ISOLATION OF *SPO12–1* AND *SPO13–1* FROM A NATURAL VARIANT OF YEAST THAT UNDERGOES A SINGLE MEIOTIC DIVISION[1]

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ABSTRACT

ATCC4117 is a strain of *S. cerevisiae* that undergoes a single nuclear division during sporulation to produce asci containing two diploid ascospores (GREWAL and MILLER 1972). All clones derived from these spores are sporulation-capable and, like the parental strain, form two-spored asci. In this paper, we describe the genetic analysis of ATCC4117. In tetraploid hybrids of vegetative cells of the ATCC4117 diploid and **a**/**a** or α/α diploids, the production of two-spored asci is recessive. From these tetraploids, we have isolated two recessive alleles, designated *spo12–1* and *spo13–1*, each of which alone results in the production of asci with two diploid or near-diploid spores. These alleles are unlinked and segregate as single nuclear genes. *spo12–1* is approximately 22 cM from its centromere; *spo13–1* has been localized to within 1 cM of *arg4* on chromosome *VIII*. This analysis also revealed that ATCC4117 carries a diploidization gene allelic to or closely linked to *HO*, modifiers that reduce the number of haploid spores per ascus and alleles affecting the total level of sporulation.

MEIOTIC mutants have been valuable tools for investigating the genetic control of meiosis in a variety of organisms (see BAKER *et al.* 1976 for review). Previous reports from our laboratory have described aspects of the genetic control and integration of landmark events of meiosis and ascosporogenesis in the yeast *Saccharomyces cerevisiae* (see ESPOSITO and ESPOSITO 1978 for review). These studies were based on the behavior of conditional sporulation-defective (*spo*) mutants that fail to produce mature asci at their restrictive temperatures. The purpose of the present investigation was to identify genes involved in the regulation of the two meiotic divisions by analysis of another type of sporulation variant, one that produces mature asci containing two diploid or near-diploid spores.

The process of sporulation in diploid cells of *S. cerevisiae* normally includes premeiotic DNA synthesis, followed by two meiotic divisions and the enclosure of the four haploid products into ascospores. A number of yeast strains that produce a large proportion of asci containing fewer than four spores have been de-

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scribed (ESPOSITO, ESPOSITO and MOENS 1974; GREWAL and MILLER 1972; JAMES 1974; TAKAHASHI 1962). A variety of physiological parameters have also been shown to influence the proportion of asci containing one, two or three spores (MILLER and HALPERN 1956; GREWAL and MILLER 1972; HABER and HALVORSON 1972; KÜNZI, TINGLE and HALVORSON 1974; DAVIDOW, GOETSCH and BYERS 1980; KLAPHOLZ, unpublished). In most cases, genetic and cytological analyses of two-spored asci indicate that the spores contains haploid genomes that have been randomly or nonrandomly packaged. For example, studies of *spo3*, a mutant that forms almost exclusively two-spored asci at its semi-permissive temperature and no asci at its restrictive temperature, indicate that this mutant is specifically defective in the coordination of the second meiotic division and ascospore packaging. The segregation of centromere markers in the two-spored asci provides evidence for random inclusion of two haploid genomes into ascospores (ESPOSITO, ESPOSITO and MOENS 1974; MOENS, ESPOSITO and ESPOSITO 1974). In another study, a strain induced to form two-spored asci by reversible thermal arrest failed to undergo typical meiosis II modification of two of the four spindle pole bodies and subsequent spore wall formation, resulting in nonrandom packaging of nonsister meiosis II products (DAVIDOW, GOETSCH and BYERS 1980).

The production of asci containing two diploid ascospores by a number of wild-type yeast strains was first reported by GREWAL and MILLER (1972). Initial electron miscroscopic studies of two of these variants, ATCC4117 and 19el, demonstrated that only one nuclear division occurs during sporulation of these strains (MOENS 1974). This division resembles meiosis II, with its characteristically modified spindle pole bodies. In a subsequent study of ATCC4117, polycomplexes and rare synaptonemal complexes were observed, indicating that this strain undergoes meiosis I prophase prior to the single meiosis II-like division (MOENS *et al.* 1977). Furthermore, all spore clones produced by ATCC4117 were sporulation-capable (GREWAL and MILLER 1972) and thus, presumably heterozygous (*MAT***a**/*MAT*α) at the mating-type locus. The term "apomictic" was used to describe this strain by analogy to apomictic plants (see STEBBINS 1950 for review) because the products of sporulation appeared to be asexual or mitotic in character (MOENS *et al.* 1977).

We undertook a genetic analysis of ATCC4117 to identify the loci responsible for its unusual sporulation phenotype and to study the relationship between these genes and sporulation functions defined by other *spo* loci. In addition to providing information about the genetic control of the two meiotic divisions, strains that produce diploid spores are potentially useful for the analysis of mutations that cause abnormal exchange or aberrant meiosis I segregation. Because reductional segregation in most organisms depends on normal meiotic recombination, recombination-defective mutants often undergo significant nondisjunction and yield a high proportion of inviable aneuploid meiotic products (reviewed by BAKER *et al.* 1976). Equational division, as in mitosis or meiosis II, does not normally require exchange of homologs. Thus, we anticipated that the genes responsible for the ATCC4117 sporulation phenotype would allow recovery of viable spores in recombination-defective genetic backgrounds.

During the course of this study, we discovered that ATCC4117 contains two recessive alleles, designated *spo12–1* and *spo13–1*, each causing the production of asci with two-diploid or near-diploid spores. ATCC4117 also contains a dominant allele of the *HO* locus, involved in mating-type switching after spore germination (HICKS and HERSKOWITZ 1976; OSHIMA and TAKANO 1971), and several sporulation modifiers. In addition, the patterns of spore viability and aberrant segregation observed in some of the crosses suggested that ATCC4117 harbors an aneuploid genome. The relevance of these defects to the two alleles causing the two-spored phenotype is discussed.

MATERIALS AND METHODS

Strains and nomenclature: The strains of *Saccharomyces cerevisiae* used in these studies are listed with their genotypes and origins in Table 1. ATCC4117 was kindly provided by J. J. MILLER. Its genotype was determined during the course of this work. It should be noted that in previous publications (ESPOSITO and ESPOSITO 1978; MOENS *et al.* 1977), the phenotype of ATCC4117 was described by the term "apomictic" meiosis and the gene symbol *apo*. Following identification and characterization of the genes responsible for this phenotype, the alleles were given *spo* locus numbers, in order to unify the nomenclature for genes governing the process of sporulation. We use the designation Spo+ to indicate the phenotype of tetrad-producing strains, and Spo- to indicate the phenotype of two-spored-ascus-producing strains, according to the nomenclature proposed by DEMEREC *et al.* (1966). Crosses are designated by the letters K or KC. Asci dissected from diploid or tetraploid strains are numbered consecutively and each spore assigned a letter, *e.g.*, K127–6C–1A represents spore A from tetrad 1, dissected from the diploid spore clone K127–6C, derived from tetraploid K127.

Media: All experiments were carried out on solid media containing 1.5% Bactoagar. Tetracycline (Squibb) was added at 20 μg/ml to complex and sporulation media to prevent bacterial contamination. All media contained 5 μg/ml Botrane and Thiobendazole (gift of E. GARBER) to retard fungal contamination. Most of the complex and synthetic media used in these experiments are described by GOLIN and ESPOSITO (1977). MG (Minimal glycerol) contains 3% glycerol, 0.17% Bacto-yeast nitrogen base (w/o amino acids and w/o ammonium sulfate) and 0.5% ammonium sulfate, pH 5.8. SPIII-21 (Sporulation) contains 2% potassium acetate, 0.1% dextrose and 0.25% Bacto-yeast extract, supplemented with 75 μg/ml of each of the following: adenine sulfate, L-arginine-HCl, L-histidine-HCl, L-leucine, L-lysine-HCl, L-methionine, L-phenylalanine, L-tryptophan, L-tyrosine, L-threonine and uracil, and 10 μg/ml PABA, pH 7.0.

