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A Conserved Checkpoint Pathway Mediates DNA Damage–Induced Apoptosis and Cell Cycle Arrest in *C. elegans*

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Summary

To maintain genomic stability following DNA damage, multicellular organisms activate checkpoints that induce cell cycle arrest or apoptosis. Here we show that genotoxic stress blocks cell proliferation and induces apoptosis of germ cells in the nematode *C. elegans*. Accumulation of recombination intermediates similarly leads to the demise of affected cells. Checkpoint-induced apoptosis is mediated by the core apoptotic machinery (CED-9/CED-4/CED-3) but is genetically distinct from somatic cell death and physiological germ cell death. Mutations in three genes—*mrt-2*, which encodes the *C. elegans* homolog of the *S. pombe rad1* checkpoint gene, *rad-5*, and *him-7*—block both DNA damage-induced apoptosis and cell proliferation arrest. Our results implicate *rad1* homologs in DNA damage–induced apoptosis in animals.

Introduction

The maintenance of genomic stability is essential for the survival of organisms. To ensure this stability, surveillance mechanisms—commonly termed checkpoints—exist to interrupt cell cycle progression when damage to the genome is detected, thereby allowing for the timely repair of offending lesions. In multicellular organisms, a second output of checkpoint signaling is the activation of the apoptotic pathway. This second pathway is used to eliminate cells whose unrepaired damage might potentially become deleterious for the entire organism. The signaling system that communicates between DNA lesions and the cell death machinery is still largely unknown.

Genetic studies in yeasts have led to the identification of key checkpoint molecules that lead to an arrest in cell cycle progression following DNA damage. Briefly, the *S. pombe* proteins rad1$^+$, rad17$^+$, rad9$^+$, and hus1$^+$ are thought to be a part of a checkpoint protein complex (Weinert, 1998; Caspari and Carr, 1999). Although rad1$^+$ shows limited homology to nucleases and PCNA (Sunnerhagen et al., 1990; Siede et al., 1996), and rad17$^+$ to replication factor C (RFC), respectively (Griffiths et al., 1995), the molecular details of their function are still unknown (Griffiths et al., 1995; Lydall and Weinert, 1995; Weinert, 1998; Caspari and Carr, 1999; Thelen et al., 1999). Checkpoint signaling further involves the rad3$^+$ lipid kinase, the rad53$^+$ kinase, and the chk1$^+$ kinase and ultimately leads to cell cycle arrest (G2 phase arrest in fission yeast and M phase arrest in budding yeast) due to the inactivation of key cell cycle proteins (Matsuoka et al., 1998; Weinert, 1998; Caspari and Carr, 1999). Unfortunately, much about the mechanisms regulating yeast cell cycle arrest has not yet been generalized to metazoans. Furthermore, DNA damage–induced apoptosis does not appear to occur in yeasts (Fraser and James, 1998). In metazoan systems, studies of damage induced checkpoints have been hampered by the lack of a genetic system. The identification of key components of checkpoint pathways is further complicated by the differential responses of various cell types to genotoxic insults (Polyak et al., 1996; Giaccia and Kastan, 1998).

To guide future studies on DNA damage–induced checkpoints, in particular apoptosis, in metazoans, we wished to develop a suitable genetic system. Genetic studies in *C. elegans* provided the framework for the genetic dissection of the core apoptotic pathway (Hengartner and Horvitz, 1994b, 1994c; Conradt and Horvitz, 1998; Metzstein et al., 1998). However, cell deaths that were examined in these studies are part of developmental programs. Previous studies in *C. elegans* on the effects of genotoxic stress suggested that there are little or no checkpoint controls exerted during embryonic development (Jones and Hartman, 1996). To analyze checkpoint responses, we thus focused our attention on the *C. elegans* germline, which proliferates both during larval development and in adult worms. In contrast to the almost invariant somatic *C. elegans* development, germline development is much more malleable (Schedl, 1997). As in mammalian tissues, populations of germ cells respond to growth factor regulation and are subjected to stochastic events, like the elimination of many cells by programmed cell death to maintain tissue homeostasis (Gumienny et al., 1999).

Here, we demonstrate the existence of DNA damage checkpoints in *C. elegans* and present a genetic characterization of these checkpoint pathways. While DNA damage–induced signaling only minimally affects cells during somatic development of *C. elegans*, DNA damage checkpoints can induce both cell cycle arrest and apoptosis in the worm germline. These two responses are spatially separated and under developmental control. Meiotic germ cells also respond to a meiotic recombination checkpoint system that triggers the apoptotic demise of cells that initiate but do not complete recombination. Checkpoint-induced apoptosis requires the apoptotic core machinery but is genetically distinct from somatic cell death and physiological germ cell death. We have identified mutations in three checkpoint genes that are required for DNA damage–dependent cell proliferation arrest and apoptosis, as well as for the meiotic

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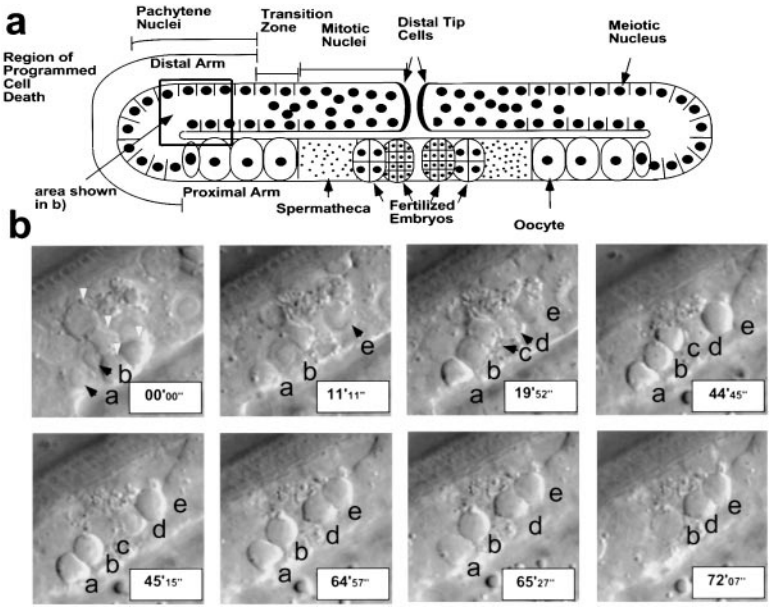


