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Mitotic Chromosome Loss Induced by Methyl Benzimidazole-2-yl-Carbamate as a Rapid Mapping Method in *Saccharomyces cerevisiae*

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Mitotic chromosome loss induced by methyl benzimidazole-2-yl-carbamate has been utilized as a rapid and simple method for assigning genes to individual chromosomes in *Saccharomyces cerevisiae*. This technique relied on the segregation of heterozygous markers in a diploid strain after methyl benzimidazole-2-yl-carbamate treatment due to loss of whole chromosomes. Correlations between the expression of an unmapped gene and that of a previously mapped recessive marker indicated chromosomal linkage. Depending on whether the unmapped gene and the marker were located in coupling or in repulsion, either positive or negative correlations were seen. The chromosomal location of several previously mapped genes were confirmed as a test of the method, and one previously unmapped gene, *nib1*, was mapped.

Although the genetic map of *Saccharomyces cerevisiae* has been extensively characterized, its long recombinational length (>4,600 centimorgans) and large number of chromosomes (16 or 17) (8) has often made assigning a new marker to a particular location on the linkage map tedious with conventional meiotic tetrad analysis. In this paper I report on the use of the high-frequency chromosome loss induced by the mitotic inhibitor methyl 2-yl-benzimidazole carbamate (MBC) as a simple and rapid method for localizing new markers to particular chromosomes in *S. cerevisiae*. This method requires only that the unmapped gene and recessive auxotrophic mutations marking the chromosomes be in a heterozygous state.

MATERIALS AND METHODS

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

Media. Minimal liquid medium contained, per liter, 1.6 g of yeast nitrogen base without amino acids or ammonium sulfate (Difco Laboratories), 10 g of succinic 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 4 μg each of adenine, arginine, asparagine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, uracil, and valine per ml were added 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.). The concentration of cycloheximide in the YEPD + cycloheximide plates was 10 μg/ml.

Genetic techniques. Standard techniques of yeast genetic analysis (5) were used for constructing strains and complementation testing of markers.

Mapping protocol. The mapping procedure utilized well-marked haploid strains which had auxotrophic mutations on 14 or 16 chromosomes (Table 1). One of these strains was mated to a strain containing the unmapped mutation of interest to produce a diploid heterozygous for both the auxotrophic markers and the unmapped gene. To make subsequent complementation tests easier, the diploid strains were X-ray treated and clones homozygous for mating type were isolated. This step was not essential since *MAT***a**/*MAT*α clones could be induced to sporulate and then the spores could be tested for complementation (G. Kawasaki, Ph.D. thesis, University of Washington, Seattle, 1979).

Cultures of the heterozygous diploid strains were grown overnight in liquid minimal medium with only required supplements at 23°C with shaking. Mid-log cells from these cultures were diluted to between 3×10^6 and 6×10^6 cells per ml, and 100 μg of MBC per ml was added (from a stock solution of 20 mg of MBC per ml of dimethyl sulfoxide). The efficacy of the MBC treatment was determined by examining the cells for the cell cycle arrest morphology characteristic of MBC-treated cells (10, 12); after 4 to 8 h of incubation with MBC, >90% of the cells were arrested as a mother and large bud. After incubation with MBC for 24 h at 23°C, the viability of the cultures was between 10 and 50% of the initial cell density; the treated cultures were then plated onto YEPD medium to

TABLE 1. List of strains

Strain	Source	Genotype
Heterozygous diploids		
6808-2[a]	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/+*
6830-1	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/+*
Well-marked strains		
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*; XII: *asp5*; XIII: *lys7*; XIV: *lys9 met2*; XV: *ade2 arg1*; XVI: *aro7*; *mal suc*
Complementation strains[b]		
N248-1A	R. K. Mortimer	*MATa ade1 gal1 leu1 his2 ura3 trp1 met14*
N248-1C	R. K. Mortimer	*MATα*, same as above
N442-4A	G. Kawasaki	*MATa his6 ade2 lys9 ura1 trp5 met2 arg4 mal suc*
N442-5C	G. Kawasaki	*MATα*, same as above
3654-5A	D. Hawthorne	*MATa his4 trp1 ura4 ade6 leu2 lys2 thr4 tyr1 arg4 MAL2 gal7*
3654-1D	D. Hawthorne	*MATα*, same as above
N435-1A	G. Kawasaki	*MATa his7 lys7 met6 arg1 gal4 MAL2 SUC*
N435-2A	G. Kawasaki	*MATα*, same as above
Other		
X2180-1B	Yeast Stock Center (Berkeley, Calif.)	Wild type, *MATα*
2136-4-1	C. Holm	*his4 ade2 lys2 cry1 MATα nib1*

