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DEGRADATION AND RESTORATION OF MITOCHONDRIA UPON DEAERATION AND SUBSEQUENT AERATION OF AEROBICALLY GROWN *SACCHAROMYCES CEREVISIAE* CELLS

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SUMMARY

Changes in the mitochondria of aerobically grown *Saccharomyces cerevisiae* cells upon deaeration and subsequent aeration of the medium were studied.

1. It is shown that removal of oxygen at the end of the exponential phase of growth (after completion of mitochondria formation) causes a decrease in activity of the respiratory enzymes. The activity of the complete respiratory system decreases much more rapidly than the activities of its fragments (NADH : ferricyanide reductase, succinate : ferricyanide reductase, NADH : cytochrome *c* reductase, succinate : cytochrome *c* reductase and cytochrome oxidase). The activities are restored to their initial level upon aeration of the cell suspension. The addition of Tween-80 and ergosterol to the medium prior to deaeration does not prevent inactivation of the respiratory system.

All the changes in mitochondria described occurred under conditions where cell division was insignificant.

2. Deaeration of the medium decreases the content of cytochromes *b* and aa_3 in the mitochondrial fraction, cytochrome aa_3 "disappearing" more quickly. The concentration of cytochromes in this fraction increases upon subsequent aeration of the cells. The total cytochromal content of the cells remains practically unchanged under the same conditions.

3. According to electron microscopic data, anaerobiosis causes a certain disorganization of mitochondrial cristal membranes. The mitochondrial structures are recovered upon aeration of the yeast cell suspension. It may be reasoned that inactivation and reactivation of the respiratory system are associated with reversible changes in mitochondrial membrane structure.

4. The effect of protein synthesis inhibitors on the restoration of mitochondria was investigated. It is shown that chloramphenicol does not suppress this process. In the presence of cycloheximide, oxygen induces reactivation of the respiratory system and simultaneously the appearance of particles resembling mitochondria. However, these particles gradually undergo morphological changes and the respiratory activity of the mitochondrial fraction decreases. Cycloheximide added to yeast cells that had not been deaerated, did not affect their mitochondria.

5. The results described suggest that the functions of oxygen in the formation of mitochondria are not restricted to the induction of mitochondrial protein synthesis

and to the participation in the synthesis of certain non protein membrane components. Evidently, oxygen has a direct effect on the assembly of the respiratory system and mitochondrial membranes as a whole.

INTRODUCTION

The study of the biogenesis of mitochondria has in recent years become one of the urgent trends of molecular biology. Important data on the formation of mitochondria were obtained as a result of investigations of the adaptive development of respiration in facultative anaerobic yeast. It was shown that if grown in the absence of oxygen, the latter does not contain cytochromes *b*, c_1, *c* and aa_3, or substantial amounts of the flavine enzymes of the respiratory system[1–4]. No fully developed mitochondrial structures are found in such cells[4–8], but they arise simultaneously with the respiratory system components upon aeration of the medium[1,2,5,7,9]. The nature of this process is not known in detail. There are grounds to think that mitochondria appear during oxygen adaptation or glucose derepression as a result of transformation of the preceding subcellular particles ("promitochondria") or specific membrane systems[8,10–12,27]. Of special interest is the role of oxygen in the formation of mitochondria. It is known to take part in the synthesis of heme[13], as well as of ergosterol and unsaturated fatty acids[14–16], which are needed for the formation of mitochondrial membranes[8,17]. It has been suggested that oxygen may be an inductor of respiratory enzyme synthesis[1,9,18]. However, it is quite possible that oxygen also fulfills other, as yet unkown functions.

One of the possible ways of ascertaining these functions is to study the changes mitochondria undergo during the incubation of aerobically grown yeast cells under anaerobic conditions. No detailed investigations have been carried out so far in this line. The only data that can be cited are those of EPHRUSSI AND SLONIMSKI[1], MEISEL *et al.*[19], and CHAPMAN AND BARTLEY[20], according to whom anaerobiosis causes certain degradational changes in mitochondria. In the present work these changes are studied in more detail. The new functions of oxygen were detected in a study of the restoration of mitochondria. The experiments showed that under certain conditions oxygen is capable of inducing reactivation of the respiratory system and the appearance of membrane structures resembling mitochondria even in the presence of protein synthesis inhibitors (chloramphenicol and cycloheximide).

MATERIALS AND METHODS

Organism

Saccharomyces cerevisiae, wild-type diploid, strain N 11 of the National Collection of Yeast Cultures was used in all the experiments. The organism was maintained aerobically on agar slopes containing: 1.5 % agar–agar, 0.2 % yeast extract (Koch–Light Laboratories), and 0.25 % glucose. The yeast was grown for 24 h at 30° and subcultured monthly.

