NEITHER RESPIRATION NOR CYTOCHROME *c* OXIDASE AFFECTS MITOCHONDRIAL MORPHOLOGY IN *SACCHAROMYCES CEREVISIAE*

CYNTHIA CHURCH AND ROBERT O. POYTON*

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA

*Author for correspondence (e-mail: poyton@spot.colorado.edu)

Summary

Previous studies have reported that mitochondrial morphology and volume in yeast cells are linked to cellular respiratory capacity. These studies revealed that mitochondrial morphology in glucose-repressed or anaerobically grown cells, which lack or have reduced levels of respiration, is different from that in fully respiring cells. Although both oxygen deprivation and glucose repression decrease the levels of respiratory chain proteins, they decrease the expression of many non-mitochondrial proteins as well, making it difficult to determine whether it is a defect in respiration or something else that effects mitochondrial morphology. To determine whether mitochondrial morphology is dependent on respiration *per se*, we used a strain with a *null* mutation in *PET100*, a nuclear gene that is specifically required for the assembly of cytochrome *c* oxidase. Although this strain lacks respiration, the mitochondrial morphology and volumes are both comparable to those found in its respiration-proficient parent. These findings indicate that respiration is not involved in the establishment or maintenance of yeast mitochondrial morphology, and that the previously observed effects of oxygen availability and glucose repression on mitochondrial morphology are not exerted through the respiratory chain. By applying the principle of symmorphosis to these findings, we conclude that the shape and size of the mitochondrial reticulum found in respiring yeast cells is maintained for reasons other than respiration.

Key words: mitochondria, cytochrome *c* oxidase assembly, respiration, yeast, *Saccharomyces cerevisiae*.

Introduction

Mitochondria are essential organelles that are present in all eukaryotic cells (Munn, 1974; Baker and Schatz, 1991). They do not form *de novo* but assemble from pre-existing mitochondria, a process that requires the products of both nuclear and mitochondrial genomes (Attardi and Schatz, 1988; Pon and Schatz, 1991). Beginning with the early observations of Lewis and Lewis (1914), several studies have demonstrated that mitochondria are dynamic structures that are constantly fusing, dividing and changing their shape and size. Other studies have revealed that mitochondria vary considerably in their structure and location in different cell types (for reviews, see Tandler and Hoppel, 1972; Bereiter-Hahn, 1990; Bereiter-Hahn and Voth, 1994). In some types of cells, mitochondria are distributed randomly throughout the cytosol as thread-like tubules, while in others they are organized into stacks or spirals, usually near energy-consuming cellular structures. For example, mitochondria are stacked between myofibrils in muscle fibers (Munn, 1974; Walton *et al.* 1981; Kirkwood *et al.* 1986) and they are wound helically around the base of the flagellum in mammalian spermatozoa (Boissin and Manier, 1966; Galangau and Tuzet, 1968; Bawa and Werner, 1988) and in the zoospores of green algae (Hitano and Ueda, 1988).

It is not yet clear how mitochondrial position, size or structure is established or maintained. However, several studies have suggested that mitochondrial morphology can be affected by cellular energy metabolism (see Bereiter-Hahn and Voth, 1983). This has been demonstrated most clearly with the facultative anaerobe *Saccharomyces cerevisiae*. In this organism, mitochondria are highly dynamic organelles that undergo rapid fusion and division (Koning *et al.* 1993). The time-averaged shape, size and number of mitochondria are variable and are regulated in response to the cell's physiological state (Stevens, 1977, 1981; Visser *et al.* 1995). The number of mitochondria can vary from one to more than 40 depending on oxygen availability, carbon source and growth phase. For example, steady-state cells that are grown in the presence of air on a carbon source (e.g. ethanol) that does not repress the expression of genes for respiratory proteins respire fully and contain many small mitochondria dispersed throughout the cell. In contrast, cells that are grown in the presence of air on a carbon source (e.g. glucose) that represses the genes for respiratory proteins have reduced levels of respiration and 2–3 long branched structures that lie near the periphery of the cell (Visser *et al.* 1995). It has been calculated that the mitochondrial volume in non-repressed cells accounts for up to 12 % of the cell volume, but in

glucose-repressed cells it accounts for only 3 % of the cell volume (Stevens, 1977, 1981). From observations of cells at various stages of growth, Yotsuyanagi (1962*a*) suggested that, in the presence of glucose, cells initially have a low level of respiration and possess few mitochondria with poorly developed cristae. However, as the culture grows, the glucose is converted to ethanol and the cells develop an increased respiratory capacity in the mid- to late-logarithmic phase. During these growth phases, the mitochondria become elongated into a reticulum. Finally, as the cells enter the early stationary phase and use ethanol as their sole carbon source, they develop a full respiratory capacity and the mitochondrial reticulum breaks up into several small mitochondria located at the periphery of the cell. *S. cerevisiae* cells grown under anaerobic conditions form poorly differentiated 'promitochondria' (Criddle and Schatz, 1969; Plattner and Schatz, 1969). These are branched, thread-like structures similar to the mitochondrial reticulum seen in glucose-repressed cells (Visser *et al.* 1995). Early studies showed that these promitochondria are structural precursors to the mitochondria which form when the cells are shifted to aerobic conditions (Plattner *et al.* 1970). More recent studies have demonstrated that the morphological differences observed between anaerobic or glucose-repressed cells and non-repressed respiring cells are fully reversible (Visser *et al.* 1995).

Considered together, the above observations have suggested that changes in the mitochondrial morphology and volume in *S. cerevisiae* are linked to cellular respiratory capacity (Stevens, 1981; Visser *et al.* 1995). This conclusion is also supported by the finding that respiratory-deficient rho^0 and rho^- mutants lacking functional mitochondrial DNA have altered mitochondrial morphologies (Stevens, 1981). It is not yet known whether it is respiration itself, or something else, that affects mitochondrial morphology. Indeed, although both oxygen availability and glucose repression affect the levels of respiratory chain proteins, they also affect the expression of genes for many non-mitochondrial proteins (de Winde and Grivell, 1993; Bunn and Poyton, 1996; Poyton and McEwen, 1996; Kwast *et al.* 1998). Similarly, rho^0 and rho^- mutations lack mitochondrial respiration but affect the expression of genes for proteins that reside in other organelles as well (Shyjan and Butow, 1993; Poyton and McEwen, 1996). Finally, it has been asserted that 'most nuclear (pet) mutations causing respiratory deficiency have little effect on mitochondrial morphology as long as these mutations do not cause the loss of the mitochondria genome' (Pon and Schatz, 1991). This latter conclusion appears to have been based entirely on an early ultrastructural analysis of three yeast strains that carry nuclear *pet* mutations and that lack respiration (Yotsuyanagi, 1962*b*). The results presented in this early study are difficult to interpret because the precise nature of the mutations carried by these strains is unclear and because at least two of these strains lack cytochrome *b* and, hence, may carry either a rho^0 or a rho^- mutation as well as a *pet* mutation.

