A NEW MAPPING METHOD EMPLOYING A MEIOTIC REC⁻ MUTANT OF YEAST[1]

SUE KLAPHOLZ[2] AND ROCHELLE EASTON ESPOSITO[2, 3]

Department of Biology[2], and Committee on Genetics,[3] University of Chicago, Chicago, Illinois 60637

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ABSTRACT

A rapid new mapping method has been developed for localizing a dominant or recessive mutation to a particular chromosome of yeast. The procedure utilizes the ability of strains homozygous for the *spo11-1* mutation to undergo chromosome segregation without appreciable recombination during sporulation. The level of sporulation in *spo11-1/spo11-1* diploids is reduced and asci are often immature or abnormal in appearance; spore viability is less than 1%. The first step of the mapping procedure is the construction of a haploid *spo11-1* strain carrying a recessive drug-resistance marker and the unmapped mutation(s). This strain is crossed to a set of three *spo11-1* mapping tester strains containing, among them, a recessive marker on each chromosome. The resulting *spo11-1/spo11-1* diploids are sporulated and plated on drug-containing medium. Viable meiotic products that express the drug-resistance marker due to chromosome haploidization are selectively recovered. These meiotic products are haploid for most, but generally not all, chromosomes. The level of disomy for individual chromosomes averages 19%. Each of the recessive chromosomal markers is expressed in approximately a third of the drug-resistant segregants. Ninety-eight percent of these segregants show no evidence of intergenic recombination. Thus, two markers located on the same chromosome, but on different homologs, are virtually never expressed in the same drug-resistant clone. The utility of this mapping procedure is demonstrated by confirming the chromosomal location of seven known markers, as well as by the assignment of a previously unmapped mutation, *spo12-1*, to chromosome *VIII*. In addition, the analysis of the products of *spo11-1* meiosis indicates that several markers previously assigned to either chromosome *XIV* or chromosome *XVII* are actually on the same chromosome.

THE most recent genetic map of *Saccharomyces cerevisiae* is composed of 17 linkage groups containing over 300 mapped genes (MORTIMER and SCHILD 1980, 1981). There remain numerous sparsely-marked regions and three groups of linked markers, termed fragments, which have not been assigned to a particular chromosome. Because of the large number of chromosomes in yeast, a rapid method for determining the chromosome on which an unmapped muta-

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tion resides is highly desirable. Current procedures for locating a gene to a chromosome include, in addition to standard tetrad analysis, meiotic trisomic analysis (MORTIMER and HAWTHORNE 1973; WICKNER 1979), chromosome transfer (DUTCHER 1981), mitotic recombination (NAKAI and MORTIMER 1969) and mitotic chromosome loss or nondisjunction (KAWASAKI 1979; MORTIMER, CONTOPOULOU and SCHILD 1981). Once a gene has been mapped to a particular chromosome, meiotic tetrad analysis, random spore analysis or mitotic mapping is used to localize the gene relative to other markers and the centromere (see MORTIMER and SCHILD 1981 for review). In this paper, we describe a new and relatively rapid method for localizing an unmapped dominant or recessive mutation to a specific chromosome. This method utilizes the haploidization-without-recombination sporulation phenotype of strains homozygous for the *spo11-1* mutation.

The recessive *spo11-1* mutation, isolated by ESPOSITO and ESPOSITO (1969), causes a reduction in both sporulation and spore viability. Diploids homozygous for *spo11-1* undergo premeiotic DNA synthesis (C. GIROUX, personal communication; S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, unpublished) and complete both meiotic divisions (ESPOSITO and ESPOSITO 1973, 1974; MOENS *et al.* 1977). The asci formed are often immature or abnormal in appearance, and the spores largely inviable.

Several lines of evidence have indicated that *spo11-1* is a meiotic Rec$^-$ mutation. First, when genetically marked sporulating cells are transferred to the appropriate selective medium, no increase in the recovery of intragenic recombinants is observed above the mitotic level (S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, unpublished). Second, rare viable meiotic products can be selectly recovered by plating a sporulated *spo11-1/spo11-1* diploid, heterozygous for a recessive drug-resistance marker, on drug-containing medium (S. KLAPHOLZ, unpublished; this study). The resulting drug-resistant colonies are haploid for most, but usually not all, chromosomes. Among these colonies, intergenic recombinants are infrequent. Thus, markers on the same chromosome show almost 100% linkage, regardless of their distance apart. Third, the meiotic Rec$^-$ phenotype conferred by *spo11-1* has been confirmed by analysis of *spo11-1/spo11-1 spo13-1/spo13-1* double mutant diploids (KLAPHOLZ 1980). The presence of the *spo13-1* mutation alone permits diploid cells to enter meiosis and execute recombination at meiotic levels. The cells then undergo a single, generally equational division, resulting in the formation of two diploid spores (KLAPHOLZ and ESPOSITO 1980a, b). In contrast to single mutant *spo11-1/spo11-1* strains, double mutant *spo11-1/spo11-1 spo13-1/spo13-1* diploids produce highly viable diploid spores. Intragenic and intergenic recombination is absent or greatly reduced in these viable meiotic products (KLAPHOLZ 1980; S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, unpublished).

In this report, we describe the rationale behind the *spo11* mapping procedure and present mapping data for several known markers and one previously unmapped mutation. Evidence that several markers assigned to either chromosome *XIV* or *XVII* are actually part of the same linkage group is also described.

MATERIALS AND METHODS

Strains: The genotypes of eight *spo11-1* strains specifically constructed for the purpose of mapping are presented in Table 1A. The remaining strains of *Saccharomyces cerevisiae* employed in this study are listed in Table 1B and below. The following strains were used for complementation and/or mating type testing (only the relevant genotypes are given): 74-1A (*spo1*); 1230 and 1232 (*met2*, *pet8*); K65-3D (*ade2*, *met13*, *tyr1*); JG49-20A (*his2*); K222-2B, K338-3A and K338-8A (*spo12*); M3 (*ade2*, *his1*); P65, S1336B, W85-8D and W87-4B (*met4*); S90-2A (*MAT*α, *met3*); S90-2D (*MAT***a**, *met3*); Tester 1 (*MAT***a**, *ade1*); Tester 2 (*MAT*α, *ade1*); Tester X and Tester Y (*ade1*, *his7*, *met3*); W60-10B (*met2*); W224-1C (*MAT***a**, his6,

TABLE 1

A. spo11 *mapping strains*

Strain	Genotype
	III VIII V V VII XV II V II
K382-23A	*MAT***a** *spo11 ura3 can1 cyh2 ade2 his7 hom3*
K382-19D	*MAT*α *spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1*
	III VIII V VII VIII XVI XII XI II XV IV
K398-4D	*MAT***a** *spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*
K381-9D	*MAT*α *spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*
	III VIII V VI VII IX XVII XIV
K399-7D	*MAT***a** *spo11 ura3 his2 leu1 lys1 met4 pet8*
K393-35C	*MAT*α *spo11 ura3 his2 leu1 lys1 met4 pet8*
	III VIII V I V III XIII X VII
K396-11A	*MAT***a** *spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*
K396-22B	*MAT*α *spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*

B. Haploid strains

Strain	Genotype
K231-1A	*MAT***a** *ade2 ade5 can1 his7 lys2 spo11 spo12 trp5*
K289-3A	*MAT***a** *ade1 his6 leu1 leu2 met4*
K321-2A	*MAT*α *ade2 cyh2 his7 leu1 lys2 met13 spo11 trp5 tyr1 ura3*
K338-3A	*MAT*α *his6 met13 spo12 trp1*
K338-8D	*MAT*α *his6 lys1 met4 trp1*
K365-18RS	*MAT*α *ade1 ade2 ade6 his6 his7 leu1 lys1 met4 met14 pet8 spo11 tyr1 ura3*
K321-3C	*MAT*α *ade2 cyh2 his7 leu1 lys2 met13 spo11 spo12 trp5 tyr1 ura3*
K366-7D	*MAT*α *ade6 arg4 aro7 asp5 met14 pet17 spo11 ura3*
K366-12A	*MAT*α *ade2 aro7 asp5 cyh2 his6 leu1 lys2 met13 met14 pet17 spo11 trp5*
K366-12D	*MAT*α *ade2 ade6 aro7 asp5 his6 lys2 met14 pet17 spo11*
K368-23RS	*MAT*α *ade2 ade6 cdc14 his2 his7 hom3 leu1 lys1 lys2 met14 spo11 trp5 tyr1 ura3*
K392-6C	*MAT***a** *ade2 can1 cyh2 his7 hom3 met13 spo1 spo11 tyr1 ura3*
K415-6A	*MAT*α *ade2 his6 his7 leu1 pet3 ura1*

Gene symbols are as follows: *MAT***a** and *MAT*α, mating type; *ade*, adenine; *aro*, aromatic amino acids; *asp*, aspartate; *arg*, arginine; *can*, canavanine; *cdc*, cell-division-cycle; *cyh*, cycloheximide; *his*, histidine; *hom*, homoserine; *leu*, leucine; *lys*, lysine; *met*, methionine; *pet*, petite; *spo*, sporulation-defective; *trp*, tryptophan; *tyr*, tyrosine; *ura* uracil (c.f. PLISCHKE, *et al.* 1976). Roman numerals designate the chromosome numbers.

met14, pet8); W224-1D (*MATα, his6, met14, pet8*) and X3455-1D (*pet8*). We obtained JG49-20A from J. GOLIN; Testers 1 and 2 from D. PLOTKIN; Testers X and Y, and M3 from R. MALONE; W60-10B, W85-8D, W87-4B, W244-1C and W244-1D from R. ROTHSTEIN; and strains 1230 and 1232 from R. WICKNER. P65, S1336B and X3455-1D were obtained from the Berkeley Yeast Genetic Stock Center. All other strains are from our laboratory collection.

