The *RAD9* Gene Controls the Cell Cycle Response to DNA Damage in *Saccharomyces cerevisiae*

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Cell division is arrested in many organisms in response to DNA damage. Examinations of the genetic basis for this response in the yeast *Saccharomyces cerevisiae* indicate that the *RAD9* gene product is essential for arrest of cell division induced by DNA damage. Wild-type haploid cells irradiated with x-rays either arrest or delay cell division in the G2 phase of the cell cycle. Irradiated G1 and M phase haploid cells arrest irreversibly in G2 and die, whereas irradiated G2 phase haploid cells delay in G2 for a time proportional to the extent of damage before resuming cell division. In contrast, irradiated *rad9* cells in any phase of the cycle do not delay cell division in G2, but continue to divide for several generations and die. However, efficient DNA repair can occur in irradiated *rad9* cells if irradiated cells are blocked for several hours in G2 by treatment with a microtubule poison. The *RAD9*-dependent response detects potentially lethal DNA damage and causes arrest of cells in G2 until such damage is repaired.

CELL DIVISION IS CONTROLLED IN RESPONSE TO VARIOUS physiological changes, including damage to DNA. Bacteria and animal cells respond to DNA damage by delaying cell division. In bacteria, the purpose of division delay has not been clearly demonstrated although the genetic and molecular basis of the response is well understood; DNA damage activates the RecA protease leading to synthesis of an inhibitor of cell septation (*1*). In eukaryotes the nature and purpose of DNA damage-sensitive division delay is understood although underlying mechanisms remain obscure. DNA damage induces arrest in the G2 phase of the cell cycle, after DNA replication and before mitosis (*2–9*). The following observations suggest that G2 arrest may simply provide ample time for the cell to repair DNA lesions and thus ensure integrity of condensed chromosomes for segregation at mitosis.

The role of G2 arrest in response to DNA damage has been demonstrated in mammalian cells through the use of agents that cause the cells to fail to arrest. Chromosomes either in S phase (undergoing replication) or in G2 (completely replicated but not condensed) could be induced to condense either by fusion with a mitotic cell or by treatment with caffeine (*4–6*). Breaks were revealed in the condensed chromosomes from cells that had been forced prematurely from the G2 arrest induced by DNA damage, whereas fewer breaks were observed in chromosomes either forced to condense after a G2 delay or permitted to progress naturally to mitosis (*4, 5*). Furthermore, cell viability was lower if DNA damaged cells were forced from G2 arrest by treatment with caffeine than when cells were permitted to delay in G2 (*6*). These results demonstrate that DNA damage repair occurs during G2 delay, that cell division in the presence of chromosome damage is lethal, and hence that G2 division delay is essential for viability in cells with damaged DNA. Furthermore, since treatment with caffeine or mitotic cell fusion bypasses G2 arrest, this arrest is likely to be mediated by a control mechanism rather than by structural constraints of the damaged DNA that directly prevent entry into mitosis.

Common features of division delay in many diverse cell types including sea urchin eggs and yeast (*7–9*) suggest the existence of common molecular mechanisms in eukaryotes. The budding yeast *Saccharomyces cerevisiae* provides an ideal subject for physiological and genetic studies of division delay. Progression of individual cells through the nuclear cell cycle can be easily followed by cell and nuclear morphology (*10*). Bud emergence occurs approximately at the time of DNA synthesis. Stage G2 and mitosis follow and are distinguishable by nuclear morphology; cells in G2 and before nuclear segregation (before anaphase) have the nucleus at the neck of the emerging bud (we refer to these cells collectively as G2 cells), whereas cells progressing through mitosis have an elongated, bipolar nucleus. Cytokinesis generates two unbudded, G1 progeny cells.

Yeast cells treated with x-irradiation or methylmethane sulfonate delay cell division when the nucleus is at the neck of a large budded cell (*8, 9, 11*), a phenotype identical to that of several mutants that block nuclear division in S or G2 (*12*) at their restrictive temperatures. Burns (*8*) and Brunborg and Williamson (*9*) also provided evidence that yeast cells irradiated in G2 delay in G2 before resuming cell division.

Genetic studies of DNA repair-deficient mutants have resulted in identification of more than 30 radiation-sensitive (RAD) mutants that have been characterized by sensitivity to different DNA damaging agents, epistasis, effects on recombination, mutation rates, and sporulation (*13*). Although cell cycle progression of several radiation sensitive mutants has been analyzed, a mutant defective for G2 arrest in response to DNA damage has not been identified (*14*).

