Transgene-mediated cosuppression and RNA interference enhance germ-line apoptosis in *Caenorhabditis elegans*

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Introduction of multiple copies of a germ-line–expressed gene elicits silencing of the corresponding endogenous gene during *Caenorhabditis elegans* oogenesis; this process is referred to as germ-line cosuppression. Transformed plasmids assemble into extrachromosomal arrays resembling extra minichromosomes with repetitive structures. Loss of the transgene extrachromosomal array leads to reversion of the silencing phenomenon. Cosuppression and RNAi depend upon some of the same genes. In the *C. elegans* germ line, about half the cells undergo a physiological programmed cell death that shares most genetic requirements with somatic apoptosis. In addition, apoptosis is stimulated by DNA damage and synaptic failure mediated through different apoptotic checkpoints. We found that both germ-line cosuppression and RNAi of germ-line–expressed genes enhance apoptosis during *C. elegans* oogenesis. In contrast, apoptosis is not enhanced by extrachromosomal arrays carrying genes not driven by germ-line–specific promoters that thus do not elicit transgene-mediated cosuppression/silencing. Similarly, introduction of doubled-stranded RNA that shares no homology with endogenous genes has no effect on apoptosis. "Silencing-induced apoptosis" is dependent upon *sir-2.1* and *cep-1* (the worm p53 ortholog), and is accompanied by a rise in RAD-51 foci, a marker for ongoing DNA repair, indicating induction of DNA double-strand breaks. This finding suggests that the DNA damage-response pathway is involved. RNAi and cosuppression have been postulated as defense mechanisms against genomic intruders. We speculate that the mechanism here described may trigger the elimination of germ cells that have undergone viral infection or transposon activation.

genome-preservation | gametogenesis | meiosis

The ability of double-stranded RNA (dsRNA) to inhibit expression of homologous genes, a process known as RNAi, was discovered over a decade ago in *Caenorhabditis elegans* (1) and proved to be a widespread phenomenon shared by many phyla. To induce interference, dsRNAs must be processed into siRNAs by the Dicer protein and amplified by RNA-directed RNA polymerase, thereby generating secondary siRNAs. Subsequent assembly of RNA-induced silencing complexes governs degradation of the corresponding endogenous mRNAs. A related phenomenon, cosuppression, had first been discovered in plants and fungi (2) and a few years later in *C. elegans*. Cosuppression protects the *C. elegans* germ-line genome from invasion by repetitive sequences, transposons, viruses, and exogenous transgenes (3, 4). The introduction of multiple copies of a homologous germ-line–specific transgene leads to silencing of both the exogenous sequences and the endogenous genomic copy of the corresponding gene. In the following decade, genes involved in both phenomena were identified. Many of the genes and mechanisms discovered are also involved in the endogenous/physiological RNAi-mediated regulation of gene expression (5–10).

RNAi and transgene-mediated cosuppression phenocopy the depletion phenotypes of the corresponding genes. RNAi has thus become a powerful reverse-genetic technique for gene depletion. The efficiency of RNAi has led to its use in genomic screening, and, being able to maintain a cosuppressed line for several generations and introduce extrachromosomal arrays in different genetic backgrounds by crosses, has furthered our understanding of meiotic pathways.

Although RNAi and transgene-mediated cosuppression share many key players [e.g., *dcr-1* (Dicer) and *rde-2* genes (4)], clear distinctions exist between the two processes. RNAi is not stably inherited for more than a few generations and can affect every cell of the nematode in which the gene is expressed. In contrast, upon introduction of dsDNA carrying a coding sequence under a genuine germ-line promoter, extrachromosomal arrays are formed that can be inherited for several generations despite their non-Mendelian segregation. Because a somatic selectable marker can be coinjected together with the germ-line gene, nematodes carrying the extrachromosomal array can be easily identified and stable cosuppressed transgenic lines selected for. Only germ-line genes can be silenced in this way. Interestingly, upon loss of the extrachromosomal array, silencing is attenuated and gradually lost in the following generations (10).

