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SPK-1, an SR protein kinase, inhibits programmed cell death in *Caenorhabditis elegans*

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To identify genes involved in protecting cells from programmed cell death in *Caenorhabditis elegans*, we performed a genetic screen to isolate mutations that cause an increase in the number of programmed cell deaths. We screened for suppressors of the cell-death defect caused by a partial loss-of-function mutation in *ced-4*, which encodes an Apaf-1 homolog that promotes programmed cell death by activating the caspase CED-3. We identified one extragenic *ced-4* suppressor, which has a mutation in the gene *spk-1*. The *spk-1* gene encodes a protein homologous to serine-arginine-rich (SR) protein kinases, which are thought to regulate splicing. Previous work suggests that *ced-4* can be alternatively spliced and that the splice variants function oppositely, with the longer transcript (*ced-4L*) inhibiting programmed cell death. *spk-1* might promote cell survival by increasing the amount of the protective *ced-4L* splice variant. We conclude that programmed cell death in *C. elegans* is regulated by an alternative splicing event controlled by the SR protein kinase SPK-1.

apoptosis | RNA splicing

Multicellular organisms eliminate unneeded or harmful cells by the process of programmed cell death or apoptosis. Apoptosis plays a fundamental role in animal development and tissue homeostasis as well as in human health (1, 2). Improper regulation of apoptosis is associated with disease, and defects in apoptosis that cause either too much death or too little have been implicated in cancers, neurodegenerative diseases, autoimmune diseases, and many other disorders (1).

Mechanisms of programmed cell death have been elucidated in part through studies of the nematode *Caenorhabditis elegans*. During the development of the *C. elegans* hermaphrodite, 1,090 somatic cells are generated by an essentially invariant pattern of divisions (3–5). Of these cells, 131 undergo programmed cell death (4, 5). At least 20 genes have been identified that regulate either the pattern or the process of programmed cell death in *C. elegans* (6). The core pathway of programmed cell death in *C. elegans* is composed of four genes: *egl-1*, *ced-9*, *ced-4*, and *ced-3*. The cloning of these four genes defined a core molecular pathway for programmed cell death later shown to be conserved in vertebrates (7–11).

egl-1, *ced-9*, *ced-4*, and *ced-3* act within dying cells to control nearly all somatic programmed cell deaths in *C. elegans* (10, 12, 13); loss-of-function mutations in *egl-1*, *ced-4*, or *ced-3* or a gain-of-function mutation in *ced-9* prevent essentially all somatic programmed cell deaths (7, 10, 14). CED-3 is a defining member of the caspase (cysteine aspartate-specific protease) family (9). The identification of *ced-3* as encoding a protease led to the discovery that caspases are required for many apoptotic cell deaths in mammals (15). *ced-4* facilitates CED-3 activation (16–19), and its human homolog Apaf-1 (apoptotic protease activating factor) was identified as a factor responsible for caspase activation in vitro (8, 20). Cells are protected from programmed cell death by *ced-9* (7), which encodes a member of the Bcl-2 family of apoptotic regulators (21). Human Bcl-2 can block programmed cell death in *C. elegans* and can substitute for *ced-9*, demonstrating a functional conservation (21, 22). Finally, *egl-1* encodes a BH3-only protein (defined as a Bcl-2 family member lacking obvious BH1, BH2, and BH4 domains) that negatively regulates CED-9 to drive cells to undergo programmed cell death (10, 23–25). Mammalian BH3-only proteins are similarly proapoptotic. Transcriptional control of *egl-1* is likely to be the major mechanism of the cell-specific regulation of programmed cell death (10, 24–28).

