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Genetic Effects of Methyl Benzimidazole-2-yl-Carbamate on *Saccharomyces cerevisiae*

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The genetic effects of the mitotic inhibitor methyl benzimidazole-2-yl-carbamate (MBC) have been studied in *Saccharomyces cerevisiae*. MBC had little or no effect on the frequency of mutation. In some experiments MBC caused an increase in the frequency of mitotic recombination; however, this effect was small and not reproducible. The primary genetic effect of MBC was to induce mitotic chromosome loss at a high frequency. Chromosome loss occurred at equal frequencies for all chromosomes tested (13 of 16). Cells which had lost multiple chromosomes were found more frequently than predicted if individual chromosome loss events were independent. The probability of loss for a particular chromosome increased with length of time cells were incubated with MBC. MBC treatment also increased the frequency at which polyploid cells were found. These results suggested that MBC acted to disrupt the structure or function of the mitotic spindle and cause chromosome nondisjunction.

Methyl benzimidazole-2-yl carbamate (MBC) is the active component of the widely used fungicide Benomyl. Interest in MBC, and in the benzimidazole fungicides in general, has come from the specificity of their action on cells. In both fungal and mammalian cells MBC has been found to cause metaphase arrest (9, 26, 55). This block in mitosis observed cytologically has been found functionally to be a stage-specific block in early nuclear division (42; J. Wood and L. Hartwell, J. Cell Biol., in press). Several other effects of MBC, probably related to the mitotic block, have been reported: disruption of apical organization in hyphal tips of *Fusarium* (26), inhibition of nuclear movement in *Aspergillus* (41), and induction of multimicronucleated cells in mammalian cell lines (12). MBC did not inhibit growth (dry weight) or glucose and acetate oxidation in *Neurospora* or *Ustilago* (5) and had no direct effect on RNA or DNA metabolism (5, 32).

The effects observed with MBC are all apparently due to the disruption of microtubules. Davidse and Flach found that MBC bound to a fungal protein closely resembling mammalian tubulin (10). Conclusive evidence that this protein was tubulin has been presented by Sheir-Neiss et al. (50); they demonstrated that benomyl-resistant strains of *Aspergillus* had β-tubulins with altered electrophoretic charge or peptide fingerprints. Resistant or supersensitive mutants also had altered binding coefficients for benomyl to tubulin. That binding was relatively weak, even with tubulin from wild-type strains, may explain why several groups found that MBC did not inhibit brain tubulin polymerization or fungal and brain tubulin copolymerization in vitro (9, 23, 27; K. Shriver, Ph.D. thesis, University of Washington, Seattle, 1978), unlike most microtubule inhibitors. This result may be an artifact, since a number of other related benzimidazole derivatives have been shown to inhibit microtubule polymerization in vitro (17, 24, 33); it is likely that MBC acts in a similar fashion in vivo. Cytological evidence suggests that this is the case; MBC-arrested cells had few or no spindle microtubles (44; B. Byers, personal communication).

The primary genetic effect of MBC has been claimed to be chromosome nondisjunction, seen as an increased frequency of mitotic segregation for heterozygous markers (1, 21, 38). However, a number of other genetic effects have also been observed. Several investigators found that it could act as a low-level mutagen in bacteria (48), *Aspergillus* (30), or mouse embryos (14). Delatour and Richard found that it was teratogenic in rats (13), whereas Tates found that it caused meiotic nondisjunction in spermatids (56). The purpose of the experiments in this study was to investigate the genetic effects of MBC quantitatively, utilizing the well-developed genetic system of *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Strains. The strains used, their genotypes, and their sources are listed in Table 1.

Media. Minimal liquid medium contained, per liter,

TABLE 1. List of strains

Strain	Source	Genotype
A364A	L. Hartwell	*MATa ade1 ade2 ura1 lys2 gal1 his7 tyr1*
500-321A	S. Fogel	chrIII: $\frac{his4\text{-}11\ \ leu2\text{-}1}{his4\text{-}290\ \ leu2\text{-}27} * \frac{cry1\ \ MAT\alpha\ \ +\ \ mal2}{+\ \ MATa\ \ thr4\ \ mal2}$ *met2 trp1 ade2 cupS*
885-1a	This study	chrIII: $\frac{his4\ \ +}{+\ \ leu2} * \frac{cry1\ MAT\alpha}{+\ \ MATa}\ ade2$
6800	This study	chrV: $\frac{can1}{+}\ \ \frac{his1}{+}\ \ \frac{iso1}{+}$ chrVII: $\frac{ade6}{+} * \frac{leu1\ \ trp5\ \ cyh2\ \ aro2\ \ lys5}{+\ \ +\ \ +\ \ +\ \ +}$ *MATa/MATα his1/+ ade1/+ ade2/ade2 ade8/+ leu2/+ thr4/+ mal2/+ ura1/+ lys2/+ gal1/+ his7/+ tyr1/+*
6808-2	This study (NH429-6a × X2180-1b)	*ade1/ade1 his7/+ gal1/+ leu2/+ MATa/MATa trp1/+ ura3/+ his2/+ ade6/+ leu1/+ arg4/+ his6/+ cdc6/+ ilv3/+ met14/+ lys9/+ met2/+ ade2/+ arg1/+ aro7/+*
6822	This study	chrV: $\frac{can1\ \ hom3\ iso1\ MATa/MAT\alpha\ leu1/+\ ura1/+}{+\ \ +\ \ +}$
6830-1	This study (NH439-5c-1 × 2136-4-1)	*ade1/+ his7/+ gal1/+ leu2/+ his4/+ MATα/MATα cry1/cry1 trp1/+ ura3/+ his2/+ ade6/+ leu1/+ cyh2/+ arg4/+ his6/+ cdc6/+ ilv3/+ met14/+ asp5/+ lys7/+ lys9/+ met2/+ ade2/ade2 arg1/+ aro7/+ nib1/+ lys2/+*
NH429-6a	G. Kawasaki	chrI: *ade1*; II: *his7 gal1*; III: *leu2 MATa*; IV: *trp1*; V: *ura3*; VI: *his2* VII: *ade6 leu1*; VIII: *arg4*; IX: *his6*; X: *ilv3 cdc6*; XI: *met14*; XIV: *lys9 met2* XV: *ade2 arg1*; XVI: *aro7*
NH439-5c-1	*cyh2* isolate from NH439-5c from G. Kawasaki	chrI: *ade1*; II: *his7 gal1*; III: *leu2 MATa*; IV: *trp1*; V: *ura3*; VI: *his2*; VII: *ade6 leu1 cyh2*; VIII: *arg4*; IX: *his6*; X: *ilv3 cdc6*; XI: *met14*; XIV: *asp5*; XIII: *lys7*; XIV: *lys9 met2*; XV: *ade2 arg1*; XVI: *aro7*; *mal suc*
X2180-1b	YGSC[a]	Wild type, *MATα*
2136-4-1	C. Holm	*his4 ade2 lys2 cry1 MATα nib1*
XG-99-1-2a	A. Hopper	*MATα leu2 his4 trp1 ura3 gal2 SAD1*
XG-99-1-2b	A. Hopper	*MATα leu2 his4 lys2 ura3 gal2 SAD1*

[a] YSGC, Yeast Genetic Stock Center, Department of Biophysics and Medical Physics, University of California, Berkeley.

