MATING-TYPE FUNCTIONS FOR MEIOSIS AND SPORULATION IN YEAST ACT THROUGH CYTOPLASM

AMAR J. S. KLAR

Cold Spring Harbor Laboratory, P.O. Box 100,
Cold Spring Harbor, New York 11724

Manuscript received September 12, 1979
Revised copy received October 24, 1979

ABSTRACT

Given a nutritional regime marked by a low nitrogen level and the absence of fermentable carbon sources, conventional **a**/α diploid cells of *Saccharomyces cerevisiae* exhibit a complex developmental sequence that includes a round of premeiotic DNA replication, commitment to meiosis and the elaboration of mature tetrads containing viable ascospores. Ordinarily, haploid cells and diploid cells of genotype **a**/**a** and α/α fail to display these reactions under comparable conditions. Here, we describe a simple technique for sporulation of α/α and **a**/**a** cells. Cells of genotype α/α are mated to haploid **a** cells carrying the *kar1* (karyogamy defective) mutation to yield heterokaryons containing the corresponding diploid and haploid nuclei. The *kar1* strains mate normally, but nuclei in the resultant zygotes do not fuse. When heterokaryotic cells are inoculated into sporulation media, they produce asci with six spores. Four spores carry genotypes derived from the diploid nucleus and the other two possess the markers originating from the haploid nucleus, *i.e.*, the diploid nucleus divides meiotically while the haploid nucleus apparently divides mitotically. Similarly, the **a**/**a** genome is "helped" to sporulate as a consequence of mating with α *kar1* strains. The results allow us to conclude that the mating-type functions essential for meiosis and sporulation are communicated and act through the cytoplasm and that sporulation can be dissociated from typical meiosis. This procedure will facilitate the genetic analysis of strains that are otherwise unable to sporulate.

IN *S. cerevisiae*, mating, meiosis and sporulation are controlled by two alternate alleles of the mating-type locus, **a** and α (LINDEGREN and LINDEGREN 1943). Diploid **a**/α cells heterozygous at the mating-type locus are unable to mate, but can undergo meiosis and sporulation. In contrast, **a**/**a** and α/α diploid cells express their respective mating types and are unable to sporulate (ROMAN and SANDS 1953). Clearly, meiosis and sporulation are closely associated, and the expression of both **a** and α alleles is ordinarily required for both processes. Understandably, conventional haploid strains cannot sporulate, since they carry and express only one of the two mating-type alleles and, being haploids, are incapable of engaging in synapsis and the ensuing first meiotic division.

The development of ascogenous cells in yeast is a process of intracellular differentiation wherein cells halt their growth by mitotic cycles and exhibit commitment to enter into meiotic development (for reviews see TINGLE *et al.* 1973).

Operationally, sporulation may be initiated by transferring cells to medium deficient in nitrogen and containing a nonfermentable carbon source such as potassium acetate. At the completion of this differentiation process, diploid **a**/α cells yield mature asci containing four haploid ascospores.

For the most part, genetic analysis in yeast is limited to cells capable of meiosis and sporulation since the meiotic products or ascosporal clones are scored and tested. However, **a**/**a** and α/α cells are incapable of meiosis and sporulation; hence, their analysis ordinarily requires the production and analysis of tetraploid hybrids. At best, this route is complex and laborious.

We describe here conjugate sporulation, a process that readily allows for the initiation and completion of meiosis and sporulation in **a**/**a** and α/α cells. In an independent study, similar observations have been made by R. MALONE (personal communication).

MATERIALS AND METHODS

Strains: The strains of *S. cerevisiae* used are listed in Table 1.

Media and techniques: The YEPD growth media contained 2% dextrose, 2% Bactopeptone, 1% Bacto-yeast extract and 2% agar. Sporulation media contained 2% potassium acetate, 2% agar and 10 mg/l of amino acid and base supplements to satisfy the auxotrophic requirement of the strain to be sporulated. The techniques for micromanipulation and tetrad analysis have been described (MORTIMER and HAWTHORNE 1969). Temperature for growth and sporulation was 30°.

