MAP POSITIONS OF YEAST GENES *SIR1*, *SIR3* and *SIR4*

JOHN M. IVY,[1] JAMES B. HICKS AND AMAR J. S. KLAR

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

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ABSTRACT

The *HML* and *HMR* loci in the yeast *Saccharomyces cerevisiae* each contain a complete copy of mating-type information. *HML* and *HMR* normally are transcriptionally inactive due to four unlinked genes, known as *MAR* or *SIR* or *CMT*. The map position of *MAR1* (*SIR2*) has been reported previously; it is located on the left arm of chromosome *IV*, 27 cM from the centromere. Using conventional meiotic and mitotic mapping combined with recombinant DNA techniques, we have mapped three other *SIR* genes. *SIR1* maps near the telomere of the right arm of chromosome *XI*; *SIR3* (*MAR2*) maps to the right arm of chromosome *XII*, 31 cM distal to *URA4*; and *SIR4* maps to the right arm of chromosome *IV*, 16 cM proximal to *LYS4*.

MATING type in the budding yeast *Saccharomyces cerevisiae*, designated **a** or α, is determined by alleles of the *MAT* locus, *MAT***a** or *MAT*α, respectively. Two other loci, *HML* and *HMR*, are loosely linked to *MAT* on chromosome *III* (HARASHIMA and OSHIMA 1976, KLAR *et al.* 1980), and each contains a complete copy of either **a** or α mating-type information (HICKS, STRATHERN and KLAR 1979; NASMYTH and TATCHELL 1980). Both *HML* and *HMR*, however, are maintained transcriptionally inactive by four genes, known variously as *MAR* (*ma*ting-type *r*egulator; KLAR, FOGEL and MACLEOD 1979), *SIR* (*s*ilent *i*nformation *r*egulator; RINE *et al.* 1979; RINE 1979), or *CMT* (*c*ontrol of *m*ating *t*ype; HABER and GEORGE 1979). Their gene products act in *trans* to keep *HML* and *HMR* unexpressed; mutation in any one *SIR* gene results in expression of the *HM* loci.

The map position of *MAR1* (*SIR2*) on chromosome *IV-L* (*L* for left arm), 27 cM from the centromere, has been reported previously (KLAR, FOGEL and MACLEOD 1979). This communication reports the map position of the three other *SIR* genes. *SIR1* maps to chromosome *XI-R* (*R* for right arm) and is tightly linked to *MAL4*; *SIR3* (*MAR2*, *CMT*) maps to chromosome *XII-R*, 31 cM distal to *URA4*; and *SIR4* maps to chromosome *IV-R* between *HOM2* and *LYS4*.

MATERIALS AND METHODS

Yeast culturing and genetic manipulations: Strains used in this work and their genotypes are listed in Table 1. Genetic designations are described in MORTIMER and SCHILD (1980). Standard condi-

[1] Present address: Oceanic Institute, Makapuu Point, Waimanalo, Hawaii 96795.