Figure 1. DNA Damage Induces Apoptotic Cell Death of Meiotic Germ Cells in *C. elegans*

(a) Diagram of the adult hermaphrodite gonad. The area of the germline depicted in (b) is indicated.

(b) Time lapse video microscopy of dying cells. Eight representative pictures derived from a 1.5 hr time course are shown. Various morphological stages of programmed cell death of five cells (a–e, indicated by black arrowheads) are shown. Time is indicated in the boxes at the right bottom of the images. White arrowheads indicate corpses that disappeared within the first 10 min of the time lapse analysis.

recombination checkpoint. One of these genes, *mrt-2*, encodes a homolog of the conserved *S. pombe rad1*$^{+}$ checkpoint gene (Ahmed and Hodgkin, 2000). Our results implicate that *rad1* homologs mediate DNA damage–induced apoptosis in animals.

Results

To determine the effects of genotoxic stress in *C. elegans*, we chose the hermaphrodite germline as an experimental system. Within the adult *C. elegans* hermaphrodite ovotestis, germ cells progress through various stages of differentiation (Figure 1a). The distal-most germ cells proliferate mitotically and serve as a stem cell population. During their passage through a "transition zone," germ cells cease dividing and initiate meiosis. Germ cells are only partially enclosed by the cell membrane and thus share a common cytoplasm (Schedl, 1997). In accordance with the literature, these nucleated compartments will be referred to as cells. The most abundant population of cells is in the pachytene stage of meiotic prophase and resides between the transition zone and the bend of the gonad. Upon exit from pachytene, germ cells complete meiotic prophase, cellularize, undergo the final stages of oogenesis, and finish meiosis after fertilization, which occurs as the oocyte passes through the spermatheca (Schedl, 1997).

Under normal growth conditions, approximately 50% of female germ cells are fated to die by programmed cell death (Gumienny et al., 1999). At any given time, a steady-state level of zero to four apoptotic cells can be observed within a fertile adult hermaphrodite (but not male) germline (Gumienny et al., 1999). These apoptotic deaths, termed "physiological germ cell deaths," occur under normal growth conditions and appear to ensure tissue homeostasis (Gumienny et al., 1999).

DNA Damage Induces Germ Cell Apoptosis

To determine whether genotoxic stress can induce apoptosis, we exposed worms at the fourth (final) larval stage (L4) to increasing levels of gamma radiation and analyzed the germline of matured adult worms at various time points after irradiation. We found that radiation causes a dramatic increase in the number of apoptotic germ cells, as irradiated worms contained up to 45 dead cells in the pachytene region of the germline (Figures 1b and 2a and data not shown). To confirm that the increased level of germ cell death is due to an enhanced level of apoptosis rather than an inhibition of engulfment of dead cells (Hedgecock et al., 1983), we studied the kinetics of radiation-induced germ cell death by time lapse video microscopy (Figure 1b). After "pinching off" from the surrounding syncytium, dying germ cells undergo morphological changes similar to those seen during physiological germ cell death and during somatic apoptosis (Sulston and Horvitz, 1977; Robertson and Thomson, 1982; Gumienny et al., 1999) (Figure 1b). Over approximately 20 min, the cytoplasm of dying cells becomes increasingly refractile, melds with the nucleus, and finally becomes a uniform highly refractile corpse. Following this change, the corpse persists for 20–60 min, after which it suddenly loses its refractile character within less than 1 min (Figure 1b).

To analyze how quickly apoptosis can be induced, we irradiated a synchronous population of wild-type adult worms and counted dead cells at 90 min intervals. We found that apoptosis increases significantly 2–3 hr after irradiation (Figure 2b). This rapid induction of cell death, which is similar to the kinetics of apoptosis observed in irradiated mammalian thymocytes (Lowe et al., 1993), suggests that apoptosis is likely to be a direct consequence of radiation-induced damage (Figure 2b). To support the idea that radiation-induced apoptosis is directly due to DNA damage, we also tested the ability of other DNA modifying agents to induce apoptosis in the germline. We found that the drug N-nitroso-N-ethylurea (ENU) induces apoptosis to similar levels as radiation does (Figure 2c). Ultraviolet (UV) radiation also weakly induces apoptosis, although the levels of apoptosis were difficult to score owing to the high lethality caused

