Stage-Specific Effects of X-Irradiation on Yeast Meiosis

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ABSTRACT

Previous work has shown that *cdc13* causes meiotic arrest of *Saccharomyces cerevisiae* following DNA replication by a *RAD9*-dependent mechanism. In the present work, we have further investigated the implicit effects of chromosomal lesions on progression through meiosis by exposing yeast cells to X-irradiation at various times during sporulation. We find that exposure of *RAD9* cells to X-irradiation early in meiosis prevents sporulation, arresting the cells at a stage prior to premeiotic DNA replication. *rad9* meiotic cells are much less responsive to X-irradiation damage, completing sporulation after treatment with doses sufficient to cause arrest of *RAD9* strains. These findings thereby reveal a *RAD9*-dependent checkpoint function in meiosis that is distinct from the G_2 arrest previously shown to result from *cdc13* dysfunction. Analysis of the spores that continued to be produced by either *RAD9* or *rad9* cultures that were X-irradiated in later stages of sporulation revealed most spores to be viable, even after exposure to radiation doses sufficient to kill most vegetative cells. This finding demonstrates that the lesions induced by X-irradiation at later times fail to trigger the checkpoint function revealed by *cdc13* arrest and suggests that the lesions may be subject to repair by serving as intermediates in the recombination process. Strains mutant for chromosomal synapsis and recombination, and therefore defective in meiotic disjunction, were tested for evidence that X-ray-induced lesions might alleviate inviability by promoting recombination. Enhancement of spore viability when *spo11* (but not *hop1*) diploids were X-irradiated during meiosis indicates that induced lesions may partially substitute for *SPO11*-dependent functions that are required for the initiation of recombination.

SPORULATION in *Saccharomyces cerevisiae* requires many genetically controlled functions that are shared with the vegetative cell cycle, but the order in which these functions are required with respect to various cytological landmarks often differs strikingly between the two pathways of cell division. For example, mutations that affect START–such as *cdc28*, *cdc36* and *cdc39*–cause mitotic arrest at a stage prior to DNA replication (HARTWELL 1973) and spindle pole duplication (BYERS and GOETSCH 1974; REED 1980) [reviewed in PRINGLE and HARTWELL (1981)]. In meiosis, on the other hand, these mutations permit the execution of both of these functions but then cause arrest at the stage of chromosomal synapsis, as evidenced by the persistent presence of synaptonemal complexes (SHUSTER and BYERS 1989). As a further example, mutations primarily affecting chromosomal DNA replication cause mitotic arrest in a state following spindle formation, whereas the common state of their arrest in meiosis precedes both spindle formation and synapsis (SCHILD and BYERS 1978). In light of the recent recognition that many such mitotic arrests reflect the action of checkpoint functions (HARTWELL and WEINERT 1989), it was of interest to ask how checkpoints may affect meiosis and how they may be ordered with respect to other meiotic functions. In this regard, we had previously tested whether the mutational defect in *cdc13*, which leads to a *RAD9*-dependent mitotic arrest (WEINERT and HARTWELL 1988) presumably due to the induction of chromosomal lesions (HARTWELL and SMITH 1985), could be used to generate an arrest of meiosis. We found that the mutation does, indeed, cause meiotic arrest in a *RAD9*-dependent manner, *cdc13 RAD9* cells failing to undergo either synapsis or the meiotic divisions at the restrictive temperature, while *cdc13 rad9* cells continue through meiosis and produce inviable progeny (WEBER and BYERS 1992). This being the case, we then wished to establish whether chromosomal lesions imposed at other stages of meiosis would result in a similar arrest, but found no suitable genetic deficiencies for this purpose. We therefore turned to the use of X-ray damage, which could be imposed at any stage of meiosis and which has been shown to cause mitotic arrest in G_2 in a manner similar to *cdc13* (WEINERT and HARTWELL 1988).

The impact of ultraviolet (UV) irradiation on yeast cells during meiosis had been investigated previously. SIMCHEN, SALTS and PINON (1973) found that cells heavily irradiated during premeiotic DNA synthesis were severely affected, undergoing meiotic arrest and suffering a reduction in meiotic recombination

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(SALTS, SIMCHEN and PINON 1976; RESNICK, GAME and STASIEWICZ 1983). At 30-fold lower doses of irradiation, MACHIDA and NAKAI (1980) detected no significant effect on intragenic recombination but found that intergenic recombination was enhanced. The effects of X-irradiation on meiotic yeast have also been examined, but in less detail. ROMAN (1984) described striking effects on gene conversion both in mitosis and in meiosis, but the doses tested (up to 3 krad) were insufficient to cause clear-cut effects on levels of sporulation. Using higher doses and a return-to-mitotic-growth procedure, KELLY, MERRILL and PARRY (1983) clearly demonstrated that reciprocal recombination, as measured by an *ade2* heteroallele system, is stimulated by X-irradiation. The effects of X-irradiation on ongoing meiosis merit further attention because the well-characterized lesions that are generated–single- and double-strand breaks (MCGRATH and WILLIAMS 1966; HO 1975; RESNICK and MARTIN 1976)–can be induced independently of cellular functions, such as the excision repair system, which might be required to transform the primary lesions of UV-irradiation and some types of chemical mutagenesis into interruptions of strand continuity.

X-irradiation has been shown to affect meiotic recombination in other organisms. Progeny testing in Drosophila has shown that X-irradiation leads to the induction of recombination, translocations, and mutations in meiotic cells not only of females but also in males, which normally display no recombination during meiosis [reviewed in SANKARANARAYANAN and SOBELS (1976) and HANNAH-ALAVA (1964)]. Without any effective way to stage the time of treatment with respect to the complex biology of reproduction in Drosophila, it has been difficult to establish whether it was meiosis *per se* that was being affected. Better timing has been achieved in the irradiation of Lilium (LAWRENCE 1961a) and Tradescantia (LAWRENCE 1961b) meiocytes, wherein increased frequencies of chiasmata were found at metaphase I. Significantly increased meiotic recombination in response to X-irradiation has also been reported in *Chlamydomonas reinhardi* (LAWRENCE and HOLT 1970) and *Caenorhabditis elegans* (KIM and ROSE 1987; MCKIM, HOWELL and ROSE 1988).

It seemed reasonable to expect an induction of meiotic recombination by X-irradiation because the lesions caused by X-irradiation–single-strand and double-strand breaks in DNA–are thought to be intermediates in meiotic recombination. Most models for the mechanisms of meiotic recombination invoke the formation of such breaks as a crucial initiating event and many experiments are interpreted as consistent with this concept (HOLLIDAY 1964; MESELSON and RADDING 1975; ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; RESNICK 1976; RESNICK *et al.* 1981, 1984; SYMINGTON 1991; SZOSTAK *et al.* 1983) [for review see ORR-WEAVER and SZOSTAK (1985)]. Moreover, meiosis-specific double-strand breaks have been demonstrated by physical assays not only as hotspots for meiotic recombination (CAO, ALANI and KLECKNER 1990; NICOLAS *et al.* 1989; SUN *et al.* 1989) but also more generally in meiotic chromosomes (GAME *et al.* 1989). Furthermore, double-strand breaks induced by HO cutting stimulate meiotic recombination both in *S. cerevisiae* (KOLODKIN, KLAR and STAHL 1986) and in *Schizosaccharomyces pombe* (KLAR and MIGLIO 1986).

