MATING-TYPE REGULATION OF METHYL METHANESULFONATE SENSITIVITY IN *SACCHAROMYCES CEREVISIAE*[1,2]

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ABSTRACT

Heterozygosity at the mating-type locus (*MAT*) in *Saccharomyces cerevisiae* has been shown previously to enhance X-ray survival in diploid cells. We now show that **a**/α diploids are also more resistant to the radiomimetic agent methyl methanesulfonate (MMS) than are diploids that are homozygous at *MAT* (*i.e.*, either **a**/**a** or α/α). Log-phase **a**/α cultures exhibit biphasic MMS survival curves, in which the more resistant fraction consists of budded cells (those cells in the S and G2 phases of the cell cycle). Survival curves for log-phase cultures of **a**/**a** or α/α diploids have little if any biphasic nature, suggesting that the enhanced S- and G2-phase repair capacity of **a**/α cells may be associated with heterozygosity at *MAT*. The survival of cells arrested at the beginning of the S phase with hydroxyurea indicates that *MAT*-dependent MMS repair is limited to S and G2, whereas *MAT*-independent repair can occur in G1.

THE mating-type locus (*MAT*) in the yeast *Saccharomyces cerevisiae* regulates a variety of functions throughout the life cycle of this simple eukaryote (reviewed in CRANDALL, EGEL and MACKAY 1977). The **a** or α mating type of a haploid cell, including its ability to secrete and respond to mating hormones, is specified by which of two determinants, **a** or α, is present and/or expressed at *MAT*. In the diploid state, cells heterozygous for mating type (**a**/α) are unable to mate (GUNGE and NAKATOMI 1972), whereas homozygous *MAT* diploids (*i.e.*, **a**/**a** or α/α) mate with the same high frequency as their respective haploids (ROMAN and SANDS 1953; MORTIMER 1958). Other characteristics that distinguish **a**/α diploids from homozygous *MAT* diploids include the ability of **a**/α cells to undergo meiosis and sporulation (LINDEGREN and LINDEGREN 1943; ROMAN and SANDS 1953; ROTH and LUSNAK 1970), their increased X-ray survival (MORTIMER 1958; LASKOWSKI 1960; T. R. MANNEY, personal communication) and enhanced spontaneous and UV-induced mitotic recombination (FRIIS and ROMAN 1968; HOPPER, KIRSCH and HALL 1975; J. WAGSTAFF and M. ESPOSITO,

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personal communication). Since the processes of X-ray repair, mitotic recombination and meiotic recombination are each influenced by heterozygosity at *MAT* and since all three functions are defective in certain *rad* (radiation-sensitive) mutants (GAME *et al.* 1980; PRAKASH *et al.* 1980), these pathways may have steps in common.

X-ray sensitivity in mutants has been correlated with sensitivity to the monofunctional alkylating agent, methyl methanesulfonate (MMS) in bacteria (HOWARD-FLANDERS and BOYCE 1966; KONDO *et al.* 1970; REITER *et al.* 1967), in yeast (BRENDEL, KHAN and HAYNES 1970; BRENDEL and HAYNES 1973; JACHYMCZYK *et al.* 1977) and in other organisms. Generally, wild-type and UV-sensitive yeast strains exhibited a similar response to MMS, whereas X-ray-sensitive strains showed a much greater sensitivity to MMS. Similarly, many MMS-sensitive yeast mutants were subsequently found to be sensitive to X rays or to both X rays and UV (PRAKASH and PRAKASH 1977a). These results are consistent with the observation that MMS, like X rays, produce single-strand and double-strand breaks in DNA (STRAUSS, COYLE and ROBBINS 1968; JACHYMCZYK *et al.* 1977; CHLEBOWICZ and JACHYMCZYK 1979; RESNICK 1979). Therefore, it is not unreasonable to predict a partially overlapping mechanism of repair (PRAKASH and PRAKASH 1977a).

Since heterozygosity at *MAT* increases X-ray survival and since there is a pronounced correlation between X-ray and MMS repair processes, a similar mating-type effect might be expected for MMS survival. In this paper, we show that *MAT* heterozygosity enhances MMS survival in diploid cells. Moreover, in exponentially growing cultures, the increased survival of **a**/α diploids is largely limited to those cells in the S and G2 phases of the cell cycle, a result that suggests that **a**/α diploids may have a greater capacity for G2-phase recombinational repair than do homozygous *MAT* cells.