ced-4 encodes two transcripts, *ced-4L* and *ced-4S*, that appear to have opposing functions (29). Whereas *ced-4S* (the predominant transcript) promotes cell death, *ced-4L* protects cells from death. The observation that *ced-4* has two functionally distinct splice variants originated from the identification of a 72-bp DNA sequence in the *ced-4* genomic locus not contained within the originally described *ced-4* cDNA. This element is highly conserved in two *Caenorhabditis* species, *C. briggsae* and *C. remanei*, that are distant relatives of *C. elegans* (29), suggesting that this element is functionally important. Further studies revealed that this element is contained within an alternative *ced-4* mRNA isoform, *ced-4L*. Overexpression of the CED-4L protein in *C. elegans* can protect cells against programmed cell death (29). The antiapoptotic protective effect of *ced-4* has also been observed in a sensitized genetic background: Whereas *egl-1(gf)*/+ animals display a 60% penetrant defect in egg laying (because of the ectopic deaths of the HSN neurons), *egl-1(gf)*/+ ; *ced-4(null)*/+ animals display an 85% penetrant defect in egg laying, demonstrating that the loss of *ced-4* function can increase cell death and, hence, that *ced-4* has a protective function (30). Structural and biochemical studies suggest that CED-4L prevents the formation of the CED-4 octamer, which is required for activation of CED-3 and the initiation of programmed cell death (31, 32).

In addition to *ced-4*, ≈2,500 genes in *C. elegans* (≈13% of the genes annotated in Wormbase; www.wormbase.org) are predicted to be alternatively spliced (33). Splicing is a highly regulated process that removes intronic fragments of RNA from newly transcribed pre-mRNA molecules. The spliceosome, which is composed of small nuclear ribonucleoprotein particles and proteins, performs splicing (34). Two families of proteins that assist the spliceosome in splice-site selection are the heteronuclear ribonucleoproteins (hnRNPs) and the serine-arginine-rich proteins (SR proteins) (35). These proteins are thought to function oppositely, with SR proteins binding to exonic splicing enhancer elements and promoting the inclusion of exons, and hnRNPs binding to exonic splicing silencer elements and suppressing the inclusion of exons. The SR proteins are regulated by kinases that phosphorylate serines within their SR domains, which are thought to mediate protein–protein interactions (36, 37). In this study, we describe a possible role for an SR protein

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kinase in the alternative splicing of *ced-4* RNA and the regulation of programmed cell death in *C. elegans*.

Results

A Screen for Genes That Function to Prevent Programmed Cell Death. The study of programmed cell death in *C. elegans* has primarily focused on two types of mutants: those with extra cells and those with either abnormal or persistent cell corpses. By contrast, we chose to screen for mutants with fewer cells, i.e., increased cell death, and sought mutations that increase the number of programmed cell deaths. To assay cell death, we examined the ventral nerve cord. During development, the 12 P cell neuroblasts P1–P12 generate a total of nine cells that undergo programmed cell death in the ventral cord (4). We used worms expressing the *lin-11::gfp* reporter *nIs106* to visualize a subset of these deaths, the Pn.aap cells (the posterior daughters of the anterior daughters of the anterior daughters of the 12 P blast cells) (38). In the ventral cord of wild-type animals, six Pn.aap cells survive and express *gfp* (P3.aap–P8.aap), whereas the other six undergo programmed cell death (the two anterior-most, P1.aap and P2.aap, and the four posterior-most, P9–P12.aap) (4). By contrast, in mutants defective in cell death (e.g., animals with strong loss-of-function mutations in *ced-3*) five extra Pn.aap cells survive and reliably express *gfp*, generating a total of 11 GFP-positive cells. The most anterior cell, P1.aap, does not reliably express *gfp* and, thus, was not scored in our experiments (38). The survival of Pn.aap cells can be easily monitored in strains carrying the *lin-11::gfp* reporter by using a fluorescence-equipped dissecting microscope.

To identify genes with subtle protective roles in programmed cell death, we screened for mutations that cause an increase in programmed cell death in a sensitized genetic background. Worms containing a nonnull allele of *ced-4*, such as *n3158*, are partially defective in programmed cell death. *n3158* is an early missense mutation that changes serine 163 to phenylalanine (39). On average, these animals have 4.9 extra GFP-positive Pn.aap cells (i.e., 11, compared with 6 in wild-type animals). Although *n3158* thus strongly prevents the programmed cell deaths of the Pn.aap cells, it is an allele of only medium strength as assayed for survival of cells fated to die in the anterior pharynx, an independent quantitative assay for programmed cell death (39). We observed that *ced-4(n3158)* animals have an average of 2.7 extra cells in the anterior pharynx (Table S1), whereas animals carrying a null allele of *ced-4* have an average of 13.9 extra cells (39).