Assays of arrest of cell division after DNA damage. To facilitate the search for a mutant defective in arrest of cell division by DNA damage, we characterized G2 delay in x-irradiated wild-type cells by a microcolony assay. A culture of wild-type haploid cells in the exponential stage of growth was plated on solid agar medium and then immediately exposed to x-irradiation; the number of buds in each microcolony was recorded after 5 and 10 hours. We predicted that essentially all irradiated cells would arrest in G2 and

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cells unable to repair the damage would remain arrested. G1 and M phase cells are especially sensitive to x-irradiation (*9*) and should arrest irreversibly (generating two- and four-budded microcolonies, respectively), whereas S and G2 phase cells are more resistant and should delay in G2 before resuming cell division. Unirradiated cells consisted initially of budded and unbudded cells, and the number of new buds increased exponentially with time (Fig. 1A). In contrast, 5 hours after irradiation with 8 krad, microcolonies generated from wild-type cells remained predominantly single cells with a large bud (48 percent two-budded microcolonies) or became two cells, each with a large bud (38 percent four-budded microcolonies) (Fig. 1C). About 10 hours after irradiation 69 percent of the microcolonies still retained two- and four-budded morphologies and were probably irreversibly arrested. Larger microcolonies (five to nine buds or more) at 10 hours contained cells that continued to divide and corresponded to the proportion of cells surviving radiation treatment—30 percent survival for RAD^+ cells (Fig. 1C and Table 1). At lower doses of radiation (2 krad) RAD^+ cell populations also showed evidence of cell cycle arrest (Fig. 1B). After 10 hours, about half the irradiated cells had recovered from division arrest and generated viable microcolonies, and about half of the cells remained arrested with either two or four buds (as discussed below). These observations are consistent with our expectations that essentially all exposed cells delay division and only those cells capable of repairing the DNA lesions resume cell division. The microcolony assay documents G2 arrest most clearly in cells unable to repair DNA damage, the G1 and M phase haploid cells. Terminal arrest of cells unable to repair broken chromosomes has been proposed for *Drosophila melanogaster* (*15*) and mammalian cells (*5*).

The response to radiation of strains deficient in DNA repair (*rad* strains) is consistent with this interpretation. The *rad52* cells are deficient in double-strand break repair (*16*), rendering S and G2 phase cells also sensitive to radiation. A high percentage (81 percent) of the cells arrested with two- and four-budded microcolony morphologies even after 2 krad of radiation (Fig. 1D). At the doses of radiation used, the percentage of cells remaining arrested after 10 hours for RAD^+ and *rad52* strains corresponded to that of inviable cells (Table 1).

Analysis of nuclear morphology shows that irradiated cells accumulate with a nuclear morphology indicative of cells in G2 (Fig. 2). The percentage of cells with this medial nuclear division morphology increased 3 hours after irradiation for RAD^+ and *rad52* strains and corresponded to the percentage of cells terminally arrested 10 hours after irradiation (Table 1).

***rad9* cells are defective for G2 delay.** A mutant defective in G2 delay should be sensitive to treatment by any reagent that induces DNA damage, provided that division arrest is required for efficient repair of all types of damage. Mutants sensitive to ultraviolet, x-rays, and alkylating agents exist among the collection of previously identified radiation sensitive (RAD) mutants (*13*). We analyzed several of these RAD mutants for their ability to arrest cell division in response to x-irradiation by the microcolony assay and by inspection of nuclear morphologies. Of seven *rad* mutants analyzed (*rad6*, *-8*, *-9*, *-10*, *-11*, *-12*, and *-18*), six showed normal cell cycle