[a] Although the *ade6* allele originally came in with the NH429 parent, during the construction of strain 6808-2 a mitotic recombination event occurred, switching the linkage of the *ade6* and *leu1* alleles from coupling (in strain NH429) to repulsion (6808-2). This was confirmed by tetrad analysis of a spontaneous isolate of 6808-2 which was heterozygous for mating type and able to sporulate; segregation for *ade6* and *leu1*, assuming *trans* linkage, was: 7 PD:0 NPD:12 T (PD, parental ditype; NPD, nonparental ditype; T, tetrad).

[b] All of the strains used for complementation testing are now available from the Yeast Genetic Stock Center.

obtain single colonies (except for *nib1* mapping, which were plated on YEPD + cycloheximide as described in Results). Approximately 2,500 colonies from the YEPD plates were screened to find those that had acquired or "uncovered" additional auxotrophies different from those of the untreated, heterozygous diploid. A total of 100 to 300 clones which had new auxotrophies were tested for all of the markers originally heterozygous in the parent strain, as well as for the phenotype of the unmapped mutation. Because the well-marked strains contained several mutations conferring the same auxotrophy (*his2*, *-6*, *-7*; *leu1*, *-2*; etc.), the clones picked up after MBC treatment were also tested by complementation to distinguish between these mutations. The frequency of appearance of each chromosome marker was between 0.5 and 5%. Finally, the number of times the unmapped mutant phenotype appeared or did not appear in clones that had also become auxotrophic for each chromosome marker was tabulated with a Heath H89 microcomputer. Correlations >90% or <10% for uncovering the unmapped mutant phenotype and a chromosome marker auxotrophy in the same clones were considered significant and indicative of chromosomal linkage.

RESULTS

A diploid strain was constructed that was heterozygous for recessive auxotrophic mutations marking most of the *S. cerevisiae* linkage groups and heterozygous for an unmapped mu-

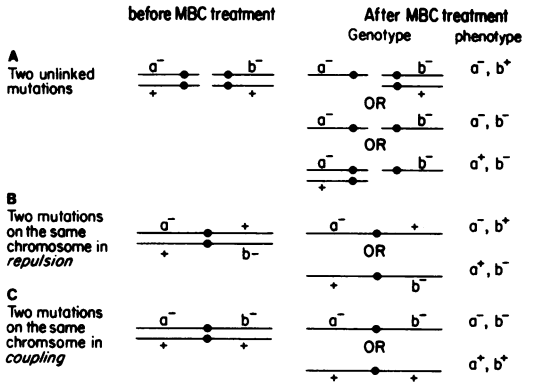

FIG. 1. Mapping genes by chromosome loss. The different types of clones which could be observed after MBC-induced chromosome loss for two heterozygous recessive mutations, depending on their linkage relationship, are shown.

tation. MBC-induced loss of a chromosome in such a diploid cell would make it hemizygous for a recessive auxotrophic mutation on the homolog of the lost chromosome, thus "uncovering" the phenotype of the mutation. The appearance of an unmapped recessive phenotype would be correlated with that of an auxotrophic marker on the same chromosome. Depending on whether the two mutations were in coupling or in repulsion in the heterozygous diploid, the correlation observed would be either positive (the two phenotypes always uncovered together) or negative (never uncovered together) (Fig. 1). Since MBC induces chromosome loss randomly and at equal frequencies for all chromosomes (12), the only correlations observed should be for genes located on the same chromosome.