In order to address the question of whether respiration influences mitochondrial morphology in *S. cerevisiae,* we chose to compare mitochondrial morphologies in a well-characterized respiratory-deficient nuclear *pet* mutant with those in its isogenic, respiratory-proficient parent. This mutant carries a defect in a newly discovered protein, PET100p, that is required specifically for the assembly of cytochrome *c* oxidase (Church *et al.* 1996; Church, 1996).

Materials and methods

Strains, media and growth conditions

The following *Saccharomyces cerevisiae* strains were used: JM43 (*MATa his4-580 trp-2891 leu2-3,112 ura3-52*) and a respiratory-deficient mutant, JM43GD100 (*MATa his4-580 trp-2891 leu2-3,112 ura3-52 PET100::LEU2*), derived from it (Church *et al.* 1996). The latter carries a *null* mutation in the *PET100* gene and therefore produces no PET100p. Cells were grown at 30 °C in YP medium (1 % Difco yeast extract, 2 % Difco Bacto-peptone) containing 0.5 % galactose, 2 % galactose or 3 % glycerol. Liquid cultures were grown on a shaker as low-density batch cultures (200 revs min^{-1}). They were routinely maintained in steady-state exponential growth for at least six generations before harvesting. This was achieved by repeated transfer of mid-exponential phase cultures to fresh growth medium. Cells were harvested in mid-exponential phase (3×10^7 to 5×10^7 cells ml^{-1}).

Confocal microscopy

Living yeast cells, grown in YP medium containing 2 % galactose, were stained with two different mitochondria-specific dyes: 2-(4-dimethylaminostyryl)-*N*-methylpyridinium iodide (DASPMI) (Bereiter-Hahn, 1976), at 100 µg ml^{-1}, or 3,3′-dihexyloxacarbocyanine iodide (DiOC$_6$) (Terasaki, 1989), at 0.1 µg ml^{-1}. The stained cells were placed directly on glass slides. To immobilize the cells for photography, 4 µl of the cell suspension was mixed with 10 µl of 2 % low-melting-point agarose and cover-slipped. The cells were examined immediately using a Molecular Dynamics confocal imaging system, with a 100× objective using excitation (488 nm) and barrier (515 nm) filters appropriate for fluorescein. Optical sections in the *z*-axis were scanned at 0.5 µm intervals.

Computer-aided three-dimensional reconstruction of images from the confocal microscope was carried out at the Boulder Laboratory for Three-dimensional Fine Structure using IMOD software (Kremer *et al.* 1996). Contours of cells and mitochondria were created in successive sections by a semi-automated procedure that creates a contour around a selected area. In this study, the areas selected were the brightly stained mitochondria and the more diffusely stained cytosol. Once the appropriate areas of each section had been selected, a three-dimensional model was created by the program IMODV. Mitochondrial lengths were determined after taking into account *z*-scale and pixel size. Cell and mitochondrial volumes were calculated using the program IMODINFO.

Electron microscopy

Yeast cells were grown in YP medium containing 0.5 % galactose, harvested in mid-exponential phase and washed once in sterile distilled water. We used lower concentrations of galactose to grow cells for electron microscopy in order to reduce the amount of stored glycogen, which sometimes interferes with the visualization of organelle ultrastructure in yeast cells. Washed cells were fixed by adding a solution of 1.5 % filtered $KMnO_4$ while rotating suspended cells gently at room temperature (28 °C) for 20 min. Cells were washed in distilled water until clear, and cell pellets were stained with 1 % uranyl acetate overnight at 4 °C with constant agitation. Before infiltration in Epon-Araldite, cell pellets were washed twice in distilled water and dehydrated with a graded series of acetone. The samples were incubated for 40 h at 60 °C. Sections (80 nm) were cut with a Microstar diamond knife and placed on single slot grids (1 mm×2 mm) covered with a Formvar film. Sections were stained with Reynolds lead citrate and examined on a JEOL transmission electron microscope at 80 kV. Photographs were taken at magnifications of 10,000× to 50,000×.

Low-temperature difference spectroscopy

Cytochrome spectra of isolated mitochondria were obtained at −70 °C, as described by Ebner *et al.* (1973). The mitochondria were suspended in $40 \, \text{mmol} \, l^{-1}$ KPO_4 (pH 7.4) at $5 \, \text{mg protein ml}^{-1}$, and the cytochromes in a 5 mg sample were reduced with 15 μl of $0.5 \, \text{mol} \, l^{-1}$ sodium dithionite for 2 min at room temperature. Ethylene glycol was added to a final concentration of 30 % v/v, and the sample was loaded into a 2 mm path length cuvette. The sample was flash-frozen in liquid nitrogen, devitrified for 2 min at room temperature and refrozen for spectroscopy. All spectra were scanned using an Aminco DW-2000 spectrophotometer with the following parameters: dual beam, wavelength acquisition, filter slow, slit width 0.8 nm, monochromater 1 from 390 to 700 nm, and monochromater 2 at 577 nm. After obtaining the reduced spectrum, the sample was thawed and the cytochromes were oxidized using 20 μl of $0.5 \, \text{mol} \, l^{-1}$ potassium ferricyanide. The freezing procedure was repeated and the oxidized spectrum was subtracted from the reduced spectrum.

Biochemistry

Mitochondria were isolated from cells grown in YP medium containing 2 % galactose at 30 °C to mid-exponential phase, as described by Allen *et al.* (1995). Respiration was measured at 30 °C using a Yellow Springs Instruments model 53 oxygen monitor. For each measurement, the rate of oxygen consumption in the presence of $1 \, \text{mmol} \, l^{-1}$ potassium cyanide was subtracted from the rate of oxygen consumption in the absence of potassium cyanide. For each strain, three measurements were performed on each of two cultures grown on YP medium containing 2 % galactose. Protein concentration was determined using a bicinchoninic acid (BCA) colorimetric assay (Stoscheck, 1990).