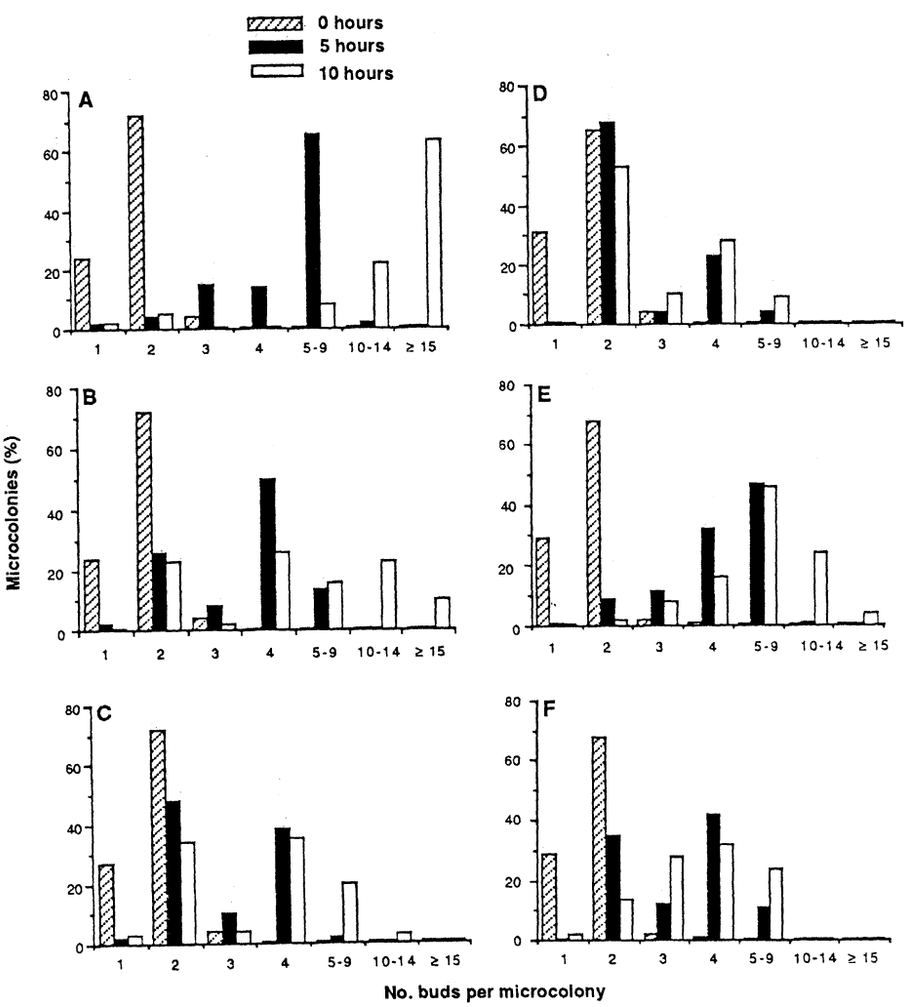

Fig. 1. Haploid yeast cells grown to logarithmic phase in YM-1 liquid medium (rich medium) were disrupted by sonication, plated on solid agar medium (YEPD, rich medium), irradiated (Machlett OEG-60 x-ray tube operated at 50 kV and 20 mA delivering a dose rate of 106 rad/s), and incubated at 23°C. The number of buds in at least 100 random microcolonies was determined by light microscopy at time of irradiation (hatched bars), 5 hours after irradiation (solid bars), and 10 hours after irradiation (open bars). (**A**) RAD^+, unirradiated; (**B**) RAD^+, 2 krad; (**C**) RAD^+, 8 krad; (**D**) *rad52*, 2 krad; (**E**) *rad9*, 2 krad; and (**F**) *rad9*, 8 krad. The genotypes of strains used are as follows: strain 7815-6-3 (*MAT*α *ura3*); strain 7815-6-4 (*MAT***a** *rad9-1 ade2 leu2 ura3*); and strain 7821-7-3 (*MAT***a** *rad9-1 rad52 can1 his3*). These are strains derived from crosses between unrelated parental strains X56-10A *MAT***a** *rad9-1 ade2* from the Berkeley Yeast Stock Center and A364a from this laboratory, and therefore are not congenic. Strains 45401b (*MAT*α *rad52 ade2 ura3 leu2 his3 his7 can1 sap3*) and 5186-1-2 (*MAT***a** *rad18 his7*) are congenic derivatives of A364a. Yeast genetic methods were standard (*29*), as were formulas for liquid and solid yeast media (*30*). Values and data in figures, tables, and text are typically from experiments from a single or the average of duplicate cultures and are qualitatively reproducible in identical strains on different days.

arrest similar to that shown for RAD$^+$ and *rad52* strains (Fig. 1 and Table 1) (*17*).

The *rad9* cells, however, failed to undergo cell cycle arrest in response to x-irradiation (Fig. 1, E and F, and Table 1). This result is dramatic after treatment with 2 krad; the amount of radiation that induced 68 percent lethality resulted in only 18 percent of the cells having the two- and four-budded morphology. In contrast, most of the inviable cells of irradiated RAD$^+$ or *rad52* strains could be accounted for in the class of two- and four-budded microcolonies (Fig. 1, B to D, and Table 1). When *rad9* cells were irradiated with 8 krad of x-rays, where viability is only 1 percent, two- and four-budded microcolonies (compare Fig. 1, F and C) were not predominant, although at this dose about half of these *rad9* cells did have large buds and a nuclear morphology characteristic of G2-arrested cells (Table 1). Possible explanations for this partial response of *rad9* cells are addressed below.

Analysis of nuclear morphology indicates further that *rad9* cells are defective in division arrest. Three hours after irradiation, RAD$^+$ and *rad52* cells showed high percentages of cells with the medial nuclear division (mND) morphology expected of cells arrested in G2 (Table 1 and Fig. 2), whereas *rad9* cells treated similarly showed less of an increase in cells with this morphology.

