Chromosomal Assignment of Mutations by Specific Chromosome Loss in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Yeast 2-μm plasmids were integrated near the centromere of a different chromosome in each of 16 cir^0 mapping strains of *Saccharomyces cerevisiae*. The specific chromosomes containing the integrated 2-μm plasmid DNA were lost at a high frequency after crossing the cir^0 strains to cir$^+$ strains. A recessive mutation in a cir$^+$ strain can then be easily assigned to its chromosome using this set of mapping strains, since the phenotype of the recessive mutation will be manifested only in diploids having the integrated 2-μm plasmid and the unmapped mutation on homologous chromosomes.

THE large number of chromosomes and the high frequency of meiotic recombination in yeast make it difficult to map a gene simply by tetrad analysis. Numerous procedures have been developed to circumvent these difficulties by first assigning a mutation to its chromosome (LIRAS *et al.* 1978; KAWASAKI 1979; WICKNER 1979; MORTIMER, CONTOPOULOU and SCHILD 1981; SCHILD and MORTIMER 1985; KLAPHOLZ and ESPOSITO 1982; WOOD 1982). Some of these methods rely on constructing a diploid heterozygous for the unmapped mutation and various chromosome markers and subsequently the random loss of chromosomes caused by chemical agents or certain mutations. However, most of these procedures require that the unmapped mutation be crossed into a particular yeast background. In addition, chromosome loss is nonspecific and multiple chromosome losses can occur, which further complicates the chromosomal assignment. This paper describes a new method for the chromosomal assignment of recessive mutations that does not require strain constructions and that involves the specific loss of individual chromosomes. The method relies on a set of cir^0 tester strains, each containing 2-μm plasmid DNA integrated at or near the centromere of a different chromosome. Plasmids containing the 2-μm inverted repeat sequence and either random or defined segments of yeast DNA were integrated into the yeast genome of cir^0 strains at the region of homology. The 2-μm plasmid DNA is stably maintained as an integrant because the plasmid DNA lacks the 2-μm *FLP* (flip) gene required for 2-μm site specific recombination and the cir^0 cells contain no resident 2-μm circles to provide the *FLP* function. Specific mitotic chromosome loss can be induced after a cir^0 × cir$^+$ cross. The *FLP* recombination function provided by the 2-μm circles of the cir$^+$ parent can recognize a site in the 2-μm DNA repeat sequence of the integrant and catalyze a site specific recombination event. This results in the loss of the integrant as well as chromosomal DNA distal to the site of integration. If the 2-μm plasmid DNA is integrated at or near the centromere, the entire chromosome is lost at a high frequency. These properties of chromosomes with integrated 2-μm plasmid DNA have been described (FALCO *et al.* 1982; FALCO, ROSE and BOTSTEIN 1983; FALCO and BOTSTEIN 1983).

A recessive mutation in a cir$^+$ strain can be assigned to its chromosome by crossing to the set of cir^0 mapping strains. Subclones of the cir^0/cir$^+$ diploids will lose the integrated 2-μm plasmid DNA plus the chromosome into which integration occurred at a high frequency. The recessive mutation will be expressed only in the diploid containing the cir^0 mapping strain with an integrant at the centromere of the chromosome that is homologous to the chromosome that the mutation is on. The mutation will remain heterozygous in all of the other diploid strains. Once a mutation has been assigned to its chromosome, the location on the chromosome can be determined by conventional meiotic analysis.

MATERIALS AND METHODS

Genetic methods: Key yeast strains and plasmids used in this study are listed, respectively, in Tables 1 and 2. The media and genetic procedures including genetic crosses, sporulation, dissection, tetrad analysis and the scoring of nutritional markers have been described (SHERMAN, FINK and HICKS 1987). The medium containing 5-fluoroorotic acid (1 mg/ml) is described by BOEKE, LACROUTE and FINK (1984).