In the *C. elegans* germ line, recombination occurs at the very early stages of meiotic prophase; the endonuclease SPO-11 induces meiotic double-strand breaks (DSBs) (11) and the recombinase RAD-51 is quickly loaded onto processed DSBs, which are rapidly resolved (12). Genome integrity during oogenesis is guarded by checkpoints. At least three checkpoints operate in the different gonad compartments: (*i*) the spindle checkpoint, which acts in the premeiotic compartment and arrests nuclear divisions in the mitotic compartment (13); (*ii*) the pachytene checkpoint, which safeguards chromosome pairing and synapsis (a prerequisite for crossing over) and acts in the transition zone/early pachytene stage (14); and (*iii*) the DNA-damage checkpoint, which preserves genome integrity throughout oogenesis (15). Activation of the latter two checkpoints leads to increased apoptosis immediately after the pachytene stage in *C. elegans*. Massive physiological germ-line cell death takes place in the wild-type germ line and requires all of the conserved apoptotic players, such as the caspase CED-3 (16). Apoptosis further increases once one or both checkpoints are activated (14, 15). Many mutants in meiotic genes involved in chromosome organization and DNA repair display increased apoptosis.

In previous studies of the repair gene *fcd-2*, we used transgene-mediated cosuppression to deplete *fcd-2*. Comparing the

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cosuppressed line with the null mutant (*tm1298*), we observed very similar phenotypes, such as an increase in pachytene RAD-51 foci, indicating a DNA repair problem, and a high incidence of developmental defects (ref. 17 and Table S1). Surprisingly, although the damage-induced apoptosis observed in *fcd-2* mutants is totally suppressed in a *spo-11*–mutant background (where meiotic DSBs are not induced), cell death remains markedly high in *spo-11* worms cosuppressed with the extrachromosomal array of the *fcd-2* gene (present study and Fig. S1*A*). This finding led us to postulate that the ongoing germ-line cosuppression per se enhances apoptosis. Therefore, the objective of our study was study this phenomenon and its genetic requirements.

Results

Does the Presence of an Extrachromosomal Array Lead to Transgene-Induced Apoptosis? To explore a possible relation between germ-line apoptosis and transgene-mediated cosuppression, we generated two independent cosuppressed lines of a different meiotic gene, *com-1*. Although the *com-1* mutation affects meiotic repair, the loss-of-function mutant of this gene does not display any increase in germ-line apoptosis compared with the wild-type (18) (Fig. 1 *A* and *D*). However, the two independent lines, both of which exhibited cosuppression of *com-1*, displayed more than twice the level of apoptosis compared with the wild-type or the *com-1* mutant (Fig. 1*A*). Importantly, siblings that have lost the transgenic array [easily identified by the lack of the associated selectable marker *rol-6(su1006)*] show an attenuated *com-1* phenotype (Table S2) and an intermediate level of apoptosis at the F1 generation that further decreases in the next generations (Fig. 1*A*). It has been reported that the epigenetic effect of cosuppression is gradually lost over the first two generations, further suggesting a link between the mechanisms of silencing and the induction of apoptosis (10).

Extrachromosomal arrays appear as small distinct chromatin structures with properties of real chromosomes. These structures are passed on to the next generation, although in a non-Mendelian fashion. We wondered whether the presence of an unpaired extrachromosomal array might itself activate the pachytene pairing checkpoint and induce apoptosis (14). To test this idea, we measured the level of apoptosis in nematode lines bearing extrachromosomal arrays that carry the plasmid pRF4*rol-6(su1006)*, which expresses a dominant mutation of the *rol-6* gene in the soma and is used as a selectable marker, or a mixed array containing the mutant *rol-6* coding sequence and the *act-5* gene. Neither of these genes is expressed in the germ line: the *rol-6* gene encodes a cuticle collagen and is required for normal cuticular morphology, and *act-5* is only expressed in microvillous intestinal cells and excretory cells. Therefore, neither of these extrachromosomal arrays is likely to induce transgene-mediated germ-line cosuppression. In both circumstances, we did not observe a significant increase in the physiological levels of apoptosis (Fig. 1*B*).

