THE OCCURRENCE IN YEAST OF CYTOPLASMIC GRANULES WHICH RESEMBLE MICROBODIES*

CHARLOTTE J. AVERS and MICHELINE FEDERMAN. From the Department of Biological Sciences, Douglass Campus, Rutgers University, New Brunswick, New Jersey 08903

INTRODUCTION

Microbodies and microbody-like cytoplasmic granules have been identified by ultrastructural criteria in liver and kidney cells (1, 7, 17) and in various cell types in all major plant groups (14). These or similar organelles have been shown to contain hydrogen peroxide-producing and -destroying enzymes in liver and kidney cells (2, 7–9) and in the protozoan *Tetrahymena pyriformis* (8). The term "peroxisome" has been introduced by de Duve and Baudhuin (11) to identify the biochemical entity which may or may not be identical with the morphologically defined "microbody" (11, 17) in all cells. Thus far, combined biochemical and morphological studies have been limited to the parenchymatous cells of rat liver (7, 11). During the course of a study on mitochondrial populations in bakers' yeast (13), we noted that a microbody-like cytoplasmic granule occurred in both wild-type and respiration-deficient, petite strains, but in different frequencies. A survey of electron micrographs from 35 yeast strains confirmed the initial observations. In addition, we conducted preliminary studies to determine the distribution of catalase activity in isolated particle fractions of one diploid strain, since this enzyme has been used as a peroxisome marker (7, 11). The data obtained from the electron microscopical and the biochemical analyses provided the basis for the present report, which shows the existence of cytoplasmic granules which differed from mitochondria in fine-structural features and in enzyme activity distribution.

MATERIALS AND METHODS

Electron micrographs of 35 diploid and haploid strains of *Saccharomyces cerevisiae* were examined to determine the numbers of microbody-like structures per cell section and to ascertain the presence or absence in these structures of reaction products deposited after cytochemical tests to localize cytochrome oxidase (5) and cytochrome-*c* peroxidase (3) activities. The strains and methods used for electron microscopy have been described elsewhere (3, 5, 6, 13).

Studies of isolated cytoplasmic particles were conducted only for the diploid strain iso-N, grown for 24 hr to stationary phase (4) under vigorous aeration on a rotary shaker kept at 25°C. Spheroplasts (12), produced by snail enzyme treatment (3), were lysed in a Vortex Genie mixer (Scientific Industries, Inc., Queens Village, N.Y.) (3, 12). Intact cells and cellular debris were removed by conventional low-speed centrifugation, and the cell-free brei was subjected to further centrifugations at 0°–4°C. The particulate sediments were collected and used for enzyme activity

TABLE I

Frequencies of Microbody Profiles from Electron Micrographs of 35 Strains of Saccharomyces cerevisiae

Ploidy level	Respiratory phenotype	No. strains examined	No. cell sections examined	Range of Nos. of microbodies per cell section	Mean No. microbodies per cell section, ±SEM
Diploid	Wild type	14	751	0–7	1.16 ± 0.04
	Respiration competent*	4	282	0–6	1.00 ± 0.06
	Petite	8	127	0–1	0.10 ± 0.03‡
Haploid	Wild type	4	105	0–3	0.52 ± 0.07§
	Petite	5	283	0–2	0.18 ± 0.03‡

* See reference 6.
‡ Significantly different from wild type, $P = < 0.01$.
§ Significantly different from diploid wild type, $P = < 0.01$.

* This paper is dedicated to Ralph E. Cleland on the occasion of his 75th birthday.