Results

Phenotype of JM43GD100

An important prerequisite for examining whether mitochondrial respiration affects mitochondrial morphology in yeast is to choose a strain that lacks respiration but is otherwise minimally perturbed. For this analysis, we have chosen to use a strain with a mutation in a nuclear *PET* gene, *PET100*, instead of a strain with a *rho*⁰ or *rho*⁻ mitochondrial mutation because of the known side-effects of these latter mutations on the expression of genes for non-mitochondrial proteins. In addition, mitochondria in strains with these latter mutations lack a high enough membrane potential to accumulate the mitochondria-specific fluorescent vital dyes DASPMI or $DiOC_6$; hence, they cannot be easily viewed in living cells (Pringle *et al.* 1989). The product of the *PET100* gene is a protein of the inner membrane and is required for the assembly of cytochrome *c* oxidase (Church, 1996; C. Church, B. P. Goehring, S. C. Fontaine, P. Wazny, C. Dagogaard and R. O. Poyton, in preparation)

The mutant strain JM43GD100 was constructed by disrupting the *PET100* gene in its isogenic parent JM43 (Church *et al.* 1996). From Table 1, it is clear that both JM43 and JM43GD100 are capable of growth on the fermentable non-repressing carbon source galactose, but that JM43GD100 is unable to grow on a medium containing the nonfermentable carbon source glycerol as the sole carbon source. When grown on galactose, JM43 cells respire vigorously, whereas galactose-grown JM43GD100 cells are essentially devoid of mitochondrial (i.e. cyanide-sensitive) respiration (Table 1). These differences are paralled by the specific activity of cytochrome *c* oxidase in mitochondria isolated from strains JM43 and JM43GD100 (Church *et al.* 1996). From the low-temperature difference spectrum shown in Fig. 1, it is clear that

Table 1. *Growth and respiration rates of yeast strains JM43 and JM43GD100*

| Strain | Growth medium | | Cyanide-sensitive respiration | |
	Galactose	Glycerol	Respiration rate (pmol O_2 min⁻¹ μg⁻¹ cell mass)	Percentage of JM43 rate
JM43	+	+	8.6±0.3	100
JM43GD100	+	−	0.046±0.008	<0.005

Values for respiration rate are for growth on medium containing galactose and are means ± S.E.M., *N*=5.
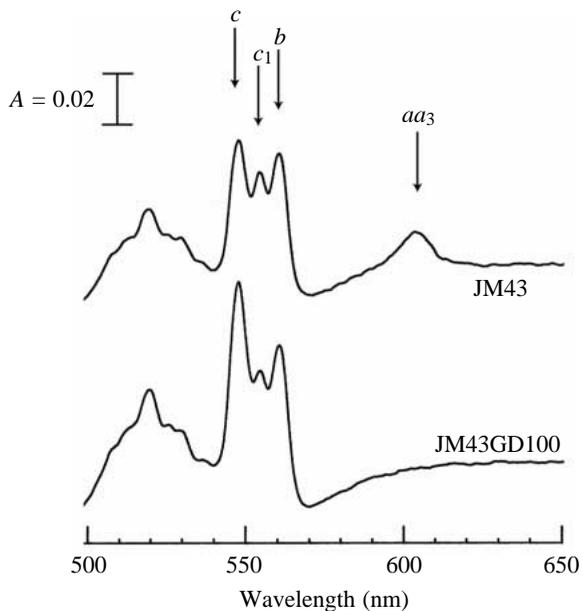
Fig. 1. Low-temperature absorption spectra of cytochromes from strains JM43 and JM43GD100. Mitochondria were isolated and low-temperature difference spectra were recorded as described in Materials and methods. The α absorption bands for cytochromes c, c_1, b and aa_3 are indicated by arrows. Top trace, JM43 ($PET100^+$); bottom trace, JM43GD100 ($pet100::LEU2$). A, absorbance in arbitrary units.

Fig. 2. Mitochondrial morphology of JM43 cells. Cells were stained with the vital fluorescent dye $DiOC_6$ and subjected to confocal laser microscopy as described in Materials and methods. The arrowheads point to mitochondria either in longitudinal section (black arrowhead) or in cross section (white arrowhead). The white arrow indicates the nuclear envelope. Scale bar, 1 μm.

the absorption band corresponding to cytochromes aa_3 is completely absent from strain JM43GD100. In contrast, both the levels and absorption maxima for other respiratory cytochromes (b, c and c_1) are unaffected. Together, these results support the conclusion (Church *et al.* 1996) that the *PET100* gene product, PET100p, is specifically required for cytochrome c oxidase biogenesis and, consequently, is essential for mitochondrial respiration.

Visualization of mitochondria in living yeast cells

Previously, several different techniques have been used to analyze mitochondrial morphology in yeast cells. These have included: ultrastructural examination of serial thin sections of fixed cells by transmission electron microscopy (Hoffman and Avers, 1973; Grimes *et al.* 1974; Stevens, 1977), examination of serial thick sections of fixed cells by high-voltage electron microscopy (Davidson and Garland, 1975) and examination of living cells stained with fluorescent vital dyes that specifically stain mitochondria (Bereiter-Hahn, 1976; Pringle *et al.* 1989) by fluorescent microscopy or confocal laser scanning microscopy. We have chosen the latter technique because it can be applied to living cells, avoids potential artifacts introduced by chemical fixation and allows observation of mitochondrial movement, fusion and division. In addition, it allows visualization of mitochondrial morphologies in many cells at the same time. To avoid morphological differences that may result from analyzing cells from different phases of growth, we have studied steady-state cells that have been
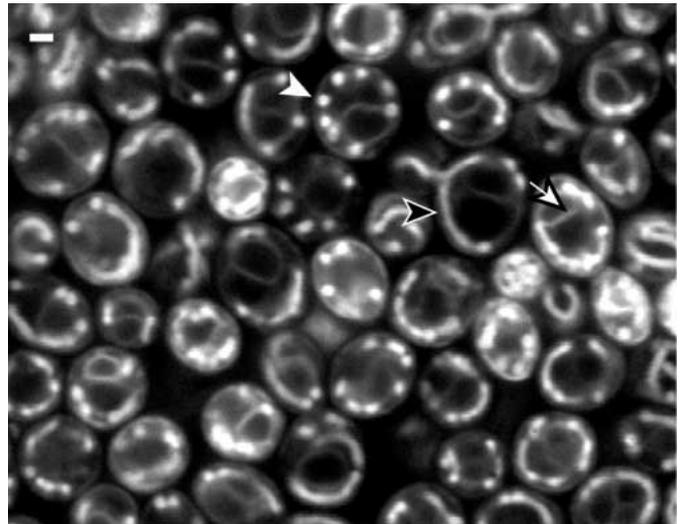
grown for at least six generations in mid-logarithmic phase by low-cell-density repeated-batch cultivation.