Construction of **a**/**a** *and* α/α *strains*: A recessive antibiotic cryptopleurine-resistant marker, *cry1*, is located about 3 cM proximal to the mating-type locus (GRANT, SANCHEZ and JIMENEZ 1974). Since the wild-type allele *CRY1* confers sensitivity and is dominant to the *cry1* allele, heterozygous *CRY1/cry1* diploids exhibit sensitivity to this drug. Spontaneous reciprocal mitotic

TABLE 1

List of strains used

Strain	Genotype*	Source
K67	α/**a** *cry1/cry1 ade6/+ ura1/+* or *ura3/+ leu2/+ his4/+ his2/+ aro1/+ thr4/thr4*	This study
JC7	**a** *leu1 kar1*	G. FINK
K68	α *cry1 ade6 ura1* or *ura3 leu2 his4 thr4*	This study
K69	**a**/α *+/cry1 thr1/+ arg4/+ ilv3/+ ura1/+ his2/+ ade6/+ trp1/trp1 lys1/lys1*	This study
K70	α/α *cry1/cry1 thr1/+ arg4/+ ilv3/+ ura1/+ his2/+ ade6/+ trp1/trp1 lys1/lys1*	This study
K71	**a**/**a** *cry1/cry1 lys2/+ leu2/+ his4/+ his2/+ ura1/+ aro1/+*	This study
JC25	α *ade2 his4 kar1*	G. FINK
227A	**a** *cry1 lys1*	J. STRATHERN
K72	*his4–4 leu2–1* α / *his4–290 leu2–27* α disome chrom. III *ade2 kar1*	This study
K73	**a** *his4 his2? aro1 ade6 thr4 ura1* or *ura3*	This study

* The genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE *et al.* 1976).

recombinants possessing the *cry1/cry1* genotype can be selected on YEPD media containing 1 mg/l cryptopleurine. Since *cry1* is closely linked to the mating-type locus, most cryptopleurine-resistant mitotic recombinants are homozygous for the mating-type alleles in *cis* array with the *cry1* allele. Hence, effectively isogenic **a**/**a** and α/α strains can be derived from parental **a**/α strains.

RESULTS

Rationale: CONDE and FINK (1976) reported a *kar1* (karyogamy defective) mutation in *S. cerevisiae*. Strains carrying this mutation mate readily with the cells of the opposite mating type, but the gametic pronuclei of the resulting zygotes do not fuse. Accordingly, zygotic heterokaryons carrying two nuclei of different genotypes can be generated. Heterokaryons with α/α diploid and **a** haploid as well as **a**/**a** diploid and α haploid nuclei, were constructed. Since the defect in *kar1* mutants appears to be nuclear-limited (CONDE and FINK 1976), only the haploid parent need carry the *kar1* mutation. Thus, by means of cytogamic fusion, we were able to provide the **a** and α functions essential for meiosis and sporulation within the same, single cell. We asked whether the diploid nucleus can be induced to sporulate by the opposite mating-type allele functions provided by the *kar1* haploid nucleus, and *vice versa*.

Sporulation of the α/α diploid cells: Zygotes were produced after 4.5 hr by mixing strains K67 (α/α) and JC7 (**a** *kar1*) on YEPD media. Then, the mating mixture was transferred directly to plates containing the sporulation media. Sporulation of the zygotic cells occurred after two days of incubation (Figure 1) Usually, the zygotic asci contained more than four spores and, frequently, unbudded zygotes produced asci containing six spores ("hexads"). Also, zygotes displaying a bud produced asci containing more than six spores. Asci with six spores were subjected to tetrad analysis. The genotypes of the six spores derived from a single ascus are presented in Table 2. Apparently, the diploid α/α genome went through a standard meiosis, since all the heterozygous markers (see Table 1 for genotype of K67 and JC7) segregated 2+:2− to produce four α haploid ascospores. The other two spores carried genotypes attributable only to the **a** *kar1* parental genome. No detectable genetic exchange occurred between the diploid and the haploid nuclei.