Growth conditions

The inoculum was grown for 24 h at 30° in a medium containing 2.5 g glucose, 2 g yeast extract, 1 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.5 g NaCl, and 0.5 g $MgSO_4 \cdot 7H_2O$

(per l of water). 50 ml of the inoculum (250–280 mg of moist yeast) was added to 6 l of fresh medium of the composition indicated above. The biomass was grown at 35° in a 10-l fermenter with constant stirring and aeration at a rate of 1 l air per l medium per min. The growth phase was determined by counting the number of cells in a Thomas–Goryayev Chamber or by weighing the biomass contained in 100 ml of suspension. The cell respiration was measured simultaneously. At the end of the exponential growth phase (12–13 h) the suspension was taken from the fermenter in the amount needed for isolation of the mitochondria and electron-microscopic control of the cells. Then argon (containing not more than 0.001 % oxygen) was passed through the fermenter for 3 h, after which the suspension was aerated for another 3 h. To avoid respiratory adaptation of the cells incubated in anaerobic conditions the samples were taken in an intensive stream of argon while cooling the suspension rapidly to 0–2°.

Isolation of mitochondria

Mitochondria were isolated by the KOVÁČ[21] method, using lyophilized snail (*Helix pomatia*) gut juice for destroying the cell envelopes, or by the TZAGOLOFF[22] method, destroying the cells in a homogenizer (14000 rev./min) after freezing them in liquid nitrogen.

Estimation of protein

Mitochondrial protein was determined after the method of LOWRY *et al.*[23].

Enzyme assays

The NADH oxidase, succinate oxidase, NADH:cytochrome *c* reductase and succinate:cytochrome *c* reductase activities of the mitochondria were measured under the same conditions as were used by MACKLER *et al.*[24]. These activities (in the order indicated above) were equal to 700–800 nmoles NADH and 300–350 nmoles succinate, oxidized at 30° per min per mg protein; 600–700 and 160–170 nmoles cytochrome *c* reduced per min per mg protein. The activities given pertain to mitochondria isolated by the TZAGOLOFF[22] method. The NADH oxidase and succinate oxidase activities of the mitochondria produced according to KOVÁČ *et al.*[21] were 250–320 nmoles NADH and 120–150 nmoles succinate oxidized per min per mg protein.

NADH:ferricyanide reductase and succinate:ferricyanide reductase activities were measured under the conditions described by RAO *et al.*[25]. For the mitochondria isolated by the method of TZAGOLOFF these activities were 1500–1900 and 400–500 nmoles of $K_3Fe(CN)_6$ reduced at 30° per min per mg protein, respectively.

The cytochrome oxidase activity measured as described in an earlier paper[26], was 120–150 nmoles cytochrome *c* oxidized at 30° per min per mg protein in the case of the mitochondria isolated by the method of KOVÁČ *et al.*[21]. The activity of mitochondria produced by the method of TZAGOLOFF[22] was 2.5–3.0 times higher.

Measurement of cytochrome concentrations

Cytochrome concentrations in the mitochondrial fractions were estimated by recording the differences in absorbance at 605 and 630 nm in the case of cytochrome aa_3 or at 562 and 575 nm for cytochrome *b*. The measurements were carried out with

a dual-wavelength Hitachi 356 spectrophotometer. The cytochromes were reduced with dithionite. $\Delta\varepsilon$ (605–630 nm) and $\Delta\varepsilon$ (562–575 nm) were taken equal to 12.0 $mM^{-1} \cdot cm^{-1}$ and 19.1 $mM^{-1} \cdot cm^{-1}$, respectively.

The difference low-temperature spectra of yeast cells were recorded with a DSF-1M (U.S.S.R.) spectrophotometer. The cell suspension taken at the times indicated from the fermenter was centrifuged in the cold. The cells were suspended in cold 10% argon-saturated sucrose. The resulting suspension (200 mg yeast per ml) was immediately frozen in special cuvettes with liquid nitrogen, dithionite being added preliminarily to the control cuvette.

Electron microscopy

The whole cells were fixed with permanganate as described earlier[5]. The conditions of fixation of the anaerobically incubated cells were the same as those for anaerobically grown *S. cerevisiae* in the study referred to above. Microphotographs of the cell sections were made with an HU-11B (Hitachi) electron microscope.

RESULTS

Changes in enzyme activities of mitochondria during deaeration and subsequent aeration of a yeast cell suspension

Fig. 1 shows the effect of anaerobiosis on the respiratory system of aerobically grown yeast cells. The medium was deaerated at the end of the exponential growth phase, when the yeast mass concentration, whole cell respiration and respiratory activity of the mitochondrial fraction had reached their limiting values. Under the