Media: The following recipes are for one liter of medium. Tetracycline (Squibb) is added at 20 μg/ml to complex and sporulation media to prevent bacterial contamination. Solid medium contains 15 gm Bacto-agar. YPDA contains 20 gm Bacto-dextrose, 20 gm Bacto-peptone, 10 gm Bacto-yeast extract and 1 ml 0.5% adenine sulfate. YPG contains 30 ml glycerol, 20 gm Bacto-peptone and 10 gm Bacto-yeast extract. Minimal contains 20 gm Bacto-dextrose, 1.7 gm yeast nitrogen base and 5 gm ammonium sulfate. Minimal plus uracil contains Minimal supplemented with 20 mg uracil. Complete contains Minimal supplemented with each of the following: 10 mg adenine sulfate; 25 mg L-arginine-HCl; 75 mg L-aspartic acid; 20 mg L-histidine-HCl; 100 mg L-leucine; 50 mg L-lysine-HCl; 20 mg L-methionine; 50 mg L-phenylalanine; 300 mg L-threonine; 50 mg L-tryptophan; 50 mg L-tyrosine and 20 mg uracil. Omission contains Complete lacking one or more amino acids or nitrogen bases. Methionine omission lacks both methionine and threonine and contains 80 mg homoserine. Cycloheximide (CYH) contains Complete with 1 ml of 0.1% cycloheximide added after autoclaving. Canavanine (CAN) contains Arginine omission with 6 ml of 1% canavanine sulfate added after autoclaving. Sporulation II (SPII-22) contains 20 gm potassium acetate, supplemented with 75 μg/ml of each of the following: adenine sulfate; L-arginine-HCl; L-aspartic acid; L-histidine-HCl; L-leucine; L-lysine-HCl; L-methionine; L-phenylalanine; L-threonine; L-tryptophan; L-tyrosine; and uracil; and 10 μg/ml PABA, pH 7.0. Sporulation III (SPIII-22) contains 20 gm potassium acetate, 1 gm Bacto-dextrose and 2.5 gm Bacto-yeast extract, supplemented as per SPII-22.

Genetic Techniques: Standard procedures for the genetic manipulation of yeast were employed (MORTIMER and HAWTHORNE 1969; SHERMAN and LAWRENCE 1974).

Mapping procedure: The general scheme for localizing an unmapped mutation to a particular chromosome is as follows (See RESULTS): A strain carrying an unmapped mutation (*mutx*) is mated to a haploid of opposite mating type containing *spo11-1*, *can1* and *cyh2*. The diploid hybrid is sporulated, and 10–20 tetrads dissected to recover a *MAT***a** *or MATα spo11-1 mutx can1 cyh2* haploid. The segregation of *spo11-1* in the tetrads is followed by complementation tests, described below. The haploid is then mated to each of three *spo11-1* tester strains, containing among them one or more markers on each chromosome (Table 1A). Each test hybrid is incubated on sporulation (SPIII-22) plates for 5 days or in liquid sporulation (SPII-22) medium for 48 hr. Sporulated cells are streaked or plated on CAN and/or CYH media and incubated for about 5 days. Drug-resistant colonies of various sizes (50-100 per cross per drug selection) are picked to a YPDA master plate, incubated for 2–3 days, and replicated to the appropriate selective media to assay marker segregation.

Marker scoring: Petite mutations are scored on YPG and auxotrophic mutations are scored on omission media, after 1 and 2 days incubation. Colonies that do not grow on a selective plate are scored as minus (−); whole colonies that grow or are sectored for growth (*i.e.*, part of the colony grows and part does not) are scored as plus (+). The mating type of each colony is determined by crossing it with *MAT***a** and *MATα* strains, and testing for the formation of diploids by prototrophic selection. Colonies that mate only with the *MATα* tester are designated **a**; those that mate only with the *MAT***a** tester are designed α. Colonies that do not mate with strains of either mating type and colonies that are sectored for mating-ability (*e.g.*, part nonmater and part **a**) are classified as nonmaters (N). Complementation tests are carried out with **a** and α colonies to score markers that are masked or not expressed in the haploid colonies (*e.g.*, an unmapped *met* mutation that cannot be phenotypically distinguished from the *met* markers in the tester strains, or a *spo* mutation not expressed in a haploid colony). The rationale for assigning an unmapped mutation to a particular chromosome is illustrated in Figure 1 and discussed in RESULTS. In the studies described here, all

strains were grown at 30°; *spo11-1/SPO11* diploids were sporulated at 30°, and *spo11-1/spo11-1* diploids were sporulated at 34°, on solid SPIII-22 medium.

Complementation testing for spo11-1: Haploid ascosporal colonies derived from crosses of the *spo11-1 can1 cyh2* strain to the strain carrying the unmapped mutation are mated to *MAT***a** or *MATα spo11-1* testers. The three pairs of *MAT***a** and *MATα spo11-1* mapping testers (Table 1A) can be used for this, and in most cases, prototrophic selection on Minimal plus uracil medium can be used to obtain the diploids. The *spo11-1/spo11-1* diploids can be identified by several criteria: (1) reduced sporulation ability (typically 30% mature asci at 25°, 20% at 30°, and 10% at 34°, on SPIII-22); (2) abnormal, immature-appearing asci; (3) poor spore viability (less than 1%); and (4) reduced haploidization. The latter phenotype can be scored by replicating the diploids from SPIII-22 to CAN and CYH plates. The two to four segregants in each tetrad that are *can1* and/or *cyh2* will produce nearly confluent patches of growth if the diploid is *SPO11/spo11-1*. Few or no papillae will be found if they are homozygous *spo11-1/spo11-1*.

Ascus dissection and micromanipulation: Asci were dissected as previously described (KLAPHOLZ and ESPOSITO 1980b). To determine the viability of intact *spo11-1/spo11-1* asci, sporulated cells of the diploid K382-19D × K393-35C (Table 1A) were obtained from a 34° SPIII-22 plate, briefly treated (4 min) with 1% glusulase to aid in ascus recognition, and streaked to YPDA. Intact asci were then micromanipulated to individual positions on the plate and incubated at 30°.

Photomicroscopy: Sporulated cultures of *spo11-1/spo11-1* (K303) and *SPO11/SPO11* (K65-3D) were fixed in 70% ethanol for 10 min and washed with distilled water. They were then stained with 0.25 μg/ml DAPI (4′, 6-diamidino-2-phenylindole), washed, and stored in 50% glycerol. Slides were prepared and photographed with fluorescence (not shown) and phase optics on a Leitz Orthoplan miscroscope.

RESULTS

Mapping rationale: Based on the haploidization-without-recombination sporulation phenotype of *spo11-1/spo11-1* diploids, we devised a simple mapping procedure to establish the chromosomal location of an unmapped dominant or recessive mutation. The mapping scheme is outlined in Figure 1. A haploid strain carrying the *spo11-1* mutation, an unmapped mutation (*mutx*), and one or more recessive drug-resistance markers (*e.g.*, *can1* and *cyh2*), is constructed and crossed to three *spo11* mapping tester strains (see Table 1A). Each resulting *MAT***a**/*MATα spo11-1/spo11-1 mutx/MUTx can1/CAN1 cyh2/CYH2* diploid is also heterozygous for recessive markers on a subset of the total yeast chromosomes, contributed by the *spo11-1* tester parent. The diploids are sporulated and then transferred to canavanine-containing (CAN) or cycloheximide-containing (CYH) media to select for rare viable meiotic products that are haploid for the chromosome carrying the drug-resistance allele. After several days of incubation, drug-resistant colonies are picked to master plates and analyzed for input marker segregation.