To further characterize the nature of the meiotic event in the diploid α/α nucleus, the map distance between the linked markers, *arg4* and *thr1*, and the segregation of the independently assorting centromere-linked markers, *arg4* and *ilv3*, were determined These values were compared to those obtained from the analysis of the standard **a**/α parent (strain K69) from which the α/α (strain K70) was derived. As displayed in Table 3, *arg4* and *thr1* exhibited comparable linkage of 13.8 cM (centi-Morgans) in the α/α derivative and 13.1 cM in the parental α/**a** control strain. Furthermore, in both cases, *arg4* and *ilv3* displayed centromere linkage, since the frequency of tetratypes observed was significantly less than 0.67 (Table 3). The map intervals, independent assortment of the unlinked markers and the second division segregation frequencies of the centromere-linked markers were comparable for the tested asci that were obtained from cytogamic sporulation of the α/α strain to that of the parental

FIGURE 1.—Sporulation of heterokaryotic cells: Strains K67 (α/α) and JC7 (**a** *kar1*) were allowed to mate for 4.5 hr on YEPD; the mating mix was then transferred to plates containing sporulation media. After 48 hr of incubation, the zygotic cells (marked by arrows) produced asci containing six or more spores.

a/α strain. Therefore, in heterokaryotic cells, the diploid α/α nucleus enters into and completes a conventional meiotic cycle in which recombination rates are effectively normal.

Are spores derived from the kar1 *nucleus haploid?* As reported by CONDE and FINK (1976), the *kar1* mutation is somewhat leaky since a small fraction of the binucleate zygotes give rise to true diploids. On selective media, hybrid diploid cells overgrow their heterokaryotic counterparts. By means of this procedure, *kar1* strains could be easily crossed with other strains for meiotic analysis. A segregant with the *kar1* parental genotype obtained from a cross described above

TABLE 2

*The analysis of K67 (α/α) × JC7 (**a**, kar1) heterokaryon six-spored ascus*

Segregant	*MAT*	*cry1*	*ade6*	*ura1* or *3*	*leu2*	*his4*	*his2*	*aro1*	*thr4*	*leu1*
A	α	+	+	+	—	—	+	—	—	+
B	α	+	—	+	—	—	+	+	—	+
C	α	+	—	—	+	+	—	—	—	+
D	α	+	+	—	+	+	—	+	—	+
E	**a**	—	+	+	+	+	+	+	+	—
F	**a**	—	+	+	+	+	+	+	+	—

TABLE 3

Comparison of map intervals and the centromere linkage in the parental **a**/α *(K69) and the derivative* α/α *(K70) strains*

Strain	Marker pair	PD†	NPD†	TT†	cM*
K69 (**a**/α)	*arg4–thr1*	72	0	25	13.0
K69 (**a**/α)	*arg4–ilv3*	23	33	41	centromere linked
K70 (α/α)	*arg4–thr1*	58	0	16	13.8
K70 (α/α)	*arg4–ilv3*	14	23	41	centromere linked

Entries in the table correspond to the numbers of asci that displayed the various segregation patterns.
† PD, parental ditype; NPD, nonparental ditype; TT, tetratype tetrads.
* The map distance in centi-Morgans (cM) was calculated according to PERKINS (1949).

(Table 2) was hybridized to strain K68, a haploid containing seven recessive genetic markers. Tetrad analysis of such a hybrid yielded 2+:2– segregation for heterozygous markers *thr4*, *cry1*, *his4*, *ade6*, *ura1* or *ura3*, *leu1*, *leu2* and the mating type locus in 17 tetrads analyzed. Thus, we conclude that the **a** *kar1* segregants are haploid.

Analysis of these results allows us to conclude that a heterokaryon containing an α/α diploid and **a** *kar1* haploid nuclei produce asci containing six spores, four of which possess markers of the diploid parent and the other two carry the genotype of the haploid parent.

*Sporulation of the **a**/**a** diploid cells:* Freshly grown cells of strains K71 (**a**/**a**) and JC25 (α *kar1*) were allowed to mate for 4.5 hr on YEPD media, and the mating mix was then transferred directly to sporulation media. After two days of incubation, structures similar to those presented in Figure 1 were observed. The zygotic cells were observed to produce asci with six or more spores. Asci with six spores were dissected and subjected to tetrad analysis. The genotypes of six spores derived from a single ascus are presented in Table 4. Clearly, four spores possess genotypes derivable from only the diploid parent and the other two only from the haploid *kar1* parent. No detectable genetic exchange between the diploid and the haploid nuclei was observed. Apparently, the **a**/**a** diploid genome underwent meiosis, since all the heterozygous markers segregated 2+:2–, yielding four haploid spores. The spore segregants with the *kar1*

