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Lipid Synthesis During Sporulation of *Saccharomyces cerevisiae*

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Lipid synthesis was studied in both sporulating (diploid) and nonsporulating (haploid) cells of *Saccharomyces cerevisiae*. Two phases of lipid synthesis occur in diploid cells transferred to sporulation medium. Phase I, which occurs during the first 12 h of exposure to sporulation medium, was also observed in the haploid strains. Phase II, occurring from the 20th to the 25th h, coincided with the appearance of mature asci and was observed only in the diploid cells. The majority of phospholipid synthesis took place during period I, whereas neutral lipid synthesis occurred during both periods. Phospholipid synthesis was virtually identical in both type and quantity in the sporulating and nonsporulating strains.

Lipid accumulation in sporulating cells of *Saccharomyces cerevisiae* has been well documented. Pontefract and Miller (14) reported an increase in lipid-containing vacuoles during sporulation. Esposito et al. (3), in examining the uptake of ^{14}C-acetate into total lipids during sporulation, observed one period of synthesis during the first 10 h and a second from 24 to 30 h of sporulation. Recently, Illingworth et al. (6) also found two periods of lipid synthesis. In addition, they demonstrated in sporulating cells increases in phospholipids, neutral lipids, and unsaturated fatty acids.

Sporulation in yeast involves two meiotic divisions in which rearrangements of nuclear and plasma membranes occur. Therefore, the possibility exists that significant changes in membrane components such as lipids might accompany the meiotic divisions and subsequent spore formation. However, any analysis of synthetic activity occurring in sporulating cells must take into account the environmental changes associated with the shift to sporulation medium. Sporulation medium is deficient in nitrogen and utilizes acetate as a source of energy. Thus, it is important to determine what metabolic processes are initiated as a response to the alteration in growth conditions, even in the absence of sporulation.

The present report deals primarily with two questions. First, what lipids are synthesized during sporulation? Particular emphasis is given to phospholipids, because they are important membrane components. Second, is lipid synthesis sporulation specific or is it also induced in nonsporulating cells?

MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* diploid Y-185 a/α $\frac{ade\ 2}{+}$ $\frac{his\ 5}{+}$ $\frac{his\ 8}{+}$ was employed in these studies. Two haploid strains, Y-185 8Cα and Y-185 11Ca were obtained by sporulation and ascus dissection of Y-185. These strains are wild type for all auxotrophic markers and grow in potassium acetate presporulation medium (17) at a rate comparable to the diploid parent.

Growth conditions. All growth and sporulation experiments were conducted at 30 C. Both diploid and haploid strains were grown in potassium acetate presporulation medium and harvested in log phase as previously described (17). The cells were washed twice by centrifugation with sterile, distilled water and resuspended (T_0) at an optical density (600 nm) of 5 in 2% potassium acetate (sporulation medium) and vigorously aerated at 30 C. Subsequent hours of exposure to the sporulation medium will be referred to as T_1, T_2, $T_3 \ldots T_N$. Samples of known quantities (10 to 25 ml) of cells were harvested from sporulation medium at intervals and washed twice with distilled water by centrifugation, and the cell pellets were frozen for later analysis.

Cell counts. Cells were counted with a hemocytometer. Cells containing a bud were counted as a single cell.

Isotope incorporation. Isotopes were added to the sporulation medium at T_0. When ^{14}C-acetate was employed, it was added at 0.30 μCi/ml to the 2% potassium acetate sporulation medium. Carrier-free

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^{32}P was added at 50 μCi/ml. Acetate-*1*-^{14}C (1 mCi/1.4 mg) and carrier-free $H_3{}^{32}PO_4$ were obtained from New England Nuclear Corp.

Lipid extraction and analysis. Lipids were extracted by the methods of Letters (10) as modified by Getz et al. (4). The lipid extracts were frozen, stored, and desiccated for no more than 1 week before analysis. The lipid extracts were separated into phospholipids, free fatty acids, and neutral lipids by thin-layer chromatography by the methods of Keith et al. (8). Phospholipids were separated by two-dimensional thin-layer chromatography as described by Getz et al. (4). Individual phospholipids were identified by comparison with known standards and by the use of the spray reagents described by Dittmer and Lester (2). Lipid spots were located on the plates by exposure to iodine or by autoradiography, or both. For two-dimensional separations it was essential to spray the plates with acetic acid and water (1:1) before exposure to iodine. Without this treatment, the entire plate turned yellow in the presence of iodine. Areas of silica-containing lipid were scraped from these plates after the iodine sublimed, suspended in Aquasol (New England Nuclear Corp.), and counted directly (9). Phosphatidic acid, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, and cardiolipin standards were obtained from General Biochemicals; phosphatidyl choline, *N,N*-dimethyl phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, and lysophosphatidyl choline were obtained from Sigma Chemical Co.