MATERIALS AND METHODS

Yeast strains: The heterothallic strains of *Saccharomyces cerevisiae* used in these experiments are listed in Table 1, with their genotypes and sources. All strains were derived originally from X2180, the standard **a**/α diploid of R. K. MORTIMER and the Yeast Genetics Stock Center (Berkeley, CA.). The XN136 diploid series was obtained from the sporulation of a tetraploid (XN136) by T. R. MANNEY (Kansas State University). Diploids homozygous for mating type (XV355-**a**/**a**, XV355-α/α, XG95-**a**/**a** and XG95-α/α) were spontaneous mitotic recombinants derived from the **a**/α diploids XV355 and XG95, respectively. Strains XG95 and XG99 were constructed to be isogenic diploids, except at *MAT*; XG95 (**a**/α) was derived from the cross of XG41–14B by XG83–20A, and independent clones of XG99 (α/α) were isolated from the rare fusions of XG41–14B and XG83—20A. Therefore, XG99–1 and XG99–3 are the products of independent fusions and are not identical. They were subsequently found to be homozygous for the dominant mutation (*SAD1*), which was carried by XG83–20A and permits α/α diploids to sporulate efficiently. *SAD1* is somewhat unstable, however, and appears to have been lost prior to or during formation of the **a**/α diploid XG95. The origin and properties of *SAD1* are discussed more fully in HOPPER and MACKAY (1980).

Media and culture conditions: YEPD (yeast extract-peptone-dextrose) was used in all experiments as indicated and was prepared as described previously (MACKAY and MANNEY 1974). All incubations were at 30°; liquid cultures were aerated by shaking at approximately 200 rpm. Cells

TABLE 1

Yeast strains

Strain	Genotype	Source
XN136-3A	$\frac{\alpha}{\alpha}\ \frac{trp5}{trp5}\ \frac{gal2}{gal2}\ \frac{ade2}{+}$	T. R. MANNEY
XN136-3B	$\frac{\mathbf{a}}{\alpha}\ \frac{ade2}{ade2}\ \frac{trp5}{trp5}\ \frac{ura1}{ura1}\ \frac{gal2}{gal2}$	T. R. MANNEY
XN136-3C	$\frac{\mathbf{a}}{\mathbf{a}}\ \frac{trp5}{trp5}\ \frac{gal2}{gal2}$	T. R. MANNEY
XV334-1A	**a** *his5 his4 leu2 lys1 ura4 gal2*	This lab
XV331-10B	α *lys1 gal2 ade2 leu1 trp5 can1*	This lab
XG41-14B	α *ade2 ura3 his4-12 leu2-27 gal2*	This lab
XG41-14C	**a** *ade2 ura3 his4-12 leu2-27 gal2*	This lab
XG83-20A	α *ura3 his4-290 leu2-1 lys2 trp1 gal2 SAD1*	This lab
XV355 (XV334-1A × XV331-10B)	$\frac{\mathbf{a}}{\alpha}\ \frac{his5}{+}\ \frac{his4}{+}\ \frac{leu2}{+}\ \frac{lys1}{lys1}\ \frac{ura4}{+}\ \frac{gal2}{gal2}\ \frac{+}{ade2}\ \frac{+}{leu1}\ \frac{+}{trp5}\ \frac{+}{can1}$	This lab
XV355-α/α	$\frac{\alpha}{\alpha}$ derived from XV355	This lab
XV355-**a**/**a**	$\frac{\mathbf{a}}{\mathbf{a}}$ derived from XV355	This lab
XG95* (XG41-14C × XG83-20A)	$\frac{\mathbf{a}}{\alpha}\ \frac{ade2}{+}\ \frac{ura3}{ura3}\ \frac{his4-12}{his4-290}\ \frac{leu2-27}{leu2-1}\ \frac{+}{lys2}\ \frac{+}{trp1}\ \frac{gal2}{gal2}$	This lab
XG95-**a**/**a**	$\frac{\mathbf{a}}{\mathbf{a}}$ derived from XG95	This lab
XG95-α/α	$\frac{\alpha}{\alpha}$ derived from XG95	This lab
XG99-1 (XG41-14B × XG83-20A)	$\frac{\alpha}{\alpha}\ \frac{ade2}{+}\ \frac{ura3}{ura3}\ \frac{his4-12}{his4-12}\ \frac{leu2-27}{leu2-27}\ \frac{+}{lys2}\ \frac{+}{trp1}\ \frac{gal2}{gal2}\ \frac{SAD1}{SAD1}$	This lab
XG99-3 (XG41-14B × XG83-20A)	$\frac{\alpha}{\alpha}\ \frac{ade2}{+}\ \frac{ura3}{ura3}\ \frac{his4-12}{his4-290}\ \frac{leu2-27}{leu2-1}\ \frac{+}{lys2}\ \frac{+}{trp1}\ \frac{gal2}{gal2}\ \frac{SAD1}{SAD1}$	This lab

Gene symbols indicate mutations leading to requirements for the following: *ade* (adenine), *his* (histidine), *leu* (leucine), *lys* (lysine), *trp* (tryptophan), *ura* (uracil). Additional symbols include the following: **a** or α (mating type), *can* (resistance to canavanine), *gal* (inability to ferment galactose), *SAD1* (suppressor of a deficiency).

