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CHARACTERISTICS OF DEVELOPING MITOCHONDRIAL GENETIC AND RESPIRATORY FUNCTIONS IN GERMINATING FUNGAL SPORES*

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SUMMARY

Spores of the fungus *Botryodiplodia theobromae* began a cyanide-sensitive oxygen consumption immediately upon exposure to a liquid medium, and spore germination and respiration were not affected by ethidium bromide, D-threochloramphenicol, and acriflavin until later during germ tube emergence. These inhibitors of the mitochondrial genetic system all inhibited total cell protein synthesis to the same intermediate degree from the outset of incubation. When spores were incubated in water under non-germinating conditions, protein synthesis and oxygen uptake proceeded at initial rates almost identical to those seen in spores germinating in the presence of the three mitochondrial system inhibitors. Although the spores respired at rapid rates from the onset of incubation, no cytochrome absorption peaks could be observed in mitochondrial fractions prepared from ungerminated spores; they were readily observed in germinated spores, however. When the spores were germinated in the presence of inhibitors of the mitochondrial system, an excess of cytochrome *c* was observed in the near absence of cytochromes *a* and *b*. The results indicate that the ungerminated spores of this organism contain a preserved, potentially functional aerobic respiratory system which requires cycloheximide-sensitive ribosome activity to become functional when the spores are inoculated into a liquid medium.

INTRODUCTION

The ordered transition of metabolically quiescent fungal spores to rapidly-growing cells provides an attractive and convenient experimental system for study of certain processes of eukaryotic cell development and metabolic regulation. In particular, these spores may be especially useful for an examination of mitochondrial biogenesis in cells which are obligately aerobic and which may be semi-synchronized in cell growth and organelle assembly.

Several studies have been reported in recent years which describe some activ-

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ities of nucleo-cytoplasmic systems for nucleic acid and protein syntheses during fungal spore germination (for a review, see ref. 1). However, little is known about the counterpart activities of the mitochondrial genetic system or about the contribution made by the products of this organelle genetic system toward mitochondrial assembly in germinating spores. At least some ungerminated fungal spores, such as the conidia of *Neurospora crassa* [2, 3] and *Trichoderma viride* [4], contain mitochondria which appear near normal morphologically, and they do not resemble the rudimentary "promitochondria" observed in anaerobic *Saccharomyces cerevisiae* cells [5]. It is not clear, however, whether these spore mitochondria are comparable metabolically to those of growing cells; nor is it understood what is required metabolically for the mitochondria of ungerminated spores to resume their normal genetic and respiratory activities.

A general, long-term objective of our study of fungal spore germination is to describe the cooperation that must exist between the nuclear and mitochondrial systems for transcription and translation of genetic information necessary for organelle assembly during germination and to identify the contributions of each system toward assembly and function of the mitochondrial respiratory apparatus. We also wish to establish whether mitochondria of ungerminated spores are functionally complete organelles and how they may differ biochemically from the mitochondria of physiologically active cells. A specific objective of this present study is to describe some of the mitochondrial genetic and respiratory activities during spore germination; these observations rely primarily upon measurement of the effects of presumed chemical inhibitors of the mitochondrial genetic system upon total spore protein synthesis, aerobic respiration, and respiratory cytochrome incorporation. Evidence is presented in this report that the ungerminated conidiospores of *Botryodiplodia theobromae* (a homothallic ascomycete) contain a potentially functional aerobic respiratory system which becomes active upon inoculation of the spores into a liquid medium. Mitochondrial genetic activity is not required for this cyanide-sensitive respiration or for germination, although function of cytoplasmic ribosomes is essential.

MATERIALS AND METHODS

Germination of spores

Conidiospores of *B. theobromae* were collected from the parent mycelium (grown for 3 weeks on V-8 juice agar) by flooding the cultures with sterile, distilled water, and, after agitation, the spores were rapidly harvested free of contaminating mycelium and pycnidia by serial filtration of the crude suspension through cheesecloth, a milk filter, and Whatman No. 4 filter paper. The ingredients of the nutrient medium (in g/l) were: glucose (10), KNO_3 (1), $MgSO_4 \cdot 7HOH$ (0.5), KH_2PO_4 (0.65), K_2HPO_4 (0.35), NaCl (0.1), a complete animal diet vitamin supplement (Nutritional Biochemicals; 1 g/l); standard trace element solution [6], 0.5 % Tween 80, and a few drops (per l) of Dow Antifoam A. The water incubation medium also contained 0.5 % Tween 80 and the antifoam. The spores were incubated or germinated in multi-baffled 500 ml Erlenmeyer flasks in a Controlled Environment Gyrotory Incubator Shaker (Model G-45) or a Gyrotory Water Bath Incubator (Model G-76) of the New Brunswick Scientific Co. The rotary platform speed was 300 rev./min and the temperature was 34 °C; the flasks each contained 150 ml of medium and the concentration of the