Fate of irradiated G1, M, and G2 phase haploid cells. Division arrest is most easily detected in cells most sensitive to radiation, including repair-deficient strains like *rad52* and wild-type G1 (and M phase) haploid cells. This finding is consistent with physical and genetic observations suggesting that double-strand DNA breaks in yeast generated in G1 haploid cells cannot be repaired because repair of double-strand breaks occurs only through use of DNA homology (*18, 19*). The higher sensitivity of G1 haploids to radiation compared to G1 diploids or G2 haploids could be due to the absence in G1 haploids of either a sister chromatid or a homologous chromosome to provide the homology necessary for repair. Thus most G1 haploid cells even in a RAD$^+$ strain should be irreversibly arrested at the two-bud stage after irradiation. In order to test this expectation, we examined by photomicroscopy the response of unbudded G1 haploid cells specifically. Analysis of the fate of irradiated G1 cells from the RAD$^+$ and *rad9* strains verify this prediction and show dramatically the arrest defect in *rad9* cells. Eighty percent (48 of 60) of the unbudded RAD$^+$ cells (Fig. 3A) arrested as two-budded microcolonies (Fig. 3B) 10 hours after irradiation whereas only 7.6 percent (4 of 52 cells) of the unbudded *rad9* cells generated two-budded microcolonies 10 hours after irradiation (Fig. 3, C and D, respectively). Most G1 *rad9* cells generated three to eight buds before ceasing division. The RAD$^+$-arrested cells are greatly enlarged, typical of continued growth of cell division cycle mutants arrested at the restrictive temperature (*10*), whereas most of the *rad9* cells are not enlarged.

Table 1. Correlation between cell division arrest and sensitivity to x-irradiation.

Strain*	Dose (krad)	Arrested† cells (%)	Lethality‡ (%)	Nuclei§ (mND) (%)
RAD$^+$	0	5	0	15
	2	49	52	46
	8	69	70	72
rad52	0	10	0	29
	2	81	95	77
rad9	0	5	0	13
	2	18	68	23
	8	46	99	41
rad9 rad52	0	20	0	15
	2	39	93	37

*Strains are described in the legend to Fig. 1. †Percent of arrested cells is the sum of two- and four-budded microcolonies formed 10 hours after irradiation as described in Fig. 1. Data are from Fig. 1. except for the double mutant *rad9 rad52* strain. ‡Percent lethality of asynchronous logarithmic cells irradiated at the doses indicated was taken from Fig. 5, except for *rad9 rad52*. The lethality was set at 0 for unirradiated cells. RAD$^+$ and *rad9* strains have plating efficiencies of 90 to 95 percent, whereas both *rad52* strains have plating efficiencies of about 70 percent without irradiation. §The percentage of nuclei with the medial nuclear division morphology was determined for unirradiated asynchronous logarithmic cultures (0 krad) and for cells irradiated with 2 or 8 krad and incubated for 3 hours after irradiation at 23°C. Cells were prepared and analyzed for nuclear morphology as described in the legend to Fig. 2. Cells were scored as having an mND morphology if the daughter bud was estimated to be at least half the size of the mother and the nucleus is across or near the neck of the bud. Arrested cells often have the nucleus stretched across the neck of the bud as seen in many cells in Fig. 2B.

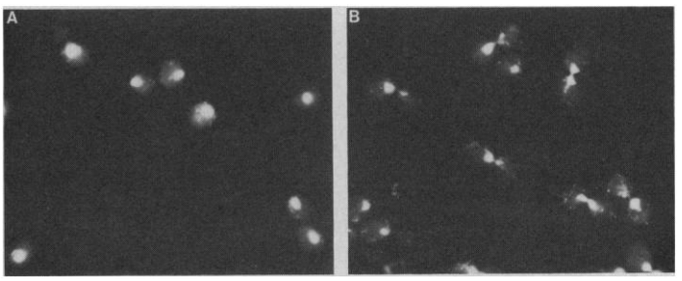

Fig. 2. Nuclear morphology of logarithmically growing and G2-arrested cells. Haploid cells grown to logarithmic phase were either fixed directly or plated on solid agar medium, irradiated with 2 krad, incubated for 3 hours at 23°C, then recovered and fixed. Nuclei were visualized by staining with 4,6-diamino-2-phenylindole (*31*) and photographed by fluorescence microscopy. (**A**) RAD$^+$ logarithmically growing; (**B**) RAD$^+$ irradiated with 8 krad.

Each segregated half of a binucleate M phase cell has the G1 content of DNA, and M phase cells are as sensitive to x-rays as G1 cells (*9*). We therefore expected that an irradiated M phase cell would generate two cells, and each would arrest in G2 of the next cell cycle to form a four-budded microcolony. Arrest of irradiated RAD$^+$ M phase cells and defective arrest in *rad9* M phase cells was demonstrated directly by photomicroscopy. In a RAD$^+$ strain 55 percent (40/72) of large, budded cells arrested with four buds after 10 hours, whereas only 3.1 percent of *rad9* cells (2/65) arrested with four buds after 10 hours (Fig. 3). (Both M phase and G2 cells have large buds and are indistinguishable in the light microscope, thus some large budded RAD$^+$ cells that were not arrested after 10 hours probably were G2 cells at time of irradiation.)