During sporulation of a *spo11-1/spo11-1* diploid, meiotic recombination is absent or greatly reduced (S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, unpublished, and this study). Thus, an unmapped mutation and a recessive tester strain marker located on the same chromosome, *in repulsion*, will rarely be expressed in the same drug-resistant meiotic segregant (Figure 1, Table 2). In contrast, a tester strain marker located on a different chromosome from the unmapped mutation will segregate independently of the unmapped muta-

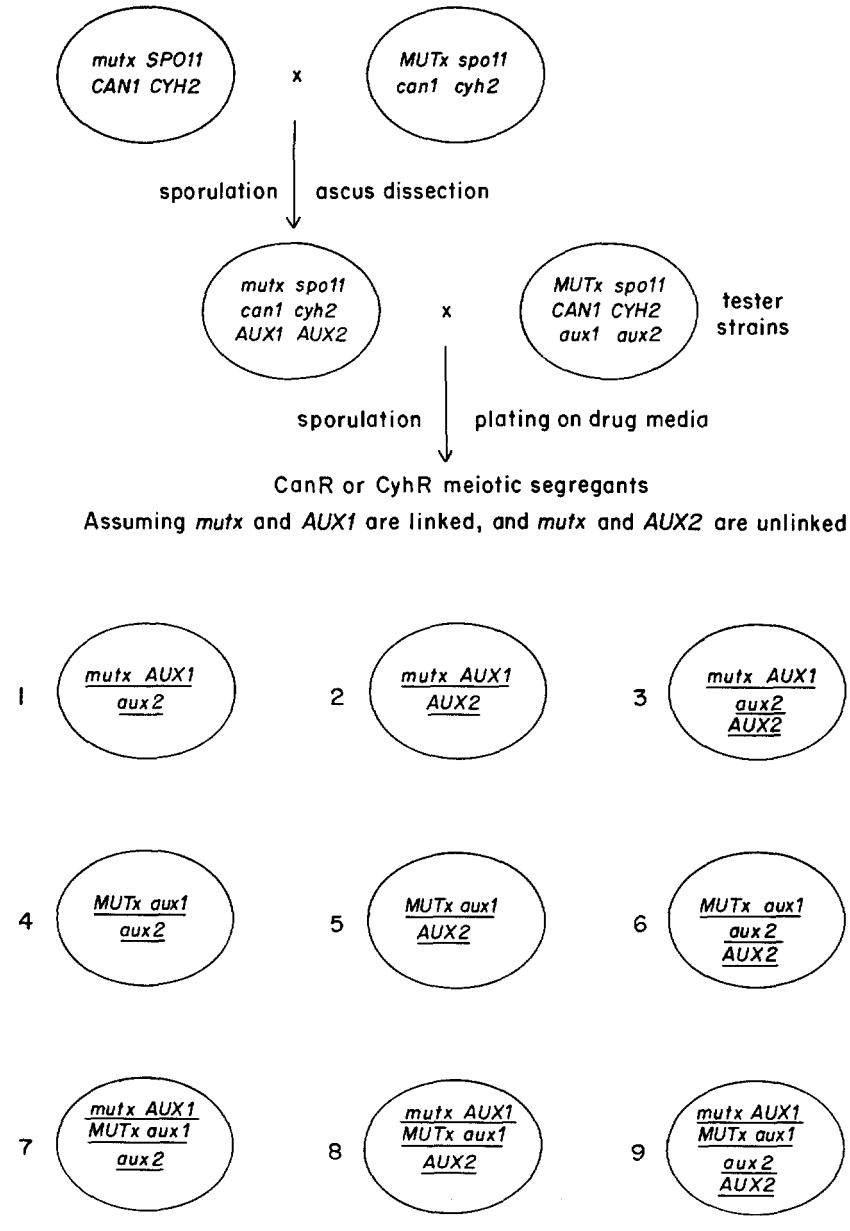

FIGURE 1.—The *spo11* mapping procedure. A haploid strain carrying an unmapped recessive mutation (*mutx*) is crossed to a *spo11-1* haploid carrying two recessive drug-resistance markers (*can1* and *cyh2*). A *spo11-1* haploid carrying the unmapped mutation and the two drug-resistance markers is derived by tetrad analysis. This haploid is then mated to a set of *spo11-1* tester strains containing, among them, a recessive marker on each chromosome. In this example, the tester strain is marked with recessive auxotrophic markers (*aux1* and *aux2*)

TABLE 2

Expected phenotypes of canavanine-resistant clones

Diploid genotype*	Phenotypes of canavanine-resistant clones†			
	Mut+Aux+	Mut-Aux+	Mut+Aux-	Mut-Aux-
Mapping a recessive mutation, mutx (*mutx AUX1 can1* × *MUTx aux1 CAN1*)				
1. markers are on different chromosomes: $\frac{mutx}{MUTx}\ \frac{AUX1}{aux1}\ \frac{can1}{CAN1}$	+	+	+	+
2. *mutx* and *AUX1* are linked: $\frac{mutx\ \ AUX1}{MUTx\ \ aux1}\ \frac{can1}{CAN1}$	+	+	+	—
3. *mutx* and *can1* are linked: $\frac{mutx\ \ can1}{MUTx\ \ CAN1}\ \frac{AUX1}{aux1}$	—	+	—	+
Mapping a dominant mutation, MUTx (*MUTx AUX1 can1* × *mutx aux1 CAN1*)				
1. markers are on different chromosomes: $\frac{MUTx}{mutx}\ \frac{AUX1}{aux1}\ \frac{can1}{CAN1}$	+	+	+	+
2. *MUTx* and *AUX1* are linked: $\frac{MUTx\ \ AUX1}{mutx\ \ aux1}\ \frac{can1}{CAN1}$	—	+	+	—
3. *MUTx* and *can1* are linked: $\frac{MUTx\ \ can1}{mutx\ \ CAN1}\ \frac{AUX1}{aux1}$	—	+	—	+

* Diploids are *MATa/MATα spo11-1/spo11-1*.

† The diploids are sporulated and plated on canavanine medium. Mut- = mutant; Mut+ = wild type; Aux- = auxotrophic; Aux+ = prototrophic. The + and — symbols indicate that a particular phenotype is expected, and not expected, to occur, respectively.

tion. Hence, the recovery of drug-resistant clones that express both the unmapped mutation and a particular tester strain marker indicates that these loci reside on different chromosomes. When the unmapped mutation and the drug-resistance marker are present on the same chromosome, *in coupling*, the mutation will be expressed in virtually all of the drug-resistant colonies. These predictions apply to both recessive and dominant unmapped mutations (see Table 2).

on two different chromosomes. The diploid is sporulated and plated on drug-containing media. Assuming that *mutx* is on the same chromosome as *AUX1*, and that neither is linked to *can1* and *cyh2*, nine types of drug-resistant colonies are expected. The absence of colonies that express both *mutx* and *aux1* indicates that the markers reside on the same chromosome (see also Table 2).

By analogy with recombination-defective mutants of other organisms (see BAKER *et al.* 1976, for review), it seems likely that in *spo11-1/spo11-1* diploids, meiosis I involves the random segregation of chromosomes to each pole and meiosis II occurs normally. For any given chromosome this mode of segregation is expected to yield either four monosomic spores or two disomic and two nullosomic spores per ascus, in equal frequency (Figure 2). Because nullosomic spores are inviable, among the potentially viable spores 67% will be monosomic and 33% disomic. Therefore, excluding other consideration, any given heterozygous recessive marker should be expressed in half of the monosomic spore clones or 33% of the total viable clones recovered from a Rec⁻ meiosis.

Ascus viability in spo11-1 *diploids*: In an organism like yeast with a large number of chromosomes, random segregation at meiosis I—generating spores nullosomic for one or more chromosomes—should result in low ascus viability. The *spo11-1/spo11-1* diploids employed in the experiments reported here form, on the average, 30% asci at 25°, 20% asci at 30° and 10% asci at 34°. These asci are often immature or abnormal in morphology (Figure 3). The viability of asci produced by *spo11-1/spo11-1* diploids at 34° was determined by micromanipulating 100 intact asci of diploid K382-19D × K393-35C onto YPDA plates. After 2 days of incubation at 30°, only one of the tetrads had formed a colony; the remaining asci showed no evidence of spore germination. This low level of ascus survival (1%) is close to that predicted for a recombination-

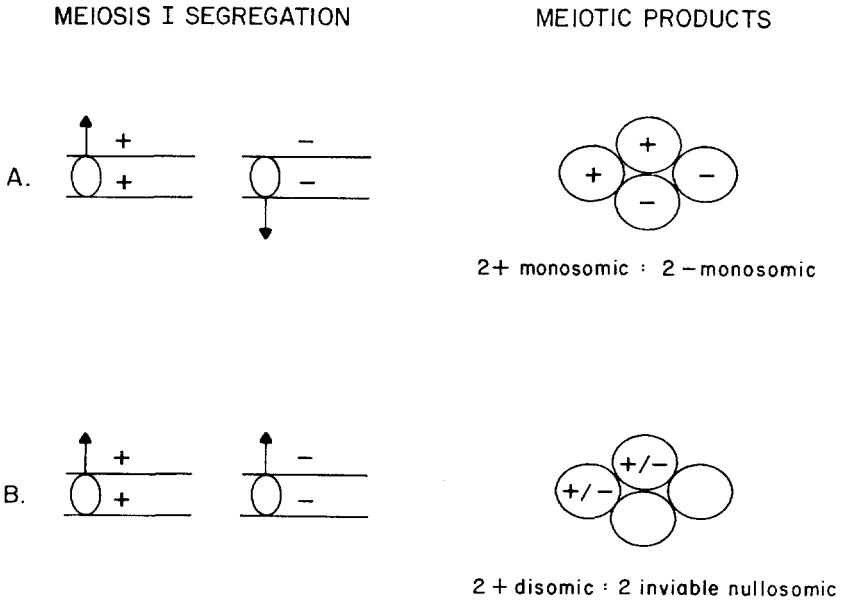

FIGURE 2.—Meiosis in a recombination-defective mutant. The + and − symbols represent dominant and recessive alleles of a gene, respectively. The meiosis I division is random: 50% of the time homologous chromosomes move to opposite poles (A); 50% of the time homologs move to the same pole (B). Meiosis II occurs normally.