* The *SAD1* mutation in XG83-20A (the α parent of XG95) is somewhat unstable (HOPPER and MACKAY 1980) and appears to have been lost prior to or during formation of XG95.

were considered to be in late stationary phase after 3 days of growth in YEPD and in early stationary phase after overnight growth in YEPD. For experiments with log-phase cultures, cultures were used at densities $< 3 \times 10^7$ cells/ml.

MMS treatment: Redistilled MMS was a gift from L. PRAKASH and F. SHERMAN (University of Rochester). Cell concentrations and percent budded cells were determined by direct counts in a hemacytometer. YEPD-grown cells were harvested by low-speed centrifugation, washed in sterile water and then resuspended in 0.067 M potassium phosphate buffer (pH 6.8) at 1×10^7 cells/ml. After the subsequent addition of 0.3% MMS (v/v), the suspensions were incubated at 30° and aliquots were removed at 10 min intervals into chilled 10% sodium thioglycollate. Cells were immediately pelleted at 4°, resuspended and diluted appropriately in sterile water and plated on YEPD agar to yield between 50 and 300 colonies/plate. All samples were plated in triplicate. Haploid cultures were sonicated to break up any clumps prior to plating. Plates were incubated for 5 days, after which no additional colonies formed. Standard deviations for survival points were calculated from the coefficient of variation and are represented in the figures as error bars, unless smaller than the plotted point.

Pretreatment with hydroxyurea (HU): From log-phase YEPD cultures, 1×10^7 cells were pelleted by low-speed centrifugation and resuspended in 10 ml prewarmed YEPD liquid medium containing 0.075 M HU (A grade, Calbiochem). Cultures were incubated with shaking for 3.5 hr, at which time 2×10^7 cells were removed, washed with sterile water and resuspended in 2.0 ml potassium phosphate buffer for MMS treatment as above. Control cells were preincubated in YEPD without HU.

X irradiation: Cells were counted, diluted appropriately in sterile water and spread on YEPD agar plates. All samples were plated in triplicate. Within 15 min of plating, plates were exposed to X rays from a Machlett OEG-60 tube equipped with a beryllium window and operated at 50 kVp and 25 mA. The dose rate was estimated to be approximately 180 rad/sec. Plates were incubated for 5 days before counting.

X-irradiation experiments were also conducted using a Picker X-ray machine with a Machlett OEG-60 tube operated at 50 kVp and 40 mA at a calibrated dose rate of 56 krad/min (located at Brooklyn College, City University of New York, and kindly made available by C. BEAM and D. HURST).

UV irradiation: Exponentially growing cells in YEPD were counted, diluted appropriately and plated on YEPD. They were irradiated immediately with UV light from germicidal lamps (General Electric) at a calibrated dose rate of 2.5 Joules/m^2/sec. The plates were incubated in the dark for 5 days before counting.

HU inhibition of DNA synthesis: DNA synthesis was assayed by a modification of the procedure of SIMCHEN, PINON and SALTS (1972). Cells were grown to log phase in YEPD + uracil (40 mg/ml) and diluted into fresh YEPD + 4 μCi/ml uracil (40 mg/ml) at a concentration of 2.5×10^5 cells/ml. After 3 hr incubation, the culture (approximately 1×10^6 cells/ml) was split into 5 aliquots, to which increasing amounts of HU were added, and the incubations were continued for 4 more hr. Samples (0.5 ml) were removed in triplicate at hourly intervals and the cells were pelleted at 4° and resuspended in 1 ml 0.4 N NaOH. After standing at room temperature for 18 to 24 hr, the samples were adjusted with 1 ml 20% tricholoroacetic acid (TCA), kept on ice for 15 min and collected on filters. The filters were washed extensively with 5% TCA and 95% ethanol, before drying and counting in a liquid scintillation counter.

Genetic methods: Sporulation, ascus dissection and tetrad analysis were performed according to standard procedures (MORTIMER and HAWTHORNE 1969).

RESULTS

Using stationary phase cultures, MORTIMER (1958) and LASKOWSKI (1960) demonstrated that **a**/α cells are more resistant to X rays than **a**/**a** or α/α diploids. Using XV355 and the **a**/**a** and α/α diploids derived from it, we have shown that **a**/α cultures exhibit enhanced resistance to MMS in late stationary phase (Fig-

ure 1A), early stationary phase (data not shown) and log phase (Figure 1B). Therefore, heterozygosity at *MAT* affects MMS survival, independent of the growth phase of the culture. With log-phase cultures, the **a**/α strain exhibited biphasic survival curves, with initial shoulders and major second shoulders; whereas, survival curves for the related **a**/**a** and α/α strains showed initial shoulders, but little biphasic nature (Figure 1B). Similar results were obtained for exponentially growing cultures of two other series of diploids: XG95 (**a**/α), XG95-**a**/**a**, and XG95-α/α (Figure 1C) and XN136–3A (α/α), –3B (**a**/α) and –3C (**a**/**a**) (data not shown), as well as many other diploid strains. Both haploid strains shown in Figure 1B and the remaining haploids listed in Table 1 were more sensitive to MMS than were diploid cells.