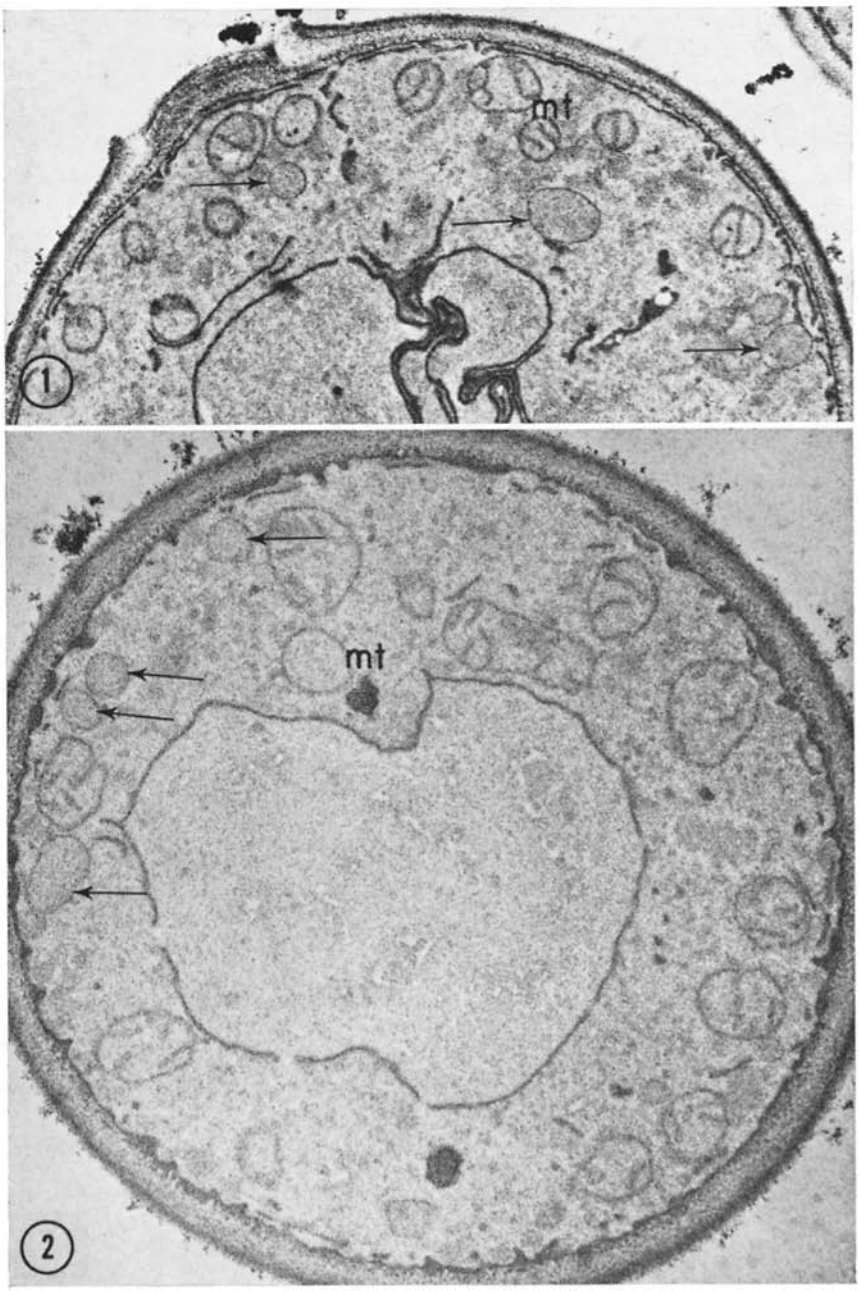

FIGURE 1 Note single limiting membrane and denser matrix of organelles resembling microbodies (at arrows) as compared with mitochondria (*mt*) in the $NaMnO_4$-fixed section of wild type iso-N. × 22,000.

FIGURE 2 The four microbody-like organelles (at arrows) in this iso-N section can be distinguished from the mitochondria, even when the latter lack cristae (*mt*), by virtue of the differences in limiting membranes and matrix density. The nonmitochondrial organelles are smaller (0.2–0.4 μ) than mitochondria. × 27,000.