spores was 1 mg/ml medium (1.5×10^5 spores/ml). When antibiotics (obtained from Sigma or CalBiochem) were present they were added to the medium immediately before spore inoculation, and unless otherwise indicated, the spores were exposed to the drugs throughout incubation or germination.

In vivo protein synthesis assays

The procedures used to measure rates of protein synthesis in the spores during germination by pulse-labeling techniques have been described previously [7]. The L-[U-^{14}C]leucine was obtained from New England Nuclear Corp., and the specific activities were 311 or 327 Ci/mol; the isotope concentration was 0.05 μCi/ml spore suspension. The liquid scintillation spectrometry was performed in a Nuclear Chicago 6848 with an efficiency of about 75 %.

Preparation of mitochondrial fraction and cytochrome absorption spectra studies

The procedures for obtaining mitochondrial fractions from ungerminated and germinated spores were identical. The spores were disrupted in a Braun MSK mechanical homogenizer (Bronwill Scientific Co.); 3–4 g of spores were combined with 27 g of 1 mm glass beads and 10 ml of extraction buffer, and the homogenizer flasks were shaken at 4000 rev./min. for 60 s with simultaneous cooling with a stream of CO_2. The homogenized cell suspensions were rinsed from the MSK flasks, and the cell debris supernatant fluid was decanted; the homogenizer beads were extracted repeatedly with extraction buffer and the washings were pooled with the first supernatant suspension and then centrifuged for 10 min at $270 \times g$ (g values are g_{av}). The resulting supernatant fluid was then centrifuged for 30 min at $27\,000 \times g$; the pelleted material was resuspended, and these two cycles of centrifugation were repeated. The extraction buffer contained sucrose (0.25 M), Na_2EDTA (0.001 M), and Tris · Cl (0.05 M); the pH was adjusted to 7.5. The final pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4) and combined with sufficient sodium deoxycholate (about 2 mg/mg protein) to lyse the mitochondria in a motor-driven Potter-Elvehjem tissue homogenizer (Arthur Thomas Co.). This suspension was centrifuged at $27\,000 \times g$ for 30 min and the resulting supernatant fluid between the meniscus skin and the loose pellet was drawn off with a pipette. All extractions and centrifugation manipulations were performed at 0–3 °C. Protein was determined by the method of Lowry et al. [8]. The lysed mitochondrial preparations used in the cytochrome spectroscopic assays contained 5–8 mg protein/ml.

The oxidized vs. reduced difference spectra of the mitochondrial fractions were recorded on a Cary 14 spectrophotometer with a 0–0.1 absorbance slide wire. Each of the two cuvettes contained 1 ml total volume with 0.5 ml of sample solution, and the oxidized and reduced cells contained 0.1 ml each of potassium ferrycianide (0.05 M) and sodium ascorbate (0.05 M), respectively; a few grains of sodium dithionite were added to the sample cell immediately before recording the spectra between 650 and 520 nm at room temperature. It was assumed that the cytochromes a, b, and c should have α absorption peaks at 609 nm, 560 nm, and 550 nm, respectively.

Oxygen uptake rate determinations

Polarographic measurements of oxygen consumption were made with oxygen electrodes installed in a dual-probe Biological Oxygen Monitor (Model 53; Yellow

Springs Instrument Co.) calibrated with air-saturated distilled water at 34 °C so that a 100 mV signal giving a full-scale deflection corresponded to a concentration of 5.1 μl of oxygen per ml of medium (at 34 °C). At 30 min intervals during germination, 3-ml aliquots of the air-saturated spore suspensions were transferred from the incubation flasks to the probe chambers, and consumption of oxygen by two samples were recorded simultaneously for 10 min in chambers sealed with the electrode probes and maintained at 34 °C with a circulating water bath. Calculations of oxygen consumption rates were derived from initial slope linearity, and all plots of these data were corrected for oxygen uptake which occurred in the presence of 0.5 mM NaCN.