Using the *lin-11::gfp* reporter, we screened for suppressors of the partial loss-of-function *ced-4(n3158)* mutation by seeking mutants with a reduced number of GFP-positive Pn.aap cells (i.e., fewer than the 11 typically found in these mutants) (Fig. S1). The screen was performed such that mutations that cause sterility or maternal-effect lethality could be isolated, because mutants with increased programmed cell death might not produce viable progeny. For example, mutations in *ced-9*, which protects against cell death, cause sterility and maternal-effect lethality (7). Thus, we saved siblings of each screen isolate so that animals heterozygous for mutations in such an essential gene would be maintained. From a screen of 5,000 mutagenized haploid genomes, we isolated a strong extragenic suppressor, *n3418*. In addition to recessively reducing the number of GFP-positive Pn.aap cells, this mutation caused recessive sterility. The decrease in the number of GFP-positive cells in the *ced-4(n3158) n3418* double mutant was not a consequence of a defect in the generation of the Pn.aap cells, because the GFP-positive cells were completely restored by the loss of function of the *ced-3* gene (*ced-4(n3158) n3418; ced-3(n3692Δ); nIs106*; $n = 25$), which is required for programmed cell death. This observation indicates that P2.aap and P9–P12.aap are generated as in wild-type animals but are more likely to undergo programmed cell death in the *ced-4(n3158) n3418* strain than in *ced-4(n3158)* animals. The *ced-3* null allele failed to suppress the sterility conferred by *n3418*, suggesting the sterility was not caused by excessive programmed cell death (*ced-4(n3158) n3418; ced-3 (n3692Δ); nIs106; n* = 25).

To determine whether suppression of *ced-4(n3158)* was specific to the Pn.aap cells, we tested the ability of *n3418* to suppress the cell-death defect in the anterior pharynges of *ced-4(n3158)* animals, which have on average 2.7 extra cells per anterior pharynx. We observed that *ced-4(n3158) n3418* double mutants have on average only 1.4 extra cells per anterior pharynx (Table S1). This result indicated the suppression of *ced-4(n3158)* by *n3418* is not limited to Pn.aap cells and that the gene defined by *n3418* has protective function in both embryonic and post-embryonic deaths.

***n3418* Is a Loss-of-Function Allele of *spk-1*, Which Encodes an SR Protein Kinase.** Using a combination of visible phenotypic markers, deficiencies, and polymorphisms, we mapped the suppressor mutation *n3418* to a 90-kb region of linkage group III containing 24 genes (Fig. 1). Database searches for information about these genes revealed that both a deletion allele of and RNAi knockdown of one of these genes, *spk-1*, caused a sterility resembling that observed in *ced-4(n3158) n3418; nIs106* animals (40, 41). We identified a mutation that changes tryptophan 142 (TGG) to an opal stop codon (TGA) in the predicted ORF of *spk-1* in *n3418* animals (Fig. 1). A deletion allele of *spk-1*, *ok706*, failed to complement the sterility of *n3418* ($n = 25$). Based on these observations, we concluded that the sterility of *n3418* was caused by the mutation in *spk-1*. To determine whether loss of *spk-1* function also suppressed the defect in programmed cell death caused by *ced-4(n3158)*, we tested the effect of the *spk-1 (ok706Δ)* deletion allele in *ced-4(n3158)* animals. *spk-1(ok706Δ)* caused suppression of the cell-death defect in *ced-4(n3158)* animals to a similar extent as did *spk-1(n3418)* (Table 1). Together, these results indicate that both the suppression of *ced-4 (n3158)* and the sterility displayed by our suppressor mutant are caused by *n3418*, a loss-of-function mutation in *spk-1*.

spk-1 encodes an SR protein kinase. The SR protein kinase family regulates alternative splicing by phosphorylating SR proteins, which are thought to be key regulators of splicing (35). Animals carrying only a loss-of-function allele of *spk-1* (either *n3418* or *ok706Δ*) in an otherwise wild-type genetic background had the wild-type number of Pn.aap cells (Table 1), showing that