In the present work, we provide evidence in *S. cerevisiae* that X-ray-induced lesions can stimulate recombination if the treatment is imposed after the initiation of premeiotic DNA synthesis, whereas the induction of X-ray damage prior to this point causes arrest in the G_1 phase of meiosis in a *RAD9*-dependent manner. In addition, assays for spore viability in *spo11* meiosis provide evidence that the lesions induced by X-irradiation at the later stage may partially substitute for this mutational deficiency in meiotic recombination and lead to improved meiotic disjunction.

MATERIALS AND METHODS

Strains: The genotypes of the strains used in these experiments are described in Table 1. Standard procedures for strain construction and genetic analysis were employed (MORTIMER and HAWTHORNE 1969). Strains LW3501 and LW3502 were generated from a diploid of 5837-2-1 and 5821-c-16, which were obtained from N. HOLLINGSWORTH. Strains LW3201, LW3202, LW3203 and LW3204 were made by crossing spore clones of 7845-8-4 and 3985-4-1b and are in the A364a background; LW3205 is a spore clone of this same diploid. Strains LW3605, LW3606, LW3607 and LW3608 were generated by crossing spore clones of a diploid of LW3501 and 5819-24-3 to LW3502, and strain LW3604 is a spore clone of this same diploid. Spore clones of a diploid of LW3604 and CAY63 were crossed to each other to generate LW3802, which (presumably because of slight differences in background) proceeds through meiosis slightly faster than the other strains used, as confirmed by fluorescence-activated cell sorter (FACS) analysis (data not shown). These strain constructions were designed to maintain a hybrid background of A364a and 131-20 while creating marked intervals that could be used to monitor reciprocal exchange. Strain LW3901 was generated by crossing spore clones from a diploid of LW3205 and LW3502 to 131-20, thus also retaining the hybrid background of A364a and 131-20. No significant differences in the rates of vegetative growth or sporulation were found among any of the strains used for the irradiation experiments (data not shown).

Genetic procedures: Materials and methods used in this study have been described, including liquid and solid media (HARTWELL 1967; WOOD 1982), sporulation procedures (SCHILD and BYERS 1978), assays of viability and commitment to recombination (SHUSTER and BYERS 1989), and the assay for haploidization (SHERMAN and ROMAN 1963; HOLLINGSWORTH and BYERS 1989). Sporulation medium (SPM) contains 3.0 g of potassium acetate and 0.2 g of raffinose per liter of water, while -N medium contains 1.61 g of yeast

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype
CAY63	*MAT***a** *ade2 ura3 can1 his3 leu2 trp1 atr::HIS3 spo11-D3*
131-20	*MATα ade2-R8 ura3 leu1 can1 cyh2*
3985-4-1b	*MATα his7 ade2*
5819-24-3	*MAT***a** *ade2 trp1 ura3*
5821-c-16	*MATα ade2 can1 leu1 leu2 trp5 cyh2 lys5 hop1::LEU2 his7*
5837-2-1	*MAT***a** *his1 leu2 trp1 ura3 spo11*
7845-8-4	*MAT***a** *cdc13-1 rad9::LEU2 his7 trp1 ura3 can1 cyh2*
LW3003	*MATα* cdc13-1 *ade2 ura1 LEU1 CYH2 his7 tyr1 CAN1 URA3 lys2* *MATα* cdc13-1 *ade2 URA1 leu1 cyh2 HIS7 TYR1 can1 ura3 LYS2*
LW3201	*MATα rad9::LEU2 ade2 trp1 URA3 CAN1 CYH2 his7* *MATα RAD9 ADE2 TRP1 ura3 can1 cyh2 his7*
LW3202	*MAT***a** *rad9::LEU2 ade2 trp1 URA3 CAN1 CYH2 his7* *MATα RAD9 ade2 TRP1 ura3 can1 cyh2 his7*
LW3203	*MAT***a** *rad9::LEU2 ade2 trp1 URA3 CAN1 CYH2 his7* *MATα rad9::LEU2 ade2 trp1 ura3 can1 cyh2 his7*
LW3204	*MAT***a** *rad9::LEU2 ade2 trp1 URA3 CAN1 CYH2 his7* *MATα rad9::LEU2 ADE2 TRP1 ura3 can1 cyh2 his7*
LW3205	*MATα ade2 his7 ura3 cdc13-1 rad9::LEU2*
LW3501	*MATα can1 cyh2 his1 hop1::LEU2 leu1 leu2 lys5 spo11 trp5*
LW3502	*MAT***a** *ade2 can1 cyh2 his1 hop1::LEU2 leu1 leu2 lys5 spo11 trp5*
LW3604	*MATα ade2 ura3*
LW3605[a]	*MAT***a** *ade2 hop1 spo11 can1 URA3 his1 leu1 trp5 cyh2 lys5 leu2* *MATα ADE2 hop1 spo11 CAN1 ura3 HIS1 LEU1 TRP5 CYH2 LYS5 LEU2*
LW3606[a]	*MAT***a** *ade2 hop1 spo11 can1 URA3 his1 leu1 trp5 cyh2 lys5 leu2* *MATα ADE2 HOP1 spo11 CAN1 ura3 HIS1 LEU1 TRP5 CYH2 LYS5 LEU2*
LW3607[a]	*MAT***a** *ade2 hop1 spo11 can1 URA3 his1 leu1 trp5 cyh2 lys5 leu2* *MATα ADE2 hop1 SPO11 CAN1 ura3 HIS1 LEU1 TRP5 CYH2 LYS5 LEU2*
LW3608[a]	*MAT***a** *ade2 hop1 spo11 can1 URA3 his1 leu1 trp5 cyh2 lys5 leu2* *MATα ADE2 HOP1 SPO11 CAN1 ura3 HIS1 LEU1 TRP5 CYH2 LYS5 LEU2*
LW3802[b]	*MAT***a** *CAN1 ura3 HIS1 TRP5 CYH2 LYS5 trp1 spo11-D3 ade2* *MATα can1 URA3 his1 trp5 cyh2 lys5 TRP1 spo11-D3 ADE2*
LW3901	*MAT***a** *ade2 cdc13-1 LEU1 CYH2 lys5 URA3 CAN1 rad9::LEU2* *MATα ade2 cdc13-1 leu1 cyh2 LYS5 ura3 can1 rad9::LEU2*

CAY63 was provided by R. E. ESPOSITO. 131-20 is described in HOPPER and HALL (1975). 5819-24-3, 5821-c-16 and 5837-2-1 were obtained from N. HOLLINGSWORTH. 5819-24-3 is a hybrid of A364a and 131-20 backgrounds. 3985-4-1b and 7845-8-4 were obtained from L. HARTWELL. LW3003 is described in WEBER and BYERS (1992).

[a] *hop1* allele is *hop1::LEU2; spo11* allele is *spo11-1.*

[b] *spo11-D3* is a deletion of *SPO11* and was kindly provided by R. E. ESPOSITO.

nitrogen base without amino acids (Difco), 11.1 g of succinic acid, and 6.7 g of sodium hydroxide per liter. All experiments were conducted at room temperature (23°) unless otherwise indicated.

