Chromosomes XIV and XVII of *Saccharomyces cerevisiae* Constitute a Single Linkage Group

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We present several lines of evidence that chromosomes XIV and XVII of *Saccharomyces cerevisiae* are not independent chromosomes, but rather constitute a single linkage group. Studies which made use of a new mapping method based on the haploidization-without-recombination meiotic phenotype of the *spoil* mutant initially indicated that markers on chromosomes XIV and XVII were linked. Tetrad analysis was used to establish gene-gene distances, and a new chromosome XIV map incorporating markers originally assigned to chromosome XVII was derived. During the course of trisomic segregation studies, we discovered that a $2n + 2$ homothallic diploid, originally believed to be tetrasomic for chromosome XVII (now XIV), carries two normal chromosome XIV homologs and two aberrant homologs which appear to be deficient for a large portion of the right arm of XIV. The previous evidence that established chromosome XVII as an independent linkage group is discussed in the light of these findings.

The yeast *Saccharomyces cerevisiae* is now widely being used for genetic, molecular, and biochemical studies of gene organization and expression. It is thus important that the chromosome map be accurate and well characterized. Efforts to determine the precise chromosome number of this yeast have been hampered by the small size of its chromosomes and their apparent failure to condense during the life cycle. The currently accepted haploid chromosome number of 17 was established by genetic analysis (10). This number is consistent with indirect evidence of 15 to 17 chromosomes obtained by electron microscopy studies of reconstructed synaptonemal complexes in meiotic cells (1). In addition to the 17 established chromosomes, there are three groups of markers, termed fragments, which have not as yet been linked to a particular chromosome (10). Because *S. cerevisiae* exhibits a high level of meiotic recombination, it is often difficult to map a gene mutation located some distance from other known markers and from its centromere. In many such cases, trisomic segregation analysis has proven to be useful in establishing linkage to a particular chromosome (9, 18). When standard tetrad and trisomic segregation analyses have failed to locate a marker to any of the known chromosomes, new chromosomes have been defined (2, 9). As pointed out by Wickner (18), however, trisomic exclusion data must be viewed with caution, as they may generate artifactual mapping results (for an example, see reference 5).

The seventeenth chromosome of *S. cerevisiae*

was originally established by trisomic and mitotic segregation studies of two centromere-unlinked markers, *met2* and *met4* (9). Trisomic analysis indicated that these markers were linked to one another but not to markers on any of the other 16 known chromosomes. They were placed on opposite arms of chromosome XVII by mitotic recombination data. Although the genome of *S. cerevisiae* is reasonably well mapped, this chromosome has remained the only one for which the centromere has not been defined by a genetic marker (10).

We first obtained evidence that markers assigned to chromosome XIV or XVII were actually located on the same chromosome during the course of developing a new rapid mapping procedure (7). This method utilizes the ability of diploids homozygous for the *spoil* mutation to undergo chromosome segregation and haploidization in the virtual absence of meiotic recombination. The *spoil* mutation causes both a reduction in the formation of asci (sporulation) and a drastic decline in ascospore viability. Rare, viable meiotic products, however, can be selectively recovered by plating for clones that express a recessive drug resistance marker present in heterozygous condition in the parental diploid. The surviving meiotic products of *spoil/spoil* diploids have been shown to arise as a consequence of the random segregation at meiosis I of essentially nonrecombinant chromosomes. Selection for viable ascospore colonies yields cells that are haploid for most, and disomic for the remaining, chromosomes; recoverable disomy for indi-

vidual chromosomes averages approximately 20%.

The key feature of the recombination-deficient (*Rec*⁻) meiotic process in *spoil/spoil* diploids with respect to its utility in genetic mapping is that markers on the same chromosome that display no linkage by meiotic tetrad analysis show nearly complete linkage among *spoil* meiotic products. Thus, genes that appear to be unlinked to their centromeres or other genetic loci by standard recombination analysis can be rapidly assigned to linkage groups. By taking advantage of this property, the location of numerous known genetic markers has been confirmed, and a previously unmapped mutation, *spol2*, was placed on the genetic map of *S. cerevisiae* (7). During these mapping studies, we found that the *met4* marker, located on chromosome XVII, did not behave as expected. Among drug-resistant meiotic segregants this marker exhibited 100% cosegregation with *pet8*, located on chromosome XIV. These results suggested that either *met4* had been previously mapped to an incorrect location or chromosome XVII was not a separate linkage group.

In this paper we report conclusive evidence derived from the *spoil Rec*⁻ mapping procedure, tetrad analysis, and trisomic linkage studies that chromosome XVII is, in fact, not an independent chromosome but part of a single linkage group composed of markers on chromosomes XIV and XVII. Classical tetrad analysis was employed to generate a new genetic map of chromosome XIV that incorporates loci previously assigned to chromosome XVII.

MATERIALS AND METHODS

Strains. The strains of *S. cerevisiae* employed in the genetic studies are listed in Table 1. Strains that were used solely for complementation testing are as follows (only the relevant genotypes are presented): 74-1A (*spol1*); W85-8D and W87-4B (*met4*); W60-10B (*met2*); and W224-1D (*pet8*). Strain 74-1A originated in this laboratory; the remaining strains were kindly provided by R. Rothstein (New Jersey College of Medicine). Strain AN33 (Berkeley Yeast Genetic Stock Center) was used as the killer-sensitive lawn for assaying the *kex2* mutation. Crosses used for meiotic tetrad analysis are shown in Table 2.

