ENVIRONMENTALLY INDUCED CHANGES IN MITOCHONDRIA AND ENDOPLASMIC RETICULUM OF *SACCHAROMYCES CARLSBERGENSIS* YEAST

CAROLINE H. DAMSKY

From the Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19174. Dr. Damsky's present address is The Wistar Institute, Philadelphia, Pennsylvania 19104.

ABSTRACT

The effects of culture environment on the volume density and surface density of mitochondria and endoplasmic reticulum in a facultative yeast were studied. When compared with cells grown aerobically on a nonrepressive substrate, cells grown in the absence of oxygen showed a sharp reduction in both volume density of mitochondria and surface density of the inner mitochondrial membrane (imm) in the remaining mitochondrial profiles. Use of fermentable (repressive) substrates under aerobic conditions restricted the volume density of mitochondria to a much greater extent than the surface density of imm. The range of mitochondrial volume densities in these experiments was 4–11%. Surface density of endoplasmic reticulum (ER) was sensitive to growth rate and in particular to changes in oxygen tension, showing large fluctuations during both anaerobic and aerobic adaptation. These fluctuations in ER are discussed in relation to the known role of this organelle in lipid metabolism.

The effects of culture environment on the structure and function of mitochondria from facultative yeast have been studied extensively (18, 19, 26, 29). In ultrastructural studies, changes in the number and the shape of individual profiles in sectioned material and in the extent and visibility of the inner mitochondrial membrane (imm)[1] have been reported as a function of oxygen tension, substrate and unsaturated fatty acid (ufa) supplementation, etc. (8, 17, 20, 23, 27, 28, 31). Only a few reports have attempted to quantitate these environmentally induced changes in the mitochondrial population of the cell as a whole (9, 10, 25).[2] Furthermore, morphological changes in other organelles in response to these same alterations have not been well studied.

This paper reports the results of a morphometric analysis of both the mitochondrial and endoplasmic reticulum (ER) membranes of yeast cells grown under conditions in which growth rate, substrate, and oxygen tension were altered independently. The data point out wide fluctuations in the volume density of mitochondria and

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; imm, inner mitochondrial membrane; omm, outer mitochondrial membrane; ufa, unsaturated fatty acid.

[2] Several reports have been published concerning the number of individual mitochondria in various yeast strains grown under different conditions. This paper will concern itself only with volume density of mitochondrial material and surface density of mitochondrial membranes. The actual number of complete mitochondria does not affect these data.

the surface density of both imm and ER membranes in response to different culture environments. Possible relationships between changes in mitochondria and ER are discussed.

MATERIALS AND METHODS

Growth and Fixation of Saccharomyces carlsbergensis (ATTC 9080) for Aerobic and Anaerobic Adaptation

All cells were grown on 2% maltose-complex medium unless otherwise indicated. This substrate is thought to be less repressive than glucose, because of the slower maximum growth rate possible on maltose (3), and therefore permits better distinction between effects on cell structure of catabolite repression as opposed to other parameters such as oxygen tension. Conditions for anaerobic growth of *S. carlsbergensis* and fixation of ruptured cells were as previously described (8). For aerobic adaptation studies, cells were harvested from an anaerobic jar (Torsion Balance Co., Clifton, N. J.) in a stationary phase in which ufa and sterols were growth-limiting constituents (24 h, six to eight cell divisions). When the anaerobic jar was opened, samples of the anaerobically grown culture were fixed immediately with permanganate or aldehyde-osmium tetroxide (8). The remainder of the culture was aerated vigorously at 30°C over a 6-h period. Samples were removed periodically and prepared for electron microscopy. These cells are referred to as aerobically adapting. In some experiments the snail gut enzyme glusulase (Endo Laboratories, Inc., Garden City, N. Y.) was used to remove the yeast cell wall (4). In unadapted cells, this procedure was performed in the presence of protein synthesis inhibitors (cycloheximide at 25 μg/ml and chloramphenicol at 4 mg/ml) to prevent aerobic induction (7). To examine cells during the transition from an aerobic to an anaerobic environment, aerobically grown inocula were placed in three anaerobic jars. One jar was harvested and fixed with permanganate after 6 h of exposure to an anaerobic atmosphere (one cell division), the second after 8 h (two cell divisions) and the third after 24 h (six cell divisions:stationary phase). The first two cultures are referred to as anaerobically adapting.

AEROBICALLY GROWN BATCH CULTURES: Cells were grown on 2% maltose-complex medium with vigorous aeration at 30°C (maximum specific growth rate for maltose in the log phase is $\mu_{max} = 0.35\ h^{-1}$; [3]). Samples were removed periodically over a 48-h period and fixed with permanganate. Culture maltose concentrations were measured on sample supernates (24).

AEROBICALLY GROWN CONTINUOUS CULTURE: Cells were grown in continuous culture on either 2% pyruvate, glucose, or maltose in complex medium at the maximum growth rate for pyruvate obtainable in our hands ($\mu = 0.12\ h^{-1}$, where μ is the reciprocal of the time constant for mass increase). Oxygen tension and optical density of the cultures were monitored and, when these parameters indicated the presence of a steady state, samples were removed and prepared for electron microscopy.

Quantitative Analysis of Electron Micrographs

Determinations of volume density[3] of mitochondria and the surface density of mitochondrial and ER membranes were performed with a Weibel multipurpose test grid (6, 30). Analysis of intact cells was performed on median cell sections at magnifications of at least 37,000. Analysis of membrane surface areas of individual mitochondria was done at magnifications exceeding 50,000. Volume density of mitochondria and surface density of imm and ER were determined according to established morphometric methods (6, 30). Errors are expressed as standard error of the mean (SEM) and are based on measurement of at least 40 cell sections from three different experiments per time period.

---

[3] The following terms will be used in the quantitative analysis of ER and mitochondrial membranes in *S. carlsbergensis*. Volume density of mitochondria: fraction of the volume of the whole cell occupied by mitochondria. Expressed as percent.

$$\frac{\mu m^3 \text{ mitochondria}}{\mu m^3 \text{ Cell}} \times 100.$$

Surface density of ER: the surface area of ER membranes per unit volume of the cell. Expressed as:

$$\frac{\mu m^2 \text{ ER}}{\mu m^3 \text{ cell}}.$$

Surface density imm is considered in two ways: as a function of a unit volume of mitochondria

$$\frac{\mu m^2 \text{ imm}}{\mu m^3 \text{ mitochondria}},$$

and as a function of a unit volume of the cell as a whole

$$\frac{\mu m^2 \text{ imm}}{\mu m^3 \text{ cell}}.$$

The former is an expression of how differentiated morphologically the inner membrane is, within the unit of mitochondrial material, whereas the latter is a morphological expression of the total respiratory capacity of the cell.