X-irradiation procedures: Vegetative cultures were grown to cell densities of 1.3×10^7 cells/ml to 1.9×10^7 cells/ml in YPA medium, washed with -N medium, resuspended in SPM, and incubated with shaking at room temperature. Subsequently cells were pelleted from 5–10-ml aliquots of larger cultures by centrifugation, resuspended in a small amount of the supernatant liquid, and plated on an agar plate of the same medium as that used for liquid growth (KAC, YEPD or minimal medium) to generate a monolayer of cells that would not be shielded from irradiation by liquid or by each other. These plated cultures were X-irradiated (with lids removed) at a dose rate of 108 rad/sec in a Picker X-ray machine set at 50 kilovolts and 20 milliamps. Unirradiated cultures were similarly subjected to replating and transport to the X-ray machine to guard against inadvertant effects of these manipulations. Treated and control cells

were then washed from the plates with appropriate liquid media and either returned to the previous growth conditions or immediately diluted for plating on test media. These plating and resuspension procedures had little effect on progression of unirradiated cells through meiosis except for those replated near the time of meiosis I (about 12–15 hr in these strains), beyond which stage responses to replating varied erratically both within and between experiments. All data presented here were gathered using cells treated at stages prior to the time of meiosis I (from 0 to 11 hr). Every experiment reported was repeated at least three times, yielding nearly identical results in each repetition. Because individual cultures varied slightly in their time of entry into the meiotic process (typically ±30 min), data are presented from single experiments rather than being pooled. FACS analysis on control meiotic time courses of these strains confirmed that cells accumulated in G_1 after transfer to SPM and later entered into S phase within about 5 hr. All values reported represent at least 100 colony-forming units (cfu) in each experiment and appropriately account for dilution factors prior to plating.

Cytological procedures: Preparation and procedures for electron microscopy were as described in BYERS and GOETSCH (1991). Serial sections were examined with a Philips EM300 electron microscope. Fluorescence microscopy procedures were as described in ADAMS and PRINGLE (1984) and BAUM, GOETSCH and BYERS (1988). DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI) (1.0 μg/ml, Sigma) for examination with a Nikon Microphot microscope equipped with epifluorescence optics and filters. Flow cytometry analysis was as described in HUTTER and EIPEL (1979) using propidium iodide to stain DNA (Sigma). A Becton-Dickinson FACScan flow cytometer and the software packages CELLFIT and LYSYS were used to collect and analyze these data. For the sake of clarity in comparison with the vegetative cell cycle parameters, we will refer to premeiotic S phase, the following interval, and meiosis I as S, G_2 and M, respectively.

Liquid holding recovery procedure: A vegetative culture was grown to the point of readiness for meiosis in YPA medium and cells were harvested by centrifugation. Half of the cells were resuspended in -N liquid medium (a starvation medium lacking any metabolic nitrogen source); the other cells were resuspended in SPM. After 5 hr of incubation with shaking, aliquots were irradiated. Serial dilutions were plated on complete solid medium at this time to assess culture viability. Dilutions were also plated from both cultures after control sporulation reached maximal levels (about 48 hr).

RESULTS

X-irradiation early in meiosis induces a *RAD9*-dependent arrest: We determined the effects of X-irradiation on yeast cells during meiosis (following transfer to SPM) by assessing the level of sporulation achieved 48 hr after the transfer, when untreated cultures had attained maximal levels. Cultures irradiated early in meiosis ultimately showed a greatly decreased level of sporulation (Figure 1, striped bars), whereas later irradiation permitted continued meiosis. For example, one portion of a culture irradiated with 15 krad at 5 hr achieved a final level of sporulation that was only 20% of control values, while another portion of the same culture that was irradiated 2 hr later sporulated to a frequency that was indistinguishable from the control. Flow cytometric analysis revealed that the earlier period of treatment, when cells were more sensitive to X-irradiation, corresponded to the period preceding DNA replication (which began at about 5 hr); cultures gained significantly in their ability to continue meiosis despite X-irradiation after they had undergone the transition into S phase (data not shown). Since we previously had shown that *cdc13* arrest in meiosis is *RAD9*-dependent (WEBER and BYERS 1992), we then wished to establish whether the X-irradiation-induced arrest also depended on the *RAD9* function. Accordingly, we subjected *rad9* strains to X-irradiation 5 hr after transfer to sporulation conditions and found that doses sufficient to cause arrest of *RAD9* cells failed to exert that effect on *rad9* cells (Figure 1, solid bars). When tested over a broad range of X-ray doses, *RAD9* strains showed a substantially greater decrease in final sporulation than did

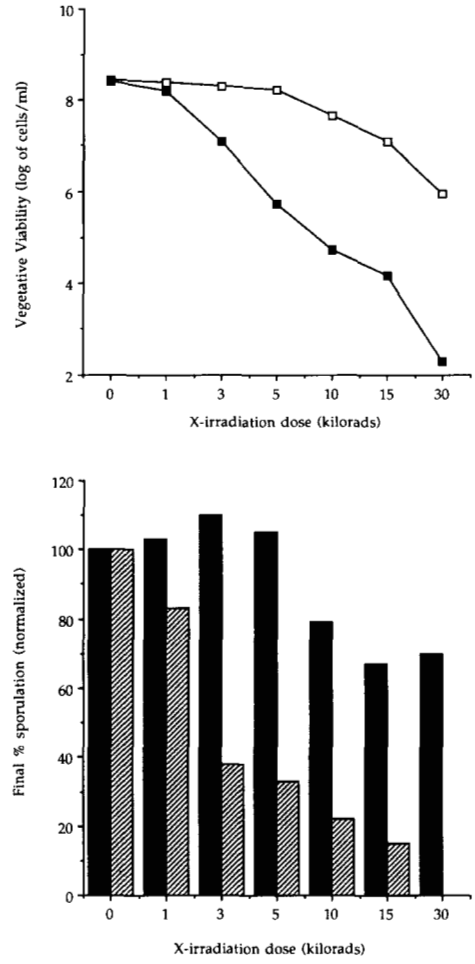

FIGURE 1.—(Upper graph) Vegetative survival of *RAD9* (open squares) and *rad9* (solid squares) in response to X-irradiation (LW3202 and LW3204, respectively). (Lower graph) Early meiotic response to X-irradiation of the same *RAD9* (striped bars) and *rad9* (solid bars) strains. Final percent sporulation for each culture is normalized to the corresponding 0 krad control value, which was 41% for LW3202 and 44% for LW3204. All treatments were given at 5 hr after transfer to sporulation conditions.

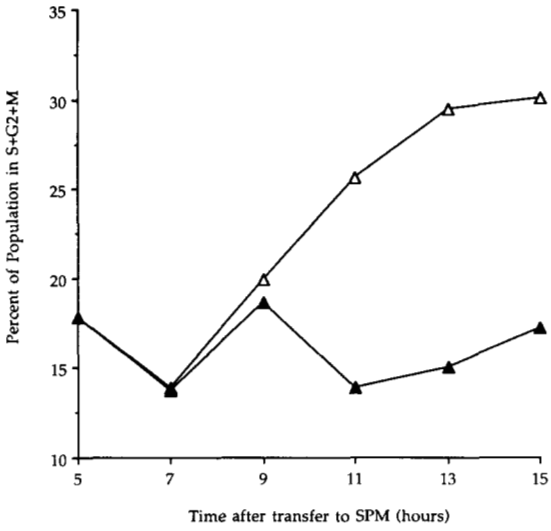

FIGURE 2.—Flow cytometric analysis of premeiotic DNA synthesis in *RAD9* control (open triangles) and irradiated (closed triangles) cultures (strain LW3201). Treatment was 20 krad of X-irradiation at 5 hr. Final percent sporulation was 54% for the control and 10% for the irradiated culture.