Molecular biology methods: Methods used in the construction of plasmids, including restriction enzyme digests, separation of plasmid DNA and restriction fragments on agarose gels, ligation of DNA fragments, and the isolation of plasmid DNA are described in MANIATIS, FRITSCH and

TABLE 1

Yeast strains

Strain	Genotype	Source
3482-16-1	*cir⁰ MATa ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R	D. LIVINGSTON
B-7926	*cir⁺ MATα cdc28-1 cyc1-72 glc1 lys2-187 tyr1*	This study
B-8013	*cir⁰ MATα ura3-52 leu2-3,112 trp1-289 met2*	This study
K382-19D	*cir⁺ MATα ura3 can1 cyh2 ade2 his7 hom3 tyr1 spo11*	KLAPHOLZ and ESPOSITO (1982)
K381-9D	*cir⁺ MATα ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1 spo11*	KLAPHOLZ and ESPOSITO (1982)
K393-35C	*cir⁺ MATα ura3 his2 leu1 lys1 met4 pet8 spo11*	KLAPHOLZ and ESPOSITO (1982)
K396-22B	*cir⁺ MATα ura3 ade1 his1 leu2 lys7 met3 trp5*	KLAPHOLZ and ESPOSITO (1982)
GT153-6A	*cir⁺ MATα ura3 ade1 ade2 arg4 aro7 asp5 gal7 his2 his6 ilv3 leu1 leu2 lys7 lys9 met2 met14 trp1*	CAMBELL *et al.* (1981)
A298-61D	*cir⁺ MATα ade8 arg4 aro1C leu2-3 pet2 rna3 trp4* Thr⁻ Ura⁻	GABER *et al.* (1983)
MC-301	*cir⁺ MATα ade5 cry1-13 his4-15 ino1-13 ino4-8 leu2 lys2 MAL2 thr4*	M. CULBERTSON

SAMBROOK (1982). Yeast transformations were performed by the spheroplasting method (BEGGS 1982) and *Escherichia coli* transformations were performed with the CaCl method (COHEN, CHANG and HSU 1972).

RESULTS

A set of cir⁰ 2μ mapping strains was constructed by integrating derivatives of the YEp24 plasmid (BOTSTEIN *et al.* 1979) at or near the centromere of each yeast chromosome in a cir⁰ strain. The derivatives of the YEp24 plasmid contained the *URA3*⁺ gene, the 2-μm circle DNA repeat sequence, pBR322 sequences and different yeast segments which correspond to regions at or near the centromere of each of the 16 chromosomes. The yeast DNA segments were obtained from either a random yeast DNA bank (CARLSON and BOTSTEIN 1982) or various cloned yeast DNA fragments encompassing centromeric regions or genes which map close to certain centromeres, as summarized in Table 2.