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

Mating procedures: For the selection of rare matings involving vegetative cells of ATCC4117, an **a**/α diploid that has no auxotrophic requirements and produces nonmating diploid spores, cytoplasmic petite derivatives were used. Petites were induced with 1 ml of 0.2 mg/ml acriflavin (Sigma Chemicals) in 95% EtOH per 35-ml YPD plate and hybrids were selected on MG. Unless otherwise noted, homothallic strains were crossed by mating spores as described by ESPOSITO and ESPOSITO (1975).

Mating-type tests: The mating ability of vegetative cells was tested as described by LIRAS *et al.* (1978), either by cross-stamping or replicating the clones to be tested to lawns of **a** and α testers, and subsequent prototroph selection of hybrids. When a clone was completely prototrophic, petite derivatives were crossed, and hybrid grande prototrophs selected. We use the term **a** or α mating type to describe those strains that mate exclusively with α or **a** mating-type testers, respectively. The general term nonmating (symbol, N) is used here to encompass a range of responses seen in vegetative cells of sporulation-capable strains (*i.e.*, both *MAT***a** and *MAT*α are present) from complete nonmating to a variable degree of "bisexual" mating with both **a** and α strains.

TABLE 1

Genotype and origin of strains

Strain	Genotype	Origin
ATCC4117	**a**/α [*HO* spo12–1 spo13–1 MAL SUC*]	J. J. MILLER
DH8–303	α *ho lys2 tyr1 HIS7 CAN1 URA3 ADE5 met13 cyh2 trp5 leu1 ade6 cly8 ade2* α *ho lys2 tyr1 his7 can1 ura3 ADE5 met13 CYH2 trp5 leu1 ADE6 CLY8 ade2*	D. PLOTKIN
DH12–466	α *ho lys2 tyr1 HIS7 CAN1 URA3 ADE5 met13 cyh2 trp5 leu1 ade6 cly8 ade2* α *ho lys2 tyr1 his7 can1 ura3 ade5 met13 CYH2 trp5 leu1 ADE6 CLY8 ade2*	D. PLOTKIN
DL171–477	**a** *ho LYS2 tyr1 his7 CAN1 URA3 ADE5 met13 cyh2 trp5 leu1 ade6 cly8 ade2* **a** *ho lys2 tyr1 his7 CAN1 ura3 ade5 met13 CYH2 trp5 leu1 ADE6 CLY8 ade2*	D. PLOTKIN
DL179–780	**a** *ho LYS2 tyr1 his7 CAN1 URA3 ADE5 met13 cyh2 trp5 leu1 ade6 cly8 ade2* **a** *ho lys2 tyr1 his7 can1 ura3 ade5 met13 CYH2 trp5 leu1 ADE6 CLY8 ade2*	D. PLOTKIN
K49–7B	**a**/α [*HO ade2 cyh2 his7 leu1 lys2 met13 spo1–1 trp5 tyr1 ura3*]	S. KLAPHOLZ
K65–3D	**a**/α [*HO ade2 ade5 can1 his7 leu1 lys2 met13 trp5 tyr1 ura3*]	S. KLAPHOLZ
K77–9C	**a**/α [*HO ade2 cyh2 his7 leu1 lys2 met13 spo1 trp5 tyr1 ura3*]	S. KLAPHOLZ
K127–6C–2B	α *ho spo12–1 ade2 ade6 cyh2 leu1 lys2 trp5 tyr1*	S. KLAPHOLZ
K127–6C–4C	**a**/α [*HO* spo12–1 can1 lys2*]	S. KLAPHOLZ
K127–6C–6A	**a**/α [*HO* ade6 his7 leu1 tyr1*]	S. KLAPHOLZ
K127–6D–6A	**a**/α *HO*/ho spo12–1/SPO12* [*spo13–1 leu1 met13 trp5 tyr1*]	S. KLAPHOLZ
K161–6A	**a** *ho spo12–1 ade2 ade5 his7 leu1 lys2 met13 trp5 tyr1*	S. KLAPHOLZ
K161–6D	α *ho spo12–1 ade2 ade6 can1 cyh2 leu1 lys2 trp5 tyr1 ura3*	S. KLAPHOLZ
K166–5D–6B	**a** *ho spo13–1 ade2 his7 leu1 lys2 met13 trp5 tyr1*	S. KLAPHOLZ
K166–10B–1B	α *ho spo13–1 ade6 his7 leu1 lys2 met13 trp5 tyr1*	S. KLAPHOLZ
K166–10B–4D	α *ho spo13–1 ade6 leu1 lys2 met13 trp5 tyr1 ura3*	S. KLAPHOLZ
K167–4C	**a** *ho spo12–1 ade2 ade6 can1 cyh2 leu1 lys2 trp5 tyr1 ura3*	S. KLAPHOLZ

TABLE 1—Continued

Strain	Genotype	Origin
K172–5B	α *ho spo12–1 ade2 can1 cyh2 his7 leu1 lys2 trp5 tyr1 ura3*	S. KLAPHOLZ
K180–15B	**a** *ho spo13–1 ade2 can1 his7 leu1 lys2 met13 trp5 tyr1 ura3*	S. KLAPHOLZ
K187–1B	**a**/α [*HO ade2 cyh2 his7 leu1 lys2 met13 trp5 tyr1 ura3*]	S. KLAPHOLZ
K187–24B	**a**/α [*HO ade2 cyh2 his7 leu1 lys2 met13 trp5 tyr1 ura3*]	S. KLAPHOLZ
K193–8B	**a**/α [*HO ade2 ade5 can1 his1 leu1 lys2 met13 trp5 ura3*]	S. KLAPHOLZ
K199–9D	α *ho spo12–1 ade2 ade5 can1 his7 leu1 lys2 met13 trp5 tyr1 ura3*	S. KLAPHOLZ
K201–9B	α *ho spo12–1 ade2 can1 cyh2 his7 leu1 lys2 trp5 tyr1 ura3*	S. KLAPHOLZ
K210–2B	α *ho spo13–1 ade2 ade5 can1 his7 leu1 lys2 met13 trp5 tyr1 ura3*	S. KLAPHOLZ
K210–6C	α *ho ade2 ade5 can1 his7 leu1 lys2 met13 trp5 tyr1 ura3*	S. KLAPHOLZ
K211–10B	**a** *ho spo13–1 ade2 ade5 his7 leu1 lys2 his7 met13 trp5 tyr1*	S. KLAPHOLZ
K211–10D	α *ho spo13–1 ade2 ade5 cyh2 his7 leu1 lys2 met13 trp5 tyr1*	S. KLAPHOLZ
K226–6D	**a** *ho spo12–1 ade2 cyh2 his7 leu1 lys2 trp5 tyr1 ura3*	S. KLAPHOLZ
K249–6A	**a**/α [*HO ade2 ade5 ade6 can1 his7 leu1 met13 tyr1*]	S. KLAPHOLZ
M90	**a**/α [*HO arg4–1 spo11–1*]	M. & R. ESPOSITO
RM26–26C	α *ho ade2 ade5 can1 his1 leu1 lys2 trp5 ura3*	R. MALONE
S41	**a**/α [*HO arg4–1*]	M. & R. ESPOSITO
W223–9A	α *ho arg4 aro7 asp5 cdc14 gal1 his2 his6 pet17 trp1*	R. ROTHSTEIN
W224–1D	α *ho aro7 his6 leu2 lys1 met14 pet8 ura3*	R. ROTHSTEIN
W230–12A	α *ho ade1 cyc2 cyc3 his6 leu1 lys7 met3 met4*	R. ROTHSTEIN

Note: Gene symbols: ***MAT*a** and ***MAT*α** (or **a** and α) mating type; *ade*, adenine; *arg*, arginine; *aro*, aromatic amino acids; *asp*, aspartate; *can*, canavanine; *cdc*, cell division cycle; *cly*, cell lysis; *cyc*, cytochrome *c*; *cyh*, cycloheximide; *gal*, galactose utilization; *his*, histidine; *HO*, homothallism; *leu*, leucine; *lys*, lysine; *met*, methionine; *pet*, petite; *spo*, sporulation-defective; *trp*, tryptophan; *tyr*, tyrosine; *ura*, uracil (*c.f.*, PLISCHKE *et al.* 1976).