Media. The media used in these experiments (YPDA, YPG, sporulation, synthetic complete, synthetic drug, and omission [6, 7] and MB [15, 20]) have been previously described.

Genetic techniques. Standard procedures for growth, sporulation, and ascus dissection were followed (8, 14). The *spoil* mapping procedure is described in detail by Klapholz and Esposito (7) and briefly below and in the legend to Fig. 1. The segregation of the killer phenotype (*kex2* versus *KEX2*) was assayed on MB medium as described by Wickner and Liebowitz (20). For constructing sporulation-capable (i.e., non-petite) *petx PET2/PETx pet2* diploids, vegetative cells of a

pet2 haploid (1893-II-5C) were positioned adjacent to individual ascospores dissected from a *petx/PETx* heterozygote (K425) on YPDA plates. By this procedure, one fourth of the resulting colonies are expected to be diploids heterozygous for *petx* and *pet2*.

RESULTS

***Rec*⁻ meiotic analysis.** The first indication that chromosomes XIV and XVII were not independent linkage groups came from *Rec*⁻ meiotic studies employing the *spoil* mutant (7; see above). These studies demonstrated that two tightly linked centromere markers on chromosome XIV, *pet8* and *spol1*, were linked to the *met4* marker on chromosome XVII. A second marker on chromosome XVII, *met2*, was then examined for its linkage to chromosome XIV markers by the same mapping procedure. Haploid K392-6C (*MATa spoil1 can1 cyh2 spol1 PET8 MET2 MET4*) was crossed to haploid K400-30C (*MATa spoil1 CAN1 CYH2 SPO1 pet8 met2 met4*) (complete genotypes are given in Table 1). The resulting diploid was sporulated and streaked onto cycloheximide-containing medium to select for rare, viable meiotic products, as previously described (7). The classes of drug-resistant meiotic products expected from this analysis are shown in Fig. 1.

The phenotypic classes of 76 drug-resistant clones from the above diploid and the data from the previous study involving only *met4* are summarized in Table 3. The *spol1* and *pet8* chromosome XIV markers present in repulsion (on opposite homologs) in both crosses showed the predicted segregation behavior for genes located on the same chromosome. All *Spo*⁻ clones were *Pet*⁺ (*spol1 PET8*), and all *Pet*⁻ clones were *Spo*⁺ (*SPO1 pet8*); those which were *Spo*⁺ *Pet*⁺ (20% of the total) were considered to be most likely disomic for chromosome XIV (*spol1 PET8/SPO1 pet8*), since recombination is negligible during meiosis in *spoil/spoil* diploids (7). As expected, no recombination was detected between *met2* and *met4* (in cross K392-6C × K400-30C), two chromosome XVII markers which show no linkage by tetrad analysis (see Table 4).

The surprising result was that the chromosome XIV and XVII markers consistently segregated as if they were part of a single linkage group (*SPO1 pet8 met2 met4/spol1 PET8 MET2 MET4*). (i) *pet8* showed 100% cosegregation with *met2* and *met4*; (ii) *spol1* always segregated away from the *met* markers; and (iii) all clones that were phenotypically diagnostic of chromosome XIV disomy were also *Met*⁺, consistent with disomy for the chromosome bearing the *met* mutations. Our interpretation of these results is shown in Fig. 1, section II.

A recombination-proficient (*Rec*⁺) diploid was analyzed next to test the possibility that