Two different fluorescent vital dyes, 2-(4-dimethylaminostyryl)-*N*-methylpyridinium iodide (DASPMI) and 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) have been used to visualize mitochondria in living yeast cells. Both are lipophilic cationic dyes that accumulate in the mitochondrial matrix as a result of the negative membrane potential (positive towards the cytosol) of the inner mitochondrial membrane (Pon and Schatz, 1991). JM43 cells stained with $DiOC_6$ are shown in Fig. 2. Depending upon the plane of the optical section, mitochondria appear as tubular structures or as spherical bodies located at the cell periphery. As observed previously (Pringle *et al.* 1989; Weisman *et al.* 1990; Koning *et al.* 1993), the low concentrations of $DiOC_6$ used here stain mitochondria and, to a lesser extent, the nuclear envelope (indicated by an arrow in Fig. 2). In contrast, DASPMI stains mitochondria exclusively (see below). Mitochondria stained with both dyes were highly mobile and divided and fused rapidly. Moreover, they projected into newly forming buds as they developed from their mother cell. Both dyes gave a low level of cytoplasmic staining that allowed for visualization of the periphery of the cellular cytosol. In our hands, the main difference between these two dyes was photostability: $DiOC_6$ photobleached faster that DASPMI.

Single optical sections such as those shown in Fig. 2 are inappropriate for examining overall mitochondrial morphology or volume because they do not reveal the three-dimensional structure of the mitochondria. To obtain both three-dimensional structures and total mitochondrial and cellular volumes, we reconstructed images from a series of
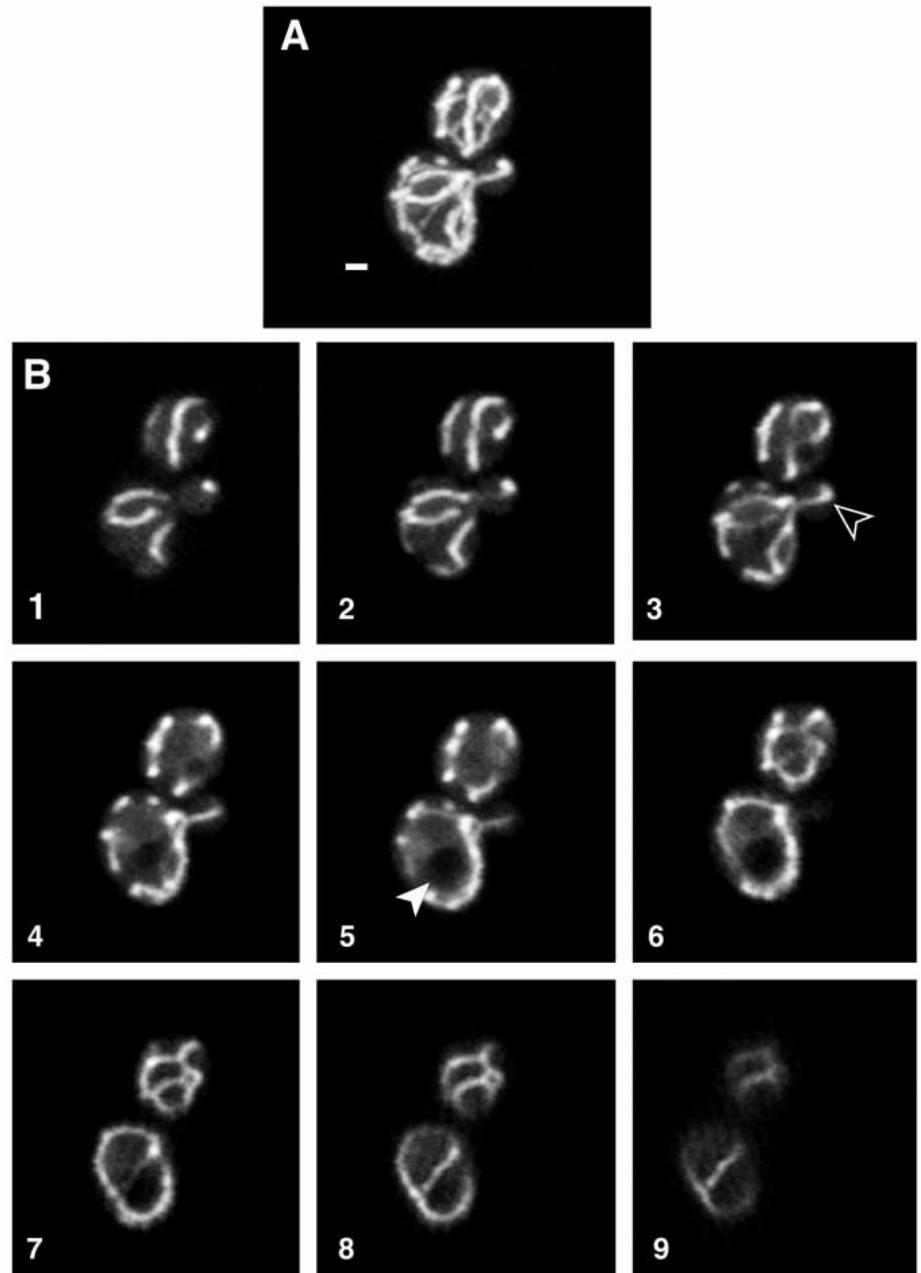
Fig. 3. Three-dimensional reconstruction of JM43 cells. Cells were stained with DASPMI, and optical sections were taken by confocal laser microscopy. (A) A three-dimensional reconstruction of a series of nine optical sections (B) taken through the cells. The thickness of each optical slice was 0.5 μm. The arrowhead in section 3 indicates a newly forming bud. The arrowhead in section 5 indicates the position of the vacuole. Scale bar, 1 μm.

optical sections through individual DASPMI-stained cells. We chose to use DASPMI, instead of DiOC₆, because it is more photostable. A three-dimensional reconstruction of JM43 cells and the nine individual 0.5 μm optical sections used to produce it is shown in Fig. 3. This illustrates the reticulate nature of mitochondria in living yeast cells and also shows the extension of part of the mitochondrial reticulum into a newly forming bud. In addition, it shows that the mitochondrial reticulum is positioned at the periphery of the cell and extends through the length of the cell. There appears to be one continuous mitochondrial reticulum in the two cells shown in Fig. 3. The area of the cell that is devoid of fluorescence (see arrow in section 5) corresponds to the vacuole.