To examine the delay in irradiated G2 cells, we prepared populations of RAD$^+$ and *rad9* cells synchronized in G2 by treatment with the microtubule inhibitory drug methyl benzimidazole-2-yl-carbamate (MBC) (*20*). MBC-arrested cells were irradiated with 0, 2, 4, or 8 krad x-rays and then immediately removed from MBC and plated on fresh agar plates. Recovery from irradiation and MBC arrest was scored as the time needed for large budded cells to generate a third bud, which signals S phase of the next cell cycle. More than 80 percent of unirradiated wild-type cells proceeded to the next cell cycle after 6 hours (Fig. 4A). Irradiated wild-type cells delayed before cells reenter the cell cycle, and the greater the radiation the longer the delay (each 2 krad of radiation resulted in about 1 hour of G2 delay). Burns also studied synchronized large budded cells and showed radiation dose-dependent delay in cell division for diploid cells (*8*). Irradiated *rad9* cells, however, failed to show a similar radiation-induced delay (Fig. 4B). These results directly demonstrate DNA damage-induced delay in G2 for wild-type cells and corroborate our studies of asynchronous cultures by showing that *rad9* cells are deficient for the G2 delay response to DNA damage.

Radiation sensitivity and defective G2 arrest due to *rad9-1*. In a test of whether the G2 delay defect and radiation sensitivity of *rad9* cells was due to the *rad9-1* lesion, diploid strains heterozygous for

rad9-1 were sporulated, and tetrads containing four viable spores were analyzed: 28 tetrads showed 2:2 segregation of radiation sensitivity, and of the ten tetrads analyzed in greater detail all showed cosegregation of radiation sensitivity with bypass of division delay as determined by the microcolony assay (Table 2). A metric that dramatically distinguishes *rad9* mutant cells from *RAD9*$^+$ cells is the ratio of the percentage of cells arrested with two- or four-budded morphology to the percentage cells killed after 2-krad irradiation; this ratio is high for RAD$^+$ spores (average 0.88) and low for *rad9* spores (average 0.22). We conclude that both defective cell cycle control and radiation sensitivity are due the *rad9-1* mutation.

The nature of the signal and the role of *RAD9* in G2 arrest. X-irradiation induces DNA damage that may be, as we propose, the signal for *RAD9*-dependent G2 arrest. Study of the DNA repair-deficient strain *rad52* supports this hypothesis. If arrest of cell division in irradiated *rad52* cells (Fig. 1D and Table 1) is in fact due to unrepairable DNA damage, and if the *RAD9*-dependent mechanism detects such DNA damage, then an irradiated *rad52 rad9* double mutant should fail to arrest. Studies of the double mutant revealed that at low doses of radiation cell viability for a *rad52 rad9* double mutant is as low as in the *rad52* strain, and most of the double mutant cells fail to arrest; of the *rad52 rad9* cells, 93 percent were killed by 2 krad but less than 40 percent of cells showed a G2 arrest morphology (Table 1). This result lends support to the proposal that unrepaired DNA damage is the signal for *RAD9*-dependent arrest of cell division.

The *RAD9* gene product might have functions other than cell cycle delay, for example, as a repair enzyme or as a controlling element of repair enzyme expression or activity. If the *RAD9* gene product only controls cell cycle progression, the radiation sensitivity of *rad9* cells might be suppressible by drug-induced G2 delay. As a test, cells were arrested with MBC and irradiated, and the MBC block was maintained to simulate a G2 delay response. Repair of DNA lesions was monitored by cell viability. The radiation sensitivity profile of an asynchronous culture of RAD$^+$ cells shows (Fig. 5A) the characteristic biphasic curve resulting from a mixed population containing (i) sensitive G1 and mitotic cells and (ii) more resistant S and G2 cells (*19*). RAD$^+$ cells irradiated after arrest in MBC no longer display the sensitive part of the curve, and continued MBC arrest after irradiation does not enhance viability. In an asynchronous culture of *rad9* cells (Fig. 5B) no G2 resistant class of cells is evident, and when *rad9* cells were irradiated and simultaneously released from MBC arrest, they failed to survive radiation (MBC release). Only *rad9* cells arrested in MBC before irradiation and held in MBC for 4 hours after irradiation showed a dramatic increase in cell viability (Fig. 5B, MBC hold). These results show (i) that the *RAD9* function is not required for DNA repair provided that cells are blocked in G2, and (ii) that, as observed in mammalian cells, delay in G2 is necessary for viability of cells with DNA damage. Radiation sensitivity of several other *rad* mutants failed to be suppressed by MBC arrest—for example, *rad52* and *rad18* (Fig. 5C) (*21*) and *rad6* (*17*)—suggesting that these mutants are defective in some aspect of DNA repair other than cell cycle control.

***RAD9*-dependent G2 arrest is a checkpoint that ensures fidelity of chromosome transmission.** We have shown that the arrest of cell division by DNA damage is under the control of the *RAD9* gene. DNA damage-induced arrest in G2 and the duration of the arrest in G2 are dependent on *RAD9*. Furthermore, DNA repair is not dependent on *RAD9* provided that irradiated cells are blocked

Table 2. Cosegregation of radiation sensitivity and defective arrest of cell division.