[ ], brackets are used to indicate the homozygous diploid genotype of homothallic *HO* strains.

* This *HO* allele was derived from ATCC4117.

Counting procedures: Sporulation ability was scored after 5 days of incubation on SPIII-21; 200 to 300 cells were counted per determination. Buds were counted as cells, providing a minimum estimate of the percent asci, since not all buds are capable of sporulation.

Ascus dissection: After 5 days of incubation on SPIII-21, sporulated cells were inoculated into 0.2 ml 1/100 dilution of Glusulase (Endo Laboratories) in distilled water and incubated for 4 to 5 minutes. Asci were dissected on YPD plates with a micromanipulator (SHERMAN 1975).

RESULTS

Phenotype of ATCC4117: The sporulation abilities of 15 independent colonies of ATCC4117 are summarized in Table 2. At three temperatures, 25°, 30° and 34°, more than 97% of the asci formed contained only two spores, and less than 2% contained three or four spores. Fifty-two two-spored asci (dyads) from a single clone of ATCC4117 were dissected. Spore viability was 98%. Each of the 102 ascosporal clones was capable of sporulation and produced more than 97% two-spored asci (Table 2). The total level of ascus production in both ATCC4117 and its ascosporal colonies was moderately temperature sensitive. These observations are in general agreement with the earlier results of GREWAL and MILLER (1972), who found that at 27° both ATCC4117 and random spores derived from ATCC4117 produced predominantly two-spored asci. Throughout this work, we have used the presence of less than 2% three- and four-spored asci among total asci as the criterion for the ATCC4117 sporulation phenotype (Spo^-).

Genetic analysis of ATCC4117: tetraploid studies: In order to analyze ATCC-4117 genetically, a series of crosses to diploid strains was designed (Figure 1). The construction of tetraploid hybrids was required because both ATCC4117 and its ascosporal clonoies are diploid. The **a**/**a** and α/α strains used in the crosses to ATCC4117 were derived from standard tetrad-producing (Spo^+) **a**/α diploids. In these tetraploids, the production of predominantly two-spored asci was recessive (Table 3). The tetraploid K127 (ATCC4117 × DL171–477) was dissected and ascosporal clones classified for the following parameters: (1) auxotrophic marker segregation, (2) the total level of sporulation at 25°, 30° and

TABLE 2

Sporulation abilities of ATCC4117 and its ascosporal colonies

Strain	Sporulation temperature	Percent sporulation	Percent of total asci		
			3+4 spores	1 spore	2 spores
ATCC4117*	25°	44.4 ± 8.0	0.1	1.1	98.8
	30°	25.0 ± 9.1	0.2	2.7	97.1
	34°	9.1 ± 3.6	1.5	0.2	98.3
ATCC4117 spore clones†	25°	52.5 ± 8.1	0.3	0.1	99.6
	30°	33.8 ± 8.4	0.4	0.2	99.4
	34°	10.6 ± 5.7	0.8	0.2	99.0

* The data represent the average of 15 independent colonies of ATCC4117.

† The data represent the average of 102 viable spore clones dissected from 52 dyads from a single sporulated colony of ATCC4117.

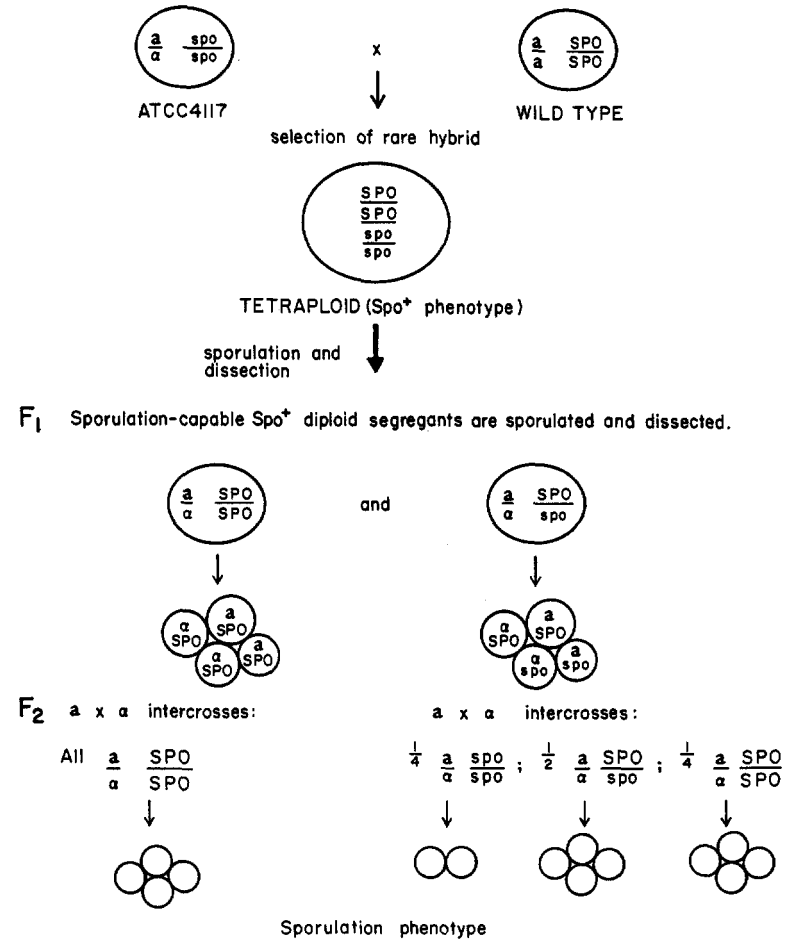

FIGURE 1.—The crossing scheme to derive haploid *spo* strains involves (1) selection of a rare mating between ATCC4117 vegetative cells and an **a**/**a** diploid, (2) dissection of the resultant tetraploid, which is presumably heterozygous *SPO/SPO/spo/spo*, (3) dissection of Spo+ diploid spore clones, some of which are *SPO/spo*, and (4) intercrosses among **a** and α haploid segregants to identify *spo* strains.

34°, (3) the percentages of one- plus two- and three- plus four-spored asci, and (4) mating ability. The spore viability of K127 was 72%, with most tetrads containing fewer than four viable products. The segregation of auxotrophic markers on four chromosomes was examined, and in each case, the percent of spores homozygous for the recessive allele was consistent with tetraploidy (Table 4).