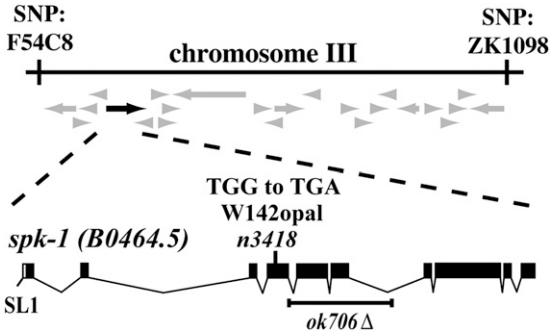

Fig. 1. Molecular identification of the *ced-4(n3158)* suppressor *spk-1 (n3418).* Schematic representation of the physical map of the *spk-1(n3418)* region and the gene structure of *spk-1*, showing the location of the allele *n3418* and the deletion allele *ok706Δ*. We mapped *spk-1(n3418)* to an interval containing 24 genes. RNAi against one gene in the interval, *B0464.5*, caused sterility resembling that of *spk-1(n3418)* (40). We determined the sequence of all exons and splice junctions of *spk-1* in the *n3418* animals and identified a nonsense mutation that changes tryptophan 142 to an opal stop codon.

Table 1. Loss-of-function alleles of *spk-1* suppress the survival of extra Pn.aap cells in *ced-4(n3158)* animals

Genotype*	No. of extra Pn.aap cells ± SE (*n*)
Wild type	0 (109)
ced-4(n3158)	4.9 ± 0.04 (50)
ced-4(n3158) spk-1(n3418)	2.0 ± 0.1 (71)
ced-4(n3158) spk-1(ok706Δ)	1.3 ± 0.1 (57)
spk-1(n3418)	0 (56)
spk-1(ok706Δ)	0 (37)

The number of extra Pn.aap cells were counted as described in *Materials and Methods*. Because *spk-1(n3418)* and *spk-1(ok706Δ)* cause recessive sterility, strains containing these alleles were derived from heterozygous parents. *n*, number of animals.
*All strains are homozygous for *nIs106*, an integrated *lin-11::gfp* reporter (38).

loss of *spk-1* does not cause ectopic Pn.aap programmed cell death in these animals.

***spk-1(n3418)* Strongly Suppresses Partial Loss-of-Function Alleles of *ced-4*.** To determine whether the loss of *spk-1* function increased death specifically in strains homozygous for the allele *ced-4 (n3158)*, we tested the ability of *spk-1(n3418)* to suppress other alleles of *ced-4* (Table 2). *spk-1(n3418)* also suppressed the cell-death defect of animals carrying *ced-4(n3141)*, which is a slightly weaker allele than *ced-4(n3158)*, the allele used in our screen. The allele *n3141* is a missense mutation that changes arginine 53 to lysine (39). Thus, *spk-1(n3418)* suppressed two partial loss-of-function alleles of *ced-4*. However, *spk-1(n3418)* did not suppress *ced-4* null alleles. Specifically, *spk-1(n3418)* failed to suppress either the strong missense allele *ced-4(n3040)* or the early nonsense allele *ced-4(n1162)*, both of which cause the complete survival of the Pn.aap cells and of all of the cells that normally die in the anterior pharynx (39).

To determine whether suppression by *spk-1* was specific to the *ced-4* gene, we tested the ability of *spk-1(n3418)* to suppress alleles of the three other genes that function in the killing step of programmed cell death. We assayed *ced-3*, *ced-9*, and *egl-1* alleles that cause a cell-death defect similar in strength to that caused by *ced-4(n3158)* and *ced-4(n3141)* in the Pn.aap cells. We observed that *spk-1(n3418)* can weakly suppress the cell-death defect observed in strains homozygous for alleles of *ced-9*, or *ced-3*, but, interestingly, not *egl-1*, the most upstream gene in the execution pathway (Table 3). Thus, *spk-1(n3418)* most strongly modified the cell-death defect of partial loss-of-function alleles of *ced-4*.