TABLE 4

The analysis of K71 (**a**/**a**) × *JC25* (α, kar1) *heterokaryon six-spored ascus*

Segregant	*MAT*	*cry1*	*lys2*	*leu2*	*his4*	*his2*	*ura1*	*uro1*	*ade2*
A	**a**	+	+	+	+	—	+	—	+
B	**a**	+	+	—	—	+	+	—	+
C	**a**	+	—	+	+	—	—	+	+
D	**a**	+	—	—	—	+	—	+	+
E	α	—	+	+	—	+	+	+	—
F	α	—	+	+	—	+	+	+	—

nucleus were also judged to be hapolid. This follows since a hybrid between an α *kar1* segregant and strain 227A segregated 2+:2− for the heterozygous makers *ade2*, *his4*, *lys1*, *cry1*, and mating type segregated 2α:2**a** in 18 tetrads analyzed.

Does sporulation of the haploid genome involve meiotic or mitotic divisions? Unexpectedly, we observed that the haploid nucleus completed a sporulation process to yield two haploid spores. Since the *kar1* nucleus comprises a haploid genome, it cannot undergo a conventional reduction division. Thus, it is highly unlikely that the sporulation of the haploid genome follows an ordinary meiotic cycle. A possible approach to analyze this phenomenon further is to study the behavior of a disomic chromosome in an otherwise haploid genome during the cytogamic sporulation. A haploid strain (K72), disomic for chromosome *III* with α/α configuration at the mating-type locus and containing the *kar1* mutation, was constructed. Chromosome *III* homologues were marked with noncomplementing *his4* and *leu2* heteroalleles. Cells from strain K72 were mated to the cells from K71 (**a**/**a**), and the resulting zygotes sporulated as detailed above. Segregants with the *kar1* genotype were derived by ascus dissection and tetrad analysis. A total of 11 segregants were characterized for disomy and for recombination between the heteroallelic markers *his4* and *leu2* situated on the left arm of chromosome *III*. Disomy and heteroallelism of the *kar1* ascosporal clones were assessed by their capacity to generate histidine and leucine prototrophic colonies due to mitotic recombination. Mitotic recombination was induced by low doses of ultraviolet radiation (38 J m^{-2}) according to ROMAN and JACOB (1958). Seven segregants gave rise to histidine and leucine prototrophic papillae; two showed papillae only for histidine. Therefore, these nine segregants were judged to be disomic. The remaining two segregants, 1D and 3B, failed to generate prototrophic papillae. However, these segregants were also judged to be disomic because the hybrids between them and strain K73 (**a** *thr4*) yielded trisomic segregations for *thr4 marker* (Table 5).

The parental disomic strain is very stable during ordinary mitotic divisions. Among 100 tested clones, all maintained heteroallelism for *his4* and *leu2* markers. Presumably, they maintained the disomic condition. Thus, the fact that at least four of 11 segregants tested had undergone some event leading to homozygosity of either *leu2*, or *leu2* and *his4*, suggests that a meiotic level of recombination occurred.

TABLE 5

A trisomic segregation of thr4 *in hybrids between K73 and 1D and 3B segregants derived from the K72 × K71 cross*

Hybrid	Tetrad classes (*Thr*+ :*thr*−) 4+:0	3+:1−	2+:2−
K73 × 1D segregant	8	9	0
K73 × 3B segregant	4	7	2

DISCUSSION

These experiments were initiated to determine whether the mating-type functions required for meiosis and sporulation are nuclear-limited and to develop a convenient approach for the genetic analysis of strains that are otherwise incapable of meiosis and sporulation, e.g., **a**/**a** and α/α. Since the expression of both mating-type alleles is essential for sporulation (ROMAN and SANDS 1953), these functions were provided by constructing heterokaryotic zygotes between diploid cells and haploid cells of opposite mating type. In these experiments, nuclear fusion was blocked by the presence of a nuclear-limited karyogamy-defective mutation (*kar1*) contributed by the haploid parent.

The diploid **a**/**a** genome was induced to sporulate as a consequence of the α functions provided by the haploid *kar1* nucleus, and *vice versa*. The principal conclusion that may be drawn from these experiments are that: (1) the mating-type functions required for meiosis and sporulation are not limited to the nucleus, but can act through the cytoplasm, and (2) in part, at least, sporulation can be dissociated from meiosis since a haploid genome is competent to sporulate.