Table 5 summarizes the combined sporulation and mating data for 87 ascosporal clones from 30 four-spored asci. Three major conclusions were drawn:

(1) *ATCC4117 is a homothallic diploid.* The low recovery of mating (5.7%) *versus* nonmating (94.3%) ascosporal clones suggested to us that ATCC4117 carries a dominant gene conferring mating-type switching and spore diploidization to yield nonmating clones. The data are compatible with the view that

TABLE 3

Sporulation ability of tetraploids derived from crosses of ATCC4117 to **a**/**a** *and* α/α *diploids*

Cross	Percent sporulation	Percent of total asci		1+2 3+4 spores
		3+4 spores	1+2 spores	
ATCC4117 × **a**/**a**:				
ATCC4117 × DL171–477	83.8	61.7	38.3	0.62
ATCC4117 × DL179–780	75.0	62.8	37.2	0.59
ATCC4117 × α/α:				
ATCC4117 × DH8–303	76.5	47.7	52.3	1.09
ATCC4117 × DH12–466	76.9	57.7	42.3	0.73
Controls				
a/**a** × α/α:				
DL171–477 × DH8–303	83.9	60.3	39.7	0.66
DL171–477 × DH12–466	86.2	93.4	6.6	0.07
DL179–780 × DH12–466	80.5	90.1	4.9	0.11
DL179–780 × DH8–303	76.5	85.0	15.0	0.18

The values represent the average of 4 or 5 independently isolated tetraploids for crosses involving ATCC4117, and 1 to 5 independently isolated tetraploids for the control crosses, at 25°. Similar results were obtained at 30° and 34°.

ATCC4117 is of genotype **a**/α *HO/HO*, and that a rare α/α *HO/HO* cell mated to form the tetraploid. A comparison of this hypothesis with the results expected, assuming that the genotype of ATCC4117 is **a**/α *HO/ho* or **a**/α *ho*/ho, is presented in Table 6. Confirmation that the diploidization gene in ATCC4117 is *HO* is described in the next section.

The presence of the *HO* gene, which results in mating-type switching, enables (1) **a** or α haploids to form **a**/α diploid clones (HICKS and HERSKOWITZ 1976;

TABLE 4

Marker segregation in the tetraploid K127

Chromosome	Marker	Marker configuration DL171-477/ATCC4117	Percent of spores homozygous for recessive marker*
II	*lys2*	−/+/+/+	1.2
	tyr1	−/−/+/+	11.6
	his7	−/−/+/+	10.5
V	*ura3*	−/+/+/+	0
VII	*leu1*	−/−/+/+	16.3
	trp5	−/−/+/+	22.1
	cyh2	−/+/+/+	1.2
	met13	−/−/+/+	18.6
XIV	*ade2*	−/−/+/+	16.3

* Values are for 86 spores of K127. The expected percent homozygosis for a recessive marker in −/−/+/+ configuration is 16.7% to 20.4% (ROMAN, PHILLIPS and SANDS 1955). In the −/+/+/+ configuration, the expected percent homozygosis for a recessive marker is 0%, assuming bivalent pairing, and from 0% to 4%, assuming tetravalent pairing and depending on gene-centromere distance.

TABLE 5

Sporulation ability of ascosporal clones from dissection of tetraploid K127(ATCC4117 × DL171-477)

Class	Percent 3+4-spored asci / total asci*	Number of spore clones	Mating phenotypes† a	α	N†
I	75–100	14	0	0	14
II	50–74	20	0	0	20
III	25–49	8	0	0	8
IV	2–24	11	0	0	11
V	< 2	27	0	0	27
VI	≤ 1% total asci	7	4	1	2
Total		87	4	1	82

The data are for 87 ascosporal clones from 30 four-spored asci, at 25°.
* All clones in classes I-V produced at least 6% total asci.
† Total percent maters is 5.7% (5 of 87).
‡ N = nonmater.

OSHIMA and TAKANO 1971, (2) **a**/**a** or α/α diploids to switch one mating-type allele to form **a**/α diploids, and (3) **a**/**a** or α/α diploids to switch both mating-type alleles to form **a**/**a**/α/α tetraploids by subsequent mating (HICKS, STRATHERN and HERSKOWITZ 1977). These possibilities complicate any interpretation of the mode of chromosome segregation (*i.e.*, meiosis I or II) and the level of

TABLE 6

Expected mating types from tetraploid K127 based on different genotypes at the HO *locus in ATCC4117*

ATCC4117 genotypes	Possible ATCC4117 contributions to K127	Expected nonmaters* (%)	Expected maters* (%)
	a/α *HO/HO*	91.7	8.3†
a/α *HO/HO*	α/α *HO/HO*	94.4	5.6‡
	α/0 *HO/HO*	88.7	11.3§
	a/α *HO/ho*	75.0	25.0†
a/α *HO/ho*	α/α *HO/ho*	83.3	16.7‡
	α/0 *HO/ho*	66.2	33.8§
	a/α *ho/ho*	50.0	50.0†
a/α *ho/ho*	α/α *ho/ho*	66.7	33.3‡
	α/0 *ho/ho*	32.5	67.5§

The observed 94.3% nonmaters and 5.7% maters in cross K127 (ATCC4117 × DL171-477) is compared with expected values for different contributions of ATCC4117 to the K127 tetraploid. DL171-477 contributed **a**/**a** *ho/ho* to K127.
* In these calculations, we assumed bivalent pairing for markers in tetrasomic configuration (ROMAN, PHILLIPS and SANDS 1955) and trivalent pairing for markers in trisomic configuration (SHAFFER *et al.* 1971; HABER 1974). Hence, the expected percent nonmaters is a maximum and the expected percent maters is a minimum estimate.
† All maters are **a**/**a** in genotype.
‡ Half of the maters are **a**/**a** and half are α/α.
§ There are approximately three times more **a** mating types than α mating types.

exchange between the mating-type locus (*MAT*) and its centromere in ATCC-4117. For example, either a single meiosis I division or recombination between *MAT* and its centromere followed by a meiosis II division could yield **a**/**a** and α/α spores. However, either event would be obscured in an *HO* background, since these spores would form nonmating sporulation-capable clones (**a**/α diploids or **a**/**a**/α/α tetraploids). It was therefore necessary to derive heterothallic strains (*ho*) to pursue the genetic analysis and characterization of the alleles in ATCC-4117 responsible for the single meiotic division.

(2) *There are two independent recessive alleles in ATCC4117, each of which alone results in the production of predominantly two-spored asci.* According to standard tetraploid analysis, if one recessive allele is present in two copies (+/+/−/−), 16.7% to 20.4% of all ascosporal clones should express the allele, depending upon gene-centromere distance and the frequency of bivalent *versus* tetravalent pairing (ROMAN, PHILLIPS and SANDS 1955). The expected frequency for two unlinked recessive alleles, each present in two copies, is between 30% and 36%. For a single dominant allele, present in one (+/−/−/−) or two (+/+/−/−) copies, the dominant phenotype should be expressed in 41.6% to 50.0% or in 79.6% to 83.3% of the ascosporal clones, respectively. The K127 data in Table 5 are in excellent agreement with the hypothesis that ATCC4117 carries two unlinked recessive *spo* alleles, each of which alone confers the Spo$^-$ phenotype. Among the K127 ascosporal clones capable of sporulation (classes I-V, Table 5), 34% (27 of 80) formed less than 2% three- and four-spored asci (class V, Table 5). Table 7 shows examples of sporulation data for tetrads with four viable spores. The presence of tetrads such as K127–22 with three segregants that produce predominantly two-spored asci further indicates that there are two independent recessive alleles segregating in K127 that confer this phenotype.