TABLE 1

Strain list

Strain	Genotype	Source
D1000-12C	*MAT***a** *cyc1-363 met14 leu2-1 lys1-1 lys2-1*	F. SHERMAN
D1000-12D	*MATα cyc1-363 met14 leu2-1 lys1-1 lys2-1*	F. SHERMAN
IX111-7D	*MAT***a** *met1 his3* and/or *his7 leu2* Ura$^+$ *(ura3? SIR1::URA3?)*	This work
IX111-11A	*MATα met1* Ura$^+$ *(ura3? SIR1::URA3?)*	This work
K382-23A	*MAT***a** *spo11 ura3 can1 cyh2 ade2 his7 hom3*	KLAPHOLZ and ESPOSITO 1982
K381-9D	*MATα spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	KLAPHOLZ and ESPOSITO 1982
K393-35C	*MATα spo11 ura3 his2 leu1 lys1 met4 pet8*	KLAPHOLZ and ESPOSITO 1982
K396-22B	*MATα spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	KLAPHOLZ and ESPOSITO 1982
C82-1785	*MATα asp5 ilv5-1 ura4*	J. G. L. PETERSEN
K103	*MAT***a**/*MATα mar2-1*/+ *leu2*/+	This work
IX83-43D	*MATα ade2 ade5 his7 met1 spo11 trp3 SIR1::URA3 ura3*	This work
IX116-19B	*MAT***a** *ade2 ade5 ade6 asp5 his3 met14 pet17 ura3*	This work
IX126-2A	*MATα ade2 ade5 ade6? his7 mak15 spo11 trp3 ura3 SIR1:URA3*	This work
1403-7A	*MAT***a** *gal3 gal4 MAL4 MGL3 suc trp1 ura3*	C. MICHELS
IX16-17A	*MATα sir4^{oc} his4 leu-2-3,2-112 trp1 ura3 can*	This work
IX64-17A	*MATα ade2 ade8 arg2 aro1 hom2 lys4 trp4 tyr1 ura3*	This work
IX57-1B	*MATα ade1? ade2 his3? his7 SIR4::URA3 ura3*	This work

tions and procedures for culture, mating, sporulation and dissection were used (MORTIMER and HAWTHORNE 1969; SHERMAN, FINK and HICKS 1983). Unless stated otherwise in the text, tetrad data were obtained from colonies grown from isolated zygotes. Mutant alleles of *MET1* and *MET14*, when segregating in a single cross, were assayed by complementation tests using strains D1000-12C, D1000-12D, IX111-7D and IX111-11A (Table 1). The inability of *can1* Arg$^-$ segregants to grow on standard synthetic media was circumvented by omitting ammonium sulfate and adding arginine to a final concentration of 200 mg/liter (WHELAN, GOCKE and MANNEY 1979).

Transformation of yeast was accomplished by the glusulase-generated spheroplast technique (BEGGS 1978; HINNEN, HICKS and FINK 1978).

The *spo11* technique for chromosome assignment of genes has been described in detail by KLAPHOLZ and ESPOSITO (1982). Following sporulation of *spo11* diploids, haploid or nearly haploid meiotic segregants were selected on medium containing canavanine or cycloheximide. At least 50 colonies from each selection were assayed for the expression of recessive markers that had been heterozygous in the diploids. Methionine auxotrophy was scored as described by KLAPHOLZ and ESPOSITO (1982).

To induce mitotic recombination with ultraviolet (UV) light, cells spread on solid, complete medium were irradiated with sufficient UV radiation (generated by a 15-watt germicidal lamp) to achieve 40–80% killing. At these levels of killing, approximately 4–20% of the surviving colonies were homozygous for *ade8*, the most distal chromosome *IV* marker used in this study.

Map distances in centiMorgans were calculated by the equation cM = 100 $[(TT + 6NPD)/2(PD + NPD + TT)]$ (PERKINS 1949) in which *PD*, *NPD* and *TT* are the frequencies of parental ditype, nonparental ditype, and tetratype tetrads, respectively. When this equation gave distances >35 cM, the equation presented by MA and MORTIMER (1983) was used to give more accurate values.

Biochemical techniques: All biochemical procedures used in this study are standard and are described in MANIATIS, FRITSCH and SAMBROOK (1982).

RESULTS AND DISCUSSION

Mapping strategies: This study used two strategies to overcome the inherent difficulties in mapping mutations that confer a nonmating phenotype. The mapping of *SIR3* (*MAR2*) made use of the fact that *sir3* mutant spores derived from a *SIR3/sir3* heterozygous diploid display a lag in expression of the mutant phenotype. Therefore, *sir3* spores may be mated within the first two generations after spore germination. Mapping of *SIR1* and *SIR4* made use of cloned *SIR1* and *SIR4* genes (J. M. IVY, A. J. S. KLAR and J. B. HICKS, unpublished results). After integrating *SIR1*- and *SIR4*-containing plasmids into the corresponding wild-type locus by homologous recombination, the selectable marker of the plasmid was mapped by conventional crosses. Integration of the plasmids was done in a *spo11* strain so that chromosome assignment could be made by the technique of KLAPHOLZ and ESPOSITO (1982).