All the experiments described in this report were performed two or more times, and the examples cited represent typical results.

RESULTS

General characteristics of B. theobromae spore germination

The term germination sequence will refer to a 240-min period which included a pre-emergence phase (about 150 min) during which none of the spores showed germ tubes and an emergence phase (about 90 min) during which 90–95 % of the spores developed elongating germ tubes under the specified incubation conditions in nutrient medium. A spore was scored as germinated when the tip of the emerging germ tube was clearly distinguishable from the surface of the surrounding spore wall. The dry weight of the spores did not change during the 240 min of incubation, but by 8.5 h the dry weight increased about 1.5 times. If the spores were incubated with inhibitors of the mitochondrial genetic system, ethidium bromide (5 μg/ml), acriflavin (20 μg/ml), or D-threochloramphenicol (3 mg/ml), the rates of germination were comparable to those of spores incubated in the absence of the drugs. However, after prolonged incubation (6–10 h) with these inhibitors, the germ tube hyphae were distorted morphologically and culture dry weight values were reduced sharply. Germination was inhibited completely by cycloheximide at 25 μg/ml if the drug was applied during the first 135 min of incubation; if cycloheximide was added to the spore suspension after this time, the spores began to germinate with normal kinetics, although the number of spores germinated was about 30 % lower than that of untreated spores. Germination was inhibited completely by treatment of the spores (from time of inoculation) with antimycin A (50 μg/ml) or with 0.5 mM NaCN. The spores germinated normally in a medium in which the glucose was replaced by either 2 % (w/v) potassium acetate or 2 % (v/v) glycerol. If the spores were incubated in distilled water or distilled water containing 0.5 % (v/v) Tween 80, fewer than 5 % of the spores developed short germ tubes (although some metabolic activities were begun, as described below), and if the water-incubated spores were collected and transferred to a nutrient medium, they germinated with kinetics identical to those of spores inoculated directly into nutrient medium (with the 150 min lag before germ tube emergence). After extended incubation (8–10 h) in water, the spores became pigmented and developed septa through a step (or series of steps) which was inhibited by cycloheximide. Cytological observations of the water-mediated spore conversion in this fungus have been published [9].

In vivo protein synthesis during germination

The kinetics of [^{14}C]leucine incorporation into acid-insoluble fractions of the

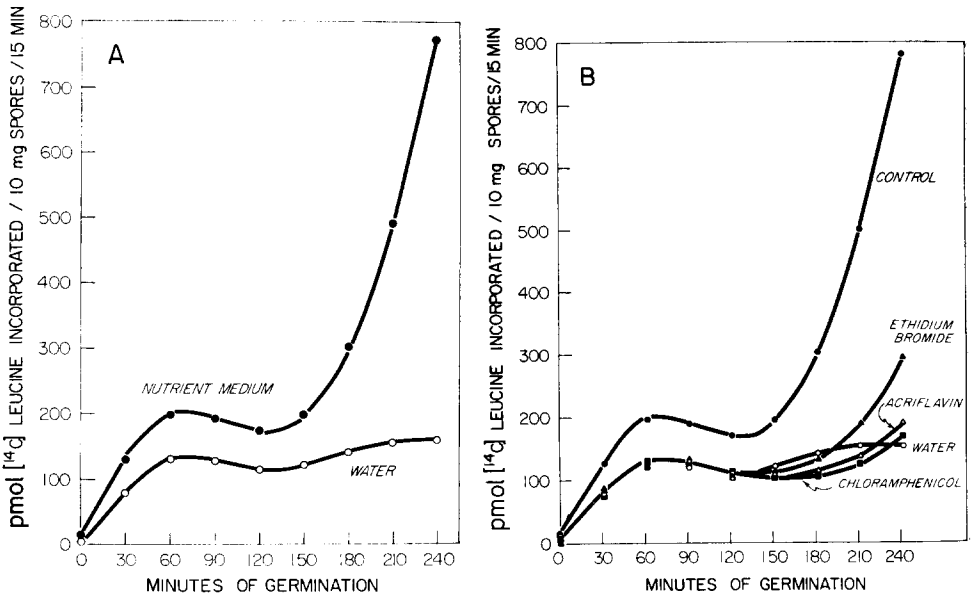

Fig. 1. Rates of incorporation (measured by pulse-labeling techniques) of [^{14}C]leucine into spores incubating (A) in nutrient medium (●) or in water (○), and (B) in nutrient medium with ethidium bromide (▲), acriflavin (△), or chloramphenicol (■) or in water (○). The correction for zero-time incorporation in all these graphs was always less than 5 pmol per assay.