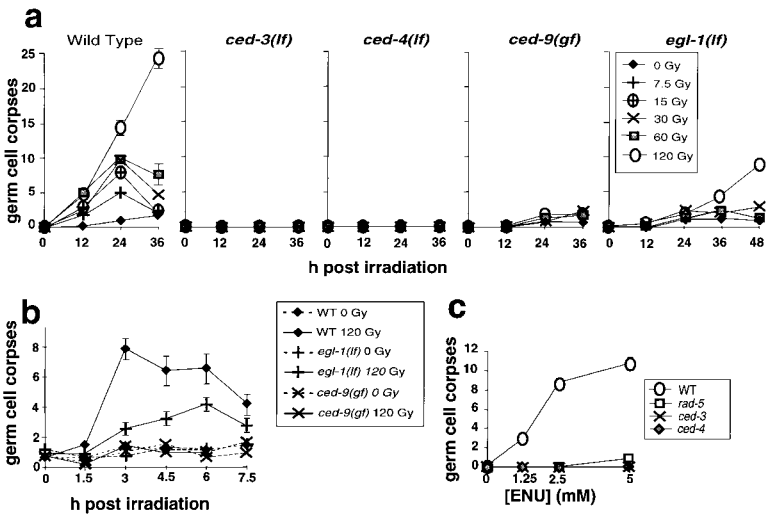


Figure 2. DNA Damage-Induced Germ Cell Apoptosis Requires the Core Apoptotic Machinery but Is Genetically Distinct from Somatic Cell Death and from Physiological Germ Cell Death

(a) Late L4 stage hermaphrodite worms were irradiated with the indicated doses of gamma radiation and scored after 0, 12, 24, and 36 hr. For each time point, 15 animals were scored for germ cell death in one gonad arm via direct observation using Nomarski optics. Data shown are mean ± SEM (standard error of the mean). Exact genotypes used were *ced-3(n717)*, *ced-4(n1162)*, *ced-9(n1950)*, and *egl-1(n3082)*.

(b) Germ cell death is induced rapidly after irradiation. Synchronized animals (24 hr after the L4/adult molt) were irradiated with 120 Gy and analyzed at 90 min intervals after irradiation. Germ cell death was scored as described in (a).

(c) ENU induces germ cell apoptosis. Hermaphrodites were synchronized in late L4 and treated in M9 buffer with the indicated concentrations of ENU for 4 hr. Germ cell death was scored after 24 hr as described in (a).

by the UV treatment (A. G., S. M., and M. O. H., unpublished data).

To determine the genetic requirements for the execution of radiation-induced cell death, we repeated our studies with animals mutant for key components of the *C. elegans* cell death machinery. Briefly, apoptotic death in *C. elegans* requires the activity of the caspase homolog CED-3 and the Apaf-1 homolog CED-4, whose oligomerization promotes CED-3 caspase activation (Metzstein et al., 1998). The ability of CED-4 to activate CED-3 is antagonized by the Bcl-2 family member CED-9 (Hengartner and Horvitz, 1994c; Metzstein et al., 1998). CED-9 has been proposed to sequester CED-4 and CED-3 in an inactive ternary complex (Metzstein et al., 1998). In cells fated to die, the prosurvival activity of CED-9 is thought to be inactivated by the proapoptotic BH3 domain–containing protein EGL-1, likely via a direct protein interaction (Conradt and Horvitz, 1998; Metzstein et al., 1998).

Germ cell death can readily be induced in a dose-dependent manner by 7.5–120 Gy of gamma radiation in wild-type (N2) animals. In contrast, no cell death is induced in the germline of animals that contain the loss-of-function (lf) alleles *ced-3(n717)* or *ced-4(n1162)* (Figure 2a), confirming that radiation-induced cell death is apoptotic in nature and requires the previously characterized core apoptotic pathway.

Somatic cell deaths and physiological germ cell deaths show dramatically different responses to the *ced-9(n1950)* gain-of-function (gf) and the *egl-1(n3082)* loss-of-function mutations: whereas both mutations completely prevent somatic cell death, they do not affect physiological germ cell death (Gumienny et al., 1999) (Table 1). We found that radiation-induced germ cell death shows a third, distinct response pattern in these mutants: radiation-induced germ cell death is completely absent in *ced-9(n1950)* (gf) mutants, while it is reduced but not abrogated in *egl-1(n3082*) (lf) (Figures 2a and 2b; Table 1). The *egl-1(n3082*) (lf) mutation introduces a frame shift in the *egl-1* open reading frame upstream of the BH3 domain and behaves like a genetic null during somatic development. Thus, while physiological germ cell death and damage-induced germ cell death use the same execution machinery (*ced-3* and *ced-4*), the differential behavior of *ced-9(gf)* and *egl-1(lf)* mutations indicates that separate death-inducing stimuli can use distinct mechanisms or pathways to activate this machinery, even within the same cell (Table 1).

Developmental Restriction of DNA Damage-Induced Germ Cell Death

To determine the potential of other cell types to undergo radiation-induced cell death, we carefully looked for induced cell death during somatic and germline development. To efficiently score for cell death during somatic development, we took advantage of a *ced-1(e1735)* corpse engulfment-deficient strain (Ellis et al., 1991). Scoring for dead cells in the head region of *ced-1(e1735)* L1 larvae (the head region contains over half of all cells in an L1 larva), we could not find any evidence of radiation-induced apoptosis (0 Gy, 18 ± 0.4 corpses; 60 Gy, 17.6 ± 0.4 corpses; see Experimental Procedures). Similar negative results were obtained in other somatic tissues (both proliferating and postmitotic; data not shown). We conclude that under normal growth conditions, DNA damage is at best a poor inducer of apoptosis in the *C. elegans* soma.

To analyze the potential of various germ cell types to undergo DNA damage–induced apoptosis, we looked at both male and hermaphrodite germlines during various stages of development. We could not identify any radiation-induced programmed cell deaths in the germline of young hermaphrodites prior to the onset of oogenesis, nor in the germline of males (data not shown). Within the adult hermaphrodite germline, cell deaths invariably occur only in the pachytene region and never in the mitotic region (data not shown). Thus, developmental controls restrict the potential to undergo radiation-

Table 1. Differential Regulation of Somatic Cell Death, Physiological Germ Cell Death, and Damage-Induced Germ Cell Death

	Wild type	*ced-3(lf), ced-4(lf)*	*ced-9(gf)*	*egl-1(lf)*	*him-7(lf), mrt-2(lf), rad-5(lf)*
Somatic cell death	+	−	−	−	+
Physiological germ cell death	+	−	+	+	+
Radiation-induced germ cell death	+	−	−	±	−
Radiation-induced germ cell proliferation arrest	+	+	+	+	−

+, Normal response.
±, Partial response.
−, Process is impaired and does not occur.
The corresponding data are shown in Figures 2–4.

induced germ cell death to the female meiotic compartment of the hermaphrodite germline.