Mapping of SIR3(MAR2)

Different mutant alleles of this locus have been given various names: *cmt* (HABER and GEORGE 1979), *ste8* (HARTWELL 1980) and *mar2* (KLAR *et al.* 1981). The temperature-sensitive *ste8* allele was subsequently renamed *sir3-8* by RINE (1979), who used it as the defining allele of this locus in his studies and who demonstrated that *sir3-8* and *cmt* are allelic. Furthermore, *mar2-1* and *cmt*, which fail to complement, are allelic (A. J. S. KLAR, unpublished results), and *sir3-8* and *mar2-1* are allelic (J. M. IVY, unpublished results). The *mar2-1* allele was used for our mapping crosses; therefore, we shall refer to the locus within this section as *MAR2*.

Location of *MAR2* on chromosome *XII* was first suggested by the occurrence of tetrads exhibiting unusual segregations for both *URA4* and *MAR2*. Three tetrads among 88 from a *URA4 mar2/ura4 MAR2* diploid gave aberrant $4^+:0^-$ segregation for *URA4* and $0^+:4^-$ segregation for *MAR2*. *URA4* maps on the right arm of chromosome *XII* distal to *RDN1*. *RDN1* is the locus of rDNA composed of approximately 120 tandem repeats, each repeat 8.4 kilobase pairs (kb) in size (PHILIPPSEN *et al.* 1978). It seemed likely that mitotic crossing over within the rDNA repeats gave rise to the observed results by causing all markers distal to *RDN1* to become homozygous.

The putative linkage was confirmed by mating spores of the diploid K103 (*MAT***a**/*MATα mar2*/+ *leu2*/+) to cells of strain C82-1785 (*MATα*) containing three markers on the right arm of chromosome *XII*: *asp5*, *ilv5-1* and *ura4*. One diploid formed by such a spore-to-cell mating segregated two nonmating (*mar2*) and two mating spore colonies in each tetrad. Tetrad data from this diploid are given in Table 2. *MAR2* was found to be linked to *URA4* (31 cM) and *URA4* to *ILV5* (42 cM). No other pairs of markers appeared to be linked. We, therefore, place *MAR2* (*SIR3*) distal to *URA4* on chromosome *XII* (Figure 1).

Mapping of SIR1 *and* SIR4

Chromosome assignment: The *SIR1* and *SIR4* cloned genes were originally selected for their ability to complement *sir1* and *sir4* mutations, respectively,

TABLE 2

SIR3(MAR2) *meiotic mapping*

Cross	Marker pair	*PD*	*NPD*	*TT*	cM
K103 spore	*URA4, MAR2*	29	0	48	31.
×	*ILV5, MAR2*	17	13	46	Unlinked
C82-1785	*ASP5, MAR2*	8	13	38	Unlinked
	ILV5, URA4	26	2	56	42.5
	ASP5, ILV5	9	9	40	Unlinked

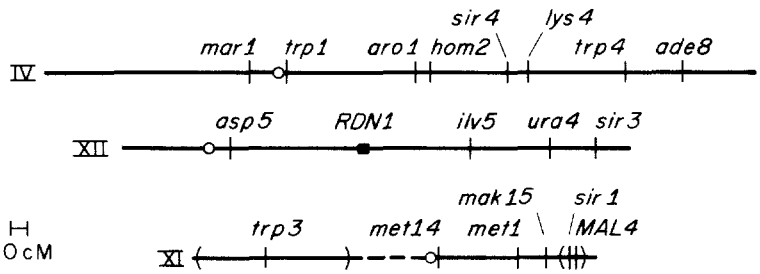

FIGURE 1.—Maps of the three yeast chromosomes to which four *MAR* and *SIR* genes have been localized. Indicated are the relative positions of markers used in this study. Chromosomes *IV* and *XI* are taken from maps published by MORTIMER and SCHILD (1982). The map position of *lys4* is as indicated (R. CONTOPOULOU, personal communication; MORTIMER and SCHILD 1980). The chromosome *XII* map and the location of *ilv5* are from PETERSEN *et al.* (1983).