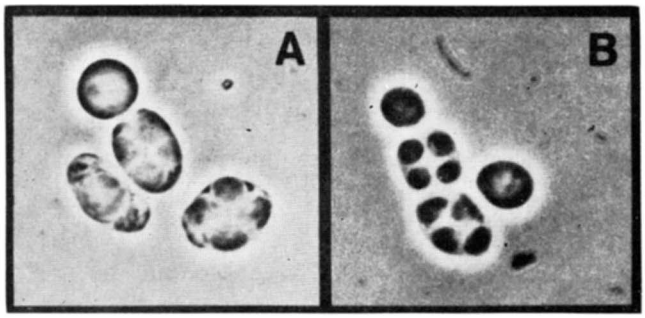

FIGURE 3.—phase micrograph of cells of *spo11-1/spo11-1* (A) and *SPO11/SPO11* (B) diploids, sporulated at 25° (magnification = 1800x).

defective mutant undergoing random chromosome segregation at meiosis I (See DISCUSSION).

DISCUSSION

Crosses to analyze the segregation of previously mapped genes in spo11-1 *diploids*: To assess the utility of this mapping procedure, we crossed a *MAT***a** *spo11 can1 cyh2* haploid strain, K392-6C, carrying several previously mapped markers on a number of chromosomes, to each of the three *MATα spo11* tester strains (Table 1). The map positions of the seven loci examined in this study are shown in Figure 4. One of these markers, a recessive sporulation-defective mutation, *spo1*, was mapped by tetrad analysis in this laboratory.

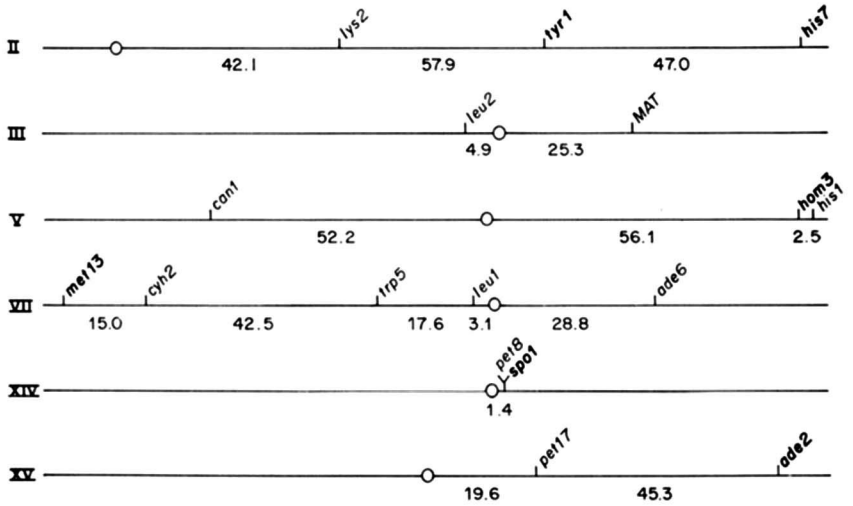

FIGURE 4.—Genetic map of *S. cerevisiae* showing the chromosomal location of K392-6C markers in relation to the *spo11* tester strain markers. K392-6C markers are indicated in bold type. Map distances, in centiMorgans, were determined by tetrad analysis (see MORTIMER and SCHILD 1980, for review).

The other map positions are from the compiled data of MORTIMER and SCHILD (1980). All of the markers are recessive, with the exception of *MAT***a**, which is condominant with *MAT*α.

Cross hybrids between K392-6C and the *spo11-1* testers were incubated on sporulation plates at 34° for five days, and streaked to drug-containing media (MATERIALS AND METHODS). Fifty to 100 canavanine-resistant (CanR) and cycloheximide-resistant (CyhR) colonies from each cross were picked to YPDA master plates after 3 to 5 days incubation at 30°. The drug-resistant colonies included a wide range of sizes and morphologies. The phenotypes of these clones were scored directly or by complementation testing of mating-capable clones.

Among the 210 CanR and 240 CyhR clones initially examined, most were of **a** or α mating type. Nonmating clones comprised 36% of all CanR clones and 20% of all CyhR clones. In each cross, more than 98% of these nonmating clones expressed at least one recessive marker on a chromosome other than the one carrying the selected drug-resistance marker. This implies that diploid CanR and CyhR mitotic recombinants do not make a significant contribution to the population of nonmating clones (cf., DISCUSSION). The segregation of markers among nonmating and mating clones was not very different; for example, *trp1* was expressed in 40% of nonmating and 51% of mating clones, and *aro7* was expressed in 30% of nonmating and 39% of mating clones. Because complementation testing was necessary to determine the presence of many of the markers in these crosses, the data presented below are derived only from mating-capable clones.

Recovery of markers on the same chromosome as the selected drug-resistance marker: As predicted (Table 2), markers *in coupling* with the selected drug-resistance markers were expressed in virtually all of the respective drug-resistant clones. The recessive *hom3* allele, located on the opposite arm from *can1* on chromosome *V*, was expressed in 123/124 CanR clones; *met13*, linked to *cyh2* on chromosome *VII*, was expressed in all of the 179 CyhR colonies

TABLE 3

Segregation of recessive markers from K392-6C in CanR and CyhR clones

Marker	Chromosome	Marker phenotypes of drug R clones CanR + : −	CyhR + : −
*his7, tyr1**	*II*	77:47	112:67
*MAT***a**†	*III*	57:67	98:83
hom3	*V*	**1:123**	129:50
met13	*VII*	67:57	**0:179**
spo1	*XIV*	63:61	86:93
ade2	*XV*	79:45	98:81

* *his7* and *tyr1* cosegregated in all clones.

† *MAT***a** and *MAT*α are codominant alleles of the *MAT* locus; the numbers in the + columns refer to α maters and the number in the — columns refer to **a** maters. Nonmaters (*MAT***a**/*MAT*α) are not included in these data.

tested (Table 3). Likewise, none of the 30 CanR clones from the cross of K392-6C to tester K396-22B, and none of the CyhR clones from all three crosses, expressed the recessive tester strain marker for chromosomes *V* or *VII*, respectively. These results indicate that the drug-resistant clones are largely monosomic for the chromosome bearing the selected drug *R* locus. In contrast, only 35% of the CyhR colonies expressed *hom3*, and 48% of the CanR colonies expressed *met13*. The other markers contributed to the crosses by K392-6C, which are not located on chromosomes *V* or *VII*, were recovered in similar proportions among CanR and CyhR clones (see Table 3).

Recovery of recessive alleles among drug-resistant clones: Table 4 summarizes the segregation data for each marker contributed to the crosses by K392-6C (asterisked) or the tester strains. Excluding cases where the drug

TABLE 4

Expression of chromosomal markers in mating-capable drug-resistant clones

Chromosome	Marker	Phenotype of drug-resistant clones +	−	Total	Percent minus
I	*ade1*	47	28	75	37
II	*his7**	189	114	303	38
	*tyr1**	189	114	303	38
	lys2	87	55	142	39
III†	*MAT***a***	153	150	303	50
	leu2	36	39	75	52
IV	*trp1*	70	72	142	51
V‡	*can1**	128	51	179	28
	*hom3**	129	50	179	28
	his1	17	28	45	62
VI	*his2*	50	36	86	42
VII‡	*cyh2**	68	56	124	45
	*met13**	67	57	124	46
	ade6	41	21	62	34
	leu1	23	9	32	28
	trp5	19	11	30	37
VIII‡	*arg4*	53	27	80	34
IX	*lys1*	59	27	86	31
X	*met3*	48	27	75	36
XI	*met14*	97	45	142	32
XII	*asp5*	129	14	143	10
XIII	*lys7*	69	6	75	8
XIV	*spo1**	149	154	303	51
	pet8	67	19	86	22
XV	*ade2**	177	126	303	42
	pet17	74	68	142	48
XVI	*aro7*	87	55	142	39
XVII	*met4*	67	19	86	22

* Markers contributed to the cross by K392–6C.

†Chromosome *III* data do not include disomes, which are **a**/α nonmaters. Numbers in the + column refer to α maters, numbers in the — column refer to **a** maters.

‡ Chromosome *VII* data are from CanR clones only; Chromosome *V* and *VIII* data are from CyhR clones only (see text).

selection prevented the recovery of a particular allele, the percent of clones expressing the recessive (or, in the case of *MAT***a**, the codominant) allele of each locus ranged from 8% to 62%, with an average value of 36%. A number of factors appear to influence how frequently a given chromosomal marker is recovered among drug-resistant clones:

1. *Relative survival of disomic versus monosomic spore clones*: It is known from studies of the meiotic products of triploid strains of yeast that disomy is tolerated less well for some chromosomes than for others (PARRY and COX, 1970; CAMPBELL *et al.* 1981). For example, disomy for chromosome *IV*, one of the longest chromosomes, is infrequently detected (PARRY and COX 1970). We found that *trp1*, marking chromosome *IV* in this study, was expressed in approximately 50% of all drug-resistant clones. If chromosome *IV* disomes were completely unstable, we would expect 50% *trp1* monosomic and 50% *TRP1* monosomic clones. A more reliable assessment of disomy for a particular chromosome can be made when both homologous chromosomes are marked (cf., item 3). Estimates of the level of disomy for a number of chromosomes are presented in a later section.

