Ordered Linear Tetrads Are Produced by the Sporulation of Newly Formed Zygotes of *Saccharomyces cerevisiae*

James H. Thomas and David Botstein

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ABSTRACT

Diploid *Saccharomyces cerevisiae* strains normally sporulate to produce tetrahedral unordered asci containing four spores (tetrads). We report that when newly formed zygotes are subjected to the same sporulation conditions, they form predominantly linear ordered tetrads. We show that the two spores from each end of such a linear tetrad invariably contain nonsister centromeres. Spore viability, recombination and independence of centromere segregation appear unaffected.

W HEN sporulated, diploid strains of *Saccharomyces cerevisiae* normally produce tetrahedral or oval four spored asci (tetrads). Certain Saccharomyces hybrids produce a fraction of asci in which the four spores are linearly arranged. When these linear tetrads from such a hybrid were dissected and analyzed as an ordered array, sister chromatids were found to have segregated in the pattern *A a A a* (HAWTHORNE 1955). HAWTHORNE showed that this pattern reflects underlying spatial patterning of the meiosis I and meiosis II chromosome segregations, resulting in a highly reproducible placement of the nuclei. Since spore wall deposition is initiated at the spindle pole bodies (embedded in the nuclear envelope), the position of the nuclei determines the position of the spore walls (MOENS 1971; DAVIDOW, GOETSCH and BYERS 1980). Whether or not standard *S. cerevisiae* strains show such spatial patterning during sporulation has not been determined because asymmetric morphological markers in the tetrahedral ascus are not yet available.

Here we report that newly formed zygotes of common laboratory *S. cerevisiae* strains, when subjected to sporulation conditions, produce predominantly linear tetrads. We show that the two spores from each end of such a linear tetrad invariably contain nonsister chromatids, consistent with the spatial patterning observed by HAWTHORNE (1955) in Saccharomyces hybrids. We also present cytological evidence that indicates that the spatial pattern of nuclear divison under these circumstances is similar to that observed by HAWTHORNE.

MATERIALS AND METHODS

Media and methods of genetic analysis: Yeast strains were grown as described by SHERMAN, FINK and LAWRENCE (1974), except that sporulation medium was 1% potassium acetate, 2% Difco agar with no additional nutrients, to achieve immediate starvation upon transfer to sporulation medium.

Efficient mating and sporulation of zygotes: Efficient mating over a short time was achieved by a method similar to that of DUTCHER and HARTWELL (1983). The strains to be mated were grown to no more than 1×10^7 cells/ml in YEPD broth. Cells (5×10^6) of each parent were mixed and filtered onto a sterile 25 mm 0.45 micron HA Millipore filter. The filter was placed face up on the surface of a YEPD + 2% agar plate at 26°. After 2 hr cells were resuspended from the filter with sterile water, pelleted, washed once and resuspended in 15 µl of sterile water. The suspension was spotted onto a sporulation plate in a 1 cm patch and allowed to dry. The plate was incubated for at least three days at 26° before counting or dissection of tetrads.

Microscopy and photography: Cells were observed and photographed using a ×100 phase contrast oil immersion objective. Nuclei were fixed, stained with 4′,6′-diamidino-2-phenylindole (DAPI, Accurate Chemical Company), and observed and photographed as described in THOMAS, NEFF and BOTSTEIN (1985).

Dissection of partially ordered tetrads: A portion of the sporulated cells was resuspended in water and applied in a line to a YEPD plate with a 25-µl micropipette. Next to the cells, a line of 2.5 mg/ml zymolyase 60,000 (Miles Laboratories, Inc.) dissolved in 0.1 M potassium phosphate (pH 7.5) was applied. Both were allowed to dry into the plate. Under a dissecting microscope, linear tetrads were located and moved from the cell mixture to predetermined positions where the zymolyase had been applied. After 3–10 min the tetrad could be gently broken up with the micromanipulation needle. About half the time the tetrad broke cleanly into its two component dyads, one derived from each end of the tetrad. The remainder broke into a dyad and two separated spores. Visual observation during dissection and analysis of genetic markers indicated that these two separated spores always derived from a terminal dyad. The results in this report are totals of the two types of tetrads. Any unseparated dyads were allowed to digest further until all spores had separated from one another. The separated spores were micromanipulated to predetermined positions on the plate such that spores from the same dyad were identifiable.