1.6 g of yeast nitrogen base without amino acids or ammonium sulfate (Difco Laboratories), 10 g of succininc acid, 6 g of sodium hydroxide, 1 g of ammonium sulfate, and 20 g of dextrose. Amino acid, purine, or pyrimidine supplements were added at 4 μg/ml (except for adenine, which was added to a concentration of 8 μg/ml) as necessary for growth of particular strains. YEPD plates consisted of 1% yeast extract (Difco), 2% peptone (Difco), 40 μg of adenine per ml, 40 μg of uracil per ml, 2% dextrose, and 2% agar. Synthetic complete plates were similar to minimal liquid medium except that adenine, arginine, asparagine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyro-

sine, uracil, and valine were added at 4 μg/ml each and the medium was solidified with 2% agar. Auxotrophic mutations were assayed on complete plates made without one or more supplements (complete − adenine, complete − arginine, etc.). In the drug-containing plates the concentrations were as follows: complete − arginine + canavanine = 60 μg of L-canavanine per ml; YEPD + cryptopleurine = 1 μg of cryptopleurine per ml; YEPD + cycloheximide = 10 μg of cycloheximide per ml. KAC plates for sporulation consisted of 1% potassium acetate, 0.25% yeast extract, 40 μg of adenine per ml, 40 μg of uracil per ml, and 2% agar.

Genetic techniques. Genetic analysis used standard techniques as described by Mortimer and Hawthorne (39).

MBC treatment procedure. All experiments were done with technical-grade MBC (98% pure) from Du Pont Co. The dry MBC powder was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg of MBC per ml to make a stock solution. When the stock solution was first made, and before each use, it was heated to 100°C to ensure that the MBC was fully dissolved. Except for experiments in which MBC concentration dependence was being tested, all experiments were performed at a final concentration of 20 or 100 μg of MBC per ml (0.1 and 0.5 mM, respectively). These concentrations were above the solubility limit of MBC in water; nevertheless, I found that they were necessary to obtain a complete cell cycle block of long duration (data not shown). At 100 μg of MBC per ml the concentration of DMSO in the medium equaled 0.5%. This concentration of DMSO alone produced only a slight inhibition of growth rate (<10% slower than a control culture without DMSO; data not shown).

Unless otherwise noted, experiments were done with diploid strains because they gave a higher percentage of cells displaying stage-specific arrest in MBC (>95%) than did haploid strains (70 to 90%). Cells were grown overnight in liquid minimal medium with only required supplements to mid-log phase. At the start of an experiment the culture was diluted to between 3×10^6 and 6×10^6 cells per ml, and supplements were added for all markers heterozygous in the strain so that there would not be any selection against cells which became auxotrophic for an originally heterozygous marker during the course of the MBC treatment. MBC treatments were done in growth medium for 12 to 48 h at 23°C; under these conditions there was little or no cell division, although individual cells continued to grow in size.

MBC was removed from a culture by filtering the cells (0.45-μm pore size, type HA; Millipore Corp.) and washing them with at least 10 volumes of isotonic saline (minimal media without glucose, ammonia, or amino acid supplements). This removed the visible MBC precipitate and allowed the washed cells to begin dividing immediately (data not shown). Washed cells were suspended in liquid medium and incubated for at least 4 h at 30°C to allow time for expression of new mutations or newly uncovered markers before plating on selective medium (subsequent experiments indicated that this step was not necessary [see Results]).

Haploid and diploid cells were distinguished by the ability of diploid cells to sporulate. Cells were plated on YEPD plates and allowed to grow into colonies. After being replica-plated to KAC plates and incubated at 30°C to induce sporulation, the colonies were replica-plated to fresh YEPD plates and treated with ether (by exposure of the plates to a saturated atmosphere of diethyl ether at 30.0 ± 0.5°C for 0.5 h) to kill haploid and diploid vegetative cells but not ascospores (11). Colonies that survived the ether treatment were able to grow during subsequent incubation at 30°C. Scrapings from these colonies from the KAC master plates were suspended in water and microscopically scanned for asci as confirmation that the colonies had sporulated.

RESULTS

Cells heterozygous for recessive auxotrophic mutations were frequently induced to express these auxotrophies after treatment with MBC. The following experiments were designed to determine whether MBC caused these genetic changes by inducing mutation, recombination, or chromosome loss.

MBC was not mutagenic. To test the mutagenic potential of MBC, a canavanine-sensitive haploid strain, A364A, was tested for the frequency at which canavanine-resistant colonies arose after MBC treatment. Mutations to canavanine resistance are recessive and occur as null alleles at a single locus, *can1*, the arginine permease gene (18, 57); thus, they provided a simple, yet sensitive, assay for mutagenicity. The frequency of canavanine resistance after MBC treatment was only slightly above that of the untreated control culture and the same as that of the DMSO-treated control culture (Table 2). These data demonstrated that MBC was not, or only very weakly, mutagenic in *S. cerevisiae*.

MBC did not induce intragenic recombination. The ability of MBC to cause mitotic recombination was assayed with strain 500-321a, a haploid strain disomic for chromosome III and carrying heteroalleles of both *his4* and *leu2* on the right arm of chromosome III (Table 1). The frequency at which mitotic recombination occurred between these heteroalleles to give histidine or leucine prototrophs was tested before and after MBC treatment (Table 3). No increase in the frequency of prototrophs was observed after MBC treatment. The induced frequencies observed after X-ray treatment were consistent with previous reports (15). These experiments indicated that MBC did not cause intragenic recombination in *S. cerevisiae*.

MBC caused chromosome loss. The ability of MBC to induce chromosome loss was tested in strains heterozygous for a recessive drug marker and heterozygous for a recessive auxotrophic marker on the opposite arm of the same chromosome, with both of the recessive markers linked in coupling (Fig. 1). The frequency of drug-resistant cells was assayed before and after MBC treatment, and then 50 to 100 independent,

TABLE 2. Mutation frequency[a]

Treatment	Initial cells/ final cells	Frequency of Can^r
Untreated	>5.0[b]	$3.65 \pm 2.9 \times 10^{-6}$
EMS	0.21	$1.69 \pm 0.44 \times 10^{-3}$
DMSO	>5.0[b]	1.91×10^{-5}
MBC (20 μg/ml)	0.58	$4.75 \pm 3.05 \times 10^{-5}$
MBC (100 μg/ml)	0.13	$7.58 \pm 2.77 \times 10^{-6}$

[a] Frequency at which canavanine-resistant clones were found after different treatments of strain A364A, a canavanine-sensitive haploid strain. All values are the average of three experiments, except for the DMSO control, which was done only once. For the ethyl methane sulfonate (EMS) treatment, cells were suspended in 0.05 M KPO_4 buffer (pH 7) plus 5×10^{-2} M EMS and incubated at 30°C for 1 h. MBC and DMSO treatment were all for 24 h; the DMSO concentration used was 0.5% (equivalent to the concentration of DMSO in a culture with 100 μg of MBC per ml).

[b] These values were due to normal cell division.

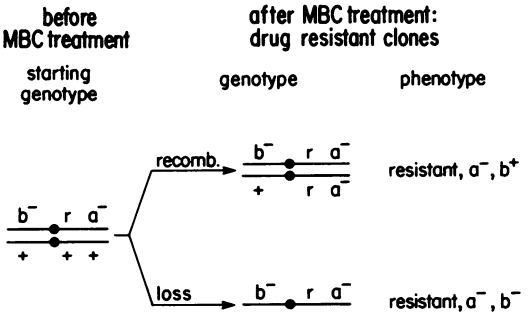

FIG. 1. Linkage relationships for using a recessive drug-resistant mutation and auxotrophic mutations to distinguish clones which become drug resistant either due to mitotic recombination or by chromosome loss.

drug-resistant clones were tested for the appearance of the auxotrophic marker located on the opposite arm of the chromosome from the drug resistance allele. This design distinguished between mitotic recombination (one chromosome arm altered) and presumptive chromosome loss (both chromosome arms altered) events.