Strain*	RAD†	Arrested‡ cells (%)	Lethality§ (%)	Efficiency of arrest‖
7815-6-1	−	16	80	0.20
6-2	+	53	60	0.88
6-3	+	49	52	0.94
6-4	−	18	68	0.26
7813-2-1	−	16	80	0.20
2-2	−	15	83	0.18
2-3	+	44	60	0.73
2-4	+	53	62	0.85
7810-1-1	−	21	78	0.27
1-2	+	53	56	0.95
1-3	−	19	88	0.22
1-4	+	61	68	0.90
7810-5-1	−	19	71	0.27
5-2	+	51	61	0.84
5-3	+	52	55	0.95
5-4	−	12	70	0.17
RAD$^+$	+	52	59	0.88
rad9	−	17	77	0.22

*Tetrads generated from diploid strains heterozygous for *rad9*. Strains were generated by crossing congenic parental strains X56-10A (*MAT***a** *rad9-1 ade2*) or X56-8D (*MAT*α *rad9-1 ade2*) with different congenic A364a strains carrying *MAT***a** *cdc13-1 ura1 his7* (strain 7810), *MAT***a** *cdc17-1 ura1 his7* (strain 7813), and *MAT*α *leu2 ura3 cyh2* (strain 7815), respectively. X56-10A and X56-8D are not congenic with A364a. †Radiation sensitivity was determined by irradiating a patch of cells with 8 krad of x-rays and comparing growth with an unirradiated replica. ‡Percent arrested determined as described in Table 1. §Percent lethality determined by exposing a logarithmically growing culture to 2-krad x-irradiation. ‖The ratio of the percent of arrested cells to the percent of cells killed (lethality) reflects the efficiency of arrest.

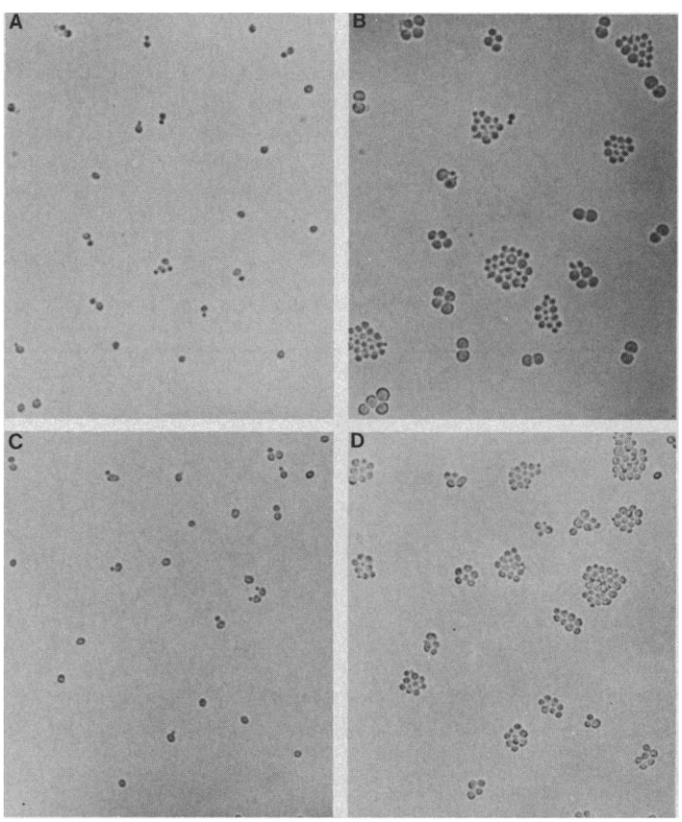

Fig. 3. Photomicroscopy of irradiated RAD$^+$ and *rad9* cells. Haploid yeast cells grown to logarithmic phase were plated on thin agar slabs, x-irradiated with 2 krad, and protected from drying with a glass cover slip. Fields of cells were photographed by phase contrast microscopy at the time of irradiation (**A** and **C**) and after a 10-hour incubation at 23°C (**B** and **D**). Fields were selected and aligned to demonstrate the fate of individual unbudded and large budded cells. (A and B) RAD$^+$; (C and D) *rad9*.