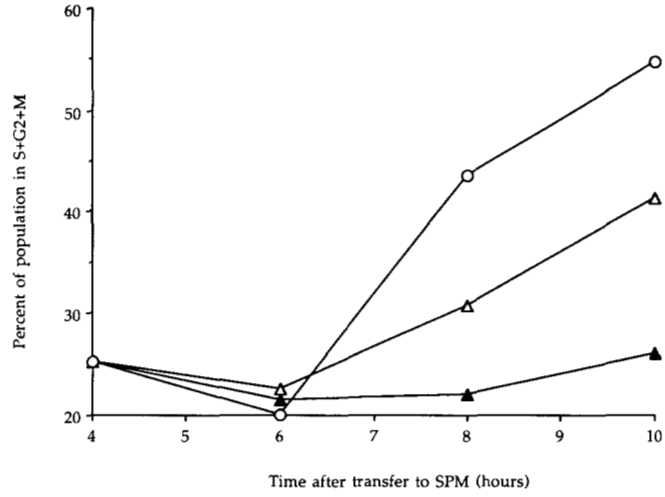

FIGURE 3.—Flow cytometric analysis of control, *cdc13* arrest and X-irradiation arrest in the same yeast strain (LW3003). Four hours after transfer to sporulation conditions, one aliquot was raised to the *cdc13* restrictive temperature (34.5°) (open triangles), another aliquot was exposed to a 40-krad dose of X-irradiation (closed triangles), and the control aliquot was plated on KAC and then resuspended in SPM (open circles).

rad9 strains. Only at very high doses (45 krad and higher), which were almost completely lethal to vegetative *rad9* cultures (data not shown), did the *rad9* cultures also fail to complete sporulation.

Characterization of the arrest induced by X-irradiation: Flow cytometric analysis was performed on the X-irradiated meiotic cultures to determine the stage of meiotic arrest. At 5 hr after transfer of LW3201 cells to sporulation conditions, subcultures were X-irradiated and returned to sporulation conditions. Aliquots taken at regular intervals were analyzed for DNA content per cell by flow cytometry (Figure 2). For the irradiated *RAD9* culture, the aggregate percentage of cells that had reached the S, G_2 and M phases of meiosis remained largely unchanged from that measured at the time of irradiation, whereas the proportion of cells in these stages continued to rise in the unirradiated *RAD9* control.

Having previously shown that a *RAD9*-dependent arrest of *cdc13* cultures occurs after the bulk of DNA replication has occurred (WEBER and BYERS 1992) and in light of the fact that X-irradiation of vegetative cells causes arrest following DNA synthesis (WEINERT and HARTWELL 1988), we were struck by the present finding that X-irradiation causes an arrest of meiosis prior to detectable premeiotic DNA synthesis. Because of our concern that the difference in the stages of arrest by *cdc13* and X-irradiation might have resulted from differences in genetic background, we decided to test for both effects in the same strain. Challenge of the *cdc13* homozygote, LW3003, by transfer to the restrictive temperature (34.5°) assayed the effect of the *cdc13* deficiency, while X-irradiation was performed at the permissive temperature (23°). Flow cytometric analysis showed that premeiotic DNA replication occurs in cells transferred to the restrictive temperature (34.5°), as evidenced by progression of the culture into the S and G_2 phases (Figure 3). In contrast, the X-irradiated culture was markedly inhibited in its progression beyond G_1. We conclude that the respective lesions caused by these two treatments do, indeed, lead to two distinct stages of meiotic arrest.

Meiosis can rescue *rad9* strains from otherwise lethal damage: We found during the course of these studies that doses of X-irradiation that are largely lethal to vegetative *rad9* cultures caused little lethality in cultures permitted to complete meiosis after irradiation. When cultures of LW3203 and LW3204 were irradiated early in meiosis (0–5 hr) and returned to sporulation conditions, aliquots plated on complete media 48 hr after the initiation of sporulation showed viabilities more than 2 orders of magnitude greater than when similar aliquots of the cultures were plated on complete media immediately after irradiation. The viability of one culture, for example, was reduced to 2.2×10^5 viable cfu/ml at the time of irradiation (5 hr) with 30 krad but increased to 3.1×10^7 viable cfu/ml after 48 hr total incubation.

Two possible explanations for this dramatic increase in X-ray resistance seemed worth considering. One possibility was that meiosis-specific repair and recombination functions could assist in the repair of breaks caused by X-irradiation. The other explanation would attribute the increased viability to the phenomenon known as "liquid holding recovery," in which vegetative cells subjected to irradiation regain viability when held in non-nutritive media before plating on growth media. This recovery presumably arises largely because the cells benefit from an increased period of

TABLE 2

Survival of *rad9* cultures after X-irradiation: LHR *vs.* sporulation

Medium	Time sampled (hr)	Viable cells/ml	
		+15 krad	Control
-N	5	1.1×10^6	2.3×10^8
	48	2.5×10^6	4.9×10^8
SPM	5	1.5×10^6	2.6×10^8
	48	31.0×10^6	3.2×10^8

LW3203 cells in log phase were transferred to -N medium or SPM and incubated at room temperature with shaking. At 5 hr, aliquots were irradiated and returned to previous culture conditions. Serial dilutions were plated both immediately after the 5-hr treatment and after 48-hr total incubation to quantitate cell viability.

LHR is liquid holding recovery; see text for discussion.

time to execute the type of repair functions that are typical of nonmeiotic cells before growth is resumed [for review, see HAYNES and KUNZ (1981)]. Wishing to establish whether the recovery of viability in irradiated *rad9* cultures resulted from meiotic functions or simply from liquid holding recovery, we also assayed survival of cells that were irradiated during incubation in starvation conditions (-N media), thus providing a prolonged period for repair in the presumed absence of meiotic functions (Table 2). Cells suffering a 200-fold loss of viability immediately after irradiation with 15 krad recovered only about twofold in plating efficiency upon continued incubation in -N medium. This twofold increase is nearly equal to the extent of increased plating efficiency that was seen in the unirradiated controls and may simply reflect a delay in separation of cells that had already undergone cytokinesis. Liquid holding recovery *per se* may therefore be less than twofold. In contrast, the culture that was returned to sporulation medium showed a 21-fold increase in viability between the time of irradiation at 5 hr and the 48-hr final time point. Even if the SPM cultures had experienced a twofold increase due to delayed cell separation, as was evident for the -N cultures, a full order of magnitude in increased viability would still be attributable to the return to sporulation conditions and therefore, presumably, to the resumption of meiosis.

The viability of spores derived from asci formed upon the meiotic rescue of irradiation in *rad9* strains was assayed by plating for recessive drug resistance phenotypes. The yield of drug-resistant segregants was found to be high, even after X-irradiation at dosages (*e.g.*, 20 krad) that would kill more than 99% of vegetative *rad9* cells. Tetrad dissection revealed, for example, that a dose of 30 krad reduced the viability of spores to only about half that shown by the unirradiated control. (Among 21 tetrads dissected, one had four viable spores, seven had three viable spores, four had two viable spores, eight had one viable spore, and one had no viable spores, for a total spore viability of 47%.) We also monitored reciprocal recombination between scored markers in this experiment, but did not detect any significant difference from the extent of recombination seen in unirradiated *rad9* cultures (Table 3).