in vivo. Confirmation of the plasmids' identities will be described elsewhere (J. M. IVY, A. J. S. KLAR and J. B. HICKS, unpublished results). Briefly, the identity of the *SIR1* plasmid was confirmed by inserting the *LEU2* gene into the *SIR1* cloned gene and replacing the genomic wild-type allele with this insertional mutation. This *sir1*::*LEU2* "gene disruption" had a Sir$^-$ phenotype, and a diploid heterozygous for *sir1*::*LEU2* and *sir1-1* (RINE *et al.* 1979) produced no recombinants between these two *sir1* mutant alleles among 26 tetrads. For the *SIR4* plasmid, a subclone in a vector containing the *LEU2* gene was integrated into the chromosome by homologous recombination. Meiotic segregation of this *SIR4*::*LEU2* locus from the *sir4-351*^{oc} mutation (RINE 1979) produced no recombinants in 42 tetrads.

The *SIR1* and *SIR4* plasmids used in this mapping study are diagramed in Figure 2A. Both *SIR* gene fragments were cloned in the plasmid YIp5 (STRUHL *et al.* 1979), which carries the marker *URA3*. Strain K382-23A (*MAT***a** *ura3 spo11*) was transformed with pJI22.11 (*SIR1*) and pJI28.10 (*SIR4*) plasmid DNAs that had been cut with *Bgl*II and *Bam*HI, respectively, before transformation. The linearization by restriction endonuclease digestion within the *SIR* gene fragment helped to ensure that chromosomal integration occurred within the *SIR* gene and not within *ura3* (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983). Genetic tests established that each plasmid had integrated at only one locus, and DNA blots (Figure 2B) confirmed that the plasmids had integrated

