A DNA-Damage-Induced Cell Cycle Checkpoint in Arabidopsis

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ABSTRACT

Although it is well established that plant seeds treated with high doses of gamma radiation arrest development as seedlings, the cause of this arrest is unknown. The *uvh1* mutant of Arabidopsis is defective in a homolog of the human repair endonuclease *XPF*, and *uvh1* mutants are sensitive to both the toxic effects of UV and the cytostatic effects of gamma radiation. Here we find that gamma irradiation of *uvh1* plants specifically triggers a G_2-phase cell cycle arrest. Mutants, termed *suppressor of gamma* (*sog*), that suppress this radiation-induced arrest and proceed through the cell cycle unimpeded were recovered in the *uvh1* background; the resulting irradiated plants are genetically unstable. The *sog* mutations fall into two complementation groups. They are second-site suppressors of the *uvh1* mutant's sensitivity to gamma radiation but do not affect the susceptibility of the plant to UV radiation. In addition to rendering the plants resistant to the growth inhibitory effects of gamma radiation, the *sog1* mutation affects the proper development of the pollen tetrad, suggesting that *SOG1* might also play a role in the regulation of cell cycle progression during meiosis.

THE ability of an organism to repair DNA damage in a timely fashion is essential for the integrity and maintenance of the genome. To this end, signal transduction pathways are involved in sensing DNA damage, pausing the cell division cycle to provide time for repair, inducing repair, and finally releasing the cell cycle from arrest. This arrest allows the cell's repair machinery time prior to S phase or M phase to mend the damage. Cells proficient in DNA repair, but deficient in their ability to arrest in response to damage, typically exhibit high levels of genomic instability, demonstrating the importance of DNA-damage-dependent cell cycle arrest in maintaining the genome (for review see Paulovich *et al.* 1997).

Half a century ago plant biologists demonstrated that when corn kernels were treated with very high levels of ionizing radiation (500 krad), the seedlings would germinate at a normal rate and respond appropriately to their environment. The plants would then produce only a limited number of leaves, which contained few cells, although these cells were much larger than those of unirradiated plants (Schwartz and Bay 1956). Further studies on the formation of "gamma plantlets" (Evans 1965) demonstrated that irradiated *Vicia faba* (fava bean) was delayed by several days in the uptake of tritiated thymidine, indicating that entry into S phase was postponed by radiation treatment. Foard and Haber (1961); and Haber *et al.* (1961) further characterized irradiated plants, this time working in wheat, and showed that although the plants were arrested there was no evidence of senescence and that protein levels appeared to be normal. These early experiments demonstrated that the treatment of seeds with high levels of gamma radiation could arrest progression through the cell cycle but did not arrest transcription, translation, or basic cellular processes.

In this article, we investigate the mechanistic basis of the gamma-plantlet phenomena in Arabidopsis and seek to determine whether plants in fact do have a checkpoint response to DNA damage. We chose to investigate the responses of Arabidopsis to gamma radiation in the gamma radiation hypersensitive *uvh1* mutant background. A screen for UV-sensitive mutants of Arabidopsis (Harlow *et al.* 1994) identified one mutation, termed *uvh1*, that rendered the plants sensitive to both UV and gamma radiation (Figure 1). Several groups (Fidantsef *et al.* 2000; Liu *et al.* 2000) demonstrated that *UVH1* is a homolog of the human repair endonuclease *XPF*, which is involved in nucleotide excision repair, the excision of intermolecular crosslinks, and the repair of single stranded 3′ DNA overhangs that are generated during the joining of double-strand breaks (Figure 2; Bardwell *et al.* 1994). Like the yeast mutants defective in double-strand-break repair, *uvh1* makes an ideal genetic background for the study of DNA-damage-induced cell cycle arrest as very low doses of gamma radiation induce the gamma-plantlet phenomenon, eliminating the varied stress responses observed in wild-type at high doses. Our studies suggest that the gamma-plantlet response in *uvh1* is due to a DNA-damage-dependent G_2 cell cycle arrest. Furthermore, the isolation of mutants defective in this response demonstrates that the forma-

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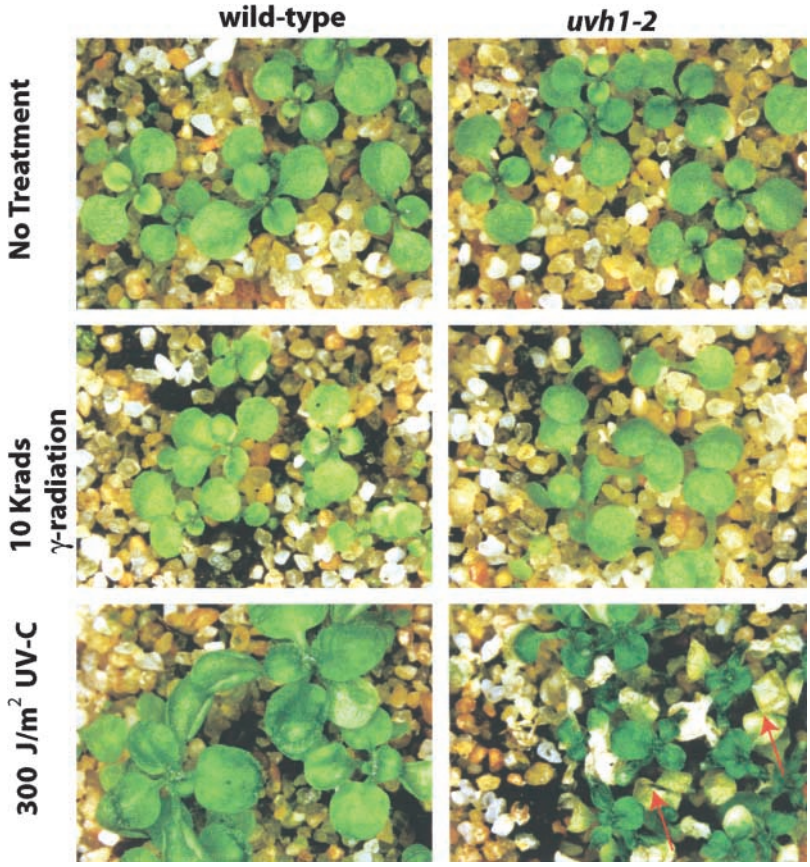

Figure 1.—Effects of UV and gamma radiation on wild-type and *uvh1-2* plants: wild-type and *uvh1-2* plants with no treatment, seeds irradiated at 10 krad, and plants treated with 300 J/m^2 UV-C radiation. Red arrows indicate necrotic leaves in UV-C-treated plants.

tion of gamma plantlets is not an intrinsic and direct effect of unrepaired damage but is instead imposed upon the cell by a genetically encoded signal transduction pathway. The genetically unstable phenotype of these mutants indicates that this checkpoint is important for the maintenance of genomic integrity.

MATERIALS AND METHODS

Stocks: *uvh1-1* was isolated in the Columbia background by Harlow *et al.* (1994) and was a gift from David Mount. *uvh1-2* was isolated in the Landsberg erecta background in our lab (Jiang *et al.* 1997). Arabidopsis plants (Columbia ecotype) containing the *cycB1:GUS* reporter construct were a gift of Peter Doerner and Adan Colon-Carmona (Colon-Carmona *et al.* 1999). The *gymnous::GUS* reporter line was a gift of Yuval Eshed (Eshed *et al.* 1999). The *alb1* line (Columbia ecotype) is listed at the *Arabidopsis* Biological Resource Center (Columbus, OH) as stock CS26.

Growth conditions: Plants were grown on either Sunshine mix 2 (SunGro, Bellevue, WA) or 1/2× MS (GIBCO, Carlsbad, CA) Phytagel (Sigma, St. Louis) agar, pH 5.2. Plants were grown under cool-white lamps filtered through Mylar at an intensity of 100–150 μmol/m^2/sec with a 24-hr day. The temperature was set at 22° and the humidity at 50%.

Mutagenesis and screening: Two grams of *uvh1-2* seeds was treated with 0.225% (w/v) methane sulfonic acid ethyl ester (EMS; Sigma) and placed on a rocking table for 16 hr at room temperature. The EMS-containing solution was decanted and the seeds were washed in 500 ml of water for 4 hr before sowing. Approximately 5000 M_1 plants were harvested in two separate batches. A total of 50,000 M_2 seeds were gamma irradiated (see below) and 12-day-old seedlings were screened for the presence of leaves. The *sog1-1* mutation was generated in the first batch of seeds while the *sog1-2–sog1-5* mutants were from the second; thus *sog1-1* was derived independently from the other mutants.