X-irradiation during the meiosis of mutant strains: The failure of X-irradiated *rad9* strains to undergo G_2 arrest or to suffer significant lethality during meiosis suggested that X-ray-induced chromosomal lesions may be subject to processing by the same mechanisms that act on recombination intermediates during meiosis. We wished to test this hypothesis by asking whether such lesions might serve as recombination intermediates. Expecting that any contribution to overall recombination by these lesions would be undetectable among a predominance of normal meiotic recombination events, we sought to assay strains in which recombination was severely inhibited by relevant mutations. Necessarily, of course, any other meiotic functions that were required for the resolution of these potential intermediates would have to remain functional in the mutant strains.

We chose to test for behavior of this sort among mutants defective either in pairing (*hop1*) or in another recombination-specific function (*spo11*). *spo11*, like many other meiotic mutations conferring defects on recombination [*e.g.*, *hop1* (HOLLINGSWORTH and BYERS 1989), *mei4* (MENEES and ROEDER 1989), *mer1* (ENGEBRECHT and ROEDER 1989, 1990), *red1* (ROCKMILL and ROEDER 1988, 1990), and *rad50* (GAME and MORTIMER 1974)] causes production of spores that rarely are viable (KLAPHOLZ, WADDELL and ESPOSITO 1985). Representative viable spore colonies could be selected by the use of two recessive drug resistance markers, *can1* and *cyh2*, that are heterozygous in the parent diploid. Preliminary tests confirmed that haploid (spore) progeny recovered from the meiosis of unirradiated *spo11* diploids by plating for resistance to both drugs displayed levels of reciprocal recombination substantially less than 10% of wild-type levels (*e.g.*, see Table 5). This confirmed previous findings (KLAPHOLZ and ESPOSITO 1982) and is consistent with the proposition that recombination is sufficiently reduced by *spo11* that the resulting nondisjunction is largely responsible for the prevalent inviability of spores.

Treatment of *spo11* diploids in sporulating conditions with X-rays enhanced the production of $can^r cyh^r$ meiotic products nearly sixfold (Table 4). The minimal levels of X-irradiation capable of causing a detectable increase in the frequency of viable spores left the viability of vegetative cultures virtually unaffected. A higher dose (30 krad), which decreased the viability of vegetative cultures by approximately half, increased the number of $can^r cyh^r$ cfu generated to

TABLE 3

Meiotic recombination (rec) in irradiated *rad9* cells

	LW3203 *can1-ura3*		LW3204 *can1-ura3*		LW3901					
					can1-ura3		*leu1-cyh2*		*cyh2-lys5*	
Dose[a]	% rec	No. scored	% rec	No. scored	% rec	No. scored	% rec	No. scored	% rec	No. scored
0	34	93	44	91	30	93	32	93	33	93
1	30	95	33	93						
3	33	92	35	88						
5	41	93	42	93						
10	37	95	33	94	34	95	37	95	38	95
15	34	77	35	46						
20					31	95	33	95	27	95

Cells in log phase were transferred to SPM and incubated with shaking at room temperature. Five hours later, aliquots were transferred to plates for irradiation and then returned to liquid culture conditions. After 48 hr total time in SPM, cultures were diluted and plated on selective media (−arg +can+cyh) as well as complete (D) media. Colonies arising on selective media were patched onto complete media and subsequently assayed for auxotrophies and mating type (nonmaters excluded from data shown). All data are from random spore analysis.

[a] X-irradiation dose in kilorads.

TABLE 4

Effect of X-irradiation on meiotic survival of strains bearing meiotic mutations

(Strain), relevant genotype	Radiation dose (krad)	*can*^{r} *cyh*^{r}/viable cell[a] at time of treatment			Control[b]	Viable cells/ml[c]
		5 hr	8 hr	10 hr		
(LW3608)						
HOP1 *SPO11*	0	0.17	0.17	0.23	0.27	1.9×10^8
hop1::LEU2 *spo11-1*	15	0.05	0.08	0.14		1.3×10^8
(LW3606)						
HOP1 *spo11-1*[d]	0	0.93×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	0.86×10^{-3}	9.3×10^7
hop1::LEU2 *spo11-1*	15	0.81×10^{-3}	5.9×10^{-3}	4.6×10^{-3}		9.1×10^7
(LW3606)[e]	0	1.3×10^{-3}	0.54×10^{-3}		0.58×10^{-3}	1.3×10^8
	15	6.7×10^{-3}	10.6×10^{-3}			1.0×10^8
(LW3802)						
HOP1 *spo11Δ*[f]	0	0.13×10^{-3}	0.13×10^{-3}	0.16×10^{-3}	0.18×10^{-3}	1.3×10^8
HOP1 *spo11Δ*	15	5.4×10^{-3}	3.6×10^{-3}	1.8×10^{-3}		1.4×10^8
(LW3607)						
hop1::LEU2 *SPO11*	0	1.6×10^{-3}	1.2×10^{-3}	1.5×10^{-3}	2.0×10^{-3}	6.1×10^7
hop1::LEU2 *spo11-1*	15	0.95×10^{-3}	1.6×10^{-3}	0.5×10^{-3}		7.0×10^7
(LW3605)						
hop1::LEU2 *spo11-1*	0	0.82×10^{-3}	0.71×10^{-3}	0.58×10^{-3}	1.2×10^{-3}	4.4×10^7
hop1::LEU2 *spo11-1*	15	0.36×10^{-3}	0.38×10^{-3}	0.17×10^{-3}		5.0×10^7
		5 hr	7 hr			
	0	1.3×10^{-3}	0.54×10^{-3}			1.3×10^8
(LW3606)[e]	15	6.7×10^{-3}	10.6×10^{-3}		0.58×10^{-3}	1.0×10^8

Cells in log phase (1.3×10^7 cells/ml to 1.9×10^7 cells/ml) were transferred to SPM and incubated with shaking. At times indicated, aliquots were plated, irradiated, and returned to liquid culture conditions. After 48 hr total of incubation, serial dilutions were plated on complete (D) and *can*^{r}*cyh*^{r}-selective media (−arg+can+cyh).

[a] Values are the frequency of *can*^{r}*cyh*^{r} cfu divided by the frequency of cfu on complete media and thus represent a measure of *can*^{r}*cyh*^{r} generation per viable cell.

[b] No resuspensions or replatings; assayed only at final time point.

[c] Viability figures from the 5-hr experimental group are included to illustrate that the doses of radiation used did not significantly affect viability. Minor fluctuations in these figures most likely reflect slight variability in cell recovery from the plates used for the irradiation procedure (see MATERIALS AND METHODS).

[d,e,f] Separate experiments fell into these classes, which were internally consistent; see text for discussion. The differences in the 5-hr values in these two sets of experiments is attributable to slight variation in rates of progression through meiosis, some 5-hr samplings preceding (*d*) and others following (*e*) the transition out of the period of X-ray induced arrest in G_1. The *spo11Δ* diploid strain LW3802 (*f*) entered premeiotic S phase about 3 hr after transfer to SPM, which is reflected in the pattern of induction of *can*^{r}*cyh*^{r} cfu.

approximately twice the level seen with the 15-krad dose. There was no further increase in the overall number of *can*^{r}*cyh*^{r} cfu generated when cells were treated with 45 krad, and even higher doses (60 and

90 krad) led to a decrease in the overall yield (see Table 6). In spite of these decreases in overall yield, the proportion of sporulating cells surviving the treatment (scored as the ratio of *can^{r}cyh^r* cfu to total cfu) continued to rise, further implicating the entry into meiosis in the development of resistance to X-irradiation.