The results of treating a *MATa/MATα cry1/CRY1* chromosome III disomic haploid strain, 885-1a, with MBC are shown in Fig. 2 and Table 4. Before MBC treatment, the frequency of cryptopleurine-resistant (Cry^r) clones was approximately 1.2×10^{-5}, and 74% of these were due to mitotic recombination. After 24 to 48 h in MBC, the frequency of resistant clones was increased more than 100-fold to 4×10^{-3} to 5×10^{-3}, and 90% of these had uncovered markers on both arms of chromosome III. Since the frequency of these presumptive chromosome III loss colonies increased more than 100-fold while viability declined less than 10-fold, MBC treatment could not be simply selecting for preexisting or spontaneous chromosome loss cells, unless such cells were resistant to division arrest by MBC. This latter possibility was tested by pooling 10 Cry^r chromosome loss colonies into one culture and 10 Cry^r mitotic recombination colonies into another and treating each of these pooled cultures with MBC. Neither culture was resistant to the inhibitory effect of MBC: the viable cell counts after 48 h in MBC were 84 and 125%, respectively, of their starting values. Thus, the increase in chromosome loss frequency caused by MBC must have been due to the induction of new events.

To see whether loss occurred for other chromosomes as well, a similar experiment was performed with a diploid strain heterozygous for both of the recessive drug resistance markers to canavanine on chromosome V and cycloheximide on chromosome VII as well as for auxotrophic markers on the opposite arms of these same chromosomes (strain 6800, Table 1). At the start of the experiment canavanine-resistant (Can^r) clones occurred at a frequency of approximately 2×10^{-4}, and 62% of these were the result of mitotic recombination (Fig. 3; Table 4). After 13 h of MBC treatment the frequency of Can^r was increased 150-fold to approximately 3×10^{-2}, and 92% of these were due to chromosome loss. In the same experiments cells were plated on cycloheximide medium to determine the frequency of cycloheximide-resistant (Cyh^r) cells (Fig. 4; Table 4). The initial frequency of Cyh^r

TABLE 3. Recombination frequency[a]

Treatment	Viability (%)	Frequency of *his*$^+$	Frequency of *leu*$^+$
Untreated	≫100[b]	$9.5 \pm 2.3 \times 10^{-5}$	$2.8 \pm 2.2 \times 10^{-5}$
X-ray	50	$4.8 \pm 0.4 \times 10^{-4}$	$2.5 \pm 1.0 \times 10^{-4}$
MCB	93	$7.8 \pm 3.3 \times 10^{-5}$	$1.6 \pm 0.9 \times 10^{-5}$

[a] Frequency of *his*$^+$ or *leu*$^+$ recombinant clones after different treatments of strain 500-321A (haploid and disomic for chromosome III carrying heteroalleles of *his4* and *leu2*). All values are the average of three experiments. The protocol for X-ray treatment was to irradiate 2 m of exponentially growing cells (5×10^6/ml) in a 90-mm petri dish with the cover removed for 60 s at 106 rads/s (6,400 rads). MBC treatments were for 24 h at either 20 (two experiments) or 100 (one experiment) μg/ml.

[b] This value was due to normal cell division.

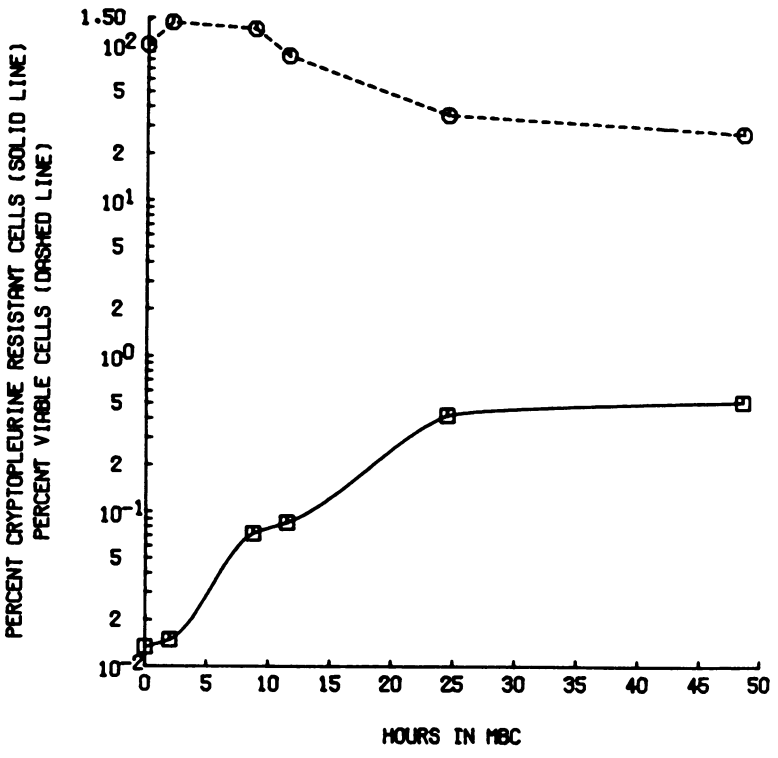

FIG. 2. Frequency of cryptopleurine-resistant cells as a function of length of MBC treatment (solid line). Also shown is the percent viable cells in the culture relative to the initial cell density (3.1×10^6/ml) (dashed line).

TABLE 4. Frequency of chromosome loss and intergenic recombination[a]

Drug resistance	Chromosome	Length of MBC treatment (h)	Frequency of chromosome loss	Frequency of recombination
cry1	III	0	$3.4 \pm 1.2 \times 10^{-5}$	$9.6 \pm 0.3 \times 10^{-5}$
		24, 48	$4.2 \pm 0.3 \times 10^{-3}$	$4.4 \pm 0.9 \times 10^{-4}$
can1	V	0	$5.1 \pm 0.1 \times 10^{-5}$	$1.3 \pm 0.1 \times 10^{-4}$
		13, 26	$2.7 \pm 0.7 \times 10^{-2}$	$1.5 \pm 1.3 \times 10^{-3}$
cyh2	VII	0	$6.6 \pm 1.0 \times 10^{-7}$	$3.8 \pm 0.1 \times 10^{-5}$
		13, 26	$3.7 \pm 1.7 \times 10^{-4}$	$1.6 \pm 1.1 \times 10^{-4}$

[a] Frequency of drug-resistant clones after MBC treatment of strains carrying heterozygous recessive resistance mutations. Clones which became resistant by chromosome loss or by mitotic recombination were distinguished with heterozygous auxotrophic mutations on the same chromosomes (see text). The chromosome III experiment was done with strain 885-1a; two experiments for chromosomes V and VII were done with strain 6800. All frequencies are pooled results for both 20 and 100 μg of MBC per ml. In addition, for chr III, cells were treated for either 24 or 48 h; for chr V and VII, cells were treated for either 13 or 26 h. Between 100 and 500 colonies were scored for each line in the table.

clones was about 4×10^{-5}, and essentially all of these (98%) were caused by mitotic recombination. After 13 h in MBC the frequency had increased 20-fold to 8×10^{-4} Cyh^r cells, and 66% of these were the result of chromosome loss.