Autoradiography. Thin-layer plates were carefully covered with Saran Wrap and placed in contact with a sheet of Kodak no-screen X-ray film. Films were exposed for 4 to 10 h, depending upon the amount of ^{32}P present.

RESULTS

Under the conditions employed, the first asci in Y-185 appeared after T_{20} and rose sharply until T_{28} (Fig. 1). None of the haploids produced asci. The cell number remained constant in all three cultures, but the optical densities increased from T_0 to T_{12} and then remained constant. When the cells were examined by phase microscopy during this incubation period, both progressive vacuolization and an increase in cell volume were observed in cells of all three strains.

The kinetics of ^{14}C-acetate and $^{32}PO_4$ incorporation into total lipids of the diploid and two haploid strains during incubation in sporulation medium is shown in Fig. 2. The biphasic nature of lipid synthesis in the sporulating diploid, suggested by the observation of others (3, 6), is clearly evident. In all three cultures the first phase (I) lasts from T_0 to about T_{12}. After this phase there is little further ^{32}P incorporation in both the diploid and the two haploid strains, and there are only slight increases of ^{14}C-acetate incorporation in the haploids. The second phase

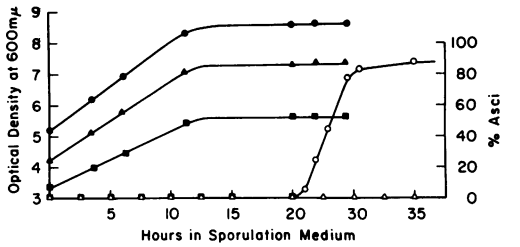

FIG. 1. *Increase in optical density and ascus formation after the shift to sporulation medium. Open symbols, ascus production; closed symbols, optical density; Y-185 diploid, O; Y-185 8C haploid, Δ; Y-185 11C haploid, □. All cultures contained 5 × 10⁷ cells/ml.*

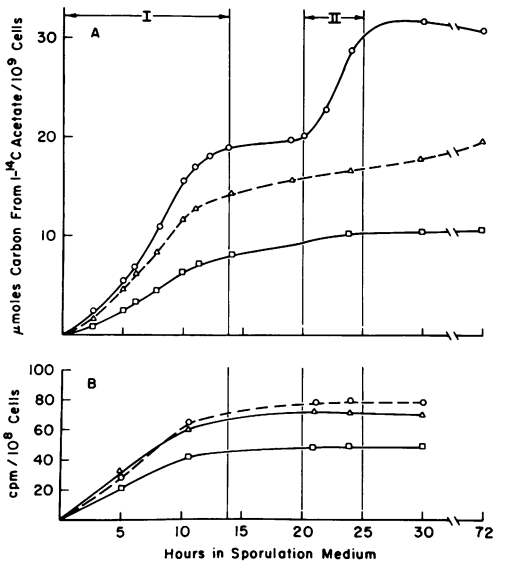

FIG. 2. *Incorporation of ^{14}C-acetate and $^{32}PO_4$ into total lipids during sporulation. See Materials and Methods for details. A, ^{14}C-acetate sporulation medium; B, $^{32}PO_4$ sporulation medium; Y-185, O; Y-185 8C, Δ; and Y-185 11C, □.*

(II) of lipid synthesis in the diploid is demonstrated by a second increase in ^{14}C-acetate incorporation into lipids at T_{20} to about T_{26}.

The difference in the pattern of ^{14}C-acetate and ^{32}P incorporation into lipids during sporulation in the diploid (Fig. 2) suggested that phase II may consist only of neutral lipid synthesis. To directly test this possibility the kinetics of ^{14}C-acetate incorporation into various lipid fractions was studied in both diploid and haploid strains. Phase II in the diploid strain consists almost entirely of neutral lipid synthesis (Fig. 3) and corresponds in time to the period when mature asci first appear in the sporulation medium (Fig. 1). The incorporation of ^{14}C-acetate into phospholipids (in all three strains)

occurs almost exclusively during phase I, and the kinetics are similar to those seen in Fig. 2 for the incorporation of ^{32}P into total lipids. Incorporation of both ^{14}C-acetate and ^{32}P into phospholipids is virtually identical in diploid Y-185 and haploid Y-185 8C strains (Fig. 2 and 3). Total incorporation is reduced in haploid Y-185 11C strain, but the kinetics of precursor incorporation into phospholipids in this strain is comparable to that of the other two strains.

