig. 1G).

Peden et al., also identified a novel protein interaction between CEH-30 and the transcriptional repressor UNC-37, the *C. elegans* Groucho homolog.[54] This interaction is mediated by a FIL/eh1 motif near the N-terminus of the CEH-30 protein. The CEH-30 FIL/eh1 motif and *unc-37* are required to prevent inappropriate CEM cell death in males. These data suggests that within the CEMs a transcriptional repressosome containing CEH-30 and UNC-37 inhibits programmed cell death (Fig. 1G). The target(s) of CEH-30/UNC-37 regulation were not identified and remain an important topic of future study.

Interestingly, Schwartz and Horvitz present genetic evidence that CEM cell death specification may not be regulated at the level of *egl-1* transcription. Rather, CEM cell death appears to be specified by factors acting in parallel to or downstream of *ced-9*, which perhaps modulate CEM sensitivity to cell death. As stated above, *ced-9(lf)* animals exhibit embryonic lethality due to widespread programmed cell death. In order to study the effect of *ced-9(lf),* the worms must be maintained with a *ced-3* reduction-of-function (rf) allele, which suppresses *ced-9(lf)* lethality and allows for survival.[4] In these experiments, *ced-9(lf); ced-3(rf)* animals exhibit relatively normal CEM cell death specification in both males and hermaphrodites. In *ced-9(lf); ced-3(rf); ceh-30(gf)* hermaphrodites, many CEMs still survive, suggesting that *ced-9* is dispensable for protection of CEMs against cell death mediated by *ceh-30(gf)*. Likewise *ced-9(lf); ced-3(rf); ceh-30(lf)* males are missing CEMs as in *ceh-30(lf)* males and inclusion of the *egl-1(lf)* allele to this background does not reduce CEM cell death. These results suggest that *egl-1* cannot affect CEM cell death in the absence of *ced-9* and that CEM cell death specification may also involve a regulatory mechanism independent of both *ced-9* and *egl-1*. However, *egl-1(lf)* mutations fully suppress ectopic CEM cell death in *ceh-30(lf)* males, indicating that *egl-1* is required for proper CEM cell death specification. In addition, *egl-1(lf)* alleles almost completely suppress CEM cell death in hermaphrodites. This is in contrast to tail-spike cell death where *egl-1(lf)* mutations only partially suppress the death event.[27] It will be interesting to find out whether *ced-3* or *ced-4* is a target of *ceh-30* transcription repression in the CEMs. This might be a difficult task, since the CEH-30 homeodomain is not required for proper CEM cell death specification[29] and other DNA binding protein(s) may be needed to direct the CEH-30/UNC-37 repressosome to its appropriate targets.

Conclusion

To date, studies of cell death specification in *C. elegans* indicate that the majority of death events are specified, as expected, by the transcriptional upregulation of the pro-apoptotic gene *egl-1*. For the death of NSM sister cells,[22] the ventral cord cell P11.aaap,[21]

and the HSNs,[20] direct regulators of *egl-1* transcription and their corresponding binding sites within the *egl-1* promoter have been identified (CES-1 and HLH-2/HLH-3 for the NSM sisters; CEH-20 and MAB-5 for P11.aaap; and TRA-1A for the HSNs). One of the striking findings regarding the *egl-1* gene is the small size of its coding region and the large size of its promoter containing multiple important *cis*-regulatory elements. Enhancer and repressor sites for the *egl-1* gene have been identified more than 6.4 kb downstream from the start codon. It seems likely that cell-type specific enhancer/repressor sites are distributed throughout this region. Full characterization of the *egl-1* promoter is critical for comprehensive understanding of cell death specification in *C. elegans* and may provide novel insight into transcriptional regulation in general.

Although the importance of *egl-1* regulation in cell death specification cannot be overemphasized, there are examples of *egl-1*-independent cell death specification. The tail-spike cell is targeted for death by the transcriptional upregulation of *ced-3*.[27] This mode of cell death specification raises important mechanistic questions. How is CED-3 auto-activation accomplished without EGL-1-induced release of CED-4 from CED-9 at the mitochondria? Is CED-4 transcriptionally upregulated in the dying tail-spike cell? Similar questions apply regarding how *ceh-30* might regulate CEM sex-specific cell death in an *egl-1* and *ced-9* independent manner.[30]

The specification of many other *C. elegans* cell death events remains unknown. More efforts obviously are required to determine the modes by which these cells are specified for death. *C. elegans* is an ideal system for dissecting the complex process by which individual cells or cell types are specified for death.

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