Radiation treatment: Prior to radiation, seeds were imbibed in water and placed at 4° for 24 hr. Gamma radiation was carried out in a ^{137}Cs reactor at the UC Davis Institute for Toxicology and Environmental Health. UV-irradiated plants were grown for 2 weeks and then treated with 250 kJ/m^2 of UV-C using a germicidal lamp, placed under orange light for 48 hr, and then returned to normal lighting conditions. UV sensitivity was assayed 72 hr after transfer to the growth chamber.

Histology: A variety of histological methods were used to analyze tissue in this study.

Paraffin embedding and 4′,6-diamidino-2-phenylindole staining: To analyze the mitotic index, seeds were gamma irradiated, grown in 1/2× MS liquid culture, pH 5.2, and 3 days later the plants were fixed in FAA and then dehydrated in an ethanol series followed by paraffin embedding using a Leica (Wetzler, Germany) tissue fixation TP1020 robot (50% ethanol 1 hr, 60% ethanol 1 hr, 75% ethanol 1 hr, 80% ethanol 1 hr, 95% ethanol 0.1% eosin 6 hr, 2× 100% ethanol 1 hr, 2× Histoclear 1 hr, Histoclear saturated with paraffin 4 hr, paraffin 5 hr, and paraffin 6 hr). Embedded tissue was cut to an 8-μm width on a Microm (Walldorf, Germany) HM-340E microtome. Wax sections were placed on microscope slides and then deparaffinized and dehydrated (2 × 10 min Histoclear, 2 × 2 min 100% ethanol, 1 min 95% ethanol, 1 min 90% ethanol, 1 min 80% ethanol, 1 min 60% ethanol, 1 min 30% ethanol, and 2 min water). The tissue was mounted in 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes, Eugene, OR), 50% glycerol, 0.1 m Tris, pH 9.2, 1 mg/

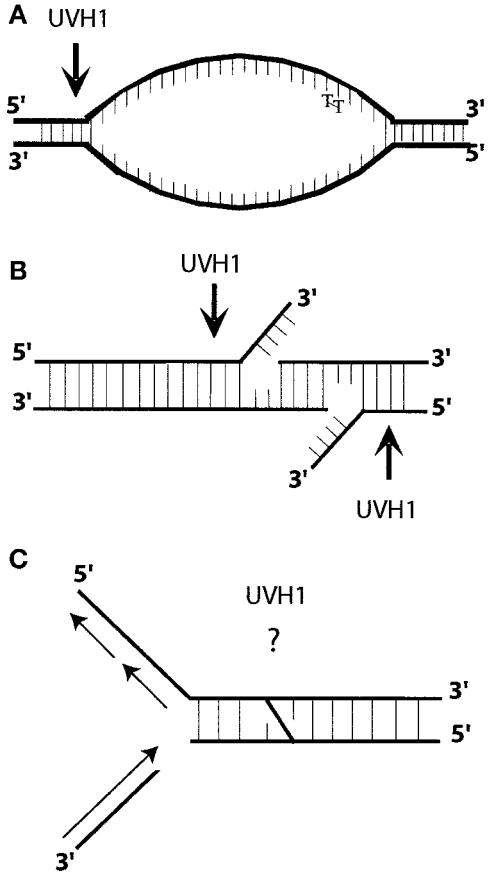

FIGURE 2.—Roles of the UVH1 protein in nucleotide excision repair (A), nonhomologous end joining (B), and the repair of intermolecular DNA crosslinks (C). (A) Nucleotide excision repair: Following the recognition of base damage by the nucleotide repair machinery, a denaturation bubble is formed. The XPF/ERCC1 heterodimer (*UVH1* is the Arabidopsis homolog of *XPF*), in conjunction with RPA, cleaves the duplex/3′ single-strand junction of the bubble while XPG cleaves the 3′ end of the bubble. The damaged oligonucleotide is then released and a new strand is synthesized across the gap (FRIEDBERG *et al.* 1995). (B) Nonhomologous end joining: In the repair of a double-strand break the 5′ end of the DNA is resected to reveal regions of microhomology. The annealing of the complementary strands then displaces one or more single-strand flaps. Again, the XPF/ERCC1 heterodimer, guided by RPA, specifically cleaves 5′ of the double-strand to single-strand DNA transition, releasing the DNA flap and allowing ligation of the ends to proceed (BARDWELL *et al.* 1994). The heterodimer plays a similar role in removing flaps during single-strand annealing, a homology-dependent (but RAD51 independent) process involving recombination between tandem repeats (PRADO and AGUILERA 1995). (C) Interstrand crosslinks: Although crosslink repair is still poorly understood, mutants defective in the homologs of XPF or ERCC are extremely sensitive to crosslinking agents.

ml phenylenediamine. The stained material was analyzed on a Zeiss (Jena, Germany) Axiphot microscope.

Staining for GUS: Plants that were to be analyzed for GUS were grown in liquid containing 1/2× MS salts at a pH of 5.2. If embryos were to be harvested the seed coat was first removed. The tissue was cleared and stained according to standard techniques (JEFFERSON *et al.* 1987; RODRIGUES-POUSADA *et al.* 1993). The stained tissue was then placed on slides with 50% glycerol and analyzed on a Zeiss Axiophot microscope.

Tetrad analysis: To visualize pollen tetrads and meiocytes, individual buds were dissected and the anthers removed. The anthers were then pierced with a fine needle, allowing the contents to flow out. The tissue was prepared according to Ross *et al.* (1996) with slight modification; the buds were digested at 37° for 30 min in 0.3% pectolyase, 0.3% cytohelicase, and 1% cellulase (Sigma) and then mounted in DAPI as above. The tissue was analyzed on a Zeiss Axiophot microscope and images were captured using a Zeiss MC100 camera with Kodak 160T film (Eastman Kodak, Cherry Hill, NJ).

Cosegregation of tetrad and gamma radiation phenotypes: To determine whether the abnormal tetrad phenotype cosegregated with the gamma-resistant phenotype we analyzed a population derived from a backcross of *uvh1-2 sog1-3* to *uvh1-2*. F_3 families from this cross were scored for their sensitivity to both UV and gamma radiation. Individuals from 10 gamma-resistant and 10 gamma-sensitive families were then analyzed for the presence of abnormal tetrads as detailed above.

Loss of heterozygosity tests: All *uvh1-2 sog1* mutants were backcrossed to *uvh1-2*, and the resulting F_3 families were screened for plants that were sensitive to UV but formed leaves in the presence of gamma radiation. Seeds from these families were sown and the resulting plants were crossed to *alb1* mutant plants (these plants carry, heterozygously, a mutation that produces an albino phenotype when homozygous). F_3 families were screened for lines that were homozygous for either *uvh1-2* or *uvh1-2* and *sog1* and were heterozygous for the *alb1* mutation.

RESULTS

Irradiated *uvh1* plants do not progress through mitosis: While it has long been established that the irradiation of plant seeds induces an arrest of seedling development, it is not known whether this developmental arrest is due to the death of meristematic cells or to an arrest of cell division. To distinguish between these two possibilities, we analyzed DAPI-stained longitudinal sections of irradiated Arabidopsis seedlings for evidence of either cell death or cell cycle arrest. Wild-type and *uvh1-2* seeds were either untreated or irradiated at 10 krad and the shoot apical meristem of DAPI-stained sectioned plants was analyzed. In no case did we find any direct evidence for cell death. There were no obviously enucleate cells, no apoptotic bodies (WANG *et al.* 1996), and no evidence of dead crushed cells (data not shown). However, when we compared the shoot apical meristem of arrested seedlings to that of nonarrested seedlings, we found a substantial difference in the ability of cells to progress through mitosis. To quantify the percentage of mitotically active cells in the different treatments, we counted the number of cells in which a mitotic figure was present (Figure 3). We found that both unirradiated wild-type and unirradiated *uvh1* plants had approximately the same fraction of cells passing through mitosis, with mitotic indices of ~4%. While irradiated wild-type plants were not different from unirradiated plants, none of the 20 gamma-irradiated *uvh1* sections had a mitotic figure, indicating that the shoot apical meristems of