The phospholipid content of vegetative cells of yeast has been previously documented (10). Because Getz et al. (4) reported that the phospholipid composition varies with strain and cultural conditions, it is important to monitor the composition during sporulation.

Figure 4 shows a typical radioautogram of a thin-layer separation of ^{32}P-labeled phospholipids of the diploid after 24 h in sporulation medium. No qualitative differences were seen in a comparison of phospholipids of the haploid and diploid strains, and no unique spots were detected in any of the three strains. The relative amounts of the various phospholipids synthesized in the sporulating diploid and the two nonsporulation haploids were similar (Table 1). During the first 5 h, the incorporation of ^{32}P into phosphatidyl inositol is greater than incorporation into phosphatidyl choline. Thus, for example, in the diploid the ratio of phosphatidyl choline to phosphatidyl inositol changes from 0.63 at 5 h to 1.77 at 24 h. These changes are more evident when the kinetics of ^{32}P incorporation into phosphatidyl choline, phosphatidyl inositol, and phosphatidyl ethanolamine are considered (Fig. 5). During phase I, the rate of accumulation of radioactivity into phosphatidyl inositol decreases after the first 5 h, whereas the rate of synthesis of phosphatidyl choline remains constant. This pattern is essentially the same in all three strains and explains the relative increase in the percentage of ^{32}P recovered from phosphatidyl choline as compared with phosphatidyl inositol during the course of sporulation (Table 1).

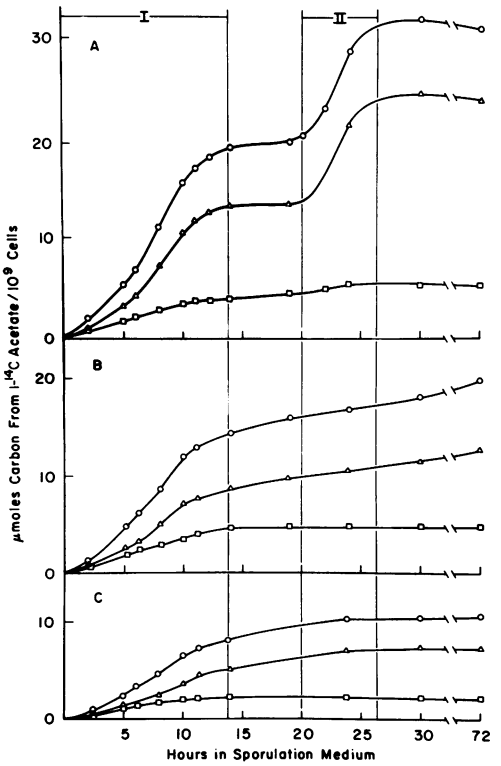

FIG. 3. *Kinetics of lipid synthesis from ^{14}C-acetate during sporulation. A, Y-185; B, Y-185 8C; C, Y-185 11C; total lipids, O; neutral lipids, Δ; and phospholipids, □. See Materials and Methods for details.*

DISCUSSION

The cytological events associated with meiosis and spore formation in *S. cerevisiae*, as revealed by both light and electron microscopy, are now understood in considerable detail (for review, see reference 20). Lipid-containing vacuoles accumulate in the cytoplasm prior to nuclear division (14). In serial-sectioning studies, the nuclear membrane in yeast has been shown to remain intact during meiosis; the spore nuclei bud from the parental nucleus (11, 12). The meiotic spindle plaques are associated with the nuclear membrane in regions resembling enlarged pores (13). Membranous vesicles observed in association with the spindle plaques during the second meiotic division are thought to be associated with the formation of the prospore wall (13).