2. *Relative survival of + versus — spores or spore clones*: Theoretically, we expect to recover between 33% (disomes fully viable) and 50% (disomes completely inviable) clones that are mutant in phenotype for a given heterozygous recessive marker (Figure 2). However, this expectation is based on the assumption that + and — monosomics are equally viable, and that the recovery of the alleles is not affected by the segregation of other markers (cf., items 3 and 4). Of the 27 recessive markers examined, only four were expressed in significantly fewer than a third of all drug-resistant colonies [*lys7* (8%), *asp5* (10%), *pet8* (22%) and *met4* (22%) (Table 4)]. At least in the case of *lys7*, we have observed that spore clones carrying this mutation grow noticeably more slowly than *LYS7* spore clones, even on rich YPDA medium. Thus, *lys7* spore clones may be at a selective disadvantage in these experiments. We have found that choosing small colonies from CAN or CYH plates at 5 days incubation enriches for these less vigorous colonies.

3. *The effect of another marker on the same chromosome*: When two markers are *in coupling*, one will be coincidently expressed in virtually all clones that express the other. For example, *tyr1* and *his7* on chromosome *II* cosegregated in all 303 clones tested (see also Table 3). Hence, it is apparent that if a marker displays a strong selective advantage or disadvantage, it will influence the recovery of other markers on the same homolog. A similar situation can arise for markers *in repulsion*, resulting in the recovery of one homolog more frequently than the other. For example, among CyhR clones from the cross K392-6C × K396-22B, the *his1*-bearing homolog of chromosome *V* was recovered twice as frequently as the one carrying *can1* and *hom3*. Of the 45 clones tested, 62% expressed *his1*, while only 31% expressed *can1* and *hom3* (the remaining 7% were disomic).

4. *The effect of another marker on a different chromosome*: The presence of certain alleles of two different genes in the same spore may result in in-

TABLE 5

Phenotypes of drug-resistant clones for various tester strain chromosomal markers

Marker on chromosome:	All drug R clones	+:− segregation of chromosomal markers in drug R clones expressing the following alleles:* *his7*, *tyr1*	*MAT***a**	*hom3*	*met13*	*spo1*	*ade2*
I	47:28	20:12	21:15	27:17	32:23	24:14	20:12
II	87:55	**47:0**	43:31	45:32	65:38	41:24	35:24
III	36:39	13:19	**36:0**	17:27	29:27	19:18	12:20
IV	70:72	21:26	35:39	39:38	49:56	28:37	29:30
V	47:38	22:10	19:17	**44:0**	28:28	27:11	20:12
VI	50:36	22:13	23:17	32:20	41:34	34:17	18:17
VII	262:41	99:15	127:27	133:41	**236:0**	135:19	108:18
VIII	115:27	40:7	65:9	**77:0**	53:27	51:14	48:11
IX	59:27	22:13	27:13	35:17	53:22	33:18	23:12
X	48:27	18:14	23:13	27:17	37:19	28:10	20:12
XI	97:45	25:22	56:18	53:24	67:36	44:21	39:30
XII	127:14	40:7	68:5	74:3	92:13	58:7	51:8
XIII	69:6	30:2	33:3	41:3	50:6	27:4	27:5
XIV	67:19	27:8	31:9	42:10	59:16	**51:0**	27:8
XV	74:68	23:24	36:38	37:39	58:47	35:30	**59:0**
XVI	87:55	26:21	42:32	47:30	65:40	38:27	30:29
XVII	67:19	27:8	31:9	42:10	59:16	**51:0**	27:8

* Data are for mating-capable CanR and CyhR clones from crosses K392–6C with K381–9D, K396–22B and K393–35C. The three letter gene symbols refer to markers contributed by K392–6C; + and — refer to markers contributed by the tester strains.

creased or decreased survival compared to spores bearing one or the other allele. One such example concerns the interaction between *arg4* (the chromosome *VIII* marker in cross K392-6C × K381-9D) and *can1*. The *CAN1* locus codes for an arginine permease (GRENSON *et al.* 1966). Arginine auxotrophs with a *can1* mutation cannot grow on our synthetic media because they are unable to take up arginine. When CanR clones were selected, all 62 were Arg$^+$, and among CyhR clones, all *arg4* clones were CanS and all *can1* clones were Arg$^+$. An artifactual mapping result that can occur as a consequence of this type of interaction between genes is described below.

Mapping data for previously mapped markers: Among clones that expressed a particular K392-6C marker, the number that were + or — in phenotype for each of the seventeen chromosomal markers contributed by one of the *spo11-1* tester strains is summarized in Table 5. The total number of clones that were + or — in phenotype for each chromosomal marker is presented for comparison. The data are clearly in agreement with chromosomal locations for *ade2* (*XV*), *his7* and *tyr1* (*II*), *MAT***a** (*III*) and *met13* (*VII*), previously determined by tetrad analysis. None of the clones that expressed any one of these alleles expressed the recessive tester strain marker for its respective chromosome. The reverse is also true: all clones that expressed the chromosomal marker were + in phenotype for the K392-6C marker (Table 6). As predicted (Figure 1 and Table 2), three classes of clones were observed: those that expressed the wild-type allele of one and the mutant allele of the other marker

TABLE 6

Segregation of linked markers in drug-resistant clones

Marker pair	Chromosome	Marker phenotypes of drug R clones* +, − (α, −)	−, + (**a**, +)	+, + (N, +)	−, − (**a**, −)	Total clones tested
his7 – lys2 *tyr1 – lys2*	*II*	55	47	40	0	142
*MAT***a** *– leu2*	*III*	39	36	26	0	101
hom3 – his1†	*V*	28	14	3	0	45
met13 – ade6‡	*VII*	25	21	16	0	62
met13 – leu1‡	*VII*	9	21	2	0	32
met13 – trp5‡	*VII*	11	11	8	0	30
spo1 – pet8	*XIV*	19	51	16	0	86
ade2 – pet17	*XV*	68	59	15	0	142

* The + and − symbols refer to the phenotypes of the auxotrophic markers: + = wild type and − = mutant. The symbols α, **a** and N refer to α mating type, **a** mating type, and non-mating, respectively. The symbols are given in the same order as the genes listed under "Marker pair." Symbols in parentheses refer to the *MAT***a** *– leu2* pair.

† Data are from CyhR clones only.

‡ Data are from CanR clones only.

(+ − and − + monosomic clones), and those that expressed both wild-type alleles (+ −/− + disomics).

The location of *hom3* on the same chromosome as *can1* (chromosome *V*) was indicated initially by the expression of *hom3* in more than 99% of all CanR clones (Table 3). However, the data shown in Table 5 also suggested that *hom3* was on chromosome *VIII* (marked by *arg4*). This means that expression of the Hom⁻ phenotype appeared to require the presence of alleles on two separate chromosomes. Our inability to detect clones that are both *arg4* and *hom3* is due to the fact that *hom3* clones are also *can1*. As noted above, the *can1* gene in combination with *arg4* prevents growth on our synthetic medium.

Unusual behavior of markers on chromosomes XIV *and* XVII: The behavior of the tester strain markers for chromosomes *XIV* (*pet8*) and *XVII* (*met4*) was most unexpected. In the cross of K392-6C with K393-35C (*spo1 PET8 MET4 / SPO1 pet8 met4*), *pet8* and *met4* exhibited 100% cosegregation: 19 clones expressed both *pet8* and *met4*; 67 clones expressed neither (Tables 5, 6). The *spo1* mutation, shown by tetrad analysis to be tightly linked to *pet8* (PD=19, NPD=0, T=0), was not expressed in any of the clones that expressed *pet8* and *met4*. Fifty-one of the 67 Pet⁺ Met⁺ clones were *spo1* in genotype; the remaining 16 (19%) were Pet⁺ Met⁺ Spo⁺ and presumably disomic. These results implied that *pet8*, *spo1* and *met4* were located on the same chromosome, raising the intriguing possibility that chromosomes *XIV* and *XVII* comprised a single linkage group. Subsequent studies, to be reported elsewhere, including meiotic tetrad analysis of markers previously thought to reside separately on chromosomes *XIV* and *XVII*, have confirmed that these chromosomes are indeed part of one linkage group (KLAPHOLZ and ESPOSITO, unpublished).