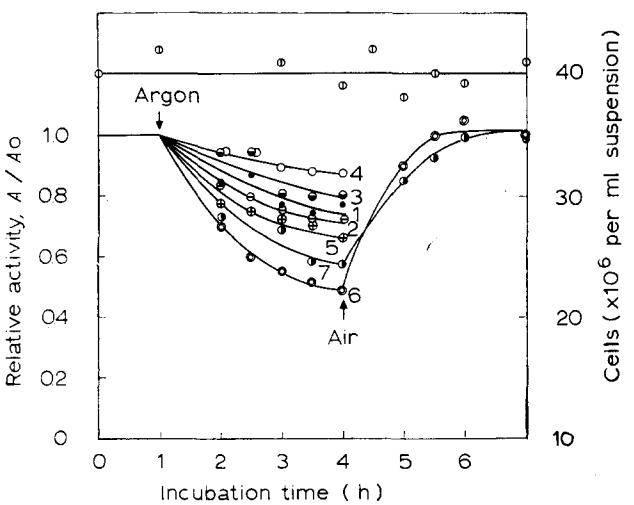

Fig. 1. Changes in activities of certain mitochondrial enzymes upon deaeration and subsequent aeration of yeast cells. Yeast cells were subjected to anaerobiosis at the end of the exponential growth phase. At the times indicated in the figure the required amounts of the cell suspension were drawn off, mitochondria were isolated and the activities indicated below were assayed. A/A_0 is the ratio of current to initial specific activity. The A_0 for all activities are given in the experimental section. Curve 1, NADH:ferricyanide reductase; 2, succinate ferricyanide reductase; 3, NADH:cytochrome *c* reductase; 4, succinate:cytochrome *c* reductase; 5, cytochrome oxidase; 6, NADH oxidase; 7, succinate oxidase.

conditions indicated the NADH oxidase and succinate oxidase activities of the mitochondria decreased considerably, whereas the succinate dehydrogenase, NADH dehydrogenase, succinate:cytochrome *c* reductase, NADH:cytochrome *c* reductase and cytochrome oxidase activities decreased more slowly. These functions could be completely restored by merely aerating the cells without transferring them to a new medium.

Anaerobic incubation of the yeast cells in a medium containing Tween-80 and ergosterol did not prevent the decrease in NADH oxidase and succinate oxidase activities of the mitochondria (Fig. 2). It follows from this experiment that inactivation of the respiratory system was not due to the shortage of unsaturated fatty acids and ergosterol in the cells. However, this fact does not eliminate the possibility of the lipid composition of mitochondrial membranes changing when the cells are incubated under anaerobic conditions.

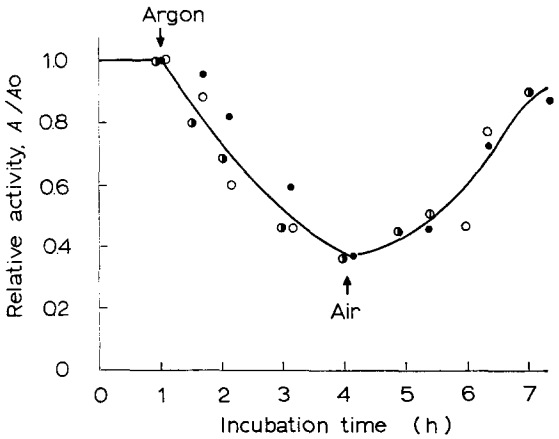

Fig. 2. Changes in NADH oxidase activity of the mitochondrial fraction upon deaeration and subsequent aeration of yeast cells in the presence of Tween-80 and ergosterol. Conditions as in Fig. 1. Tween-80 (5 ml/l) and ergosterol (20 mg/l) were added to the medium just before starting the delivery of argon. The figure gives the results of different experiments. Temperature, 30°.

In contrast to previous studies of adaptive changes in yeast cells[20,27] this experiment was carried out under conditions where the yeast mass concentration remained practically unchanged, *i.e.* under conditions of very insignificant cell division (see Fig. 1). Enzyme activities were measured not in cell suspensions or cell homogenates, as previously described[1,2,5,18,20], but directly in the mitochondrial fraction. The results were independent of the method by which the mitochondria were isolated. It may therefore be considered that they reflect the actual changes in enzyme activities of the mitochondrial fraction.

The decrease in the respiratory activity shown in Fig. 1 was observed at the end of the exponential phase when glucose had been exhausted and the ethanol accumulated could not be utilized due to the absence of oxygen. One could believe that the changes in the respiratory system are the result of starvation of the cells rather than of the specific effect of anaerobiosis. However, it was shown by special experiments that deaeration of yeast at the beginning of the exponential phase, when the glucose concentration is high enough, causes greater degradation of the respiratory system as compared to what happens at the end of this phase.

The results obtained could be associated either with certain degradational changes in the mitochondria or with dilution of the mitochondrial fraction with extraneous particles. The second assumption seems rather improbable. If follows from Fig. 1 that 3 h of anaerobiosis decreased the specific activities of certain mitochondrial respiratory enzymes (*e.g.* NADH dehydrogenase and succinate dehydrogenase) by not more than 20–25 %. This means that under our experimental conditions the mitochondrial fraction was diluted by not more than 20–25 %, if at all. Since the rate of the respiratory chain inactivation was much higher, it might have been due to certain changes in the multi-enzyme system.

According to CHAPMAN AND BARTLEY[20], respiratory enzyme activities assayed in cell homogenates decrease when *S. cerevisiae* cells are grown under conditions repressing their respiratory system.