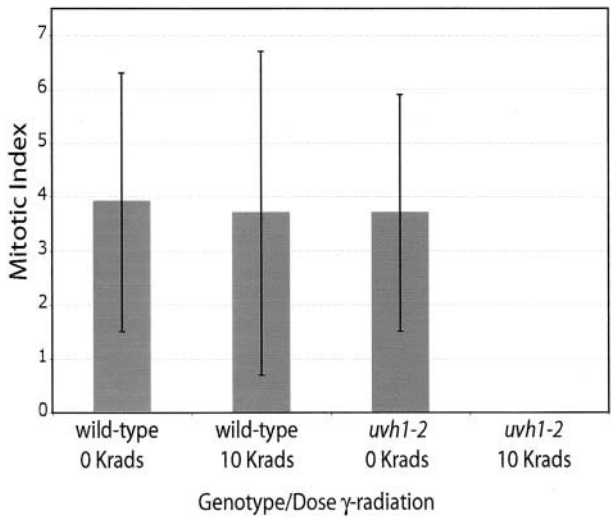

FIGURE 3.—Mitotic index of irradiated and unirradiated wild-type and *uvh1-2* seedlings at the shoot apical meristem. Seeds of wild type and *uvh1-2* were gamma irradiated at 10 krad. Twelve days following gamma radiation the tissue was fixed and sectioned, and the nuclei were stained with DAPI. Mitotic figures were present in 3.9 ± 2.4% ($n = 20$) and 3.7 ± 3.0% ($n = 19$) of unirradiated and irradiated wild-type shoot apical meristem cells, respectively. In *uvh1-2* plants, 3.7 ± 2.2% ($n = 25$) of the unirradiated shoot apical meristem cells contained mitotic figures, while none of the irradiated *uvh1-2* cells were in mitosis ($n = 20$; n = number of plants screened). Each L*er* apical meristem contains ~100 cells at germination; a minimum of 50 cells were scored from each plant.

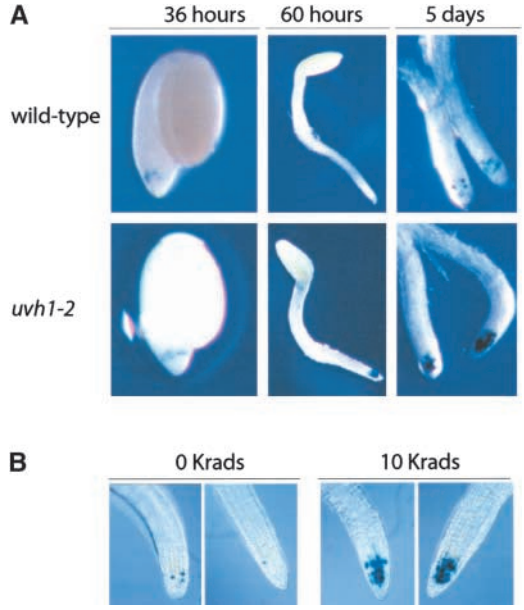

FIGURE 4.—Shoot apical meristem of irradiated *uvh1-2* roots arrest in G_2 phase. Seeds were imbibed in water for 24 hr and then gamma irradiated at 10 krad. Seedlings were harvested either 36 hr or 5 days after gamma irradiation and then stained for the presence of the GUS protein. (A) Both wild-type and *uvh1-2* plants showed an accumulation of GUS and, therefore, an entry into G_2 36 hr after imbibition. While wild type never had more than a few cells staining for GUS at any one time, a large proportion of the *uvh1-2* root apical cells stained for the presence of GUS. (B) A ×20 magnification of 5-day-old *uvh1-2* roots, either unirradiated or treated with 10 krad of gamma radiation. Two representative roots are displayed at each dose.

these plants were arrested at some point outside of M phase.

Meristematic cells of *uvh1-2* enter G_2 but do not proceed to M: To pinpoint in which phase of the cell cycle the gamma plantlets were arrested, we crossed a late G_2-/early M-phase specific reporter construct in the Columbia background into the *uvh1-2* line. This construct consists of the Arabidopsis *cycB1* promoter (formerly designated *cyc1aAt*; see FERREIRA *et al.* 1994) driving a protein fusion of the *cycB1* mitotic destruction box and the β-glucuronidase gene. β-Glucuronidase reacts with X-GlcU to create a blue semi-cell-autonomous precipitate. The *cycB1* promoter drives expression of β-glucuronidase in late G_2/early M while the mitotic destruction box targets the protein for degradation as the cell begins to exit M phase (COLON-CARMONA *et al.* 1999).

As the plant embryo becomes fully mature, the cells cease division and prepare for long-term quiescence. Across the plant kingdom there is variation as to whether these cells completely arrest in G_1 or arrest in both G_1 and G_2 (DELTOUR 1985). On the basis of previous work we expected that the bulk of the Arabidopsis embryo's cells would be arrested in G_1 (LAUFS *et al.* 1998). To obtain a qualitative estimate of the frequencies of cells in G_2, we looked for expression of the *cycB1::GUS* construct in the mature embryo. We removed the seed coat of mature *cycB1::GUS* seeds, imbibed the embryos in X-GlcU, and could find no GUS staining cells in the Arabidopsis embryo. To ensure that the product of the GUS gene was stable in the seeds, we also looked at the same reporter driven by the embryo-specific *Gymnos* promoter (ESHED *et al.* 1999) and found ample staining (data not shown). This observation is consistent with previously published observations (LAUFS *et al.* 1998) suggesting that the Arabidopsis embryo is a naturally "synchronized" G_1 population of cells.

We next analyzed the effects of radiation on cell cycle progression in *uvh1-2 cycB1::GUS vs. UVH1 cycB1::GUS* plants. Seeds, having been soaked in water for 24 hr, were either untreated or gamma irradiated at 10 krad. Three days after irradiation the plants were analyzed for expression of the reporter. We found no evidence for a substantial G_1 arrest; at 24 hr after irradiation (48 hr after imbibition) no cells had entered G_2, while at 36 hr after irradiation the cells of both wild-type and *uvh1* root tips had begun to progress into G_2. However, at later time points the apical and root tip meristem cells of irradiated *uvh1-2* plants accumulated in G_2 while irradiated wild-type or unirradiated wild-type and *uvh1-2* meristem cells continued through the cell cycle (Figure 4). These data, taken together with the mitotic index

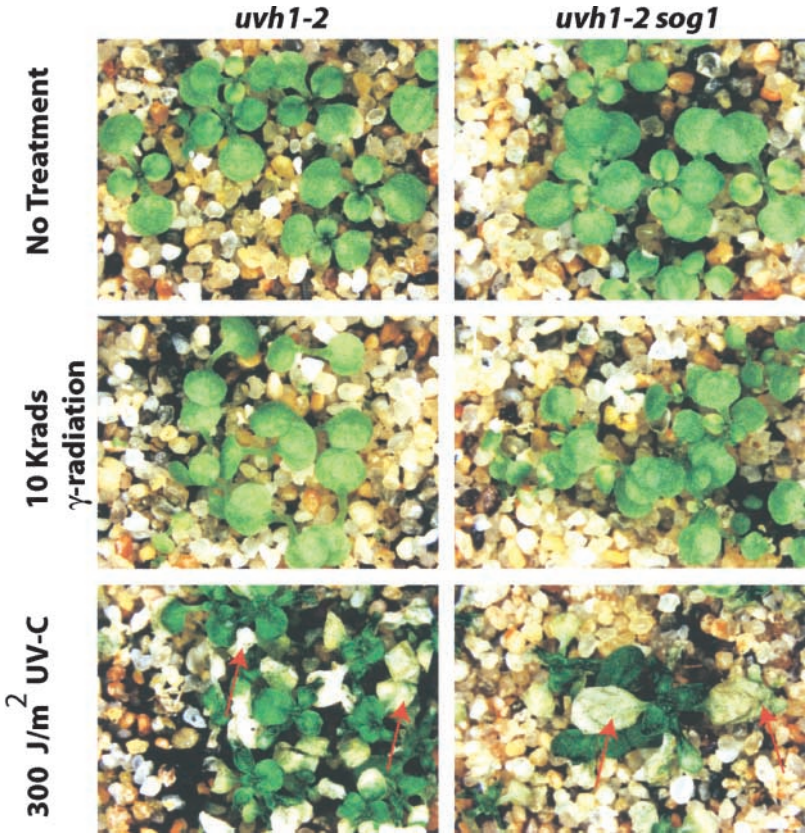

FIGURE 5.—*sog1 uvh1* mutants are gamma resistant but UV hypersensitive. *uvh1-2* and *uvh1-2 sog1* plants from untreated seeds, seeds gamma irradiated at 10 krad, or seedlings treated with 300 J/m^2 UV-C radiation. Red arrows indicate necrotic leaves in UV-C-treated plants.

investigations, suggest that irradiation of *uvh1-2* seeds triggers a G_2 arrest. In contrast, undamaged or repair-proficient cells continue to cycle normally.