Mapping a previously unmapped mutation: The *spo11* mapping procedure was used to determine the chromosomal location of the recessive *spo12-1* mutation. Tetrad analysis had indicated that *spo12-1* was centromere-linked (less than 55% second-division segregation), but no linkage was established between *spo12-1* and centromere-linked markers on 15 chromosomes (KLAPHOLZ and ESPOSITO 1980a; S. KLAPHOLZ, unpublished). The *spo11* mapping data compiled from 11 crosses, which places *spo12-1* on chromosome *VIII*, is summarized in Table 7. Tetrad analysis has confirmed this result, placing *spo12-1* less than 1 cM from *pet3* (Table 8). The positioning of *spo12-1* near *pet3*, some 80 cM from the centromere, was surprising because the second-division segregation frequency for *spo12-1* had indicated that it was centromere-linked. In this study both *spo12-1* and *pet3* exhibited 60% second-division segregation, again slightly less than the expected value of 67% for centromere-unlinked markers.

In an earlier section we described an artifactual mapping result that occurred for *hom3*, a marker in coupling with *can1* on chromosome *V*, due to the presence of an *arg* mutation in one of the tester strains. The *hom3* marker appeared to be on both chromosomes *V* and *VIII*, linked to *his1* and *arg4*, respectively (Table 5). In contrast, this type of artifact is not observed for

TABLE 7

Segregation of spo12–1 *among drug R clones expressing tester strain markers**

Marker on chromosome	Total	Drug R clones expressing tester strain marker *spo12–1*	Percent *spo12–1*
I	12	2	17
II	19	9	47
III	54	26	48
IV	20	10	50
V	5	2	40
VI	12	3	25
VII	59	25	42
VIII†	**11**	**0**	**0**
IX	27	15	56
X	10	3	33
XI	41	17	41
XII	11	5	45
XIII	2	1	50
XIV	7	2	29
XV	37	20	54
XVI	36	16	44
XVII	7	2	29

* The data are based on the analysis of **a** and α CanR or CyhR clones from crosses of *spo11–1 spo12–1 can1* haploid K231–1A with each of the following *spo11–1* testers: K321–2A, K365–18RS, K366–12A, K366–12D, K368–23RS, K381–9D, K393–35C and K396–22B, and from crosses of *spo11–1 spo12–1 cyh2* haploid K321–3C with K366–7D, K396–11A and K398–4D (see Table 1 for complete genotypes).

† Of 30 **a** or α CyhR clones from cross K321–3C × K396–11A, 14 were Arg+ *spo12–1*, 11 were *arg4* Spo+ and 5 were Arg+ Spo+. No *arg4 spo12–1* clones were observed; 4 are expected if *arg4* and *spo12–1* are on different chromosomes.

TABLE 8

Tetrad analysis of spo12–1 *versus* pet3 *on chromosome* VIII

Cross	Genotype	Ascus type* PD	NPD	T	Total	Percent spore viability
K415–6A × K338–3A	*pet3 SPO12* / *PET3 spo12–1*	39	0	0	39	96
K415–6A × K321–3C	*pet3 SPO12* / *PET3 spo12–1*	36	0	0	36	92

* PD = parental ditype, NPD = nonparental ditype, and T = tetratype, ascus.

markers located on chromosome *VIII*. The *spo12-1* gene, for example, does not appear to reside on both chromosomes *V* and *VIII*. This is because chromosome *VIII* disomes are relatively stable. The *spo11* mapping cross that placed *spo12-1* on chromosome *VIII*, K321-3C (*cyh2 spo12-1 ARG4*) × K398-4D (*CYH2 SPO12 arg4*) generated 11 *arg4* Spo$^+$, 14 Arg$^+$ *spo12-1*, 5 Arg$^+$ Spo$^+$ and zero *arg4 spo12-1* clones that were **a** or α and CyhR. We estimate from these data that 17% (5/30) of the clones were disomic for chromosome *VIII*. In the cross K231-1A (*can1 spo12-1 ARG4*) × K381-9D (*CAN1 SPO12 arg4*), although none of the CanR clones expressed *arg4*, as expected, only 60% expressed *spo12-1*. This observation clearly eliminated chromosome *V* as the location of *spo12-1*. The remaining clones that were Spo$^+$ in phenotype were most likely disomic for chromosome *VIII* (*spo12-1 ARG4/SPO12 arg4*). Based on our estimated disomy frequency, we would expect 72% of all Arg$^+$ clones to be *spo12-1*; this is in reasonable agreement with the value observed in this cross.

Disomy levels for various chromosomes: Because the level of recombination is negligible during sporulation of *spo11-1/spo11-1* diploids (see below), we were able to closely estimate the frequency of disomy for several chromosomes carrying recessive markers *in repulsion* (+ —/— +). In these cases, the level of disomy is equal to the frequency of clones expressing the wild allele of each locus, *i.e.*, + + clones. The frequency of disomy for chromosome *III* is equal to the frequency of nonmaters (*MAT***a**/*MAT*α) among the initial populations of drug-resistant clones. The level of disomy for the seven chromosomes, determined in this and in other studies, ranged from 7% to 28% and averaged 19% (Table 9).

Forty-six percent of all CyhR clones from cross K392-6C × K381-9D were disomic for one or more of the three chromosomes examined (*II*, *XII* and *XV*), indicating that the frequency of aneuploidy among the meiotic products of *spo11-1* is quite high, although the number of disomic chromosomes per clone appears to be small. We compared the individual disomy frequencies for chromosomes *II*, *VII* and *XV* with the frequency of coincident disomy for two or three of these chromosomes (Table 10). The multiple disomy

TABLE 9

Disomy for individual chromosomes in spo11-1 *meiotic products*

Chromosome	Percent disomics among drug R clones			
	CanR	CyhR	Average	Source*
II	26	30	28	1
III	42	26	27	1
	26	19		1
	39	14		1
	33	—		2
	—	19		3
V	—	7	7	1
VII	6	—	24	1
	27	—		1
	26	—		1
	34	—		2
	24	—		3
	14	—		4
	33	—		3
VIII	—	17	17	3
XV	10	11	12	1
	—	9		3
	7	—		3
	—	23		3
	—	14		3
XIV	19	19	18	1
	—	17		3

* Source: 1. This study; 2. S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, in preparation; 3. S. KLAPHOLZ, unpublished; 4. S. KLAPHOLZ and R. MALONE, unpublished.

frequencies observed were equal to or slightly higher than those expected, based on the product of the individual frequencies.

The level of aneuploidy among the *spo11-1* meiotic products was also analyzed by crossing drug-resistant clones to marked haploid testers. Eight **a** or α CanR clones from diploids K231-1A $\times$ K366-12A and K231-1A $\times$ K366-12D were mated with haploid strains K338-8D or K289-3A, respectively. Diploids were sporulated and 10 tetrads per cross were dissected. All of the diploids exhibited between 70% and 90% sporulation (at 30°), except for one, which produced

TABLE 10

Coincident disomy in drug-resistant colonies from cross K392-6C $\times$ K381-9D

Drug R colonies	Disomy for at least chromosomes:*						
	II	*VII*	*XV*	*II,VII*	*II,XV*	*VII,XV*	*II,VII,XV*
CanR	0.26	0.27	0.10	0.10(0.07)	0.07(0.03)	0.03(0.03)	0.03(0.01)
CyhR	0.30	—	0.11	—	0.04(0.03)	—	—

* Expected disomy based on the product of the individual disomy frequencies is given in parenthesis.

40% asci. Spore viability was generally high, suggesting that the CanR clones were near-haploid in ploidy: 93%-98% in six of the crosses and 70% in two. (Diploids typically show greater than 90% spore viability and triploids exhibit only 20% to 30% spore viability.)

There was no evidence from these crosses for meiosis II nondisjunction, which would give rise to homozygous disomes. All **a** clones were *MAT***a**, all α clones were *MAT*α, and all CanR clones were *can1*, in genotype. Five of the eight clones were disomic for one (two clones) or two (three clones) of the nine chromosomes monitored. Table 11 summarizes the frequency of disomy for the different chromosomes tested. Disomy was monitored in two ways. For chromosomes carrying a heterozygous recessive marker in diploids K231-1A $\times$ K366-12A and K231-1A $\times$ K366-12D, the segregation of the — allele in a cross between a phenotypically + CanR segregant and a + tester strain indicated disomy. For unmarked chromosomes in the *spo11-1/spo11-1* diploids, trisomic segregation patterns in crosses of the + CanR segregants to — haploid testers indicated disomy. These results support the data presented in Tables 9 and 10, indicating that disomy for a small number of chromosomes is common among the *spo11-1* meiotic products.

The stability of disomes for various chromosomes was tested by examining the frequency with which a disomic colony gave rise to monosomic clones when streaked to nonselective medium (YPDA). An average of 15 colonies per disomic clone were analyzed. Disome stability varied with the particular chromosome. As might be expected, the frequency with which a given disome was initially recovered was reflected in its stability. Chromosome *XV* disomes, for example, which were recovered at a relatively low frequency (0.12), were highly unstable: all 10 disomes segregated clones that expressed one of the two marked homologs. Chromosome *II* disomes, on the other hand, which were recovered at a relatively high frequency (0.28) were quite stable; only one of six generated monosomic derivatives.