spores during germination are shown in Fig. 1A; although measured under modified germination conditions, this incorporation kinetics curve is very similar to that which we previously described for this organism [7]. The rates of incorporation of [^{14}C] leucine indicate that this protein synthesis began immediately upon inoculation of the spores into the germination medium, and it was abolished if cycloheximide was present throughout incubation. This protein synthesis continued for a period in the apparent absence of RNA synthesis, and, based on evidence reported earlier [7], these spores contain a latent messenger RNA (in polyribosomes) which is translated upon the onset of spore incubation. The labeled leucine was incorporated almost entirely into the cellular fractions chemically identified as protein.

Such pulse-labeling experiments necessarily measure rates of whole cell synthetic activity, and they do not discriminate between activities of the nucleocytoplasmic and the mitochondrial genetic systems. However, because treatment of the germinating spores with presumed inhibitors of the mitochondrial genetic system might help to identify the contribution of the mitochondrial translational system to the total observed protein synthesis, the spores were germinated in the continuous presence of ethidium bromide, acriflavin, or chloramphenicol and the rates of total protein synthesis were measured at 30-min intervals. As shown in Fig 1B, the presence of any one of these three inhibitors reduced appreciably the amount of [^{14}C] leucine incorporated into the spores as compared to the untreated spores; the eventual degree of inhibition at 240 min differed somewhat among the drugs, but in all cases the degree of inhibition of total protein synthesis during the first 120 min of germination were identical.

In other experiments (not described here), the fraction of the total spore pro-

tein synthesis resistant to a 2-min pre-incubation with cycloheximide increased from 6 % at 15 min to 11 % at 60 min and 13 % at 240 min of germination.

If the spores were incubated in water under conditions which did not permit germination, they immediately began to incorporate [^{14}C]leucine into protein, but at a rate somewhat lower than that of the spores incubated from the outset in a nutrient medium, and the second, more rapid protein synthesis was not begun. The rate of this protein synthesis in water-incubated spores also is shown in Figs. 1A and B, and it can be seen that the kinetics of protein synthesis of these spores incubated under nutritionally restrictive conditions are very similar to those of spores germinating in a nutrient medium but with a chemically-inhibited mitochondrial genetic system (Fig. 1B). The rates of protein synthesis in water-incubated spores could not be reduced further by treatment with ethidium bromide, chloramphenicol, or acriflavin. In the water, neither nuclear nor mitochondrial DNA syntheses occur (unpublished data, Brambl, R.), and as we reported previously [7] no RNA synthesis is begun.

Cytochrome spectra

Oxidized vs. reduced difference spectra of mitochondrial fractions were recorded in order to establish whether significant differences existed in the cytochrome content of germinated and ungerminated spores and whether inhibition of the mitochondrial genetic system with ethidium bromide, acriflavin, or chloramphenicol would alter the patterns of development of respiratory cytochromes early in growth.

In Fig. 2A are shown the spectra obtained from detergent-lysed mitochondria

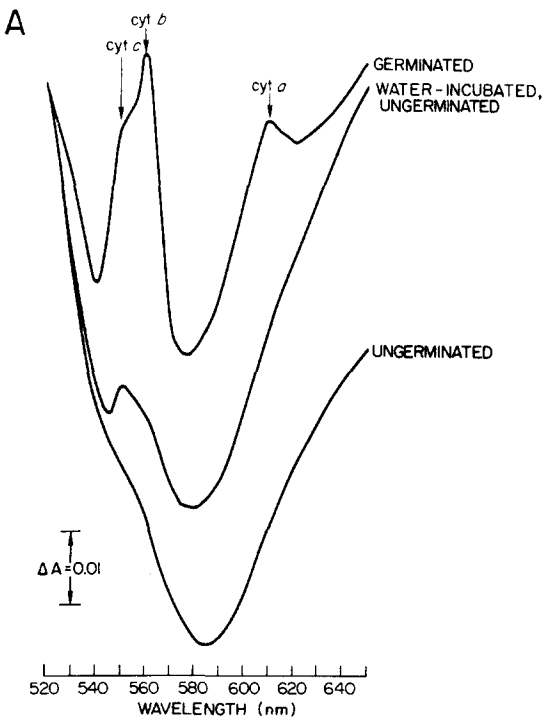

Fig. 2A See opposite page for legend.

