MODIFICATION OF SPORULATION IN YEAST STRAINS WITH TWO-SPORED ASCI
(SACCHAROMYCES, ASCOMYCETES)

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SUMMARY

Electron-microscope observations indicate that the single nuclear division of sporulating yeasts which form 2-spored asci resembles, in several aspects, the nuclear behaviour during sporulation of the more common 4-spored yeasts. Specifically the parental nucleolus remains behind in the ascus cytoplasm, a characteristic nucleolus-associated spherical structure is formed, and the nucleus divides by budding rather than by elongation and medial constriction.

INTRODUCTION

Among 15 strains of Saccharomyces which produce predominantly 2-spored asci in acetate sporulation medium, Grewal & Miller (1972) found 3 strains which differ from the others in the following respects. (1) Four-spored asci were never observed. (2) The asci have 4C DNA content, that is 2C per ascospore relative to the DNA content of a haploid strain (X901-35, 51 µg per 3 × 10^8 cells). Asci with germinating spores have an 8C DNA content. (3) Conjugation was not observed. Clones derived from single spores are capable of sporulation. (4) Sporulation is not inhibited by the addition of glucose to the acetate medium. (5) The spores of each doublet have a connecting structure and they are difficult to separate. On the basis of these observations and on observations of Giemsa-stained material, Grewal & Miller conclude that it is uncertain whether the single nuclear division should be designated as a mitosis or a meiosis I.

Because the nuclear behavior in budding yeast is different from that in sporulating yeast (Moens & Rapport, 1971a, b), an electron-microscope study was undertaken to determine the fine-structural nuclear morphology of the yeast strains with 2-spored asci. The results indicate that the nuclear division, with some modifications, has the characteristics of those observed during sporulation of 4-spored yeast strains. In terms of cell-cycle regulation these strains are of interest since they demonstrate that the normal developmental sequence of meiosis I followed by meiosis II is not a necessary condition for sporulation to take place.
MATERIALS AND METHODS

The following 2-spored Saccharomyces strains were obtained from Dr J. J. Miller (McMaster University, Hamilton, Ontario, Canada): S. cerevisiae io,ei; and S. cerevisiae American type culture collection 4098 and 4117. The pre-sporulation medium consisted of 10 g yeast extract, 20 g peptone, 10 g potassium acetate and 20 mg tetracyclin per litre (Fast, 1973). After 12 h at 30 °C in pre-sporulation medium, a sample of logarithmic-phase cells was transferred to fresh pre-sporulation medium. After 12 h the logarithmic-phase cells were spun down and washed twice in distilled water and suspended in sporulation medium (Fast, 1973). Samples of cells were taken at 0, 2, 4, 6, 8, 12 and 24 h. The cells were immediately spun down and 2 % glutaraldehyde in phosphate buffer was added. After 12 h the cells were washed in buffer and postfixed in buffered 2 % osmium tetroxide for 1 h. The cells were washed in buffer and collected by draining the suspended cells from a pipette on to filter paper. The pellet of cells was suspended in agar, trimmed, dehydrated in a graded ethanol and propylene oxide series and embedded in Epon according to Luft's 1:1 mixture (Luft, 1961). After polymerization at 60 °C for 2 days, series of about 100 sections were cut and placed on Formvar-covered single-hole grids. The sections were stained with saturated aqueous uranyl acetate solution for 5 min, washed and stained for 1 min in lead citrate.

RESULTS

Light microscopy. After 12 h on sporulation medium, strain io,ei had practically 100 % refractile 2-spored asci, strain 4117 had some 2-spored asci and 4098 had none. At 24 h, 4117 had completely sporulated but 4098 still had no spores.

Electron microscopy. Strains io,ei and 4117 have similar ultrastructural characteristics of nuclear division and spore formation and they are therefore discussed together. Strain 4117, however, was about 2 h behind the development of io,ei. Strain 4098 is not considered here because it did not sporulate under the conditions described in the Methods section.

The 6-h culture of io,ei (and 8-h of 4117) had asci with single nuclei, dividing nuclei, and early spore formation. In 33 completely examined nuclei, 13 had no observable spindle pole bodies (SPB), 5 nuclei had a single SPB (Fig. 1), 2 nuclei had divided side-by-side SPBs and 13 nuclei had metaphase (Fig. 2) or later spindles (Figs. 3–5). Prior to metaphase a distinct round body, which was identified previously in sporulating cells of S. cerevisiae Hansen strain CBS 5525 (Moens & Rapport, 1971), was present in strains io,ei and 4117.

Short metaphase spindles were observed in 4 nuclei (0·6, 0·7, 0·8 and 0·9 μm). The inner plaque of the spindle pole bodies is well developed but the outer plaque at the cytoplasmic side is not pronounced. The spindle pole bodies resemble those of mitosis or meiosis I, but not meiosis II. Two more advanced 1·μm spindles had large outer plaques, characteristic of meiosis II, and prospore wall formation had been initiated at the cytoplasmic side of the outer plaques (Fig. 3). Spindles of 1·1, 1·1, 1·2, 1·3, 1·5 and 2 μm (Fig. 4) had progressively larger prospore walls. The nuclear material of the parent nucleus and cytoplasm moves into the 2 sacs formed by the prospore walls. The parental nucleolus is sequestered into a nuclear evagination (Fig. 5A, B) and does not enter either ascospore (Fig. 5A, B) (Moens, 1971).