Identification of *C. elegans* Checkpoint Mutants

Given the above results, we reasoned that gene products might exist that sense DNA damage and transmit this information to the apoptotic machinery. We postulated that *C. elegans* checkpoint mutants might have already been isolated in previous genetic screens for mutants defective in various signaling pathways, radiation-sensitive mutants, and mutants with a high rate of chromosome nondisjunction (Hodgkin et al., 1979; Hartman and Herman, 1982; Weinert and Hartwell, 1988). Screening through a panel of such mutants, we found three strains defective in radiation-induced apoptosis: *rad-5(mn159)* (*rad*iation sensitive; Hartman and Herman, 1982), *him-7(e1480)* (*h*igh *i*ncidence of *m*ales [meiotic nondisjunction in XX hermaphrodites leads to a high incidence of XO males]; Hodgkin et al., 1979) (Figures 3a and 3b), and *mrt-2(e2663)* (*mort*al germline [*mrt-2* is required for telomere maintenance]; Ahmed and Hodgkin, 2000). Importantly, *mrt-2* encodes the *C. elegans* homolog of the *S. pombe rad1*$^+$ and *S. cerevisae RAD17* checkpoint genes (Ahmed and Hodgkin, 2000). This observation suggests that the function of the *rad1/mrt-2* family in DNA damage checkpoint has remained conserved between yeasts and animals.

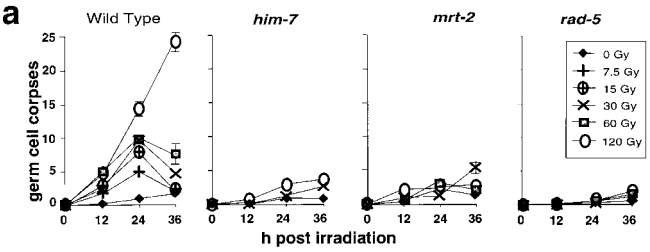
To show that *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* specifically affect DNA damage–induced germ cell apoptosis rather than all programmed cell deaths, we tested the ability of these mutants to prevent radiation-induced germ cell death and developmental apoptosis in the pharynx. Whereas strong and weak *ced-3* mutations prevented developmental cell death and radiation induced cell death to approximately the same extent, *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* strongly prevent radiation-induced apoptosis but have no effect on developmental cell death (Figure 3c).

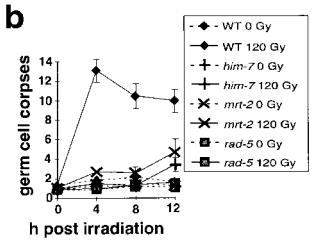
DNA Damage Induces a Checkpoint-Dependent Mitotic Germ Cell Cycle Arrest

Besides DNA damage-induced apoptosis, a transient halt in cell cycle progression is the second defining output of checkpoint signaling (Weinert and Hartwell, 1988; Morgan and Kastan, 1997). To determine whether this is also true in *C. elegans*, we examined the effects of radiation on cell proliferation. Previous studies, focused on embryonic cell divisions, had revealed only minor radiation-dependent cell cycle delays (Jones and Hartman, 1996). Since mitotic germ cells proliferate throughout the life of adult worms, we analyzed the effect of radiation on mitotic germ cells.

Under normal growth conditions, the total number of syncytial germ cell nuclei increases steadily over time (Figure 4a, top left panel, 0 Gy). After irradiation, we found that the number of germ cell nuclei in wild type decreased significantly. This decrease could not be ascribed only to the elimination of damage meiotic germ cells through apoptosis, as irradiated *ced-3(n717)* animals had significantly fewer germ cells than their nonirradiated counterparts, even though apoptosis is completely blocked in these mutants (Figure 4a). We suggest that this residual effect observed in *ced-3* mutants is caused by an arrest or delay in proliferation in the mitotic compartment.

Interestingly, because cellular and nuclear growth





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Genotype	Extra cells pharynx	n	Germ cell corpses	n
Wild Type	0	15	24.6 ± 1.4	15
ced-3(n717)	12.0 ± 2.6	11	0.1 ± 0.1	15
ced-3(n2438)	1.7 ± 1.2	12	15.4 ± 2.5	15
ced-9(n1950)	13.3 ± 0.3	40	2.1 ± 0.3	15
egl-1(n3082)	11.4 ± 0.4	19	4.3 ± 0.4	15
him-7(e1480)	0	20	3.4 ± 0.6	15
mrt-2(e2663)	0	20	1.8 ± 0.5	15
rad-5(mn159)	0.8 ± 0.3	19	1.8 ± 0.4	15

Figure 3. *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* Are Checkpoint Mutants Specifically Defective in Radiation-Induced Cell Death

(a and b) Late L4 stage (a) or adult (b) hermaphrodite *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* worms were irradiated and germ cell death assessed as described in Figure 2. Note that physiological germ cell death (0 Gy) is unaffected in these mutants.

(c) Checkpoint mutants do not affect developmental deaths. Extra cells in the pharynx, resulting from the inhibition of programmed cell death, were quantified as described previously (Hengartner et al., 1992). Results shown are mean ± SEM; n, number of animals counted.