Measurement of cytochrome contents in whole cells and mitochondria

The experimental data obtained in measuring the cytochromes *b* and aa_3 content in the mitochondrial fraction isolated from aerobically grown yeast cells before and after incubation in the absence of oxygen, disclose to a certain extent one of the reasons for the decrease in respiratory activity of the mitochondria. It follows from Table I that 3 h anaerobiosis lowers the content of cytochromes *b* and aa_3 in the mitochondrial fraction by 34 and 42 %, respectively. Subsequent aeration enriches this fraction in cytochromes.

TABLE I

EFFECT OF ANAEROBIOSIS AND SUBSEQUENT AERATION OF YEAST CELLS ON THE CYTOCHROME CONTENT IN MITOCHONDRIA

The table gives the data of 4 experiments. Mitochondria were isolated after TZAGOLOFF[22] in all cases. Experiments with mitochondria isolated after KOVÁČ *et al.*[21] gave similar results.

Conditions of yeast cell growth	*NADH oxidase activity of mitochondrial fraction* (%)	*Cytochrome content of mitochondrial fraction (nmoles/mg protein)*	
		Cytochrome b	*Cytochrome aa_3*
Aerobic cells (12th h of growth)	100	0.55–0.60	0.28–0.30
Cells after 3 h anaerobiosis (15th h of growth)	45–50	0.38–0.39	0.16–0.18
Cells after 3 h aeration (18th h of growth)	100	0.57	0.24–0.27

Spectrophotometric analysis of cytochromes in yeast cells gave different results. Fig. 3 shows the difference low-temperature spectra of *S. cerevisiae* cells and of the same cells incubated for 4 h in anaerobic conditions. The spectra practically do not differ from each other. It follows that when the medium is deaerated cytochromes *b*, *c* ($+c_1$), and aa_3 are not destroyed. This conclusion was confirmed by control dual-wavelength spectrophotometric measurements of cytochrome aa_3 content in the cell homogenates under conditions similar to those described above for the mitochondrial fraction. Our results correlate with the data of CHIN[2], according to

which aeration of resting brewer's yeast cells results in synthesis of respiratory chain cytochromes, which do not disappear upon subsequent deaeration.

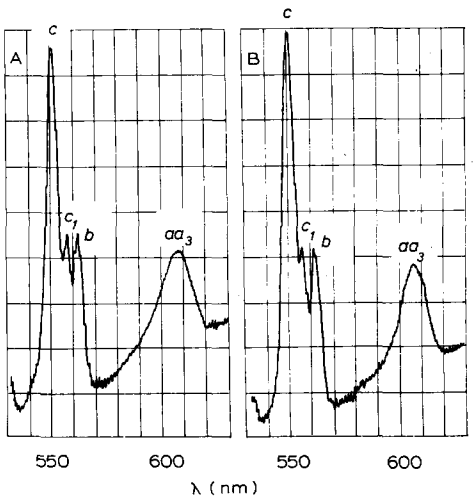

Fig. 3. Difference low-temperature spectra of *S. cerevisiae* cells. A. Cell collected at the end of the exponential growth phase. B. After 4 h incubation in anaerobic conditions.

As was indicated in the previous section, under anaerobic conditions the mitochondrial fraction is diluted by not more than 20 or 25 %, if at all. Hence spectrophotometric data suggest that under the conditions indicated mitochondria lose a certain amount of cytochromes. The effect described is not due to the destruction of the cytochromes and is evidence of some degradational changes in the cristal membranes.

Effect of cycloheximide and chloramphenicol on restoration of the respiratory system

It has been shown that protein synthesis inhibitors (cycloheximide and chloramphenicol) depress adaptive development of the respiratory system in yeast cells[34,35]. Specifically inhibiting the cytoplasmic protein-synthesizing system, the former prevents incorporation of amino acids into soluble mitochondrial proteins[28–31]. The latter predominantly suppresses the synthesis of mitochondrial membrane proteins[29–33].

It follows from Fig. 4 that chloramphenicol does not hinder recovery of NADH oxidase activity of the mitochondria of yeast cells aerated after 3 h anaerobiosis. The results given in Fig. 4, like the spectrophotometric data described above, are evidence that restoration of the respiratory system does not require additional cytochrome synthesis*.

In contrast to chloramphenicol, cycloheximide had a substantial and peculiar effect on the restoration of the respiratory system of mitochondria (Fig. 5). When the yeast cells were incubated with cycloheximide at the end of the exponential growth phase under aerobic conditions, the respiratory activity of the mitochondria remained unchanged during at least 6 h. The addition of cycloheximide simultaneously with the

* When added to the medium in the beginning of the exponential phase of growth, chloramphenicol suppressed cytochrome synthesis in the yeast culture used exactly as was observed earlier by HUANG *et al.*[33].