Comparison of mitochondrial morphologies in JM43 and JM43GD100 cells

From the three-dimensional reconstructions shown in Fig. 4, it is clear that the morphologies of mitochondria in strains JM43 and JM43GD100 are indistinguishable. In both cases, the mitochondria form a reticulum that lies near the periphery of the cell and that projects into newly forming buds as a single tubule. In addition, the overall level of staining of the two strains was comparable, suggesting that mitochondria from each strain accumulate equivalent levels of dye and, by implication, had comparable membrane potentials across the inner membrane.

To examine the mitochondria in both strains more closely, we analyzed thin sections of cells from each by transmission
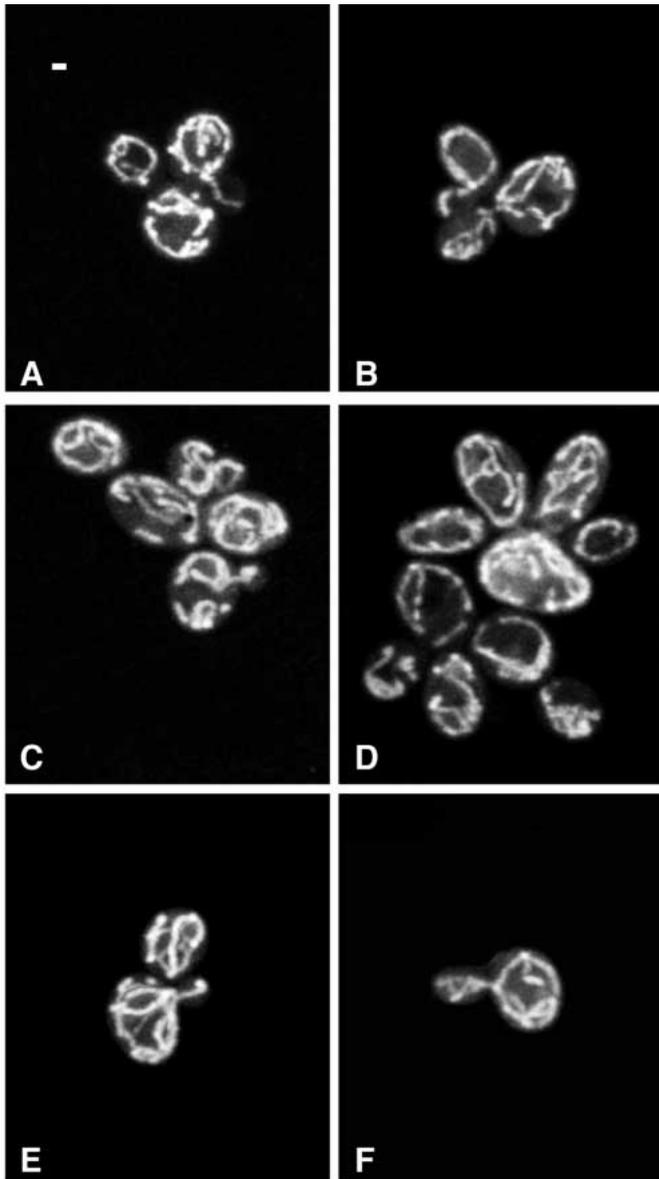
Fig. 4. Comparison of mitochondrial morphologies in JM43 and JM43GD100 cells. Three-dimensional reconstructions of DASPMI-stained JM43 cells (A,C,E) and JM43GD100 cells (B,D,F). Scale bar, 1 μm.

electron microscopy (Fig. 5). Examination of several thin sections of JM43 and JM43GD100 cells revealed long thread-like mitochondria in longitudinal section and small round mitochondria in cross section (Fig. 5A,B). Mitochondria from both strains possess easily observable inner and outer membranes and inner membrane cristae (Fig. 5C,D). The morphology of cellular structures other than mitochondria also appeared comparable in both strains.

Comparison of mitochondrial volumes in JM43 and JM43GD100 cells

Mitochondrial volumes were calculated as a percentage of total cell volume from three-dimensional reconstructions of

both strains. For this purpose, we used the bright fluorescence that labeled the mitochondrial matrix to estimate mitochondrial volume and the diffuse fluorescence of the cytosol to estimate total cell volume. The percentage of total cell volume occupied by the mitochondrial reticulum in JM43 varied from 13 to 21 %, with a mean of 18±3 % (mean ± S.D., N=8), whereas the percentage of total cell volume occupied by the mitochondrion in JM43GD100 varied from 13 to 18 % with a mean of 16±2 % (mean ± S.D., N=8). The difference in the mean volumes occupied by mitochondria between the two strains is not significant as judged by a Student's t-test (P=0.27).

Discussion

The results presented here demonstrate that neither assembled cytochrome c oxidase nor cellular respiration is required for normal mitochondrial morphology, distribution or volume in the yeast *S. cerevisiae*. These findings are of interest because they show that the previously reported alterations in mitochondrial morphology and volume brought about by changes in oxygen availability and glucose repression (Stevens, 1977, 1981; Visser *et al.* 1995) are unrelated to the presence or absence of respiration *per se*. While both oxygen concentration and a repressing carbon source, such as glucose, affect the level of respiration in yeast, they affect other non-respiratory functions as well. For example, oxygen affects the level of expression of proteins involved in oxidative stress and in the synthesis of unsaturated fatty acids, sterols and heme (Zitomer and Lowry, 1992; Bunn and Poyton, 1996; Kwast *et al.* 1998). Similarly, the glucose repression system affects the transcription of genes involved in gluconeogenesis, the Krebs cycle, peroxisome biosynthesis and β-oxidation (Entian and Barnett, 1992; Gancedo, 1992; Johnston and Carlson, 1992; Ronne, 1995). Thus, although respiration is not involved in the maintenance of mitochondrial morphology, oxygen availability and glucose repression may affect mitochondrial morphology *via* one of several other metabolic processes.