Integration of 2-μm plasmid DNA containing random yeast DNA sequences: Mapping strains for chromosomes *II*, *III*, *VII*, *VIII*, *IX*, *XI* and *XV* were constructed by transforming the cir⁰ strain, 3482-16-1 with the YEp24 plasmid library containing inserts of random yeast DNA segments (CARLSON and BOTSTEIN 1982). *URA3*⁺ transformants were selected on medium lacking uracil, and screened by first plating on nonselective medium, and then spotting on 5-fluoroorotic acid (FOA) medium. FOA medium inhibits *URA3*⁺ cells, but allows growth of *ura3*⁻ cells (BOEKE, LACROUTE and FINK 1984). Those transformants which contained 2-μm plasmid DNA integrated into the genome, about 1%, were unable to grow on FOA medium, since the 2-μm plasmid DNA containing the *URA3*⁺ gene remained stably integrated in the genome in cir⁰ cells. However, confluent growth on FOA medium is observed for transformants containing autonomously replicating 2-μm plasmids, which are unstable and lost at a high frequency in cir⁰ cells. Out of 6183 *URA3*⁺ transformants, 46 contained 2-μm plasmid DNA stably integrated into the genomes. These 46 transformants were further characterized by identifying the chromosome into which the 2-μm plasmid DNA was integrated in each strain. Each transformant was crossed to the following cir⁺ multiply marked mapping strains containing recessive auxotrophic mutations linked to the centromeres of all 16 chromosomes: K382-19D, K381-9D, K393-35C, K396-22B, GT153-6A, MC-301 and A298-61D. Diploids from these crosses were isolated on selective medium, the diploids were plated on nonselective medium at a concentration resulting in 50–100 colonies per plate, and then assayed on various types of omission media for the expression of auxotrophic markers. The chromosome into which integration had occurred was identified for a particular transformant when an auxotrophic marker was expressed. Since cir⁰/cir⁺ diploids will lose the integrated 2-μm plasmid DNA plus all or part of the chromosome containing the integrant at a high frequency, an auxotrophic marker will only be expressed in a diploid containing a transformant with 2-μm plasmid DNA integrated in the homologous chromosome. Of the 46 transformants characterized by this method, 7 were found to have integrations in the following chromosomes: *II*, *VII*, *VIII*, *IX* (2), *XI* and *XV* (Table 2). The remaining transformants were not identified because no auxotrophic markers were expressed in diploids containing these strains. This suggests that integration in these strains did not occur near the centromere. In this case, the loss of the 2-μm plasmid DNA would not destabilize the entire chromosome at high frequency, but only chromosomal sequences distal to the point of integration. If integration occurred near a telomere or on a chromosomal arm which is not well marked by auxotrophic mutations, the chromosome carrying the integrant would not be identified.

The transformation of strain 3482-16-1 using the random DNA bank also allowed for the direct selection of a strain with an integrant near the centromere on chromosome *III*. Transformants complementing the *leu2-3, 112* mutation were selected on medium

lacking both leucine and uracil. These transformants were tested on medium containing FOA to detect a strain with 2-μm plasmid DNA stably integrated at the *LEU2* gene near the centromere of chromosome *III*.

Integration of 2-μm plasmid DNA containing cloned yeast centromeric-linked DNA sequences: Mapping strains for chromosomes *I*, *IV*, *V*, *VI*, *X*, *XII*, *XIII*, *XIV* and *XVI* were constructed using the defined yeast DNA sequences shown in Table 2. The 2-μm DNA containing plasmids ZA50 (TEEM and ROSBASH 1983) and pYE51B (ABOVICH and ROSBASH 1984), which contain clones of the *RP51A* and *RP51B* genes, respectively, were used to transform strain 3482-16-1. Integrants of the plasmid ZA50 containing the *RP51A* gene integrated into chromosome *XIII*, while integrants of the plasmid pYE51B containing the *RP51B* gene integrated into chromosome V. All other defined sequences were transferred into the plasmid pAB659 which was constructed by replacing the *Eco*RI-*Hin*dIII fragment of YIp5 with the 2.1-kb *Eco*RI-*Hin*dIII 2-μm B fragment containing the 2-μm DNA repeat sequence. The *Hin*dIII-*Hin*dIII centromeric regions for chromosomes *I*, *IV*, *X*, *XIV* and *XVI* were transferred from plasmids p181, p113, p180, p203 and p179, respectively (HIETER *et al.* 1985; P. HIETER, personal communication), to the *Hin*dIII site of pAB659, resulting in, respectively, the plasmids pAB660, pAB661, pAB662, pAB663 and pAB664. Also fragments encompassing the genes *GCN4*, *SUP11-1* and *PPR1* were inserted into pAB649. A 4.5–5.0-kb *Bam*HI-*Hin*dIII fragment containing the *GCN4* gene was transferred from pAH20 (HINNEBUSCH and FINK 1983) and ligated in the *Bam*HI and *Hin*dIII sites of pAB649, resulting in pAB665. Part of the *PPR1* gene contained in a 2.5-kb *Eco*RI-*Eco*RI fragment (LILJELUND *et al.* 1984; F. LACROUTE, personal communication) was transferred into the *Eco*RI site of pAB645, resulting in pAB666. The plasmid pAB667 was constructed by replacing the 4.0-kb *Sal*I-*Nde*I fragment of pAB645 with the 5.4-kb *Sal*I-*Nde*I fragment of p179 (HIETER *et al.* 1985). The two fragments are the same except the fragment from p179 also contains a 1.35-kb fragment encompassing the *SUP11-1* gene.