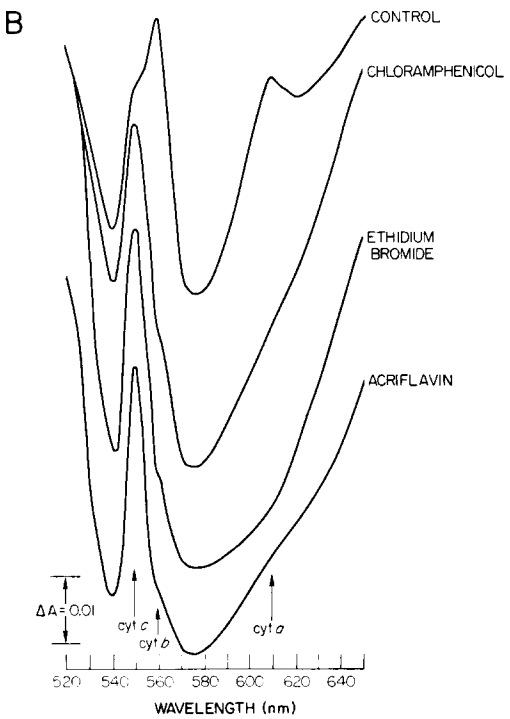

Fig. 2. Oxidized vs reduced cytochrome absorption difference spectra of mitochondrial fractions prepared (A) from germinated, ungerminated, or ungerminated water-incubated spores, or (B) from spores germinated in the continuous presence of chloramphenicol, ethidium bromide, or acriflavin; the control spectrum refers to untreated spores. These spectra were recorded at room temperature, and only the α absorption peaks of the cytochromes are shown.

fractions of ungerminated and germinated (15 h) spores and of spores incubated in water (10 h). The germinated spore mitochondria possessed the α absorption peaks which are characteristic of the cytochrome fractions observed in other mycelial fungi [10, 11]. The ungerminated spores, however, had levels of respiratory cytochromes which were undetectable by the present technique. If the spores were incubated for a prolonged period (10 h) in distilled water under standard conditions of temperature and aeration, an absorption peak of cytochrome *c* was seen; no absorption peaks of cytochromes a or b were observed unequivocally in this spore fraction.

In other pulse-labeling experiments (data not shown), δ-[^{14}C]aminolevulinic acid was incorporated at high rates into the spores after 150 min of germination. The incorporation into acid-insoluble material was sensitive to ethidium bromide, acriflavin, and chloramphenicol and did not occur in the water-incubated spores. Provided that this label was incorporated primarily into mitochondrial heme, this information suggests that new respiratory cytochromes may not be synthesized until after the second phase of accelerated protein synthesis was begun.

Mitochondria from spores which had been germinated (15 h) in the presence of presumed inhibitors of the mitochondrial genetic system exhibited the patterns of cytochrome absorption shown in Fig. 2B. In the presence of ethidium bromide (5 μg/ml), acriflavin (20 μg/ml), or chloramphenicol (3 mg/ml), the responses were

similar in that excesses of cytochrome *c* were present in the mitochondrial fractions in comparison to the apparently diminished quantities of cytochromes *a* and *b*. These results suggest that in the absence of a completely functional mitochondrial genetic system, the germinating spores incorporated only cytochrome *c* into the mitochondria and that the other cytochromes were incompletely synthesized or, if synthesized, were not assimilated to the same extent.