RESULTS

Permanganate and Aldehyde-Osmium Tetroxide Fixation of Yeast Cells: General Considerations

Throughout this study, two fixing and staining procedures have been used. Permanganate fixation penetrates the intact yeast cell and stains all cellular membranes well under all of the aerobic growth conditions employed in this study. Therefore, all morphometric measurements on mitochondrial and ER membranes of aerobically grown cells have been performed on permanganate-fixed cells. Permanganate does not preserve mitochondrial membranes in anaerobically grown, ufa-sterol limited cells although all other cellular membranes including ER are adequately preserved. This is presumably due to the especially low ufa-sterol content of mitochondrial membranes after anaerobic growth (8, 11, 22). Since permanganate reacts with and stains double bonds in ufa and sterols almost exclusively (see reference 8 for discussion), mitochondria do not become visible until their membranes have acquired sufficient ufa and sterol during exposure to air to be preserved by permanganate. Therefore, the visibility of existing mitochondria during aerobic adaptation is a reflection of their ufa content. Morphometric analysis of ER surface density and mitochondrial volume density during aerobic adaptation have been performed on permanganate-fixed cells only for the purpose of correlating the synthesis of ER with the appearance of visible (that is, ufa-sterol containing) mitochondrial membranes (see Fig. 1). The measurements on ER surface density in aerobically adapting cells are considered to be valid, as will be described later in the text. The mitochondrial volume density as determined after permanganate fixation in the first 2–3 h of adaptation, however, is not considered valid because of the inability of permanganate adequately to preserve mitochondrial membranes during this period.

Glutaraldehyde and osmium tetroxide penetrate the yeast cell wall with difficulty and do not produce adequate contrast in the cell unless the cell wall is ruptured or removed. If this is done, aldehyde-osmium tetroxide fixation gives excellent preservation of all cellular structures including mitochondria in anaerobically grown as well as aerobically grown cells. Rupturing the cell wall

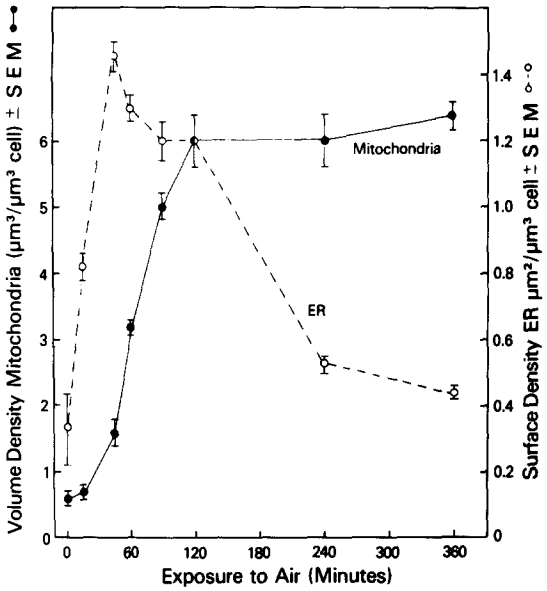


FIGURE 1 Surface area of endoplasmic reticulum and volume density of mitochondria in permanganate-fixed cells during adaptation of anaerobically grown, lipid-limited yeast to air.

or completely removing the cell wall enzymatically, however, distorts the cell boundaries and makes it difficult to measure volume or surface densities of organelles as a function of cell volume. Therefore, morphometric analysis on glutaraldehyde-osmium tetroxide-fixed cells was restricted to (*a*) measurement of imm surface density per unit volume mitochondria during aerobic adaptation in preparations which were first fixed with glutaraldehyde and then ruptured, and (*b*) volume density of mitochondria during aerobic adaptation in cells which were fixed lightly before partial removal of the wall with enzymes. These preparations outline the mitochondrial boundaries sufficiently to obtain accurate measurements. The surface density imm as a function of cell volume was then obtained by multiplying the mitochondrial volume density by the figure obtained for imm surface density per unit volume mitochondria.

Structure of Mitochondria during Aerobic Adaptation of Anaerobically Grown Yeast

The morphometric and ultrastructural data for cells fixed during the first 6 h of aerobic adapta-

TABLE I

Morphometric Analysis of Mitochondria and Endoplasmic Reticulum during Aerobic Adaptation of Anaerobically Grown, Lipid-limited Yeast

Time of exposure to air	Volume density of mitochondria $\mu m^3/\mu m^3$ cell ± SEM		Surface density inner membrane: aldehyde-osmium tetroxide fixation		Surface density ER $\mu m^2/\mu m^3$ cell ± SEM
	Permanganate fixation	Aldehyde fixation	$\mu m^2/\mu m^3$ mitochondria ± SEM	$\mu m^2/\mu m^3$ cell*	
min					
0	0.7 ± 0.05	4.0 ± 0.5‡	16.7 ± 5.3	0.64	0.34 ± 0.02
15	0.8 ± 0.1	4.5 ± 0.3	–	–	0.82 ± 0.04
45	1.6 ± 0.2	–	–	–	1.43 ± 0.05
60	3.2 ± 0.2	–	–	–	1.3 ± 0.04
90	5.0 ± 0.2	5.8 ± 0.5	–	–	1.2 ± 0.07
120	6.0 ± 0.4	6.2 ± 0.6	–	–	1.2 ± 0.1
240	6.0 ± 0.4	7.0 ± 0.3	34.4 ± 6.8	2.38	0.53 ± 0.03
360	6.4 ± 0.2	7.2 ± 0.4	34.3 ± 7.5	2.44	0.44 ± 0.02
Inoculum cell, aerobically grown, stationary phase (Fig. 2)	10.5 ± 1.7	10.9 ± 1.5	34.4 ± 4.5	3.8	0.14 ± 0.05

* Value in this and subsequent tables obtained by multiplying mean surface density inner membrane per volume mitochondria by the mean volume density of mitochondria.

‡ Measured on cells prefixed in glutaraldehyde followed by removal of the cell wall in the presence of protein synthesis inhibitors.

tion are displayed in Table I and Figs. 1–13. Both permanganate- and aldehyde-osmium tetroxide-fixed cells were examined. Mitochondrial membranes are not visible after permanganate fixation during the first 15 min of adaptation (Fig. 3). Their presence in sections of anaerobically grown cells is demonstrated only after aldehyde-osmium tetroxide fixation of ruptured cells (8) or cells whose walls have been partially removed by glusulase in the presence of protein synthesis inhibitors. With these techniques, mitochondria in anaerobically grown, lipid-limited cells are shown to occupy about 4% of the cell volume (Table I, Fig. 9). Both the outer and inner membranes of these mitochondria stain poorly. The few cristae are defined by the limits of the dense matrix rather than by the visibility of their membranes (Figs. 6 and 9). After 240 min of aeration, the mitochondria occupy 6–7% of the cell volume after either aldehyde-osmium tetroxide or permanganate fixation. The inner and outer membranes stain prominently (Figs. 5, 7, and 8). Measurements on the imm in aldehyde-osmium tetroxide-fixed cells at 0 and 6 h of aeration show a significant increase of about twofold in the surface density of imm per unit volume of mitochondria. Coupled with the 1.75-fold increase in mitochondrial volume density, this results in an increase of 3.5-fold in the imm surface density as a function of total cell volume over the 6-h aeration period. Although of interest, it is beyond the capacity of the data to determine whether the increase in imm surface density per mitochondrion and the increase in mitochondrial volume density proceed in a coordinate fashion or whether one precedes the other.