The accuracy of this procedure for mapping genes to particular chromosomes was tested with two auxotrophic mutations whose chromosomal locations have been previously established: *ade6* and *his4* (Fig. 2 and 3). Both of these mutations were located in repulsion to the chromosomal markers used; hence, their linkage relationships were identified by negative correlations with particular chromosomal markers.

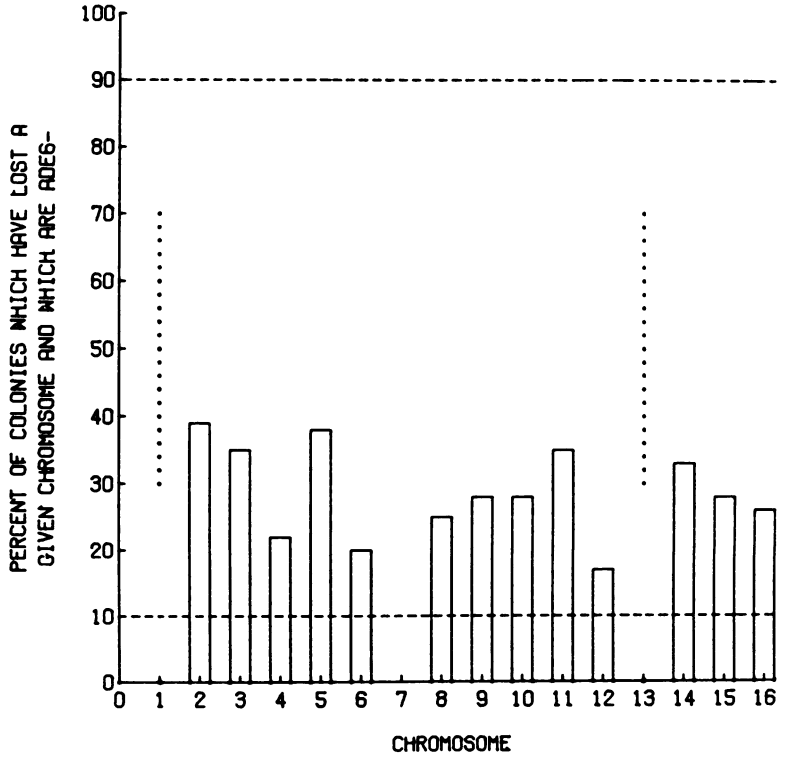

FIG. 2. Mapping *ade6*. A total of 2,500 MBC-treated clones of strain 6800 were screened for the appearance of *ade6* and recessive chromosome marker auxotrophies; 83 *ade6* colonies were found. The percentage of colonies that had uncovered a particular chromosome marker and which were *ade6* was tabulated for each chromosome and plotted as shown. Chromosomes I and XIII did not carry heterozygous markers, and therefore loss of either of these chromosomes was not scored (dotted lines). The 10 and 90% correlation levels considered significant (see text) are shown as dashed lines. The absence of colonies that were both *ade6* and *leu1* (the chromosome VII marker) indicated that *ade6* was on chromosome VII.