Extrachromosomal arrays can be integrated into the genome and kept in homozygosis, resulting in gene silencing of both the transgene and the endogenous locus (19). We integrated the *com-1* array by γ-irradiation and out-crossed the integrated line several times. In worms homozygous for the *com-1*–integrated array, the germ-line apoptosis level was still significantly higher than in the wild-type after 10 generations and remained elevated over 50 generations, suggesting that it is the mechanism of gene silencing itself (and not the presence of an unpaired additional chromosome-like structure) that is responsible for apoptosis up-regulation (Fig. 1 *B* and *E*).

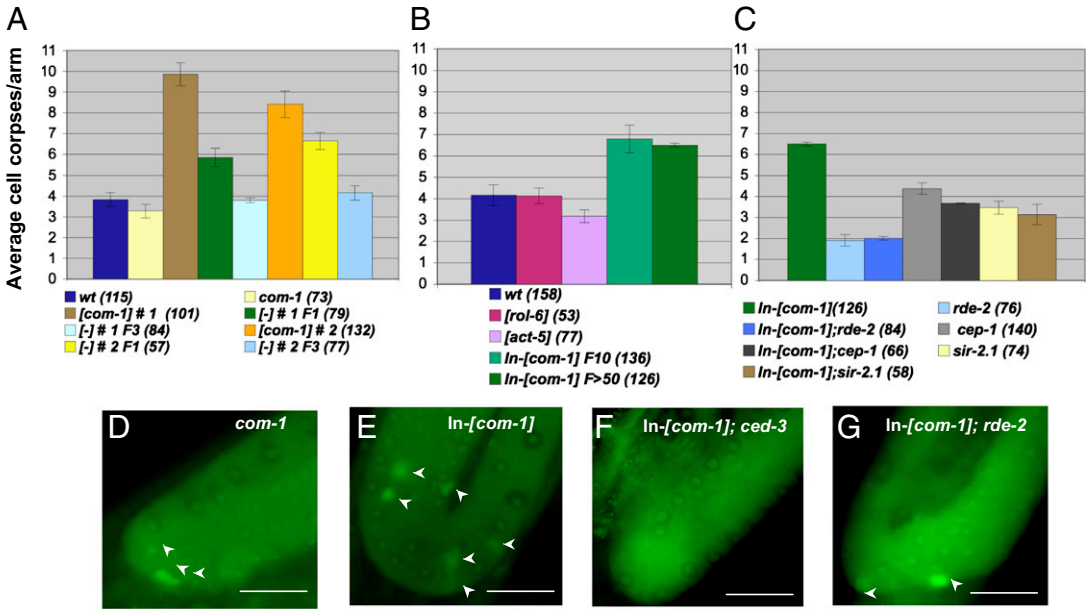

Fig. 1. Transgene-mediated cosuppression of the *com-1* gene induces up-regulation of germ-cell apoptosis. (*A*–*C*) Average apoptosis levels were scored in the indicated genotypes. The *y* axis shows the average number of SYTO12-labeled nuclei per gonadal arm. Genotypes are shown in the color legend at the bottom of each chart. Cosuppressed transgenes are indicated in squared parenthesis. The numbers of gonadal arms analyzed are written as numbers in parenthesis next to the genotype color legend. The two independent *com-1* cosuppressed lines are labeled #1 and #2. Siblings of cosuppressed nematodes that have lost the extrachromosomal arrays and their descendants are indicated as "*[-]* #1" and "*[-]* #2" followed by the generation (F1 or F3). [*rol-6*] Are nematodes carrying the extrachromosomal array somatically expressing the dominant mutation (*su1006*) in the gene *rol-6* as selectable marker. [*act-5*] Are nematodes carrying a mixed extrachromosomal array of the two genes, *rol-6*(*su1006*) and *act-5,* both expressed in the soma. The integrated line is derived from line #2 and indicated as In-*[com-1]*. Error bars correspond to SEMs calculated from at least three independent experiments. (*D*–*G*) SYTO12-stained gonads of the indicated genotype. (Scale bar, 25 μm.)