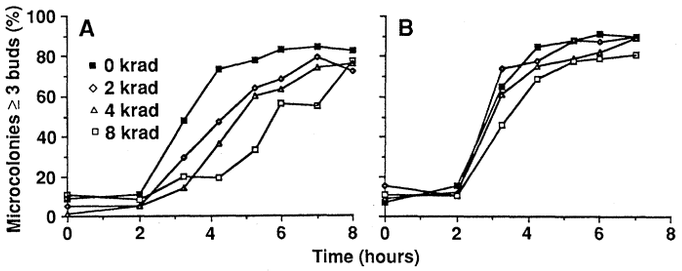

Fig. 4. Division delay of irradiated cells. Cells grown to logarithmic phase were plated on solid agar medium containing methylbenzimide-2-yl-carbamate (MBC) at 100 μg/ml and incubated for 3 hours at 23°C to arrest cell division. Cells were then x-irradiated at the doses indicated, immediately washed off the plates, sonicated, replated on fresh agar medium, and observed by light microscopy. At least 80 percent of the cells in each experiment had the large budded MBC-arrest morphology after 3 hours in the presence of drug. The number of buds in at least 100 random microcolonies was recorded at the times indicated, allowing determination of the percentage of microcolonies with three buds or more. (**A**) RAD$^+$; (**B**) *rad9*. Although MBC treatment killed some cells, more than 80 percent of the unirradiated cells from both strains divided at least once before ceasing cell division, showing that MBC-induced lethality does not drastically alter entry into the first cell division after arrest.

in G2 by use of a microtubule poison. On the basis of these results we propose that *RAD9* performs solely a control function in cell division; the integrity of chromosomes is checked by the *RAD9*-dependent mechanism that blocks entry into mitosis if the DNA is broken. Thus *RAD9* performs the "surveillance mechanism" proposed by Tobey (*3*) to account for the G2 arrest response to DNA damage in mammalian cells.

The use of x-irradiation permits study of cell division arrest since virtually every cell experiences DNA damage and the response of individual cells can easily be followed. We suggest, however, that the *RAD9*-dependent control function plays a role in the cell cycle of unirradiated cells. DNA damage probably accounts for the low but detectable spontaneous mitotic recombination observed in wild-type cells (*22*), and thus a *RAD9*-dependent G2 delay may ensure that DNA repair is complete before chromosome segregation in those rare cells that experience spontaneous damage. Such a mechanism would ensure maximum fidelity in chromosome transmission. We expect that other checkpoints, in addition to the *RAD9*-dependent mechanism, may be responsible for the very high fidelity of mitotic chromosome transmission (one chromosome loss per cell per 10^5 generations) (*22*). Genomic stability of a population of cells can be reduced by three orders of magnitude with little effect on growth rate (*22, 23*); thus checkpoints may not be essential for growth of a cell population but rather for the occasional cell that experiences intrinsic damage (incurred during DNA replication, for example). Genetic analysis of strains completely deficient for *RAD9* show that, in fact, *RAD9* is nonessential for growth and that genomic instability in *RAD9*-deficient strains is increased (*17*). The nature and frequency of genomic rearrangements in *RAD9*-deficient cells should further define the role of G2 arrest in maintaining genomic stability.

At high doses of radiation about half of the *rad9* cells from an asynchronous culture accumulated with a nuclear morphology characteristic of G2 division delay (Table 1) and *rad9* cells presynchronized with MBC did exhibit some delay in G2 (Fig. 5B). This partial response might have one of three possible explanations. (i) The *rad9* allele used in these studies may have been leaky. (ii) Other checking functions that are sensitive to higher levels of damage or function earlier in the cell cycle (or both) may exist. For example, DNA replication is inhibited by irradiation in normal mammalian cells, yet inhibition fails to occur in the mutant cells from patients with ataxia telangiectasia (*24*); defective control mechanisms may account for this failure. (iii) Extensive damage to chromosomes may itself impede progression of the cell cycle. *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* cells deficient in topoisomerase II arrest with a nuclear morphology suggestive of a physical impediment to nuclear division (*25*).

Irradiation may also cause inhibition of DNA synthesis in yeast as has been demonstrated in mammalian cells (*24*). A role, if any, for *RAD9* in effects of DNA damage on DNA synthesis in yeast remains to be determined.

Both G1 haploids and *rad52* cells are unable to repair double-strand breaks (for reasons described above), and both types of cells arrest division efficiently after x-irradiation. This arrest is *RAD9*-dependent, suggesting that double-strand breaks in DNA act as an inducer, either directly or indirectly, of G2 delay. Analysis of other conditions in which cells arrest in G2 may be informative as to the nature of the signal. For example, we have observed that deficiencies in the temperature-sensitive cell division cycle gene *CDC13* lead to high levels of mitotic recombination (*22*) and to *RAD9*-dependent division arrest (*17*); both phenomena can be attributed to DNA damage.

DNA damage may be the only type of chromosomal damage detected by the *RAD9*-dependent cell division arrest mechanism. Yeast cells defective for topoisomerase II or for histone 2B apparently traverse the *RAD9*-dependent arrest point, and therefore distortions of chromatin structure expected in these mutants are not detected by *RAD9* (*12, 25, 26*). Damaged DNA itself may play a central role in cell cycle arrest as it does in the SOS response in bacteria (*1*).