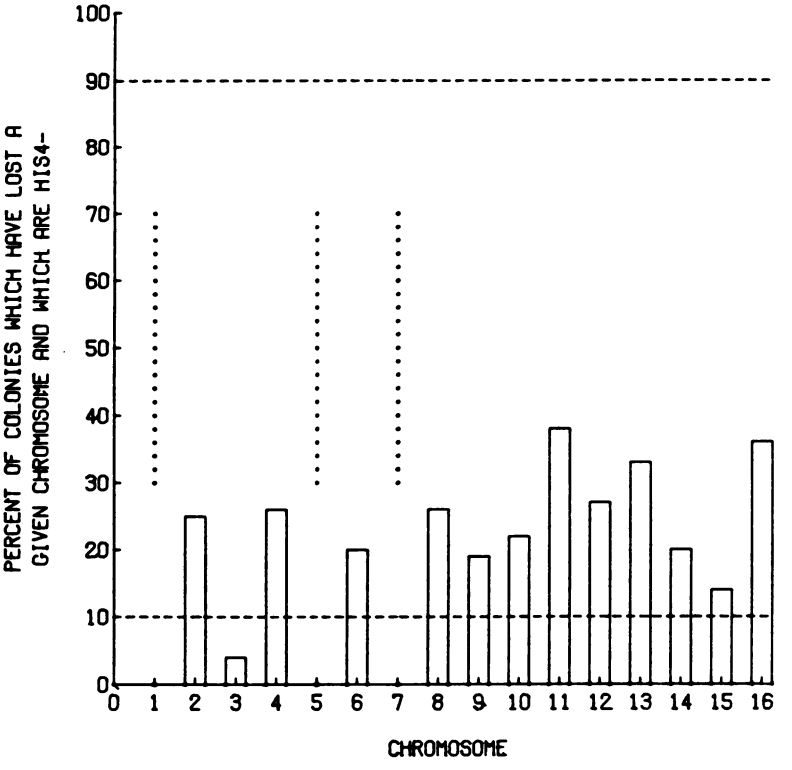

FIG. 3. Mapping *his4*. A total of 255 MBC-treated clones of strain 6830 which had uncovered *cyh2* were screened for the appearance of *his4*; 21 colonies were found. The percentage of colonies that had uncovered a particular chromosome marker and that were *his4* was plotted as in the legend to Fig. 2. Chromosome I did not carry a heterozygous marker, and therefore loss of this chromosome was not scored (dotted line). Since some, but not all, of the clones selected for resistance to cycloheximide (loss of chromosome VII) were *his4*, *his4* must not be on chromosome VII; the actual level of correlation observed between the appearance of *his4* and chromosome VII loss was not meaningful for chromosomal linkage (also shown as a dotted line). There were insufficient data for chromosome V (only one chromosome V loss colony found) to look for correlations between loss of chromosome V and appearance of *his4* (dotted line). The very low frequency of *his4* colonies which were also *leu2* (the chromosome III marker) indicated that *his4* was on chromosome III.

After MBC treatment of a heterozygous diploid, no colonies which were both *ade6* and *leu1* were seen, even though both were found separately many times. This result agrees with the known location of the *ade6* and *leu1* genes on chromosome VII (8). *his4* was verified to be on chromosome III in a similar manner: a strong negative correlation (<10%) was observed with *leu2* on chromosome III. The *his4* auxotrophic colonies were screened for among cells which had been first selected for resistance to cycloheximide (uncovering the *cyh2* allele). Although this preselection was done to facilitate scoring *nib1* in the same experiment (see below), it also reduced the number of MBC-treated colonies which needed to be screened for *his4* (see Discussion).

A test of the method that used a positive correlation for markers in coupling was performed with *arg1*. Occurrence of *arg1* displayed a strong positive correlation with appearance of *ade2* (>90%), the chromosome XV marker (Fig. 4). Both of these auxotrophic mutations have been mapped to chromosome XV (8).

Strain NH429-6A was originally thought to carry *lys9* on chromosome XIV and *met2* on chromosome XVII. However, the very strong positive correlation seen between *lys9*, the chromosome XIV marker, and *met2* (>95%; Fig. 5) suggested that these genes were both on the same chromosome. This has now been confirmed; markers previously identified as being on chromosome XVII are now known to be located on the left arm of chromosome XIV (S. Klapholtz and R. Esposito, submitted for publication).

From these results, I concluded that a positive or negative correlation for the joint appearance of recessive markers after MBC treatment indicated chromosomal linkage for the markers.

MBC-induced chromosome loss was used to

locate the unmapped mutation *nib1* (a "nibbled" colony morphology phenotype; 1). Scoring the *nib1* allele is most easily performed when the *nib1* gene and chromosome VII are at the same ploidy level (C. Holm, personal communication). Therefore, after MBC treatment cells were first selected for loss of chromosome VII (by uncovering resistance to cycloheximide [*cyh2*] on YEPD − cycloheximide plates). These clones, which were all *cyh2* and *leu1*, were then screened for appearance of the *nib1* allele by complementation testing (1). The *nib1* clones showed a negative correlation with *aro7* clones (Fig. 6), indicating that *nib1* was probably located on chromosome XVI. Meiotic mapping subsequently confirmed this; *nib1* was found to be tightly linked to *rad1* on the left arm of chromosome XVI (1).