Complementation tests among segregants from K127 that formed less than 2% three- and four-spored asci (all produced greater than 16% total asci), confirmed the presence of more than one independent *spo* allele. A total of 13 intercrosses employing vegetative cells (rare matings) from nine different ascosporal clones were made. Two of the crosses showed complementation, generating hybrids that produced more than 2% three- and four-spored asci. The isolation of the two *spo* alleles into haploid backgrounds is described below. Demonstration that the asci contain diploid or near-diploid genomes is presented in the accompanying paper (KLAPHOLZ and ESPOSITO 1980).

(3) *Other modifiers that affect either the proportion of 1+2/3+4-spored asci or total ascus production are present in ATCC4117.* Sporulation-capable segregants that exhibited reduced numbers of spores per ascus but, unlike ATCC4117, more than 2% three- and four-spored asci, comprise classes III and IV (Table 5). In control crosses of DL171–477 to α/α diploids derived from Spo$^+$ strains, these classes were rarely observed (only one out of 43 sporulation-capable clones examined had a 1+2/3+4-spored asci ratio of greater than 1.0). Genetic analysis of representative segregants from these classes indicates that they form haploid spores resulting from the nonrandom packaging of nonsister meiosis II products, rather than from leaky behavior of variants that form two-spored asci containing diploid spores (KLAPHOLZ, unpublished).

TABLE 7

Sporulation in K127 tetrads with four viable spores

Tetrad number	Percent sporulation	Percent of total asci* 3+4 spores	Percent of total asci* 1+2 spores	Tetrad number	Percent sporulation	Percent of total asci* 3+4 spores	Percent of total asci* 1+2 spores
6 A	69	74	26	36 A	78	0	100
B	40	0	100	B	10	50	50
C	55	89	11	C	44	91	9
D	59	81	19	D	55	0	100
11 A	69	88	12	41 A†	1	0	100
B	15	20	80	B‡	1	0	100
C	58	0	100	C	61	34	66
D	39	46	54	D	6	33	67
22 A	81	86	14	43 A	62	50	50
B	83	1	99	B	20	75	25
C	22	1	99	C	petite: no sporulation		
D	7	0	100	D	32	6	94
32 A	86	57	43	45 A	53	81	19
B	33	85	15	B	42	60	40
C	49	35	65	C	52	4	96
D	79	61	39	D	54	0	100

The sporulation ability at 25° of ascosporal clones from eight K127 tetrads are shown above.
* When 0% 3+4-spored asci is indicated, no 3- or 4-spored asci were observed among 200 to 300 sporulated cells.
† α mating type; ‡ Nonmater.

Among the segregants that formed less than 2% three- and four-spored asci at 25° (class V, Table 5), the total percent of asci ranged from 7% to 83% (mean 49%) at 25°, and from 0% to 62% (mean 25%) at 34°. Among the remaining sporulation-capable segregants (classes I-IV), the total percent of asci ranged from 6% to 88% (mean 56%) at 25° and from 0% to 87% (mean 47%) at 34°. Meiotic segregants from control tetraploids from crosses between the **a**/**a** *SPO*/SPO parent of K127 (DL171–477) and α/α *SPO/SPO* diploids, did not exhibit this wide array of sporulation capabilities. These data indicate that ATCC4117 is the source of additional temperature-sensitive and nontemperature-sensitive alleles affecting total ascus production.

In order to isolate the two alleles controlling the production of asci containing two diploid spores free of other sporulation defects, the segregation of the modifiers described above was followed in all crosses. We have not yet determined whether expression of the two-diploid-spored ascus phenotype specifically favors the accumulation of such modifiers, or simply obscures their presence. However, since their segregation in crosses had no direct bearing on the isolation of the two major *spo* alleles, the details of their behavior are not presented.

Evidence that ATCC4117 is homothallic (HO): Analysis of sporulation-capable diploid segregants from K127 led to the isolation of the gene for homothallism present in ATCC4117. Approximately half of the meiotic products of K127–6C (see Table 7) were nonmaters and sporulation-capable, while most of

the remaining products were **a** or α in mating type (Table 8). These results suggested that K127–6C was heterozygous for a locus controlling homothallism. Ascospores from one of the sporulation-capable progeny, K127–6C–6A, were crossed to an *ho* haploid, K210–6C, and the resulting diploid (K249) dissected. Spore viability was 80%, and among the 30 viable products examined, 14 were **a**/α, eight were **a** and eight were α, indicating the segregation of a single gene conferring homothallic behavior. The spores of an **a**/α ascosporal clone, K249–6A, which exhibited 75% sporulation at 30°, where crossed to an *ho* haploid, RM26–26C (K256) and to spores of an *HO* diploid K193–8B (K257). Homothallism segregated 2:2 among 13 tetrads with four viable spores in K256 (93% spore viability) and 4:0 among 14 tetrads with four viable spores in K257 (94% spore viability). These results demonstrate that the homothallism gene originating from ATCC4117 is allelic to or closely linked to *HO*.

Isolation of spo12–1: Three of the four-viable-spore clones from tetrad 6 (K127–6A, 6C and 6D) were Spo^+; one (K127–6B) was Spo^- (Table 7). Assuming that two recessive alleles each resulting in the two-spored phenotype are present in the tetraploid, at least two of the Spo^+ clones should be heterozygous, and the Spo^- clone homozygous, for one or both of the *spo* alleles. Thus, the meiotic products of each member of the K127–6 tetrad were examined to isolate haploids carrying these *spo* alleles. The crosses involved in the isolation of these haploids are shown in Figure 2.

K127–6A and K127–6B were not useful in the isolation of haploid *spo* strains because neither produced haploid ascospores. K127–6A appeared to be a tetraploid, arising perhaps by mating-type switching and subsequent mating within an **a**/**a** or α/α *HO*/*ho* diploid spore clone (data not shown), and K127–6B produced only two-spored asci containing diploid spores. However, both K127–6C and K127–6D segregated haploid and diploid meiotic products due to heterozygosity for *HO* (Table 8). K127–6C was also heterozygous for at least one of the spo alleles (Table 8), and for several markers on chromosomes *II*, *VII* and *XV*. K127–6D exhibited extremely poor spore viability (7%). However, among the viable spores, *HO*, *MAT* and markers on chromosomes *II*, *VII* and *XV* segre-

TABLE 8

Phenotypes of K127–6C and K127–6D meiotic products

Parental clone	Percent spore viability	No. viable spores analyzed	Phenotypes of meiotic products				
			Mating type			Sporulation-capable *N* clones	
			a	α	N	3+4-spored asci/total asci $< 2\%$	3+4-spored asci/total asci $\geq 2\%$
K127–6C	32	12	1	3	8	4	2
K127–6D	7*	14	4	5	5	1	3

* Initial dissection of nine tetrads of K127–6D yielded 6% viable spores, including K127–6D–6A (see RESULTS) To obtain more viable products of K127–6D, 55 undigested tetrads were placed at isolated positions on a YPD plate with a micromanipulator. Sixteen colonies arose, indicating a minimum of 7% (16 of 220) spore viability, since each clone could represent the germination and growth of 1 to 4 spores.

