Formation of Two-spored Asci by Interrupted Sporulation in *Saccharomyces cerevisiae*

By PRAMOD K. SRIVASTAVA, SATOSHI HARASHIMA AND
YASUJI OSHIMA*

Department of Fermentation Technology, Osaka University, Yamadakami, Suita-shi,
Osaka 565, Japan

(Received 25 June 1980)

Sporulation in *Saccharomyces cerevisiae* was disturbed by transferring cells to water after 4 to 8 h incubation in potassium acetate medium, giving rise to almost exclusively two-spored asci at a high frequency. The (+ –) asci for a given auxotrophic marker (+ and – indicate the phenotypes due to the dominant and recessive alleles of the marker, respectively) predominated over the (+ +) and (– –) asci, and the (+ +) asci predominated over the (– –) asci. The frequencies of these three ascus types were related to the distance of the marker from the centromere. Most of the segregants showed a mating reaction with either a or α haploids, while a few were non- or omni-maters. These observations, along with statistical data on observed and expected frequencies of various ascus types, indicate that the two-spored asci are not generated from normal meiotic products by random abortion of two spores. An alternative mechanism is proposed in which the first meiotic division including crossing over at the four-chromatid stage occurs normally, but the second meiotic division is interrupted, possibly at centromere splitting and/or formation of the spindle apparatus. On this basis, equations describing theoretical frequencies of various ascus types were derived. Expected frequencies of the different ascus types for nine auxotrophic markers having various centromere distances and for mating types were calculated from the equations and compared with the data observed in 188 dyads from three different diploids. A uniformly good fit was found between expected and observed frequencies.

INTRODUCTION

Two-spored asci commonly occur during sporulation of *Saccharomyces cerevisiae*. Their genetic nature was first described by Takahashi (1962), who observed that the two-spored asci arise by random abortion of two spores from each ascus after normal meiotic development. Esposito *et al.* (1974) reported a similar finding in *spo3/spo3* diploids. In contrast, Grewal & Miller (1972) found three naturally occurring yeast strains producing exclusively two-spored asci with diploid spores under normal sporulation conditions. Klapholz, Esposito & Esposito (1978, Rochester, New York, Yeast Genetics Conference) assigned these to two *spo* mutations, *spo12* and *spo13*, responsible for an apomictic mode of development. In studies with *S. cerevisiae* in which sporulation was interrupted by transferring cells to sterilized water after incubation in potassium acetate sporulation medium for a few hours, we observed significant induction of two-spored asci.

This communication describes the specific sporulation regimen and mode of formation of two-spored asci, with segregation data for several genetic markers. We conclude that the two-spored asci arose due to interruption of the formation of the spindle apparatus at the second meiotic division and random chromosome loss, and not to random abortion of haploid meiotic products.

Table 1. *Yeast strains*

Strain	Ploidy	Genotype	Source
43763	<i>n</i>	a <i>his2 leu1</i>	Stock culture
46611	<i>n</i>	<i>α</i> <i>lys1 trp1</i>	Stock culture
S1586C	<i>n</i>	a <i>ura1</i>	Stock culture
732	<i>n</i>	<i>α</i> <i>his1 trp2</i>	Stock culture
PH53	<i>2n</i>	$\frac{\mathbf{a}}{\alpha} \frac{\text{his2}}{+} \frac{\text{leu1}}{+} \frac{+}{\text{lys1}} \frac{+}{\text{trp1}}$	Cross of 43763 × 46611
PH55	<i>2n</i>	$\frac{\mathbf{a}}{\alpha} \frac{+}{\text{his1}} \frac{+}{\text{trp2}} \frac{\text{ura1}}{+}$	Cross of S1586C × 732
131	<i>2n</i>	$\frac{\mathbf{a}}{\alpha} \frac{\text{ac-21}^+}{+} \frac{\text{ad-2}^-}{\text{ad-2-R8}^-} \frac{\text{can}^+}{+} \frac{\text{leu1}}{+} \frac{+}{\text{met8}} \frac{\text{ura3}}{+}$	G. Simchen

METHODS

Yeast strains. These are listed in Table 1. Strain 131 was obtained from G. Simchen, Department of Genetics, The Hebrew University, Jerusalem, Israel. The other strains were selected or constructed from our stock cultures. All strains were heterothallic. The genetic symbols used are those proposed by the nomenclature committee for yeast genetics (Plischke *et al.*, 1976), except for mating type and the symbols *ac-21*⁺, *ad-2*⁻, *ad-2-R8*⁻ and *can*⁺, used in strain 131, which follow the original description of Simchen *et al.* (1972).