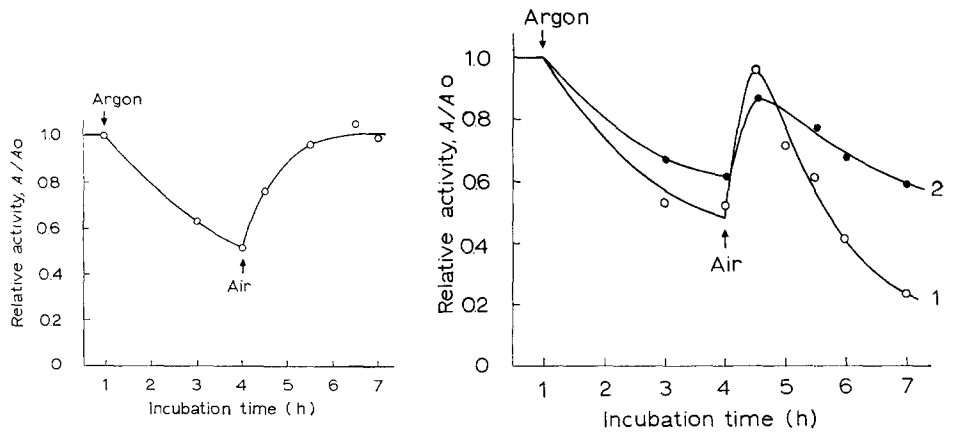

Fig. 4. Restoration of respiratory system in the presence of chloramphenicol. Conditions as in Fig. 1. Chloramphenicol (4 g/l) was added to the medium before starting to deliver air. The NADH oxidase activity of the mitochondrial fraction was measured.

Fig. 5. Effect of cycloheximide on the restoration of the respiratory system. Conditions as in Fig. 1. Cycloheximide (25 mg/l) was added to the medium before starting to deliver air. Curve 1, NADH oxidase activity of mitochondria; 2, cytochrome oxidase activity.

start of argon delivery or directly before subsequent delivery of air (see Fig. 5) resulted in specific suppression of restoration. In any of these experiments the respiratory activity of the mitochondria at first grew to its normal level and then decreased rapidly. A similar decrease was observed when cycloheximide was used together with chloramphenicol. Since the effect of cycloheximide depended little on the moment it was added to the medium, the increase in activity at the initial stage of restoration cannot evidently be associated with the time it takes the inhibitor to penetrate into the yeast cells. Most probably these facts, together with previous results (see Figs. 3 and 4), mean that the initial stage of restoration of the respiratory system, being induced by oxygen, may be accomplished under conditions excluding protein synthesis. The effect of cycloheximide is discussed in greater detail in the next section.

CRIDDLE AND SCHATZ[11] have shown that incorporation of [^{14}C]lysine in the pro-mitochondrial fraction during respiratory adaptation of anaerobically grown *S. cerevisiae* cells is suppressed under certain conditions by cycloheximide and is resistant to chloramphenicol. However, according to later data of ROUSLIN AND SCHATZ[34] and PINUS *et al.*[35], adaptation is accomplished neither in the presence of cycloheximide, nor of chloramphenicol. Both inhibitors, as HENSON *et al.*[30] discovered, also prevent incorporation of amino acids into the mitochondria of derepressing yeast cells. The discrepancies between the data mentioned and the results of this study are due to the fact that the development of respiration in cells grown anaerobically (or aerobically under conditions of glucose repression) is impossible without synthesis of cytochromes and other respiratory system components, whereas restoration of the respiratory system probably does not require synthesis of these components.

Morphological changes in mitochondria during deaeration and subsequent aeration of yeast cells. Effect of cycloheximide

Fig. 6A is an electron microphotograph of a *S. cerevisiae* cell grown under aerobic conditions (end of exponential growth phase). The microphotograph shows distinctly