ER during Aerobic Adaptation

The ER in yeast has two primary components: a peripheral (pER) series of single flat cisternae which lie just inside the plasma membrane, and a more internal set of single cisternae (ER) scattered throughout the cytoplasm. Our present results show that the surface area of this latter component is strongly affected by changes in growth rate and, in particular, by changes in oxygen tension. Table I and Figs. 3–5 show that, during aerobic adaptation, a rapid rise in ER surface area is detectable before the appearance of visible mitochondrial profiles. Its value reaches a maximum after 45 min. After 2 h, the surface area of the ER decreases, while the volume density of visible mitochondrial profiles continues to increase over the next 2 h (Fig. 1).

The de novo elaboration of the ER membrane during early adaptation can be demonstrated by

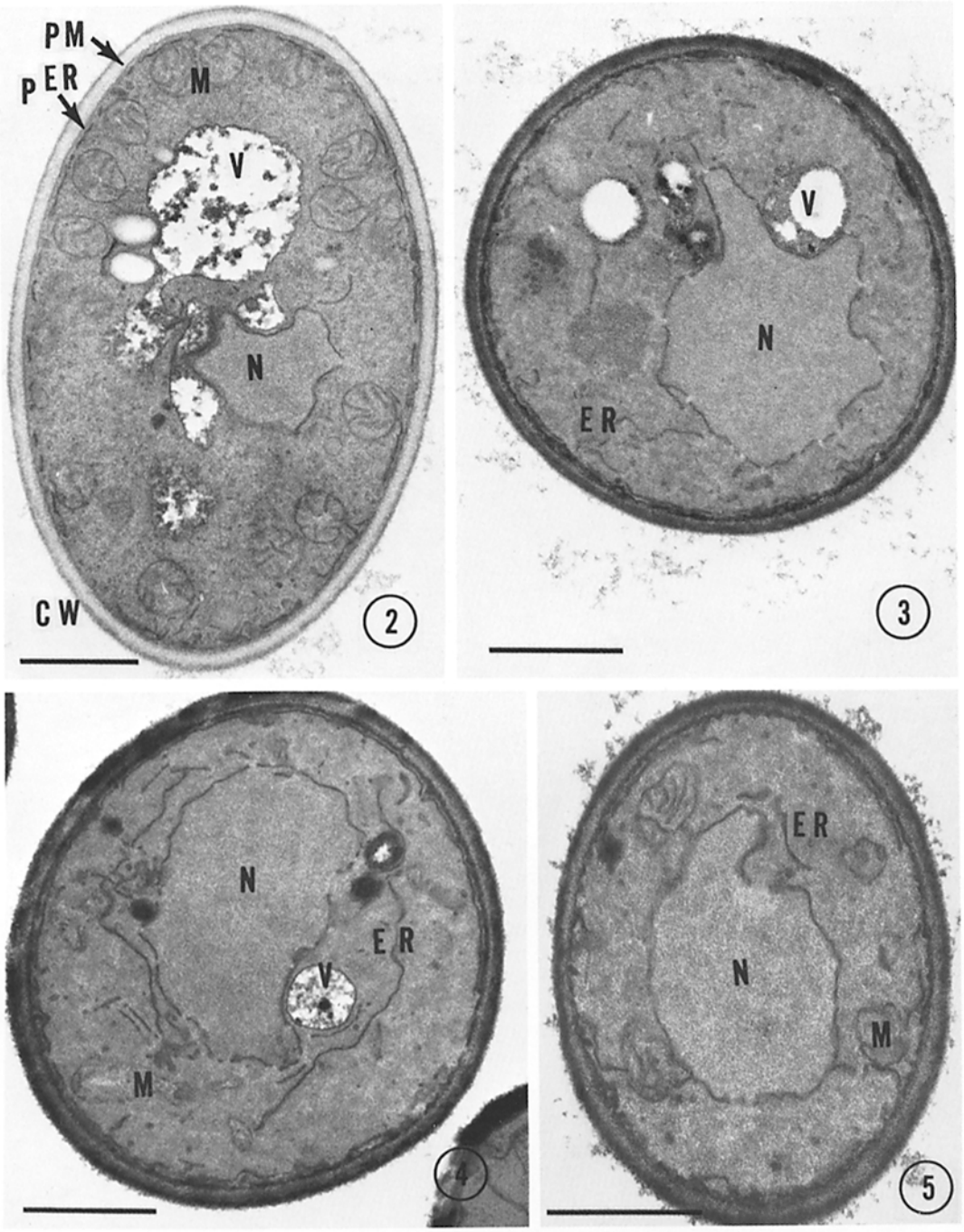


FIGURE 2 Aerobically grown, stationary-phase *S. carlsbergensis* yeast cell. Such cells were used as the inoculation culture for all experiments. Notice well-defined plasma, nuclear, and mitochondrial membranes. Peripheral endoplasmic reticulum (pER) lies inside the plasma membrane (PM). A small amount of endoplasmic reticulum is present. *N*, nucleus; *M*, mitochondrion; *V*, vacuole; *CW*, cell wall. Permanganate fixed; magnification, 18,000. Line = 1 μm unless otherwise indicated.

FIGURE 3 Anaerobically grown, lipid-limited cell 15 min after exposure to air. ER has started to proliferate but mitochondrial profiles are not visible. Permanganate fixed. Magnification, 20,000.

FIGURE 4 Permanganate fixed after 45 min of aeration. The amount of reticulum membrane has increased dramatically. Mitochondrial profiles are faintly visible: their internal structure is poorly defined and their membranes stain much less distinctly than nuclear and reticulum membranes. Magnification, 20,000.

FIGURE 5 Permanganate fixed after 240 min of aeration. The amount of cytoplasmic reticulum membrane has decreased. The mitochondria contain well-defined outer and inner membranes. Magnification, 23,000.