RESULTS

Production of linear tetrads: DBY1517 (*MATα lys2-801 ura3-52 trp1*) and DBY1561 (*MATa ade6 leu1*
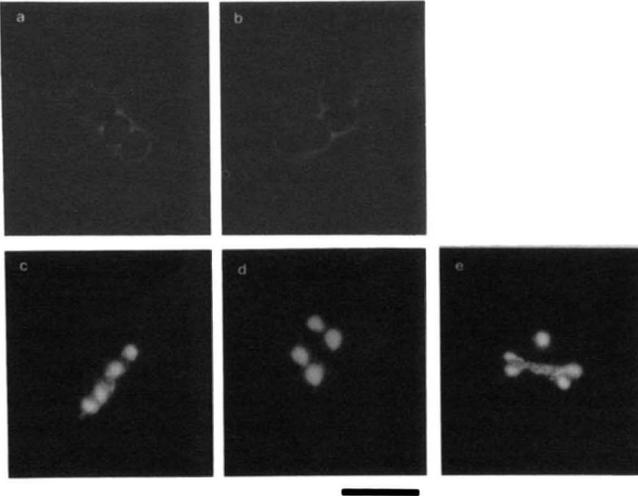
FIGURE 1.—Linear tetrads produced by the sporulation of newly formed zygotes. Newly formed zygotes were shifted to sporulation medium as described in the text. Panels a and b show cells after sporulation was complete (4 days), viewed by phase contrast microscopy. They show the most common forms of the linear tetrads, either fully in line or with the two terminal dyads at an angle to one another. Panels c through e show cells that were fixed and DAPI stained (MATERIALS AND METHODS) after 24 hr in sporulation medium, and are viewed for DAPI fluorescence with a low level of phase contrast illumination. Panel c shows a cell, clearly an unbudded zygote on the basis of shape, that has apparently completed both meiotic divisions but not yet formed its spore walls. Panel d shows two unbudded zygotes that have completed meiosis I. Many such cells were observed and the two regions of nuclear DNA were always arranged along the long axis of the zygote. Panel e shows a large budded zygote in which the bud has remained attached to the zygote but only the zygote has undergone meiosis (this was clear from the phase contrast view of this cell, not shown). The bud nucleus is at the top. The zygote appears to be in late meiosis II, judging from the trails of DNA that connect the nuclei (see text). Size bar = 10 μm.

lys2-801 cyh2) were mated under conditions that result in efficient production of zygotes over a short time (MATERIALS AND METHODS). The mating mixture was transferred to sporulation medium after only 2 hr of mating. At this time 36% of the cells in the mixture were zygotes, the majority of which were unbudded. After 4 days of sporulation at 26°, the cells were observed in the microscope to determine whether any zygotes had sporulated. About 5% of all the cells appeared to have sporulated. The remainder consisted of unsporulated zygotes and other cells (presumably unmated haploids). Of the tetrads in the mixture about 70% were linear or nearly linear tetrads (Figure 1) and the remainder were primarily tetrads that appeared to consist of two half-tetrads (dyads) oriented perpendicularly to one another. A few percent tetrahedral and oval tetrads were also observed.

To be certain that the observation of linear tetrads was not simply a characteristic of this particular cross, the same cells were mated and sporulated by the conventional method, which involves growth for some time as a mitotic diploid. The resulting cells sporu-

lated very efficiently and produced no detectable linear tetrads (<0.5%). Indeed, the tetrads they formed were typical tetrahedral or oval forms, indistinguishable from other wild-type crosses we have observed.