In contrast to the behavior of the *spo11* diploids, no significant increase in *can^{r}cyh^r* cfu generation was seen in *hop1* diploids or *hop1 spo11* diploids (Table 4). Therefore, *hop1* strains seem incapable of being stimulated to higher survival by X-irradiation, and this deficiency of *hop1* is epistatic to the effect shown for *spo11*.

A crucial parameter in the induction of increased spore viability during *spo11* meiosis was the time of X-irradiation. If irradiated before the beginning of premeiotic DNA synthesis (about 5 hr in these strains), *spo11* cultures failed to show an increase in *can^{r}cyh^r* cfu. This is consistent with the observation (data not shown) that *spo11* diploids subjected to irradiation early in meiosis become arrested to the same extent as wild-type cells. Similarly, delaying the time of X-irradiation until a late stage of meiosis also prevented the treatment from effectively increasing the frequency of *can^{r}cyh^r* cfu (Table 4). A maximally responsive stage was found to lie in the interval of about 5–8 hr after transfer to sporulation medium. In these strains, this represents a period extending from early in premeiotic S phase until the latter part of meiotic prophase. The greatest induction of *can^{r}cyh^r* cfu detected in the course of these experiments was obtained for strain LW3606 after irradiation at 7 hr. It remains to be resolved whether this is indicative of there being only a narrow span of time in which a culture is maximally susceptible to induction.

Induction of meiotic survival and recombination by X-irradiation: The origin of the *can^{r}cyh^r* progeny obtained upon X-irradiation of *spo11* strains was examined by undertaking further genetic characterization. Having found sporulation generally to be incomplete in these strains, we wished to ascertain first whether these drug-resistant colonies represented spore colonies or arose from unsporulated diploids that had simply been rendered homozygous for the drug resistance markers by virtue of high levels of gene-centromere recombination in response to the irradiation treatment (for review see HAYNES and KUNZ 1981). Furthermore, genotypic analysis of haploid colonies would serve to assess the level of reciprocal recombination that had occurred during the meiosis leading to their formation. To permit evaluation of mating capability and reciprocal exchange, *can^{r}cyh^r* cfu were patched individually onto complete medium and assayed for mating ability.

Many of the nonmaters were prototrophic for unselected markers and were therefore presumed to be either hyperploid products of meiotic nondisjunction or diploids that had not undergone meiosis. The frequency of nonmaters was higher among the *can^r cyh^r* products of the recombination-deficient mutants than those from wild type (Table 5), as had previously been seen for *hop1* strains (HOLLINGSWORTH and BYERS 1989), and this frequency failed to show a dosage-dependent response to X-irradiation. The absence of dosage dependency might be explained by positing that meiosis had occurred but that full levels of recombination (as indicated by marker segregation) had not been achieved upon irradiation, so the progeny were still largely subject to the pattern of nondisjunction that is typical of recombination-deficient meiosis. It also seemed possible that some of the observed recombination events occurred during vegetative growth rather than meiosis, but the fact that an increased production of *can^r cyh^r* cfu could be induced only during a limited time after transfer to SPM argued in favor of their origin in meiosis. Regardless, nonmating cfu were eliminated from the recombination analysis in order to focus specifically on those progeny that most likely were true haploids.

The haploid maters were assayed for reciprocal exchange in five intervals: *can1-ura3* and *ura3-his1* on chromosome *V*; and *leu1-trp5*, *trp5-cyh2* and *cyh2-lys5* on chromosome *VII* (Table 5). X-irradiation caused an increased incidence of reciprocal exchange in every interval assayed in the *spo11* diploid. Little or no induction was detected in the wild-type strain or the *hop1* homozygote. Limited induction detected in certain intervals in the *hop1 spo11* diploid was largely restricted to the later times. Further, this induced recombination was not accompanied by the pattern of increase in viable *can^{r}cyh^r* cfu that was seen for *spo11* diploids (Table 4). The failure of irradiation to cause increased spore viability in the doubly mutant strain suggested that the limited induction of recombination was ineffective in promoting improved disjunction.

Having found that X-ray-induced lesions are capable of enhancing recombination in *spo11* strains, we then wished to ask whether one might attain normal meiotic levels of recombination at sufficiently high doses. Having tested several strains and culture conditions for a combination yielding the highest level of induction, we chose for further study *spo11Δ* homozygote LW3802, which proceeds rapidly through meiosis and becomes quite susceptible to induction (and insensitive to *RAD9*-dependent arrest) 5 hr after transfer to SPM. High dose irradiation treatments of LW3802 at this time (Table 6) yielded a percentage of recombinants among the *can^{r}cyh^r* segregants that was at least as high in most intervals tested as that found among spores produced by an unirradiated wild-type strain. Similar results were obtained with

TABLE 5

X-irradiation-induced recombination in strains bearing meiotic mutations

(Strain), genotype	Time (hr)[a]	X-ray dose (krad)	Percent recombination[b] *can1-ura3*	*ura3-his1*	*leu1-trp5*	*trp5-cyh2*	*cyh2-lys5*	No. scored	Nonmater frequency (%)
(LW3608)	5	0	17	40	14	52	32	92	2/94 (2)
HOP1 SPO11		15	23	37	12	36	33	92	3/95 (3)
hop1 spo11		30	16	37	14	39	30	86	4/89 (5)
	8	0	20	38	8	47	24	92	2/94 (2)
		15	27	37	15	41	24	92	0/94 (0)
		30	22	31	11	32	30	93	3/96 (3)
	10	0	25	35	9	45	40	91	5/96 (5)
		15	25	36	7	46	31	91	5/96 (5)
		30	20	40	12	51	32	92	3/95 (3)
	Control[c]		11	46	9	47	18	91	5/96 (5)
(LW3607)	5	0	5	14	1	6	5	81	15/96 (16)
hop1::LEU2		15	3	10	1	5	3	73	23/96 (24)
hop1::LEU2		30	1	17	3	11	3	70	24/94 (26)
SPO11	8	0	5	16	1	11	4	82	12/94 (13)
spo11		15	0	10	1	13	6	77	19/96 (20)
		30	4	13	5	28	5	76	19/95 (20)
	10	0	6	15	0	15	3	79	16/95 (17)
		15	0	13	0	25	5	64	31/95 (33)
		30	5	17	1	17	5	76	19/95 (20)
	Control[c]		0	10	1	7	0	88	8/96 (8)
(LW3606)	5	0	0	2	0	6	0	82	14/96 (15)
HOP1		15	5	16	4	15	5	73	21/94 (22)
hop1		30	5	13	8	20	24	75	21/96 (22)
spo11-1	8	0	1	6	0	8	4	83	12/95 (13)
spo11-1		15	8	16	5	16	9	85	10/95 (11)
		30	7	23	6	27	27	81	12/93 (13)
	10	0	0	4	0	12	0	77	19/96 (20)
		15	7	21	4	18	11	85	11/96 (11)
		30	9	26	15	22	21	81	14/95 (15)
	Control[c]		0	0	0	5	1	78	18/96 (19)
(LW3605)	5	0	0	0	0	0	1	75	21/96 (22)
hop1::LEU2		15	0	5	0	5	3	60	34/94 (36)
hop1::LEU2		30	9	9	0	9	0	22	10/32 (31)
spo11-1	8	0	0	0	0	0	0	73	23/96 (24)
spo11-1		15	7	5	0	8	2	61	33/94 (35)
		30	5	2	2	5	4	57	35/92 (38)
	10	0	0	0	0	0	0	78	18/96 (19)
		15	0	5	0	5	3	39	39/78 (50)
		30	5	16	2	14	4	56	38/94 (40)
	Control[c]		0	0	0	0	0	75	21/96 (22)

Cells in log phase of growth were transferred to SPM and incubated with shaking. At the indicated time after this transfer, aliquots were plated, irradiated, and returned to liquid culture conditions. After 48 hr total incubation, serial dilutions were plated on complete and *can^{r}cyh^r*-selective media. Colonies arising on selective media were patched onto complete media and subsequently assayed for auxotrophies and mating type.