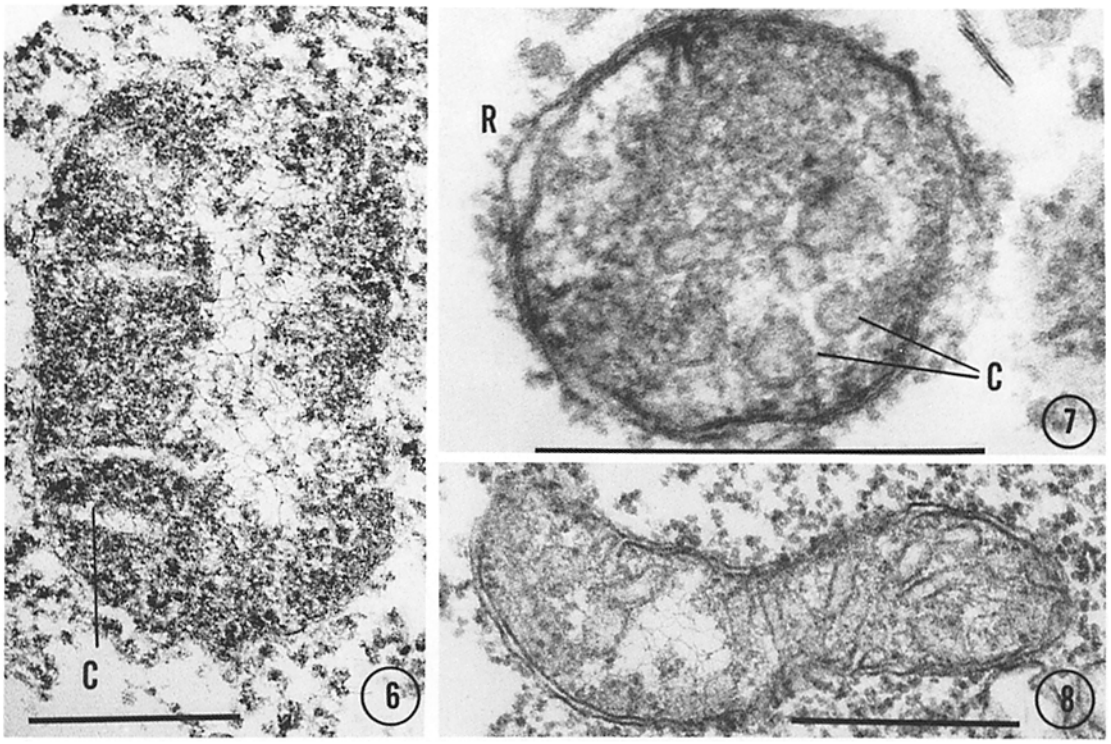


FIGURE 6 Mitochondrion from an anaerobically grown, lipid-limited *S. carlsbergensis* yeast, glutaraldehyde fixed after no aeration and ruptured in a ball mill. Limiting membranes are poorly defined. Cristae (*C*) are few in number (see also Fig. 9). Magnification 60,000. Line = 0.5 μm.

FIGURE 7 As above, but fixed after 180 min of aeration. Tripartite structure of inner and outer membranes is visible, and extent of inner membrane has increased. Ribosomes are seen to be attached to outer membrane. Magnification 120,000. Line = 0.5 μm.

FIGURE 8 As above, but fixed after 240 min of aeration. Tripartite structure of limiting membranes and cristae is visible. The extent of the inner membrane has increased further. Magnification, 60,000. Line = 0.5 μm.

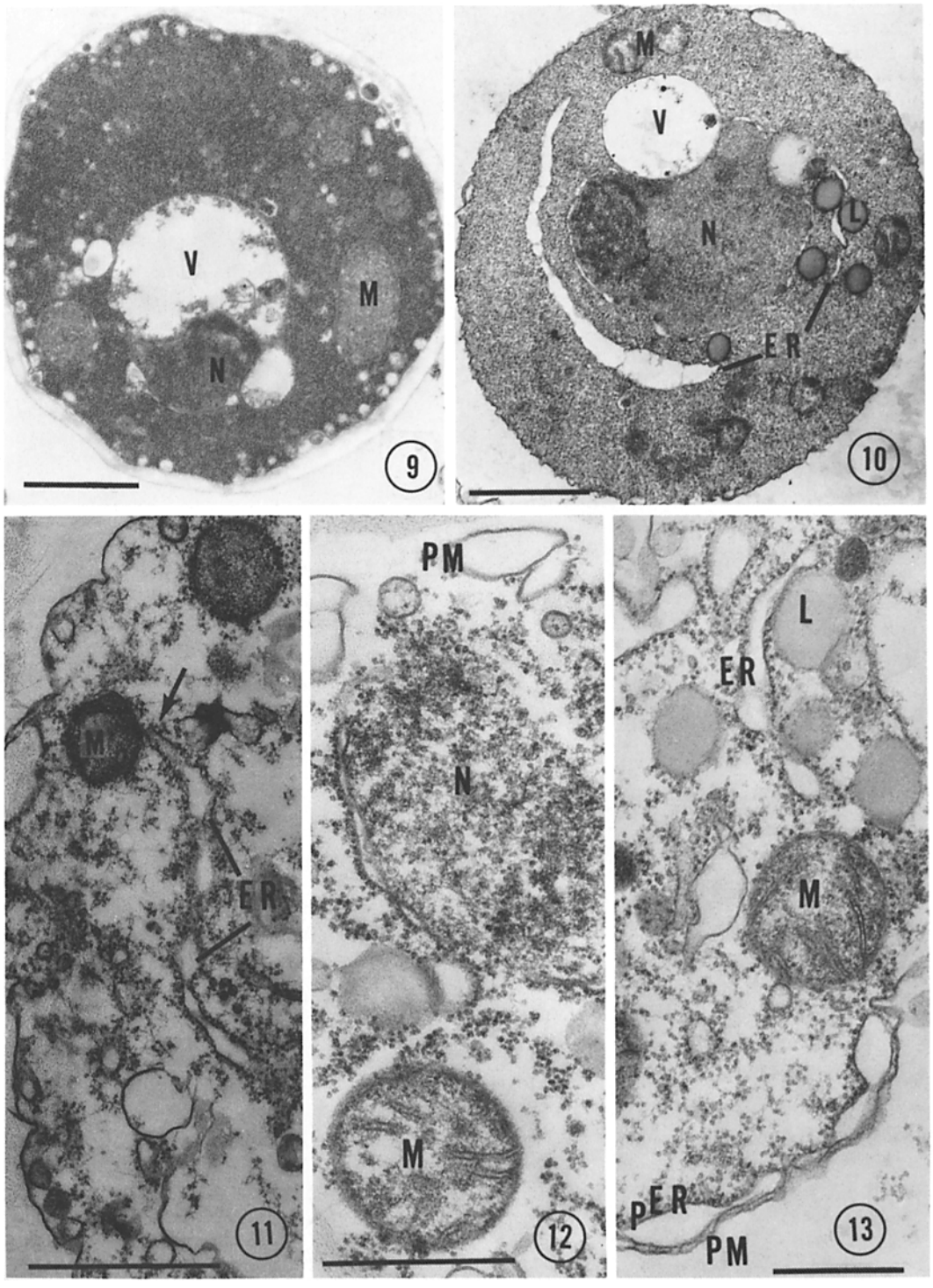
FIGURE 9 Anaerobically grown, lipid-limited yeast. No aeration. Cell wall was partially removed by glusulase in the presence of protein synthesis inhibitors. Mitochondrial profiles with no cristae are visible. Very little ER is present. Aldehyde fixed. Magnification, 18,000.

FIGURE 10 Anaerobically grown, lipid-limited *S. carlsbergensis*. 60-min aeration. Cell wall was removed with glusulase. Intact cell showing mitochondria with cristae and long strands of endoplasmic reticulum (ER). ER is often associated with lipid droplets (L). Magnification, 18,000.

FIGURE 11 Prepared as in Fig. 10 and osmotically ruptured, showing strands of ER associated with ribosomes. ER is closely associated with mitochondrion (arrow). Magnification, 30,000.

FIGURE 12 Prepared as in Fig. 10 and osmotically ruptured, showing nuclear membrane studded with ribosomes. Ribosomes do not associate with plasma membrane. Magnification, 60,000. Line = 0.5 μm.

FIGURE 13 Prepared as in Fig. 10 and osmotically ruptured, showing association of ribosomes with cytoplasmic surface of peripheral ER (pER) and with internal ER cisternae. Lipid droplets (*L*) are closely associated with ER. Magnification, 40,000. Line = 0.5 μm.