To demonstrate that the production of linear tetrads is a phenomenon not restricted to the strains used above, newly formed zygotes from two other crosses with different parent strains (both of which produce tetrahedral and oval tetrads under normal sporulation conditions) were sporulated. Both crosses produced predominantly linear tetrads, although no genetic analysis of their order was performed.

The linear tetrads are ordered: To determine whether the spores in the linear tetrads were arranged in any fixed order, the linear tetrads were dissected into their component halves (which we call "terminal dyads") as described in MATERIALS AND METHODS. It was found to be technically difficult to achieve a more fully ordered analysis. Thus our data address only the pattern of markers found in pairs of spores derived from the two ends of a linear tetrad. The spores were all viable (48/48 asci had four viable spores) and segregated all of the expected heterozygous markers predominantly in a 2:2 ratio. Every tetrad showed recombination between at least one pair of markers, indicating that all of the tetrads derived from zygotes that had undergone karyogamy and meiosis. ade6 and leu1 showed the expected degree of linkage (25:0:19, PD:NPD:TT, indicating an observed linkage of 22 cM, within statistical error of the actual linkage value of 35 cM (MORTIMER and SCHILD 1982)). This result indicates that intergenic meiotic recombination was at least roughly normal. The three centromere linked markers trp1, leu1 and ura3 segregated roughly equal numbers of parental and nonparental ditypes (accumulated data 67:55, PD:NPD) indicating that different centromeres were randomly segregated during the meiosis I division.

The two spores from each terminal dyad of a linear tetrad could, in principle, be of two types: sisters derived from one pole of meiosis I, or nonsisters derived from each pole of meiosis I. To conserve markers, each tetrad must consist of two dyads both of which consist of either two sister or two nonsister spores. If nuclei were packaged into spores in random order, one-third of the tetrads should consist of dyads with sister centromeres. Analysis of the three centromere linked markers in our cross indicated that every linear tetrad consisted of dyads whose spores were nonsisters. trp1, which is less than 1 cM from its centromere, always segregated 1 Trp+:1 Trp⁻ in each terminal dyad (Table 1). leu1 and ura3, about 3 and 8 cM from their respective centromeres, segregated predominantly 1 Leu+ (Ura+):1 Leu⁻ (Ura⁻), but showed the expected number of 2 Leu+ (Ura+) and 2 Leu⁻ (Ura⁻) dyads that would result from a single

TABLE 1

Observed and expected marker segregation in the terminal dyads from linear tetrads

| Marker | Percent nonsisters in terminal dyads: | |
	Observed	Expected[a]
trp1	>98	>99
leu1	94	97
ura3	91	95
MAT	77	82
ade6	75	70
cyh2	52	62

Data shown are for the 44–48 tetrads that segregated 2:2 for the indicated marker.

[a] Expectation is calculated on the assumption that all terminal dyads contain nonsister centromeres. For *trp1*, *leu1* and *ura3* the expectation was calculated as (100 − [%SDS × 1/2]) based on SDS values from MORTIMER and HAWTHORNE (1966), and similar SDS values obtained from our strains. For *MAT*, *ade6* and *cyh2*, %SDS was calculated from map distances (MORTIMER and SCHILD, 1982) by the method of SNOW (1979), assuming an interference value of 0.3. SDS = second division segregation.

recombination event between the gene and its respective centromere. The more centromere distal markers *ade6*, *cyh2*, and *MAT* also showed about the expected frequency of recombination with their respective centromeres (Table 1).

Morphological observations of sporulating zygotes: In order to determine the morphological events underlying the production of the linear tetrads, we fixed cells (from DBY1517 × DBY1561 sporulated by the regimen described above) at a time when they should have been undergoing meiotic chromosome segregations (20–24 hr after shift to sporulation medium) and stained them for nuclear DNA with DAPI (see MATERIALS AND METHODS). At this stage the outline of the zygote cell wall was still readily recognizable (Figure 1, panel c), but sporulating cells could be recognized in many cases by the presence of four regions of nuclear DNA, indicating completion of both meiotic divisions. Our observations demonstrated that only zygotes themselves give rise to the linear tetrads, since the characteristic zygote cell shape was invariably observed in the cells undergoing meiosis. Judging from whether the linear tetrads were arising from budded or unbudded zygotes, about one-third of the tetrads were derived from zygotes that had not undergone any mitotic divisions. The remainder arose from budded zygotes that had undergone a single mitotic division as a diploid and segregated one nucleus to the zygote bud (which remained attached to the zygote). In these cases the linear tetrad was always observed to form in the zygote and not in the mitotic bud (which did not undergo meiosis).