[a] Time of treatment.

[b] Numbers shown are from a representative experiment: the 5- and 8-hr time points were taken in a total of three experiments and the 10-hr time point was taken in two experiments; in each case the values were nearly identical. The *can^{r}cyh^r* cells examined were from experiments also described in Table 4.

[c] Control values were obtained from an undisturbed culture, as opposed to the 0 krad data points, for which aliquots were plated and resuspended exactly as were the irradiated aliquots with the exception of irradiation (see MATERIALS AND METHODS). Minor differences between the two are probably attributable to stress imposed by the plating procedure.

TABLE 6

X-irradiation-induced recombination in a *spo11* diploid is dosage-dependent

X-ray dose (krad)	*can*^{r}*cyh*^{r} (cfu/ml)	*can*^{r}*cyh*^{r}(cfu/viable cfu)[a]	Percent recombinants[b]				
			can1-ura3	*ura3-his1*	*trp5-cyh2*	*cyh2-lys5*	Total scored[c]
0	1.9×10^4	1.8×10^{-4}	1	0	0	0	87
15	6.8×10^5	5.4×10^{-3}	4	22	11	17	76
30	1.3×10^6	1.5×10^{-2}	15	34	23	15	88
45	1.4×10^6	8.0×10^{-2}	14	39	24	27	93
60	6.9×10^5	1.8×10^{-1}	16	33	24	25	91
90	1.8×10^4	1.3×10^{-1}	28	45	36	39	94
Wild type[d]			21	38	48	32	275

LW3802 cells in log phase were transferred to SPM and incubated with shaking. At 5 hr after this transfer, aliquots were removed, irradiated, and returned to liquid culture conditions. After 48 hr of total incubation, serial dilutions were plated on complete and *can*^{r}*cyh*^{r}-selective media. Colonies arising on selective medium were patched onto complete medium and subsequently assayed for auxotrophies and mating type.

[a] Viable cfu determined from cfu growth on solid complete media.

[b] Only maters were scored.

[c] Colonies growing on *can*^{r}*cyh*^{r}-selective media.

[d] Wild-type values are pooled data from the LW3608 0-krad controls presented in Table 5.

spo11-1 homozygote strain LW3606 (data not shown). Assuming the behavior of the rare viable spores from these *spo11* strains to be representative of the entire population, the level of reciprocal recombination that can be induced by X-irradiation differs little from that occurring normally in wild-type meiosis.

DISCUSSION

We have demonstrated here that X-irradiation of wild-type yeast cells early in meiosis prevents entry into premeiotic S phase, whereas irradiation at later stages has relatively little effect on progression through the pathway to sporulation. In contrast to these findings, WEINERT and HARTWELL (1988) have shown that X-irradiation of vegetative cells at any stage of the mitotic cell cycle leads to arrest, the arrest occurring in G2 and being dependent on *RAD9*. Although the G_1 meiotic arrest reported here differs from the vegetative arrest in its stage of response, it shares with it a dependency on the *RAD9* function, as *rad9* cultures continued through the sporulation process after receiving the same X-ray doses that blocked sporulation of *RAD9* strains. Strikingly, sporulating *rad9* diploids were also found to be considerably less sensitive to killing by X-irradiation than vegetative cultures. This latter finding suggested that entry into the meiotic pathway leads to the induction of functions that might treat X-ray-induced lesions as recombination intermediates. Testing the ability of X-irradiation to promote recombination in diploids homozygous for recombination-defective mutations has revealed that such treatment of cells bearing the meiosis-specific mutation *spo11* increased the yield of viable spores, while no such induction was seen in diploids homozygous either for *hop1* or for both *hop1* and *spo11*.

***RAD9*-dependent arrests occur in both G_1 and G_2:** Although transfer of a *cdc13* strain to nonpermissive conditions causes meiotic arrest in G_2, X-irradiation at an early stage of meiosis is shown here to result in G_1 arrest. We note in this regard that, whereas X-irradiation damage can be imposed at any time, *cdc13* damage may only become manifest when the *CDC13* function normally should be performed. Thus, the failure of *cdc13* to cause an arrest prior to premeiotic DNA synthesis may result from an absence of any associated chromosomal damage, such as that proposed by HARTWELL and SMITH (1985), rather than from insensitivity of the cell to chromosomal damage if any such damage had been present at that stage. It has been possible, on the other hand, to impose X-ray-induced damage at other stages of the meiotic pathway. Doing so has revealed that early damage causes G_1 arrest, while later treatments of similar dosage fail to lead to the type of G_2 arrest shown by *cdc13*. This failure of X-irradiation damage to cause meiotic arrest in G_2 suggests that the damage caused by *cdc13* may differ qualitatively, leading to a greater stimulation of the *RAD9*-mediated response mechanism. Specifically, we might imagine that X-ray-induced lesions are structurally more similar to normal intermediates of meiotic recombination and therefore are at least partially amenable to being processed by meiosis-specific functions.

The finding that X-irradiation can cause a G_1 arrest of meiosis is consistent with previous work with other agents that affect DNA metabolism. KUPIEC and SIMCHEN (1985) showed that methyl methanesulfonate, which causes single-strand breaks in DNA and induces recombination in mitotic cells, causes arrest of sporulating cells before premeiotic DNA replica-

tion. Hydroxyurea also causes meiotic arrest at the same stage (SIMCHEN, IDAR and KASSIR 1976; SCHILD and BYERS 1978), but this agent is thought to impinge more directly on DNA replication by inhibiting precursor pools (ELFORD 1968; SLATER 1973).

The finding that the *RAD9*-dependent arrests of meiosis and mitosis in response to DNA damage by X-ray differ in relation to DNA replication, occurring in G_1 and G_2, respectively, is not unprecedented in comparisons of the two pathways. Studies of the *cdc* genes in mitosis and meiosis have revealed many differences in the ordering of essential functions. *cdc7*, for example, was examined together with a group of other mutations having similar vegetative arrest points (*cdc2*, *cdc8* and *cdc21*) by SCHILD and BYERS (1978). Cytologically, all these mutations caused meiotic arrest at a stage characterized by the presence of duplicated-but-unseparated spindle pole bodies and the absence of synaptonemal complex, as was also found for *cdc13* (WEBER and BYERS 1992). Strains bearing *cdc8* and *cdc21* failed to undergo DNA replication before arresting in meiosis (as in mitosis), whereas premeiotic DNA synthesis was largely completed before the points of arrest in both mitosis and meiosis for *cdc2*. Surprisingly, *cdc7*, which causes mitotic arrest at the initiation of DNA replication (see PRINGLE and HARTWELL 1981) caused meiotic arrest at a stage following the completion of DNA replication, but prior to synaptonemal complex assembly. Another precedent for differences in the ordering of *CDC* functions is found in the behavior of *cdc28* strains (SHUSTER and BYERS 1989). Although *cdc28* causes a vegetative arrest in G_1 at START, mutants are blocked in meiosis at pachytene, prior to the SPB separation event that leads to spindle formation in preparation for meiosis I. This mutation also partially blocks spindle formation for meiosis II. Thus, the finding that *RAD9* acts not only in G_1 after X-irradiation but also in the G_2 arrest of *cdc13* mutants simply expands a growing list of examples showing that the order of common functions differs between mitosis and meiosis.