V
M
N
9
M
V
N
L
ER
10
M
ER
11
PM
N
M
12
L
ER
M
PER
PM
13

looking at cells fixed in glutaraldehyde after removal of the cell wall in the presence of protein synthesis inhibitors. Unadapted cells show very little reticulum membrane (Fig. 9). Cells fixed after 1 h in the presence of oxygen show long sacs of membrane (Fig. 10). If these cells are lysed before fixation (Figs. 11–13), the reticulum membranes appear studded with ribosomes. Ribosomes also appear attached to the outer nuclear membrane and to the cytoplasmic side of the pER. Ribosomes are not attached to the plasma membrane or vacuole membrane. Small groups of ribosomes are sometimes seen attached to outer mitochondrial membranes in adapting cells (Fig. 7).

To determine the extent to which fluctuations in mitochondrial volume density and surface density of ER and mitochondrial membranes during aerobic adaptation were due to changes in growth rate rather than to changes in oxygen tension, cells from three other conditions of growth were examined: (*a*) cells during the transition from aerobic to anaerobic growth (anaerobic adaptation); (*b*) cells during different stages of an aerobic growth cycle on maltose; and (*c*) cells growing at the same rate in continuous culture on nonfermentable (pyruvate) as opposed to fermentable (glucose or maltose) substrates.

Adaptation of Aerobically Grown Cells to Anaerobic Conditions

After 6 h of growth in an oxygen-free environment, yeast cells had a reduced volume density of identifiable mitochondrial profiles and a dramatically increased surface density of ER (Table II). In addition, there was considerable extraneous membrane with the appearance of disintegrating mitochondria (Fig. 14). After 8 h, the surface density of ER remained elevated and the extraneous membrane disappeared (Fig. 15).

Mitochondria and ER during Aerobic Batch and Continuous Culture

Table III shows the morphometric data for mitochondria and ER at several stages in the aerobic growth cycle on maltose. Two points of particular interest are noted if the data in this Table are compared with those from aerobically and anaerobically adapting cells (Tables I and II). (*a*) The increase in ER surface density documented during early log phase of the aerobic growth cycle is less pronounced than that in early aerobic and anaerobic adaptation. (*b*) Aerobically growing cells are capable of altering significantly the volume density of mitochondria during a growth cycle on maltose. However, this is not accompanied by a significant change in the surface density of the imm per unit volume of mitochondria (Fig. 16). This point was further explored by holding growth rate at a constant, low value ($\mu = 0.12\ h^{-1}$) and comparing the effects of repressive (maltose and glucose) and nonrepressive (pyruvate) substrates (see Table IV). The pyruvate-grown cells had the high volume density of mitochondria and high surface density of imm typical of cells in the early stationary phase of batch culture (metabolizing ethanol; Fig. 17). The glucose- and maltose-grown cells showed both the low volume density of mitochondria and the high surface density imm per unit volume of mitochondria typical of cells in the log phase of aerobic batch culture (metabolizing maltose; Fig. 18).

TABLE II

Anaerobic Adaptation of Aerobically Grown S. carlsbergensis (Permanganate Fixation)

Time after removal of air	Volume density mitochondria $\mu m^3/\mu m^3$ cell ± SEM	Surface density ER $\mu m^2/\mu m^3$ cell ± SEM
h		
0 (inoculum cell)	10.5 ± 1.7	0.14 ± 0.05
6 (one cell division)	4.6 ± 0.8*	1.1 ± 0.1*
8 (two cell divisions)	3.1 ± 0.6	1.47 ± .16
24 (six to eight cell divisions)	0.7 ± 0.05	0.34 ± 0.19
24 (aldehyde fixation)	4.0 ± 0.5	–

* Due to large amount of membrane giving the appearance of disintegrating mitochondria, identification of membrane as mitochondria or endoplasmic reticulum was not definitive.

For all substrates in the continuous culture experiments, the values for the surface density of ER were similar, and less than that found in more rapidly growing log phase batch cultures using maltose as a substrate.

DISCUSSION

The results of the morphometric data presented above point to the following conclusions on the membrane systems of *S. carlsbergensis*.

There appears to be an upper and a lower limit beyond which the volume density of mitochondria does not go. Derepressed cells, whether grown on pyruvate or on the products of maltose fermentation (ethanol), produce a maximum value of 10–11%. This value is in substantial agreement with that found by Grimes et al. (9) for S. cerevisiae. The minimum value is about 4% and is docu-

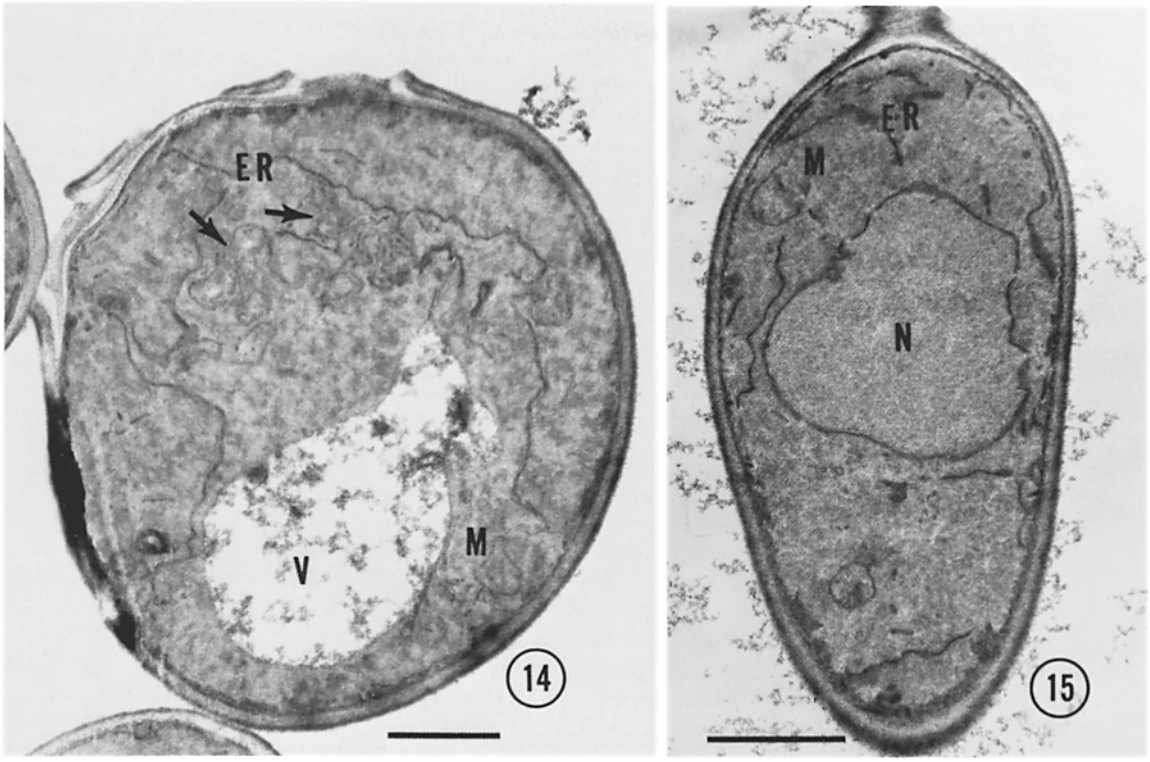


FIGURE 14 Aerobically grown, stationary phase cell grown on 2% maltose and placed in an aerobic jar in fresh medium and harvested after 6 h (one cell division). Note reduced number of intact mitochondria, elevated ER level, and membrane resembling disintegrating mitochondria (arrows). Magnification, 15,000.