To demonstrate that uncovering of recessive markers on both arms of a chromosome was due to the loss of a chromosome, a number of MBC-induced Can^r clones from strain 6822 (marked on both arms of chromosome V [Table 1]), both presumptive chromosome loss and presumptive mitotic recombination clones, were sporulated and dissected (Table 5). Two Can^r clones which were still prototrophic for homoserine, and thus the result of mitotic recombination events, gave nearly perfect spore germination. Of 10 chromosome loss clones (*can1* and *hom3*) dissected, 3 produced only tetrads, with two or fewer viable spores (clones ii, iii, and iv). The spore lethality was tightly centromere linked, as expected for diploid cells which were monosomic for a chromosome. The other seven chromosome loss clones produced predominantly four viable spore tetrads; however, all of these did show a $0^+:4^-$ segregation for *hom3*. This result suggested that these clones may have undergone two

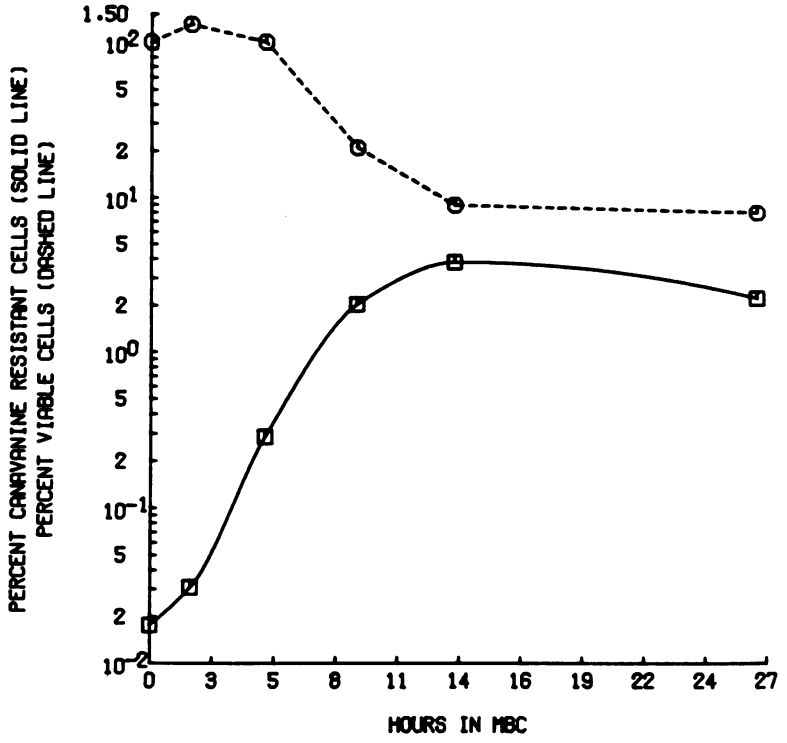

FIG. 3. Frequency of canavanine-resistant cells as a function of length of MBC treatment (solid line) and the percent viable cells in the culture relative to the initial cell density (3.1×10^6/ml) (dashed line).

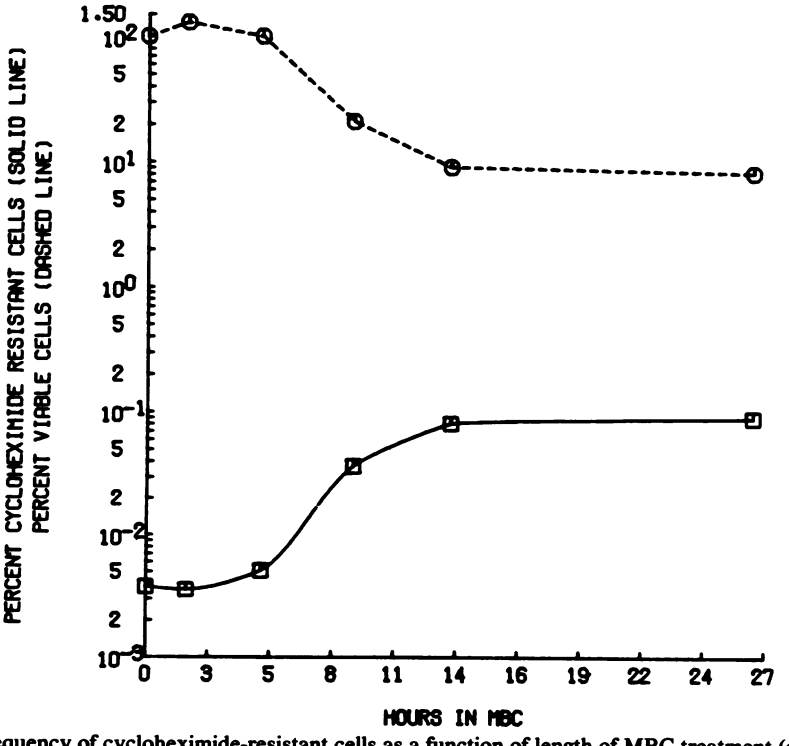

FIG. 4. Frequency of cycloheximide-resistant cells as a function of length of MBC treatment (solid line) and the percent viable cells in the culture relative to the initial cell density (3.1×10^6/ml) (dashed line).

TABLE 5. Marker segregation after chromosome loss[a]

Strain	Phenotype	Clone no.	No. of tetrads	Segregation pattern (no. of tetrads with indicated pattern)						
				Viable:Lethal				$hom3^+$:$hom3^-$[b]		
				4:0	3:1	2:2	1:3	2:2		0:4
6822	Can^r $hom3^+$ (mitotic recombination)	i	16	14	1	1	0	13	:	1
		ii	15	12	3	0	0	12	:	0
	Can^r $hom3^-$ (chromosome loss)	i	9	7	2	0	0	0	:	7
		ii	16	0	0	13	3	–	:	–
		iii	11	0	0	9	2	–	:	–
		iv	15	0	0	15	0	–	:	–
		v	12	8	1	2	1	0	:	8
		vi	11	7	2	1	2	0	:	7
		vii	15	12	1	2	0	0	:	12
		viii	16	12	2	1	1	0	:	12
		ix	16	13	2	0	1	0	:	13
		x	16	8	5	3	0	0	:	8

Strain	Phenotype	Clone no.	No. of tetrads	MATa:MATα			$his4^+$:$his4^-$		
				2:2		Non-2:2[c]	2:2		4:0, 1:3, or 3:1
885-1a	Cry^r $his4^+$ (recombination)	i	16	2	:	14	4	:	12
		ii	10	1	:	9	0	:	10
		iii	6	2	:	4	1	:	5
		iv	12	10	:	2	4	:	8
	Cry^r $his4^-$ (loss)	i	22	19	:	3	22	:	0
		ii	7	7	:	0	6	:	1
		iii	11	11	:	0	9	:	2
		iv	13	13	:	0	11	:	2
		v	11	11	:	0	9	:	2
		vi	12	12	:	0	12	:	0
		vii	10	10	:	0	6	:	4
		viii	9	1	:	8	5	:	4

[a] Meiotic segregations patterns for recessive markers among putative chromosome loss clones. Canavanine-resistant clones of strain 6822 (a diploid strain) were sporulated and dissected directly. Cryptopleurine-resistant clones of strain 885-1a (*MATα* or *MATα*/*MATα*) were mated to strain 174-1-3 (*MATa his*$^+$ *ural leu1*) to produce diploids, which were then sporulated and dissected with the results as shown.

[b] Segregation patterns for *hom3* were scored only in tetrads with four viable spores.