The findings reported here also reveal that fully respiring mitochondria in steady-state cells growing on the non-repressing carbon source galactose are in a reticulate structure. In general, these findings are in agreement with earlier studies (Yotsuyanagi, 1962*a*; Stevens, 1977) that have demonstrated that mitochondrial morphology is related to growth phase and that, in mid- to late-exponential phase, mitochondria are in a reticulum, irrespective of whether cells are grown on a repressing or non-repressing carbon source. Our findings do not support the conclusion that the mitochondrial reticulum occurs only in cells that have low levels of respiration and that fully respiring cells have many small individual mitochondria (Visser *et al.* 1995). As a fermentable but non-repressing carbon source, galactose does not repress the expression of mitochondrial proteins (de Winde and Grivell, 1993) and does not reduce the levels of respiration. Indeed, the galactose-grown JM43 cells used here have levels of respiration that are comparable to those found in glycerol- or ethanol-grown cells (R. O. Poyton, unpublished observations). The mitochondrial
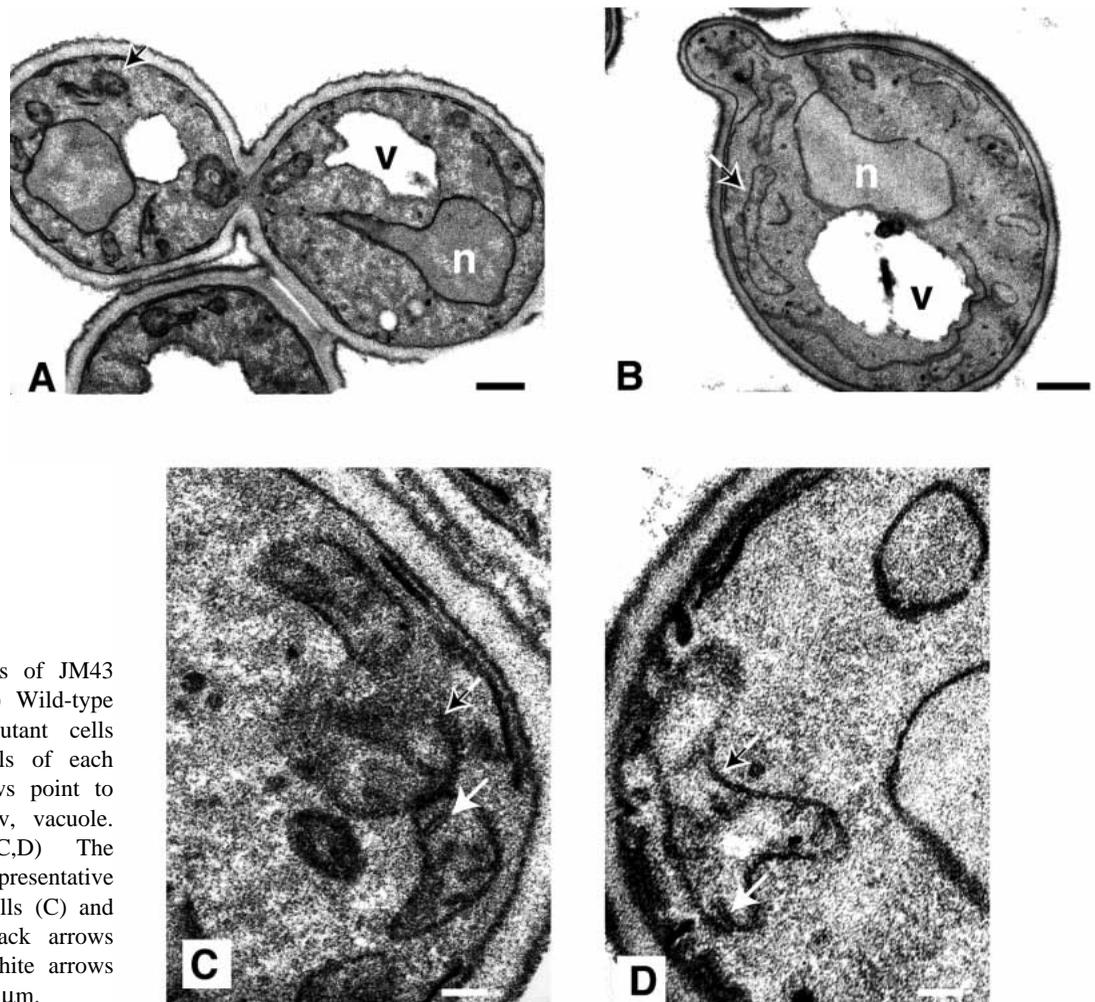
Fig. 5. Electron micrographs of JM43 and JM43GD100 cells. (A) Wild-type cells (JM43) and (B) mutant cells (JM43GD100). Budded cells of each type are shown. The arrows point to mitochondria. n, nucleus; v, vacuole. Scale bars, 0.5 μm. (C,D) The morphology of individual representative mitochondria from JM43 cells (C) and JM43GD100 (D) cells. Black arrows denote mitochondria and white arrows denote cristae. Scale bars, 0.1 μm.

volume in these cells is 18 % of the total cell volume. This is somewhat higher than the value reported previously for derepressed respiring cells (Stevens, 1977). This difference is probably attributable to differences in the variables chosen to calculate mitochondrial and cell volume. Here, we have chosen to use the fluorescence of the mitochondrial matrix as a marker for mitochondrial volume and the fluorescence of the cytosol as a marker for cell volume. In contrast, the previous study (Stevens, 1977) used digitized tracings of the entire mitochondrial and cell volumes.

Our observation that mitochondrial morphology is normal in the absence of respiration or functional cytochrome *c* oxidase is interesting in the context of the principle of symmorphosis (Weibel *et al.* 1991), which states that 'no more structure is formed and maintained than is required to satisfy functional needs'. Indeed, this finding suggests that mitochondrial structure is formed and maintained for reasons other than the respiratory function of mitochondria. What function could necessitate the formation and maintenance of the unusual reticulate mitochondrial structure observed in yeast cells? In considering this question, it is useful to note that in addition to being the intracellular site for oxidative phosphorylation

mitochondria also function in the synthesis of heme, amino acids and pyrimidines. These functions make mitochondria essential components of eukaryotic cells, whether they are grown oxidatively or anaerobically (Baker and Schatz, 1991). It is possible that the unusual morphology exhibited by yeast mitochondria is linked to one of these functions. However, it seems more likely that mitochondrial morphology is linked to mitochondrial inheritance, i.e. to the passage of mitochondria from one generation to the next (Yaffe, 1991; McConnell *et al.* 1990). This conclusion is supported by the recent discovery of several genes (*MMM1*, *MDM10*, *MDM12*, *YME1*, *MRS11*, *MDM1* and *MDM2*) that are required for mitochondrial inheritance and the observation that the mutants in each of these genes alter mitochondrial morphology dramatically. Mutants in many of these genes are temperature-sensitive for the transfer of mitochondria into growing buds. At the non-permissive temperature, the mitochondrial reticulum fragments and the mitochondrial vesicles aggregate into a large mitochondrion in the mother cell. One of these genes (*MDM1*) encodes an intermediate filament-like protein that is distributed throughout the cytosol (McConnell and Yaffe, 1992, 1993; Fisk and Yaffe, 1997), while others (*MMM1*, *MDM10* and

MDM12) encode proteins of the outer mitochondrial membrane (Burgess *et al.* 1994; Sogo and Yaffe, 1994; Berger *et al.* 1997), the inner mitochondrial membrane (*YME1*) (Campbell *et al.* 1994) and the intermembrane space (*MRS11*) (Jarosch *et al.* 1997). In addition, *MDM2* encodes fatty acid desaturase (Stewart and Yaffe, 1991). Current studies are focused on how mitochondrial morphology and division are linked and how these proteins function in these processes (Yaffe, 1996).