***sog* mutants in the *uvh1-2* background lack the gamma-plantlet response:** Radiation-induced G_2 arrest of *uvh1-2* seedlings could be due to one of two causes. DNA damage induced by gamma radiation might directly impede progression through the cell cycle by physically blocking the cellular machinery. For example, damaged bases might block the advancement of RNA polymerase and therefore prevent the expression of genes required to positively regulate cell cycle progression. Alternatively, an extrinsic signaling network may sense even very small amounts of DNA-damage and enforce an arrest of cell division, providing time for the cell to repair this damage before the chromosomes segregate into separate daughter cells and the opportunity for repair is lost. Isolation of mutants that are defective in DNA-damage-induced arrest provides evidence that this arrest is due to an extrinsic signaling mechanism. A signal transduction network that senses DNA damage and arrests cell division is well documented in the yeast and mammalian literature (for example, see WEINERT and HARTWELL 1988; ZHOU and ELLEDGE 2000).

To differentiate between the two possible causes of arrest we screened for mutants in the *uvh1-2* background that did not arrest cell division following gamma irradiation. Since *uvh1-2* is defective in DNA repair, we could then screen for suppressors of arrest using a very low dose of gamma radiation without generating excessive damage throughout the cell. Because Arabidopsis is diploid, we reasoned that some loss of chromosome arms resulting from segregation of unrepaired chromatids would be tolerated. Interpolating from the yeast and mammalian data on the rate of induction of double-strand breaks (DSBs) by gamma radiation, a 10-krad dose should induce ~70 DSBs per haploid Arabidopsis genome (WARD 1990), and it is unclear what fraction of double-strand breaks (or other gamma-induced lesions) are repaired via a *UVH1*-dependent pathway. Approximately 5000 *uvh1-2* seeds were EMS mutagenized. As we assumed that most of the mutations would be recessive, we allowed the M_1 plants to self-fertilize and then screened the M_2 generation. Of 50,000 M_2 plants, 52 were able to form leaves following γ-radiation. The M_3 progeny of these 52 plants were retested for their ability to form leaves following gamma radiation. Six families were found to reproducibly develop true leaves after radiation. These mutants were termed *sog* (*s*uppressor *o*f *g*amma radiation; Figure 5).

To determine whether the *sog* mutations were dominant or recessive we backcrossed the *sog uvh1-2* mutants to *uvh1-2*, selfed the resulting F_1, and scored the segregating F_2 generation for resistance to gamma radiation. We found that the F_2 progeny segregated in a 3:1 ratio [3 gamma sensitive (arrested) to 1 resistant], indicating that the *sog* alleles are recessive (Table 1). We then determined how many genes the six radiation-resistant

TABLE 1

Segregation of the gamma-radiation-resistant phenotype in F_2 progeny from backcrosses of *sog uvh1-2* to *uvh1-2* plants

Cross (female × male)	Radiation^R	Radiation^S	χ^2 for 3:1
uvh1-2 sog1-1 × *uvh1-2*	16	42	0.21
uvh1-2 sog1-2 × *uvh1-2*	22	53	0.76
uvh1-2 sog1-3 × *uvh1-2*	20	66	0.14
uvh1-2 sog1-4 × *uvh1-2*	16	57	0.37
uvh1-2 sog1-5 × *uvh1-2*	15	69	2.23
uvh1-2 sog1-6 × *uvh1-2*	27	85	0.04

families represented by performing a complementation test. Individuals from each family were crossed in a pairwise manner, the F_1 was selfed, and the F_2 plants were scored for radiation resistance. Crosses between mutants affecting the same gene should produce 100% gamma-sensitive progeny, while mutants in different genes, when crossed, will produce an F_2 generation that segregates for wild-type *vs.* mutant phenotypes [in an expected ratio of 9 sensitive to 7 resistant (*sog/sog*) plants, if unlinked]. At 10 krad, occasional "pseudosensitive" individuals (plantlets that produced no true leaves at the time of scoring) are observed in even homozygous *uvh1 sog* mutants, and chi-square tests were performed to distinguish between the presence of these rare "escapers" and the higher frequency of segregation expected from noncomplementation. This test revealed that the mutants fell into a single complementation group (Table 2). The *sog1* mutants were generated from two independently mutagenized populations (and thus might represent only two alleles). *sog1-6* has severe fertility defects and therefore has proved recalcitrant to genetic analysis; for this reason this article focuses on the *sog1-1* through *1-5* alleles.

It is possible that the *sog1* mutation did not represent a second-site suppressor of the gamma-sensitive phenotype of *uvh1*, but instead was merely a reversion of the *uvh1* mutation. To test this possibility we utilized the dual role of *UVH1* in DNA repair; as well as being hypersensitive to gamma radiation *uvh1* is also hypersensitive to the effects of UV light. The *UVH1* gene encodes a homolog of the human repair endonuclease *XPF* and the ensuing sensitivity to UV light derives from the persistence of toxic pyrimidine dimers (Fidantsef *et al.* 2000; Liu *et al.* 2000). These UV-induced lesions can impair the progression of DNA and RNA polymerase and therefore lead to cell death. UV toxicity is observed as the browning and necrosis of exposed tissue. To determine whether the UV-sensitive phenotype of *uvh1* mutation was still present, the *uvh1-2 sog1* mutants were grown for 2 weeks on soil and then irradiated with UV-C light. The plants were subsequently shielded from photoreactivating blue light for 3 days and then placed in the growth chamber. All of the mutants exhibited the typical

TABLE 2

Segregation of the gamma-radiation-resistant phenotype in F_2 progeny from crosses between the different *sog uvh1-2* isolates

Cross (female × male)	Radiation^R	Radiation^S	χ^2 for 9:7
uvh1-2 sog1-1 × *uvh1-2 sog1-2*	132	4	157
uvh1-2 sog1-1 × *uvh1-2 sog1-3*	50	0	64.2
uvh1-2 sog1-1 × *uvh1-2 sog1-4*	81	6	85.9
uvh1-2 sog1-1 × *uvh1-2 sog1-5*	60	0	77.14
uvh1-2 sog1-1 × *uvh1-2 sog1-6*	22	8	10.66
uvh1-2 sog1-2 × *uvh1-2 sog1-3*	76	3	88.33
uvh1-2 sog1-2 × *uvh1-2 sog1-4*	130	3	158
uvh1-2 sog1-2 × *uvh1-2 sog1-5*	51	3	56.5
uvh1-2 sog1-2 × *uvh1-2 sog1-6*	59	34	14.6
uvh1-2 sog1-3 × *uvh1-2 sog1-4*	40	0	51.4
uvh1-2 sog1-3 × *uvh1-2 sog1-5*	42	0	53.9
uvh1-2 sog1-3 × *uvh1-2 sog1-6*	25	17	4.22
uvh1-2 sog1-4 × *uvh1-2 sog1-5*	43	7	36.2
uvh1-2 sog1-4 × *uvh1-2 sog1-6*	24	22	1.34
uvh1-2 sog1-5 × *uvh1-2 sog1-6*	24	14	5.86

necrotic browning of the leaves that is the hallmark of UV hypersensitivity (Figure 5). Therefore, the *sog* mutation does not represent a complete reversion of *uvh1*.

The possibility remained that the *sog1* mutation represented a partial reversion of the gamma-radiation-induced damage repair capacity of *uvh1-2*. To test this possibility we analyzed the segregation ratio of *uvh1-2 sog1* plants crossed to wild type. If the *sog1* phenotype were due to reversion of the gamma sensitivity conferred by the *uvh1-2* allele, one would expect all F_2 progeny to be gamma resistant. Rather, four independently generated F_2 families segregated 13:3 for resistance to gamma radiation ($\chi^2 = 0.35 \sim 2.4$), confirming the genetic separability of the *uvh1-2* and *sog1* mutations.