[c] Tetrads which did not segregate mating type 2:2 gave an excess of *MATα* and *MATa*/*MATα* spores.

events: either two mitotic recombinations or chromosome loss followed by chromosome duplication (by endo-reduplication or nondisjunction) to produce cells homozygous for both *can1* and *hom3*$^-$ (see Discussion). MBC-induced Cry^r clones from strain 885-1a were also analyzed meiotically. Cells of this strain that became crypto-pleurine resistant due to chromosome loss would be euploid haploids and, therefore, more stable than the aneuploid chromosome loss clones of strain 6822. Eight Cry^r *his4*$^-$ clones (from chromosome loss and presumed to be MATα) and four Cry^r *his4*$^+$ clones (by mitotic recombination and presumed to be *MATα*/*MATα*) were crossed with a *MATa* haploid, and the resulting diploids were sporulated and dissected (Table 5). Clones i through vii from the chromosome loss colonies all showed the 2:2 segregation for mating type and *his4* expected of euploid diploid strains. Mitotic recombination clones i through iii did not show 2:2 segregation for either of these markers, but produced an excess of *MATα*, nonmating, and His^+ spores, indicating trisomy for chromosome III. The fourth recombinant clone, clone iv, gave 2:2 segregation for mating type but a trisomic pattern for *his4*, suggesting that it was a partial trisome for chromosome III. Clone viii of the chromosome loss clones did not show 2:2 segregation for mating type as the other presumptive chromosome loss colonies did. This suggested that its Cry^r His^- phenotype was due to two crossover events, which homozygosed both Cry^r and *his4*$^-$, rather than due to loss of a

chromosome III homologe. These results with strain 885-1a confirmed that the simultaneous appearance of markers that were originally heterozygous on both arms of a chromosome was almost always due to loss of a chromosome.

Intergenic recombination was affected by MBC treatment. The data of Table 4 also showed a 5- to 20-fold increase in the frequency of mitotic recombinants after MBC treatment. This was somewhat surprising, since MBC did not cause an increase in the frequency of intragenic recombination (Table 3). The increase seen in intergenic recombination was highly variable, ranging from 0- to 40-fold (Table 4 and other experiments not shown) and could not be correlated with any experimental parameter such as concentration of MBC, length of treatment, or use of a particular strain or marker combination. In all cases, however, the increase in mitotic recombination was at least 20-fold lower than the increase in chromosome loss in the same experiment. When the increase in recombination frequency occurred, a sufficient number of recombinant clones were scored that the increase was significant, although the effect was not reproducible between experiments. Similar differences in effect on intragenic and intergenic recombination frequencies have been observed before (45).

All chromosomes were lost at the same frequency. Chromosome loss was observed in a strain which was heterozygous for auxotrophic markers on 13 chromosomes (6808-2) to examine the relative frequencies of loss of each of these chromosomes after MBC treatment. I found that all chromosomes were lost at approximately equal frequencies (Table 6). This result contrasted with the frequencies of loss previously observed for chromosomes III and VII (Table 4). However, in the experiments of Table 4, cells that had undergone a chromosome loss were selected by drug resistance; in these experiments the plating efficiency of MBC-treated cells on drug-containing media was not known. In contrast, the chromosome loss frequencies in Table 6 were from unselected clones and, therefore, more likely to represent the total frequencies of loss events for particular chromosomes. I concluded that MBC induced loss for all chromosomes at equal frequencies.

Loss occurred randomly among all chromosomes. The chromosome loss events of the experiment in Table 6 were compared in pairwise combinations to determine whether there were any correlations (Table 7). With only three exceptions (chromosome III/VII, chromosome VI/VII, and chromosome VII/VII pairs), no correlations were found. The first exception was an absolute negative correlation for uncovering *ade6* and *leu1*, both on chromosome VII. This proved to be an artifact of a recombinational event during the construction of strain 6808-2, which switched these two markers from their original *cis* linkage in strain NH429-6a to a *trans* configuration in strain 6808-2. This recombination event had the fortuitous effect of demonstrating that both homologs of chromosome VII were lost at equal frequencies during MBC treatment. The other correlations seen were the loss of chromosome III/VII and VI/VII pairs, which were observed more frequently than predicted from the individual loss frequencies of these chromosomes. The excess of chromosome III/VII pairs was likely to have been due to chance, since the correlation was significant only at the 95% level and not for a 99% confidence limit, and the excess was seen with only one of the chromosome VII homologs. The observed high frequency for joint loss of the chromosome VI/VII(*leu1*) pair was highly significant ($P < 0.001$) and might indicate nonrandom chromosome loss. However, this effect was specific for the chromosome VII homolog carrying *leu1*, since an excess of chromosome VI/VII(*ade6*) joint loss colonies was not seen and, therefore, probably indicated a growth advantage (which would increase the probability of recovery) of cells which lost both chromosomes VI and VII over cells which had lost only one or the other of these chromosomes. Thus, except possibly for chromosome VI and a particular homolog of chromosome VII, the likelihood of MBC-induced loss for any particular chromosome was

TABLE 6. Frequency of loss for different chromosomes[a]

Chromosome	Loss frequency (% of viable)
I	NT[b]
II	1.7
III	2.4
IV	2.3
V	2.0
VI	3.6
VII (*ade6*)	3.1
VII (*leu1*)	3.4
VIII	2.4
IX	1.4
X	3.0
XI	2.2
XII	NT
XIII	NT
XIV	3.4
XV	4.1
XVI	3.4

[a] Frequency at which different chromosomes in a single strain were lost after MBC treatment. Exponentially growing cells of strain 6808-2 were treated with 20 μg of MBC per ml for 24 h; viability was 40% of the initial value. A total of 2,700 independent clones were scored for all markers. The average frequency of loss for all chromosomes was 2.7%.

[b] NT, Not tested.

TABLE 7. Pairwise combinations of chromosome loss events[a]

Chromosome	Total	Chromosome															
		II	III	IV	V	VI	VII *ade6*	VII *leu1*	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
II	156		8	7	13	19	18	10	8	10	15	12	–	–	10	16	10
III	184			6	17	13	23[b]	11	10	10	26	12	–	–	17	18	12
IV	191				12	19	14	22	11	5	26	11	–	–	16	13	16
V	203					13	20	19	15	10	25	14	–	–	16	13	16
VI	273						19	39[c]	17	15	29	15	–	–	20	31	24
VII *ade6*	246							0[c]	16	11	22	21	–	–	29	31	22
VII *leu1*	241								16	8	31	16	–	–	22	26	21
VIII	201									9	22	13	–	–	19	24	21
IX	130										12	9	–	–	8	15	8
X	317											22	–	–	27	28	31
XI	199												–	–	20	16	17
XII	0													–	–	–	–
XIII	0														–	–	–
XIV	258															26	21
XV	295																32
XVI	256																

[a] The number of clones observed that lost a particular pair of chromosomes (without regard to whether other chromosomes were also lost in the same clone). The data are from the same experiment as the data of Table 6. The probability of the observed value for loss of a particular chromosome pair occurring by chance was calculated for both 95 and 99% confidence intervals, using summed terms of a binomial distribution as follows:

$$\alpha/2 < \left\{ \sum_{k=0}^{n_{i,j}} \left[\binom{N_i}{k} (p_j)^k (1 - p_j)^{(N_i - k)} \right] \right\} < 1 - \alpha/2$$

where: $n_{i,j}$ = observed number of clones which lost chromosome i and chromosome j
N_i = observed number of clones which lost chromosome i and any other marked chromosome $[= \Sigma_j n_{i,j}]$
p_j = probability of observing a clone which lost chromosome j and any other marked chromosome $[= N_j/(1/2\ \Sigma_j N_j)]$
α = 0.05 for a 95% confidence limit or 0.01 for a 99% confidence limit

This formulation did not assume independence of individual chromosome loss events within a single clone when loss events were tabulated without regard to chromosome identity (Table 8). The calculated probabilities for the observed frequencies of all chromosome pairs were within the 95% confidence limits for chance occurrence, except for those indicated otherwise.

[b] Probability of observed frequency outside 95% confidence limit.

[c] Probability of observed frequency outside 99% confidence limit.

not affected by the loss of any other particular chromosome.