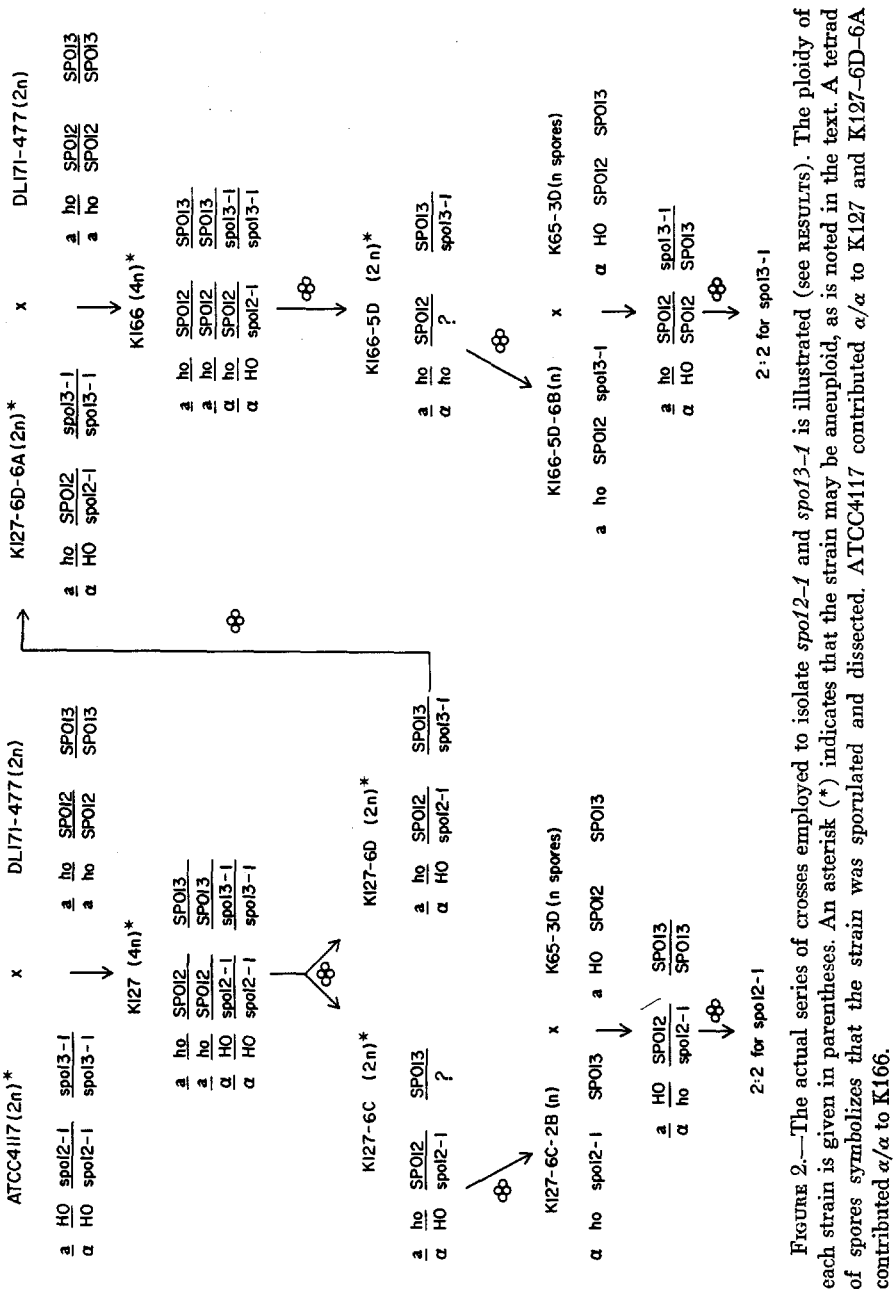

FIGURE 2.—The actual series of crosses employed to isolate *spo12-1* and *spo13-1* is illustrated (see RESULTS). The ploidy of each strain is given in parentheses. An asterisk (*) indicates that the strain may be aneuploid, as is noted in the text. A tetrad of spores symbolizes that the strain was sporulated and dissected. ATCC4117 contributed a/α to K127 and K127-6D-6A contributed a/α to K166.

gated as expected for a diploid. K127–6D also carried at least one *spo* allele; one fourth of the sporulation-capable meiotic products expressed the two-spored phenotype (Table 8). Both K127–6C and K127–6D ascosporal clones are analyzed in further detail below.

Several haploid meiotic products of K127–6C were crossed with spores from

an *HO/HO* diploid, K65–3D, since such hybrids should permit direct scoring of the sporulation phenotype among the *HO* meiotic segregants. We expected the resulting diploids (**a**/α *HO/ho*) to yield half *ho* **a** or α and half *HO* **a** or α spores upon sporulation and dissection. Since the *HO* spores will diploidize during germination, giving rise to *HO/HO* **a**/α clones homozygous for all genes except the *MAT* locus, the resulting diploid spore clones could then be sporulated and monitored for their sporulation phenotype.

The segregation analysis of one such cross, K127–6C–2B × K65–3D (Figure 2), indicated that the haploid derivative, K127–6C–2B, harbors one recessive allele that causes the production of predominantly two-spored asci. This allele was designated *spo12–1*. The hybrid exhibited a Spo$^+$ phenotype at 25° and 34°; approximately 60% of the total cells formed asci, the majority of which contained three or four spores. Among the *HO* segregants, 10 were *spo12–1* (with less than 2% three- and four-spored asci at all temperatures) and 10 were *SPO12*.

Table 9 presents the data from seven crosses of *spo12–1* haploid strains to *SPO12* haploids. The segregation of the *spo12–1* allele was scored by complementation testing of all haploid meiotic products with **a** or α *spo12–1* testers and by direct scoring of *HO* diploid ascosporal clones. The *spo12–1* allele segregated 2:2 in 78 tetrads and 3:1 in one tetrad, indicating that the *spo* phenotype is due to a single Mendelian gene. Crosses were made between *spo12–1* strains and strains with markers on chromosomes *I* through *XVII*, many of which are

TABLE 9

Segregation of spo12–1 *in heterozygous diploids*

Cross	Genotype	*SPO12:spo12–1* 4:0	3:1	2:2	1:3	0:4	Total	Spore viability (%)
K167–4C / W224–1D	**a** *ho* *spo12–1* / α *ho* *SPO12*	0	0	13	1	0	14	95
K167–4C / W230–12A	**a** *ho* *spo12–1* / α *ho* *SPO12*	0	0	16	0	0	16	90
K172–5B / K65–3D	**a** *ho* *spo12–1* / α *HO* *SPO12*	0	0	11	0	0	11	95
K187–1B / K201–9B	**a** *ho* *spo12–1* / α *HO* *SPO12*	0	0	15	0	0	15	93
K199–9D / K49–7B	**a** *ho* *spo12–1* / α *HO* *SPO12*	0	0	7	0	0	7	90
K199–9D / K77–9C	**a** *ho* *spo12–1* / α *HO* *SPO12*	0	0	9	0	0	9	95
K266–6D / K187–24B	**a** *ho* *spo12–1* / α *HO* *SPO12*	0	0	7	0	0	7	93
Total		0	0	78	1	0	79	Avg. 93

The data are from 79 tetrads with four viable spores.

centromere-linked. Table 10 shows that *spo12–1* is about 22 cM from its centromere. We have eliminated all but the following chromosome arms as the location for *spo12–1*: *I(L)*, *VI(L)*, *XII(L)*, *XV(L)* and *XVI(L)* (data not shown). Marker segregation in these crosses showed that the *spo12–1* strains that we have derived from successive outcrosses to our laboratory strains are euploid for chromosomes *I* through *XVII*.

Isolation and analysis of spo13–1: The isolation of *spo13–1* proved to be more complicated than that of *spo12–1*. As described above, the diploid segregant, K127–6D, was thought to be **a**/α *HO/ho* and heterozygous for one or more *spo* alleles. Only one sporulation-capable meiotic segregant from K127–6D produced less than 2% three- and four-spored asci. Vegetative cells of this segregant, K127–6D–6A, were crossed to two *spo12–1* diploids (K127–6C–1D and K127–6C–4C), which were obtained from K127–6C tetrad dissection. The resulting tetraploids complemented to give the Spo$^+$ phenotype. The results shown in Table 11 indicate that the *spo* allele in K127–6D–6A is not in the same complementation group as *spo12–1*. This new allele was designated *spo13–1*.