Plasmids ZA50, pYE51B, pAB660, pAB661, pAB662, pAB663, pAB664, pAB665, pAB666 and pAB667 were used to transform strain 3482-16-1 to uracil prototrophy. Transformants, in which plasmids had stably integrated into the genome, were selected by first enriching for integrants. Transformants from each transformation were pooled in lots containing about 100 transformant colonies and each lot was used to inoculate an overnight culture in YPD nonselective medium. Samples were diluted and plated on medium lacking uracil to select for *URA3*$^+$ transformants. Ten *URA3*$^+$ colonies were isolated from each overnight culture, subcloned on a nonselective medium, then plated on medium containing FOA to select for transformants which contained integrated 2-μm plasmid DNA. The stable integrants isolated from transformations with each of the plasmids described above were crossed to strains K382-19D, K381-9D, K393-35C, K396-22B and A298-61D. A diploid from each cross was isolated, subcloned on a nonselective medium and then diluted and plated on a nonselective medium at a concentration to produce 50–100 colonies per plate. These plates were replica-plated on various omission media to test that only auxotrophic markers on the same chromosome containing the integrated 2-μm plasmid DNA were expressed.

Genetic analysis of 2μ mapping strains: The strains containing integrated 2-μm plasmid DNA were crossed to the cir^0 strain, B-8013; the diploids were sporulated and dissected, and the tetrads from each cross analyzed. The *URA3*$^+$ marker segregated 2:2 in all crosses, consistent with the view that the 2-μm plasmid DNA (*URA3*$^+$) is stably integrated into the yeast genome. A set of *MAT***a** and *MATα* 2μ mapping strains were chosen from among the tetrads of crosses with B-8013, as shown in Table 3. The strains B-7179 and B-7590 are separate strains which contain 2-μm plasmid DNA integrated at different sites in chromosome *V*. Similarly, the strains B-7175 and B-7176 contain 2-μm plasmid DNA integrated at different sites in chromosome *IX*. The strains B-7603 and B-7607 are the *MATα* equivalents of B-7590 and B-7175, respectively.

The frequency at which the integrated 2-μm plasmid DNA is lost from the genome in each of the 2μ mapping strains was determined by crossing each strain to the *cir*$^+$ *ura3*$^-$ strain K381-9D and assaying for the percentage of Ura$^-$ diploid subclones. The loss of the integrated 2-μm plasmid DNA also results in the loss of the *URA3*$^+$ gene contained in the plasmid DNA. The 2μ mapping strains lose the integrated 2-μm plasmid DNA at frequencies ranging from 10 to 50% (Table 2), and lose the entire chromosome containing the integrated 2-μm plasmid DNA at frequencies ranging from 1 to 50% (Figure 1). The variation in the frequency of chromosomal loss in the different mapping strains may be due to the difference in the sites of integration on different chromosomes. In addition, the frequency of chromosomal loss of each mapping strain can also vary somewhat when they are crossed to different cir$^+$ strains (data not shown).

Each mapping strain was examined for its efficiency in the chromosomal assignment of mutations along the entire length of the chromosome containing the integrated 2-μm plasmid. The strains were crossed to 28 multiply marked mapping strains, some of which are listed in Table 1 and which included the 56

TABLE 2

Plasmids integrated into the 2μ mapping strains and the frequencies of loss of the plasmid DNA