TABLE 1. Strains

Strain	Genotype ^a	Source
A433	<i>MATα ade2 his4C lys9 trp5 tsm5</i>	J. Haber
CG89	<i>MATα can1 gal2 SUC2</i>	C. Giroux
K262-28D	<i>MATα ade2 ade6 cyh2 his7 hom3 leu1 lys2 met13 trp5 tyr1 ura3</i>	S. Klapholz
K326-4A	<i>MATα ade1 his6 leu1 leu2 lys1 met3 met4 met14 pet8 ura3</i>	S. Klapholz
K338-8A	<i>MATα aro7 cyh2 his6 leu2 lys1 pet8 spo12 ura3</i>	S. Klapholz
K365-13A	<i>MATα his7 leu1 lys1 met4 pet8</i>	S. Klapholz
K368-8D	<i>MATα ade2 ade6 cdc14 his2 lys1 met14 spo11 ura3</i>	S. Klapholz
K389-15A	<i>MATα ade2 lys1 met4 ura3</i>	S. Klapholz
K389-27C	<i>MATα his2 leu1 lys1 met14 pet8 spo11 ura3</i>	S. Klapholz
K392-6C	<i>MATα ade2 can1 cyh2 his7 hom3 met13 spo1 spo11 tyr1 ura3</i>	S. Klapholz
K393-2D	<i>MATα his2 lys1 met4 pet8 ura3</i>	S. Klapholz
K393-27C	<i>MATα his2 leu1 lys1 met4 pet8 ura3</i>	S. Klapholz
K393-35C	<i>MATα his7 leu1 lys1 met4 pet8 spo11 ura3</i>	S. Klapholz
K393-38A	<i>MATα his2 leu1 lys1 met4 spo11 ura3</i>	S. Klapholz
K399-7D	<i>MATα his2 leu1 lys1 met4 pet8 spo11 ura3</i>	S. Klapholz
K400-3C	<i>MATα his2 lys2 met2 pet8 spo11 ura3</i>	S. Klapholz
K400-25B	<i>MATα his7 leu1 lys1 met2 met4 pet8</i>	S. Klapholz
K400-30C	<i>MATα arg1 his7 leu1 lys1 met2 met4 pet8 spo11</i>	S. Klapholz
K402-4A	<i>MATα lys9 met2 met14/MET14</i>	S. Klapholz
K402-4B	<i>MATα arg1 his4C his7 lys9 met2 met14/MET14</i>	S. Klapholz
K411-7D	<i>MATα ade arg leu1 lys2 (lys7 or LYS7) pha2 met2</i>	S. Klapholz
K411-9C	<i>MATα ade1 leu1 lys2 lys9 met2 pet2 pha2 met14/MET14</i>	S. Klapholz
K425-4A	<i>MATα ade lys met2 petx</i>	S. Klapholz
W224-1C	<i>MATα his6 leu2 lys1 met14 pet8 ura3</i>	R. Rothstein
1230	<i>MATα arg1 his7 met2 met14 pet8</i>	R. Wickner
1232	<i>MATα arg1 his7 met2 pet8 met14/MET14</i>	R. Wickner
1464	<i>MATα ade1 ade2 his7 lys2 ski3 tyr1 ura1</i>	R. Wickner
1595	<i>MATα kex2 petx ura1</i>	R. Wickner
1596	<i>MATα ade2 lys petx</i>	R. Wickner
1893-II-5C	<i>MATα ade1 ade arg gal7 leu1 lys2 lys7 met2 pet2 pha2 ura3</i>	Berkeley Yeast Genetic Stock Center
1933-2A (S41)	<i>MATα/MATα [HO arg4 met4/MET4]</i>	D. Hawthorne (M. and R. Esposito)
K10-2D	<i>MATα/MATα [HO ade2 ade5 can1 his7 leu1 lys2 met13 spo1 trp5 tyr1 ura3]</i>	S. Klapholz
K49-7B	<i>MATα/MATα [HO ade2 cyh2 his7 leu1 lys2 met13 spo1 trp5 tyr1 ura3]</i>	S. Klapholz

^a Gene symbols: *MATα* and *MATα*, mating type; *ade*, adenine; *arg*, arginine; *aro*, aromatic amino acids; *can*, canavanine; *cdc*, cell division cycle; *cyh*, cycloheximide; *gal*, galactose; *his*, histidine; *HO*, homothallism; *hom*, homoserine; *kex*, killer expression; *leu*, leucine; *lys*, lysine; *met*, methionine; *pet*, petite; *pha*, phenylalanine; *ski*, super killer; *spo*, sporulation defective; *suc*, sucrose; *trp*, tryptophan; *tsm*, temperature-sensitive lethal; *tyr*, tyrosine; *ura*, uracil (10, 12). All genes within brackets for *HO*-containing strains are present in homozygous condition.

Pet⁺ Met⁻ and Pet⁻ Met⁺ drug-resistant clones failed to be recovered due to a strong selective disadvantage in this protocol. We reasoned that if these classes were absent because of genetic linkage rather than selection, then they should be recovered among drug-resistant meiotic products derived from a meiosis in which recombination was permitted to take place. The same parental strain that was used in the two previous crosses, K392-6C (*MATα spo11 can1 cyh2 spo1 PET8 MET2 MET4*), was mated to haploid K400-25B (*MATα SPO11 CAN1 CYH2 pet8 met2 met4*). The resulting Rec⁺ *spo11/SPO11*

heterozygote was examined in the same manner as the Rec⁻ *spo11/spo11* homozygotes described above. Among 55 drug-resistant *a* or *α* meiotic products, approximately equal numbers of *pet8 MET4*, *PET8 met4*, and *pet8 met4* colonies were obtained, eliminating selection as a factor in the previous results.

Marker confirmation. We further ruled out the possibility that the markers used in these studies were not, in fact, *pet8*, *met2*, and *met4* but some other linked *pet* and *met* markers by complementation testing and tetrad analysis. The following pertinent complementation test results

Construction of *spo11* / *spo11* diploid

Can R or Cyh R meiotic segregants

Genotypes if chromosomes **XIV** and **XVII** are:

I. Independent chromosomes

II. One chromosome

TABLE 2. Crosses used for meiotic tetrad analysis

Cross no.	Parental strains	Spore viability (%)	Total no. of complete tetrads
1	K402-4B × 1596	85	41
2	A433 × 1230	94	16
3	K402-4A × K393-2D	96	23
4	K400-30C × 1596	96	98
5	K10-2D × W224-1C	100	10
6	K49-7B × W224-1C	100	9
7	K400-30C × 1464	93	30
8	K411-9C × K399-7D	91	69
9	K393-38A × 1232	96	24
10	K411-7D × 1595	95	44
11	K402-4A × 1893-II-5C	85	19
12	CG89 × K393-35C	100	10
13	K326-4A × K262-28D	98	11
14	K365-13A × K368-8D	96	21
15	K389-15A × K389-27C	90	105
16	K425-4A × 1893-II-5C	93	34