In order to determine whether any of the haploid meiotic products of K127–6D carried one or more *spo* alleles, **a** $\times$ α intercrosses were made. These intercrosses failed to identify any two-spored-ascus-producing strains. Because no haploid *spo* segregants were identified from 12 intercrosses, the strategy undertaken to isolate *spo13–1* haploids was to cross the single diploid *spo13–1* segregant (K127–6D–6A) to a diploid strain. Vegetative cells of K127–6D–6A (inferred genotype: **a**/α *HO/HO spo13–1/spo13–1*) were crossed with DL171–477 (**a**/**a** *ho/ho SPO13/SPO13*), and the tetraploid K166 were selected. This tetraploid was examined in a manner analogous to the K127 tetraploid analysis (Figure 2). K166 exhibited 59% sporulation at 25° and 55% sporulation at 34°, with the majority of asci containing three or four spores.

TABLE 10

Centromere linkage of spo12–1

Cross	Markers	Number of asci* P:N:T	*spo12*-centromere distance (cM)†
K167–4C / W224–1D	*spo12–1 leu1*	2:7:8	20.5
	spo12–1 leu2	5:5:8	16.2
	spo12–1 met14	3:7:8	22.0
	spo12–1 pet8	6:4:8	22.2
K167–4C / W230–12A	*spo12–1 ura3*	8:2:17	25.3
	spo12–1 ade1	5:4:16	26.0
K167–4C / W223–9A	*spo12–1 trp1*	3:2:5	25.0
			Avg. 22.5

* P:N:T = Parental:nonparental:tetratype asci.

† The map distances were calculated from half the frequency of tetratype asci minus the gene-centromere distance of the known marker. Gene-centromere distances are from MORTIMER and HAWTHORNE (1975).

TABLE 11

Identification of the spo13-1 *complementation group*

Strain	Genotype	Percent sporulation (25°)	Percent of total asci	
			3+4 spores	1+2 spores
K127-6C-1D	*spo12-1 SPO13* / *spo12-1 SPO13*	6.2	<1.8	>98.2
K127-6C-4C	*spo12-1 SPO13* / *spo12-1 SPO13*	52.0	<0.5	>99.5
K127-6D-6A	*SPO12 spo13-1* / *spo12-1* spo13-1*	16.2	<0.9	>99.1
K127-6C-1D / K127-6D-6A	*spo12-1 SPO13* / *spo12-1 SPO13* / *SPO12 spo13-1* / *spo12-1* spo13-1*	37.0	79.2	20.8
K127-6C-4C / K127-6D-6A	*spo12-1 SPO13* / *spo12-1 SPO13* / *SPO12 spo13-1* / *spo12-1* spo13-1*	43.0	72.1	27.9

* *spo12-1* heterozygosity in K127-6D-6A is inferred from analysis of cross K166.

The genetic analysis of K166 yielded several unexpected results. We had assumed that K127-6D-6A arose by diploidization of a haploid HO ascospore from K127-6D and was homozygous at all loci except *MAT*. Genetic analysis, however, indicated that although K127-6D-6A was homozygous for *spo13-1*, it was actually heterozygous for *HO* and *spo12-1*. K166 yielded 16.7% maters (three **a** maters plus two α maters per 30 spores) and 83.3% nonmaters (25/30), expected from an *HO/ho/ho/ho* **a**/**a**/α/α tetraploid (see Table 6). Among the sporulation-capable ascosporal clones of K166, the frequency of clones with the ATCC4117 two-spored phenotype was 28% (7 of 25). Although it was clear that K127-6D-6A was not homozygous for *spo12-1*, since it complemented the *spo12-1/spo12-1* diploids from K127-6C, this latter result indicated that K127-6D-6A very likely was of genotype *HO/ho SPO12/spo12-1 spo13-1/spo13-1*. Complementation tests between six Spo$^-$ segregants from K166 and an *spo12-1/spo12-1* diploid tester indeed revealed that four of six contained *spo12-1*. This observation indicated that the original segregant, K127-6D, must have been heterozygous not only for *HO*, but for both *spo12-1* and *spo13-1*. These findings, as well as the poor spore viability of K127-6D (7% survival) and the cross K166 (51% survival), raised the possibility that K127-6D and its meiotic derivates are aneuploid (see DISCUSSION).

Independent Spo$^+$ meiotic products of K166 were dissected with the aim of obtaining haploid *spo13-1* strains from any of the diploids that were heterozygous for *spo13-1*. Table 12 presents the dissection and segregation data for two of these segregants. Strains K166-5D and K166-10B each generated only mating-capable meiotic products with a 1:1 ratio of **a**:α mating types. Intercrosses be-

TABLE 12

Dissection analysis of K166 ascosporal clones

Ascosporal clone number	Phenotype of parental spore clone: Percent sporulation (25°)	Percent 3+4-spored asci / total asci	Percent spore viability	Phenotype of meiotic products: Mating type a	α	N
K166-5D	65	79	29 (7/24)	3	4	0
K166-10B	61	56	29 (4/14)	2	2	0

tween the presumed haploid segregants of K166–5D and K166–10B revealed one **a** and two *α spo* strains. To determine whether these haploids were *spo12–1*, *spo13–1*, or *spo12–1 spo13–1*, they were crossed to *spo12–1* haploid testers. Table 13 shows that the strains are all *spo13–1*, *i.e.*, they fully complement *spo12–1*.

Three crosses between the *spo12–1* and *spo13–1* strains (Table 13) were dissected. In one cross (K169), spore viability was high (92%), indicating that the *spo13–1* parent (K166–5D–6B) was near-haploid. The segregation of both *spo* alleles was scored by complementation tests. The two *spo* alleles are completely unlinked (P:N:T = 1:3:8) and each segregated 2:2 in 11 tetrads. The two other crosses had poor spore viability and are not described further.

A cross of K166–5D–6B (*ho* **a** *spo13–1*) with spores of K65–3D (*HO/HO* **a**/*α* *SPO13/SPO13*) was made to monitor the segregation of sporulation phenotypes among the *HO* products (Figure 2). The total percent sporulation of the diploid

TABLE 13

Demonstration of two spo *complementation groups by intercrosses between* spo *haploids*

Cross	Genotype*			Percent sporulation	Percent of total asci: 3+4 spores	1+2 spores
K166-5D-6B / K166-10B-1B	**a** / *α*	*SPO12* / *SPO12*	*spo13-1* / *spo13-1*	68	0.4	99.6
K166-5D-6B / K166-10B-4D	**a** / *α*	*SPO12* / *SPO12*	*spo13-1* / *spo13-1*	74	0.4	99.6
K161-6A / K161-6D	**a** / *α*	*spo12-1* / *spo12-1*	*SPO13* / *SPO13*	65	0.7	99.3
K166-5D-6B / K161-6D	**a** / *α*	*SPO12* / *spo12-1*	*spo13-1* / *SPO13*	64	85	15
K161-6A / K166-10B-1B	**a** / *α*	*spo12-1* / *SPO12*	*SPO13* / *spo13-1*	68	61	39
K161-6A / K166-10B-4D	**a** / *α*	*spo12-1* / *SPO12*	*SPO13* / *spo13-1*	56	73	27

* The sporulation values presented were determined at 25°; at 34°, all strains produced more than 45% total asci with proportions of 1+2- and 3+4-spored asci similar to those found at 25°.