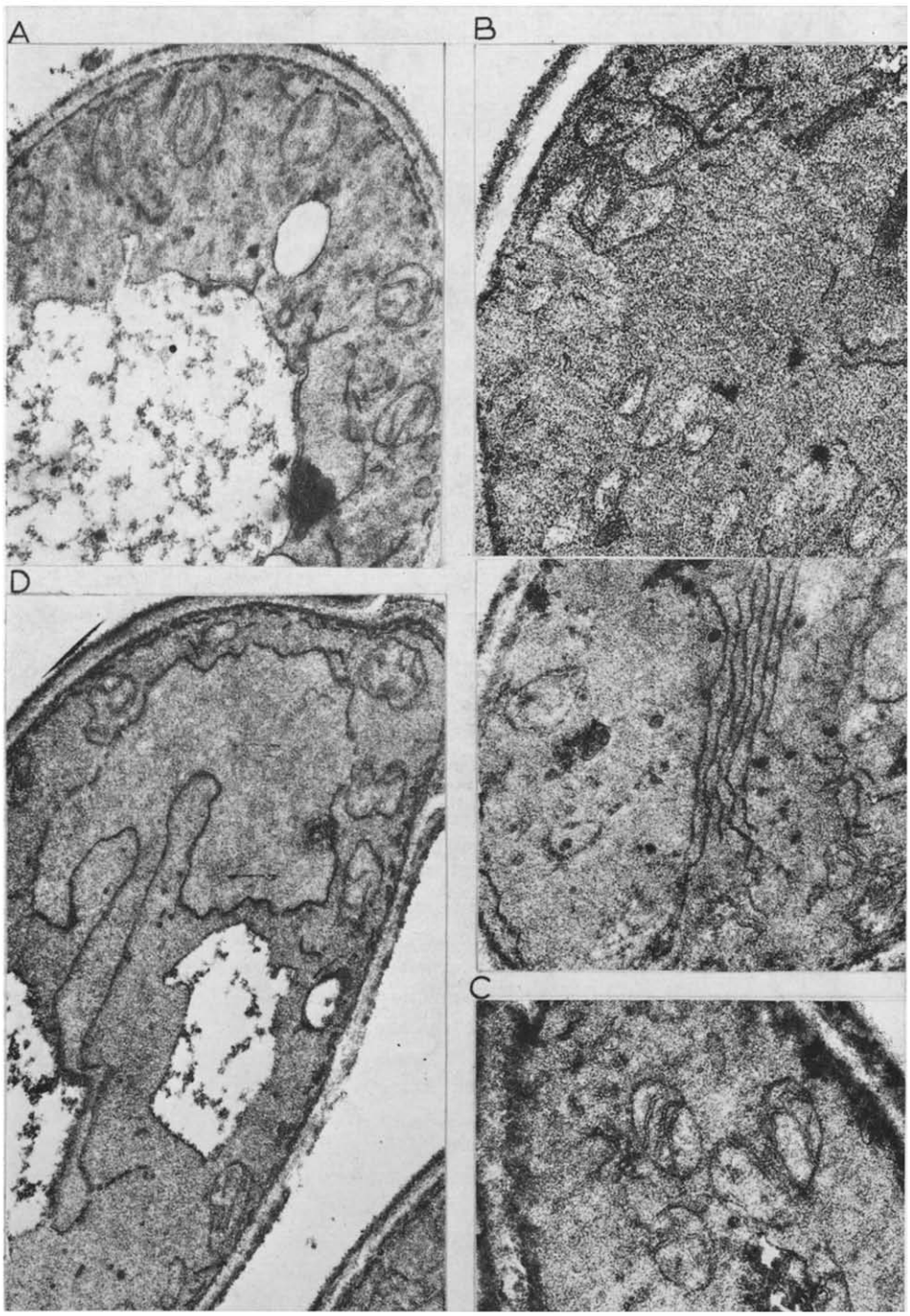

Fig. 6. Degradation and restoration of mitochondria of yeast cells according to electron microscopic data. A. Cells collected at the end of the exponential phase of growth. Mitochondria are arranged around the periphery of the cells. B. After 3 h incubation in anaerobic conditions. Cristae in mitochondria are reduced. The amount of mitochondrial structures is decreased. C. After 0.5 h aeration. Characteristic mitochondrial structures appear. D. After 3 h aeration. Restored mitochondria are arranged around the cell periphery. × 48000.

the cell wall, the plasma membrane, the nucleus, the vacuole, some free cytoplasmic membranes and mitochondria situated on the cell periphery. The cristae can be seen in the mitochondria. Analogous data regarding the ultrastructure of yeast cells were given previously by other authors[36–38]. After 3 h anaerobic incubation the cell morphology changed substantially (Fig. 6B). In most mitochondria the cristae became indiscernible and the number of organelles that could be qualified as mitochondria decreased. At the same time free cytoplasmic membranes appeared in a large quantity. The cells subjected to deaeration resembled *Candida parapsilosis* cells grown at low oxygen pressure[39]. Right after the delivery of air was started the cytoplasmic membranes began to rearrange themselves. In as little as 30 min after the beginning of aeration, structures could be distinguished, resembling mitochondria (Fig. 6C). After 3 h aeration the cells practically differed no longer from the initial ones (Fig. 6D).

These data are in accord with the results given in Fig. 1 and in Table I. The decrease in specific respiratory activity of the mitochondria upon incubation of aerobically grown yeast cells in the absence of oxygen appears to be correlated directly with certain degradational changes in the cristal membranes. The increase in respiratory activity upon subsequent aeration is related to the restoration of the mitochondrial structures.

Fig. 7 gives the results obtained in a study of the effect of cycloheximide on the restoration of mitochondria. Aeration of yeast cells in the presence of cycloheximide for 30 min gave rise to membrane structures (Fig. 7A) similar to those observed in the previous experiment (Fig. 6C). It is evident from Figs. 1, 4, 5, 6C and 7A that the initial stage of restoration occurred so rapidly that it is difficult to imagine that any biosynthetic processes are needed to accomplish it. However, subsequently cycloheximide began to show a considerable effect. After 4 h elongated irregularly shaped "giant mitochondria" appeared in the cytoplasm (Fig. 7B). Longer incubation gave rise mainly to free cytoplasmic membranes in the cells (Figs. 7C and 7D). Control experiments showed that the mitochondria did not undergo such changes on prolonged incubation of aerobically grown cells with cycloheximide*, unless the cells were preliminary deaerated.