Genetic Requirements for Cosuppression-Mediated Apoptosis. Silencing-induced cell death is *ced-3*–dependent, because we barely detected any apoptotic corpses in worms stably cosuppressed for *com-1* and lacking the CED-3 caspase (Fig. 1*F*) (one germ-cell corpse from 60 scored gonadal arms compared with one corpse from 64 arms in the *ced-3* mutant alone). Furthermore, when the *com-1*–integrated array was crossed to the *rde-2* mutant (in which cosuppression does not take place) (3, 4), we did not detect any increase in apoptosis compared with the wild-type (Fig. 1 *C* and *G*). All of the *com-1* meiotic phenotypes (i.e., maternal effect lethality and fragmented and decondensed diakinesis chromosomes) were reproduced both in the *ced-3* and in the wild-type, but not in the *rde-2*–mutant background (Table S2).

The experiments shown so far excluded activation of the pairing checkpoint as a cause for the apoptosis enhancement. The other checkpoint responsible for apoptotic induction during pachytene is the DNA-damage checkpoint. We tested whether lack of the CEP-1 protein (the ortholog of p53, an essential player in DNA damage-induced apoptosis) abrogates cosuppression-induced apoptosis (15) by crossing the integrated *com-1* array into a *cep-1*–deficient background. All of the *com-1* meiotic phenotypes (Fig. S2 and Table S2) were reproduced in the *cep-1* mutant. However, enhancement of apoptosis was suppressed in the absence of CEP-1, suggesting that the DNA-damage apoptosis pathway is activated during cosuppression (Fig. 1*C*). It has been suggested that the SIR-2.1 protein (ortholog of the yeast Sir2 deacetylase) acts at the chromatin level and is also involved in damage-dependent apoptosis (20, 21). We found that cosuppression-mediated apoptosis is also abolished in *sir-2.1*–mutant background (Figs. 1*C* and Fig. S1*B*).

Does Silencing of Meiotically Expressed Genes Induce Apoptosis in General? A concern at this point was that both genes used in the cosuppression experiments (i.e., *fcd-2* and *com-1*) are involved in meiotic DNA repair. It is possible that suppression of these genes might somehow induce more DNA damage than those present in the null mutants.

To address this concern, we cosuppressed an ectopically germline–expressed transgene unrelated to DNA repair. We used the strain AZ212 carrying a copy of the *Ppie-1::gfp-his-11* transgene (10). In this strain, the transgene is integrated in a single copy, and therefore it is not silenced and does not induce silencing of the endogenous *his-11* gene. In strain AZ212 the transgene expresses histone H2B fused to the GFP coding sequence under a genuine germ-line promoter (22). The endogenous *his-11* gene is intact, and the GFP-HIS-11–fused protein is expressed only in the germ line being observed in the gonad and during early embryonic divisions. To specifically cosuppress the *gfp-his-11* transgene, we injected a construct carrying the GFP coding sequence (but not the histone sequence) under a different germline promoter, *Prad-51* (23). The resulting cosuppressed line lost meiotic chromosome fluorescence (Fig. 2*A*, *Upper*), and at the same time we observed a net increase in germ-line apoptosis associated with the silencing effect (Fig. 2*B*). As expected, chromosome fluorescence was gradually regained in some individuals in the following generations upon loss of the extrachromosomal array (Fig. 2*A*, *Lower*). Apoptosis also gradually decreases in these worms (Fig. 2*B*). These experiments clearly demonstrate that cosuppression of a meiotically expressed gene unrelated to the DNA repair pathway induces additional germ-cell death events.

Cosuppression Induces RAD-51 Foci in the Absence of Meiotic DSBs. Based on the activation of the DNA damage pathway during cosuppression, we wondered whether the silencing mechanism may cause DNA damage. The recombinase protein RAD-51 binds processed DSBs during meiotic prophase, promoting recombination and crossovers; therefore, increase of RAD-51 foci (detected by immunolocalization) may be an indication of an increase in DSBs (24). We, therefore, quantified and compared the levels of meiotic RAD-51 foci in the strain AZ212 and in the strain AZ212 cosuppressing GFP expression. RAD-51 foci appeared significantly more abundant in the germ line of cosuppressed worms, confirming that cosuppression per se induces DSBs (Fig. 2 *C* and *D* and Table S3). We also observed a discrete number of brightly staining apoptotic nuclei in middle/late pachytene stages of AZ212 worms cosuppressed with the extrachromosomal array of the GFP coding construct. These