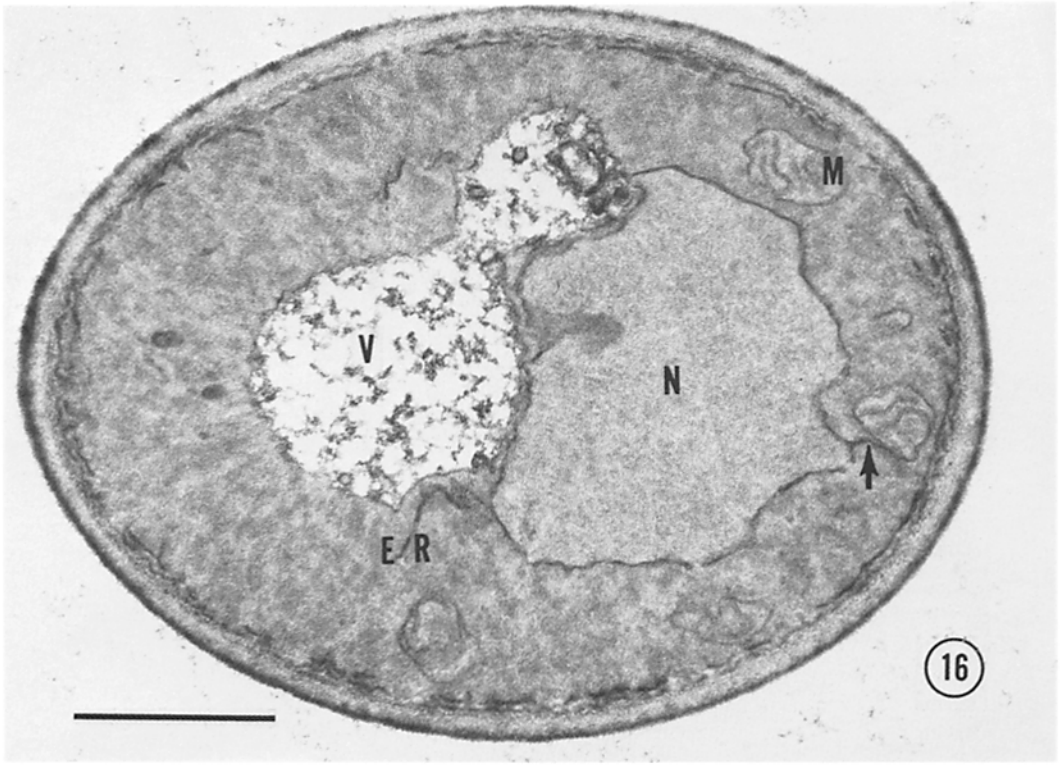
FIGURE 15 As above, after 8 h (two divisions) under anaerobic conditions. Patches of disorganized membrane are gone. Elevated ER levels remain. Magnification, 19,000.

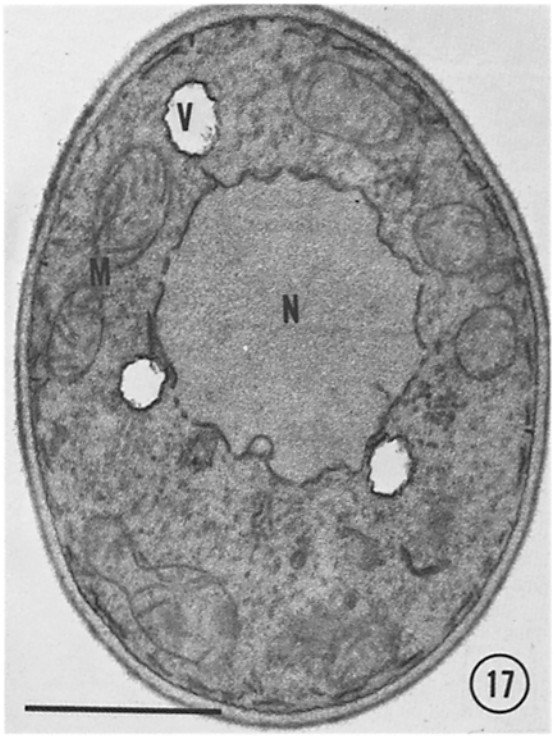
TABLE III

Aerobic Growth of S. carlsbergensis in Batch Culture on 2% Maltose (Permanganate Fixation)

Time in culture	Volume density mitochondria	Surface density inner mitochondrial membrane	Surface density inner mitochondrial membrane	Surface density ER
h	*$\mu m^3/\mu m^3$ cell ± SEM*	*$\mu m^2/\mu m^3$ mitochondria ± SEM*	*$\mu m^2/\mu m^3$ cell*	*$\mu m^2/\mu m^3$ cell ± SEM*
2 } log	5.5 ± 0.5	32.5 ± 6.4	1.78	0.61 ± 0.03
10 } phase	5.9 ± 0.6	30.4 ± 4.8	1.56	0.53 ± 0.04
15 } early	7.0 ± 0.3	33.8 ± 6.8	2.36	0.28 ± 0.06
17 } stationary	7.3 ± 0.6	37.3 ± 5.2	2.72	0.19 ± 0.02
20 } phase	7.9 ± 1.5	–		–
24 } mid	10.1 ± 1.1	–		
27 } stationary	10.5 ± 1.7	34.4 ± 4.5	3.8	0.14 ± 0.05
} phase				

mented here for anaerobically grown, lipid-limited cells. Other attempts (not illustrated here) were made to produce cells with a lower mitochondrial volume density. For instance, anaerobically grown, lipid-limited cells which were subcultured anaerobically for another 24 h in the presence of


M
V
N
E R
16


V
M
N
17

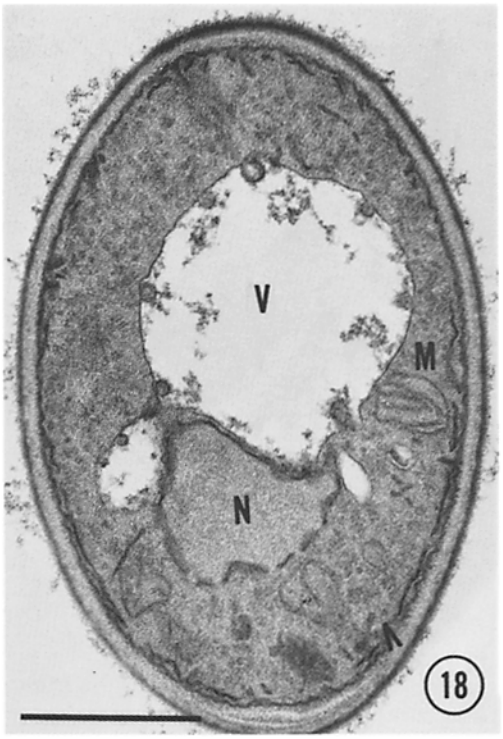

V
M
N
18

TABLE IV

Aerobic Growth of S. carlsbergensis in Continuous Culture

Substrate	Volume density mitochondria	Surface density inner membrane	Surface density inner mitochondrial membrane	Surface density ER
$\mu = 0.12\ h^{-1}$	$\mu m^3/\mu m^3$ *cell* ± *SEM*	$\mu m^2/\mu m^3$ *mitochondria* ± *SEM*	$\mu m^2/\mu m^3$ *cell*	$\mu m^2/\mu m^3$ *cell* ± *SEM*
2% pyruvate	11.5 ± 1.5	34.8 ± 6.8	4.0	0.3 ± 0.03
2% glucose (data were similar for 2% maltose)	4.1 ± 0.4	30.1 ± 4.5	1.2	0.23 ± 0.05

supplemental ufa and sterol showed no further significant reduction in mitochondrial volume density.