***sog1* mutants produce leaves at wild-type rates:** To understand the extent of *sog1* suppression of the gamma-plantlet phenomena we compared the rate of leaf production in irradiated wild-type, *uvh1*, and *uvh1 sog1* plants. It is possible that irradiated plants with only a partial checkpoint defect would have a growth rate intermediate between wild-type and *uvh1*. On the other hand, if the *sog1* mutation was pleiotropic and was involved in regulating the development of new organs, it is possible that the *sog1* mutants would show growth rates in excess of wild type. To investigate these two possibilities we irradiated wild-type, *uvh1-2*, and *uvh1-2 sog1* seeds and measured the rate of true leaf development over 2.5 weeks. On average, the first pair of leaves from irradiated wild-type plants was visible to the naked eye 11 days after irradiation while on average *uvh1-2* plants displayed their first pair of leaves 20 days after irradiation. The five different *sog1* alleles showed a range of developmental rates with the first pair of leaves emerging from 10 to 14 days. The growth curves of the

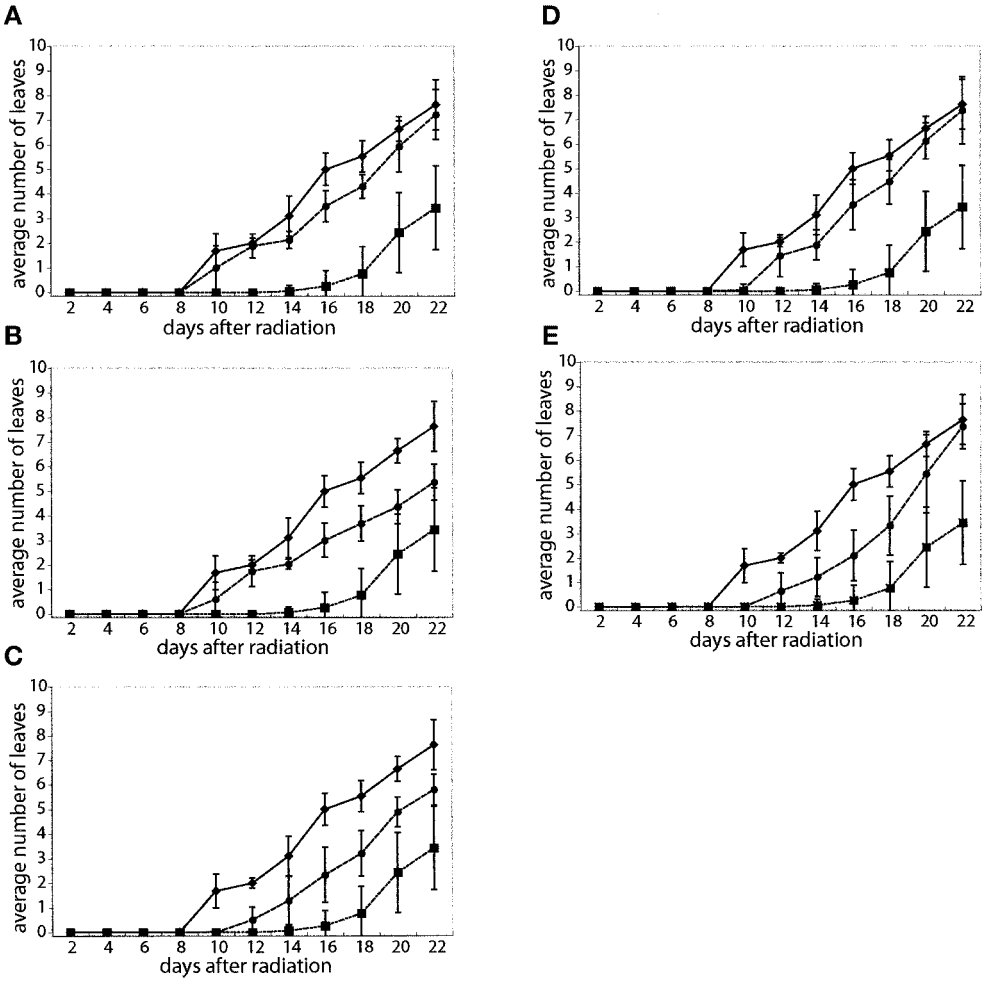

FIGURE 6.—Rates of leaf production in wild-type, *uvh1-2*, and *uvh1-2 sog1* plants. Seeds from wild-type, *uvh1-2*, and *uvh1-2 sog1* were irradiated at 20 krad and sown on soil, and the rate of leaf production was followed. Diamonds denote wild-type, squares are *uvh1-2*, and circles are the *uvh1-2 sog1* lines. (A) *uvh1-2 sog1-1*, (B) *uvh1-2 sog1-2*, (C) *uvh1-2 sog1-3*, (D) *uvh1-2 sog1-4*, and (E) *uvh1-2 sog1-5*.

sog1 mutants demonstrated that the mutant plants did not grow faster than irradiated wild-type plants; rather, the *sog1 uvh1* seedlings grew at the same or at a slightly slower rate than did wild-type plants (Figure 6). Although the rate of leaf production in the *uvh1-2 sog1* lines was essentially unchanged from wild-type, we noticed that at the higher dose of 20 krad the leaves were misshapen, with a ragged appearance, perhaps indicating the death of certain cell files. This phenotype worsened in *uvh1 sog1* plants as we further increased the dosage of radiation, whereas in wild-type plants the leaves became progressively smaller with increasing doses of radiation but did not appear to be "ragged" or missing files of cells.

The fact that leaves developed in all of the irradiated *sog1 uvh1-2* double mutants suggests that the G_2 arrest that was seen in the single mutant has been abolished. To further test this hypothesis we crossed the *cycB1::GUS* reporter construct into lines *uvh1-2 sog1-1* and *uvh1-2 sog1-2*. We irradiated seeds homozygous for *uvh1-2*, *sog1-1*, and *cycB1::GUS*, grew the plants for 3 days on agar plates, and then stained for GUS expression (Figure 7). In contrast to the irradiated *uvh1-2 cycB1::GUS*, which showed extensive GUS staining, these plants showed an accumulation of GUS similar to that of irradiated wild-type plants. As expected, approximately half a dozen cells expressed the construct, but there was no large-scale accumulation of GUS-expressing cells. These findings confirmed our hypothesis that the *sog1* mutation prevents a DNA-damage-induced G_2 arrest.

***uvh1-2 sog1* plants show increased levels of genomic instability:** Although our data suggest that the irradiated *sog1* mutants develop leaves because of the lack of a G_2 arrest, it is also possible that the cells were no longer arresting because there was a second-site mutation that partially rescued the repair deficiency of the *uvh1* mutation. In this case the mutation might specifically increase the ability of the plant to repair ionizing radiation-induced damage while not promoting the excision repair of UV-induced dimers. To differentiate between an upregulation of repair and the loss of a DNA-damage-dependent cell cycle checkpoint, we analyzed the impact of the *sog1* mutation on genomic integrity by both indirect and direct methods. We scored plants heterozygous for an albino mutation for sectors representing loss of the wild-type *Albino* allele. We also used fluorescence microscopy to look at DAPI-stained pollen meiocytes for evidence of chromosome loss and/or improper meioses.

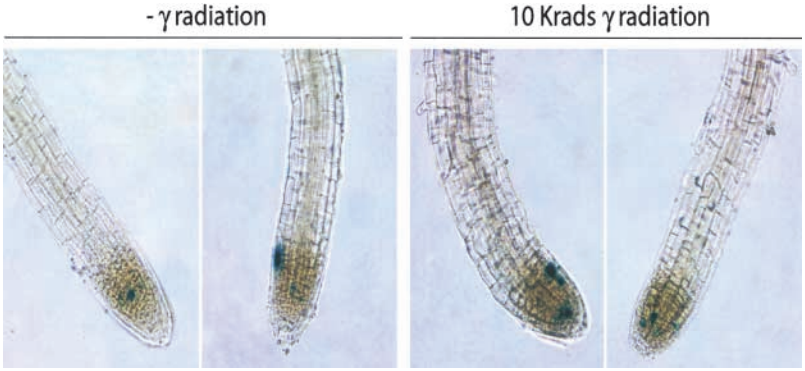

FIGURE 7.—*uvh1-2 sog1* plants no longer arrest in G_2. *uvh1-2 cycB1::GUS* plants were crossed to *uvh1-2 sog1-1* and *uvh1-2 sog1-2* plants to generate *uvh1-2 sog1 cycB1::GUS* plants. Seeds from *uvh1-2 sog1-1 cycB1::GUS* plants were imbibed in water for 24 hr and then were either left untreated or gamma irradiated at 10 krad. Five days after gamma irradiation seedlings were stained for the presence of GUS. Two representative *uvh1-2 sog1-1 cycB1::GUS* roots are displayed at each dose. There was no apparent difference in the staining pattern between irradiated and unirradiated plants. The *uvh1-2 sog1-2* plants did not stain differently from the *uvh1-2 sog1-1* plants.