were obtained. The *met2* marker used in these experiments, which originated from strain 1232 (from R. Wickner, National Institutes of Health), failed to complement the *met2* mutation in strains W60-10B (R. Rothstein, New Jersey Medical College) and 1893-II-5C (Berkeley Yeast Genetic Stock Center). The *met4* marker, derived from strain W230-12A (R. Rothstein), did not complement the *met4* mutation in Berkeley Yeast Genetic Stock Center strains S1336B and P65. In addition, tetrad analysis of *pet8* versus *lys9* (chromosome XIV) and of *met2* versus *pha2* and *pet2* (chromosome XVII) gave results consistent with the previously published map (10; Table 4). Furthermore, crosses of our strains to those from other laboratories generally gave excellent ascospore viability (average, 93%), eliminating the presence of a simple chromosomal aberration, such as a translocation, uniquely present in our strains as an explanation of the data (Table 2).

Meiotic tetrad analysis. To establish specific linkage relationships and construct a new chromosome XIV-XVII map, we undertook meiotic tetrad analysis. In all cases our tetrad data (Table 4) were in good agreement with previously determined map distances between pairs of markers on chromosome XIV and pairs of markers on chromosome XVII (reviewed by Mortimer and Schild [10]). In addition, we detected linkage between four chromosome XIV-chromo-

some XVII marker pairs: *kex2-met2* (28.0 centi-Morgans [cM]), *petx-met2* (38.1 cM), *petx-pet2* (35.3 cM), and *pet8-met4* (61.9 cM).

***pha2-met2-pet2-kex2-petx* linkage group.** We had originally suspected that *petx*, a nuclear petite mutation isolated and mapped to chromosome XIV by Wickner and Liebowitz (20), might be a mutation in the same gene as *pet2* on chromosome XVII, since the two mutations previously had neither been complementation tested nor mapped relative to one another. Indeed, intercrosses of *petx* and *pet2* haploids resulted in hybrids that were petite, i.e., they failed to grow on YPG medium and were incapable of sporulation (data not shown). However, because certain petite mutations lead to rapid loss of mitochondrial DNA (13), two mutations in distinct *pet* genes may fail to exhibit complementation. To circumvent this problem, we performed spore-to-cell matings between *petx* and *pet2* strains (see above). One cross-hybrid, K425-4A × 1893-II-5C, was analyzed. This diploid grew normally on YPG and sporulated, indicating that *petx* and *pet2* define separate loci. However, subsequent tetrad analysis of this diploid demonstrated that these two markers were, in fact, located on the same chromosome, approximately 35 cM apart (Table 4).

The relative order of *met2*, *kex2*, and *petx* was determined from a three-point cross between haploids K411-7D (*KEX2 met2 PETx*) and 1595 (*kex2 MET2 petx*). Three tetratype (T) asci for *kex2-petx* were recovered from this cross; all were parental ditYPE (PD) for *kex2-met2*, and one was PD and two were T for *petx-met2*. These data support the gene order determined from the calculated gene-gene map distances alone: *met2-kex2-petx*. The additional fact that *petx* does not show linkage to *pha2*, located 40 cM from *met2*, places *petx* and *pha2* on opposite sides of and approximately equidistant from *met2* (Table 4). The final map for this linkage group based on these data is thus *pha2-40-met2-14-pet2-21-kex2-6-petx*. The *pet2-kex2* distance, not determined directly by tetrad analysis, was calculated by subtracting the *pet2-met2* and the *petx-kex2* distances from the *petx-met2* value.

***lys9-pet8-spo1-met4* linkage group.** The *pet8-lys9* map distance obtained in our study (36.8 cM) is in agreement with previously published data (37.5 cM) (Table 4) (10), as is our calculated *pet8-centromere* distance of less than 1 cM (data not shown). The tight *pet8-spo1* linkage (<2.6

FIG. 1. The *spo11* mapping scheme. A *Rec⁻ spo11/spo11* diploid, heterozygous for the two drug resistance markers *can1* and *cyh2*, chromosome XIV markers *pet8* and *spo1*, and chromosome XVII markers *met2* and *met4*, was constructed. The diploid was then sporulated, and *CanR* or *CyhR* meiotic segregants were selected on the appropriate medium. These segregants will either be monosomic or disomic for any unselected chromosome. The relative order of markers on the same chromosome was arbitrarily chosen. *Spo⁺* and *Spo⁻* refer to the phenotype at the *spo1* locus; *Met⁺* and *Met⁻* refer to the phenotype at both the *met2* and *met4* loci, and *Pet⁺* and *Pet⁻* refer to the phenotype at the *pet8* locus.

TABLE 3. Rec⁻ meiotic analysis of markers previously assigned to chromosome XIV or XVII

Cross	Relevant genotype ^a	No. of drug-resistant clones expressing the following phenotype ^b					
		Spo ⁻	Pet ⁺	Met ⁺	Spo ⁺	Pet ⁻	Met ⁻
K392-6C × K393-35C	<i>spo1 PET8 MET4</i> × <i>SPO1 pet8 met4</i>	51			19		16
K392-6C × K400-30C	<i>spo1 PET8 MET4</i> × <i>MET2 × SPO1 pet8 met4 met2</i>	35			24		17

^a All cross diploids are *spo11/spo11* and heterozygous for the recessive drug resistance markers *can1* and *cyh2*. Complete genotypes are listed in Table 1.