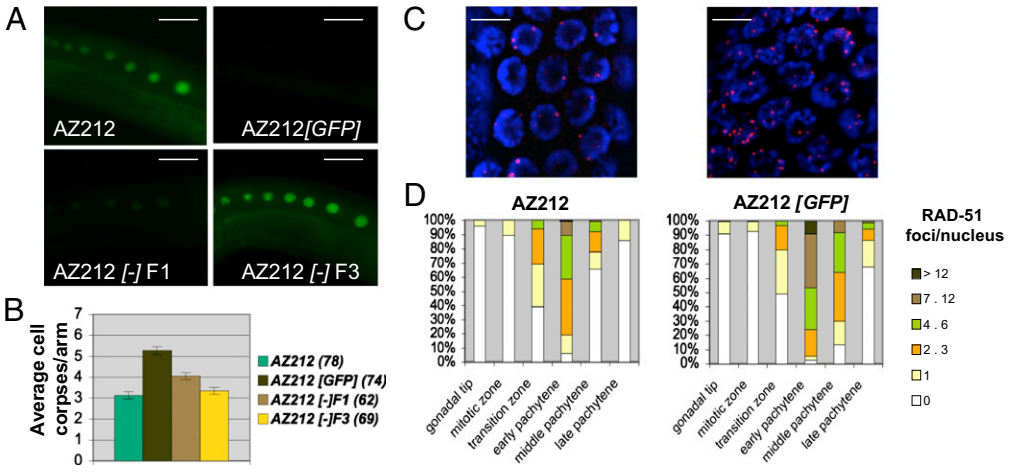

Fig. 2. Cosuppression of a meiotically expressed transgene induces additional germ-cell death events. (*A*) GFP-labeled diakinesis chromosomes in strain AZ212. (Scale bar, 25 μm.) Labeling is lost in worms carrying the extrachromosomal array expressing GFP under the *rad-51* promoter (indicated as AZ212*[GFP]*) and regained after loss of the extrachromosomal array in the following generations (AZ212*[-]* F1 and AZ212*[-]* F3). (*B*) Apoptosis levels in AZ212 and AZ212 *[GFP]*. The *y* axis represents the average number of SYTO12-labeled nuclei present per gonadal arm. Each genotype is indicated in the color legend at the bottom of the chart. Numbers in parenthesis correspond to the number of scored gonadal arms. Error bars correspond to SEM calculated from at least three independent experiments. (*C*) AZ212*[GFP]* show elevation of RAD-51 foci compared with AZ212. (Scale bars, 5 μm.) Representative image of germ lines immunostained with anti-RAD-51 (24) and DNA counterstained with DAPI (blue). (*D*) Histograms represent quantification of RAD-51 foci in germ lines of animals of the indicated genotypes. The *y* axis represents the percentage of nuclei with the indicated number of foci. The *x* axis represents the position (zone) along the germ line. Statistical analysis was carried out using the Student *t* test (Table S3).

brightly staining nuclei may be interpreted as apoptotic nuclei containing fragmented DNA because of the action of the *nuc-1* endonuclease; their number, in fact, is proportional to the apoptotic levels in any strain analyzed so far (17, 24, 25) and absent in the *ced-3* mutant.

We also compared RAD-51 foci in *spo-11*, *fcd-2* double mutants and *spo-11* worms cosuppressed with the extrachromosomal array of the *fcd-2* gene. SPO-11 is the meiotic endonuclease responsible for physiological DSB induction, and is essential for crossover and proper chromosome segregation. Therefore, in the absence of SPO-11, almost no RAD-51 foci are generated and chromosomes do not properly segregate. We again observed a biologically and statistically significant increase in RAD-51 foci in early pachytene stage of cosuppressed worms (Fig. S3 and Table S4).

Does RNAi of Germ-Line–Expressed Genes Have a Similar Effect on Germ-Cell Death? Because the mechanisms of cosuppression and RNAi partially overlap, we performed RNAi by injecting dsRNA homologous to the GFP coding sequence into the AZ212 strain, thus inhibiting expression of the *Ppie-1::gfp-his-11* integrated construct, and then measured germ-cell death. Active ongoing RNAi also enhanced germ-line RAD51 foci and *ced-3*–dependent apoptosis (Fig. 3 *A* and *D*). GFP RNAi induced enhancement of RAD-51 foci also in an AZ212 strain carrying the *ced-3* allele, although brightly staining late pachytene nuclei disappeared because of the absence of apoptosis (Fig. S4 and Table S3). On the other hand, simple injection of GFP-dsRNA into wild-type worms did not elevate apoptosis (Fig. 3*A*). We therefore conclude that an excess of dsRNA per se does not cause apoptosis induction.