Thus, under the experimental conditions described cycloheximide did not suppress the initial stage of restoration, but hindered the appearance of stable mitochondrial structures. It is usually assumed in *in vivo* experiments that cycloheximide acts only on the protein- and RNA-synthesizing systems[11,30,40]. If this is so, the effect of cycloheximide on the restoration of mitochondria in yeast cells was due to suppression of the synthesis of certain proteins, which play an important part in integration of the mitochondrial structure. It is interesting that under certain conditions chloramphenicol also prevents mitochondria formation, and the effects of both protein synthesis inhibitors at the morphological level are very similar. As KELLERMAN *et al.*[39] have shown, *C. parapsilosis* cells grown in the presence of chloramphenicol contain defective mitochondria, resembling those visible in Fig. 7B.

However, other explanations of the effect of cycloheximide are not impossible. In particular, it should be noted that cycloheximide can influence the synthesis of

* Since the respiratory activity and structure of the mitochondria did not change upon incubation of aerobically grown yeast cells with chloramphenicol and/or cycloheximide, it may be thought that no substantial renewal of mitochondrial proteins occurred after 12 h of growth of the culture.

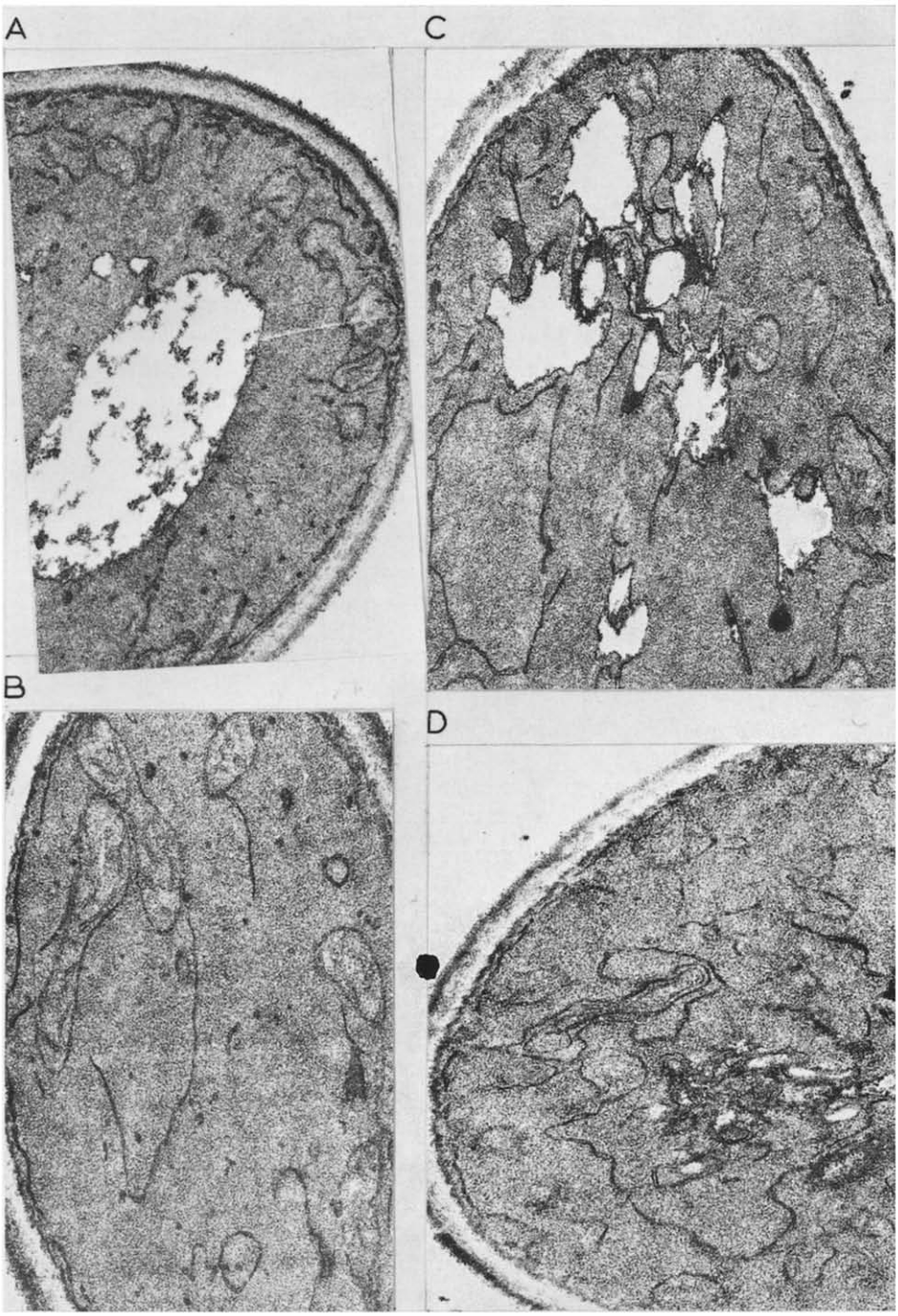

Fig. 7. Effect of cycloheximide on the restoration of mitochondria according to electron microscopic data. Conditions as in Fig. 5. A. After 30 min aeration in the presence of cycloheximide following 3 h anaerobiosis. Structures resembling mitochondrial structures can be seen (for comparison see Figs. 6B and 6C). B. After 4 h aeration. "Giant mitochondria" appear. C and D. After 5 and 6 h aeration. Content of free cytoplasmic membranes increases. Mitochondrial structures are practically invisible. × 48000.

certain lipids[14,41]. Besides, REILLY *et al.*[42] found recently that cycloheximide is capable of acting in some way on the cell membrane, disturbing the uptake of K^+ and phosphate ions by yeast cells. If cycloheximide is able to modify other membranes as well, it may be assumed that intact mitochondria are stable against cycloheximide, whereas the membrane structures arising at the first stage of restoration of the mitochondria, are destroyed in its presence. This assumption seems rather speculative at present, since there are as yet no detailed data on the interaction of cycloheximide with membrane system components.

Interpretation of the electron microscopic data given in this section encounters certain difficulties. On the one hand, these data may indicate that cristal membranes are partially fragmented in anaerobic conditions. On the other, it is quite possible that the membranes are preserved but lose their ability to bind permanganate owing to certain changes in composition (*e.g.* owing to the splitting of phospholipids[43,44]). This may be the case with "pro-mitochondria", which are difficult to detect when yeast cells are fixed with permanganate[6,8,11,17,45]. Finally, it is not impossible that anaerobiosis labilizes the structure of the membranes and they are decomposed upon fixation[8,45]. Despite these difficulties it can be asserted definitely that deaeration of yeast cells results in reversible disorganization of the fine structure of mitochondrial membranes. This can be seen particularly vividly in the experiments with cycloheximide, which, while not affecting the respiratory system functions, prevents restoration of mitochondrial structures in aerobic conditions.