Several critical aspects of the technique merit emphasis. Matings between diploid and haploid *kar1* strains should not extend beyond 4.5 hr, and the sporulation media should not permit subsequent growth. Matings of longer duration generate zygotes that frequently contain more than two nuclei. When sporulated, such cells contain numerous and largely inviable spores. Similarly, asci with a large number of inviable spores are produced by sporulation on media that allows growth.

The procedure given above is useful for isolating haploid derivatives of diploid strains originated by endomitosis, analyzing diploids with mutations at the mating-type locus that are defective for the sporulation functions and analyzing sporulation-defective mutants. Three recessive mutations have been reported that allow α/α or **a**/**a** cells to sporulate: *sca* (GERLACH 1974), *csp* (HOPPER and HALL 1975) and *rme* (KASSIR and SIMCHEN 1976). Monitoring these mutations in crosses is difficult, due to their recessive nature, and because their diagnostic phenotypes are observable in diploids that are homozygous at the mating-type locus. Our technique for sporulating **a**/**a** and α/α cells (or cells of higher ploidy) could be applied to any strain that expresses its mating type. For routine use, only two strains of opposite mating type carrying the *kar1* mutation (*e.g.*, JC7 and JC25) are needed. During the ordinary mitotic growth of heterokaryotic cells, a low level of chromosomal interchange between the nuclei has been observed (DUTCHER and HARTWELL, personal communication). However, in the protocol used here, such interchanges were not detected. Thus, the conjugate sporulation procedure may be utilized with reasonable assurance that the nuclear informational content of each parental nucleus is maintained and conserved.

That the haploid genome could undergo sporulation to yield two haploid spores was rather surprising. In an experiment where the haploid genome disomic for chromosome *III* was sporulated, all eleven spore segregants tested were disomic. The chromosome *III* homologues carried heteralleles at *his4* and *leu2*

markers. A majority of the disomic segregants possessed heteroalleles at both markers, but a significant fraction (two of 11 for *his4* and *four* of 11 for *leu2*) became homoallelic. Though the number of tested spores is small, it is clear that a level of recombination approximating that of meiosis occurs between chromosome *III* markers in the *kar1* nucleus. An increased level of recombination should not be considered as the proof of meiotic division, since *Saccharomycodes ludwigii* undergoes typical meiosis essentially without any recombination (YAMAZAKI, OHARA and OSHIMA 1976). The possibility exists that the haploid genome undergoes abortive first meiotic division where a "unipolar" spindle is formed to which all the chromosomes are attached. The second meiotic division, which is a simple mitosis, then follows and gives rise to two spores that are disomic for chromosome *III*. A related phenomenon has been reported by GUTZ (1967), who observed that, in crosses of diploid and haploid cells of *Schizosaccharomyces pombe*, a small number of asci contained six haploid spores; four possessed the markers of the diploid parent and the other two carried markers of the haploid nucleus mitotically. Also, a mitotic sporulation in Saccharomyces yeasts, although in diploids, has been suggested in strains producing 2-spored asci (GREWAL and MILLER 1972; S. KLAPOLZ and R. ESPOSITO, personal communication).

The discovery of the atypical sporulation of the haploid genome in the heterokaryon cells may allow one to predict that haploid cells should be competent to sporulate should the mating type **a** and α functions be provided. However, chromosome *III* disomes with **a**/α constitution at the mating type locus are incapable of sporulation (ROTH and FOGEL 1971). Such strains initiate the process of sporulation, but no spores are produced. Similarly, we have recently described a mutation (KLAR, FOGEL and MACLEOD 1979) that provides the **a** and α functions by allowing the expression of silent mating-type information proposed to exist at *HM***a** and *HM*α (HICKS, STRATHEKEN and HERSKOWITZ 1977).The haploid strains possessing this mutation are sterile, but unable to sporulate. Apparently, providing **a** and α functions is not enough to allow the haploid cells to sporulate. Consistent with this suggestion is the observation that heterokaryons constructed by mating the *kar1* haploid and standard haploid strains are unable to sporulate. This result also rules out the possibility that the haploid genome sporulates due to the *kar*1 mutation. In the experiments involving the heterokaryons, the diploid nucleus undergoing typical meiosis and sporulation must be providing additional functions. A possible explanation is that the haploid genome divides mitotically and the resulting nuclei are "wrapped" into spores under the influence of the diploid nucleus, which is undergoing typical meiosis and sporulation. However, the issue of meiotic and mitotic division in the haploid nucleus remains unresolved. Further genetical and cytological studies are needed for a clear understanding of this interesting phenomenon.