As shown previously by T. R. MANNEY (personal communication), X-ray survival of log-phase diploid cultures is similarly influenced by heterozygosity at *MAT* (Figure 2A). Survival curves for homozygous *MAT* diploids have only slight (if any) second shoulders (T. R. MANNEY, personal communication) and are clearly biphasic for exponentially growing **a**/α cultures (DE LANGGUTH and BEAM 1973a). These results suggest that the same or similar survival/repair pathways may handle MMS and X-ray damage in **a**/α diploids. As shown in Figures 1B and 2A, however, haploid survival curves for MMS and X-ray damage are quite distinct and indicate some significant differences in the overall survival/repair mechanism(s) in these cells. In contrast to the MMS and X-ray results, UV survival of diploid cells is not influenced by *MAT* in log-phase diploids (Figures 2B and 2C) or in stationary phase cultures (R. K. MORTIMER, personal communication), although a slight *MAT* effect on UV survival would probably not be detected in these wild-type, repair-proficient strains.

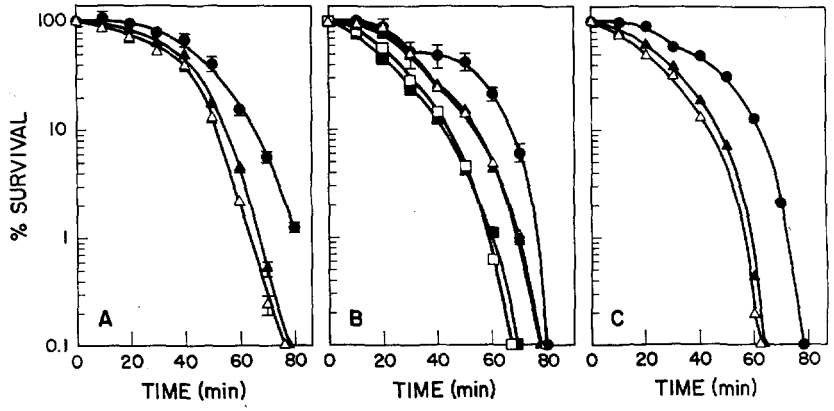

FIGURE 1.—Effect of heterozygosity of *MAT* on MMS survival. (A) Late stationary-phase cultures of the XV355 series: XV355 (**a**/α), 16% budded cells, ●—●; XV355-**a**/**a**, 8% budded, ▲—▲; XV355-α/α, 12% budded, △—△. (B) Log phase cultures of the XV355 diploid series and the haploid parents: XV355 (**a**/α), 71% budded, ●—●; XV355-**a**/**a**, 70% budded, ▲—▲; XV355-α/α, 67% budded, △—△; XV344–1A (**a**), 56% budded, ■—■; XV331–10B (α), 59% budded, □—□. (C) Log phase cultures of the XG95 diploid series: XG95 (**a**/α), 64% budded, ●—●; XG95-**a**/**a**, 55% budded, ▲—▲; XG95-α/α, 52% budded, △—△. Standard deviations are not shown in Figure 1C.

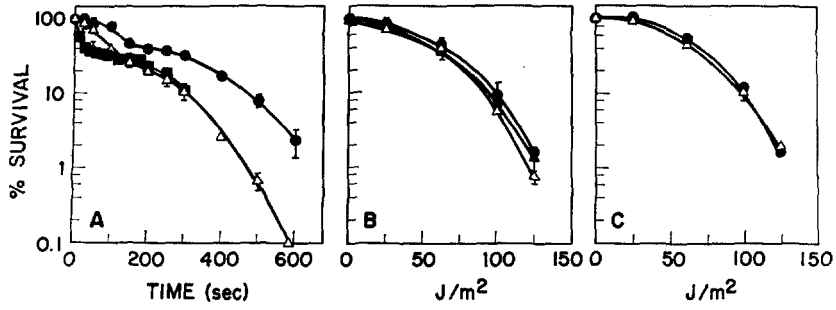

FIGURE 2.—Mating-type effect on radiation survival in log phase cells. (A) X-ray survival for XV355 (**a**/α), 51% budded cells, ●—●; XV355-α/α, 54% budded, △—△; XV334-1A (**a**), 50% budded, ■—■. The dose rate was approximately 180 rads/sec. (B) UV survival for XV355 (**a**/α), ●—●; XV355-**a**/**a**, ▲—▲; XV355-α/α, △—△. (C) UV survival for XN136-3B (**a**/α), ●—●; XN136-3A (α/α), △—△.