DISCUSSION

These results demonstrate that chromosomal linkage of genes can be inferred from correlations between the appearance of two recessive markers after MBC treatment of a diploid strain initially heterozygous for these markers. The correlations observed were not absolute for several reasons. Aneuploid clones frequently grew slowly, making some of the auxotrophic markers difficult to score. Occasional mitotic recombination events changed the linkage relationships of the unmapped gene and the chromosomal markers. Finally, although the average frequency of loss for each chromosome was equal, random fluctuations about this average sometimes yielded an insufficient number of observations for particular chromosomes. Nevertheless, the observed correlations limited a gene to one or, at most, a few chromosomes, greatly reducing the amount of work needed to map it accurately with conventional tetrad analysis. Empirically, for a mutation located in repulsion to the chromosomal markers, between 25 and 75 colonies showing the phenotype of the unmapped mutation were sufficient to obtain a negative correlation (<10% joint appearance of the unmapped mutation and a chromosomal marker) and assign

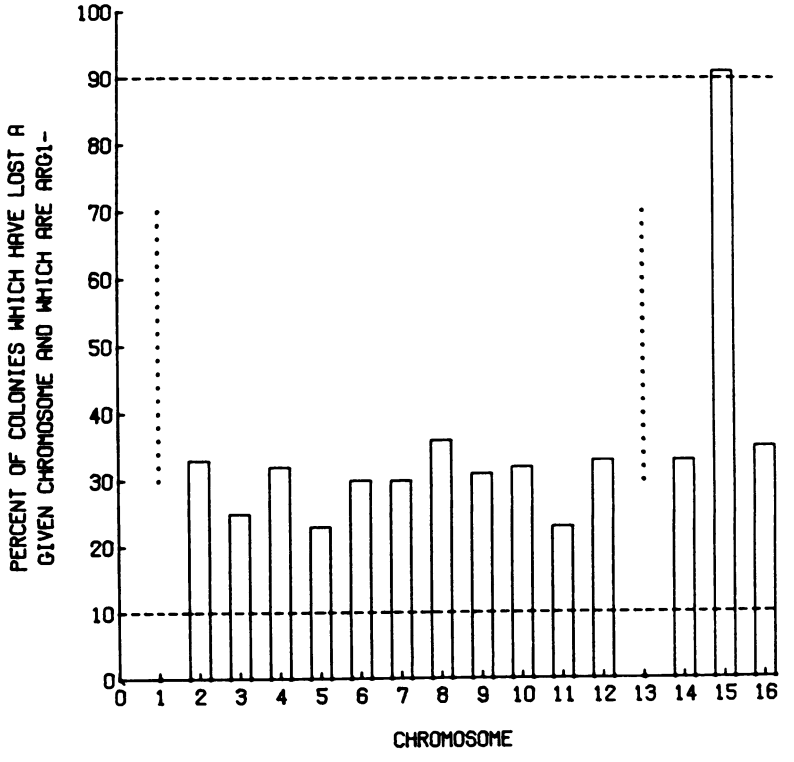

FIG. 4. Mapping *arg1*. A total of 2,500 MBC-treated clones of strain 6808 were screened for the appearance of *arg1* and recessive chromosome marker auxotrophies; 108 *arg1* colonies were found. The percentage of colonies that had uncovered a particular chromosome marker and which were *arg1* was plotted as in the legend to Fig. 2. Chromosomes I and XIII did not carry heterozygous markers, and therefore loss of either of these chromosomes was not scored (dotted lines). The very high frequency of *arg1* colonies which were also *ade2* (the chromosome XV marker) indicated that *arg1* was on chromosome XV.