I thank JEAN MCINDOO for excellent technical assistance, J. STRATHERN, J. BROACH and J. HICKS for thoughtful discussions and their help in the preparation of the manuscript, S. FOGEL and J. GAME for critically reading the manuscript and G. FINK for providing the *kar1* strains. LOUISA DALESSANDRO prepared the manuscript. This investigation was supported by Public Health Service Grant No. GM25678.

LITERATURE CITED

CONDE, J. and G. FINK, 1976 A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. U.S. **73**: 3651–3655.

GERLACH, W. L., 1974 Sporulation in mating type homozygotes of *Saccharomyces cerevisiae.* Heredity **32**: 241–249.

GRANT, P., L. SANCHEZ and A. JIMENEZ, 1974 Cryptopleurine resistance: Genetic locus for a 40S ribosomal component in *Saccharomyces cerevisiae.* J. Bacteriol. **120**: 1308–1314.

GREWAL, N. S. and J. J. MILLER, 1972 Formation of asci with two diploid spores by diploid cells of *Saccharomyces.* Can. J. Microbiol. **18**: 1897–1905.

GUTZ, H., 1967 Zwillings meiose-eine neue Beobachtung bei einer. Hefe. Ber. Deut. Bot. Ges. **80**: 555–558.

HICKS, J. B., J. STRATHERN and I. HERSKOWITZ, 1977 The cassette model of mating-type interconversion. pp. 457–462. In: *DNA Insertion Elements, Plasmids and Episomes.* Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

HOPPER, A. K. and B. D. HALL, 1975 Mating type and sporulation in yeast. I. Mutations which alter mating-type control over sporulation. Genetics **80**: 41–59.

KASSIR, Y. and G. SIMCHEN, 1976 Regulation of mating and meiosis in yeast by the mating-type region. Genetics **82**: 187–206.

KLAR, A. J. S., S. FOGEL and K. MACLEOD, 1979 *MAR1*—A regulator of the *HM***a** and *HMα* loci in *Saccharomyces cerevisiae.* Genetics **93**: 37–50.

LINDEGREN, C. C. and G. LINDEGREN, 1943 A new method for hybridizing yeast. Proc. Natl. Acad. Sci. U.S. **29**: 306–308.

MORTIMER, R. K. and D. C. HAWTHORNE, 1969 Yeast genetics. pp. 385–460. In: *The Yeasts,* Vol. I. Edited by A. H. ROSE and J. S. HARRISON. Academic Press, Inc. New York.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis.* Genetics **34**: 607–626.

PLISCHKE, M. E., R. C. VON BORSTEL, R. K. MORTIMER and W. E. COHN, 1976 Genetic markers and associated gene products in *Saccharomyces cerevisiae.* pp. 765–832. In: *Handbook of Biochemistry and Molecular Biology,* 3rd edition. Edited by G. D. FASMAN. Chemical Rubber Co. Press, Cleveland, Ohio.

ROMAN, H. and F. JACOB, 1958 A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. Cold Spring Harbor Symp. Quant. Biol. **23**: 155–160.

ROMAN, H. and S. M. SANDS, 1953 Heterogeneity of clones of *Saccharomyces* derived from haploid ascospores. Proc. Natl. Acad. Sci. U.S. **39**: 171–179.

ROTH, B. and S. FOGEL, 1971 A system selective for yeast mutants deficient in meiotic recombination. Mol. Gen. Genet. **112**: 295–305.

TINGLE, M., A. J. S. KLAR, S. A. HENRY and H. O. HALVORSON, 1973 Ascospore formation in yeast. Symp. Soc. Gen. Micro. **XXIII**: 209–243.

YAMAZAKI, T., Y. OHARA and Y. OSHIMA, 1976 Rare occurrence of the tetratype tetrads in *Saccharomycodes ludwigii.* J. Bacteriol. **125**: 461–466.

Corresponding editor: F. SHERMAN