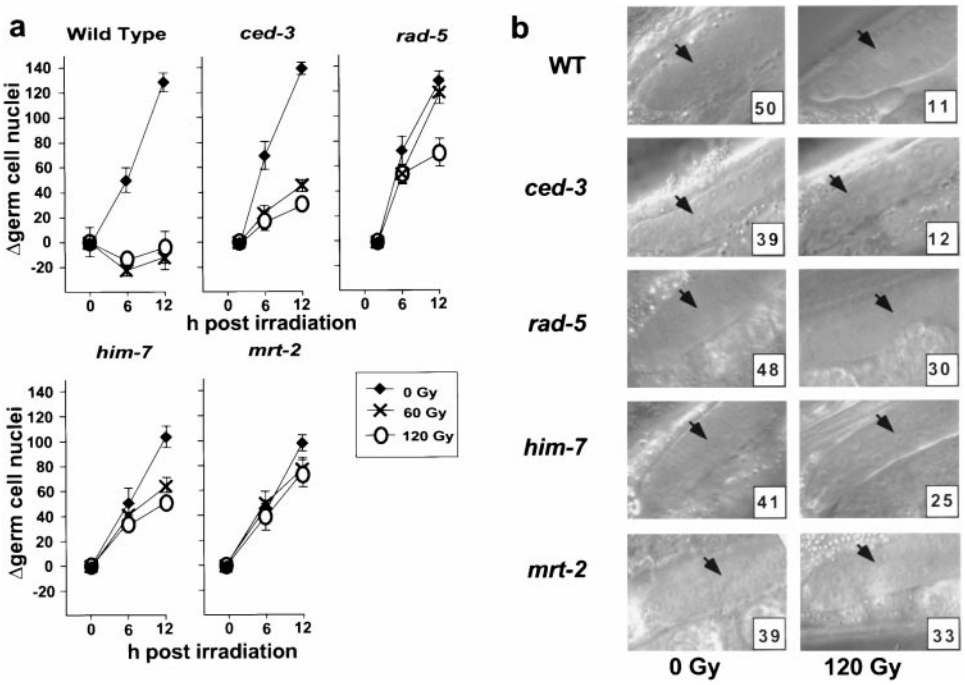

Figure 4. Radiation Induces a Checkpoint Gene–Dependent Cell Proliferation Arrest

(a) Synchronized worms were irradiated at the mid–L4 stage, and the total number of surviving germ cell nuclei (including mitotic and meiotic germ cell nuclei) were counted after DAPI staining at the indicated time points. Note that data shown in this figure represent surviving cells, not dying cells. After irradiation, the total number of germ cell nuclei in wild-type animal decreases, as mitotic cells stop proliferation and meiotic cells undergo apoptosis. The number of nuclei increases only slightly following irradiation in *ced-3* mutants, for germ cells in these mutants do not undergo apoptosis but still do undergo arrest (less death = more surviving cells). Nuclei are most numerous in irradiated checkpoint mutants, as germ cells neither undergo apoptosis nor proliferation arrest. Graph shows changes in cell number from time = 0; initial germ cell numbers were similar to each other and adjusted to 0 at the first time point: 137 ± 13, *N2*; 113 ± 2, *ced-3(n717)*; 121 ± 5, *him-7(e1480)*; 144 ± 6, *mrt-2(e2663)*; and 115 ± 6, *rad-5(mn159)*. Results are mean ± SEM; n = 5.

(b) Representative pictures of the mitotic germ cell compartment. Pictures were taken as described in Figure 1. Arrowheads indicate representative nuclei. The number of mitotic germ cell nuclei visible in each frame are indicated in the lower right corner. Because cell growth is not blocked by DNA damage, mitotic cell nuclei are much larger in animals undergoing proliferation arrest (wild type and *ced-3* mutants). Meiotic germ cells do not increase in size following irradiation (data not shown).

continues during radiation-induced cell proliferation arrest, the volume of mitotic germ cell nuclei as well as their surrounding cytoplasm becomes greatly enlarged (Figure 4b). The finding that regulation of cellular growth and division can be separated is in accordance with classic yeast cell cycle literature, as well as with recent findings in *Drosophila* (Pringle and Hartwell, 1981; Neufeld et al., 1998; Weinert and Hartwell, 1988).

To test whether *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* affect radiation-induced cell proliferation arrest, we analyzed mitotic germline compartments of these mutants upon irradiation. We found that the radiation-dependent reduction of wild-type and *ced-3(n717)* germ cell numbers is reduced or abolished in all three checkpoint mutants (Figure 4a). Furthermore, the radiation-dependent increase in cell size is also largely alleviated in the checkpoint mutants as compared to wild type (Figure 4b). As expected, the radiation response of mitotic germ cells is normal in *ced-3* mutants (Figure 4b). We conclude that, as is the case with p53 in mammals, *mrt-2*, *rad-5*, and *him-7* mediate both apoptosis and proliferation arrest in *C. elegans*.

Checkpoint Mutants Are Radiation Sensitive

In yeasts and mammals, loss of DNA damage checkpoints results in increased genomic instability and reduced long term survival following genotoxic stress (Weinert and Hartwell, 1988; Morgan and Kastan, 1997). To test whether *him-7*, *mrt-2*, and *rad-5* also promote genomic stability and long-term survival in *C. elegans*, we irradiated mutant and wild-type worms and determined the survival rate of their progeny (developing *C. elegans* embryos are highly sensitive to genomic aberrations and are the direct "descendents" of mutagenized germ cells). Embryos laid from checkpoint mutants had a dramatically decreased survival rate as compared to those from wild-type animals (Table 2). Embryos died necrotically (no signs of increased apoptotic cell death) in a radiation dose-dependent manner at various stages of embryonic development, presumably as a delayed consequence of unrepaired DNA damage (data not shown) (Hartman and Herman, 1982). This necrotic death of embryos, which occurs several cell generations after exposure to radiation, is reminiscent of the necrotic death of yeast cells (Weinert and Hartwell, 1988) and to the "clonogenic death" of mammalian cells following treatment with radiation or DNA damage–inducing chemotherapeutic agents.