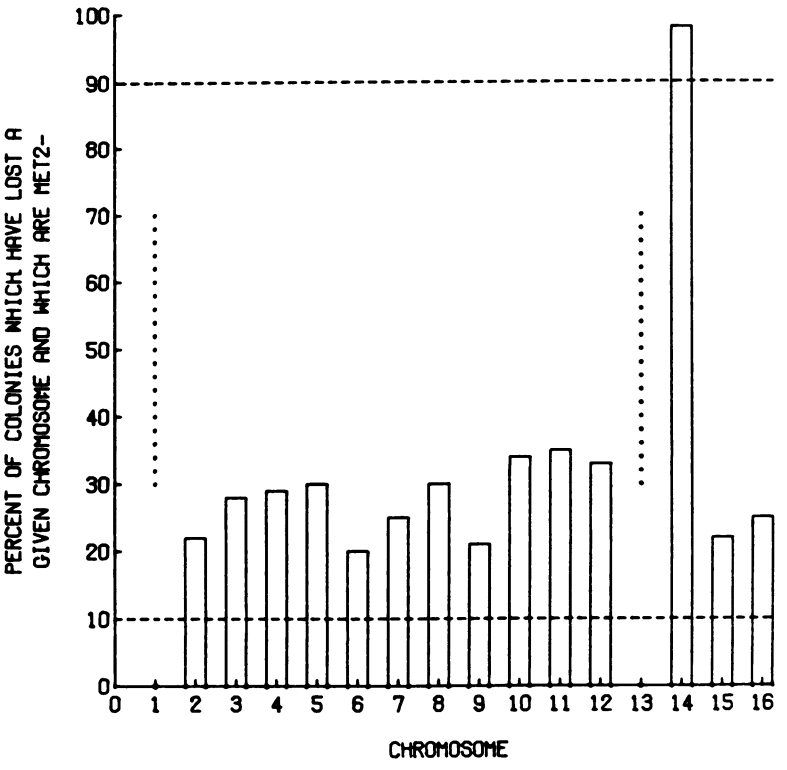

FIG. 5. Mapping *met2*. A total of 2,500 MBC-treated clones of strain 6808 were screened for the appearance of *met2* and recessive chromosome marker auxotrophies; 92 *met2* colonies were found. The percentage of colonies that had uncovered a particular chromosome marker and that were *met2* was plotted as in the legend to Fig. 2. Chromosomes I and XIII did not carry heterozygous markers, and therefore loss of either of these chromosomes was not scored (dotted lines). The very high frequency of *met2* colonies that were also *lys9* (the chromosome XIV marker) indicated that *met2* was on chromosome XIV.

the gene to a linkage group. For a mutation located in coupling to the chromosomal markers, fewer clones were required to detect a positive correlation (>90%) between an unmapped mutation and a chromosome marker. Since multiple loss events within a single cell occur more frequently than expected on the basis of independence (although which chromosomes are lost is random) (12), it was not possible to make any statistical calculation of the minimum number of clones necessary to obtain an unambiguous chromosome assignment.

Nonindependence of chromosome loss within a single cell also reduced the total number of colonies that had to be screened for the unmapped phenotype from the total required if events were independent. By using a drug resistance marker to first select cells that had already undergone at least one chromosome loss, the frequency of cells that had lost multiple chromosomes was increased. This result greatly increased the frequency of cells in which the unmapped gene and the chromosomal markers were uncovered. The total number of colonies screened for *his4* or *nib1*, which were first selected for loss of chromosome VII (uncovering *cyh2*), was 10-fold less than the number screened for the mapping of other genes, where a preselection for loss of one chromosome was not carried out (*ade6*, *arg1*, and *met2* in Fig. 2, 4, and 5).

A dominant mutation could be mapped with this technique in the same manner as a recessive mutation, except that one would look for a correlation between the loss of the dominant phenotype and uncovering of a recessive auxotrophy, mapping the location of the recessive, wild-type allele of the gene rather than the dominant, mutant allele.