Techniques. Media composition, general methods for ascus dissection, mating type determination by microscopic observation of zygote formation with the standard **a** and *α* haploid cells, and testing for sporulation in genetic analysis and for characterization of genetic traits were as described by Takano & Oshima (1967). In interrupted sporulation experiments, the regimen described by Fast (1973) was adopted as the standard condition. Cells grown at 30 °C on a glucose nutrient agar slant were inoculated into yeast extract (1%, w/v), peptone (2%, w/v), potassium acetate (1%, w/v) medium (YPA) and shaken (150 strokes of 5 cm amplitude min⁻¹) at 30 °C for 8 to 10 h to give a concentration of 10⁷ cells ml⁻¹. One ml of the culture was inoculated into 100 ml fresh YPA medium. The fresh culture was incubated at 30 °C for 12 h, by which time it had attained a cell concentration of 10⁷ cells ml⁻¹. The cells were then harvested, washed with sporulation medium consisting of 2% (w/v) potassium acetate solution in distilled water (this concentration of potassium acetate had been determined to be optimal for sporulation) and suspended in 100 ml of the same sporulation medium. The sporulation culture was shaken at 30 °C for the periods indicated in Results. Sporulation was monitored by the appearance of asci containing refractile spores, using a haemocytometer. At least 400 cells were counted for each observation. Buds were counted separately from the mother cells.

RESULTS

Induction of two-spored asci by interruption of sporulation

To examine the kinetics of differential appearance of two- and four-spored asci under normal sporulation conditions, cells of strain PH55 were shaken continuously in sporulation medium at 30 °C. Two- and four-spored asci were counted (three-spored asci were scored as four-spored) in samples taken at intervals from the culture. The meiotic cycle took 26 h in this strain. The major phase of sporulation took place between 20 and 26 h after suspension in sporulation medium; during this period approximately 10% of the cells were transformed into four-spored asci and another 5% into two-spored asci (Fig. 1). The sporulation peak of four-spored asci observed in the samples taken after 26 and 28 h incubation probably signifies only a fluctuation in the data.

To investigate the effect of interruption of the sporulation process, cell samples were taken at appropriate intervals from the sporulation medium. The cells were harvested and suspended in distilled water. The cell suspension was shaken at 30 °C until the control culture had been in sporulation medium for 30 h. The numbers of one-, two- and four-spored asci in each culture were then scored (Fig. 2). Four-spored asci first appeared in the water suspension prepared from cells exposed to sporulation medium for 6 h and increased to a

Fig. 1. Kinetics of appearance of two-spored (O) and four-spored (or three-spored) (●) asci under the normal sporulation regimen in strain PH55. Cells were suspended in sporulation medium and the culture was shaken at 30 °C as described in Methods. Samples were taken at intervals and the frequency of ascus formation was scored.

Fig. 2. Appearance of one-spored (▲), two-spored (O) and four-spored (●) asci under interrupted sporulation conditions in strain PH55. Cells in the sporulation medium were transferred to distilled water at various intervals and the frequency of ascus formation was scored 30 h after the start of incubation in the sporulation medium.

plateau after 12 h. In contrast, two-spored asci first appeared in the water suspension from the sporulation culture interrupted after 2 h, and increased to 13% in the culture interrupted at 8 h. Thereafter, the frequency of two-spored asci decreased to approximately 5%, i.e. the same level as in the normal sporulation culture (Fig. 1), while that of four-spored asci increased. A significant number of one-spored asci were observed in sporulation cultures interrupted in the initial period (within 4 h). It is noteworthy that the frequency of two-spored asci in the water suspensions of cells from the sporulation culture interrupted at 4 or 8 h (approximately 13% at 8 h) was appreciably higher than that under normal sporulation conditions (approximately 5%; Fig. 1), and that the cultures interrupted at 4 to 8 h showed almost exclusively two-spored asci. These observations indicate that the interruption of sporulation at 4 to 8 h promotes abortion of two spores during the subsequent development of the meiotic process.