Interestingly, *ced-3* mutants presented an intermediate sensitivity to radiation (Table 2). Because *ced-3* animals show normal cell proliferation arrest following DNA damage (Figures 3 and 4), the increased sensitivity of *ced-3* mutants is likely due to the absence of DNA damage–induced apoptosis, consistent with the hypothesis

Table 2. Checkpoint Mutants Show Reduced Survival Following Genotoxic Stress

	0 Gy		60 Gy		120 Gy	
Genotype	Eggs/hr	Percent of Survival	Eggs/hr	Percent of Survival	Eggs/hr	Percent of Survival
Wild type	4.1	98%	3.4	63%	2.8	41%
ced-3(n717)	4.5	91%	4.0	41%	4.2	25%
him-7(e1480)	4.7	76%	4.0	22%	3.6	6%
mrt-2(e2663)	4.4	76%	3.5	24%	2.5	3%
rad-5(mn159)	2.2	74%	3.3	34%	3.5	4%

L4 hermaphrodites (six to ten animals) were irradiated as indicated. Eggs laid 24–36 hr after irradiation were counted. Surviving animals at various stages of larval development were counted 2 days later to assess survival. Numbers shown are eggs laid per hour per adult hermaphrodite and the percent of survival of embryos.

that apoptosis following genotoxic insults significantly contributes to the removal of cells with unrepaired DNA.

A Meiotic Checkpoint Monitoring Recombination I Intermediates Triggers Apoptosis in *C. elegans*

Double-strand breaks occur not only following DNA damage, but also normally during meiotic prophase as the initiating events in meiotic recombination (Roeder, 1997). Several yeast mutants that initiate but fail to complete meiotic recombination arrest or delay cell cycle progression at the pachytene stage in a checkpoint-dependent fashion (Lydall et al., 1996; Roeder, 1997). This cell cycle arrest is alleviated by mutations that prevent the initiation of recombination (e.g., mutations that eliminate the double-strand break generating enzyme Spo-11) (Roeder, 1997). To test whether an intermediate block in the recombination process would elicit checkpoint-induced apotosis in *C. elegans*, we inhibited the function of the only nematode homolog of Rad-51 (Rinaldo et al., 1998; Takanami et al., 1998). Rad-51 is a member of the RecA–strand exchange protein family that catalyze the invasion of DNA single-strand overhangs into a recipient double-strand DNA to initiate the formation of D loops and the later steps of meiotic recombination (Bishop et al., 1992; Roeder, 1997). Inactivation of *C. elegans rad-51* by RNA-mediated interference (RNAi) not only produced a set of phenotypes diagnostic of recombination defective mutants (data not shown; see Experimental Procedures; Dernburg et al., 1998), but also resulted in a dramatic increase in germ cell death (Table 3). This increase in germ cell apoptosis is not observed when *rad-51* is inactivated in a *spo-11* background, which is defective in initiating meiotic recombination, suggesting that the increased apoptosis is indeed triggered by accumulation of recombination intermediates (Table 3). The *him-7(e1480)*, *mrt-2(e2663)*, and *rad-5(mn159)* checkpoint mutants suppress the *rad-51* inactivation dependent apoptosis, consistent with the hypothesis that apoptosis is the result of a DNA damage checkpoint pathway (Table 3).

Discussion

C. elegans has provided a very powerful system for the genetic analysis of molecules and pathways that regulate apoptosis. However, these studies have been restricted to cell deaths that are part of developmental programs. Here we show DNA damage can induce apoptosis (Figures 1 and 2) and cell proliferation arrest (Figure 4) in germ cells via an evolutionarily conserved checkpoint pathway (Figure 3).

Cell Type Specificity of Checkpoint Response

As is the case in mammalian systems, the response of *C. elegans* cells to DNA damage is cell type–specific: DNA damage induces a proliferation arrest in mitotic germ cells (Figure 4), whereas female, but not male, meiotic cells can be induced to undergo programmed cell death (Figures 1–3 and data not shown). Interestingly, somatic cells appear to be resistant to DNA damage–induced apoptosis. While we do not know the molecular basis of this resistance, it makes teleological sense: unlike mammals, *C. elegans* development follows a very strict developmental lineage, and there is little ability to replace lost cells (Sulston, 1988). Therefore, it might be advantageous for *C. elegans* to keep damaged

Table 3. A Meiotic Recombination Checkpoint Triggers *him-7*-, *mrt-2*-, and *rad-5*-Dependent Apoptosis

Genotype	Corpses per Germline	n	Number of Worms Injected
Wild type	1.2 ± 0.3	15	N/A
rad-51(RNAi)	13.2 ± 1.4	83	11
rad-51(RNAi) ced-3(n717)	0	100	10
rad-51(RNAi) him-7(e1480)	2.5 ± 0.5	87	9
rad-51(RNAi) mrt-2(e2663)	3.1 ± 0.4	58	7
rad-51(RNAi) rad-5(mn159)	1.7 ± 0.3	49	8
spo-11(ok79)	0.8 ± 0.3	15	N/A
rad-51(RNAi) spo-11(ok79)	3.0 ± 0.6	23	4

To inactivate *rad-51* by RNAi, worms were injected with double-stranded RNA corresponding to the *rad-51* EST clone yk241d12 (for details, see Experimental Procedures). P0 worms with the indicated genotypes were injected, and the progeny of injected worms was collected 16–24 hr after injection. Successful inactivation of *rad-51* by RNAi was confirmed by the high rate (more than 95%) of embryonic lethality, which is due to random meiotic chromosome disjunction. F1 animals with RNAi-inactivated *rad-51* were scored for germ cell death 24 hr after the L4 molt. The number of analyzed F1 worms is indicated (n). N/A, not applicable.

cells, rather than to loose them completely by having them undergo apoptosis. In contrast, the germline contains scores of equivalent cells; eliminating damaged germ cells therefore is of little cost to the animal and might provide a significant evolutionary benefit.