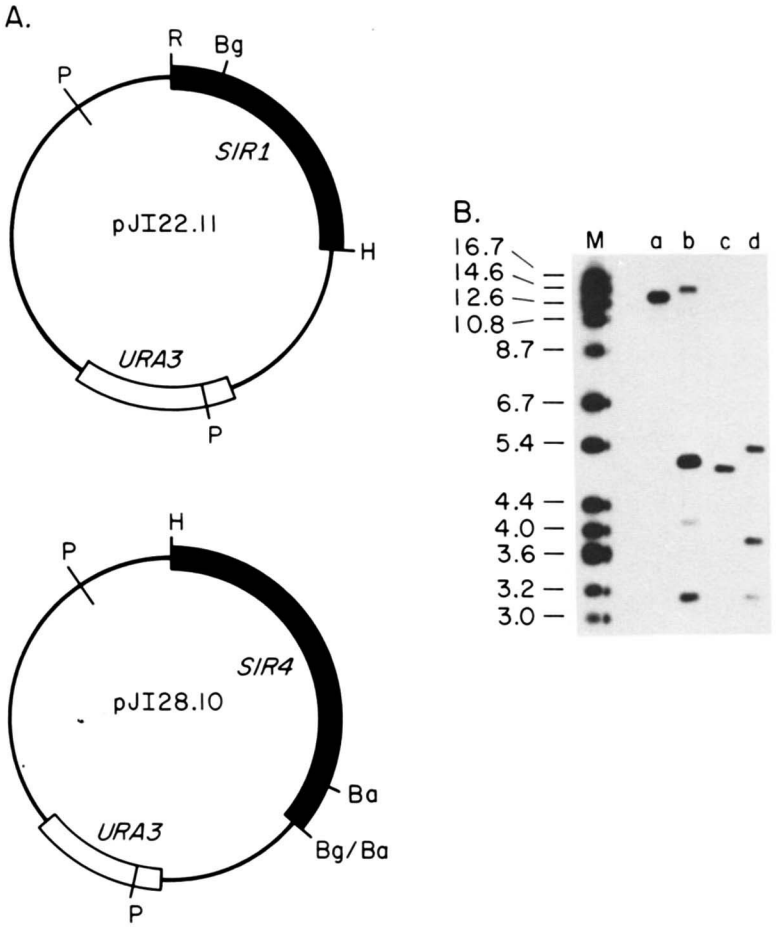

FIGURE 2.—A, Cloned *SIR1* and *SIR4* genes used to mark their corresponding genomic loci. A 1.9-kb *Eco*RI-*Hin*dIII *SIR1* fragment and a 3.0-kb *Hin*dIII-*Bgl*II *SIR4* fragment from their original YEp13 clones (J. M. IVY, A. J. S. KLAR and J. B. HICKS, unpublished results) were transferred to YIp5 (STRUHL *et al.* 1979). This yeast-*E. coli* shuttle vector carries the yeast *URA3* gene (open box) as a selectable marker, and because it supplies no origin of replication, it must integrate into the chromosome to be maintained. Solid boxes represent *SIR* gene sequences, and the thin line represents pBR322. Restriction enzyme sites are *Eco*RI(R), *Bgl*II(Bg), *Hin*dIII(H), *Pst*I(P), and *Bam*HI(Ba). B, Blot analysis of yeast genomic DNA. Genomic DNA from strain K382-23A untransformed (lanes a and c) and transformed with pJI28.10 (lane b) or pJI22.11 (lane d) was prepared, digested with *Pst*I and analyzed by the method of SOUTHERN (1975). The *SIR4* probe used (lanes a and b) was a fragment cloned into pBR322 that spanned the insert in pJI28.10. The *SIR1* probe (lanes c and d) was the *Eco*RI-*Hin*dIII fragment of pJI22.11 cloned into pBR322. It can be seen that the normal *SIR*-gene *Pst*I fragments are absent in the transformants. The smallest band in lanes b and d is the *URA3*-pBR322 *Pst*I vector fragment. The other two bands of lane d represent the *SIR1*-vector junction fragments. Similarly, the 14.6-kb and 4.1-kb bands in lane b represent *SIR4*-vector junction fragments.The 5-kb band from this transformant is the plasmid *Pst*I fragment containing *SIR4*. Its presence suggests the existence of extrachromosomally replicating copies of the plasmid in addition to the integrated copy [it is known that this *SIR4* fragment contains an autonomously replicating sequence (J. M. IVY, A. J. S. KLAR and J. B. HICKS, unpublished results)]. Alternatively, the plasmid may have integrated multiple times, generating tandem duplications of the insert. If the latter is correct (see text), we can estimate from the intensities of the two plasmid fragments relative to the junction fragments that there are at least five tandem duplications. Size of markers in kilobases is given in the left margin.

TABLE 3

SIR1 *meiotic mapping*

Cross	Parents	Gene pair	*PD*	*NPD*	*TT*	cM
IX119	IX83-43D	*MET14, MET1*	22	1	44	39.
	IX116-19B	*MET1, SIR1::URA3*	31	1	35	31.
		MET14, SIR1::URA3	7	2	59	58.
IX127	IX126-2A	*SIR1::URA3, MAL4*	52	0	0	0
	1403-7A	*MAK15, SIR1::URA3*	30	1	19	25.
		MAK15, MAL4	31	1	19	25.

at the appropriate *SIR* locus. These loci containing integrated plasmids will be referred to as *SIR1::URA3* and *SIR4::URA3*.