To confirm that ongoing RNAi promotes germ-line cell death, we also used an endogenous gene. We reasoned that depletion of SPO-11 (11) should decrease the number of DNA breaks in the germ line; therefore, the *spo-11* gene is an ideal substrate upon which to perform RNAi to test whether RNAi per se can induce germ-cell death. Mothers subjected to RNAi were selected for lethal (aneuploid) progeny and for univalent diakinesis chromosomes. F1 progeny of injected mothers were stained for apoptosis. Only nematodes with a fully penetrant *spo-11* phenotype were analyzed (see *Materials and Methods* and Table S5 for details). Enhancement of cell death was similarly observed in this experiment (Fig. 3*B*). Furthermore, we confirmed that, also in this context, apoptosis was *ced-3*–dependent (only one germ-cell corpse detected from 42 scored gonadal arms), and that SPO-11 dsRNA injection did not enhance apoptosis in the *rde-2* or *cep-1* mutant backgrounds (Fig. 3 *B* and *C*).

Discussion

Evolution may select common genetic components to be shared by different pathways; different input signals make use of such common genetic components to activate functions that are sometimes very different (26). The phenomenon of RNAi is thought to protect the cell from viral infection, and it is related to pathways of gene regulation by small RNAs. In addition, RNAi overlaps in part with the phenomenon of transposon silencing. It is therefore not totally surprising that in the germ line, where genome preservation is crucial for species survival, several layers