TABLE II

Enzyme Activity Distribution in Differential Centrifugate Fractions of 24-hr Cultures of iso-N.
Cytochrome oxidase and catalase activities are expressed as the first-order reaction rate constant ($k \cdot sec^{-1}$) per mg protein used in an assay or per ml of total cell-free homogenate.

Centrifugate fraction	Cytochrome *c* oxidase		Catalase	
	Specific activity	Total activity	Specific activity	Total activity
	$k(sec^{-1}) \cdot mg^{-1}$	$k(sec^{-1}) \cdot ml^{-1}$	$k(sec^{-1}) \cdot mg^{-1}$	$k(sec^{-1}) \cdot ml^{-1}$
10,000-*g* particles	0.104	0.516	0.031	0.153
15,000-*g* particles*	0.012	0.035	0.083	0.165
20,000-*g* particles*	0.003	0.008	0.023	0.060
25,000-*g* particles*	0.002	0.004	0.016	0.037
25,000-*g* supernatant‡	—	0.0005	—	0.121

* Particles sedimented at indicated velocity from supernatant of the previous centrifugation step.
‡ Supernatant remaining after final centrifugation step.

assays or for rate separation in the Z-15 zonal rotor (International Equipment Co., Needham Heights, Mass.) in a continuous linear sorbitol gradient.

For differential centrifugation experiments, four different particle fractions were collected from the cell-free brei by subjecting each supernatant remaining from a previous centrifugation step to increasingly higher centrifugal forces (3). Thus, particles were collected successively by centrifugations for 30 min each time at 10,000, 15,000, 20,000, and 25,000 *g*. For density gradient separation in the zonal rotor, a single particle fraction first was obtained by centrifuging a cell-free brei for 10 min at 20,000 *g* in a fixed-angle rotor. This sediment then was layered on a continuous linear gradient containing 800 ml of 0–30% (w/w) sorbitol and 0.1 mM EDTA, adjusted to pH 6.5, and fractionated during a 30 min interval with the rotor spinning at 8,000 rpm. The centrifugal field-time integral was calculated at the sample zone as 1.4×10^5 g·min. After centrifugation the rotor speed was reduced to 2,000 rpm, and the density gradient was displaced with 60% (w/w) sucrose. The effluent from the rotor was led through a 2 mm flow cell in a spectrophotometer for a profile of absorbance at 280 mμ and then through a flow refractometer for a profile of the sorbitol concentrations (15). The effluent was then collected manually in 25-ml fractions for use in spectrophotometric assays for cytochrome-*c* oxidase (6), NADH–cytochrome-*c* reductase (19), and catalase (10) activities.

Samples from density gradient and differential centrifugation separations also were prepared for electron microscopy (3, 18) to identify the isolated particles.

RESULTS

The frequencies of microbody-like granules followed a Poisson distribution in all strains, with different mean or median values for diploids and haploids and for wild-type respiration-deficient vegetative petite cultures (Table I). These cytoplasmic granules sometimes were near an element of the sparse endoplasmic reticulum (Fig. 1). Crystalloid inclusions, such as those present in rat liver microbodies (1, 7), invariably were absent from the homogeneous matrix of the organelle bounded by a single limiting membrane (Figs. 1 and 2).

A total of 1,548 cell sections (Table I) were scored for the frequencies of nonmitochondrial particles resembling microbodies. Each of these electron micrographs had been examined previously for mitochondrial traits, including 821 photographs in which cytochemical reaction products indicated cytochrome-*c* oxidase or cytochrome-*c* peroxidase activities (3, 5, 6, 13). Among these 821 sections, 705 contained mitochondria with deposits due to cytochrome oxidase activity (5, 6, 13), but none of the microbody-like granules contained reaction deposits. All the remaining 116 of these 821 cell sections were of diploid iso-N after cytochrome *c* peroxidase cytochemical tests (3). Only 23% of the microbody-like structures were reaction-positive, whereas all the mitochondria in the same photographs contained the peroxidase reaction product (3).