In addition to inducing meiotic germ cell death, we found that DNA damage also transiently blocks proliferation of the mitotic germ stem cells (Figure 4 and data not shown). DNA damage specifically induces cell cycle arrest in species as divergent as *S. cerevisiae*, *S. pombe*, *Drosophila*, and mammals (Weinert and Hartwell, 1988; Hari et al., 1995; Morgan and Kastan, 1997; Caspari and Carr, 1999). While we strongly suspect that a similar mechanism is functioning in *C. elegans*, we have not yet been able to dissect the exact nature of the cell proliferation arrest phenotype (e.g., whether cells arrest in G1, S, or G2/M).

Checkpoint-Mediated Germ Cell Death Is Distinct from Physiological Germ Cell Death

We have previously shown that many *C. elegans* germ cells die under normal growth conditions (Gumienny et al., 1999). The DNA damage–induced germ cell deaths that we describe here appear to be regulated via a distinct pathway from these "physiological germ cell deaths." For example, mutations in *ced-9* and *egl-1* that significantly impair radiation-induced death have no effect on physiological germ cell death (Gumienny et al., 1999; Figure 2). Similarly, mutations in the checkpoint genes *rad-5*, *him-7*, and *mrt-2* only prevent DNA damage–induced apoptosis: physiological germ cell death continues unabated in these mutants (Figure 2, 0 Gy). These data are consistent with the hypothesis that physiological germ cell death is not the result of extensive damage to germ cells, but rather part of a developmental program used to eliminate excess germ cells (Gumienny et al., 1999). However, we cannot exclude the possibility that a small fraction of germ cell deaths are due to activation of the DNA damage checkpoint.

Indeed, we have found that aberrant meiotic recombination intermediates can efficiently induce germ cell death (Table 3). These deaths are mediated by the same pathway as radiation-induced cell death (Table 3). Our findings generalize the presence of meiotic recombination checkpoints throughout evolution. Similar checkpoints have been described to operate during budding yeast meiosis (Lydall et al., 1996; Roeder, 1997) and during mammalian spermatogenesis (Barlow et al., 1997; Plug et al., 1997; Roeder, 1997; Odorisio et al., 1998). In budding yeast, meiotic damage sensing, which like *C. elegans* needs *S. cerevisaie RAD17/S. pombe rad1*$^+$*/mrt-2*, induces a transient meiotic cell cycle arrest (Lydall et al., 1996), whereas during mammalian spermatogenesis, it triggers either apoptosis or cell cycle arrest (Barlow et al., 1997; Plug et al., 1997; Roeder, 1997; Odorisio et al., 1998; Yoshida et al., 1998). Consistent with the general involvement of *S. cerevisae RAD17/S. pombe rad1/mrt-2* in meiotic checkpoint regulation, human *rad-1*, which partially complements *S. pombe rad-1*, has recently been shown to be highly expressed during spermatogenesis and to be associated with meiotic chromosomes (Freire et al., 1998).

C. elegans rad1$^+$ Homolog *mrt-2* Is Required for DNA Damage–Induced Apoptosis and Cell Proliferation Arrest

We have identified three *C. elegans* checkpoint genes—*rad-5*, *mrt-2*, and *him-7*—which function in DNA damage response, as well as in a checkpoint pathway monitoring meiotic recombination intermediates (Table 1). Since only a single mutant allele of each gene has been identified, we suspect that many additional checkpoint genes remain to be identified in *C. elegans*.

mrt-2(e2663) affects a splice junction in the evolutionarily conserved *rad-1* checkpoint gene family (Ahmed and Hodgkin, 2000), which includes *S. pombe rad1*$^+$ and *S. cerevisiae RAD17* (Weinert, 1998). These results strongly suggest that this gene family also mediates DNA damage response in animals. Because of the extensive conservation of the apoptotic program between nematodes and mammals, our findings suggest that *rad1*$^+$ homologs might also be required to induce apoptosis following DNA damage in humans.

The DNA Damage Checkpoint Pathway Is Evolutionary Conserved

How did the ability of *C. elegans* cells to die in response to DNA damage evolve? Our findings suggest that this ability was not acquired by the generation of a de novo pathway, but rather that it evolved by linking up the more ancient cell cycle response pathway to the apoptotic machinery. We do not yet know how long this "link" is, but it might involve only one or a few genes.