The log-phase **a**/α populations in Figures 1 and 2A appear to be heterogeneous, consisting of a sensitive fraction (the initial shoulder, approximately 40%) and a resistant one (the second shoulder, approximately 60%). As noted in the figure legends, 50–70% of the cells in log-phase YEPD cultures have detectable buds that are smaller than mature cells. HARTWELL (1974) has shown that these budded cells are in the S and G2 phases of the cell cycle. Therefore, the MMS-resistant **a**/α fraction might consist of S- and G2-phase cells, as found for X-ray resistance of **a**/α diploids (DE LANGGUTH and BEAM 1973a, b). This interpretation was tested by determining MMS survival in cultures that were arrested near the beginning of the S phase by preincubation with hydroxyurea (HU), a reversible inhibitor of DNA synthesis, but not the budding cycle (SLATER 1973; HARTWELL 1976). As illustrated in Figure 3A, DNA synthesis in growing cells was inhibited approximately 90% for at least four hours by the addition of 0.075 M HU; SLATER (1973) reported that this concentration had little effect on RNA and protein synthesis and none on cell viability (also this study, data not shown). After 2.5 hours, cell division in the HU-treated culture stopped, but resumed synchronously approximately 1.5 to two hours after transfer to fresh YEPD (Figure 3B), indicating that the HU-treated cells are arrested near the beginning of the S phase (HARTWELL 1976). A round of cell division did not occur, however, when HU-treated cells were transferred to buffer; most of the cells apparently remained arrested at the S phase. When control cells (not preincubated with HU) were shifted from YEPD to buffer, cell division ceased immediately, yielding an asynchronously arrested population. HU-treated and control cultures of α/α cells behaved similarly. The effect of HU preincubation on MMS survival is shown in Figure 4. Pretreatment of **a**/α cultures (Figures 4A and 4C) specifically reduced the MMS-resistant fraction, yielding nearly monophasic survival curves similar to those of α/α diploids instead of the biphasic curves of the untreated cells. In contrast, HU-treated α/α cultures (Figures 4B and 4D) exhibited only a slight reduction in the overall monophasic MMS survival curves. These results suggest that most of the *MAT*-dependent MMS repair

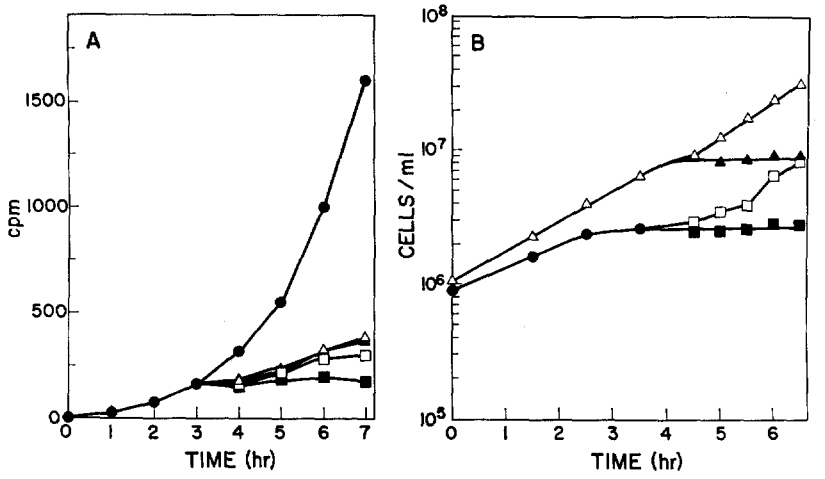

FIGURE 3.—HU inhibition of DNA synthesis and cell division. (A) DNA synthesis: HU was added at 3 hours to a growing culture of XV355 at 0 (●—●), 0.05 M (△—△), 0.075 M (▲—▲), 0.1 M (□—□) and 0.2 M (■—■) concentrations. DNA synthesis was measured as described in MATERIALS AND METHODS. (B) Cell division: at 0 hours, YEPD-grown log-phase cells were transferred into prewarmed fresh YEPD (△—△) or YEPD + 0.075 M HU (●—●). Cultures were incubated with shaking at 30° and cell number was determined by direct counting. At 4 hours, the cultures were split, pelleted and resuspended in fresh YEPD (△—△, □—□) or 0.067 M KPO_4 (pH 6.8) (▲—▲, ■—■). Incubation at 30° and cell number determinations were continued.

in exponentially growing **a**/α cells is limited to the 50 to 70% of the cells in the S and G2 phases of the cell cycle. An alternative interpretation that HU, which directly inhibits the synthesis of DNA precursors, might also affect repair of MMS damage appears unlikely, since the cells are washed and resuspended in HU-free buffer prior to incubation with MMS and subsequent plating on HU-free medium. HU-treated cells that are washed and resuspended in HU-free growth medium rapidly resume DNA synthesis (SLATER 1973; R. POLSTER and V. MACKAY, unpublished) and undergo a round of cell division after approximately 1.5 to two hours, one generation time (Figure 3B), as expected if the effects of HU are rapidly and completely reversible (SLATER 1973).