DISCUSSION

The purpose of our work was to establish certain details of mitochondrial transformations during deaeration and subsequent aeration of aerobically grown yeast cells. According to enzyme assays, spectrophotometric measurements and electron microscopic data, the fine structure of intact mitochondrial cristal membranes is disorganized in anaerobic conditions. More precise information on the nature of these changes is lacking.

In a general way the phenomena described above resemble the data of JAYARAMAN *et al.*[27] obtained in a study of glucose repression and subsequent derepression of the respiratory system of *S. cerevisiae*. However, at present it is difficult to say whether there is any definite inherent relation between these effects.

The results of this study are evidence that stabilization of mitochondria by deaeration of the medium, which HUNTER *et al.*[46] observed *in vitro*, is impossible under the cytoplasm conditions. It was found recently that ischemia induces inactivation of the oxidative phosphorylation system and gradual destruction of mammalian cell mitochondria[43,44]. However, it is difficult to suppose that these experiments were carried out strictly *in vivo*.

Of definite interest are data concerning the restoration of mitochondria. Upon aeration of the medium, *i.e.* under conditions where the rate of respiration in the mitochondrial fraction increased and particles with the characteristic structure of mitochondria appeared in the previous amount, we did not find the large dividing mitochondria which JAYARAMAN *et al.*[27] observed upon derepression of the respiratory system of *S. cerevisiae*. The results of this work as a whole favour the idea that restoration of mitochondria involved only reorganization of the membrane structures,

synthesis of the lacking components and transformation of "defective" mitochondria into normal particles. The experimental conditions proposed hold promise for studying certain interesting details of mitochondria assembly. In particular, they make it possible to extend the ideas of the role played by oxygen in this process. Previously it was assumed that during respiratory adaptation of yeast cells oxygen induces synthesis of respiratory enzymes[1,18], and takes part in the synthesis of heme[13], unsaturated fatty acids and ergosterol[14–16], contained in mitochondrial membranes. However, in the case considered in this investigation all these components were evidently already present in the cytoplasm before the second aeration of the medium began. Hence, the functions of the oxygen differed from those enumerated above. It can be seen distinctly in Figs. 6C and 7A that oxygen (or the processes in which it is involved) in some way induce rapid reconstruction of membrane structures, the synthesis of new proteins not being necessary at this stage of mitochondria restoration. The mechanism of this phenomena is still obscure. It is true to say that one might refer to the results of model experiments, from which it follows that conditions favouring electron transfer are necessary for active respiratory ensembles to form[47,48] and stabilize[49–51]. However, despite their definite relation to the problem under discussion, these data seem insufficient for a complete understanding of the reasons for the effect of oxygen on the formation of mitochondrial structures. The problem unquestionably deserves further study.

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