Despite the demonstration of structures unique to meiosis in *S. cerevisiae*, very few sporulation-specific metabolic processes have been reported. The metabolism of acetate (3) and the synthesis of ribonucleic acid (RNA) (1, 3, 7), deoxyribonucleic acid (DNA) (1, 3, 18), protein (1, 3), lipid (3, 6), and carbohydrate (16, 17) have all been studied in sporulating cells, but in few of these have nonsporulating controls been included. Roth and Lusnak (18) detected a round of DNA synthesis preceeding meiosis only in sporulating a/α diploids and nonsporulating a/a and α/α diploid cells exposed to sporulation medium. Similarly, Kadowaki and Halvorson (7) observed the accumulation of a 20*S* species of RNA in a/α diploid and disomic cells undergoing sporulation, but not in vegetative cells or

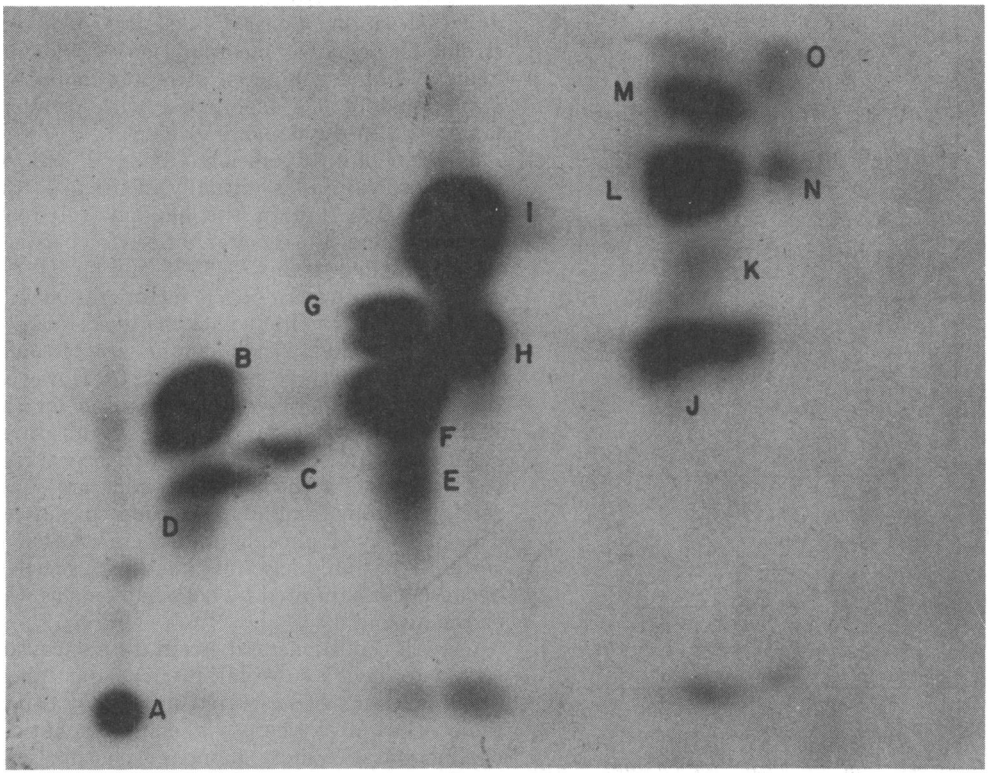

FIG. 4. *Separation of ^{32}P-labeled phospholipids. Thin-layer radioautogram of ^{32}P-labeled phospholipids of Y-185 after 24 h of exposure to sporulation medium. A, origin; B, lysophosphatidyl choline; C and D, lysophosphatides ?; E, dimethylphosphatidyl ethanomine ?; F, phosphatidyl inositol; G, lysophosphatidyl ethanolamine; H, phosphatidyl serine; I, phosphatidyl choline; J, cardiolipin; K, phosphatidyl glycerol ?; L, phosphatidyl ethanolamine; M, phosphatidic acid ?; and N and O, unknown. (?, Indicates minor components whose identity was not absolutely established.)*

TABLE 1. *Incorporation of ^{32}P into phospholipids during incubation in sporulation medium*[a]

Phospholipid fractions	T_6 distribution (%)			T_{11} distribution (%)			T_{24} distribution (%)		
	Y-185	Y-185 8C	Y-185 11C	Y-185	Y-185 8C	Y-185 11C	Y-185	Y-185 8C	Y-185 11C
PC	25.1	22.3	28.6	34.6	33.4	36.3	39.4	36.4	42.7
PI	39.4	37.6	32.8	28.9	31.5	25.9	22.1	27.8	24.1
PE	13.1	16.2	13.6	12.1	15.3	14.2	15.2	16.2	13.2
PS	9.4	9.0	8.5	8.5	8.3	9.4	7.4	7.8	6.9
CL	4.0	5.2	3.5	3.0	3.4	3.0	3.2	3.0	2.5
Lysophosphatides	4.1	3.6	5.3	5.4	2.8	3.2	5.2	3.0	4.4
Others[b]	3.9	5.4	5.6	6.4	3.1	6.6	6.3	5.3	5.3
Residue at origin	1.0	0.7	2.1	1.1	0.3	1.5	1.2	0.5	0.9