The *C. elegans* and mammalian genomes contain homologs of virtually all known yeast checkpoint genes (Bao et al., 1998; Dean et al., 1998; Freire et al., 1998; Marathi et al., 1998; Matsuoka et al., 1998; Weinert, 1998; Volkmer and Karnitz, 1999). *mrt-2(e2663)* contains a mutation in the *C. elegans* homolog of *S. pombe rad1*$^+$ and *S. cerevisiae RAD17* (Ahmed and Hodgkin, 2000). In yeasts and humans, many checkpoint proteins are part of large protein complexes that include, according to the *S. pombe* nomenclature, *rad1*$^+$, *rad3*$^+$, *rad17*$^+$, *rad9*$^+$, and *hus1*$^+$ (Weinert, 1998; Caspari and Carr, 1999; Volkmer and Karnitz, 1999). In yeasts, these complexes have been shown to act upstream of a signaling cascade that ultimately results in cell cycle arrest (Weinert, 1998; Caspari and Carr, 1999). Besides *rad3*$^+$, which shows homology to the mammalian ATR and to a lesser extent to the mammalian ATM kinase, molecular details of checkpoint gene function are still largely elusive in higher eukaryotes.

Our finding that the *C. elegans* homolog of *S. pombe rad1*$^+$ regulates both checkpoint induced apoptosis as well as cell cycle arrest, suggests that *mrt-2/rad-1*$^+$*/RAD17* may act together with other conserved checkpoint genes throughout evolution to maintain genomic stability. These proteins might be part of an ancient checkpoint pathway, which was expanded by the incorporation of p53 and its family members during vertebrate evolution. As is the case with p53, mutations in these more ancient checkpoint genes might contribute to tumor progression and/or acquisition of resistance to treatment with genotoxic chemotherapeutic agents.

Experimental Procedures

Strains

All used strains are described by J. Hodgkin (1997) unless otherwise stated. *ced-3(n2438)* is described by Hengartner and Horvitz (1994a), *spo-11(ok79)* by Dernburg et al. (1998), *egl-1(n3082)* by Conradt and Horvitz (1998), and *mrt-2(e2664)* by Ahmed and Hodgkin (2000).

Quantification of Radiation-Induced Germ Cell Corpses and Mitotic Germ Cell Proliferation Arrest

For corpse and mitotic germ cell counting, animals were synchronized as described in figure legends and mounted under standard conditions (Epstein and Shakes, 1995). Corpses were counted under Nomarski optics. Mitotic germ cells were counted after staining with DAPI (worms suspended in 1 μl water were fixed with Carnoy's fixative [6 parts ethanol, 3 parts chloroform, and 1 part glacial acidic acid], air dried, and stained with a small drop of DAPI solution [2 μg/ml in M9 buffer]). For irradiation, a Cs^{137} source (J. L. Shepherd and Associates, model 69 A irradiator; 3.984 Gy/min) was used. To induce germ cell death with ENU, hermaphrodites were synchronized in late L4 and treated in M9 buffer with the indicated concentrations of ENU for 4 hr. To induce germ cell death with UV, worms were irradiated with 6,000–24,000 μJ of UV light (254 nm) using a Stratalinker (Model 1800) cross-linker.

Quantification of Somatic Cell Death

Corpses in the head of *ced-1(e1735)* corpse engulfment-defective animals at the first larval stage (Hedgecock et al., 1983) were counted 3 hr after irradiation with 60 Gy.

Video Time Lapse Analysis

Animals were mounted under standard conditions in M9 buffer containing 2 mM levamisole (levamisole prevents animals from moving but does not affect the germline or the gonad). For video analysis, a Cohu 4910 series camera attached to a Zeiss (Axioskope) microscope was used. Images were processed using the NIH image program.

rad-5(mn159)

rad-5(mn159) is likely to be a hypomorphic allele (Hartman and Herman, 1982). However, at 20°C the only significant phenotype of *rad-5(mn159)* is a defect in radiation-induced germ cell death. The brood size of *rad-5(mn159*) animals is approximately 50% of wild type at 20°C but rapidly drops at higher temperatures due to embryonic lethality at various stages of development (A. G., S. M., and M. O. H., unpublished data) (Hartman and Herman, 1982). Furthermore, *rad-5(mn159)* animals do not induce apoptosis upon radiation at 15°C (A. G., S. M., and M. O. H., unpublished data).

RNAi of *rad-51*

The *C. elegans* genome contains only one *recA/rad51/dmc1* homolog encoded by Y43C5A.6 on chromosome IV (Rinaldo et al., 1998; Takanami et al., 1998). The consequence of *spo-11* and *recA/dmc1* inactivation in yeast can be assayed because meiotic chromosomes have to be linked to each other at metaphase of meiosis I via intact recombination intermediates to ensure proper meiotic chromosome segregation. Blocking the initiation of meiotic double-strand breaks (e.g., *spo11*), or blocking subsequent stages of recombination (e.g., by *dmc1*), causes random meiotic chromosome segregation (Roeder, 1997). In *C. elegans*, *spo-11* or *rad-51* inactivation leads to more than 98% embryonic lethality, due to random chromosome segregation; and only very few affected worms grow to adulthood because they receive by chance a correct set of chromosomes (Dernburg et al., 1998; data not shown). Approximately one-third of the surviving worms are males (Dernburg et al., 1998; data not shown) because of the random segregation of sex chromosomes (X0 are males, XX and XXX are hermaphrodites). To inactivate *rad-51*, RNA was synthesized from both orientations (T3 and T7 primers) of the *rad-51* encoding EST clone yk241d12 using an Promega RNA synthesis kit. Single-stranded RNAs were annealed and injected as described (Fire et al., 1998).

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Note Added in Proof

Since the acceptance of this paper, we have found that the CB1480 *him-7(e1480) V* strain described in this paper contains a second, previously unrecognized mutation, *op241*. We have now separated this mutation from *him-7(e1480)* and found that it, and not *e1480*, faithfully reproduces the checkpoint defects observed in the CB1480 strain. Thus, all the checkpoint defects that we observed in the CB1480 strain and have ascribed to *e1480* in this paper are in fact due to the *op241* mutation. *op241* maps to the left arm of *LG1*.