Yeast strain	Integrated plasmid: Chromosome	Plasmid: No.	Plasmid: Reference	Source of yeast segment: Segment	Source of yeast segment: Plasmid	Source of yeast segment: Reference	Frequency of plasmid loss (% *ura3*⁻)
B-7588	*I*	pAB660	This study	*CEN1*	p181	HIETER *et al.* (1985)	44
B-7170	*II*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	18
B-7171	*III*	Not recovered		*LEU2*	DNA bank	CARLSON and BOTSTEIN (1982)	20
B-7589	*IV*	pAB661	This study	*CEN4*	p113	HIETER *et al.* (1985)	40
B-7590	*V*	pAB665	This study	*GCN4*	YpAH20	HINNEBUSCH and FINK (1983)	21
B-7179	*V*	pYE51B	ABOVICH and ROSBACH (1984)	*RP51B*	pYE51B	ABOVICH and ROSBASH (1984)	12
B-7591	*VI*	pAB667	This study	*SUP11-1*	p179	HIETER *et al.* (1985)	34
B-7173	*VII*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	33
B-7174	*VIII*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	17
B-7175	*IX*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	18
B-7176	*IX*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	11
B-7593	*X*	pAB662	This study	*CEN10*	p180	HIETER *et al.* (1985)	25
B-7178	*XI*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	12
B-7595	*XII*	pAB666	This study	*PPR1*	puc8-ppr1	LILJELUND *et al.* (1984)	48
B-7255	*XIII*	ZA50	TEEM and ROSBASH (1983)	*RP51A*	ZA50	TEEM and ROSBASH (1983)	50
B-7596	*XIV*	pAB663	This study	*CEN14*	p203	HIETER *et al.* (1985)	45
B-7180	*XV*	Not recovered		Undefined	DNA bank	CARLSON and BOTSTEIN (1982)	12
B-7598	*XVI*	pAB664	This study	*CEN16*	p179	HIETER *et al.* (1985)	43

markers shown in Figure 1. Diploids from each cross were assayed for the frequency at which various recessive auxotrophic mutations which map on both arms of a chromosome were expressed. A summary of the frequencies at which mutations along each chromosome arm are expressed in the appropriate mapping strain are shown in Figure 1. In most cases, the strains express mutations on both arms of the chromosome containing the integrated 2-μm plasmid DNA. The frequency of loss of the left arms of chromosomes *VIII* and *XII*, in strains B-7174 and B-7595, respectively, were not determined because easily scored recessive-mutations on these two chromosome arms were not available. In general, strains with an integration very near the centromere lose both arms of the chromosome containing the integrant at a similar frequency (*e.g.*, B-7589, B-7590, B-7591, B-7593, B-7596 and B-7598). We believe both arms of chromosome *I* are lost at a frequency of loss of 10 to 50% in B-7588, even though only the *ade1* marker was tested; the *ade1* marker maps on the right arm of chromosome *I*, closely linked to the centromere. Strains with an integration, which is not as near to the centromere, tend to lose the chromosomal arm containing the integrated DNA at a higher frequency than the opposite arm. For example, *ura3*⁻ diploids from crosses of B-7171 and *cir*⁺ *ura3*⁻ *leu2*⁻ strains, were always *leu2*⁻, since the integrated 2-μm plasmid in B-7171, which integrated at the *LEU2* gene (left arm of chromosome *III*), contained both the *URA3*⁺ and *LEU2*⁺ genes. All *leu2*⁻ diploids from a cross of B-7171 and MC-301 were *his4*⁻ (left arm of chromosome *III*), while only 15% were both *his4*⁻ and *thr4*⁻ (right arm of chromosome *III*). This also suggests that mutations on the same chromosome arm and closely linked to the site containing the 2-μm plasmid are lost at the same frequency as the integrated DNA. Mutations which are on the same chromosome arm, but further from the site of integration, may be lost at lower frequencies. In crosses of B-7170 and B-7926, 32 out of 294 diploids were *lys2*⁻ *cdc28*⁻ *tyr1*⁻; 4 diploids were *lys2*⁻ *cdc28*⁻ *TYR1*⁺ and 1 diploid was *lys2*⁻ *CDC28*⁺ *TYR1*⁺. Although the exact site of integration of the 2-μm plasmid is unknown in this strain, it probably lies between the centromere and the *LYS2* gene since the integration must be close enough to the centromere to result in both chromosome arms being lost.