Loss events within a single cell were not independent. The chromosome loss clones in the experiment in Table 6 were also tabulated for the total number of chromosomes each had lost (Table 8). That cells which had lost eight or nine chromosomes were detected at a significant frequency strongly suggested that MBC-induced loss events within a single cell were not independent; a quantitative analysis demonstrating that this was true is given below.

Loss occurred subsequent to division arrest. From Fig. 2 to 4 it was seen that the frequency of chromosome loss started at a low value and increased steadily during the MBC treatment, until reaching a plateau after 12 to 24 h. However, all cells were division arrested after 4 to 6 h.

TABLE 8. Frequency of cells with multiple chromosome losses[a]

No. of marked chromosomes lost	No. of colonies observed	% of loss colonies
1	171	45
2	84	22
3	42	11
4	33	9
5	25	7
6	11	3
7	6	2
8	5	1
9	1	0.3

[a] Frequency at which chromosome loss clones of strain 6808-2 lost more than one chromosome after MBC treatment. These data are from the same experiment as the data of Table 6.

Thus, chromosome loss was not an immediate consequence of cell cycle arrest, but was due to some more slowly occurring event, subsequent to arrest of cell division. These data also indicated that the kinetics of chromosome loss were similar for different chromosomes and for cells which progressed from euploid to aneuploid ($2n \rightarrow 2n - 1$) or vice versa ($n + 1 \rightarrow n$).

Loss occurred during or immediately after MBC treatment. Whether chromosome loss occurred during or after MBC treatment was tested by allowing treated cells to grow for some time after removal of MBC before selection for cells which had lost a chromosome. For this experiment loss of chromosome VII was studied, using a strain that was heterozygous for *cyh2* on chromosome VII (strain 6830, Table 1). Cells that are heterozygous for cycloheximide resistance die after less than two generations when plated on cycloheximide medium (Wood, unpublished data; S. Dutcher, Ph.D. thesis, University of Washington, Seattle, 1980). Thus, any cells of strain 6830 that became Cyh^r after MBC treatment must have lost the chromosome carrying the cycloheximide-sensitive allele (*CYH2*) before, or very shortly after, they were removed from MBC. The frequency of Cyh^r before treatment with MBC was 1.4×10^{-4}; after the cells had been incubated with MBC for 24 h the frequency was 5.5×10^{-3}. This frequency did not change when the cells were washed, suspended in medium without MBC, and allowed to grow for 0, 6, or 24 h (stationary phase) before plating on cycloheximide medium (Table 9). Similar results were found for loss of chromosome V (to become Can^r) (data not shown). These results indicate that MBC-treated cells did not continue to lose chromosomes after they were removed from MBC. Thus, MBC did not have a long-term effect on the fidelity of chromosome segregation.

MBC induced polyploidy. The possibility that MBC treatment might cause polyploidy was examined by treating a haploid strain with MBC and assaying for the induction of diploid cells. A method was devised to select for cells that had become polyploid during MBC treatment: haploid *MATα* cells cannot sporulate, whereas *MATα/MATα* diploids carrying the dominant *SAD1* mutation can (25), and spores are more resistant to killing by ether than either haploid or diploid vegetative cells (11). Only cells with a diploid or near-diploid chromosome number would be able to form viable spores. Thus, the frequency of diploids in a population of *MATα SAD1* haploid cells could be measured by the frequency of cells able to sporulate and grow after ether treatment. An untreated control culture of a *MATα SAD1* haploid strain, XG-99-1-2a, sporulated at a frequency of approximately

TABLE 9. Frequency of chromosome loss after removal of MBC[a]

Length of incubation time after MBC treatment (h)	Total viable cells/ml	Frequency of Cyh^r
0	2.54×10^6	5.5×10^{-3}
6	1.20×10^7	7.0×10^{-3}
24	5.5×10^7	5.5×10^{-3}
Before MBC treatment	3.10×10^6	1.4×10^{-4}

[a] Frequency of chromosome loss during growth subsequent to MBC treatment. Cells of strain 6830 were treated with 100 μg of MBC per ml for 24 h and then washed by filtration to remove the MBC and incubated in fresh medium as indicated before plating on cycloheximide medium.

10^{-4}; after cells had been exposed to MBC for 24 h, this frequency increased 20-fold to 2×10^{-3}. The possibility that this increase was due to fusion of two cells, rather than to induction of polyploidy in a single cell, was eliminated by treating a mixed culture of strains XG-99-1-2a and XG-99-1-2b with MBC for 24 h. The frequency of diploid cells (prototrophic for lysine and tryptophan) after MBC treatment was $<10^{-5}$. Thus, the increase in sporulation frequency with XG-99-1-2a alone, in the first experiment, demonstrated that MBC treatment induced diploid cells in a haploid culture.

DISCUSSION

The results in Table 2 show that MBC is at most only weakly mutagenic in *S. cerevisiae*. Similar observations have been made in *Aspergillus* (1, 21), *Streptomyces* (4), and mammals (36, 51). Contrasting reports that benomyl or MBC is mutagenic in some organisms have been attributed to the ability of these compounds to act as purine analogs that are incorporated into DNA (14, 30, 46, 47). This low level of mutagenicity was dependent on the efficiency of DNA repair synthesis: excision repair-deficient strains of *Escherichia coli* or *Salmonella typhimurium* had a higher frequency of mutation than excision-proficient strains when treated with benomyl (30). Failure to detect the induction of mutations in eucaryotes was probably due to both their generally more efficient DNA repair systems (30) and the more pronounced effects on chromosome segregation of benomyl or MBC (48). This conclusion is in agreement with my own observations; any induction of mutations by MBC was 1,000-fold less frequent than chromosome loss (cf. Tables 2 and 4).

The effect of MBC on mitotic recombination was small. Intragenic recombination assayed between heteroalleles (Table 3) was unaffected by MBC treatment. Siebert et al. obtained similar results by using benomyl with an *S. cerevisiae* strain heteroallelic for mutations in two dif-

ferent genes, *ade2* and *trp5* (52). However, I observed significant increases in intergenic recombination after MBC treatment in a number of experiments. Since the increase in intergenic recombination was not reproducible, it might have been due to some aspect of the experimental protocol rather than to a direct effect of MBC on cells. Regardless of its cause, the increase in intergenic recombination was small relative to that of MBC-induced chromosome loss.

Simultaneous uncovering of heterozygous markers on both arms of a chromosome was interpreted as a presumptive chromosome loss event. To determine whether an actual physical loss of a chromosome had occurred, a number of chromosome loss colonies were sporulated and dissected (Table 5). Some of the clones from the diploid strain 6822 segregated lethality 2:2 as expected for a true monosome. The clones that did not segregate lethality may have resulted from chromosome loss and subsequent selection for regaining euploidy during growth of the clone into a colony or upon sporulation of the clone. There is substantial evidence for selection against aneuploidy in yeast: in my experiments chromosome loss was frequently associated with the smallest colonies after MBC treatment. Similar studies of chromosome loss, using *cdc6* and *cdc14* (G. Kawasaki, Ph.D. thesis, University of Washington, 1979), frequently found presumptive chromosome loss clones that did not segregate lethality after sporulation. Bruenn and Mortimer (2), Parry and Zimmerman (43), and Campbell et al. (3) all found that aneuploid cells were unstable and frequently reverted to euploidy. Chromosome V monosomes appear to be particularly unstable (Wood, unpublished data); there have been no reports demonstrating monosomy for chromosome V by dissection. However, the results of my experiments with a disomic haploid strain, 885-1a (Table 5), were strong evidence in favor of induction of chromosome loss by MBC. In this strain, where loss of chromosome III would produce a euploid haploid, almost all MBC-induced loss clones showed the expected 2:2 segregation of markers on chromosome III when they were crossed with another haploid strain and the resulting diploid was sporulated. Therefore, although MBC does have some highly variable effect on mitotic recombination, this effect is sufficiently smaller than that of MBC-induced chromosome loss that in nearly all MBC-induced uncovering of heterozygous markers there was loss of a chromosome.