Characteristics of oxygen uptake during germination

Measurements of oxygen consumption were made during germination to obtain a functional index for the development of aerobic respiratory competence during the germination sequence. In Fig. 3A are shown the oxygen uptake rates of spores germinating in the nutrient medium and of spores incubated in water. The spores which germinated showed a biphasic curve of oxygen uptake rates, and the initial acceleration began to increase at about 150 min of germination. These data are corrected for low amounts of uptake (0.02 μl/mg spores/min) which occurs in the presence of 0.5 mM NaCN and which is constant during this germination period. Spores under both conditions of incubation began to consume increasing amounts of oxygen immediately upon the onset of incubation, and this increase in consumption continued at the same rate until about 120–150 min, at which time the spores nutritionally stimulated to germinate began to respire at an accelerated rate and those water-incubated spores nutritionally restricted from germinating showed no further increase and the rates subsequently leveled off.

The oxygen uptake rates of spores germinated in ethidium bromide (5 μg/ml)

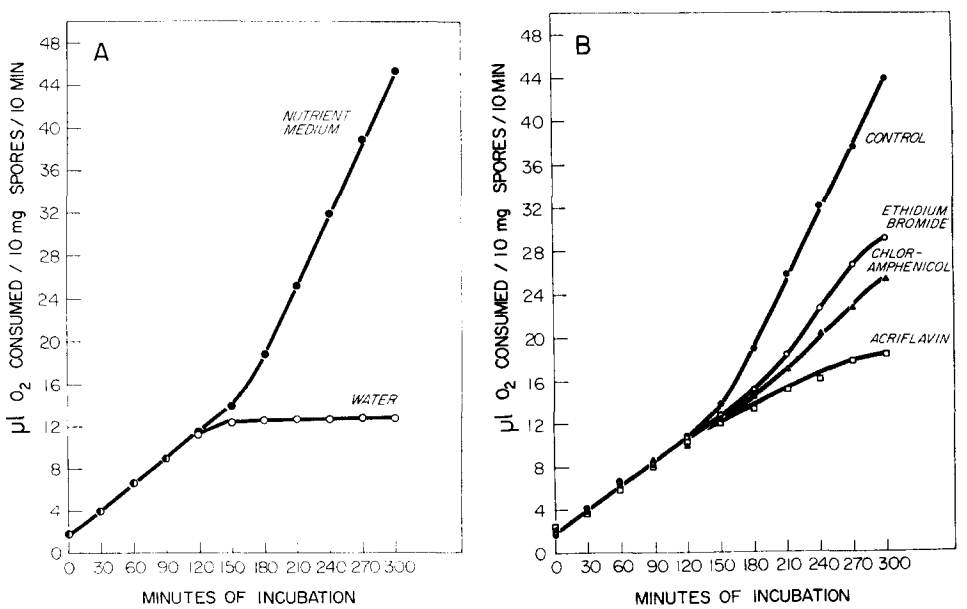

Fig. 3. Rates of oxygen consumption by spores incubating (A) in nutrient medium (●) or in water (○), and (B) in nutrient medium with ethidium bromide (○), chloramphenicol (▲), or acriflavin (□).

acriflavin (20 μg/ml), or chloramphenicol (3 mg/ml) are shown plotted in Fig. 3B. Although by 300 min the degree of inhibition varied among these drugs, in all cases the presence of these inhibitors caused substantial reduction of oyxgen uptake in comparison to the untreated spores. However, the initial accelerations of oxygen uptake in the presence of ethidium bromide, acriflavin, or chloramphenicol were not different from the initial acceleration of the untreated, control spores. Since the spores are permeable to these drugs during the first 120 min of germination (these drugs depress spore protein synthesis and/or mitochondrial DNA synthesis during this period), it is noteworthy that the early phase of oxygen uptake is resistant to these inhibitors of the mitochondrial genetic system and that they began to have an effect upon oxygen uptake only after the initial phase of respiratory activity was completed. It is this same initial phase of oxygen uptake which occurs in spores incubated in water under non-germinating conditions.