S. carlsbergensis has the ability to manipulate the surface density of imm in the cell as a whole by altering the volume density of mitochondria independently of the imm surface density per unit volume of mitochondrial material. Anaerobic growth of cells on maltose restricts the synthesis of the inner membrane as well as the volume density of mitochondria. Both an aerobic growth cycle on maltose and continuous culturing of cells on maltose or pyruvate, however, appear to produce a significant fluctuation in the volume density of mitochondria only, and not in the surface density of imm in the remaining mitochondria. These data are not meant to suggest that there are no significant differences in the biochemical composition of the imm in these populations of cells. Furthermore, the considerable variation in the values for imm within each sample time period is such that small but real differences in imm surface area would not be detectable. Despite these reservations in the interpretation of the morphometric data, it should be clear that catabolite repression of this particular yeast strain by maltose operates much more dramatically at the level of the organelle as a whole than at the level of membrane differentiation within the organelle. As added support for the morphometric data, when samples of the two continuous cultures were analyzed for cytochrome oxidase content the level was found to be 2.5–3-fold greater in the pyruvate-grown cells than in the maltose (glucose)-grown cells.[4] This correlates well with the ratio of the total surface density of imm per unit volume of cellular material in the two cultures (Table IV).

These data appear to conflict with studies (27, 31) which report significant reductions in the extent of imm in *S. cerevisiae* during the log phase of growth on glucose, but in which no quantitative measurements on membranes were presented. However, it must be emphasized that there is considerable variation among yeast strains in their susceptibility to catabolite repression and in the effect of anaerobiosis or substrate on the cellular

[4] Damsky, C. H. Unpublished experiments.

FIGURE 16 Aerobically growing cell after 2 h of growth in batch culture (early log phase $\mu = 0.35\ h^{-1}$) on 2% maltose. The mean fractional volume of mitochondria has decreased by one-half. Inner mitochondrial membranes are well defined when compared with those of anaerobically adapting (Figs. 14, 15) or anaerobically grown, lipid-limited cells (Figs. 6, 9). The mean surface density of ER is above that of stationary phase cells (Table III) but well below that found in aerobically or anaerobically adapting cells (see (Figs. 4, 14, 15). Note proximity of mitochondrial and ER membranes (arrow). Permanganate fixed. Magnification, 28,000.

FIGURE 17 Cell from a 2% pyruvate continuous culture ($\mu = 0.12\ h^{-1}$). Notice high mitochondrial volume density and well-defined imm. Permanganate fixed. Magnification, 26,000.

FIGURE 18 Cell from a 2% glucose continuous culture ($\mu = 0.12\ h^{-1}$). The volume density of mitochondria is similar to that found in Fig. 16, and imm is well defined. ER surface density in this figure is similar to that in the previous figure and similar to those found in batch culture during early stationary phase and is less than levels of ER found in log phase of batch culture (Fig. 16). Magnification, 20,000.

concentration of mitochondrial DNA (see reference 9 for discussion). Strain differences rather than technical differences may therefore explain most of these discrepancies.

ER surface area is particularly responsive to changes in oxygen tension. Changes in growth rate and oxygen tension both produce fluctuations in the surface density of ER, whereas choice of substrate does not appear to be of significant import, at least at low growth rates. The fluctuations are the most extreme under conditions which either increase or decrease the availability of oxygen. The elevated surface density of ER observed in the present study during aerobic and anaerobic adaptation may be a morphological demonstration of its known role in fatty acid and sterol metabolism (5, 15, 16). Yeast are unable to synthesize ufa or sterols in the absence of molecular oxygen (1, 2). During anaerobic adaptation, the extensive ER membranes elaborated may be involved both in the synthesis of lipids from the shorter chain saturated fatty acids which are characteristic of anaerobically grown, lipid-limited cell membranes (11, 22), and in the recycling of mitochondrial membrane material which may be used as a source of ufa and sterol for other cell membranes which have a greater survival value during anaerobic growth. During aerobic adaptation, the restoration of oxygen initiates the synthesis of longer-chain ufa. Linnane and Haslam (18) and Watson et al. (29) report rapid synthesis of ufa, but not of cytochromes during the 1st h of aerobic adaptation. Analysis of cytochrome oxidase levels during aerobic adaptation in the samples of the same cultures used for morphometry in this study also reveals about a 1-h lag before the onset of cytochrome oxidase synthesis (21). This 1st h of aerobic adaptation is the period during which the elaboration of ER reaches its peak and mitochondrial profiles start to become visible in permanganate-fixed cells (see Table I and Fig. 1). These observations are consistent with the suggestion that ER is involved in the synthesis of lipids for cell membranes including mitochondria and that these lipid components are responsible for the increased visibility of mitochondrial membranes in permanganate-fixed cells during aerobic adaptation (8) (Figs. 1-5).

The fact that yeast ER is intimately associated with ribosomes clearly suggests that it is involved in some aspects of cellular protein synthesis. The possibility that ER may be involved in the cytoplasmic synthesis and transport of mitochondrial polypeptides is an attractive one. Recently, however, morphological (see also Fig. 7) and biochemical evidence has been accumulating which indicates the existence of mitochondrion-associated cytoplasmic ribosomes (80S) which are capable of synthesizing polypeptides in vitro and transporting them into the interior of mitochondria (12-14). These data suggest that it is mitochondria-associated ribosomes, not ER-associated ribosomes, which are the site of cytoplasmic synthesis of mitochondrial polypeptides in yeast.

SUMMARY

This paper reports on extensive changes in the surface area of both mitochondrial and endoplasmic reticulum membranes of *S. carlsbergensis* in response to changes in culture environment. In examining the effect of growth rate, substrate, and oxygen tension on mitochondria and endoplasmic reticulum, we have demonstrated possible maximum and minimum values for the volume density of mitochondria, the ability of yeast to titrate their respiratory capacity by altering independently the development of the inner membrane as well as the volume density of the organelle as a whole, and the particular responsiveness of the surface area of endoplasmic reticulum to changes in oxygen tension. It is suggested that part of the extensive elaboration of ER during both aerobic and anaerobic adaptation reflects a role for the endoplasmic reticulum in the synthesis, desaturation, and recycling of mitochondrial and other membrane fatty acids and lipids.

The author acknowledges the support and encouragement of Drs. Lee D. Peachey and E. Kendall Pye.