Chromosome nondisjunction or loss, both spontaneous and induced, have been previously used as mapping tools in a number of organisms: a number of workers have used the chromosome instability of human-mouse hybrid cell lines to locate a large number of human genes (4, 9). In both *Aspergillus* and *Schizosaccharomyces*, both chromosome loss and mitotic haploidization induced by fluorophenylalanine have been

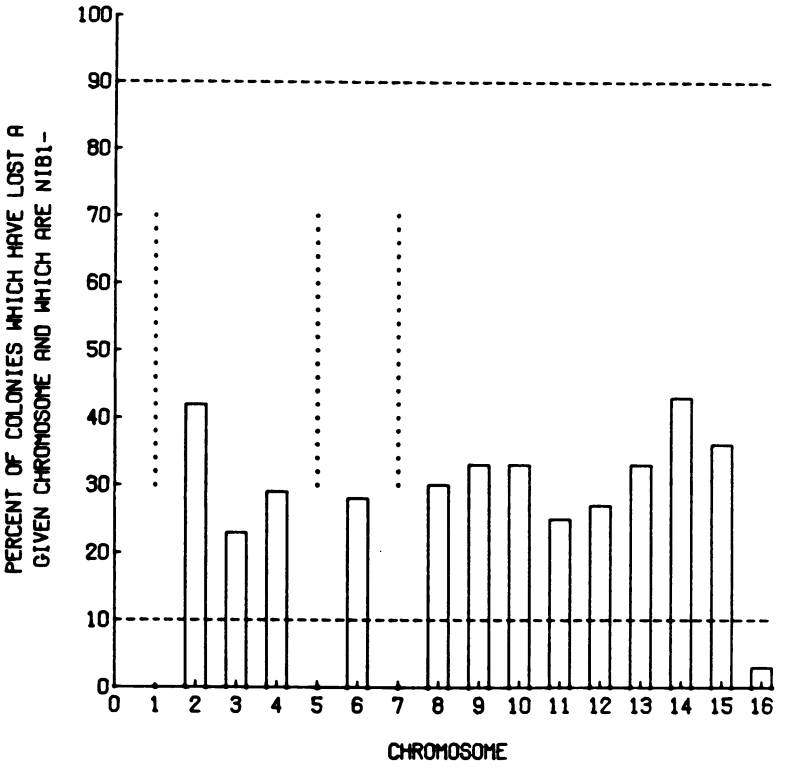

FIG. 6. Mapping *nib1*. A total of 255 MBC-treated clones of strain 6830 that had uncovered *cyh2* were screened for the appearance of *nib1* and recessive chromosome marker auxotrophies; 24 *nib1* colonies were found. The percentage of colonies that had uncovered a particular chromosome marker and that were *nib1* was plotted as in the legend to Fig. 2. Chromosome I did not carry a heterozygous marker, and therefore loss of this chromosome was not scored (dotted line). Since some, but not all, of the clones selected for resistance to cycloheximide (loss of chromosome VII) were *nib1*, *nib1* must not be on chromosome VII; the actual level of correlation observed between the appearance of *nib1* and chromosome VII loss was not meaningful for chromosomal linkage (also shown as a dotted line). There were insufficient data for chromosome V (only one chromosome V loss colony found) to look for correlations between loss of chromosome V and appearance of *nib1* (dotted line). The very low frequency of *nib1* colonies which were also *aro7* (the chromosome XVI marker) indicated that *nib1* was on chromosome XVI.

used extensively to assign mutations to linkage groups (2, 3). *cdc6* (Kawasaki, Ph.D. thesis, 1979) and *rad52* (D. Schild, personal communication) have been used in *Saccharomyces* to induce chromosome loss for mapping genes. A number of other methods for mapping genes in *Saccharomyces* that rely on mitotic recombination (7), triploid segregation patterns (11), disome analysis (6), or abolition of meiotic recombination (Klapholtz and Esposito, submitted for publication) have also been developed. These techniques have facilitated mapping new markers; however, most still require some tetrad analysis, or crossing the marker to be mapped into a particular genetic background. Unlike these methods, mapping with MBC does not rely on a homozygous mutation to cause chromosome loss and does not require extensive strain construction. Genes can be mapped in any genetic background; no crosses or dissections are necessary other than making a heterozygous diploid with one of the multiply marked strains available (Yeast Genetic Stock Center, Berkeley, Calif). Furthermore, the high frequency of multiple-loss events greatly reduces the amount of work necessary to map a mutation with a nonselectable phenotype.

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