Segregation of genetic markers in the two-spored asci

To study the genetic events that occurred in the interrupted sporulation cultures, we investigated the dyad segregations with the diploid strains PH53 and 131 as well as PH55, each of which had several auxotrophic markers (Table 1). Strains PH53 and 131 showed patterns of sporulation in the normal and interrupted regimens similar to those of strain PH55. The frequencies of ascus formation differed slightly depending on the strain, being 5 to 20% for two-spored asci and 10 to 50% for four-spored asci. The data on dyad segregation of auxotrophic markers (Table 2) showed two striking features. First, the frequency of asci having + and – spores for a given auxotrophic marker [(+ –) asci] appeared to be a function

Table 2. *Phenotypic segregation of auxotrophic traits in two-spored asci produced by interrupted sporulation*

Observed numbers of the (+ +), (+ -) and (- -) ascus types were compared with values expected from the mechanisms of random abortion of two spores and of aberrant meiosis II produced by the interrupted sporulation. Details of the calculations are given in the text.

Marker*	Centromere distance (cM)†	No. of asci tested	No. of each ascus type									
			Observed			Expected						
			(+ +)	(+ -)	(- -)	Random abortion			Aberrant meiosis II			
			(+ +)	(+ -)	(- -)	(+ +)	(+ -)	(- -)	(+ +)	(+ -)	(- -)	P (%)‡
<i>trp1</i>	1.5	83	7	75	1	13.8	55.3	13.8	<1	1.0	81.7	0.3
<i>leu1</i>	3.0	134	12	118	4	22.3	89.2	22.3	<1	3.3	129.7	1.0
<i>ura3</i>	12.5	51	3	46	2	8.5	34.0	8.5	<1	2.7	47.4	0.8
<i>lys1</i>	28.5	83	23	53	7	13.8	55.3	13.8	<1	19.1	57.9	6.0
<i>his2</i>	31.5	83	23	53	7	13.8	55.3	13.8	<1	21.4	54.8	6.8
<i>his1</i>	47.5	54	12	36	6	9.0	36.0	9.0	30-50	21.0	26.3	6.7
<i>met8</i>	>50	51	18	26	7	8.5	34.0	8.5	<1	20.9	23.5	6.6
<i>trp2</i>	>50	54	23	27	4	9.0	36.0	9.0	<1	22.1	24.8	7.0
<i>ura1</i>	>50	54	20	27	7	9.0	36.0	9.0	<1	22.1	24.8	7.0

* Of the 12 markers available in this study, 9 (not *ac-2I⁺*, *can^r* and *ad-2⁻*) were scored for dyad data.

† Distances longer than 50 centimorgans (cM) were treated as 50 cM for calculation of the values for the aberrant meiosis II hypothesis.

‡ Each term of the ascus type in which the expected occurrence of asci is less than five was excluded from the calculation of the χ^2 statistics.

Table 3. *Phenotypic segregation of mating types in two-spored asci produced by interrupted sporulation*

Observed numbers of six different categories of two-spored asci were compared with values expected from the mechanisms of random abortion of two spores and of aberrant meiosis II produced by the interrupted sporulation. Details of the calculations are given in the text.

Ascus type	No. of each ascus type		
	Observed	Expected	
		Random abortion	Aberrant meiosis II†
Non-mater : non-mater*	4	0.0	5.9
Non-mater : a	15	0.0	15.2
Non-mater : α	10	0.0	15.2
a : a	14	31.4	9.7
a : α	131	125.3	132.2
α : α	14	31.4	9.7
Total	188	188.1	187.9
χ^2		19.54	6.22
Degrees of freedom		2	5
Probability (%)		$P < 1$	$20 < P < 30$

* Omni-maters were included in the non-mater class for the reason described in the text.

† Values were calculated with a centromere distance of 20 cM for the mating-type locus.

of the distance of the marker from its centromere. For example, those closely linked to their respective centromeres, such as *leu1*, *trp1* and *ura3*, showed an extremely high frequency of (+ —) asci in comparison with the markers loosely linked or unlinked to their centromeres, *his1*, *lys1* and *met8*. Second, the frequency of the (+ +) asci (asci containing two spores both having the dominant phenotype for a certain marker) was higher than that of the (— —) asci (in which both spores have the recessive phenotype for the marker). That this is due to differential survival of these spores can be excluded by the good recovery (80 to 90%) of two-spored asci in which both spores had survived in the diploids used. In addition, the segregation of markers in these diploids was normal in four-spored tetrads with the decreased recovery (25 to 50% depending on the strain) of asci in which all four spores had survived.