In light of the fact that meiotic cultures can survive vegetatively lethal doses of X-irradiation and generate viable spores if progression through meiosis is not blocked by the *RAD9* function, this early meiotic arrest in response to X-irradiation seems counterproductive for the cells. That is, one might think it disadvantageous for the cell to become arrested immediately before it would otherwise gain the recombinational benefits enjoyed by its *rad9* counterpart. On the other hand, we note that sporulating *RAD9* cultures arrested by X-irradiation do not die. Even when incubated for several days, the cells remaining capable of reverting to vegetative growth (data not shown). Consequently, there may be no selective advantage for the cell to retain the ability to resume meiosis once arrested by radiation damage. In fact, because diploids can tolerate much higher doses of X-irradiation than can haploids (MORTIMER 1952), it may be advantageous for the cell to remain diploid if there is a risk of continued or repeated exposure to ionizing irradiation.

Replacement of meiotic function by X-ray-induced damage: Although the high levels of recombination normally occurring during meiosis make it difficult to detect any recombinogenic effects of X-irradiation during normal meiosis, stimulatory effects on recombination have become evident in strains mutant for a recombinational function. We have shown not only that spore viability is enhanced by X-irradiation during *spo11* meiosis, but also that reciprocal recombination is increased in the production of these spores.

It was of special interest in this regard to compare the behavior of two *spo11* alleles which had been reported to differ in their ability to form synaptonemal complex. KLAPHOLZ, WADDELL and ESPOSITO (1985), using thin-section electron microscopy, reported the appearance of synaptonemal complex in a strain homozygous for the *spo11-1* mutation. In contrast, GIROUX, DRESSER and TIANO (1989) found by examination of spread preparations that strains deleted for *SPO11* formed no detectable synaptonemal complex. In the present work, these two alleles were tested in the same background for their proficiency in the parameters measured here. A strain bearing the *spo11* deletion displayed a lower meiotic background level of $can^r cyh^r$ cfu generation than strains bearing *spo11-1* but was similarly capable of being induced to produce an increased yield of viable ($can^r cyh^r$) progeny upon X-irradiation (Table 4). By this measure of meiotic success, the two *spo11* alleles do not differ.

The enhancement of reciprocal recombination and spore viability by X-irradiation after the beginning of S phase clearly suggests that the nondisjunctional deficiency of *spo11* meiosis is partially ameliorated by the induction of lesions that may substitute in some manner for an absent *SPO11* function. It is of interest to consider the probable number of lesions that may have been induced to exert this stimulatory effect on viability. X-ray-induced lesions appear to consist of single- and double-strand breaks that follow the Poisson distribution (see GAME *et al.* 1989). Computations from values for the size of the yeast genome (LAUER, ROBERTS and KLOTZ 1977; LINK and OLSON 1991) and extrapolation from data on double-strand breaks induced in yeast by ^{60}Co γ-rays (RESNICK and MARTIN 1976), which creates double-strand breaks at a rate similar to that caused by X-irradiation (ROOTS *et al.* 1990), leads to an estimate that about 0.58 double-strand breaks would be induced per krad in a diploid

genome. Single-strand breaks would be induced at a 20-fold higher rate (ROOTS *et al.* 1990), yielding about 11.6 breaks per krad per diploid genome. Therefore, a 15-krad dose of X-irradiation, shown in Table 4 to increase the viability of *spo11* meiotic products roughly fivefold, would represent the creation of a mean of 18 double-strand breaks and 360 single-strand breaks per G_2 diploid genome. Perhaps it is significant that the predicted number of double-strand breaks, which are thought to play an important role in synapsis and recombination [for review see ORR-WEAVER and SZOSTAK (1985)], does not markedly differ from the number of bivalents that must form and undergo disjunction. However, the distribution of induced breaks should follow the Poisson distribution (GAME *et al.* 1989), making it likely that only a fraction of the bivalents would receive double-strand breaks. Single-strand breaks, on the other hand, should be present abundantly on all chromatids, perhaps indicating that these lesions are sufficient for the induction of reciprocal recombination events.

It is interesting to note that although increased viability was induced by X-irradiation in this experiment, the recombination events that arose from this treatment failed to substitute fully for the role of *SPO11*. The pattern of induced recombination differed from that occurring in wild-type meiosis, as may be seen by comparing induced recombination in specific intervals with that occurring in untreated wild-type meiosis (Table 6). Also, the frequency of viable spores remained far below that for the wild type at all doses tested. Note, for example, the different behavior of *spo11* diploid LW3606 when irradiated before (about 5 hr) or after entry into S phase (about 8 hr; Table 4, experiment "d"). Recombination among can^r cyh^r cfu was induced at both times and was stimulated to nearly the same level (Table 5), but only the latter treatment resulted in an increased yield of can^r cyh^r cfu (Table 4). The failure to supplant normal recombination fully is not surprising since the lesions induced at either stage may not fall within sites that might be normally specified by the synaptonemal complex and other meiotic functions. In addition, the timing of their induction may well be critical. In fact, given the partial asynchrony of the cellular population in progression through meiosis, the most effective time for induction would probably not be shared by all cells. Here, as in the studies of the *MER1* and *MER2* functions by ENGEBRECHT, HIRSCH and ROEDER (1990), the occurrence of reciprocal exchange *per se* need not generate the type of crossovers that would most effectively promote homolog disjunction.

GAME *et al.* (1989) have reported that double-strand breaks arise in meiotic cells well before the first recombinant molecules can be detected and PADMORE, CAO and KLECKNER (1992) have confirmed this finding, also showing that double-strand breaks are present before the initiation of chromosomal synapsis. It is of particular interest in this regard to note that the formation of meiosis-specific breaks at a hot spot for reciprocal recombination is dependent on *SPO11* function (CAO, ALANI and KLECKNER 1990). It is consistent both with this result and with the findings of the present report to postulate that the specific defect of *spo11* in synapsis and recombination is rather directly related to the failure of such breaks to arise. Similar to the finding that HO-induced double-strand breaks can stimulate meiotic recombination (KOLODKIN, KLAR and STAHL 1986), provision of artificially created lesions by X-irradiation may partially substitute for the missing breaks, thereby leading to improved disjunction. Finally, we propose that X-irradiation during ongoing meiosis may serve more widely to help us determine which other meiotic mutations may be defective in recombination and/or disjunction because of a primary failure to create break. We must note, however, that the applicability of this assay will be constrained by the fact that many mutations which confer recombinational defects in meiosis also cause radiation sensitivity [*i.e.*, the "*RAD52* group": *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55* and *RAD57* (RESNICK 1987; HAYNES and KUNZ 1981; PETES, MALONE and SYMINGTON 1990; SAEKI, MACHIDA and NAKAI 1980)]. Stimulatory effects might be assayable only for mutations that lack such radiation sensitivity. Nevertheless, the possibility that induced lesions may serve as functional intermediates in meiotic recombination provides a potentially valuable tool for dissection of this complex process.

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