Loss of a chromosome from a cell could occur by failure to replicate, failure to segregate to either pole, or mitotic nondisjunction. Nondisjunction is distinguishable from failure to segregate or nonreplication since in a diploid cell it would produce both a daughter cell monosomic for a chromosome (a chromosome loss) and a daughter trisomic for the same chromosome (a chromosome gain), whereas failure to replicate or segregate to either pole would produce only a monosomic daughter and a normal diploid daughter. Although MBC has been claimed to cause nondisjunction in *Aspergillus* (28, 29), this conclusion was based solely on induction of chromosome loss; since no attempt to score trisomy was made, this conclusion is injustified. In my screening of MBC-treated cells for chromosome gain, no clones were found that could be confirmed to be trisomic by sporulation and dissection (data not shown). However, trisomes might have been missed had they occurred as they are known to be unstable (22).

The production of diploid cells from haploids after MBC treatment (with a *MATα SAD1* strain [see Results]) indicated that MBC caused polyploidy. Since MBC could induce both chromosome loss and polyploidy (chromosome gain), it may indeed cause chromosome nondisjunction. If this conclusion is correct, then, given the stringency of selection for viability after sporulation (only cells with a diploid or near diploid chromosome number would produce viable spores), the fact that the induced frequency of diploids (2×10^{-3}) was only 10-fold lower than the frequency of loss of any one particular chromosome (approximately 2×10^{-2}) suggested that all MBC-induced chromosome loss events might result from nondisjunction. Induction of polyploidy could be independent of chromosome loss; however, the simplest hypothesis is that these two effects were due to a single cause: MBC-induced chromosome nondisjunction. Proof of this hypothesis will require the recovery of both products of a single nondisjunction event.

MCB induced loss of all chromosomes at equal frequencies (Table 6). This observation is similar to that of Kawasaki, using *cdc14* to produce chromosome loss (Kawasaki, Ph.D. thesis, 1979). Both of these experiments demonstrated that there cannot be strong selection in *S. cerevisiae* against a diploid cell monosomic for any of the 13 chromosomes monitored. This conclusion could be extended to all pairwise combinations of monosomes as well, since nearly all pairwise combinations of chromosomes occurred at random frequencies (Table 7). These results supported the idea that MBC-induced chromosome loss was completely random with respect to individual chromosomes in a population of treated cell. Thus, loss was not a function of any gene or sequence on a particular chromosome.

Analyzing whether chromosome loss events within individual cells were independent (wheth-

er a cell that had lost one chromosome had a higher probability of losing a second) was complicated by two factors. First, not all chromosomes were marked in these experiments; only 13 of 32 chromosomes could be followed. Second, it was not possible to know what loss events had occurred in the nonviable cells, which ranged from 60 to 90% of the initial cells. However, by making two assumptions it was possible to correct for these factors and calculate the expected frequencies of cells with multiple loss events if individual loss events were independent. The assumptions made were that (i) the probability of loss was the same for marked and unmarked chromosomes, and (ii) cells which had lost both homologs of a particular chromosome were lethal; otherwise, the distribution of chromosome loss events was the same among both the viable and nonviable cells. From these assumptions a formula for the expected frequencies of different classes of marked chromosomes lost was calculated and compared with the experimental data (see Appendix). This analysis indicated that MBC-induced loss events were not independent; by using the average frequency of loss measured experimentally for a single chromosome (2.7%; see Table 6), it was apparent that multiple loss events occurred more frequently than predicted relative to the single-loss class. Kawasaki found a similar excess of multiple chromosome events with *cdc14*-induced chromosome loss (Kawasaki, Ph.D. thesis, 1979). *cdc6*-induced loss, on the other hand, seemed to cause a deviation from independence in the opposite direction, fewer than predicted multiple events (Kawasaki, Ph.D. thesis, 1979).

Comparing the experimental and calculated curves in Fig. 5 suggested that the departure from independence for MBC-induced loss might be due to two or more subpopulations of cells with different sensitivities to MBC: for example, two groups of equal size, where one had a low probability of loss for any single chromosome and the other group had a high probability. With two groups of cells this model was not sufficient to account for the data; however, the unlikely possibility that there were three or more groups with different sensitivities could not be ruled out. An alternative way to explain the data would be to assume that each succeeding chromosome loss within a cell had a higher probability than the previous loss. This model predicted that the probability of loss of any particular chromosome would increase with the length of time in MBC. When the data for Can^r in Fig. 3 was replotted as the absolute number of resistant colonies versus time (the cells did not divide in MBC), the initial rate of increase of drug resistance was exponential (Fig. 6), which was

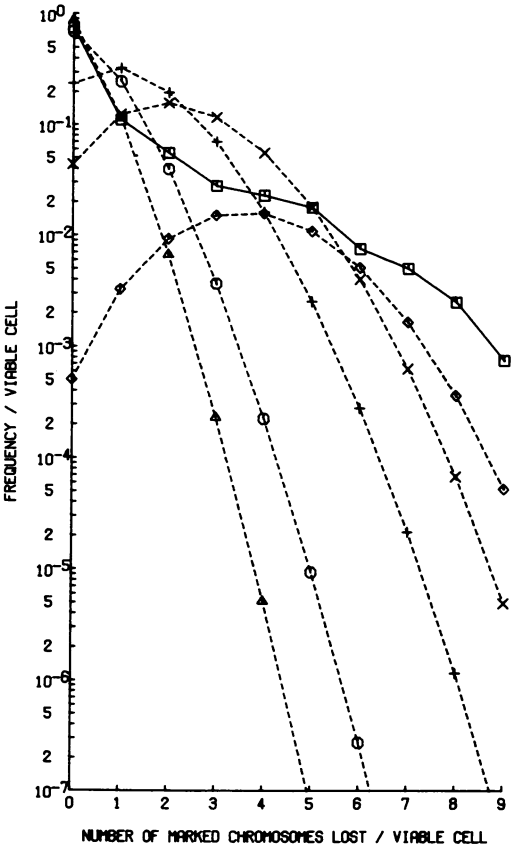

FIG. 5. Expected frequencies of cells that have lost different numbers of marked chromosomes for different values of p (the probability of loss for any single chromosome) if individual chromosome loss events were independent (calculated as described in the Appendix). Symbols: △, $p = 0.01$; ○, $p = 0.027$ (the observed average frequency of loss for a single chromosome [Table 6]); +, $p = 0.1$; ×, $p = 0.2$; ◇, $p = 0.4$. The solid line and the symbol □ show the observed frequencies of multiple chromosome loss clones.

consistent with an increasing probability of loss of chromosome V with time. Similar exponential increases in drug-resistant colonies were found when the data for chromosomes III (Fig. 2) and VII (Fig. 4) were replotted (not shown). These results suggested that the lack of independence of chromosome loss might be due to progressive disruption of the mitotic spindle during MBC treatment.