In contrast to all other diploid strains examined, two homozygous *MAT* diploids (XG99–1 and XG99–3) initially exhibited biphasic MMS (Figure 5) and X-ray survival curves (data not shown), identical in shape and level of resistance to **a**/α curves. The **a**/α-like survival was thought to be attributable to the *SAD1* lesion that enables these α/α diploids to sporulate as efficiently as **a**/α diploids, unlike normal **a**/**a** and α/α strains, which do not sporulate (HOPPER and MACKAY 1980). However, as XG99–1 and XG99–3 were repeatedly cloned and subcultured over 2 to 3 years, their MMS and X-ray survival capacity gradually diminished. When ten different stocks of XG99–1 and XG99–3 were recently tested for X-ray survival, one (an XG99–3 stock) was as sensitive as XG95–α/α and the others were somewhat more X-ray resistant than XG95–α/α,

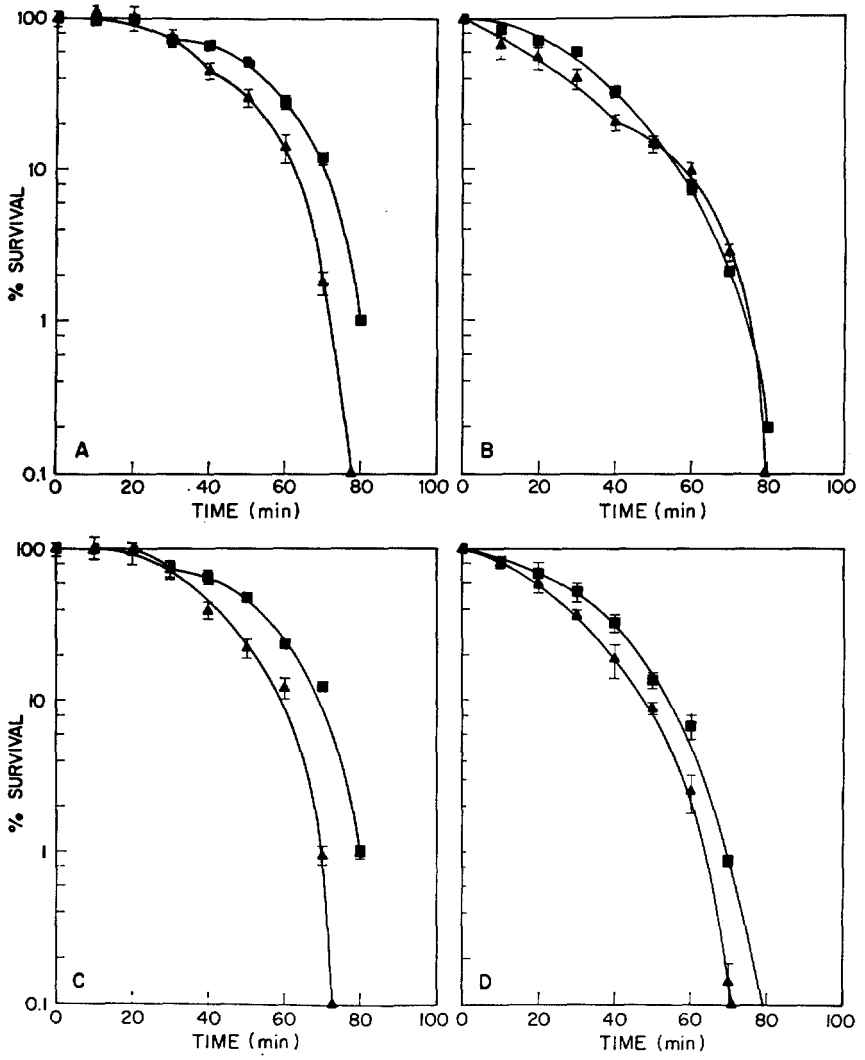

FIGURE 4.—MMS survival curves for diploid cultures preincubated with HU. Log-phase YEPD-grown cells were subcultured into YEPD (■—■) or YEPD + 0.075 M HU (▲—▲) for 3.5 hr preincubation. As described in METHODS, cells were washed free of medium and HU before transfer to buffer + MMS. See METHODS for additional experimental details. (A) XV355 (**a**/α). (B) XV355-α/α. (C) XG95 (**a**/α). (D) XG95-α/α.