^b The diploids were sporulated at 34°C and plated to select for haploidized drug-resistant colonies. Only mating-capable (α or α) clones were chosen for analysis to facilitate complementation tests. All of the colonies analyzed could be classified into the three phenotypic classes shown. Spo⁺ and Spo⁻ refer to the phenotype at the *spo1* locus. In the second cross, all Met⁻ colonies were *met2 met4* in genotype. See the text for details.

cM) was originally demonstrated in this laboratory. Loose linkage was observed between *pet8* and *met4* ($\chi^2 < 0.005$), based on 219 tetrads from several crosses. The bulk of this data comes from cross K389-15A × K389-27C (PD = 24; non-PD (NPD) = 6; T = 72). The lack of linkage between *met4* and *lys9* (XIV, right arm) places *met4* on the left arm of chromosome XIV.

New chromosome XIV map. The proposed new map of chromosome XIV, indicating the positions of markers analyzed in this study, is shown in Fig. 2 along with the original chromosome XIV and chromosome XVII maps for comparison. Toh-E et al. (17) showed that the cluster of genes including *ski3*, *petx*, and *kex2* was located on the opposite arm of chromosome

TABLE 4. Tetrad analysis of markers previously assigned to chromosome XIV or XVII

Marker pair	No. of tetrads that were:				Map distance (cM) ^a	Crosses analyzed ^b
	PD	NPD	T	Total no.		
Chromosome XIV × XIV						
<i>lys9 - petx</i>	2	8	30	40		1
<i>pet8 - lys9</i>	15	1	22	38	36.8 (37.5)	2, 3
<i>pet8 - petx</i>	12	12	62	77		4
<i>pet8 - spo1</i>	19	0	0	19	<2.6	5, 6
<i>petx - kex2</i>	24	0	3	27	5.6 (7.0)	10
Chromosome XVII × XVII						
<i>met2 - met4</i>	40	36	151	227		3, 4, 7, 8, 9
<i>met2 - pet2</i>	45	0	18	63	14.3 (15.2)	8
<i>met2 - pha2</i>	36	3	66	105	40.0 (37.6)	8, 10
<i>met4 - pet2</i>	17	7	43	67		8
<i>met4 - pha2</i>	11	8	50	69		8
<i>pet2 - pha2</i>	16	3	68	87	49.4 (51.9)	8, 11
Chromosomes XIV × XVII						
<i>lys9 - met2</i>	22	19	98	139		1, 2, 3, 8
<i>lys9 - met4</i>	9	13	63	85		3, 8
<i>lys9 - pet2</i>	11	17	52	80		8, 11
<i>lys9 - pha2</i>	9	16	56	81		8, 11
<i>kex2 - met2</i>	13	0	13	26	25.0	10
<i>kex2 - pha2</i>	0	6	21	27		10
<i>pet8 - met2</i>	12	16	63	91		2, 3, 7, 9
<i>pet8 - met4</i>	48	20	151	219	61.9	3, 7, 9, 12–15
<i>pet8 - pet2</i>	7	3	18	28		8
<i>petx - met2</i>	55	5	104	164	40.8	1, 4, 10
<i>petx - met4</i>	15	11	51	77		4
<i>petx - pet2</i>	15	1	18	34	35.3	16
<i>petx - pha2</i>	2	8	31	41		10

^a Map distance was calculated by the formula $(T + 6 \text{ NPD})/[2(\text{PD} + \text{NPD} + T)] \times 100$ (11). In all cases where map distance was determined, the number of PD tetrads was significantly greater than the number of NPD tetrads ($\chi^2 < 0.005$). Map distances from the compiled data of Mortimer and Schild (10) are given in parentheses.

^b Numbers refer to crosses listed in Table 2.

FIG. 2. The original and new chromosome XIV and XVII maps. The original maps are based on the compiled data of Mortimer and Schild (10) and on the chromosome XIV mapping data of Wickner (19); the new map is based on the data presented in Table 4. Symbols: ○, Centromeres; ..., trisomic linkage; ---, mitotic linkage. All other linkage values, in centiMorgans, were determined by tetrad analysis.

XIV from *lys9* by mitotic recombination analysis. Taking this into account, we would place both the *pha2-met2-pet2-kex2-petx* cluster and *met4* on the left arm of chromosome XIV. The right arm, containing *pet8*, *spo1*, and *lys9*, remains unchanged by this study. Since neither *petx* nor *pha2* is linked to *met4*, our data cannot distinguish between the orders (*petx-met2-pha2*)-*met4*-centromere versus (*pha2-met2-petx*)-*met4*-centromere (see below).