Several other microtubule inhibitors are known to produce chromosome segregation aberrations similar to those seen with MBC. With diploid or near-diploid Chinese hamster cell lines, two groups discovered that with low colcemid concentrations and short exposure times, a high frequency of aneuploid (rather than polyploid) cells were induced (6, 7, 31). Monosomic and trisomic cells were found for all chromo-

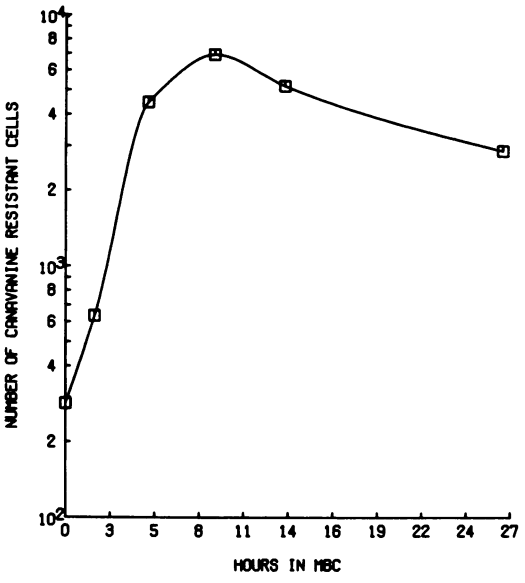

FIG. 6. Number of canavanine-resistant cells observed as a function of length of MBC treatment. These data are from the same experiment as the data of Fig. 3.

somes, either at random frequencies or with a tendency for small chromosomes to be involved somewhat more frequently. This suggested that Colcemid induced chromosome nondisjunction similar to that postulated for MBC. That aneuploids were produced only at low concentrations of colcemid, or with short exposure times, suggested that aneuploidy resulted from a leaky or short-duration block of mitosis, with a more complete or longer block producing a complete failure of mitosis and polyploidy. Larizza et al. used cultured mammalian cells to show that griseofulvin induced similar karyotypic changes (35). Uncovering of genetic markers was demonstrated with hybrid cell lines heterozygous for 8-azoguanine resistance. After treatment of cells with griseofulvin, an increase in the frequency of 8-azoguanine resistance segregation was seen in five of six hybrid lines (34). *p*-fluorophenylalanine has also been shown to cause chromosome nondisjunction (49, 54) or karyotypic abnormalities (53, 56). The similarity in genetic effects seen with these inhibitors is not surprising since most act in a similar fashion on the mitotic spindle preventing microtubule polymerization by binding or incorporation into tubulin dimers: MBC and colchicine are known to be competitive binding inhibitors (9), and Morris and Oakley found that benomyl-resistant mutants of *Aspergillus* frequently were also resistant to *p*-fluorophenylalanine (38).

The experiments reported here suggested several conclusions about the effect of MBC arrest on mitotic cells of *S. cerevisiae*. The genetic effects of MBC arrest were specific: treated cells lost chromosomes at a high frequency. MBC must act at a site or sites independent of the identity of particular chromosomes, since all chromosomes were lost randomly and at equal frequencies. The maximum levels of induced loss were found well after division had been arrested, indicating that cells could recover and properly segregate their chromosomes for some time after they had stopped dividing. Consistent with this idea was the observation that the probability of loss for particular chromosomes increased with length of time in MBC. However, this cumulative effect on chromosome loss frequency must be quickly reversible because cells did not continue to lose chromosomes after removal of MBC; all induced events occurred during, or in the first division after, MBC treatment. Induction of polyploid cells suggested that MBC-induced chromosome loss occurred by mitotic nondisjunction. The lower frequency of polyploid cells relative to aneuploid cells induced by MBC suggested that MBC did not produce a complete failure of mitosis as does colcemid.

MBC provides a useful extension to the numerous cytological or in vitro biochemical studies done with other microtubule inhibitors, since none of the well-characterized inhibitors are active on *Saccharomyces*. The ability to easily manipulate and determine chromosome composition has made it possible to use selective genetic techniques to examine the effect of a mitotic inhibitor on chromosome segregation. Extensive work on the *Saccharomyces* cell cycle has also made it possible to examine the effect of MBC within the context of the mitotic cell cycle (Wood and Hartwell, in press). The high frequency of chromosome loss induced by MBC has been utilized as a rapid method for localizing unmapped genes to particular chromosomes (58).

APPENDIX

Testing Independence of Chromosome Loss

If the probability of loss for each chromosome was the same and individual loss events were independent, then the distribution of the number of cells with different numbers of loss events would be binomial:

$$P_x = \binom{n}{x} p^x (1 - p)^{(n-x)}$$

If the probability of loss was the same for both marked and unmarked chromosomes, then the distribution of marked chromosomes lost (observable) among the total number of loss events (marked + unmarked) would be hypergeometric:

$$P\begin{pmatrix} x \text{ marked chromosomes lost, given} \\ n \text{ total chromosomes lost} \end{pmatrix} = \frac{\begin{pmatrix} \text{number of marked chromosomes} \\ \text{number of marked chromosomes lost} \end{pmatrix}\begin{pmatrix} \text{number of unmarked chromosomes} \\ \text{number of unmarked chromosomes lost} \end{pmatrix}}{\begin{pmatrix} \text{number of total chromosomes} \\ \text{number of total chromosomes lost} \end{pmatrix}}$$

Any number of assumptions could be made about the viability of cells with different numbers of loss events; however, the minimum assumption was that any cell that had lost both homologs of a chromosome must be lethal. For greater than 16 chromosomes lost, the probability of lethality must be 100%; for less than 16, it could be calculated as follows. The probability that a particular chromosome lost would be the second member lost of a homologous chromosome pair depended on both the total number of chromosomes lost and the probability that two of the other chromosomes lost did not already form a homologous pair:

p = probability that a particular chromosome lost would be homologous with another chromosome which has been lost

$$= [1 - P(n-1)]\,\{(n-1)\}/[32 - (n-1)]$$

where n = total number of chromosome lost and $1 - P(n-1)$ = probability that the other chromosomes lost do not contain a homologous pair.

Therefore:

$P(n)$ = the total probability of at least one pair of homologous chromosomes lost for n total chromosomes lost

$$= \sum_{i=1}^{n} \{[1 - P(i-1)]\,[(i-1)/(32 - (i-1))]\}$$

$$= \sum_{i=1}^{n} \{[1 - P(i-1)]\,[(i-1)/(33 - i)]\}$$

where $P(0) = 0$.

$1 - P(n)$ = probability of not getting a pair for n total chromosomes lost

$$= 1 - \left(\sum_{i=1}^{n} \{[1 - P(i-1)]\,[(i-1)/(33 - i)]\}\right)$$

where $P(0) = 0$.

Combining all of these:

$F(x)$ = expected frequency of x marked chromosomes being lost among the viable cells

$$F(x) = \sum_{i=x}^{16} \left[\begin{matrix}(\text{probability of } n \text{ total lost})\,(\text{probability that of } n,\ x \text{ are marked}) \\ (\text{probability that } n \text{ lost is viable})\end{matrix}\right]$$

$$F(x) = \sum_{n=x}^{16} \left\{p^{(n)}\,(1-p)^{(32-n)}\begin{pmatrix}13\\x\end{pmatrix}\begin{pmatrix}19\\n-x\end{pmatrix}\left[1 - \left(\sum_{i=1}^{n} \{[1 - P(i-1)]\,[(i-1)/(33 - i)]\}\right)\right]\right\}$$

where: $P(0) = 0$; p = probability of loss of any single chromosome.

With this equation, the frequencies of different classes of marked chromosomes lost could be calculated for different values of p. These curves are plotted, together with the experimental results, in Fig. 5. It is clear that the data were not well matched by any of the theoretical curves. The fit between the theoretical curves and the data was not improved by altering the third assumption above, that the probability of loss was the same for both viable and nonviable cells. If lethal cells had a greater frequency of chromosome loss than the viable cells, this only increased the apparent value of p in the equation. Likewise, the opposite assumption, that lethal cells lost fewer chromosomes than the viable cells, made the p value less. Neither altered the basic shape of the theoretical curves. This analysis, therefore, indicated that MBC-induced events were not independent.

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