Table 2. *spk-1(n3418)* suppresses the cell-death defect of partial, but not complete, loss-of-function alleles of *ced-4*

Genotype*	No. of extra Pn.aap cells ± SE (*n*)
ced-4(n3141)	4.6 ± 0.1 (50)
ced-4(n3141) spk-1(n3418)†	1.2 ± 0.1 (55)
ced-4(n3158)	4.9 ± 0.04 (50)
ced-4(n3158) spk-1(n3418)	2.0 ± 0.1 (71)
ced-4(n3040)	5.0 ± 0 (52)
ced-4(n3040) spk-1(n3418)†	5.0 ± 0 (50)
ced-4(n1162)	4.9 ± 0.03 (50)
ced-4(n1162) spk-1(n3418)†	4.9 ± 0.04 (58)

The number of extra Pn.aap cells were counted as described in *Materials and Methods*. Because *spk-1(n3418)* causes recessive sterility, strains containing this allele were derived from heterozygous parents. *n*, number of animals.
*All strains used to count extra Pn.aap cells are homozygous for *nIs106*, an integrated *lin-11::gfp* reporter (38).
†*spk-1(n3418)* was *cis*-marked with *lon-1(e185)* in each of these strains to facilitate identification of homozygous animals.

Table 3. *spk-1(n3418)* weakly suppresses the survival of extra Pn.aap cells caused by partial loss-of-function alleles of some additional Ced genes

Genotype*	No. of extra Pn.aap cells ± SE (*n*)
ced-3(n2443)	0.9 ± 0.1 (50)
spk-1(n3418); ced-3(n2443)†	0.3 ± 0.1 (52)
ced-3(n2427)	3.4 ± 0.2 (50)
spk-1(n3418); ced-3(n2427)†	1.2 ± 0.1 (60)
ced-3(n2436)	4.8 ± 0.1 (50)
spk-1(n3418); ced-3(n2436)†	4.0 ± 0.1 (52)
ced-9(n3377)	2.3 ± 0.1 (50)
spk-1(n3418) ced-9(n3377)†	1.6 ± 0.1 (67)
egl-1(n4045)	3.8 ± 0.1 (50)
spk-1(n3418); egl-1(n4045)†	3.6 ± 0.1 (60)
egl-1(n3331)	4.7 ± 0.1 (50)
spk-1(n3418); egl-1(n3331)†	4.5 ± 0.1 (48)

The number of extra Pn.aap cells were counted as described in *Materials and Methods*. Because *spk-1(n3418)* causes recessive sterility, strains containing this allele were derived from heterozygous parents. *n*, number of animals.
*All strains are homozygous for *nIs106*, an integrated *lin-11::gfp* reporter, except those containing *egl-1(n3331),* which are homozygous for the related reporter *nIs96* (38).
†*spk-1(n3418)* was *cis*-marked with *lon-1(e185)* in each of these strains to facilitate identification of homozygous animals.

***spk-1* Might Regulate the Alternative Splicing of *ced-4*.** Because *spk-1* most strongly suppressed *ced-4*, we hypothesized that *spk-1* might have a specific interaction with *ced-4*. This hypothesis is attractive given that SR protein kinases are thought to regulate alternative splicing, and *ced-4* is the only *C. elegans* cell-death gene known to be regulated by alternative splicing (29, 35). *ced-4* encodes two different transcripts that can function oppositely in programmed cell death: the shorter *ced-4* splice product, *ced-4S*, promotes programmed cell death, whereas the longer product, *ced-4L*, inhibits programmed cell death (29).

To test whether *spk-1* inhibits programmed cell death by modifying the splicing of *ced-4*, we asked whether an *spk-1* mutation can suppress an allele of *ced-4* that is not properly spliced. The *ced-4(n2273)* mutation is located 5′ of the *ced-4S* splice acceptor site and disrupts *ced-4* splicing (29). We hypothesized that if *spk-1* exerts its effect on *ced-4* by modifying splicing, then the perturbed splicing in *ced-4(n2273)* mutants might not be modified by a loss of *spk-1* function. We observed that the cell-death defect of *ced-4(n2273)* mutants is not modified by the loss of *spk-1* function (Table 4). *ced-4(n2273)* is the only partial-loss-of-function allele of *ced-4* we tested that is not suppressed by the loss of *spk-1* function. This result suggests that *spk-1* affects programmed cell death by regulating the splicing of *ced-4*.