The sog mutation increases the frequency of sectoring in irradiated plants: To assay genomic instability we crossed plants containing the *albino1* mutation (*alb1*) to *uvh1-2 sog1* plants. The *alb1* mutation is recessive and homozygous albino plants are white and die at the seedling stage. The *ALB1* gene lies close to the tip of chromosome 1, at a map position of 12 cM (VAN-DER-VEEN 1973). In a heterozygote, loss of the wild-type *ALB1* allele in a cell lineage results in a white or light green sector (Figure 8A). We hypothesized that if wild-type *SOG1* is required for maintaining genomic stability, plants that were homozygous for *uvh1-2* and *sog1-5* and heterozygous for *alb1* should exhibit a rate of sectoring higher than that of *alb/ALB* or *uvh1-2/uvh1-2 alb1/ALB1* plants. This loss of heterozygosity might be due to breakage of the *ALB1* chromosome between the locus and centromere, with subsequent loss of the acentric fragment, or it might be due to mitotic crossing over or the formation of smaller deletions that include the wild-type locus. The instabilities observed at *ALB1* would presumably be typical of instabilities occurring throughout the genome. In fact, it is possible that some of the sectors scored might be due to loss of other loci required for chlorophyl production, although sectors were extremely rare in irradiated plants that were not already heterozygous for *alb1*.

After crossing *uvh1-2 sog1-5* homozygous plants to *ALB1/alb1* plants, we screened for F_3 families that were either *uvh1-2/uvh1-2 SOG1/SOG1 ALB1/alb1* or *uvh1-2/uvh1-2 sog1-5/sog1-5 ALB1/alb1*. These two families and the original *alb1/Alb1* family allowed us to analyze the relative rates of genomic instability in wild-type, *uvh1-2*, and *uvh1-2 sog1-5* backgrounds. F_3 families of each genotype were either untreated or irradiated at 10 krad and leaves were analyzed for sectors over the vegetative life of the plants. In no instance did we find sectors in any of the unirradiated plants ($n > 1000$).

In plants derived from seeds treated with 10 krad of gamma radiation we found two different types of sectors, white and pale green, presumably representing sectors that span all the inner layers of the leaf or those that occurred in only one of the layers (the plant epidermis is colorless). Plants that carried the *albino* mutation alone or both *albino* and *uvh1* had a low frequency of sectoring (Figure 8B). In contrast, *uvh1-2 sog1-5* plants carrying the *albino* mutation had a rate of sectoring approximately fivefold greater than that of *uvh1* (Figure 8B). This high level of chromosomal instability in the *alb1* heterozygous, *sog1 uvh1-2* mutant plants is consistent with the notion that the cells of *sog1* plants are progressing through the cell cycle in spite of the persistence of damage.

The sog1 mutation causes meiotic defects: In the course of investigating the *sog1* mutation it became apparent that several of the *uvh1-2 sog1* mutant lines were significantly less fertile than the *uvh1-2* progenitor, even in the absence of gamma radiation. These observations led us to believe that there may be problems in proper development of the pollen meiocytes. To better understand the nature of the fertility defect, we analyzed DAPI-stained *sog1-3 uvh1-2* pollen precursor cells as well as the resulting pollen tetrads. We first analyzed cells in the process of division. After a thorough screening of 50 meiotic anaphase cells (a small subset of the total number of meiotic cells) we found only one dicentric bridge in the *uvh1-2 sog1-3* pollen and none in *uvh1-2* or wild-type pollen. Because these bridges are both rare and transient, we could not make any conclusions as to the frequency of such lesions. We then analyzed the products of pollen meiosis in the least fertile line, *uvh1-2 sog1-3*, and found that this line displayed a high frequency of abnormal meioses (Figure 9). Anthers were gathered from individual buds of unirradiated wild-type, *uvh1-1*, and *uvh1-1 sog1-3* plants. The anthers were dissected and stained with DAPI. One hundred percent of wild-type tetrads contained four nuclei ($n = 153$). In contrast, 91.5% ($n = 206$) of *uvh1-2* tetrads contained four nuclei and only 65% ($n = 170$) of *uvh1-1 sog1-3* tetrads contained four nuclei. To confirm that this meiotic defect is due to the *sog1* mutation, we analyzed the cosegregation of the abnormal meiosis with the gamma-resistant phenotype. Ten of 10 plants that were gamma resistant also bore abnormal tetrads. In contrast, 0 of 10

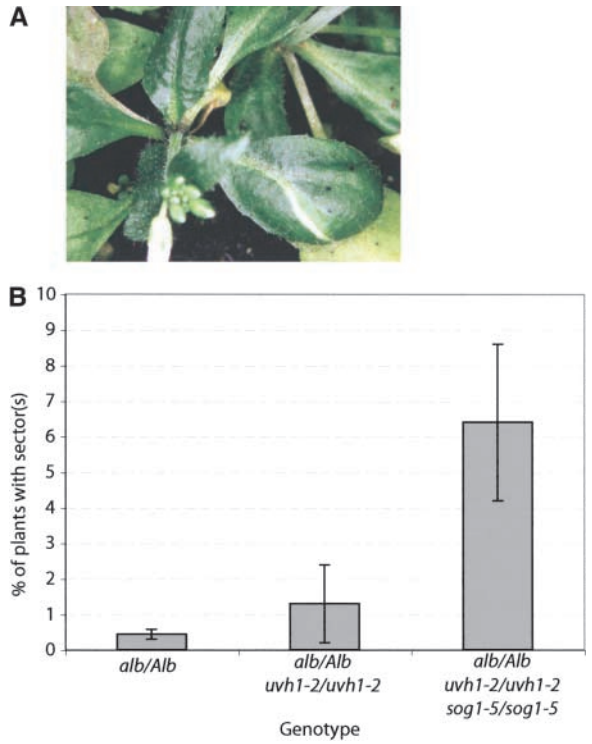

FIGURE 8.—*uvh1-2 sog1-5* plants show increased genomic instability. Wild-type, *uvh1*, and *uvh1-2 sog 1-5* seeds carrying a heterozygous *albino* mutation were gamma irradiated, grown, and screened for somatic sectoring due to the loss of the wild-type *Albina* (*Alb1*) allele. (A) Loss of the *Alb1* allele generates a white sector. (B) A total of 0.92 ± 0.2% of plants wild-type for *SOG1* and *UVH1* but heterozygous for *albino* were sectored, 1.3 ± 0.9% of the *alb/Alb uvh1-2/uvh1-2* were sectored, and 6.4 ± 1.5% of the *alb/Alb uvh1-2/uvh1-2 sog1-5/sog1-5* plants contained sectors.

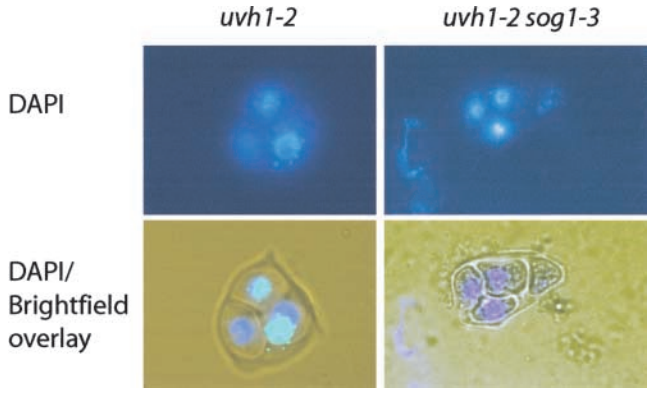

FIGURE 9.—*uvh1-2 sog1-3* plants are defective in meiosis. *uvh1-2* and *uvh1-2 sog1-3* pollen meiocytes (tetrads) were dissected from immature anthers. The tetrads were stained with DAPI to determine the number of intact nuclei. Additionally, the cells were visualized under bright field to determine both the shape and the number of the spores. In the photograph of *uvh1-2*, two nuclei (in one focal plane) were false colored dark blue while the other two (in another focal plane) were colored light blue. The *uvh1-2* tetrads conformed both to the typical number of nuclei and to cell shape. The *uvh1-2 sog1-3* had a variable number of nuclei and the cells were misshapen. The lightly fluorescing material in the *uvh1-2 sog1-3* tetrad may be either organellar DNA or chromatid fragments.

gamma-sensitive plants had abnormal tetrads, demonstrating that the two phenotypes are tightly linked. These findings indicate that either the *sog1* mutation may interfere with the proper progression of meiosis or the accumulation of defects throughout somatic development can lead to a high frequency of improper meioses.