TABLE 3

2μ mapping strains

Strain[a]	Insert	Genotype
B-7588	CHR1::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 met2*
B-7170	CHR2::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7171	CHR3::*URA3*$^+$ *LEU2*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7589	CHR4::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7590	CHR5::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7179	CHR5::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7591	CHR6::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2*
B-7173	CHR7::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7174	CHR8::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7175	CHR9::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7176	CHR9::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7593	CHR10::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2*
B-7178	CHR11::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7595	CHR12::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 met2* Cyh^R
B-7255	CHR13::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7596	CHR14::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7180	CHR15::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7598	CHR16::*URA3*$^+$	*cir*^{0} *MAT***a** *ura3-52 leu2-3,112 trp1-289 met2* Cyh^R
B-7599	CHR1::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 met2*
B-7600	CHR2::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7601	CHR3::*URA3*$^+$ *LEU2*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7602	CHR4::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7603	CHR5::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7604	CHR6::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7605	CHR7::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7606	CHR8::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7607	CHR9::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7608	CHR10::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7609	CHR11::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7610	CHR12::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 met2* Cyh^R
B-7611	CHR13::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7612	CHR14::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 met2*
B-7613	CHR15::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 his3-Δ1 met2* Cyh^R
B-7614	CHR16::*URA3*$^+$	*cir*^{0} *MATα ura3-52 leu2-3,112 trp1-289 met2* Cyh^R

[a] These strains have been deposited with the Yeast Genetic Stock Center (Donner Laboratory, University of California, Berkeley, California 94720).

In the studies described above with *cir*$^+$ *ura3*$^-$ *mut*$^-$ strains, the following classes of diploids were found: *URA3*$^+$ *MUT*$^+$; *ura3*$^-$ *MUT*$^+$; and *ura3*$^-$ *mut*$^-$ (where *mut*$^-$ denotes various auxotrophic mutations). Only auxotrophic mutations on the chromosome homologous to the 2-μm plasmid containing chromosome were expressed. In addition, no *URA3*$^+$ *mut*$^-$ diploids were found and no auxotrophic mutations on chromosomes nonhomologous to the 2-μm plasmid containing chromosome were expressed (<0.1%). Thus, it is possible to enrich for *mut*$^-$ diploids by first selecting for diploids which are *ura3*$^-$.

Mapping procedure: A hypothetical recessive mutation, *mut*$^-$, in a cir$^+$ strain is crossed to each of the cir^0 2μ mapping strains of opposite mating type listed in Table 3. A diploid for each of the 16 cir^0 × cir$^+$ crosses is isolated on selective medium, and then subcloned on nonselective YPD medium. An isolated subclone is diluted and plated on nonselective YPD medium at a concentration resulting in about 50–100 colonies per plate. Several hundred colonies of each isolated diploid are replica-plated onto a medium which detects the phenotype of the unmapped mutation, *mut*$^-$. The recessive *mut*$^-$ mutation will only be manifested in the diploid having the integrated 2 μm plasmid and the *mut*$^-$ mutation on homologous chromosomes. A recessive mutation should be expressed at a frequency of between 1 to 50%, depending on both the particular chromosomal mapping strain used and the position of the mutation on the chromosome as shown in Figure 1. The frequency at which the *mut*$^-$ mutation is manifested can be increased substantially if the strain containing the *mut*$^-$ mutation is also *ura3*$^-$ and if only *ura3*$^-$ diploids are tested. Diploid isolates, which have lost the integrated 2-μm plasmid, can be first selected on FOA medium, and then tested for expression of the unmapped mutation. In addition, mutations on chromosome *IX* can be assigned to either the left or right chromosome arm by using both strains B-7175 and B-7176. In diploids containing B-