[a] Strains Y-185, Y-185 8C, and Y-185 C11 were incubated at T_0 in sporulating medium and $^{32}PO_4$ and harvested at T_6, T_{11}, and T_{24}, and the lipid fraction was extracted and analyzed for ^{32}P incorporation into phospholipids as described in Materials and Methods and Fig. 4. The numbers represent the percent distribution of ^{32}P. Phospholipids are abbreviated as follows: phosphatidyl choline, PC; phosphatidyl inositol, PI; phosphatidyl ethanolamine, PE; phosphatidyl serine, PS; and cardiolipin, CL.

[b] Unidentified and minor components.

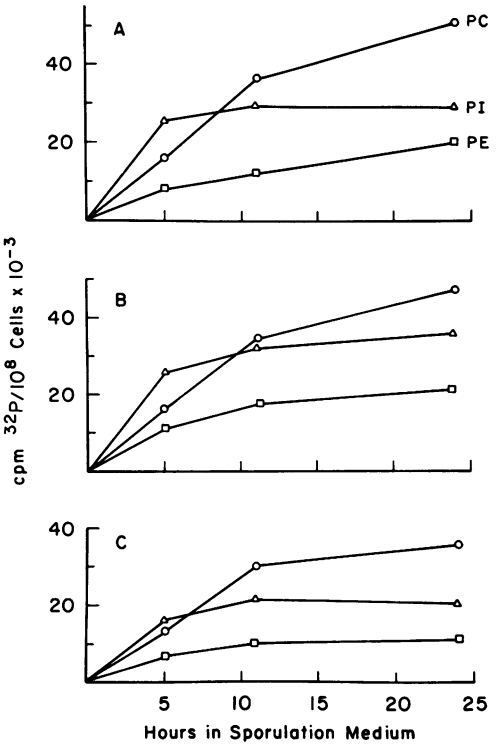

FIG. 5. *Kinetics of ^{32}P incorporation into phospholipids during sporulation. Incorporation of ^{32}P into phosphatidyl choline* (○), *phosphatidyl inositol* (△), *and phosphatidyl ethanolamine* (□) *into strains Y-185 (A), Y-185 8C (B), and Y-185 11C (C) placed into sporulation conditions.*

haploids exposed to sporulation medium. Because it is likely that pleitropic regulatory mechanisms govern sporulation in yeast, an appropriate choice of controls is an essential problem in the study of this process.

The biphasic nature of lipid synthesis in the sporulating diploid was originally suggested in the experiments of Esposito et al. (3) employing a different method of extraction. In examining the uptake of ^{14}C-acetate into total lipids, Illingworth et al. (6) observed two periods of synthesis. In the present experiments, phase I of ^{14}C-acetate incorporation occurred at T_0 to T_{13} and phase II at T_{20} to T_{26}. These two phases differ slightly from those reported by Esposito et al. (3) and Illingworth et al. (6), differences probably due to changes in strains and culture conditions employed.

The increase in lipid content in the diploid as compared with the haploid strain throughout sporulation can be attributed entirely to its accumulation of neutral lipids (Fig. 3). Although this is particularly true during phase II, and even during phase I (T_{10}) in the diploid strain, the ratio of incorporation of ^{14}C into neutral lipid as compared with phospholipid is approximately 3:1 (compared with approximately 2:1 in the haploid Y-185 8C).

The rate of incorporation of both ^{32}P and ^{14}C into phospholipids is virtually identical in the diploid Y-185 and in the haploid Y-185 8C strains. There is a greater difference between the two haploid strains Y-185 8C and 11C (Fig. 2 and 3). The kinetics of ^{32}P incorporation into phosphatidyl choline, phosphatidyl inositol, and phosphatidyl ethanolamine are virtually identical in the three strains (Fig. 5). However, the different kinetics of incorporation for the various phospholipids observed in all three strains may indicate that the synthesis or turnover, or both, for the different lipids are separately controlled under sporulation conditions. This pattern of phospholipid accumulation is certainly not uniquely associated with meiosis, because it is exhibited by both haploids as well as the diploid.

Growth conditions and strain differences are known to have a substantial effect upon phospholipid compositions (4). Under most conditions reported by these workers, phosphatidyl choline was the most abundant phospholipid, constituting between 48 and 26% of total phospholipid, and this percentage increased with growth rate. Only in the late stationary phase in a respiratory-sufficient strain was phosphatidyl inositol found in excess of phosphatidyl choline. Comparison of our results with those of other investigors are difficult because of differences in strain and culture conditions. Nevertheless, the relative proportions of the various phospholipids synthesized under sporulation conditions are similar to those found in vegetative cells.