Chromosome assignment was made using the *spo11* technique (KLAPHOLZ and ESPOSITO 1982). K382-23A (*SIR1::URA3*) and K382-23A (*SIR4::URA3*) were crossed to K381-9D, K393-35C and K396-22B (Table 1). Homozygosity for *spo11* causes the almost total absence of meiotic recombination and poor spore viability. The absence of recombination allows all markers on entire chromosomes to segregate as intact linkage groups, thus forming the basis of the mapping technique. Within a set of three diploids, each known linkage group is marked at least once by a heterozygous, recessive marker. Chromosome assignment is based on the correlated segregation of the gene in question with one of the known markers. Plating on drug-containing media allowed only the growth of haploid or almost haploid spores containing either of two recessive drug-resistance mutations that were heterozygous in the diploids. *SIR1::URA3* assorted independently from all markers except the chromosome *XI* marker *met14*; among 46 cyh^r spore colonies derived from the K382-23A × K381-9D (*SIR1::URA3/SIR1*) cross, 19 were Ura^+ (*SIR1::URA3*) Met^+, 27 were Ura^- Met^- and none were Ura^+ Met^- or Ura^- Met^+. Similarly, *SIR4::URA3* appeared linked to *trp1* on chromosome *IV*. Among 104 can^r or cyh^r spore colonies obtained from the K382-23A × K381-9D (*SIR4::URA3/SIR4*) cross, 49 were Ura^+ (*SIR4::URA3*) Trp^+, 51 were Ura^- Trp^-, two were Ura^+ Trp^- and two were Ura^- Trp^+.

Meiotic mapping of SIR1: To determine which arm of chromosome *XI* contains the *SIR1* locus, we made a three-point cross with the left arm marker *trp3*, the right arm marker *met1* and *SIR1::URA3*. This cross (data not shown) indicated that *TRP3* and *SIR1* are unlinked and that *MET1* and *SIR1* are linked. A second three-point cross using the right arm markers *met14*, *met1* and *SIR1::URA3* (cross IX119, Table 3) established the gene order as diagramed in Figure 1. The 31-cM distance of *SIR1* from *MET1* indicated that *SIR1* should map near *MAL4*, the most distal known genetic marker on the right arm of chromosome *XI*. One final three-point cross with the markers *MAL4*, *SIR1::URA3* and *mak15* (cross IX127, Table 3) indicated that *SIR1* is tightly linked to *MAL4* (Figure 2). Because of the absence of exchange between *SIR1* and *MAL4*, gene order for these two markers cannot be determined. The observed lack of recombination, however, might be deceiving. We know that

TABLE 4

SIR4 *meiotic mapping*

Cross	Parents	Gene pair	*PD*	*NPD*	*TT*	cM
IX88	IX16-17A	*SIR4, LYS4*	56	0	26	16.
	IX64-17A	*HOM2, SIR4*	20	7	55	70.
		HOM2, LYS4	19	10	54	94.

the *SIR1*::*URA3* allele used in this cross has a 5.5-kb vector insertion plus a 1.9-kb *SIR1* duplication. Furthermore, in characterizing the *MAL2* locus of chromosome *III*, it was noted that *mal2* strains completely lack *MAL2* sequences as opposed to having mutant *mal2* sequences (C. MICHELS, personal communication). Therefore, it is possible that the *mal4* allele also is missing *MAL* sequences resulting in a second sequence heterogeneity. In Drosophila, meiotic exchange is reduced in regions flanking inversion breakpoints (STURTEVANT and BEADLE 1936). If such a phenomenon occurs in Saccharomyces, it might suppress recombination around insertions and, therefore, would reduce recombination between *SIR1*::*URA3* and *MAL4* in cross IX127.