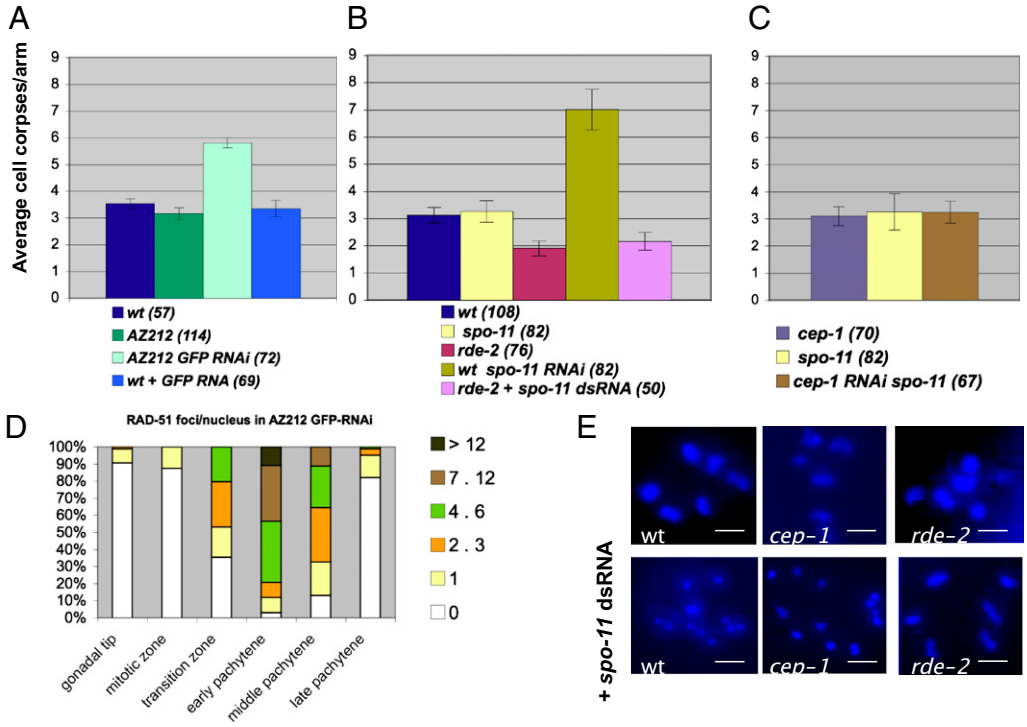

Fig. 3. RNAi of germ-line–expressed genes enhances germ-cell apoptosis. (*A*) GFP RNAi induces apoptosis in the strain AZ212. dsRNA of GFP was injected in the strain AZ212 and in wild-type. (*B*) *spo-11* RNAi elicits apoptosis. dsRNA of *spo-11* was injected in the wild-type and *rde-2* strains. (*C*) Lack of apoptosis enhancement after *spo-11* RNAi in *cep-1* strain. The *y* axis represents the average number of SYTO12-labeled nuclei present per gonadal arm. Each genotype is indicated in the color legend at the bottom of the chart. Numbers in parenthesis correspond to the number of scored gonadal arms. Error bars correspond to SEM calculated from at least three independent experiments. (*D*) GFP RNA interference induces DSBs in the strain AZ212. Histograms represent quantification of RAD-51 foci in germ lines of animals of the indicated genotypes. The *y* axis represents the percentage of nuclei with the indicated number of foci. The *x* axis represents the position (zone) along the germ line. Statistical analysis was carried out using the Student *t* test (Table S3). (*E*) Diakinesis chromosomes of indicated genotypes: wild-type, *cep-1*, *rde-2* nuclei show six bivalents held together by chiasmata. *spo-11* dsRNA injection abrogates chiasmata, and therefore nuclei show 12 univalents in wild-type and *cep-1* strains, but not in the *rde-2* mutant. (Scale bars, 2 μm.)

of responses share the same goal. DNA damage induces cell-cycle arrest and DNA repair, and ultimately culminates in programmed cell death, if the damage persists. Therefore, it is conceivable that transposon mobilization or viral attack, which can seriously alter genomic information, may induce gene silencing and ultimately lead to cell death. In support of this idea, when RNAi was first studied in mammalian cells, it was apparent that the response to a large amount of dsRNA led to IFN-mediated apoptosis (27, 28). This effect might be envisioned as the result of coevolving mechanisms similar to the one described in this work (silencing-dependent germ-line apoptosis).

The results of the experiments described above demonstrate that gene silencing due to cosuppression or RNAi is coupled with enhanced germ-cell death. The presence of an additional meiotically unpaired chromosome per se does not induce higher apoptotic levels unless active silencing is ongoing. Furthermore, neither transgene multicopy arrays nor introduction of dsRNA up-regulates apoptosis in a genetic background in which silencing is blocked, such as in the *rde-2* mutant.

The silencing-mediated apoptosis pathway takes advantage of crucial death regulators also used in the DNA damage-induced apoptotic pathway; in fact, both the CEP-1 (p53) and SIR-2.1 (sirtuin) proteins are required. Although enhancement of apoptosis is abrogated in the *sir-2.1* mutant, SIR-2.1 does not seem to be involved in silencing of the endogenous gene copy per se, because in a *sir-2.1* deletion mutant the *com-1* cosuppressed construct maintains the *com-1*–mutant phenotype, such as embryonic lethality and fragmented and decondensed diakinesis chromosomes (Fig. S2 and Table S2).

When the cosuppressing extrachromosomal array is lost during consecutive chromosome segregation cycles, the somatically expressed selectable marker disappears at the first generation, whereas the effects of the cosuppressed phenotype gradually decrease over more than two generations and the level of apoptosis declines in parallel. It will be interesting to find out whether the apoptosis-triggering signal is linked to sensors at the chromatin level. It is worth noting that the deacetylase sirtuin SIR-2.1, which is known to operate at the chromatin level (21) and to participate in DNA damage-induced apoptosis (20), is required for the increase in germ-cell death during cosuppression. In fact, the yeast *sir2* gene is known to alter the genomic distribution of DSBs (29, 30).

Activation of the DNA-damage apoptosis pathway is accompanied by *spo-11*–independent DSBs induction during cosuppression and RNAi. A significant increase in RAD-51 foci can be observed in the silencing experiments. Silencing-induced apoptosis, but not DSB induction, is *ced-3*–dependent. This observation strongly suggests that increase in DSBs is the cause and not the consequence of apoptosis enhancement. Further investigations will be necessary to identify the pathway leading to this increase in DNA damage.