DISCUSSION

Our analysis of the effects of gamma radiation on DNA repair-defective plants demonstrates that the *uvh1-2* gamma-plantlet response is caused by a DNA damage-dependent G_2-phase arrest. Our ability to identify mutants that eliminate the checkpoint response shows that the arrest is not a direct effect of DNA damage. Rather, the gamma-plantlet response is triggered by a signal-transduction mechanism that monitors the cell for damage and then arrests the cell cycle. Checkpoint mutants, defective in this response, show high levels of genomic instability and meiotic defects.

The gamma plantlet response is the result of a G_2-specific cell cycle arrest: We found that when we gamma-irradiated repair-defective *uvh1-2* seeds the plants germinated normally, but the meristematic cells arrested in the G_2 phase of the cell cycle. Our findings are similar to those of earlier investigators (FOARD and HABER 1961, 1970; EVANS 1965), who observed that unrepaired DNA damage triggers an arrest of plant development. While the early work indicated that gamma radiation interfered with cell division, it remained unclear as to where and why the plants arrested. Our data suggest that the gamma-plantlet response is induced in our repair-defective line by lesions that require expression of *UVH1* for their repair or processing. Unfortunately, the broad range of lesions induced by ionizing radiation, combined with the broad substrate specificity of the *XPF/ERCC1* repair endonuclease, makes it difficult to positively identify the nature of the arrest-inducing lesion(s); these might be double-strand breaks, intermolecular crosslinks, or even modified bases that require this endonuclease for their repair.

Our investigations of the *sog1* mutation demonstrate that the G_2 arrest is imposed on the cell by a DNA-damage-sensing signal transduction mechanism. Although studies on the effects of radiation on plants indicate that the cell cycle is arrested in response to radiation, it was previously unclear whether plants, like humans and yeast, harbor a classic checkpoint response to DNA damage. In fact, some data suggest that plants may lack some of the DNA-damage-dependent checkpoint responses that exist in other organisms (COUTEAU *et al.* 1999). The lack of any noticeable meiotic arrest in Arabidopsis meiotic recombinase-deficient *dmc1* mutants highlights the difference between the response of plants and animals to DNA damage, as discussed below.

DMC1 encodes a meiosis-specific homolog of the RAD51 recombinase and is required for strand exchange during recombination (Masson and West 2001). Yeast and mouse *dmc1* mutants are unable to repair these meiotic DSBs through homologous recombination and arrest in a checkpoint-dependent manner without completing meiosis (Lydall *et al.* 1996; Pittman *et al.* 1998; Yoshida *et al.* 1998). In Arabidopsis, the *DMC1* gene is also required for meiotic recombination (Couteau *et al.* 1999). However, rather than undergoing arrest, Arabidopsis *dmc1* cells proceed through anaphase without establishing chiasma and the chromosomes migrate randomly to the poles (Couteau *et al.* 1999). Further evidence of a lack of a meiotic checkpoint comes from the large collection of maize meiotic mutants (for examples, see Liu *et al.* 1993; Chan and Cande 1998). The different mutants are defective in various stages of meiosis but none exhibit a checkpoint response (Z. Cande, personal communication).

In both animals and yeast, unprocessed meiotic double-strand breaks are sensed by checkpoint proteins and division is arrested (Chin *et al.* 1999). Why does Arabidopsis seem to arrest in response to DNA damage generated by gamma radiation but not to breaks generated by other means? One possibility is that plants have a robust DSB-dependent mitotic checkpoint, as seen in our irradiated *uvh1* plants, but lack a meiotic DSB checkpoint. While this would explain the fact that irradiated *uvh1* plants arrest while *dmc1* plants do not arrest, it is also possible that Arabidopsis entirely lacks a DSB-dependent G_2 arrest and that the G_2-phase checkpoint observed here is a response to lesions other than double-strand breaks.

The damage induced by ionizing radiation is complex. In addition to strand breaks, ionizing radiation induces oxidative damage at sugars and bases, often in the form of multiply damaged sites (MDSs; Ward 1998). MDSs that include closely spaced lesions on opposing strands are similar to interstrand crosslinks in that they are recalcitrant to excision repair due to the lack of an undamaged template strand, can block replication of both strands of DNA, and may be processed into double-strand breaks during S phase. Crosslinks and lesions like them block both transcription and DNA replication and can trigger a checkpoint response (Friedberg *et al.* 1995). The repair of a crosslink is a multistep process involving both the creation of a double-strand break 5′ of the crosslink and the resolution of the crosslinked strands (De Silva *et al.* 2000). The processing of interstrand crosslinks in mammals requires both *XPF* (of which *UVH1* is a homolog) and *ERCC1* (of which *UVR7* is a homolog). Both *uvh1* and *uvr7* mutants exhibit a G_2 checkpoint response to gamma radiation (Jiang *et al.* 1997; Hefner *et al.* 2003). Similarly, *Saccharomyces cerevisiae rad1* (the yeast homolog of *UVH1*) mutants arrest in G_2/M in response to the crosslinking agent cisplatin (Grossmann *et al.* 1999).

The argument for a DNA-damage-dependent but not DSB-dependent checkpoint is buttressed by a collection of gamma-radiation-hypersensitive mutants recently identified in our laboratory (Hefner *et al.* 2003). Only those lines that were sensitive to both a crosslinking agent (mitomycin C) and gamma radiation arrested in G_2. In contrast, the ionizing *radiation-sensitive 1* (*irs1*) mutant (defective in the DSB-specific ligase *LIG4*; E. Hefner, J. Friesner and A. B. Britt, unpublished data) is sensitive to gamma radiation but not to MMC, and does not exhibit a G_2 arrest response to ionizing radiation. Thus two separate and independent experiments, the *dmc1* reverse genetic study and our analysis of gamma-sensitive lines, suggest that Arabidopsis may have a less stringent response than other organisms to double-strand breaks.

Genomic instability of *sog* mutants: In addition to an abrogation of the DNA-damage-dependent checkpoint response, Arabidopsis *uvh1-2 sog1* double mutants also displayed a number of hallmarks of chromosomal instability. Irradiated *uvh1-2 sog1* plants showed a dramatic increase in loss of the *ALB1* locus, although in the absence of radiation none of the plants were sectored. The *uvh1-2 sog1* plants also showed gross defects in meiotic segregation in the absence of radiation. While the loss of the *albino1* gene is likely due to a failure to correctly repair damage induced in irradiated mitotic cells, an explanation of the meiotic defect is less obvious. It is possible that either the aberrant tetrads arose as an accumulation of genetic abnormalities during mitotic development that prevents the proper progression of meiosis or the *sog1* mutation causes a defect in the regulation of meiosis *per se*. Although we cannot rule out the possibility that accumulated chromosomal aberrations would lead to improper meioses, we did look for evidence of meiotic bridges in the *uvh1-2 sog1* mutant and found only 1 among 50 anaphase figures, while we found none in *uvh1* or wild-type plants. We also failed to observe any sectoring in the unirradiated *ALB1/alb1 sog1 uvh1* mutants, suggesting that the instability-inducing lesions are rarely generated spontaneously. In contrast, the consistent meiotic defect in the distribution of chromosomes combined with the aberration in "tetrad" shape and cell number suggest a cytokinetic defect resembling the Arabidopsis *tardy asynchronous meiosis* (*tam*; Magnard *et al.* 2001) or *ask1* (Yang *et al.* 1999) mutants. The *tam* mutants behave similarly to the *uvh1 sog1* mutants in that they commonly form from one to four spores per tetrad, while occasional "polyads" have more than four spores. Additionally, *tam* mutants are no longer meiotically synchronized, each spore proceeding through meiosis independently of its neighbor, suggesting a defect in cell cycle regulation. *ask1* mutant pollen commonly has from one to six spores per polyad and a varying amount of DNA per cell, and the polyads resemble the abnormal shapes observed in *uvh1-2 sog1*. On the basis of its homology to the yeast *SKP1* gene,

ASK1 is thought to be involved in controlling the transition of the meiotic spindle from metaphase to anaphase (Yang *et al.* 1999). The similarity of the *uvh1-2 sog1* phenotype to these pollen mutants and the proposed role of the *ask1* and *tam* mutants in controlling the transition between the stages of meiosis suggest that *sog1* might also be a regulator of progression through meiosis.