Some of the crosses for mapping *SIR1* involved diploids heterozygous for the meiotic recombination-deficient mutation *spo11* (KLAPHOLZ and ESPOSITO 1982). ESPOSITO *et al.* (1972) reported that *spo11-1* is recessive for its inability to complete sporulation. That the *spo11*/+ heterozygotes had normal levels of meiotic recombination, and, hence, did not affect our mapping, can be ascertained by comparing the size of intervals from this study (Table 3) with those reported by MORTIMER and SCHILD (1980, Table 11). (Because *SIR1*::*URA3* and *MAL4* were recombinationally inseparable, the *met1-SIR1*::*URA3* interval is compared to *met1-MAL4*). Those distances for three intervals, respectively, are: *met14-met1*, 39 cM *vs.* 47.5 cM; *met1-SIR1*::*URA3*(*MAL4*), 31 cM *vs.* 32.6 cM; and *mak15-MAL4*, 25 cM *vs.* 19.3 cM. We conclude that *spo11*/+ heterozygosity had no detectable effect on meiotic recombination in our mapping crosses.

Mapping of SIR4: To determine the location of *SIR4* on chromosome *IV*, we mitotically mapped *SIR4*::*URA3* relative to four markers (*aro1, hom2, trp4* and *ade8*) on the right arm. The data (not shown) indicated that *SIR4* is on the right arm between *HOM2* and *TRP4*. Initial attempts to map meiotically *SIR4*::*URA3* relative to *hom2* and *lys4* gave discrepant results (data not shown). Figure 2B shows that the original *SIR4*::*URA3* integrant possibly contained at least five tandem duplications of the plasmid. It is possible that mitotic or meiotic recombination among the tandem duplications had altered their number, thus affecting meiotic exchange to different extents in different diploids. To avoid that complication, strain IX16-17A (*sir4*^{oc}) containing the autonomously replicating plasmid YEp13-*SIR4* (J. M. IVY, A. J. S. KLAR and J. B. HICKS, unpublished results) was crossed to IX64-17A (*hom2 lys4*). The YEp13-*SIR4* plasmid complements the *sir4*^{oc} mutation allowing the transformant to mate. Before sporulation, the diploid was grown on rich medium to allow for loss of the plasmid. The data from this cross (IX88, Table 4) indicated that

TABLE 5

SIR4 *mitotic mapping*

Diploid IX70 (IX64-17A × IX57-1B):							
ura3	*ade2*	*aro1*	*hom2*	+	*lys4*	*trp4*	*ade8*
ura3	*ade2*	+	+	*SIR4::URA3*	+	+	+

Inferred genotype of markers[a]					
ARO1	*HOM2*	*SIR4::URA3*	*LYS4*	*TRP4*	No.
+	+	+	+	+	56
+	+	+	+	−	76
+	+	+	−	−	7
+	+	−	−	−	50
+	−	−	−	−	29
−	−	−	−	−	53
−	+	+	+	−	3
+	+	+	−	+	2
+	−	+	+	−	2
+	+	−	−	+	1
−	+	+	+	+	1

[a] White, *ade8/ade8* UV-induced recombinants were scored for other chromosome *IV* markers.

SIR4 and *LYS4* are separated by approximately 16 cM. Although map distances suggest the gene order given in Figure 1, analysis of individual tetrads failed to confirm that order.

To obtain the gene order of *SIR4* and *LYS4*, mitotic mapping data were obtained from diploid IX70 (*ade2/ade2 ura3/ura3 aro1 hom2 SIR4 lys4 trp4 ade8*/+ + *SIR4::URA3* + + +). This diploid has five markers on the right arm of chromosome *IV* in addition to *SIR4::URA3*; it forms red colonies because of the homozygous *ade2* mutation. Following UV irradiation, white colonies (indicating homozygosis of the most distal *IV-R* marker, *ade8*) were picked and scored for the expression of other recessive chromosome *IV* markers. The more distal a gene is on the chromosome, the more frequently it should become homozygous. Thus, the data from this diploid (Table 5) establish the gene order from the centromere as *HOM2 SIR4 LYS4* (Figure 1).

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