Mating type segregation data from diploid strains PH53, PH55 and 131 are summarized in Table 3. Most of the segregants mated with the standard haploid strain having either a or α mating type, but there were occasional non-maters and omni-maters which could mate with the a and α mating types. As the non-maters could not sporulate and had the size and shape of haploid cells, it is possible to speculate that they were disomic for chromosome III and heterozygous for the mating-type alleles. To confirm this, a few cells of the primary spore cultures showing the non-mater phenotype were inoculated into nutrient medium and incubated for 24 h. Mating tests with the standard a and α testers were repeated: in all cases the cells showed the omni-mater phenotype. Thus it appears that the non-maters were initially disomic for chromosome III, but that they lost one of the two chromosomes during subsequent mitotic cell divisions, so that the culture could mate with both a and α cells. The same explanation would hold for the occurrence of the omni-maters in the segregants.

None of the segregants were able to sporulate, but one segregant of diploid cell size and shape was detected in dyad analysis of strain 131 (in over 400 spore cultures analysed). It, however, showed α mating type. To confirm its ploidy, it was crossed with an a haploid tester strain and the resultant hybrid was sporulated. Approximately 50% (26 spores among 40 isolated) of spore cultures from the hybrid could sporulate without prior cell conjugation. These facts strongly suggest that the original segregant was either a diploid monosomic for chromosome III or an α/α diploid. All the other segregants seemed to be haploids on the basis of the segregation of auxotrophic markers, the mating reaction with the standard strains and

their cell size and shape. These results, however, do not exclude the possibility that the segregants were originally monosomic diploids or haploids disomic for one or a few chromosomes.

THEORY

Two hypotheses to explain the occurrence of two-spored asci and the segregation pattern of the genetic markers in the dyad analyses were investigated: the random abortion of two spores, and the interrupted formation of the spindle apparatus at the second meiotic division. The theoretical frequencies of the (+ -), (+ +) and (- -) asci expected for the two hypotheses were calculated and their fit with the observed frequencies was tested by χ^2 statistics.

Random abortion of two spores

Suppose that X and x are a single pair of alleles located on a chromosome. When an X/x diploid undergoes normal meiosis, two spores in a tetrad bear the X allele and the other two the x allele. If two spores in this tetrad are randomly eliminated during the maturation of asci, the two surviving spores can pair in six different ways in dyads. Four of the six pairings result in an ($X x$) phenotype and the remaining two divide equally between ($X X$) and ($x x$) dyads. Hence, the frequencies of the ($X x$), ($X X$) and ($x x$) asci should be $\frac{2}{3}$, $\frac{1}{6}$ and $\frac{1}{6}$, respectively, of the total asci tested for any heterozygous marker in the diploid, irrespective of centromere distance. This mechanism also predicts that all the spores should be normal haploids.

These predictions did not accord with the observed frequencies of the three ascus types produced by interrupted sporulation, which varied with the genetic markers (Table 2), or with the appearance of the supposed disomic haploid and monosomic diploid segregants (Table 3); whereas agreement with the predictions was observed for two-spored asci arising from the diploids used in this study under normal sporulation conditions. The probabilities (P) calculated by χ^2 statistics (Tables 2 and 3) were all less than 1%, except for the *his1* marker; the reason for this exception is unknown. Thus, we conclude that the mechanism of random abortion of two spores is unlikely in the present case.

Interrupted formation of the spindle apparatus at the second meiotic division

In looking for an alternative mechanism, we focused on the observations that (i) the frequency of the (+ -) asci appeared to be related to the centromere distance of the marker, (ii) the (+ +) asci appeared with higher frequency than the (- -) asci, and (iii) possible disomic haploid and monosomic diploid segregants appeared with a frequency of 8.8% (33 clones among the 376 spore cultures) for chromosome III as evidenced by the appearance of non-maters (Table 3). With these findings in mind, we postulated that meiotic recombination among four chromatids and the migration of homologous centromeres to opposite poles at the first meiotic division occur normally, but that the spindle apparatus for the second meiotic division is not formed and, for an unspecified reason, one of the two chromatids for each chromosome at each pole (which is formed by the first meiotic division) is eliminated with a certain probability.