Table 4. *spk-1(n3418)* does not suppress the cell-death defect of *ced-4(n2273)*

Genotype*	No. of extra Pn.aap cells ± SE (*n*)
ced-4(n2273)	1.3 ± 0.1 (28)
ced-4(n2273) spk-1(n3418)	1.2 ± 0.1 (55)

The number of extra Pn.aap cells were counted as described in *Materials and Methods*. Because *spk-1(n3418)* causes recessive sterility, strains containing this allele were derived from heterozygous parents. *n*, number of animals.
*All strains used to count extra Pn.aap cells are homozygous for *nIs106*, an integrated *lin-11::gfp* reporter (38).
†*spk-1(n3418)* was *cis*-marked with *lon-1(e185)* in each of these strains to facilitate identification of homozygous animals.

Discussion

From a genetic screen to identify regulators of programmed cell death in *C. elegans*, we isolated a mutation in the splicing regulator *spk-1*, which encodes an SR protein kinase. Loss-of-function alleles of *spk-1* increase the amount of cell death that occurs in strains containing partial loss-of-function alleles of *ced-4* and also weakly increase cell death in animals with mutations in *ced-3* or *ced-9*. We conclude that SPK-1 promotes cell survival.

We suggest that the SPK-1 SR protein kinase regulates the splicing of *ced-4* to modulate programmed cell death. We propose a model in which SPK-1 normally promotes the generation of *ced-4L* transcripts that protect cells from dying and that the loss of *spk-1* function decreases the level of *ced-4L* transcript, thereby increasing the likelihood that cells will die (Fig. 2). We attempted to compare the levels of *ced-4L* transcript in wild-type and *spk-1* animals. However, because of the low levels of *ced-4L* expression and the sterility of the *spk-1* mutants, we were unable to quantify *ced-4L* levels by RT-PCR or to isolate sufficient amounts of RNA to perform northern blot experiments. Additionally, it is possible that *spk-1* affects *ced-4* splicing in only a subset of cells, which would make it difficult to detect differences in isoform abundance in RNA preparations from whole animals.

We suspect that SPK-1 functions cell autonomously to control *ced-4* splicing, because CED-4L protects against cell death by disrupting the ability of CED-4S to activate CED-3 (42), both of which are required in dying cells (12); however, it is also possible that *spk-1* functions cell nonautonomously. SPK-1 might control *ced-4* splicing by regulating the phosphorylation of SR proteins. In support of this hypothesis, we observed that alleles of *rsp-3*, *rsp-6*, and *rsp-8* partially suppressed the cell-death defect of *ced-4 (n3158)* (Table 5). However, eliminating the function of no single SR protein in *C. elegans* suppressed the cell-death defect of *ced-4(n3158)* to the same extent as eliminating *spk-1*, suggesting that a number of SR proteins might function redundantly in regulating programmed cell death (Table 5).

Although there is increased cell death in *spk-1(lf) ced-4(lf)* animals, we have not observed ectopic cell deaths in *spk-1* null animals with otherwise wild-type genetic backgrounds. One possible explanation is that the regulation of *ced-4* splicing is one of several determinants of whether cells live or die. The pathway for programmed cell death in *C. elegans* generally has been described as linear: EGL-1 promotes cell death by inhibiting the function of CED-9 (10) and, in the absence of EGL-1, CED-9 prevents activation by CED-4 of the caspase CED-3 (11). However, it has been observed that cell death occurs in a relatively normal pattern in *ced-9(null)*; *ced-3* partial loss-of-function animals (43). A linear model in which EGL-1 is essential for programmed cell death and functions only through CED-9 would predict that cell death would not occur normally in the absence of CED-9 (10). Thus, there must be additional mechanisms that regulate programmed cell death independently of *egl-1*. Perhaps, the alternative splicing of *ced-4* is one such mechanism that contributes to, but is not essential for, the decision of cells to live or die.

spk-1 is likely required for the embryonic development of *C. elegans*, because RNAi with a high concentration of *spk-1* dsRNA causes embryonic lethality (40, 41). If so, why are *spk-1*

Table 5. Loss-of-function alleles of *rsp* genes, which encode SR proteins, might weakly suppress the survival of extra Pn.aap cells in *ced-4(n3158)* animals