Particles collected after differential centrifugation showed different distributions for cytochrome oxidase and catalase activities (Table II). The 10,000-*g* particles were greatly enriched in cytochrome oxidase, whereas catalase activity occurred in substantial amounts in all four particle fractions.

In particles which were fractionated according

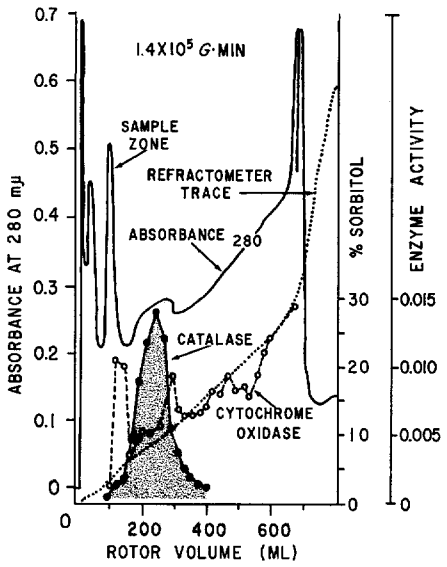

FIGURE 3 Absorbancy and total enzyme activity (per milliliter gradient) profiles from zonal density gradient experiment 46/130. Cytochrome oxidase and catalase activities are expressed as the first-order reaction rate constant (6), k(sec^{-1}) per milliliter.

to rate in a sorbitol density gradient during zonal centrifugation, catalase activity was confined to less rapidly sedimenting particles centering at about 275 ml (Fig. 3). Mitochondrial enzyme activity appeared in relatively low amounts (3, 6; Avers, C. J., C. A. Price, and A. Szabo. Data in preparation.) throughout the gradient.

Electron micrographs from thin sections of density-gradient fractions showed a scattering of mitochondrial profiles among numerous membranous materials of unknown nature and damaged organelles which closely resembled some that were described for rat liver microbody preparations (7). Thin sections from the 25,000 *g* sediment collected after differential centrifugation contained possible microbody-like structures (Fig. 4).

DISCUSSION

The ultrastructural data provide evidence for the occurrence of a distinct group of nonmitochondrial organelles. In addition to morphological features which were similar to those described for animal (1, 17) and plant (15) microbodies, there was no cytochrome oxidase activity demonstrable by cytochemical methods, whereas mitochondria in the same sections did contain reaction product deposits. The microbody-like organelles occurred in lower frequencies in respiration-deficient petites than in wild type, which also is a characteristic of mitochondrial populations in such strains (13). But, unlike mitochondria which occur in similar numbers in diploid and haploid wild-type cells (13), there were twice as many microbody-like structures in diploid as in haploid strains. Further investigation is required to determine whether this latter phenomenon is a reflection of a gene- or genome-dosage effect (16).

Although it was not possible to determine whether the morphologically-defined microbody-like organelle was the same particle containing the enriched catalase activity, it is significant that a group of particles which sedimented more slowly than mitochondria also were enriched in catalase activity. The particular conditions used for rate-zonal centrifugation were designed for resolution of the slowly sedimenting particles. The larger mitochondria migrated to the "cushion" after 30 min, thus preventing their proper analysis (Avers et al. Data in preparation).