References

ALLEN, L. A., ZHAO, X. J., CAUGHEY, W. AND POYTON, R. O. (1995). Isoforms of yeast cytochrome *c* oxidase subunit V affect the binuclear reaction center and alter the kinetics of interaction with the isoforms of yeast cytochrome *c*. *J. biol. Chem.* **270**, 110–118.

ATTARDI, G. AND SCHATZ, G. (1988). Biogenesis of mitochondria. *A. Rev. Cell Biol.* **4**, 289–333.

BAKER, K. P. AND SCHATZ, G. (1991). Mitochondrial proteins essential for viability mediate protein import into yeast mitochondria. *Nature* **349**, 205–208.

BAWA, S. R. AND WERNER, G. (1988). Mitochondrial changes in spermatogenesis of the pseudoscorpion *Diplotemnus* sp. *J. Ultrastruct. molec. Structure Res.* **98**, 281–293.

BEREITER-HAHN, J. (1976). Dimethylaminostyrylmethylpyridinium-iodine (DASPMI) as a fluorescent probe for mitochondria *in situ*. *Biochim. biophys. Acta* **423**, 1–14.

BEREITER-HAHN, J. (1990). Behavior of mitochondria in the living cell. *Int. Rev. Cytol.* **122**, 1–63.

BEREITER-HAHN, J. AND VOTH, M. (1983). Metabolic control of shape and structure of mitochondria *in situ*. *Biol. Cell* **47**, 309–322.

BEREITER-HAHN, J. AND VOTH, M. (1994). Dynamics of mitochondria in living cells: shape changes, dislocations, fusion and fission of mitochondria. *Microsc. Res. Tech.* **27**, 198–219.

BERGER, K. H., SOGO, L. F. AND YAFFE, M. P. (1997). Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* **136**, 545–553.

BOISSIN, L. AND MANIER, J. F. (1966). Spermatogenese d'*Hysterochelifer meridianus* (L. Koch) (Arachnida, Pseudoscorpion: Cheliferidae). II. Etude de l'evolution du chondriome. *Bull. Soc. Zool. Fr.* **91**, 697–706.

BUNN, H. F. AND POYTON, R. O. (1996). Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* **76**, 1–47.

BURGESS, S. M., DELANNOY, M. AND JENSEN, R. E. (1994). *MMM1* encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* **126**, 1375–1391.

CAMPBELL, C. L., TANAKA, N., WHITE, K. H. AND THORSNESS, P. E. (1994). Mitochondrial morphological and functional defects in yeast caused by yme1 are suppressed by mutation of a 26S protease subunit homologue. *Molec. Biol. Cell* **5**, 899–905.

CHURCH, C. (1996). The role of a nuclear *PET* gene in the assembly of cytochrome *c* oxidase in *Saccharomyces cerevisiae*. PhD dissetation, University of Colorado.

CHURCH, C., CHAPON, C. AND POYTON, R. O. (1996). Cloning and characterization of *PET100*, a gene required for the assembly of yeast cytochrome *c* oxidase. *J. biol. Chem.* **271**, 18499–18507.

CRIDDLE, R. S. AND SCHATZ, G. (1969). Promitochondria of anaerobically grown yeast. I. Isolation and biochemical properties. *Biochemistry* **8**, 322–334.

DAVIDSON, M. T. AND GARLAND, P. B. (1975). Mitochondrial structure studied by high voltage electron microscopy of thick sections of *Candida utilis*. *J. gen. Microbiol.* **91**, 127–138.

DE WINDE, J. H. AND GRIVELL, L. A. (1993). Global regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*. *Prog. Nucleic Acids Res. molec. Biol.* **46**, 51–91.

EBNER, E., MASON, T. L. AND SCHATZ, G. (1973). Mitochondrial assembly in respiration-deficient mutants of *Saccharomyces cerevisiae*. II. Effect of nuclear and extrachromosomal mutations on the formation of cytochrome c oxidase. *J. biol. Chem.* **248**, 5369–5378.

ENTIAN, K. D. AND BARNETT, J. A. (1992). Regulation of sugar utilization by *Saccharomyces cerevisiae*. *Trends biochem. Sci.* **17**, 506–510.

FISK, H. A. AND YAFFE, M. P. (1997). Mutational analysis of Mdm1p function in nuclear and mitochondrial inheritance. *J. Cell Biol.* **138**, 485–494.

GALANGAU, V. AND TUZET, O. (1968). Les mitochondries pendant la spermatogenese d'*Octopus vulgaris* L: Recherche au microscope electronique. *C.R. hebd. Séanc. Acad. Sci. Paris D* **267**, 1735–1737.

GANCEDO, J. M. (1992). Carbon catabolite repression in yeast. *Eur. J. Biochem.* **206**, 297–313.

GRIMES, G. W., MAHLER, H. R. AND PERLMAN, P. S. (1974). Nuclear gene dosage effects on mitochondrial mass and DNA. *J. Cell Biol.* **61**, 565–574.

HITANO, K. AND UEDA, K. (1988). Changes in shape of mitochondria during the asexual reproductive cycle in *Hydrodictyon reticulatum*. *Eur. J. Cell Biol.* **47**, 193–197.

HOFFMAN, H. P. AND AVERS, C. J. (1973). Mitochondrion of yeast: ultrastructural evidence for one giant, branched organelle per cell. *Science* **181**, 749–751.

JAROSCH, E., RODEL, G. AND SCHWEYEN, R. J. (1997). A soluble 12-kDa protein of the mitochondrial intermembrane space, Mrs11p, is essential for mitochondrial biogenesis and viability of yeast cells. *Molec. gen. Genet.* **255**, 157–165.