Conclusion: Our investigation into the radiation-induced arrest response of *uvh1* plants and the isolation of mutants defective in this response demonstrate that plants, like animals, harbor a classic checkpoint response to DNA damage. Mutants deficient in this checkpoint response exhibited both gamma-induced somatic instability and consistent meiotic defects, indicating that this checkpoint plays an important role in the maintenance of genomic integrity. These findings, coupled with the fact that the Arabidopsis genome contains many of the same checkpoint-related genes that are known in humans, demonstrate that plants can react as animals do to DNA damage. We suspect, however, that this G_2 arrest is not a response to double-strand breaks, but instead to another gamma-induced lesion.

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LITERATURE CITED

Bardwell, A. J., L. Bardwell, A. E. Tomkinson and E. C. Friedberg, 1994 Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. Science **265:** 2082–2085.

Chan, A., and W. Z. Cande, 1998 Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. J. Cell Sci. **111** (Pt. 23): 3507–3515.

Chin, L., S. E. Artandi, Q. Shen, A. Tam, S. L. Lee *et al.*, 1999 p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell **97:** 527–538.

Colon-Carmona, A., R. You, T. Haimovitch-Gal and P. Doerner, 1999 Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J. **20:** 503–508.

Couteau, F., F. Belzile, C. Horlow, O. Grandjean, D. Vezon *et al.*, 1999 Random chromosome segregation without meiotic arrest in both male and female meiocytes of a dmc1 mutant of Arabidopsis. Plant Cell **11:** 1623–1634.

Deltour, R., 1985 Nuclear activation during early germination of the higher plant embryo. J. Cell Sci. **75:** 43–83.

De Silva, I. U., P. J. McHugh, P. H. Clingen and J. A. Hartley, 2000 Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol. Cell. Biol. **20:** 7980–7990.

Eshed, Y., S. F. Baum and J. L. Bowman, 1999 Distinct mechanisms promote polarity establishment in carpels of Arabidopsis. Cell **99:** 199–209.

Evans, H. J., 1965 Effects of radiation on meristematic cells. Radiat. Bot. **5:** 171–182.

Ferreira, P. C., A. S. Hemerly, J. D. Engler, M. van Montagu, G. Engler *et al.*, 1994 Developmental expression of the Arabidopsis cyclin gene cyc1At. Plant Cell **6:** 1763–1774.

Fidantsef, A. L., D. L. Mitchell and A. B. Britt, 2000 The Arabidopsis UVH1 gene is a homolog of the yeast repair endonuclease RAD1. Plant Physiol. **124:** 579–586.

Foard, D. E., and A. H. Haber, 1961 Anatomic studies of gamma-irradiated wheat growing without cell division. Am. J. Bot. **48:** 438–446.

Foard, D. E., and A. H. Haber, 1970 Physiologically normal senescence in seedlings grown without cell division after massive gamma-irradiation of seeds. Radiat. Res. **42:** 372–380.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.

Grossmann, K. F., J. C. Brown and R. E. Moses, 1999 Cisplatin DNA cross-links do not inhibit S-phase and cause only a G2/M arrest in Saccharomyces cerevisiae. Mutat. Res. **434:** 29–39.

Haber, A. H., W. L. Carrier and D. E. Foard, 1961 Metabolic studies of gamma-irradiated wheat growing without cell division. Am. J. Bot. **48:** 351–429.

Harlow, G. R., M. E. Jenkins, T. S. Pittalwala and D. W. Mount, 1994 Isolation of uvh1, an Arabidopsis mutant hypersensitive to ultraviolet light and ionizing radiation. Plant Cell **6:** 227–235.

Hefner, E., S. B. Preuss and A. B. Britt, 2003 Arabidopsis mutants sensitive to gamma radiation include the homologue of the human repair gene ERCC1. J. Exp. Bot. **54:** 669–680.

Jefferson, R. A., T. A. Kavanagh and M. W. Bevan, 1987 GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6:** 3901–3907.

Jiang, C. Z., C. N. Yen, K. Cronin, D. Mitchell and A. B. Britt, 1997 UV- and gamma-radiation sensitive mutants of *Arabidopsis thaliana.* Genetics **147:** 1401–1409.

Laufs, P., O. Grandjean, C. Jonak, K. Kieu and J. Traas, 1998 Cellular parameters of the shoot apical meristem in Arabidopsis. Plant Cell **10:** 1375–1390.

Liu, Q., I. Golubovskaya and W. Z. Cande, 1993 Abnormal cytoskeletal and chromosome distribution in po, ms4 and ms6; mutant alleles of polymitotic that disrupt the cell cycle progression from meiosis to mitosis in maize. J. Cell Sci. **106** (Pt. 4): 1169–1178.

Liu, Z., G. S. Hossain, M. A. Islas-Osuna, D. L. Mitchell and D. W. Mount, 2000 Repair of UV damage in plants by nucleotide excision repair: Arabidopsis UVH1 DNA repair gene is a homolog of Saccharomyces cerevisiae Rad1. Plant J. **21:** 519–528.

Lydall, D., Y. Nikolsky, D. K. Bishop and T. Weinert, 1996 A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:** 840–843.

Magnard, J. L., M. Yang, Y. C. Chen, M. Leary and S. McCormick, 2001 The Arabidopsis gene tardy asynchronous meiosis is required for the normal pace and synchrony of cell division during male meiosis. Plant Physiol. **127:** 1157–1166.

Masson, J. Y., and S. C. West, 2001 The Rad51 and Dmc1 recombinases: a non-identical twin relationship. Trends Biochem. Sci. **26:** 131–136.

Paulovich, A. G., D. P. Toczyski and L. H. Hartwell, 1997 When checkpoints fail. Cell **88:** 315–321.

Pittman, D. L., J. Cobb, K. J. Schimenti, L. A. Wilson, D. M. Cooper *et al.*, 1998 Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. Mol. Cell **1:** 697–705.

Prado, F., and A. Aguilera, 1995 Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the *RAD1, RAD10* and *RAD52* genes. Genetics **139:** 109–123.

Rodrigues-Pousada, R. A., R. De Rycke, A. Dedonder, W. Van Caeneghem, G. Engler *et al.*, 1993 The Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene 1 is expressed during early development. Plant Cell **5:** 897–911.

Ross, K. J., P. Fransz and G. H. Jones, 1996 A light microscopic atlas of meiosis in Arabidopsis thaliana. Chromosome Res. **4:** 507–516.

Schwartz, D., and C. Bay, 1956 Further studies on the reversal in the seedling height dose curve at very high levels of ionizing radiation. Am. Nat. **90:** 323–327.

Van-Der-Veen, J. L., 1973 Double reduction in tetraploid Arabidopsis thaliana, studied by means of a chlorophyll mutant with a distinct simplex phenotype. Arabidopsis Inf. Serv. **10:** 11–12.

Wang, H., J. Li, R. M Bostock and D. G. Gilchrist, 1996 Apoptosis: a functional paradigm for programmed plant cell death induced

by a host-selective phytotoxin and invoked during development. Plant Cell **8:** 375–391.

Ward, J. F., 1990 The yield of DNA double-strand breaks produced intracellularly by ionizing radiation: a review. Int. J. Radiat. Biol. **57:** 1141–1150.

Ward, J. F., 1998 Nature of lesions formed by ionizing radiation, pp. 65–84 in *DNA Damage and Repair*, Vol. 2, edited by J. A. Nickoloff and M. F. Hoekstra. Humana Press, Totowa, NJ.

Weinert, T. A., and L. H. Hartwell, 1988 The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. Science **241:** 317–322.

Yang, M., Y. Hu, M. Lodhi, W. R. McCombie and H. Ma, 1999 The Arabidopsis SKP1-LIKE1 gene is essential for male meiosis and may control homologue separation. Proc. Natl. Acad. Sci. USA **96:** 11416–11421.

Yoshida, K., G. Kondoh, Y. Matsuda, T. Habu, Y. Nishimune *et al.*, 1998 The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. Mol. Cell **1:** 707–718.

Zhou, B. B., and S. J. Elledge, 2000 The DNA damage response: putting checkpoints in perspective. Nature **408:** 433–439.

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