In addition to many cells that had completed both meiotic divisions, a smaller fraction of cells appeared to have completed the first meiotic segregation only, since they had two regions of nuclear DNA stain.

Many such cells were observed, and all had the spatial pattern shown in Figure 1, panel d, indicating that they have undergone the first meiotic chromosome segregation parallel to the long axis of the zygote (see DISCUSSION). These cells are not zygotes prior to nuclear fusion (karyogamy) because they are far too frequent, and because they are frequently found in cells that have already completed the first diploid mitosis (as evidenced by the zygotic bud containing a nucleus).

Although cells actually undergoing the meiosis II chromosome segregation should be very rare, since the process is rapid, one putative such cell was observed (Figure 1, panel e). This cell was a large-budded zygote with the nucleated but unsporulated bud still attached (the nuclear DNA seen at the top of the zygote is from this bud). The pattern of staining is reminiscent of mitotic late nuclear division, in which the nuclear DNA is visibly extended between two poles where most of the DNA is concentrated. This observation suggests that the second meiotic division is indeed longitudinal, as expected from the genetic evidence.

DISCUSSION

By considering both the genetic evidence that the linear tetrad's terminal pairs of spores are invariably nonsisters, and the morphological evidence for a first meiotic chromosome segregation parallel to the axis of the zygote, we can infer the spatial patterning that gives rise to the linear arrangement of spores. The first meiotic chromosome segregation occurs on the long axis of the zygote, placing homologous chromosomes near the two ends of the zygote. In order for the observed arrangement of chromosomes after the completion of meiosis to arise, both second meiotic divisions must also be parallel to the long axis of the zygote, resulting in intercalation of the nuclei to produce an alternating array of sister chromosomes. Observation of one cell apparently engaged in the meiosis II chromosome segregation confirms this inference.

In order to give rise to the nearly linear arrangement of spores, the nuclei must become slightly rearranged after meiosis II, to come into line with the axis of the zygote. We hypothesize that as the spore walls are deposited within the zygote, they are forced by the geometry of the zygote cell wall to take up a linear or nearly linear arrangement. The observation of a substantial fraction of tetrads in which the two dyads are not well aligned (Figure 1, panel b) supports this hypothesis, since the lobed ends of the typical zygote might allow some freedom in the packing of the spores.

In support of the idea that the elongated shape of zygotes is responsible for the production of linear tetrads, we note that HAWTHORNE (1955) described

Saccharomyces hybrid strains that form elongated cells when induced to sporulate and subsequently give rise to linear tetrads. Both cases show the same pattern of nonsister spores in terminal dyads, although Hawthorne was able to discern even more order by completely dissecting ordered tetrads. In other related organisms that produce ordered tetrads cases of both sister and nonsister pairing of the end spores are known. In *Schizosaccharomyces pombe*, the two spores at the end of each ascus are sisters (LEUPOLD 1950). However, in *Saccharomycodes ludwigii* the two spores at the end of each ascus are nonsisters (WINGE and LAUSTEN 1939), as in *S. cerevisiae*.

The technique described here should permit the application of ordered tetrad analysis to most or all strains of *S. cerevisiae*. Our results indicate that meiotic chromosome segregations in *S. cerevisiae* are spatially ordered with respect to at least some cytoplasmic or cell wall determinants. This fact suggests that a similar spatial pattern may exist in normal spherical or oval meiosis, but unfortunately we currently lack the physiological markers required to detect it.

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