Genotype*	No. of extra Pn.aap cells ± SE (*n*)
Wild type	0 (109)
ced-4(n3158)	4.9 ± 0.04 (50)
rsp-1(Δ); ced-4(n3158)	4.6 ± 0.1 (21)
rsp-2(Δ); ced-4(n3158)	4.5 ± 0.1 (50)
ced-4(n3158) rsp-3(Δ)	3.5 ± 0.2 (19)
rsp-4(Δ); ced-4(n3158)	4.5 ± 0.1 (50)
rsp-5(Δ); ced-4(n3158)	4.5 ± 0.1 (50)
ced-4(n3158); rsp-6(Δ)	3.7 ± 0.2 (50)
rsp-7(Δ); ced-4(n3158)	NA
ced-4(n3158) rsp-8(Δ)	3.9 ± 0.2 (36)

The number of extra Pn.aap cells were counted as described in *Materials and Methods*. *n*, number of animals. NA, Not applicable because the animals died before *lin-11::gfp* expression.
*All strains are homozygous for *nIs106*, an integrated *lin-11::gfp* reporter (38).

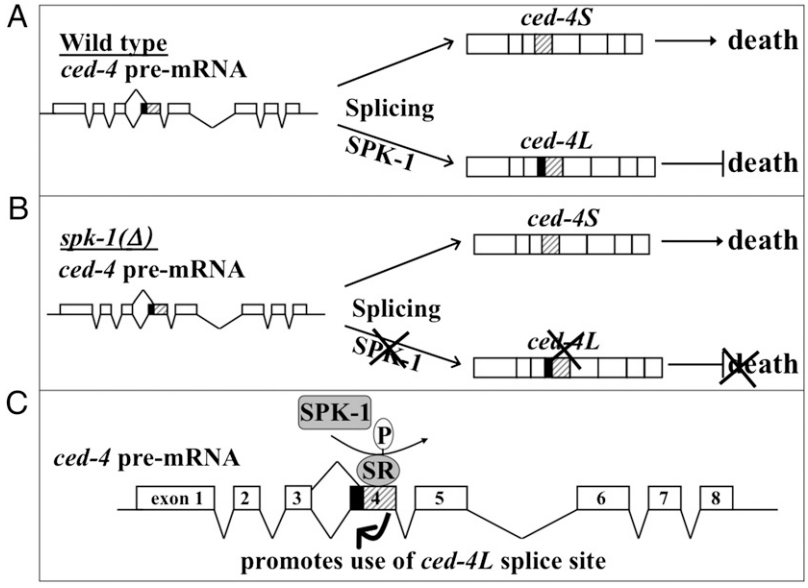

Fig. 2. A model for the regulation of *ced-4* RNA splicing and programmed cell death by SPK-1. (*A*) In wild-type animals, SPK-1 promotes the generation of the protective *ced-4L* alternative splice variant, which protects against programmed cell death. (*B*) A loss of SPK-1 decreases the production of *ced-4L*, thereby decreasing protection against programmed cell death. (*C*) SPK-1 might promote the use of the *ced-4L* splice site in the *ced-4* premRNA by phosphorylating an SR protein.

null homozygotes viable and sterile? It is possible that these animals are sustained by SPK-1 provided by their heterozygous mothers. Maternal stores of *spk-1* mRNA might be sufficient to keep the homozygous progeny of heterozygous mothers alive but not sufficient to inhibit all embryonic or Pn.aap cell deaths or to allow the gonad to develop normally. The sterility caused by *spk-1* alleles is not a consequence of inappropriately increased cell death, because *spk-1; ced-3(null)* mutant animals, in which essentially all cell deaths are blocked, were still sterile. We conclude that *spk-1* is essential for processes in addition to programmed cell death.

ced-4 is the only gene in *C. elegans* thought to produce alternative splice variants with opposing roles in programmed cell death (29). By contrast, in mammals, alternative splice forms have been described for many regulators of apoptosis, although the biological significance of these various isoforms remains largely unknown (44). For example, the gene *caspase-2*/*Ich-1* produces functionally distinct alternative splice variants (45). The long *caspase-2* transcript promotes apoptosis, whereas the short transcript protects cells from death. The alternative splicing of mammalian *caspase-2* can be influenced by SR proteins and/or SR protein kinases. Studies in tissue culture of a *caspase-2* minigene, a plasmid construct consisting of a small portion of the genomic locus surrounding the introns involved in splicing, suggest that overexpression of the SR proteins SC35 and ASF/SF2 promotes the skipping of a specific exon and the generation of the proapoptotic isoform *caspase-2L* (46). Conversely, overexpression of hnRNP A1 promotes inclusion of this exon (which results in an earlier stop codon) and the generation of the antiapoptotic *caspase-2S* isoform (46).