This work was done in partial fulfillment of the requirements of the degree of Doctor of Philosophy at the University of Pennsylvania.

Work was supported by grants from the National Science Foundation, GI32498X, GB8270 and GB6975X3.

Received for publication 23 March 1976, and in revised form 10 June 1976.

REFERENCES

1. ANDREASSEN, A. A., and T. J. B. STIER. 1953. Anaerobic nutrition of *S. cerevisiae*. I. Ergosterol requirement for growth in a defined medium. *J. Cell. Comp. Physiol.* **41**:23-36.
2. ANDREASSEN, A. A., and T. J. B. STIER. 1954. Anaerobic nutrition of *S. cerevisiae*. II. Unsaturated fatty acid requirement for growth in a defined me-

dium. *J. Cell. Comp. Physiol.* **43**:271-281.

3. BECK, C., H. KASPAR, and H. K. VON MEYERBURG. 1968. Enzyme patterns and aerobic growth of *S. cerevisiae* under various degrees of glucose limitation. *J. Bacteriol.* **96**:479-486.
4. BECK, J. C., J. R. MATTOON, D. C. HAWTHORNE, and F. S. SHERMAN. 1968. Genetic modification of energy conserving systems in yeast mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* **60**:186-193.
5. BLOOMFIELD, D., and K. BLOCH. 1960. The formation of Δ^9 unsaturated fatty acids. *J. Biol. Chem.* **235**:337-345.
6. BOLENDER, R. 1974. Stereological analysis of the guinea pig pancreas. I. Analytical model and quantitative description of non-stimulated pancreatic exocrine cells. *J. Cell Biol.* **61**:269-286.
7. CHEN, W., and F. C. CHARALAMPOUS. 1969. Mechanism of induction of cytochrome oxidase in yeast. I. Kinetics of induction and evidence for accumulation of cytoplasmic and mitochondrial precursors. *J. Biol. Chem.* **244**:2767-2776.
8. DAMSKY, C. H., W. M. NELSON, and A. CLAUDE. 1969. Mitochondria in anaerobically grown lipid-limited brewer's yeast. *J. Cell Biol.* **43**:174-179.
9. GRIMES, G., P. PERLMAN, and H. MAHLER. 1974. Nuclear gene dosage effects on mitochondrial mass and DNA. *J. Cell Biol.* **61**:565-574.
10. HOFFMAN, H. P., and C. AVERS. 1973. Mitochondrion of yeast: ultrastructural evidence for one giant, branched organelle per cell. *Science (Wash. D. C.)* **181**:749-750.
11. JOLLOW, D. G., G. M. KELLERMAN, and A. W. LINNANE. 1968. The biogenesis of mitochondria. III. Lipid composition of aerobically and anaerobically grown *Saccharomyces cerevisiae* are related to the membrane systems of the cells. *J. Cell Biol.* **37**:221-230.
12. KELLEMS, R., V. F. ALLISON, and R. A. BUTOW. 1974. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. III. Evidence for the association of cytoplasmic ribosomes with the outer mitochondrial membrane *in situ*. *J. Biol. Chem.* **249**:3797-3803.
13. KELLEMS, R., V. F. ALLISON, and R. A. BUTOW. 1975. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. IV. Attachment of ribosomes to the outer membrane of isolated mitochondria. *J. Cell Biol.* **65**:1-14.
14. KEYHANI, E. 1972. Ribosomal granules associated with outer mitochondrial membrane in aerobic yeast cells. *J. Cell Biol.* **58**:480-484.
15. KLEIN, H. P., C. M. VOLKMANN, and M. A. LEAFER. 1967. Subcellular sites involved in lipid synthesis in *S. cerevisiae*. *J. Bacteriol.* **94**:61-65.
16. KLEIN, H. P., C. M. VOLKMANN, and J. WEIBEL. 1967. Membranes of *S. cerevisiae*. *J. Bacteriol.* **94**:475-481.
17. KOVAC, L., J. SUBIK, G. RUSS, and K. KOLLAR. 1967. On the relationship between respiratory, activity and lipid composition of the yeast cell. *Biochim. Biophys. Acta.* **144**:94-101.
18. LINNANE, A., and J. M. HASLAM. 1970. The biogenesis of yeast mitochondria. *In* Current Topics in Cellular Regulation. B. L. Horecker and E. R. Stadtman, editors. Academic Press Inc., New York. **2**:101-172.
19. LINNANE, A. W., J. M. HASLAM, H. B. LUKINS, and P. NAGLEY. 1972. Biogenesis of mitochondria in microorganisms. Annual Review of Microbiology. **26**:163-191.
20. LUKINS, H. B., D. JOLLOW, P. G. WALLACE, and A. W. LINNANE. 1968. The biogenesis of mitochondria. VII. The effects of glucose repression on the lipid and enzyme composition of mitochondria from yeast. *Aust. J. Exp. Biol. Med. Sci.* **46**:651-665.
21. NELSON, W. M. 1971. Cytochromes in anaerobic and aerobic yeast. Ph.D. Thesis. University of Pennsylvania, Philadelphia, Pa.
22. PALTAUF, F., and G. SCHATZ. 1969. Promitochondria of anaerobically grown yeast. II. Lipid composition. *Biochemistry.* **8**:335-339.
23. PLATTNER, H., and G. SCHATZ. 1969. Promitochondria of anaerobically grown yeast. III. Morphology. *Biochemistry.* **8**:339-343.
24. SOMOGYI, M. 1952. Notes on sugar determination. *J. Biol. Chem.* **195**:19-23.
25. STEVENS, B. 1974. Variation in mitochondrial numbers and volume in yeast according to growth conditions. *J. Cell Biol.* **63**(2, Pt. 2):336 a. (Abstr.).
26. SUOMALAINEN, H., T. NURMINEN, and E. DURA. 1973. *In* Progress in Industrial Microbiology. D. D. Hockenhull, editor. Churchill-Livingstone Ltd., Edinburgh. **2**:109-167.
27. WALLACE, P. G., M. HUANG, and A. W. LINNANE. 1968. The biogenesis of mitochondria. II. The influence of medium composition on the cytology of anaerobically grown *S. cerevisiae*. *J. Cell Biol.* **37**:207-229.
28. WATSON, K., J. M. HASLAM, and A. W. LINNANE. 1970. Biogenesis of mitochondria. XII. The isolation of mitochondrial structures from anaerobically grown cells. *J. Cell Biol.* **46**:88-96.
29. WATSON, K., J. M. HASLAM, B. VEITCH, and A. W. LINNANE. 1971. *In* Autonomy of Mitochondria and Chloroplasts. N. K. Boardman, A. W. Linnane, and R. M. Smilie, editors. North Holland Publishing Co., Amsterdam. 162.
30. WEIBEL, E., and R. BOLENDER. 1973. Stereological techniques for electron microscope morphology. *In* Principles and Techniques of Electron Microscopy. M. A. Hayat, editor. Van Nostrand Company, Inc., New York **3**:237.
31. YOTSUYANAGI, Y. 1962. Etudes sur le chondriome au cours du cycle de la croissance aerobie. *J. Ultrastruct. Res.* **7**:121-140.