Suppose the original diploid is heterozygous for the X and x alleles. The genotype of chromatids at the two poles at the end of the first meiotic division would thus be Xx and xX or XX and xx , respectively, depending on whether or not crossing over occurred between the non-sister chromatids in the region between the centromere and X . Thus the frequency of $Xx-xX$ asci [having the ($X X$) phenotype when X is dominant over x] is the frequency of second division segregation (SDS) for X and the frequency of $XX-xx$ asci [showing the ($X x$) phenotype] is $1 - \text{SDS}$. In line with this mechanism, we can calculate the expected frequencies of the dyad ascus types. These frequencies will be changed by random loss of chromatids.

Table 4. *Various expected genotypes and phenotypes of two-spored asci and their expected frequencies if produced by interrupted formation of the spindle apparatus*

The original genotype of the diploid is X/x . The symbols k and r represent, respectively, the probability of chromatid loss at the first and second meiotic divisions, and the frequency of second division segregation.

Configuration after meiosis I	No. of chromosomes lost	Ascus type		Frequency
		Genotype	Phenotype	
$XX-xx$	0	$XX-xx$	$X-x$	$(1-r)(1-k)^2$
	1	$X-xx$	$X-x$	$(1-r)k(1-k)$
		$XX-x$	$X-x$	$(1-r)k(1-k)$
	2	$X-x$	$X-x$	$(1-r)k^2$
$xX-Xx$	0	$xX-Xx$	$X-X$	$r(1-k)^2$
	1	$X-Xx$	$X-X$	$r.k(1-k)/2$
		$x-Xx$	$x-X$	$r.k(1-k)/2$
		$xX-X$	$X-X$	$r.k(1-k)/2$
		$xX-x$	$X-x$	$r.k(1-k)/2$
	2	$X-X$	$X-X$	$r.k^2/4$
		$X-x$	$X-x$	$r.k^2/4$
		$x-X$	$x-X$	$r.k^2/4$
		$x-x$	$x-x$	$r.k^2/4$

It is possible to speculate that all patterns of chromatid loss in the $XX-xx$ genotype will give rise to an $(X\ x)$ ascus due to the homozygosity of the X and x alleles at both poles (Table 4). Thus the frequency of the $(X\ x)$ dyad from the $XX-xx$ genotype would be $(1-r)$ irrespective of the type of chromosome loss. Here, r is the SDS value of the X gene, i.e., r is the centromere distance of X (in cM)/50, since the map distance, D (in cM), was calculated by $D = 100(T + 6NPD)/2(PD + NPD + T)$ (Perkins, 1949), where PD, NPD and T indicate the number of parental ditype, non-parental ditype and tetratype asci, respectively. For rough estimation of the r values, the centromere distances may be obtained by direct reading of distances on a chromosome map (e.g. Mortimer & Hawthorne, 1973).

On the other hand, the $Xx-xX$ genotype will give rise to eight different configurations depending on the manner of chromatid loss. In addition to these eight configurations, it is possible that some fraction of the mature spores will retain both chromatids of any chromosome. If all the chromatids have the same probability of elimination, k , during the second meiotic division, the frequencies of all possible genotypic configurations and resulting phenotypes with respect to the X chromosome will be as listed in Table 4. With the assumption that X is dominant over x , the nine genotypic configurations can be compiled into three categories of dyads with respect to their phenotypes. The frequencies of these three dyads, i.e., $f(X\ x)$, $f(X\ X)$ and $f(x\ x)$, can be obtained by summing the respective frequencies. Thus:

$$f(X\ x) = r.k(1-k/2) + (1-r) \quad (1)$$

$$f(X\ X) = r(1-k/2)^2 \quad (2)$$

$$f(x\ x) = r.k^2/4 \quad (3)$$

To calculate the expected values of the three classes of dyads from these equations, the k value is necessary. From equations (2) and (3), the frequencies of the $(X\ X)$ and $(x\ x)$ dyads are each a function of k and r , but their ratio is independent of r and is a function only of k . This is confirmed by the data in Table 2; the ratio of the $(+ +)$ to $(- -)$ asci appears to be constant and roughly equal to 3 for all the auxotrophic markers, irrespective of their distances from the centromere. To obtain a more generalized value of k , the $(+ +)/(- -)$ ratio was

calculated for all the asci listed in Table 2. This value, 3.13, was used for calculation of the k value by the equation:

$$f(+ +)/f(- -) = (1 - k/2)^2/(k^2/4) = 3.13$$

The k value obtained was 0.72. This value was substituted into equations (1) to (3), allowing calculation of the expected frequencies of ascus types shown in Table 2, with the respective r values being calculated from centromere distances. For markers more than 50 cM from the centromere, $r = 1.00$ (=50/50) was adopted, since the maximum distance for two loci in direct calculation is 50 cM.