Further studies now are in progress to determine the distribution of hydrogen peroxide-producing oxidases in particle fractions, which may permit a

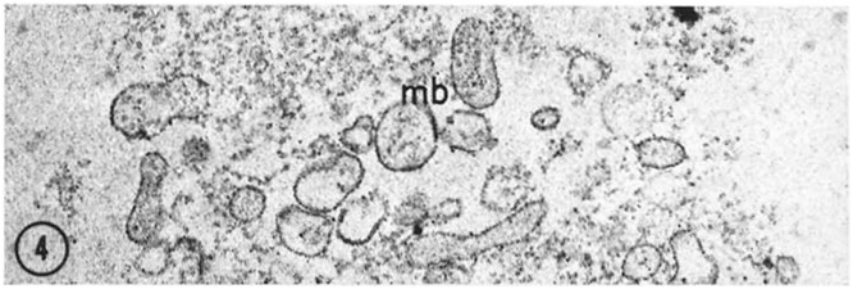

FIGURE 4 Thin section from 25,000 *g* particle fraction isolated by differential centrifugation. The microbody-like structures (*mb*) are the only recognizable organelle type in the preparation. × 42,000.

more definitive statement regarding the "peroxisome" nature of the slowly sedimenting fraction showing catalase activity. The identification of these organelles in yeast would provide increased opportunities to analyze the contributions made by peroxisomes to cellular metabolism (11), since yeast cultures can be manipulated both genetically and physiologically.

We wish to express our gratitude to Dr. C. A. Price for making available the zonal centrifuge facility and to acknowledge the excellent technical assistance rendered by Miss Anna Kovacs, Mrs. Raquel Sarmiento, and Mrs. Arlene Szabo.

This study was aided in parts by grants from the National Institutes of Health (AI 07272) and the National Science Foundation (GB 4717).

Received for publication 21 September 1967, and in revised form 22 December 1967.

REFERENCES

1. AFZELIUS, B. A. 1965. *J. Cell Biol.* **26**:835.
2. ALLEN, J. M., M. E. BEARD, and S. KLEINBERGS. 1966. *J. Exptl. Zool.* **160**:329.
3. AVERS, C. J. 1967. *J. Bacteriol.* **94**:1225.
4. AVERS, C. J., F. H. LIN, and C. R. PFEFFER. 1965. *J. Histochem. Cytochem.* **13**:344.
5. AVERS, C. J., C. R. PFEFFER, and M. W. RANCOURT. 1965. *J. Bacteriol.* **90**:481.
6. AVERS, C. J., M. W. RANCOURT, and F. H. LIN. 1965. *Proc. Natl. Acad. Sci. U.S.* **54**:527.
7. BAUDHUIN, P., H. BEAUFAY, and C. DE DUVE. 1965. *J. Cell Biol.* **26**:219.
8. BAUDHUIN, P., M. MULLER, B. POOLE, and C. DE DUVE. 1965. *Biochem. Biophys. Res. Commun.* **20**: 53.
9. BEAUFAY, H., P. JACQUES, P. BAUDHUIN, O. Z. SELLINGER, J. BERTHET, and C. DE DUVE. 1964. *Biochem. J.* **92**:184.
10. BEERS, R. F., and I. W. SIZER. 1952. *J. Biol. Chem.* **195**:133.
11. DE DUVE, C., and P. BAUDHUIN. 1966. *Physiol. Rev.* **46**:323.
12. DUELL, E. A., S. INOUE, and M. F. UTTER. 1964. *J. Bacteriol.* **88**:1762.
13. FEDERMAN, M., and C. J. AVERS. 1967. *J. Bacteriol.* **94**:1236.
14. MOLLENHAUER, H. H., D. J. MORRÉ, and A. G. KELLEY. 1966. *Protoplasma.* **62**:44.
15. PRICE, C. A., and A. P. HIRVONEN. 1967. *Biochim. Biophys. Acta.* **148**:531.
16. RITOSSA, F. M., and S. SPIEGELMAN. 1965. *Proc. Natl. Acad. Sci. U.S.* **56**:496.
17. RHODIN, J. 1954. *Aktiebolaget Godvil.* Stockholm.
18. SCHNAITMAN, C., V. G. ERWIN, and J. W. GREENAWALT. 1967. *J. Cell Biol.* **32**:719.
19. SHERMAN, F., and P. P. SLONIMSKI. 1964. *Biochim. Biophys. Acta.* **90**:1.