JOHNSTON, M. AND CARLSON, M. (1992). Regulation of carbon and phosphate utilization. In *The Molecular and Cellular Biology of the Yeast* Saccharomyces. *Genome Dynamics, Protein Synthesis and Energetics* (ed. J. R. Broach, J. R. Pringle and E. W. Jones), pp. 193–281. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

KIRKWOOD, S. P., MUNN, E. A. AND BROOKS, G. A. (1986). Mitochondrial reticulum in limb skeletal muscle. *Am. J. Physiol.* **251**, C395–C402.

KONING, A. J., LUM, P. Y., WILLIAMS, J. M. AND WRIGHT, R. (1993). DiOC$_6$ staining reveals organelle structure and dynamics in living yeast cells. *Cell Motil. Cytoskel.* **25**, 111–128.

KREMER, J. R., MASTRONERDE, D. N. AND McINTOSH, J. R. (1996). Computer visualization of three-dimensional image data using IMOD. *J. Struct. Biol.* **116**, 71–76.

KWAST, K. E., BURKE, P. V. AND POYTON, R. O. (1998). Oxygen

sensing and the transcriptional regulation of oxygen-responsive genes in yeast. *J. exp. Biol.* **201**, 1177–1195.

LEWIS, M. R. AND LEWIS, W. H. (1914). Mitochondria (and other cytoplasmic structures) in tissue cultures. *Am. J. Anat.* **17**, 339–401.

MCCONNELL, S. J., STEWART, L. C., TALIN, A. AND YAFFE, M. P. (1990). Temperature-sensitive yeast mutants defective in mitochondrial inheritance. *J. Cell Biol.* **111**, 967–976.

MCCONNELL, S. J. AND YAFFE, M. P. (1992). Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the MDM1 protein. *J. Cell Biol.* **118**, 385–395.

MCCONNELL, S. J. AND YAFFE, M. P. (1993). Intermediate filament formation by a yeast protein essential for organelle inheritance. *Science* **260**, 687–689.

MUNN, E. A. (1974). *The Structure of Mitochondria*. New York: Academic Press Inc. 1–465pp.

PLATTNER, H., SALPETER, M. M., SALTZGABER, J. AND SCHATZ, G. (1970). Promitochondria of anaerobically grown yeast. IV. Conversion into respiring mitochondria. *Proc. natn. Acad. Sci. U.S.A.* **66**, 1252–1259.

PLATTNER, H. AND SCHATZ, G. (1969). Promitochondria of anaerobically grown yeast. III. Morphology. *Biochemistry* **8**, 339–343.

PON, L. AND SCHATZ, G. (1991). Biogenesis of yeast mitochondria. In *The Molecular and Cellular Biology of the Yeast* Saccharomyces. *Genome Dynamics, Protein Synthesis and Energetics* (ed. J. R. Broach, J. R. Pringle and E. W. Jones), pp. 333–406. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

POYTON, R. O. AND MCEWEN, J. E. (1996). Crosstalk between nuclear and mitochondrial genomes. *A. Rev. Biochem.* **65**, 563–607.

PRINGLE, J. R., PRESTON, R. A., ADAMS, A. E., STEARNS, T., DRUBIN, D. G., HAARER, B. K. AND JONES, E. W. (1989). Fluorescence microscopy methods for yeast. *Meth. Cell Biol.* **31**, 357–435.

RONNE, H. (1995). Glucose repression in fungi. *Trends Genet.* **11**, 12–17.

SHYJAN, A. W. AND BUTOW, R. A. (1993). Intracellular dialogue. *Curr. Biol.* **3**, 398–400.

SOGO, L. F. AND YAFFE, M. P. (1994). Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* **126**, 1361–1373.

STEVENS, B. J. (1977). Variation in number and volume of mitochondria in yeast according to growth conditions: A study based on serial sectioning and computer graphic reconstruction. *Biol. Cell* **28**, 37–49.

STEVENS, B. J. (1981). Mitochondrial structure. In *The Molecular Biology of the Yeast* Saccharomyces. *Life Cycle and Inheritance* (ed. J. R. Broach, J. R. Pringle and E. W. Jones), pp. 471–504. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

STEWART, L. C. AND YAFFE, M. P. (1991). A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J. Cell Biol.* **115**, 1249–1257.

STOSCHECK, C. M. (1990). Quantitation of protein. *Meth. Enzymol.* **182**, 50–68.

TANDLER, B. AND HOPPEL, C. L. (1972). *Mitochondria*. New York: Academic Press. 59pp.

TERASAKI, M. (1989). Fluorescent labeling of endoplasmic reticulum. *Meth. Cell Biol.* **29**, 125–135.

VISSER, W., VAN SPRONSEN, E. A., NANNINGA, N., PRONK, J. T., GIJS KUENEN, J. AND VAN DIJKEN, J. P. (1995). Effects of growth conditions on mitochondrial morphology in *Saccharomyces cerevisiae*. *Antonie Van Leeuwenhoek* **67**, 243–253.

WALTON, J., YOSHIYAMA, J. M. AND PACKER, L. (1981). The existence of mitochondrial networks in rat skeletal muscle. *J. Cell Biol.* **91**, 285a.

WEIBEL, E. R., TAYLOR, C. R. AND HOPPELER, H. (1991). The concept of symmorphosis: a testable hypothesis of structure–function relationship. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10357–10361.

WEISMAN, L. S., EMR, S. D. AND WICKNER, W. T. (1990). Mutants of *Saccharomyces cerevisiae* that block intervacuole vesicular traffic and vacuole division and segregation. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1076–1080.

YAFFE, M. P. (1991). Organelle inheritance in the yeast cell cycle. *Trends biochem. Sci.* **1**, 160–164.

YAFFE, M. P. (1996). The division and inheritance of mitochondria. *Adv. molec. cell. Biol.* **17**, 337–346.

YOTSUYANAGI, Y. (1962a). Etudes sur le chondriome de la levure. I. Variation de l'ultrastructure du chondriome au cours du cycle de la croissance aerobie. *J. Ultrastruct. Res.* **7**, 121–140.

YOTSUYANAGI, Y. (1962b). Etudes sur le chondriome de la levure. II. Chondriomes des mutants a deficience respiratoire. *J. Ultrastruct. Res.* **7**, 141–158.

ZITOMER, R. S. AND LOWRY, C. V. (1992). Regulation of gene expression by oxygen in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **56**, 1–11.