but significantly less than XG95, the related **a**/α strain. Even the most X-ray-sensitive stock of XG99–3, however, still underwent meiosis and sporulated as efficiently as XG95 (approximately 40 to 50% asci, predominantly four-spored). In asci derived from this stock, meiotic recombination appeared to be normal since recombination frequencies and map distances were as expected for all markers in the diploid, including the *his4* and *leu2* heteroalleles that, like *MAT*, are on chromosome *III* (Table 2). The properties of these strains indicate that

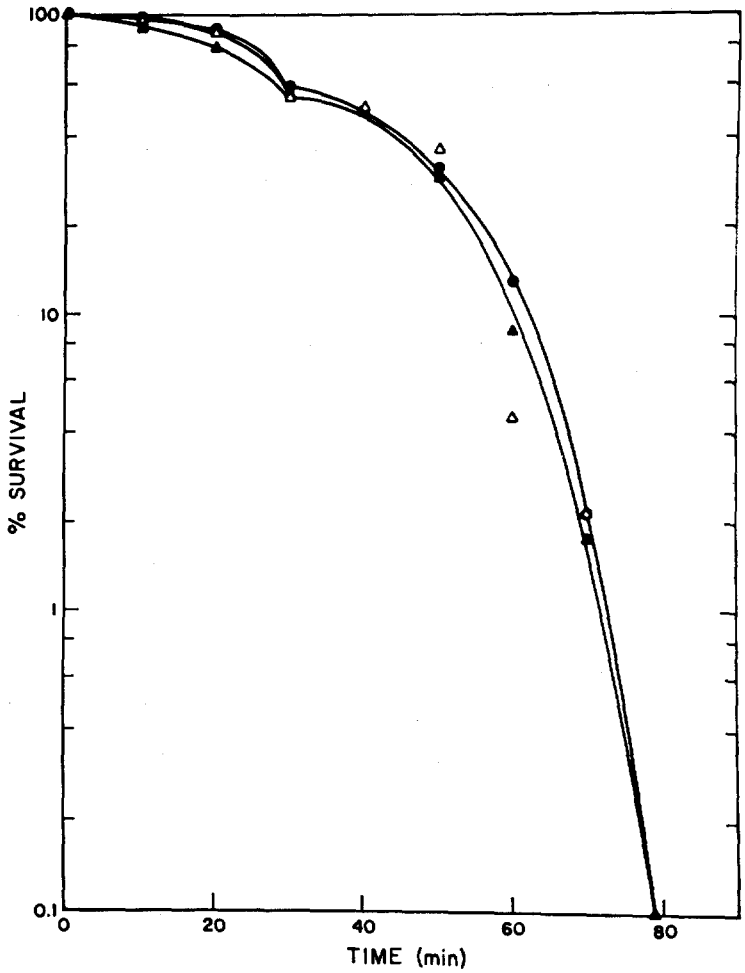

FIGURE 5.—MMS survival curves for log phase cultures of the XG99 strains and XG95. XG99–1, 58% budded cells, △—△; XG99–3, 62% budded cells, ▲—▲; XG95 (a/α), 64% budded cells, ●—●. The survival curve for XG95 is from Figure 3. Standard deviations are not shown. The 60-min survival point for XG99–1 in this experiment is lower than in other MMS survival curves for this strain.

the gene products involved in meiotic recombination may be necessary, but are not sufficient, for the enhanced MMS and X-ray survival of a/α diploids and that some additional gene product needed for DNA repair may require heterozygosity at *MAT* for its synthesis, activity, etc.

DISCUSSION

The mating-type locus in *S. cerevisiae* influences several cellular functions in diploids that involve DNA metabolism: meiosis and the high frequency of meiotic recombination characteristic of this yeast, both spontaneous and induced

TABLE 2

Marker segregations in asci derived from a stock of XG99–3 that exhibits α/α levels of X-ray survival

Ascus types $\left(\frac{\text{PD:NPD}}{\text{T}}\right)^*$

	his4	*leu2*	*lys2*	*ade2*	*trp1*
his4	—				
leu2	17:0 / 4	—			
lys2	3:3 / 17	2:4 / 15	—		
ade2	2:4 / 17	1:3 / 17	3:2 / 18	—	
trp1	8:8 / 5	11:8 / 2	0:7 / 16	4:3 / 16	—

Gene pair	Gene-gene or gene-centromere linkage†
his4–leu2	9.5 cM (13.4 cM)
his4–centromere	12 cM
leu2–centromere	4.8 cM (6.4 cM)

* PD = parental ditype; NPD = nonparental ditype; T = tetratype.