Intergenic recombinants are rarely detected among drug-resistant colonies: Intergenic recombination can be measured in drug-resistant colonies by changes in the coupling of markers on the same chromosome. It is not possible, however,

TABLE 11

Disomy in can1 *meiotic products derived from* spo11-1 *diploids*

Ploidy of + clones	Chromosome tested								
	*I**	*II*†	*IV**	*VII*†	*IX**	*XII*†	*XV*†	*XVI*†	*XVII**
No. + clones: n	1	3	6	0	4	5	4	3	2
No. + clones: n + 1	1	1	0	1	2	0	1	2	0
Total + clones	2	4	6	1	6	5	5	5	2

* Disomy diagnosed by crosses to haploid tester strain with — marker; *spo11-1/spo11-1* parental diploid was +/+.

† Disomy diagnosed by crosses to haploid tester strain with + marker; *spo11-1/spo11-1* parental diploid was +/—.

to discern whether the recombination event occurred during meiosis or during a preceding mitosis. For two markers *in repulsion* (+ −/− +), monosomic recombinants will be of two types: + + and − −. The + + recombinants are not phenotypically distinguishable from nonrecombinant + −/− + disomics. To estimate the percentage of recombinant clones, twice the number of − − clones divided by the total will give a minimum estimate. To correct for disomic recombinants, which will largely go undetected, twice the − − clones are divided by the total number of clones minus the number estimated to be disomic (*i.e.*, minus the + + clones). By these criteria, no recombination was detected between markers ranging from 45 to 124 cM apart on four chromosomes (Table 12). Based on the sample sizes examined, the maximum frequency of recombination between any of the marker pairs is estimated to be less than 3.2%. By random spore analysis, we would normally expect to see close to 50% meiotic recombination in each of the intervals examined.

Recombination can also be measured for markers *in coupling* (+ +/− −) as the frequency of phenotypically + − and − + clones (Table 12). To correct for disomic recombinants, half of which will go undetected, one-half the estimated number of disomic clones (based on data in Table 9) is subtracted from the total sample size. Only seven recombinants were detected among the 303 drug-resistant clones tested: three clones were recombinant in the *met13* — *cyh2* interval on chromosome *VII* (1.0% recombination), four were recombinant between *hom3* and *can1* on chromosome *V* (1.3% recombination) and none were recombinant in the *his7*—*tyr1* interval on chromosome *II* (<0.4% recombination). At least one of the chromosome *VII* and one of the chromosome *V* recombinant colonies were disomic for the recombinant chromosome. Three of the four *hom3*—*can1* recombinants were Hom$^+$ CanR in phenotype and were derived from the same sporulated diploid; these are likely to be the clonally related products of a cell that underwent a prior mitotic recombination event (DISCUSSION).

DISCUSSION

The utility of the spo11 *mapping procedure*: The *spo11* mapping procedure has a number of advantages for rapid assignment of a gene to a particular chromosome. First, it involves a minimal amount of strain construction and complementation testing. Tetrad dissection is used only in the initial construction of a *spo11-1* haploid carrying the unmapped mutation(s). We recommend tetrad, rather than random spore, analysis, to insure that the unmapped mutation(s) segregates as expected, and to facilitate the identification of the *spo11-1* spore clones (MATERIALS AND METHODS). The mapping strains (Table 1A) were specifically constructed to eliminate the need to complementation test to distinguish tester strain markers from one another. Complementation testing is required only if a marker brought into the cross confers the same requirement as, or masks the expression of, a tester strain marker, or if it is not expressed in haploid colony (*e.g.*, *spo1* in this study). The tester

TABLE 12

Recombination in spo11-1 *diploids*

Marker pair	Distance cM	Number of clones tested	Total clones corrected*	Recombinants Number	Recombinants Percent†
Coupling: $\frac{+\ -}{-\ +}$					
ade2 – pet17	45	142	127	0	—
met13 – trp5‡	61	75	63	0	—
met13 – leu1‡	85	86	83	0	—
his7 – lys2	103	142	102	0	—
can1 – his1‡	108	75	73	0	—
met13 – ade6‡	124	142	121	0	—
Coupling: $\frac{+\ +}{-\ -}$					
met13 – cyh2‡	15	303	290	3	1.0
tyr1 – his7	47	303	251	0	—
can1 – hom3‡	105	303	297	4	1.3
Coupling: $\frac{+\ MAT\alpha}{-\ MAT\mathbf{a}}$					
*leu2 – MAT***a**	30	101	75	0	—

* The total number of clones among which recombinants could be phenotypically detected was determined as follows. For these calculations, we assume that recombination occurs by a single crossover event between the two genetic markers, and that chromosome segregation is random at meiosis I (Figure 2) and equational at meiosis II. For markers *in repulsion* (+ —/— +), a single crossover followed by segregation of the two homologs to opposite poles (Figure 2A) will yield four monosomic spores, two of which are recombinant (+ + and — —) and two of which are parental (+ — and — +). The + + recombinant cannot be distinguished phenotypically from the + —/— + nonrecombinant disomic. If both homologs segregate to the same pole (Figure 2B), the resulting disomic spores will be of two types (depending upon the relative orientation of the two chromosomes at meiosis II): (1) + —/— — and + +/— + and (2) + —/— + and + +/— —. None of these is phenotypically diagnostic of recombination; the former spore resembles the nonrecombinant parental type and the latter three spores resemble the nonrecombinant disomic type Thus, to correct for the undetected disomic recombinants, the estimated number of disomic clones, or the number of phenotypically + + clones, is subtracted from the total number of clones (see further, note †) .

For markers *in coupling* (+ +/— —), following a single exchange, monosomic spores will be of four types: two recombinant (+ — and — +) and two parental (+ + and — —). Both recombinant types are phenotypically distinguishable from the parentals. Disomic spore pairs will be of two types (depending upon the chromosomal orientation at meiosis II): (1) + +/— — and + —/— + and (2) + +/— + and + —/— —. In the first case, both spores are nonrecombinant in phenotype. In the second case, only the latter spore is recombinant. To correct for the undetected disomic recombinants, one half of the number of clones estimated to be disomic for the chromosome in question (based on data in Table 9) is substracted from the total number of clones (see further note, ‡).

† These values are based on the corrected totals. For markers *in repulsion*, the percent recombinants is equal to twice the frequency of — — clones. For markers *in coupling*, the percent recombinants is equal to the frequency of + — plus — + clones. For + *MAT*α/– *MAT***a** the recombinants are equal to the frequency of +**a** and —α clones.

‡ CyhR clones are monosomic for chromosome *VII* (*leu1*, *met13*, *trp5* and *cyh2*) and CanR clones are monosomic for chromosome *V* (*his1*, *hom3* and *can1*) (Results; E. KLAPHOLZ, unpublished). This is taken into account in estimating the number of disomics among the total.

strains, which contain a total of 16 centromere-linked markers, can also be used in standard crosses since *spo11-1* is recessive to wild type.

Second, both dominant and recessive mutations can be mapped by this procedure (see Table 2). We present data for several recessive and one semidominant marker. The *spo11* mapping method can also be used to map two (or more) mutations which in combination confer a particular phenotype. In this case, clones that express the mutant phenotype will not express two or more of the tester strain chromosomal markers. Meiotic trisomic analysis, the most commonly used method for assigning a gene to a specific chromosome when linkage to known markers has not been established, is only useful for mapping single recessive alleles (MORTIMER and HAWTHORNE 1973; WICKNER 1979). In addition to its utility for mapping dominant and recessive mutations, all of the *spo11-1* strains are marked with the *ura3-1* mutation to permit one to conveniently determine the chromosomal location of integrated recombinant DNA vectors carrying the *URA3* gene.

Third, the yield of informative meiotic colonies for linkage analysis is high. Each chromosomal marker is expressed in approximately a third of all drug-resistant clones (Table 4). Since recombination is virtually absent during sporulation of *spo11-1/spo11-1* diploids, very few diagnostic clones are needed to eliminate a given chromosome as the location of an unmapped mutation (*e.g.*, Table 7).

Finally, the mapping strains were constructed to provide an internal control for the level of recombination. If *cyh2* (chromosome *VII*) is chosen as the marker to provide a drug selection for meiotic products, the chromosome *VII* marker in each tester strain (Table 1A) provides a means to monitor recombination on that chromosome. If *can1* (chromosome *V*) is selected, *hon3* can be included in the initially constructed *spo11-1* mutant haploid to permit monitoring of recombination on chromosome *V*.

Several potential sources of error in interpretation of data may be encountered with the *spo11* mapping procedure. One of these is illustrated by the mapping data for *hom3* (chromosome *V*). The *hom3* marker was not expressed in clones that expressed either the chromosome *V* or the chromosome *VIII* tester strain marker, suggesting that the Hom$^-$ phenotype was the combined effect of two independent alleles (Table 5). The explanation for this result, however, is trivial. All of the *hom3* clones were *can1*, due to the nearly complete linkage of markers on the same chromosome. The presence of *can1* and *arg4*, the chromosome *VIII* marker, in the same clone is incompatible with growth on our synthetic media, as described in RESULTS.