In order to help establish the temporal coordination in synthesis of products of cytoplasmic and mitochondrial ribosomes and the degree of independence of the two translational systems during germination, a series of experiments was performed to measure indirectly the function of these two systems by the ability of the germinating spores to consume oxygen in the presence or absence of cycloheximide (25 μg/ml) and chloramphenicol (3 mg/ml). The presumption is that at least some of the ribosome products are involved in development of respiratory competency in germinating

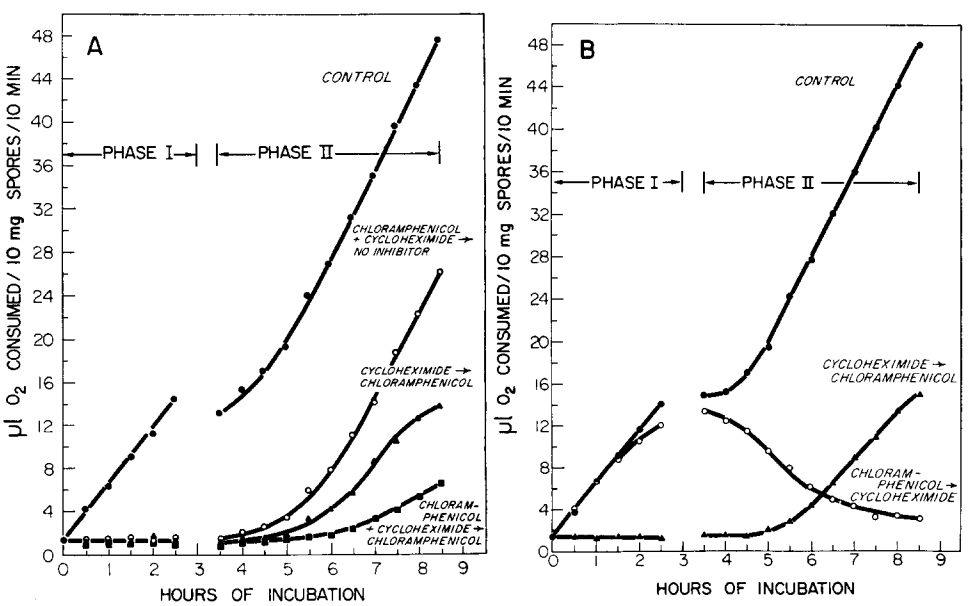

Fig. 4. Rates of oxygen consumption by spores incubated in absence or presence of chloramphenicol and/or cycloheximide in several sequences. In the control curve, no antibiotics were present in either phase of the experiment. In Fig. 4A, the spores were incubated with chloramphenicol and cycloheximide or with cycloheximide in phase I, and in phase II (after being washed free of phase I inhibitors), they were incubated further in media containing either no inhibitors or chloramphenicol. In Fig. 4B, in addition to the control uptake measurements, the spores were incubated either in chloramphenicol or cycloheximide in phase I, and in phase II the inhibitor treatments were reversed.

spores and that if ribosome activity is inhibited in germinating spores which are assembling new mitochondria, then respiratory activity may be indirectly inhibited. In Fig. 4A are shown rates of oxygen uptake by *B. theobromae* spores under three different regimens of inhibition during two phases of incubation. The control curve refers to the oxygen consumption of untreated spores, and the interruption in measurement and temporary drop in oxygen uptake rates are due to the washing of the spores in iced water between phases I and II. If the spores were incubated during the first 180 min (phase I) with cycloheximide plus chloramphenicol, no oxygen consumption was observed; after these spores were washed thoroughly and transferred to a fresh medium containing no antibiotics, in phase II the spores began to consume oxygen at an acceleration comparable to that of the untreated spores, indicating that there were no irreversible effects of treatment with cycloheximide or chloramphenicol. However, if the spores were incubated in phase I in a medium containing both cycloheximide and chloramphenicol and then, after washing, transferred to a medium containing only chloramphenicol, the subsequent acceleration of oxygen uptake in phase II was diminished severely. The most instructive pattern was observed when the spores were incubated with cycloheximide in phase I, collected and washed free of this drug, and then incubated further during phase II in a medium containing chloramphenicol. In this second phase the spores began to consume oxygen at an acceleration which suggests that, in the absence of cytoplasmic ribosome activity during phase I, mitochondrial ribosome products accumulated, which then complemented newly-synthesized cytoplasmic ribosome products in phase II and consequently permitted development of mitochondrial respiration in the presence of chloramphenicol in phase II. Identical observations have been reported previously for yeast cells undergoing adaptation to aerobic growth [13, 14]. The reverse of this experiment (chloramphenicol → cycloheximide) is shown in Fig. 4B, and as would be expected, the phase I oxygen uptake was resistant to chloramphenicol treatment; however, when the spores were incubated in cycloheximide in phase II, the capacity for oxygen uptake decreased rapidly.