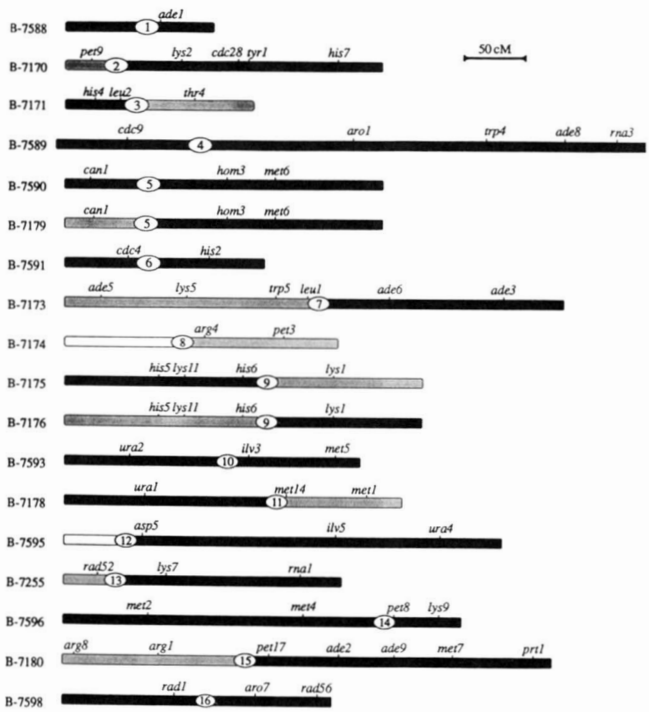

FIGURE 1.—The frequency of expression of auxotrophic markers located along the arms of each chromosome in each of the 2μ mapping strains listed on the left of the figure. The 2μ mapping strains were crossed to 28 multiply marked strains and the diploids from each cross were assayed for expression of the various auxotrophic markers shown. The numbered ellipses represent chromosomal centromeres. The frequencies at which the various mutations were expressed are denoted as follows: solid areas denote a frequency of 10–50%; stippled areas denote a frequency of 1–10%; and open areas denote untested regions of the chromosome.

7175, mutations on the left arm of chromosome *IX* are expressed at frequencies greater than 10%, while mutations on the right arm are expressed at frequencies of less than 10%. In contrast, in diploids containing B-7176, mutations on the left and right arm of chromosome *IX* are expressed at frequencies of less than and greater than 10%, respectively.

After assigning the *mut*$^-$ mutation to its chromosome, its exact location on the chromosome can be determined by conventional meiotic analysis with genes whose mapping position on the chromosome is known.

Chromosomal assignment of unmapped mutations by the 2μ mapping method: Several different laboratories have successfully used the 2μ mapping strains described in this paper to assign previously unmapped mutations to their chromosome. The mutation *ptl1* was assigned to chromosome *XV* using the 2μ mapping strains, since only a cross of B-7180 with a strain containing *ptl1* yielded temperature sensitive *ptl1*$^-$ diploid subclones. Its position on chromosome *XV* was confirmed by meiotic mapping which showed that the *PTL1* gene maps about 25 cM distal to the *HIS3* gene (J. TOYN, personal communication; TOYN *et al.* 1988). A *pet54* mutant was assigned to chromosome *VII* because about 21% of the diploid colonies from a cross of *pet54* with B-7605 were Pet$^-$. Meiotic mapping confirmed that the *PET54* gene maps on chromosome *VII*, 3.2 cM distal to *SUF15* (M. COSTANZO, personal communication; COSTANZO, SEAVER and FOX 1989). The *PMR1* gene was also assigned to chromosome *VII* using the 2μ mapping method. This chromosomal assignment was confirmed by hybridization of the cloned *PMR1* gene to a chromosomal blot prepared by OFAGE and by meiotic mapping of the *PMR1* gene, which located it 8.2 cM distal to *LYS5* (RUDOLPH *et al.* 1989). In addition, the *CYS2* and *CYS4* genes, which are closely linked to each other (ONO *et al.* 1988), were assigned to chromosome *VII* since a *cys2*$^-$ *cys4*$^-$ double mutation was manifested only in diploids constructed with B-7173. Subsequent mapping localized the *CYS2* and *CYS4* genes on the right arm of chromosome *VII*, centromere proximal to *ADE3* (B. ONO, personal communication).