An added cautionary note is that in some genetic backgrounds it may be more difficult to obtain a high yield of meiotic products. Some *spo11-1/spo11-1* diploids exhibit poorer sporulation efficiency (*i.e.*, fewer cells enter sporulation) than the strains used in the studies reported here, resulting in a higher background of mitotic nonsporulated cells among drug-resistant clones. This problem can be reduced by selecting for meiotic products on medium containing two drugs (*i.e.*, CAN plus CYH), thus requiring haploidization or

recombination events on two chromosomes. In addition, we have observed that sporulation of cells at 34°, as opposed to 25°, results in a several-fold reduction in surviving diploid cells.

Is there recombination during sporulation of spo11 *diploids?* Liquid plating experiments with *spo11-1/spo11-1* diploids, heteroallelic at *trp5* (chromosome *VII*) and *ura3* (*V*), reveal that mitotic gene conversion occurs at wild-type levels at both 25° and 34° (S. KLAPHOLZ, C. WADDELL and R. E. ESPOSITO, unpublished). No increase in the production of recombinant prototrophs at these loci, or at *lys2* (*II*), is detected when *spo11-1/spo11-1* diploids undergo meiosis, compared to the 10^3 to 10^4 -fold rise observed in wild-type cells.

In this study, intergenic recombinants were only rarely detected among the selected meiotic products of *spo11-1/spo11-1* diploids sporulated at 34° (Table 12). In this case, it was not possible to determine whether the recombination events took place during sporulation or during a preceding mitosis. Two lines of evidence suggest that at least some of the recombinants were mitotic in origin. First, three of the four *can1* — *hom3* recombinants were derived from a single sporulated diploid, K392-6C × K393-35C, and had the same recombinant genotype. The clonal relationship among the recombinants suggests that the recombinant chromosome arose during vegetative development of the diploid. Second, at least two of the seven recombinants were disomic for the recombinant chromosome. This implies that if recombination does occur during sporulation, it does not lead to proper meiosis I disjunction, as one might expect. Clones that exhibited a recombination event were nonrecombinant in all other intervals tested, indicating that they do not represent a small subpopulation of fully recombination-proficient cells.

The consequences of recombinationless meiosis in yeast: Our data are consistent with the view that in *spo11-1/spo11-1* diploids, chromosomes segregate randomly during meiosis I and then divide equationally at meiosis II, yielding for each chromosome, 50% monosomic, 25% disomic and 25% nullosomic (inviable) spores. We would expect to recover clones that express a given heterozygous recessive marker at a frequency of 33%. In our studies, we observed a range of 8% to 62% for individual markers and an average value of 36%. If all disomes were fully stable (which we know they are not), we would expect a third of all clones to be disomic for any unselected chromosome. We found a range in the recovery of disomes, from 7% to 28% (average 19%) for seven chromosomes. The random segregation of unpaired chromosomes during meiosis I followed by a normal meiosis II has been observed in some asynaptic or desynaptic mutants of plants (see BAKER *et al.* 1976 for review), as well as in monosomic diploids of yeast (BRUENN and MORTIMER 1970) and in yeast strains carrying a single copy centromere-containing plasmid (CLARKE and CARBON 1980; HSIAO and CARBON 1981). Based on our genotypic analysis of a small sample of **a** or α CanR clones by crosses to haploid testers (Table 11), we conclude that meiosis II nondisjunction accounts for less than 1/8 of all clones that express the recessive *can1* allele on chromosome *V* or either of the codominant *MAT* alleles on chromosome *III*.

One of the consequences of random chromosome segregation during the first division of meiosis in an organism with a high number of chromosomes is the production of largely inviable asci. The term $[(1/2)^n + \sum_{i=1}^{n} (1/2)^i (3/4)^{n-i}]$ (Figure 5) defines the frequency with which an ascus will give rise to a potentially viable colony (n=haploid number of chromosomes). For n=16, we expect approximately 2% of all asci to give rise to a viable colony. Furthermore, viable asci should contain either four $[(1/2)^n]$ or two $[\sum_{i=1}^{n} (1/2)^i (3/4)^{n-i}]$ viable spores. In the latter case, the surviving spores should be sisters (Figure 2). In diploids, where ascus survival is extremely poor, these predictions are difficult to test. To address this problem we have been analyzing *spo11-1* tetraploids, where ascus survival is increased because of the higher chromosome copy number. Preliminary studies of the meiotic products of *spo11-1* tetraploids indicate that the majority of surviving asci contain two viable spores.

In addition to the ascus inviability expected from random meiosis I chromosome segregation, several other factors will influence the recovery of viable *spo11* meiotic products. By selecting specifically for the expression of a recessive drug-resistance marker, the number of viable ascal clones is reduced by half. In addition, based on studies of triploid meiosis (PARRY and COX 1970; CAMPBELL *et al.* 1981), it is clear that only a small portion of aneuploid spore clones are viable. Based on our studies thus far, we estimate that ascus viability is approximately 1% on nonselective medium.

Some chromosomes were recovered as disomes more frequently than others. Of several chromosomes studied, disomes for *II* and *III* were most frequent, approaching the theoretical expectation of 33%, disomes for *VII*, *VIII* and *XIV* were intermediate in frequency (17%–24%), and those for *V* and *XV* were least frequent (7% and 12%, respectively. It is important to note that these values are likely to depend to some extent on the particular markers used to monitor disomy, as well as other markers present in the cross, as discussed in RESULTS. The recovery of particular disomes correlated well with the stability of the disomes. This is consistent with the view that disomy for each chromosome arises at the same frequency but that the recovery of an aneuploid clone depends upon how well a given disome is tolerated.

Mapping results: The mapping data for five of the seven previously mapped markers (*i.e.*, *tyr1*, *his7*, *MAT***a**, *met13* and *ade2*) were unambiguous. Of the 101 to 142 clones tested in each case, none expressed both the marker being mapped and the respective tester strain chromosomal marker (Tables 5 and 6). We had previously been unable to map the *spo12-1* mutation by tetrad analysis even though its second-division segregation frequency (less than 55%) indicated that it was centromere-linked (see KLAPHOLZ and ESPOSITO 1980a). The *spo11* mapping procedure placed *spo12-1* on chromosome *VIII*. Additional tetrad analysis showed that it was <1 cM from *pet3* and thus, approximately 80 cM from the centromere on the right arm.

The *spo11* mapping data that assigned the Hom$^-$ phenotype to two chromosomes had a trivial explanation, as noted above. There appear to be no other

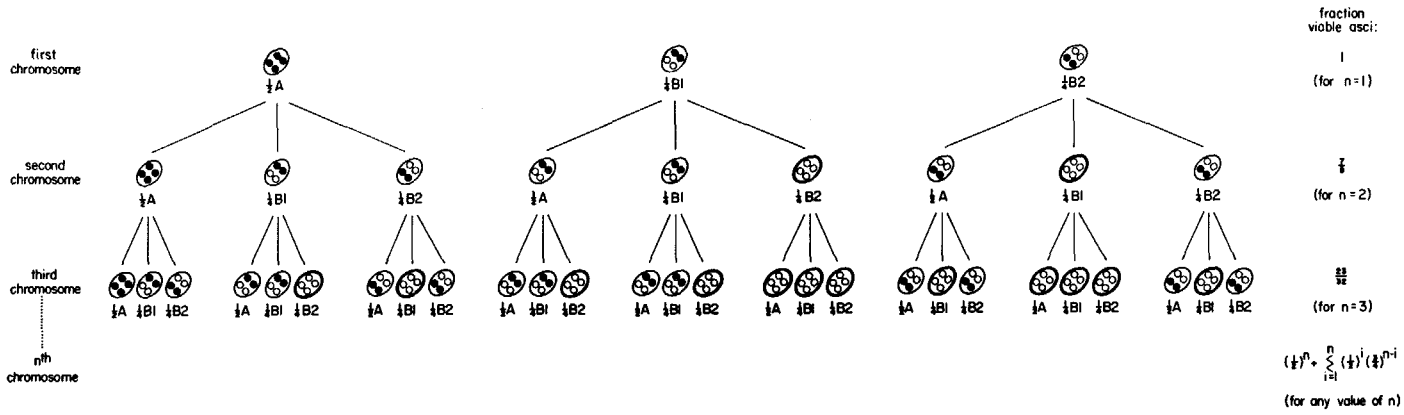

FIGURE 5.—The effect of random meiosis I chromosome segregation on ascus viability. A and B represent the two possible modes of chromosome segregation at meiosis I (see FIGURE 2). B is subdivided into B1, in which both homologs move to the top pole (top is arbitrarily chosen), and B2, in which both homologs move to the bottom pole. The ovals represent asci containing four spores; solid circles are potentially viable (n or n + 1 or more) spores and open circles are inviable (n—1 or more) spores, where n = number of chromosomes. An ascus is considered to be viable if it contains one or more viable spores (solid circles).

marker incompatibilities in our mapping strains besides the one between *can1* and *arg4*. The surprising finding that *spo1* mapped to both chromosome *XIV*, as previously determined by tetrad analysis, and chromosome *XVII*, is explained by the fact that chromosomes *XIV* and *XVII* comprise a single linkage group. In addition to the initial evidence presented here, linkage between markers on chromosomes *XIV* and *XVII* has now been established by conventional trisomic and tetrad analyses, and will be presented elsewhere.

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Corresponding editor: F. SHERMAN