We consider it likely that *RAD9* is only one member of a multicomponent control system controlling the cell cycle in response to DNA damage. Deficiencies in other genes involved in the *RAD9* control system should also confer radiation sensitivity and thus may be identified among other RAD mutants. A strategy to

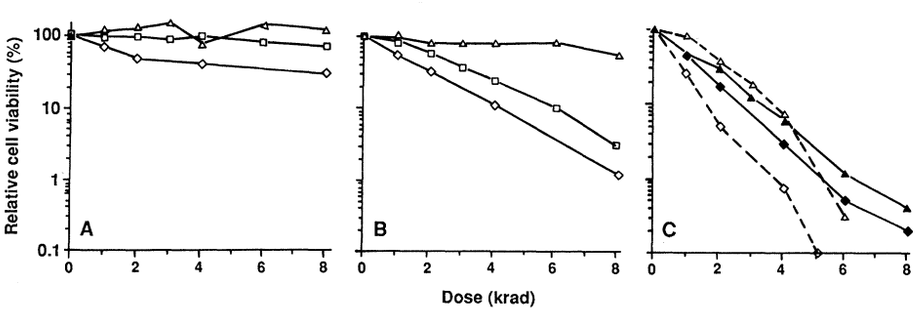

Fig. 5. Suppression of radiation sensitivity of *rad9* cells by arrest in MBC. Cell survival after x-irradiation was determined for cells in asynchronous growth (◇, ◆); for cells arrested in MBC and released from arrest at the time of irradiation (MBC release, □, ■); and for cells arrested in MBC, irradiated, and held in MBC for 4 hours (MBC hold, △, ▲). When each regime was completed, cell viability to various doses of x-irradiation was determined by plating cells on solid agar medium, incubating at 23°C, and counting colonies formed after 2 to 3 days. Each data point represents the average of duplicate experiments which typically vary less than 20 percent and always less than 50 percent of the average value. The viability of unirradiated cells was set at 100 percent for each set of experiments to normalize for lethality of MBC treatment that varies reproducibly from strain to strain and with time of incubation with MBC. (**A**) RAD$^+$; (**B**) *rad9*; (**C**) *rad52* (dashed lines) and *rad18* (solid lines). Similar results were obtained for one other RAD$^+$ and *rad9* spore.

identify the other genes involved in G2 arrest based on the characteristics of *rad9* cells may be possible.

In several different organisms, components—such as cyclin A, maturation promoting factor (MPF), and certain gene products in *Schizosaccharomyces pombe* and *Aspergillus nidulans*—have been identified that participate in the commitment to mitosis (*27*, *28*). *RAD9* may interact with similar, and as yet unidentified, components in *Saccharomyces cerevisiae* to prevent the transition of G2 to mitosis.

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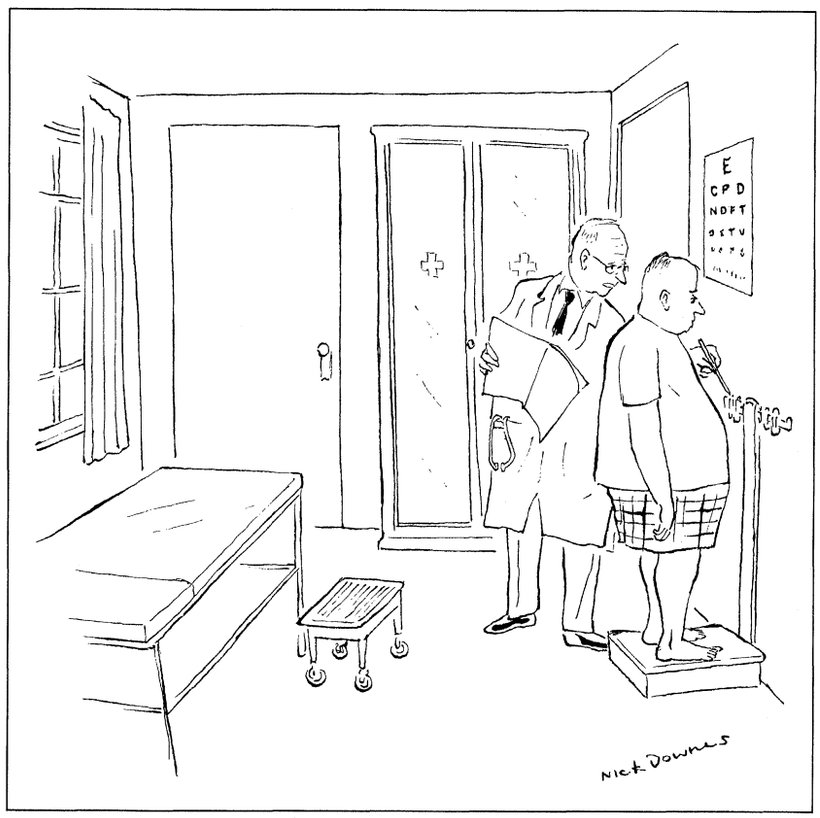
32. Supported by NIH research grant G17709 from the Institute of General Medical Sciences, a grant from the American Business Foundation for Cancer Research, and (to T.A.W.) a fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

22 February 1988; accepted 31 May 1988



"You've established an annual 20% growth rate."