As in 4-spored S. cerevisiae the early prospore wall is shoe-shaped with the spindle pole body at the heel of the shoe. As the spindle extends to 2 μm and longer the spindle
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pole bodies come to lie at the top of the spore while the last nuclear material enters at the open base (Fig. 5). In several immature spores the location of final spore closure coincided with the location of the belt which connects the 2 mature ascospores. In other cases, however, the correspondence was not clear.

DISCUSSION

Morphological evidence from electron microscopy demonstrates that the nuclear division which produces the 2-spored asci is in several respects similar to the nuclear behaviour in sporulating 4-spored yeasts. It is unlike the type of nuclear division seen during vegetative growth.

1. In 4-spored yeasts the parental nucleolus is eliminated from the division products at meiosis II (Moens, 1971) but it is not lost in mitotic divisions. Similarly, in the yeast strains with 2-spored asci the parental nucleolus is not incorporated in either of the spores and it remains behind in the cytoplasm of the ascus after spore closure (Figs. 4, 5).

2. In many yeast strains a finely granular round body occurs in association with the nucleolus in the nuclei of cells which have been on sporulation medium for about 4 h (Moens & Rapport, 1971). In strain CBS 5525, clone 10 (Engels & Croes, 1968) this body contains synaptonemal complex-like structures. It is not present in vegetatively growing cells. Sporulating cells of the yeasts which produce 2-spored asci also contain the finely granular round body.

3. During anaphase of mitosis and meiosis I the spindle elongates, the nucleus becomes extended and then takes on the shape of an hourglass. During meiosis II of 4-spored yeasts the nucleus as a whole does not elongate as the spindle elongates. Instead, lobes of nuclear material develop on the nuclear mass (Moens, 1971; Moens & Rapport, 1971a; Guth, Hashimoto & Conti, 1972; Moens, Esposito & Esposito, 1974). The anaphase of the sporogenic nuclear division in the yeasts with 2-spored asci resembles in this respect a second meiotic division (Fig. 4).

Grewal & Miller (1972) argue that the sporogenic division of the 2-spored yeast strains has some characteristics of a mitotic division because it is not suppressed by glucose and because the products are all competent to sporulate without fertilization. Presumably they have mating type a/a. On the other hand the authors' personal communication to Dr C. Robinow, who did the cytology of strains 191 and 4117, suggests that ‘... the single division that provides the nuclei for the two diploid spores of these yeasts closely resembles the first of the two meiotic divisions that furnish the nuclei for the ascospores of ordinary strains of Saccharomyces’.

Electron-microscope observations suggest that in yeasts with 2-spored asci the sporulation cycle is initiated when the cells are placed in sporulation medium. No further budding occurs, no nuclear divisions occur for the first 4 h and, presumably, DNA synthesis takes place, possibly of the prolonged type found in meiotic prophase of eukaryotes (Callan, 1972). The nucleolus-associated round body is formed. If genetic recombination occurs at this stage it would be of interest to know whether it is at low mitotic or high meiotic frequency. The nuclear division starts with a short
spindle which could be a mitotic or a first meiotic spindle, but soon the spindle, the spindle pole bodies, and the nuclear behaviour take on the characteristics of meiosis II in 4-spored yeasts. This division is also unlike meiosis I of 4-spored yeasts in that it is not suppressed by 1% glucose in the sporulation medium (Grewal & Miller, 1972). Finally, when division is completed the nucleolus remains behind, a characteristic event of meiosis in 4-spored yeasts. In the absence of genetic data, the nature of the assortment of genetic material during the division remains unsolved.

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REFERENCES


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Fig. 1 A, B. Consecutive sections of an undivided spindle pole body of a cell on sporulation medium for 6 h. x 65000.
Fig. 2. A 0.7-μm metaphase spindle of a cell exposed to sporulation medium for 6 h. The outer plaques of the spindle pole bodies are not developed yet. x 115000.
Fig. 3. A 1.0-μm spindle with well developed spindle bodies characteristic of meiosis II in yeast with 4-spored asc. ip, inner plaque of spindle pole body; n, nucleus; op, outer plaque; pwi, prosopore wall; sp, spindle. The 2 spindle pole bodies were the only ones on this nucleus. x 80000.
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Fig. 4. A 2-μm spindle, sp, with well developed prospore walls, piv. The nucleolus, no, lies in an evagination of the nuclear envelope marked by arrowheads. The parent nucleus, n, flows into the 2 sacs formed by the prospore walls. In yeasts with 4-spored ascii there would be 4 such prospore walls associated with the parent nucleus. ip, inner plaque of spindle pole; op, outer plaque; sp, spindle. × 90,000.
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4

pw

no

sp

0.2 μm
Fig. 5A, B. Skipped serial sections of advanced formation of 2 ascospores. As is the case in meiosis, but not in mitosis of yeasts with 4-spored asci, the parent nucleolus (np) is not incorporated in either spore nucleus, sn1 or sn2. It remains in the cytoplasm of the ascus after spore-wall closure at the points marked by brackets. The solid bar marks the position of the spindle pole body. np, nuclear pore; pwc, prospore wall; sp, spindle. × 80000.