† The map distance between *his4* and *leu2* was calculated by the PERKINS (1949) equation. Centromere linkage was determined, using *trp1* as a closely-linked centromere marker. Map distances shown in parentheses are those calculated from large pooled samples, summarized by R. K. MORTIMER and D. SCHILD (manuscript in preparation). Other markers in the cross are not linked to centromeres or other markers in the diploid and show random assortment as expected if recombination occurs during meiosis as frequently as found in **a**/α diploids.

mitotic gene conversion and reciprocal recombination, and X-ray survival. In this study we have demonstrated that MMS survival is also affected by heterozygosity at *MAT*; *i.e.*, **a**/α diploids are more resistant to MMS than homozygous *MAT* diploids, independent of the growth phase of the culture. Furthermore, X-ray and MMS survival curves are biphasic for log-phase cultures of **a**/α diploids and predominantly monophasic for **a**/**a** or α/α cultures. These results suggest that double-strand breaks produced by either X irradiation or MMS treatment are repaired at least in part through a similar recombinational repair pathway (RESNICK 1979). In contrast, most UV damage (*e.g.*, pyrimidine dimers) is repaired by excision repair (UNRAU, WHEATCROFT and COX 1971; RESNICK and SETLOW 1972) and the *RAD6* pathway (COX and PARRY 1968; GAME and MORTIMER 1974), which appear to be independent of *MAT* control (Figure 2B and C).

We are assuming that the mating-type effect represents repair of MMS damage rather than metabolic protection against it, for example, a decreased permeability to MMS in **a**/α diploids. Such a protective mechanism seems unlikely since **a**/α cells are also more resistant to X-rays. Moreover, DE LANGGUTH and

BEAM (1973b) have shown that the increased X-ray resistance of budded (*i.e.*, S- and G2-phase) **a**/α cells *vs.* unbudded **a**/α cells can be reduced by post-irradiation liquid holding in the presence of caffeine, which interferes with DNA repair. Therefore, we conclude that the MMS resistance of budded **a**/α cells also results from an increased capacity for DNA repair.

MAT-dependent repair of MMS damage, as seen in log-phase survival curves of **a**/α diploids and of the XG99 mutants, appears to be correlated with the budded cell population of the culture. HU treatment that arrests cells at the beginning of S specifically reduces the resistant second shoulder in the survival curves, similar to those obtained for **a**/**a** and α/α diploids. By this analysis, *MAT*-dependent MMS repair seems to be limited to S and G2. Since arresting α/α diploids in early S with HU had little effect on MMS survival (Figure 4), *MAT*-independent MMS repair in diploids is not confined to S and G2. Recently, CHLEBOWICZ and JACHYMCZYK (1979) have shown that in haploids repair of MMS-induced double-strand breaks is similarly associated with the S and G2 phases of the cell cycle, presumably because a duplicated genome is required for the recombinational repair of these lesions.

The only strains examined that initially appeared to be inconsistent with our interpretations about the *MAT* effect on MMS and X-ray survival were XG99–1 and XG99–3. However, the enhanced survival seen in these strains has been gradually lost. Other work has demonstrated that the *SAD*1 lesion in XG99–1 and XG99–3 is unstable and is frequently lost before or during meiosis (HOPPER and MACKAY 1980; KASSIR and HERSKOWITZ 1980; these studies have used the sporulation ability conferred by *SAD*1 as an assay for its presence in a strain. The lack of continual selection for the enhanced survival capacity of the initial isolates of XG99–1 and XG99–3 may have resulted in its loss, whether this property was a function of *SAD*1 or some other unstable element.

The effects of *MAT* heterozygosity on DNA repair in diploids could result from at least two conditions. First, the DNA replication apparatus may be slightly different in **a**/α cells, allowing a coordination of replication and repair that would result in S-phase resistance. Second, the G2 phase in **a**/α cells could be similar to a meiotic state (except for the absence of pairing of homologous chromosomes), so that the enzymes necessary for successful meiosis and meiotic recombination might also be responsible for G2 recombination repair, involving sister-chromatid exchange. The meiotic recombination proficiency of the XG99–3 isolate that exhibited α/α-like X-ray repair suggests, however, that at least one other function regulated by heterozygosity at *MAT* is required for **a**/α levels of X-ray and MMS survival. Furthermore, such interpretations seem too simple to explain the complex meiotic and mitotic properties of the various *rad* and *mms* (*m*ethyl *m*ethanesulfonate *s*ensitive) mutants (GAME and MORTIMER 1974; BORAM and ROMAN 1976; PRAKASH and PRAKASH 1977a, b; GAME *et al.* 1980; PRAKASH *et al.* 1980).

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