An aberrant chromosome XIV. During the course of trisomic segregation studies of mark-

ers originally assigned to chromosomes XIV and XVII, we obtained evidence that a chromosomal aberration may have been responsible for the initial misdesignation of chromosome XVII as an independent linkage group. These studies employed a homothallic diploid, designated S41, thought to be tetrasomic for chromosome XVII and heterozygous for *met4*. Because of its unusual genetic background a brief history of S41 is given here. S41 (obtained from D. Hawthorne) arose from self-diploidization of spore 1933-2A and was employed in the isolation of sporulation-defective (*spo*) mutants in this laboratory (4). Generally, all markers except for the mating-type locus are present in homozygous condition in diploids originating from homothallic spores. Unexpectedly, however, when S41, a *Met*⁺ diploid, was sporulated and dissected, it segregated *Met*⁻ spores at a low frequency (R. E. Esposito and M. Esposito, unpublished data); the *met* marker in these spores was identified as *met4* (D. Hawthorne, personal communication; M. Esposito, personal communication; R. Rothstein, personal communication).

Two observations indicated that S41 was aneuploid for the chromosome bearing *met4*: (i) the segregation of *met4* in S41 tetrads and (ii) aberrant segregation of *met2*, *pet2*, and *pha2*, located on the same chromosome as *met4*, in crosses of S41 ascospores to marked haploids (R. Rothstein, personal communication) (Table 5). The simplest interpretation of these findings is that S41 is a $2n + 2$ tetrasomic diploid (*MET4/MET4/met4/met4*) and thus that its progenitor ascospore 1933-2A was an $n + 1$ disomic haploid (*MET4/met4*). However, the absence of any $2^{+}:2^{-}$ segregation for *met4* among tetrads derived from S41 and atypical trisomic segregation ratios for *met4* compared with other markers in crosses involving S41 ascospores suggested a more complex constitution of the *met4* chromosome in S41 (Table 5).

In a cross of S41 ascospores to a strain bearing the *petx* marker on the left arm of chromosome XIV, trisomic segregation patterns were observed for both *petx* and *met4* (Table 5). This finding is consistent with the data described earlier from the *spo11* and tetrad mapping studies, which showed that chromosomes XIV and XVII comprise a single linkage group. In apparent contradiction to these results, however, crosses of S41 ascospores to strains carrying markers on the right arm of chromosome XIV, i.e., *pet8* and *lys9*, gave $2^{+}:2^{-}$ segregation for these markers (Table 5). Similar observations have been made independently by D. Hawthorne (personal communication) and R. Mortimer (personal communication).

These results, together with the abnormal segregation patterns noted above, led us to

TABLE 5. Tetrasomic and trisomic analysis of markers previously assigned to chromosome XIV or XVII

Diploid	Marker ^a	Marker segregation ^b (+:-)					Spore viability (%)
		4:0	3:1	2:2	1:3	0:4	
S41	<i>met4</i>	36	13	0	0	0	95
S41 × 1596	<i>met4</i>	28	17	0	0	0	90
	<i>petx</i>	6	32	6	0	1	
S41 × K338-8A	<i>met4</i>	21	18	0	0	0	95
	<i>pet8</i>	0	0	39	0	0	
S41 × K400-3C	<i>met4</i>	18	7	0	0	0	89
	<i>met2</i>	9	13	3	0	0	
	<i>pet8</i>	0	0	28	0	0	
S41 × K402-4A	<i>met4</i>	16	13	0	0	0	90
	<i>met2</i>	6	17	6	0	0	
	<i>lys9</i>	0	0	32	0	0	
S41 × K411-9C	<i>met4</i>	14	5	1	0	0	85
	<i>met2</i>	7	12	1	0	0	
	<i>pet2</i>	8	20	2	0	0	
	<i>pha2</i>	8	17	5	0	0	
	<i>lys9</i>	0	0	27	0	0	
S41 × K393-27C	<i>met4</i>	0	0	13	2	0	85
	<i>pet8</i>	0	0	15	0	0	
S41 × K402-4B	<i>met4</i>	4	0	0	0	0	64
	<i>met2</i>	0	4	0	0	0	
	<i>lys9</i>	0	1	3	0	0	

^a The *met4* marker is present in +/+/- trisomic configuration except in diploid S41, where it is +/+/-/- tetrasomic, and in diploid S41 × K393-27C, where it is +/-/- trisomic in configuration. All other markers are present in +/+/- or +/-/- condition; in the latter case we propose that the actual configuration is +/Δ/-, where Δ represents a deletion covering the recessive allele (see Table 1 for the complete strain genotypes).

^b In crosses of S41 to marked haploids, 4:0 and 3:1 segregation patterns are diagnostic of +/+/- trisomy, 2:2 marker segregation is consistent with either +/- disomy, +/+/- trisomy, or +/-/- trisomy, and 1:3 segregation is indicative of +/-/- trisomy.

propose that S41 is a tetrasomic diploid with two normal homologs and two aberrant homologs of chromosome XIV. The aberrant chromosomes appear to lack genetic information on the right arm of chromosome XIV, extending from *pet8* to *lys9*, but have a functional centromere and a left arm that includes the *MET4*, *MET2*, *PET2*, *PETx*, and *PHA2* loci. Because of the extent of the proposed deficiency, covering at least one known essential gene, *RNA2* (Fig. 2), this chromosome is not expected to be viable in the absence of the normal homolog. The high spore viability of S41 alone (Table 5) suggests that during meiosis the two normal homologs disjoin and the two deficient chromosomes disjoin preferentially. The behavior of trisomic diploids derived from crosses of S41 ascospores to marked haploid strains is consistent with preferential pairing of structurally similar homologs. If random two-from-one chromosome segregation occurred at meiosis I in trisomic diploids with two normal homologs and one aberrant homolog, then one third of the tetrads

should have contained two live and two dead spores. This follows from the fact that the deficient chromosome is expected to behave as a centromere-linked lethal. Although we observed a wide range of spore viability in crosses involving S41, no evidence for this nonrandom pattern of spore death was obtained (data not shown).