The frequencies of the six ascus types with respect to the mating types can also be calculated according to the mechanism of interrupted formation of the spindle apparatus. When **a** and α are arbitrarily denoted as X and x , respectively, the XX , xx and Xx genotypes will be disomic for chromosome III and have the **a**, α and non-mating types, respectively. If this set of notations is substituted in Table 4, the 13 genotypic classes can be classified into six ascus types. The frequencies of the six ascus types can be calculated, with the value of 20 cM for the centromere distance of the **a**/ α locus (Mortimer & Hawthorne, 1973), by the following equations:

$$f(\text{non-mater} : \text{non-mater}) = r(1 - k)^2 \quad (4)$$

$$f(\text{non-mater} : \mathbf{a}) = r.k(1 - k) \quad (5)$$

$$f(\text{non-mater} : \alpha) = r.k(1 - k) \quad (6)$$

$$f(\mathbf{a} : \mathbf{a}) = r.k^2/4 \quad (7)$$

$$f(\mathbf{a} : \alpha) = (r.k^2/2) + (1 - r) \quad (8)$$

$$f(\alpha : \alpha) = r.k^2/4 \quad (9)$$

These calculations gave the expected values shown in Table 3.

Comparison of the expected values with the observed values by χ^2 statistics (Table 2 for auxotrophic markers and Table 3 for mating type) demonstrated a uniformly good fit ($P > 20\%$, except for *his1* for which $5\% > P > 2\%$) irrespective of the centromere distance of the markers.

DISCUSSION

We have described here a simple method to obtain almost exclusively two-spored asci of *S. cerevisiae*. The method consists in interrupting the meiotic process by transferring sporulating cells from sporulation medium to water. Since the frequency of the $(+ -)$ asci is higher for genetic markers closely linked to the centromere than for those further from the centromere, it is reasonable to infer that the first meiotic division and chromatid crossing over at the four-chromatid stage occur normally. However, the segregation pattern of various ascus types clearly indicates that the event leading to the formation of two-spored asci is not the random abortion of two spores from the normal four-spored asci. Since the $(+ -)$ ascus type predominated over the other types, especially for the centromere markers, homologous centromeres might be segregated in a $(+ -)$ configuration in a two-spored ascus, as in normal meiotic division, and the $(+ +)$ and $(- -)$ asci might originate from crossing over at the four-chromatid stage. The ultimate products in the two-spored asci were probably haploid spores with some frequency of disomic haploid and monosomic diploid spores. This strongly suggests that the second meiotic division is disturbed by the interruption of meiosis by transfer of cells from sporulation medium to water. The disturbance might have occurred at centromere splitting and/or formation of the spindle apparatus, because no further migration of chromatids occurred.

This mechanism is strikingly different from that by which two-spored asci are produced in

the normal sporulation process, which Takahashi (1962) imputed to random abortion of two spores in each two-spored ascus. We calculated the expected frequencies of ascus types according to both mechanisms, random abortion of two spores and interrupted formation of the spindle apparatus, and compared the expected values with Takahashi's results. Although the results of the calculations were ambiguous, as the total number of asci (16) described by Takahashi was too small, we found that the values predicted by the random abortion mechanism fitted the observed data more consistently than those predicted by the interrupted formation of the spindle apparatus (data not shown). The other case of two-spored ascus formation in wild-type *S. cerevisiae*, described by Grewal & Miller (1972), also differs from the present case, as all the spores in their two-spored asci were probably diploids. With the special sporulation mutant, *spo3*, Esposito *et al.* (1974) observed exclusive formation of two-spored asci at a semi-permissive temperature. They tested the frequency of occurrence of (+-), (++) and (--) asci for *ade6*, *his7* and *leu1* and concluded that the distribution was not significantly different from the values expected if the meiotic products incorporated into the spores were a random sample of the four haploid products of the ascogenous cell. We made the same calculation with the two alternative mechanisms and found that the data obtained by Esposito *et al.* (1974) showed good accord with the random abortion mechanism (probability of fitness was $30\% > P > 20\%$ for *ade6*, $50\% > P > 30\%$ for *his7* and *leu1*, and $98\% > P > 95\%$ for *ura1*) but did not fit with the mechanism of interrupted formation of the spindle apparatus ($5\% > P > 2\%$ for *leu1* and $P < 1\%$ for the other three markers). The *spo3* gene is probably concerned with the maturation process of asci after the meiotic division, since the mutation affects a function which is specifically involved in the coordination of nuclear budding and prespore wall formation in the post-meiotic ascogenous cells (Esposito *et al.*, 1974). In contrast, the interruption of the sporulation process described here probably affects the second meiotic division of the chromosome. The interruption of sporulation appears to disturb specifically the segregation of genetic material, while the effect of the *spo3* mutation is not specific in this regard. In the former case non-random abortion occurs, while in the latter the abortion of the tetrad is random.