DISCUSSION

The 2μ mapping method described in this paper is a rapid and efficient procedure for the chromosomal assignment of unmapped recessive mutations. Compared to other chromosomal assignment procedures, the 2μ mapping method has several advantages. First, each 2μ mapping strain can be induced to specifically lose a particular chromosome. This eliminates ambiguities due to multiple chromosome loss events which occur in other chromosomal assignment methods relying on mitotic chromosome loss (LIRAS *et al.* 1978; KAWASAKI 1979; MORTIMER, CONTOPOULOU and SCHILD 1981; SCHILD and MORTIMER 1985; WOOD 1982). Secondly, the 2μ method does not require preliminary strain constructions to couple required alleles with the unmapped mutation (LIRAS *et al.* 1978; KAWASAKI 1979; MORTIMER, CONTOPOULOU and SCHILD 1981; SCHILD and MORTIMER 1985; KLAPHOLZ and ESPOSITO 1982). The 2μ mapping method is most useful for genes which have not been cloned. A cloned gene can be conveniently assigned to its chromosome by hybridization to electrophoretically separated chromosomes or by mapping methods which involve either chromosome fragmentation (VOLLRATH *et al.* 1988) or the integration of a 2-μm plasmid containing a cloned yeast fragment into the yeast genome (FALCO and BOTSTEIN, 1983).

Each 2μ mapping strain loses the arms of the chromosome containing the integrated 2-μm plasmid DNA at frequencies ranging from 1 to 50% (Figure 1). The frequency of loss depends both on the mapping strain and the chromosomal arm. Mapping strains, which were constructed by targeting the 2-μm plasmid DNA to integrate very near the centromere, as in the strains B-7588, B-7589, B-7590, B-7591, B-7593, B-7596 and B-7598, resulted in strains in which both arms of

the particular chromosome were lost at a similar frequency ranging from 10 to 50%. The strains constructed using a random yeast DNA bank (*e.g.*, B-7170, B-7171, B-7173, B-7174, B-7175, B-7176, B-7178 and B-7180) lose one chromosome arm at a higher frequency than the other. This suggests that integration in these strains occurred further from the centromere than in the strains which lose both arms at a similar frequency. Even though the exact sites of integration in these strains are unknown, the sites were sufficiently close to the centromere so that both chromosome arms were still lost. Since integration occurred at the *LEU2* gene, which is located on the left arm of chromosome *III*, and because strain B-7171 loses the left chromosome arm at a higher frequency than the right arm, the chromosome arm which is lost at a higher frequency apparently contains the integrated 2 μm plasmid DNA. Presumably, the 2-μm plasmid DNA was integrated into the left and right arm of chromosome *IX* in, respectively B-7175 and B-7176. The results with strains B-7179 and B-7255, which were constructed by integrating 2-μm plasmids containing the *RP51B* and *RP51A* genes, respectively, suggests that the *RP51B* and *RP51A* genes are located on the right arms of, respectively, chromosome *V* and chromosome *XIII*.

The 2μ mapping method has been successfully used to show that the gene *PTL1* (TOYN *et al.* 1988); and the genes *PET54* (COSTANZO, SEAVER and FOX 1989), *PMR1* (RUDOLPH *et al.* 1989), *CYS2* and *CYS4* (ONO *et al.* 1988) map on chromosomes *XV* and *VII*, respectively. Subsequent meiotic mapping confirmed these assignments. Since *PET54* (COSTANZO, SEAVER and FOX 1989), *CYS2* and *CYS4* (ONO *et al.* 1988) are located on the right arm and *PMR1* (RUDOLPH *et al.* 1989) is located on the left arm of chromosome *VII*, this method has been used to assign genes which are located on either arm of a chromosome.

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