The observation that DSBs are indeed induced during silencing is consistent with the observation that the phenotypic effects of cosuppression or RNAi of the *spo-11* gene are much less pronounced than RNAi of other genes coding for more abundant meiotic proteins, such as *rad-51* (12) or the *his-11::gfp* transgene. Only a fraction of the dsRNA-injected mothers displayed a fully penetrant *spo-11* phenotype (aneuploid lethal progeny caused by crossover failure) that, in turn, was inherited by only a fraction of the offspring (Table S5). It is known that exogenous induction of DSBs by ionizing radiations (11) is able to rescue the *spo-11* phenotype by promoting crossovers and segregation. The weak penetrance of the *spo-11* RNAi can be explained by the fact that, although depleting SPO-11, the RNAi process also induces DSBs that may similarly suppress in part the *spo-11* phenotype (11). In support of this idea, in a blinded experiment, we observed that apoptotic levels throughout the entire *spo-11 RNAi* F1 population were higher than expected (average of 5.15 SYTO12-positive nuclei per gonadal arm).

We think that the present observations are highly relevant to a comprehensive understanding of how sexual organisms protect their germ line from attacks from viruses and repetitive sequences, not only by neutralizing the invaders, but also by eliminating affected nuclei. Our findings also indicate that particular caution should be used when interpreting depletion phenotypes of germline genes obtained by RNAi and cosuppression.

Materials and Methods

Quantification of Germ-Line Apoptosis. Adult nematodes (24–36 h after larval stage L4) were suspended in M9 solution and stained by incubating with 33 μM SYTO-12 (Molecular Probes) for 1 h 30 min at room temperature in the dark. The worms were then transferred to seeded plates to allow stained bacteria to be purged from the gut. After 30 min, the animals were mounted on 2% agarose pads in 2 mM levamisole. For the time course, germ-line apoptosis were quantified at 12, 24, and 36 h postlarval stage L4 (Fig. S1*C*).

The quantitative analysis was performed using a Leica DM6000 fluorescence microscope, Leica DC 350 FX camera under the control of Leica LAS AF 6000 software. The numbers of gonadal arms scored for each genotype are indicated in the legends to the charts.

Immunostaining. RAD-51 immunostaining was carried out as described by Colaiácovo et al. (24). Quantitative analysis of RAD-51 foci was carried out on *z*-series of images acquired using a Leica DM5000 fluorescence microscope, Leica DC 350 FX camera under the control of Leica LAS AF 6000 software. Optical sections were collected at 0.25-μm increments. An average of 100 nuclei per region per genotype were scored.

RNAi. The dsRNA was injected at a concentration of 500 μg/mL into the gonad (1). Young AZ212 adult hermaphrodites were injected with GFP dsRNA (corresponding to nucleotides 253–697 of GFP coding sequence). Efficiency of interference was controlled by chromosome fluorescence loss.

Young wild-type *cep-1*, and *ced-3* adult hermaphrodites were injected with *spo-11* dsRNA (corresponding to nucleotides 593–1500 of cosmid T05E11). Injected worms (P0) were individually cloned and then transferred to seeded plates every 12 h. The F1 laid 12–48 h after injection were transferred to fresh plates for 24 h and screened for embryonic lethality (Table S5). Only F1 laid by P0 mothers that produced over 90% lethal progeny in the time window corresponding to 60–84 h after injection (12) were further analyzed. Those F1 nematodes that showed the fully penetrant *spo-11* phenotype (F2 embryonic lethality > 92%) were treated with SYTO-12 and checked for apoptosis. Sibling F1 nematodes laid 12–24 h after injection were stained with DAPI and diakinesis chromosomes were analyzed (Fig. 3*E*). We also performed a blind experiment testing the average number of SYTO12-positive nuclei in the entire F1 population (irrespectively of the *spo-11* phenotype).

Young *rde-2* adult hermaphrodites were similarly injected with *spo-11* dsRNA to simulate RNA interference, progeny laid 24–48 h after injection (corresponding to the peak of *spo-11* interference penetrance in wild-type) were treated with SYTO-12 and corpses were scored.

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