Whereas these studies of *caspase-2* present evidence that SR proteins can regulate the alternative splicing of genes involved in programmed cell death, the conclusions of these studies are opposite to the established roles of SR proteins and hnRNPs in the promotion of exonic inclusion and exclusion, respectively. As described above, SR proteins normally promote inclusion of exons, whereas hnRNPs play the opposite role. One explanation for this discrepancy might be that SR proteins are not properly regulated by phosphorylation when they are overexpressed, as they were in the *caspase-2* studies. Genetic studies of the *Drosophila* SR protein kinase gene *Doa* in *Drosophila* suggest that phosphorylated SR proteins promote the inclusion of exons (47). If we assume that SPK-1 phosphorylates SR proteins in *C. elegans* in vivo as it does in vitro (40), then our predictions are consistent with the *Drosophila* findings that suggest the phosphorylation of SR proteins promotes the inclusion of exons. We predict that in *C. elegans*, the incorporation of the *ced-4L* exon decreases when *spk-1* is reduced, just as in *Drosophila* the incorporation of the female-specific exon of *dsx* decreases when *Doa* function is reduced.

In addition to *caspase-2*, other mammalian genes involved in programmed cell death are alternatively spliced (44). For example, the gene *bcl-x*, which encodes a protein homologous to CED-9, generates a long splice isoform, Bcl-x_L, which inhibits apoptosis and contains all four of the BH domains found in Bcl-2 (48). The protein generated from the shorter isoform, Bcl-x_S, promotes apoptosis and contains only the BH3 and BH4 domains (48). Consistent with their proposed functions, Bcl-x_S protein and mRNA are widely expressed and found in tissues that contain cells undergoing apoptosis (48, 49). Several recent studies have implicated SR proteins in the control of *bcl-x* splicing (50–52).

Although alternative splice forms of mammalian genes that function in apoptosis, such as *bcl-x* and *caspase-2*, have been described, relatively little is known about the biological functions of the alternative splice forms and the mechanisms that regulate the splice-site selection of these genes (44). Our studies of *C. elegans* establish an assay in a genetically tractable system that can be used for further investigations into how alternative splicing is regulated in general and how, in particular, alternative splicing interfaces with programmed cell death. We propose that splicing regulation mediated by an SR protein kinase modulates programmed cell death in *C. elegans* and suggest that the protective function of *ced-4* mediated by CED-4L is likely regulated by SPK-1. We further propose that the regulation of apoptosis by SR protein kinases is functionally important in other organisms.

Materials and Methods

Strains and General Techniques. A description of the strains and techniques used in this study is provided in *SI Materials and Methods*.

Screen for Suppressors of *ced-4(n3158).* We mutagenized L4 *ced-4(n3158); nIs106* hermaphrodites with ethyl methanesulfonate as described by Brenner (53). After allowing the animals to recover, each P_0 was individually transferred to a 5-cm plate (one animal per plate). After 3–5 d, 12 F_1 L4 hermaphrodites were individually transferred to 5-cm plates (one animal per plate), and F_2 animals on these plates were subsequently screened to identify individuals with the wild-type number of GFP-positive Pn.aap cells. We screened ≈5,000 mutagenized haploid genomes for zygotic defects in cell survival by examining the progeny of 2,500 F_1 animals. Six siblings were picked from any plate on which a suppressor was isolated to ensure that heterozygous siblings were maintained for isolates that were inviable.

Quantitation of Cell Death of the VC-Like Neurons and in the Anterior Pharynx. Pn.aap cells expressing GFP were visualized by using a dissecting microscope equipped with fluorescence optics (M^2BIO; Kramer Scientific). Animals late in the fourth larval stage were picked by using a standard dissecting microscope and observed using the fluorescence-equipped dissecting microscope for counting their surviving VC-like neurons (38). We scored for the presence of extra cells in the anterior region of the pharynx as described by Schwartz (54).

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