The meiotic divisions associated with sporulation in *S. cerevisiae* (11, 12) occur during phase I of lipid synthesis (Fig. 2). During this period, there was very little difference in lipid synthesis between sporulating and nonsporulating cultures. The coincidence of the neutral lipid synthesis in the diploid during phase II with the appearance of mature asci in the culture may, however, indicate the involvement of these lipids in spore wall and membrane formation.

The fact that much of the lipid synthesis in sporulating cells is not sporulation specific does not, of course, mean that it is not necessary for sporulation. Auxotrophs for saturated (5, 19) and unsaturated (15) fatty acids are available, and if the necessary diploids were constructed these strains could be used to determine the

lipid requirement during various stages of meiosis and sporulation.

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LITERATURE CITED

1. Croes, A. F. 1967. Induction of meiosis in yeast. I. Timing of cytological and biochemical events. Planta Med. **76**:209-226.
2. Dittmer, J. C., and R. L. Lester. 1964. A simple, specific spray for detection of phospholipids on thin-layer chromatograms. Lipid Res. **5**:126-127.
3. Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson. 1969. Acetate utilization and macromolecular synthesis during sporulation of yeast. J. Bacteriol. **100**:180-186.
4. Getz, G. S., S. Jakovcic, J. Heywood, J. Frank, and M. Rabinowitz. 1970. A two dimensional, thin-layer chromatographic system for phospholipid separation. The analysis of yeast phospholipids. Biochim. Biophys. Acta **218**:441-452.
5. Henry, S. A., and S. Fogel. 1971. Saturated fatty acid mutants in yeast. Mol. Gen. Genet. **113**:1-19.
6. Illingworth, R. F., A. H. Rose, and A. Beckett. 1973. Changes in lipid composition and fine structure of *Saccharomyces cerevisiae* during ascus formation. J. Bacteriol. **113**:373-386.
7. Kadowaki, K., and H. O. Halvorson. 1971. Appearance of a new species of ribonucleic acid during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **105**:826-830.
8. Keith, A. D., A. S. Waggoner, and O. H. Griffith. 1968. Spin-labelled mitochondrial lipids in *Neuspora crassa*. Proc. Nat. Acad. Sci. U.S.A. **61**:819-826.
9. Kritchevsky, D., and S. Malhotra. 1970. Recovery of lipids from thin-layer chromatography for radio assay. J. Chromatogr. **52**:498-499.
10. Letters, R. 1966. Phospholipids of yeast. II. Extraction, isolation and characterization of yeast phospholipids. Biochim. Biophys. Acta **116**:489-499.
11. Moens, P. B. 1971. Fine structure of ascospore formation in the yeast, *Saccharomyces cerevisiae*. Can. J. Microbiol. **17**:507-510.
12. Moens, P. B., and E. Rapport. 1971. Spindles, spindle plaques and meiosis in yeast. *Saccharomyces cerevisiae*. J. Cell Biol. **50**:344-361.
13. Peterson, J., R. Gray, and H. Ris. 1972. Meiotic spindle plaques in *Saccharmyces cerevisiae*. J. Cell Biol. **53**:837-841.
14. Pontefract, R. D., and J. J. Miller. 1962. The metabolism of yeast sporulation. IV. Cytological and physiological changes in sporulating cells. Can. J. Microbiol. **8**:573-584.
15. Resnick, M. R., and R. K. Mortimer. 1966. Unsaturated fatty acid mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **92**:597-600.
16. Roth, R. 1970. Carbohydrate accumulation during the sporulation of yeast. J. Bacteriol. **101**:53-57.
17. Roth, R., and H. O. Halvorson. 1969. Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. **98**:831-832.
18. Roth, R., and K. Lusnak. 1970. DNA synthesis during yeast sporulation: genetic control of an early developmental event. Science **168**:493-494.
19. Schweizer, E., and H. Bolling. 1970. A *Saccharomyces cerevisiae* mutant detective in saturated fatty acid biosynthesis. Proc. Nat. Acad. Sci. U.S.A. **67**:660-666.
20. Tingle, M., A. Klar, S. Henry, and H. O. Halvorson. 1973. Ascospore formation in yeast. 23rd Symp. Soc. Gen. Microbiol., p. 209-243.