Although the viability data are thus most easily interpreted as reflecting preferential pairing of structurally similar homologs, such pairing conditions should preclude recombination between dissimilar homologs, which clearly is not the case. Exchange between a normal and a deficient chromosome was detected by the presence of 3⁺:1⁻ tetrads for *met4* in both S41 and the trisomic diploids S41 × 1596, S41 × K400-3C, S41 × K402-4A, and S41 × K411-9C (Table 5). In cross S41 × 1596, for example, the S41 parental ascospore must have contributed a deficient homolog carrying *PETx* and *met4* and a normal homolog carrying *PETx* and *MET4*; the other parent donated a normal homolog marked with *petx* and *MET4*. If the *met4* locus was

located on the normal homolog in the ascospore from S41, then among asci with four viable spores $2^+ : 2^-$ tetrads should have been found for *met4*, and *petx* and *met4* segregation patterns should have been similar. Neither of these predictions was fulfilled (Table 5). It is not possible from this cross alone to infer that the deficiency and *met4* are coupled in the parental S41 tetrasomic diploid, as recombination could have placed *met4* on the deficient homolog during meiosis and ascospore formation. Nevertheless, it seems most likely that *met4* marks the deficient homologs in S41, since in each of the crosses examined above *met4* showed a segregation pattern distinctly different from the chromosome XIV left-arm markers contributed by the haploid parent.

To reconcile the evidence for preferential disjunction of similar homologs with the occurrence of genetic recombination between dissimilar homologs, we must assume that trivalent (or tetraivalent in the case of S41) associations occur in a proportion of meiotic cells. The ratios of the various tetrad types containing four viable spores (i.e., $4^+ : 0^-$, $3^+ : 1^-$, and $2^+ : 2^-$) observed in crosses of S41 to marked haploids do not agree with our calculated expectations based upon either 100% trivalent pairing (i.e., 4:10:1 for the various tetrad types, respectively, for *petx*, *pet2*, *pha2*, *met2*, and *met4*) or 100% bivalent:univalent pairing, in which the two normal homologs pair and the aberrant homolog is unpaired (i.e., 1:4:1 for the various tetrad types, respectively, for *petx*, *pet2*, *pha2*, and *met2*, and 1:0:0 for *met4*). These initial results suggest that trivalent associations are, however, occurring in at least 50% of the meiotic cells. Additional data are required to obtain a more reliable estimate of the proportion of each pairing configuration. The relevance of the findings reported in this paper to the data that originally defined XVII as a separate chromosome is considered below.

DISCUSSION

Using a new mapping method based on the ability of *spo11/spo11* diploids to undergo haploidization in the absence of appreciable recombination (7), we showed that chromosomes XIV and XVII of *S. cerevisiae* comprise a single linkage group. The proposed new map of chromosome XIV, derived by tetrad analysis, incorporates chromosome XVII markers into the left arm; the genetic map of the relatively short right arm has not been altered (Fig. 2).

The existence of chromosome XVII was originally based on the trisomic segregation studies of Mortimer and Hawthorne (9) which: (i) eliminated *met2* and *met4* from chromosomes I through XVI and (ii) demonstrated that these markers were located on the same chromosome.

Although meiotic linkage of several other markers to *met2* expanded the genetic map of chromosome XVII, this chromosome has remained the only one in *S. cerevisiae* without a marker-defined centromere (10).

Chromosome XIV was specifically eliminated as a possible location for *met4* by trisomic studies employing strains descended from strain 1933-2A (D. Hawthorne, personal communication). Crosses of these derivatives, disomic for the chromosome bearing the *met4* marker (i.e., $n + 1$, *met4/MET4*), to marked haploids gave trisomic segregation for *met4* and *met2* and $2^+ : 2^-$ segregation for markers on the right arm of chromosome XIV (9). The results of our studies with S41 (identical to strain 1933-2A) are consistent with the findings of Mortimer and Hawthorne (9). However, we also observed trisomic segregation patterns for *petx*, known to be located on the left arm of chromosome XIV. The fact that markers on the left arm of chromosome XIV showed trisomic linkage whereas markers on the right arm segregated normally in crosses involving S41 suggested that S41 carries an extra copy of chromosome XIV which has a large deficiency on the right arm. The deficiency covers *pet8*, located within 2 cM of the centromere, and *lys9*, located approximately 40 cM from *pet8*. Based on correlations of map distance with physical distance on several other chromosomes, we can estimate that this deficiency covers at least 100 kilobases of DNA (16). The fact that the aberrant chromosome has a functional centromere can be inferred from its mitotic stability and its ability to segregate during meiosis. The appearance of *met4* segregants among the meiotic progeny of S41 and in crosses of S41 ascospores to other strains, as well as the high spore viability in many of these crosses, favor the view that the deficient chromosome(s) is capable of pairing and recombination with normal homologs but then usually segregates independently of them as a separate univalent (or bivalent in S41). Although overall spore viability in one cross involving S41 was poor (Table 5), random two-from-one segregation in trisomic crosses would yield 33% asci with two live and two dead spores; this pattern has not been found. The fact that *pet8* segregates $2^+ : 2^-$ in crosses to S41 supports the positioning of *pet8* on the right arm of chromosome XIV, since if it were located on the left arm it would exhibit trisomic segregation. Previous studies were consistent with either the order *SUF10-pet8-centromere-rna2* or *SUF10-centromere-pet8-rna2* (3).