TABLE 14

Segregation of spo13-1 *in heterozygous diploids*

Cross	Genotype			*SPO13:spo13-1* 4:0	3:1	2:2	1:3	0:4	Total	Spore viability (%)
K166-5D-6B / W230-12A	**a** / α	*ho* / *ho*	*spo13-1* / *SPO13*	0	0	21	1	0	22	92
K166-5D-6B / S41	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	0	1	23	0	0	24	94
K180-15B / K65-3D	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	0	0	9	0	0	9	98
K210-2B / K49-7B	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	0	0	9	0	0	9	95
K211-10B / K187-16B	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	0	0	16	0	0	16	92
K211-10D / K187-16B	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	0	0	18	0	0	18	88
Total				0	1	96	1	0	98	Avg. 93

The data are from 98 tetrads with four viable spores.

was 87% at 25° and 80% at 34°, with more than 80% of the asci containing three or four spores. This cross exhibited 91% spore viability. Among the *HO* spores that diploidized to form *HO/HO* **a**/α ascosporal colonies, 14 were *spo13-1* and 13 were *SPO13*. A haploid *spo13-1* segregant from this cross was used to initiate a series of crosses to derive genetically marked strains.

Segregation analysis of 98 tetrads from six **a**/α *spo13-1/SPO13* heterozygotes (Table 14) further shows that *spo13-1* segregates as a single gene. Ninety-six

TABLE 15

Linkage of spo13-1 *and* arg4

Cross	Genotype				Number of asci P:N:T*
K165-5D-6B / S41	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	*ARG4* / *arg4-1*	27:0:1
K166-5D-6B / M90	**a** / α	*ho* / *HO*	*spo13-1* / *SPO13*	*ARG4* / *arg4-1*	14:0:0
K166-5D-6B / W223-9A	**a** / α	*ho* / *ho*	*spo13-1* / *SPO13*	*ARG4* / *arg4*	11:0:0
				Total	52:0:1 (0.94 cM)

The data are taken from 27 tetrads with four viable spores and 26 tetrads where it was possible to infer the genotype of the inviable spores.

* P:N:T = Parental:non-parental:tetratype asci. The map distance is calculated as one half the frequency of tetratype tetrads, since no nonparental asci were observed.

tetrads show 2:2 segregation for *spo13–1* and two are apparently convertant asci (one was 3:1 and the other 1:3 for *SPO13:spo13–1*). The *spo13–1* allele has been mapped to less than 1 cM from *arg4* (Table 15). Crosses to strains with chromosomal or centromere markers on chromosomes *I* through *XVII* indicated that the *spo13–1* haploid strains derived from outcrosses to our laboratory strains are euploid.

A genetic analysis of the single nuclear division that occurs in *spo12–1* and *spo13–1* strains is described in the accompanying paper (KLAPHOLZ and ESPOSITO 1980).

DISCUSSION

This paper reports the initial characterization of two new recessive alleles affecting meiosis in *Saccharomyces cerevisiae*. The alleles, designated *spo12–1* and *spo13–1*, were recovered from American Type Culture Collection strain ATCC4117. Each segregates as a single Mendelian gene and confers a two-spored ascus phenotype resulting from a single division during meiosis. The spore clones are diploid or near-diploid, and primarily sporulation-capable.

The discovery of two alleles in ATCC4117, each *alone* capable of conferring this unusual phenotype was surprising. We have recently demonstrated that 19e1 (provided by J. J. MILLER), another natural variant with a phenotype similar to that of ATCC4117 (GREWAL and MILLER 1972; MOENS 1974), is *spo12 SPO13* in genotype (KLAPHOLZ, unpublished). Definition of the roles of the *spo12–1* and *spo13–1* alleles and their interactions with each other and with other sporulation-specific genes has been undertaken to determine whether there is any apparent advantage to the presence of the two alleles together. A study of the meiotic behavior of single *spo12–1* and *spo13–1* strains is presented by KLAPHOLZ and ESPOSITO (1980). The interaction of the *spo12–1* and *spo13–1* alleles with other mutations affecting the process of sporulation will be reported elsewhere.

The observation that all ascosporal colonies derived from ATCC4117 are sporulation-capable and, thus, presumably *MAT***a**/*MAT*α led GREWAL and MILLER (1972) to propose that the single nuclear division is equational. This observation also implies that recombination between the mating-type (*MAT*) locus and its centromere (25 cM), which should result in a/a and α/α spore clones incapable of sporulation, does not occur at appreciable levels. Furthermore, previous analysis of crosses of ATCC4117 to haploid and diploid strains raised the possibility of a duplicated (**a**α mating-type configuration) (MOENS *et al.* 1977). The absence of **a**/**a** and α/α clones can now be explained by the fact that the original strain, ATCC4117, is homothallic. Such clones, which may arise from either reductional segregation or crossing over between the mating-type locus and its centromere, will become heterozygous for *MAT* due to mating-type switching following spore germination (HICKS, STRATHERN and HERSKOWITZ 1977). Since the presence of *HO* prevents determination of both the mode of chromosome segregation and the level of exchange between *MAT* and its cen-

tromere during sporulation, we have reexamined the nature of the single nuclear division in heterothallic strains (KLAPHOLZ and ESPOSITO 1980).

Our analysis of ATCC4117 also revealed that it harbors a number of modifiers that have no obvious phenotype in the original genetic background. These include alleles that reduce the number of haploid spores packaged per ascus. Such alleles may have accumulated in ATCC4117 without selective disadvantage if they are not expressed during sporulation in this strain. For example, a gene required for the first meiotic division may not be expressed in a strain that bypasses this division. Alternatively, while these modifiers have no apparent influence on the number of diploid spores per ascus in ATCC4117, they may be expressed and play a role in other aspects of this atypical sporulation process.

The moderate temperature sensitivity of ATCC4117 (Table 2) was shown to result from alleles other than *spo12–1* or *spo13–1*. The wide array of sporulation capabilities, which included both temperature-sensitive and nontemperature-sensitive low sporulation phenotypes, observed in crosses involving ATCC4117 and its derivatives (see Table 7 for examples) suggested that a number of alleles were segregating that affected the sporulation process. These alleles, in combination, provided a genetic background that resulted in a relatively high level of ascus production in the original strain. Disruption of this combination of genes through outcrosses caused a reduction in sporulation ability. While the interaction between these modifiers and *spo12–1* and *spo13–1* is presently unclear, we have derived *spo12–1* and *spo13–1* strains free of such alleles to avoid confusion in evaluating the effects of the single *spo* alleles.

Throughout this study, hints arose that ATCC4117 is aneuploid and perhaps carries chormosomes structurally different from those in our laboratory strains. These include: (1) poor spore viability of crosses involving ATCC4117 and its derivatives; (2) poor spore viability upon dissection of tetrads produced by ATCC4117 sporulation-capable derivatives; (3) aberrant segregation and unexpected marker heterozygosity in crosses employing ATCC4117 and its derivaties; and (4) trisomic segregation in certain crosses involving K127–6C–6A (data not presented). In the accompanying paper, we provide evidence that a significant degree of aberrant chromosome segregation, yielding monosomic and trisomic spore pairs, occurs during sporulation of *spo12–1/spo12–1* and *spo13–1/spo13–1* diploids. This finding provides a plausible explanation for the apparent aneuploidy of ATCC4117. Moreover, we have recently found that the level of aneuploidy generated during meiosis in *spo13–1* strains can be greatly reduced by the presence of another *spo* mutation (KLAPHOLZ 1980). These results raise the interesting question of whether the additional sporulation modifiers are present in ATCC4117 for this purpose. We are currently investigating other wild-type strains that form two-diploid spored asci to determine whether they possess similarly complex genotypes.

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Note added in proof: We have recently demonstrated that *spo13-1* is an ochre-suppressible allele.

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