We have calculated the probability of chromatid loss at the poles after the first meiotic division by the interrupted sporulation to be 0.72. Hence, the probability of retaining both the chromatids, or in other words, the probability of disomicity arising for any chromosome in a spore, is 0.28 ($=1 - 0.72$). From these values, the probability of obtaining a diploid or a haploid segregant can be calculated as 0.28^{17} or 0.72^{17} , as the haploid genome contains 17 chromosomes (Wickner, 1979). We have not found a definite diploid segregant in 400 segregants tested; this does not disagree with these considerations. The two-spored asci produced by interrupted sporulation provide an easy way to detect centromere linkage of a marker. It is also suggested that the aneuploidy expected through this mechanism will be a useful tool for mapping studies.

We gratefully acknowledge Professor S. K. Sen, Botany Department, Bose Institute, Calcutta 700 009, India, for providing facilities for performing the initial part of this work. We thank T. Takahashi for discussion on the work. P. K. Srivastava was a trainee in the International Post-Graduate University Course in Microbiology, October 1978 to September 1979, supported by the Japanese Government, UNESCO and ICRO.

REFERENCES

- ESPOSITO, M. S., ESPOSITO, R. E. & MOENS, P. B. (1974). Genetic analysis of two spored asci produced by the *spo3* mutant of *Saccharomyces*. *Molecular and General Genetics* **135**, 91-95.
- FAST, D. (1973). Sporulation synchrony of *Saccharomyces cerevisiae* grown in various carbon sources. *Journal of Bacteriology* **116**, 925-930.
- GREWAL, N. S. & MILLER, J. J. (1972). Formation of asci with two diploid spores by diploid cells of *Saccharomyces*. *Canadian Journal of Microbiology* **18**, 1897-1905.
- MORTIMER, R. K. & HAWTHORNE, D. C. (1973). Genetic mapping in *Saccharomyces*. IV. Mapping of temperature sensitive genes and use of disomic strains in localizing genes. *Genetics* **74**, 33-54.
- PERKINS, D. D. (1949). Biochemical mutants in the smut fungus *Ustilago maydis*. *Genetics* **34**, 607-626.
- PLISCHKE, M. E., VON BORSTEL, R. C., MORTIMER, R. K. & HAWTHORNE, D. C. (1973). Genetic mapping in *Saccharomyces*. IV. Mapping of temperature sensitive genes and use of disomic strains in localizing genes. *Genetics* **74**, 33-54.

- R. K. & COHN, W. E. (1976). Genetic markers and associated gene products in *Saccharomyces cerevisiae*. In *Handbook of Biochemistry and Molecular Biology, Nucleic Acids* vol. II, 3rd edn, pp. 765–832. Edited by G. D. Fasman. Cleveland, Ohio: Chemical Rubber Co. Press.
- SIMCHEN, G., PIÑON, R. & SALTS, Y. (1972). Sporulation in *Saccharomyces cerevisiae*: premeiotic DNA synthesis, readiness and commitment. *Experimental Cell Research* **75**, 207–218.
- TAKAHASHI, T. (1962). Genetic segregation of two-spored ascus in *Saccharomyces*. *Bulletin of Brewing Science* **8**, 1–9.
- TAKANO, I. & OSHIMA, Y. (1967). An allele specific and a complementary determinant controlling homothallism in *Saccharomyces oviformis*. *Genetics* **57**, 875–885.
- WICKNER, R. B. (1979). Mapping chromosomal genes of *Saccharomyces cerevisiae* using an improved genetic mapping method. *Genetics* **92**, 803–821.