The proposed existence of a deficient chromosome in S41 also provides an explanation for a hitherto puzzling question concerning the isolation of *spo1*, a recessive sporulation-defective mutation closely linked to *pet8* on chromosome

XIV. This mutation was initially detected after mutagenesis of S41 ascospores; it segregated $2^+ : 2^-$ in backcrosses to homothallic S41 derivatives and to haploid strains (4; S. Klapholz, unpublished data). The recovery of a recessive mutation from a genetic background containing two homologs for the chromosome on which it is located can now be simply understood. These findings are consistent with the view that the haploid ascospore which gave rise to *spo1* contained a right-arm-deficient homolog and a normal homolog of chromosome XIV and that the *spo1* mutation was induced on the right arm of the normal homolog.

A combined genetic map of the new chromosome XIV, showing the positions of all known markers mapped to chromosomes XIV and XVII from this and other studies, is shown in Fig. 3. Mitotic recombination studies originally placed *met4* and *met2* on opposite arms of chromosome XIV (cited in reference 9, but data not presented). This result conflicts with our location of *met2* and *met4* on the same arm of chromosome XIV (Fig. 2). We favor the positioning of *met2* and *met4* on the same chromosome arm for several reasons: (i) *met4* is linked to *pet8* but not to *lys9*, clearly placing it on the left arm of chromosome XIV; (ii) *met2* is linked to *kex2* and *petx*, two markers located on the left arm of chromosome XIV by the mitotic linkage studies of Toh-E et al. (17); (iii) crosses with S41, which exhibit trisomic segregation for *petx* and *met4* on the left arm of chromosome XIV and disomic segregation for *pet8* and *lys9* on the right arm also give trisomic segregation for *pet2*, *pha2*, and *met2*, arguing in favor of locating these markers on the left arm. Recent support for the location of *met4* on the left arm of chromosome XIV has also been obtained by Klein and Petes (manuscript in preparation), who mapped a Ty insertion marked by *LEU2* to a position between *pet8* and *met4*. Moreover, strong evidence that *met2* and *met4* are located on the same chromosomal arm has been independently reported by Culbertson et al. (in press), who mapped a new centromere-unlinked marker, *suf14*, to a position between *pet2* and *met4*. Their data further argue that the gene order *pha2-met2-petx-suf14-met4* is the correct one (Fig. 2 and 3).

The new map predicts that the *ski3* mutation, located 75 cM from *kex2* (17) and on the opposite side from *mak26* (19), should be very closely linked to *pha2*. In addition, the *lap3* mutation, previously assigned to chromosome XVII (10), should be located within the cluster defined by *kex2* and *ski4* on chromosome XIV. It should be noted that in the aneuploid mapping study that placed *kex2* and *petx* on chromosome XIV, chromosome XVII was ruled out as the location

Proposed
Chromosome XIV

FIG. 3. The proposed new chromosome XIV map. The proposed map is based on data from this study (Table 4), data from Culbertson et al. (in press), data previously reported by Wickner (19), and data reviewed by Mortimer and Schild (10). In constructing this map we have assumed that *kar1*, *pet2*, and *lap3* maintain the same positions relative to *pet2*, *met2*, and *pha2* as were previously determined (9, 10). Similarly, combining the maps of chromosomes XIV and XVII should not alter the relative positions of *rna2* (9), *SUF10* (3), *mak26* (19), or *ski4* (17). See text for further explanation of the location of the other markers shown.

of these markers on the basis of trisomic segregation of *kex2* and *petx*, coincident with normal segregation of *lys10*, located on chromosome XVII (18). It has now been shown, however, that the *lys10* marker used in the above study, which was obtained from the Berkeley Yeast Genetics Stock Center (R. Wickner, personal communication), is actually *lys11*, located on chromosome IX (Berkeley Yeast Genetic Stock Center, catalog, 4th ed.). Hence, the conclusion that *kex2* and *petx* are located on a chromosome independent from XVII is no longer valid. The data at hand are not sufficient to suggest a new location for the *lys10* marker, linked to *met4* solely on the basis of mitotic recombination analysis (9). Since the *lys10* marker is presently unavailable from the Berkeley Yeast Genetic Stock Center and could not be studied further, we have omitted it from the map shown in Fig. 3.

In summary, we used a novel mapping procedure to establish linkage between markers previously mapped to chromosome XVII and markers on chromosome XIV. Tetrad analysis was then used to construct a new chromosome XIV map. In the course of this study we found that a diploid strain believed to be tetrasomic for chromosome XVII (now part of XIV) appears to contain two right-arm-deficient homologs of chromosome XIV in addition to two normal homologs. The use of a derivative of this strain in trisomic linkage studies (9) was most likely responsible for the original misdesignation of chromosome XVII as a new linkage group.

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