Similar experiments were performed by substituting ethidium bromide for chloramphenicol, and the capacity for development of phase II respiration (ethidium bromide+cycloheximide → no inhibitor) was sharply reduced; this observation indicates that the inhibitory effects of ethidium bromide are probably irreversible under the conditions of this experiment.

DISCUSSION

Previous studies indicated that in the ungerminated conidiospores of *B. theobromae* the earliest activity of the nucleo-cytoplasmic genetic system is resumed at the level of translation; RNA synthesis apparently is not initiated until about 90 min after the translation of a latent messenger RNA is begun [7]. The syntheses of nuclear and mitochondrial DNA begin approximately simultaneously between 120 and 180 min of germination [15], and the synthesis of mitochondrial DNA is inhibited completely with ethidium bromide while spore germination and synthesis of nuclear DNA are largely unaffected by the drug [12]. The results in this present report indicate that the ungerminated spores of this organism also contain a potentially functional aerobic respiratory system which may require cytoplasmic ribosome activity to become

functional when the spores are inoculated into a liquid medium. When activated, the latent aerobic respiratory apparatus probably accounts for initial spore respiration until new mitochondria are organized. Function of the mitochondrial genetic system does not appear to be essential for initiation of spore germination or germ tube emergence and its translation products (although ordinarily synthesized early in germination) are not required until a new mitochondrial respiratory apparatus is assembled later during germ tube emergence.

In this report, several metabolic activities of germinating spores are compared with those of spores treated with chemical inhibitors or with those of spores incubated in water under non-germinating, nutritionally-restricted conditions. Where it was experimentally appropriate, multiple inhibitors were compared, and the effects observed were substantially redundant. Nevertheless, uncritical acceptance of results obtained through use of such inhibitors is hazardous, and any interpretation of these experiments should take into account the inherent possibility that the drugs also may affect cell metabolism at unsuspected sites.

Continuous treatment of the spores with ethidium bromide, acriflavin, or chloramphenicol, presumed inhibitors of the mitochondrial transcriptional or translational systems, did not reduce the rate or extent of germination, although treatment continued after all spores had germinated eventually caused substantial reductions in acceleration of aerobic respiration, rates of protein synthesis, and accumulation of mycelial mass. The possibility that the spores are not permeable to these drugs early in germination is excluded by evidence that all of them depressed the rates of spore protein synthesis from the outset of germination and that ethidium bromide inhibited mitochondrial DNA synthesis when it began between 120 and 180 min of germination [12]. This conclusion that a functional mitochondrial genome is not essential for germination is supported by our previous report [12] and by the report of Tingle et al. [16] in which ethidium bromide-induced petite yeast ascospores devoid of mitochondrial DNA were found to germinate with kinetics similar to those of wild-type ascospores.

The incubation of spores in water yields a gross effect upon rates of total spore protein synthesis which mimics the effects of the mitochondrial genetic system inhibitors. It seems possible, therefore, that the reduced rate of protein synthesis which occurs in drug-treated, germinating spores may be due to activity of the cytoplasmic ribosomes; the difference in protein synthesis between chemically- or nutritionally-inhibited (water-incubated) spores and the untreated, germinating spores may represent (at least partially) the contribution of the mitochondrial translational system to total spore protein synthesis.

Although spectroscopically detectable amounts of the respiratory cytochromes could not be observed in mitochondria of ungerminated spores, the oxygen uptake studies indicate that these spores must contain a potentially functional aerobic respiratory apparatus. It is likely that the first phase of oxygen consumption reflects the activity of this latent respiratory apparatus preserved in the ungerminated spores while the second, accelerated phase of oxygen consumption probably reflects the respiratory activity of newly-organized mitochondria. Chemical inhibition of the mitochondrial genetic system during the first 120 min of germination altered only the second phase of oxygen uptake by the spores. Untreated spores incubated in the water medium exhibited only the capacity for the initial phase of oxygen consumption, and the second, accelerated phase of respiration did not begin.

Results of these respiration experiments imply that the two translational systems of the spores respond independently either to a single stimulus for germination or that there are at least two separate stimuli which may act upon the two translational systems independently. The experiments demonstrate, also, that function of the mitochondrial genetic system alone is not sufficient for development of respiratory activity in the pre-emergence phase of germination. Products of the cytoplasmic ribosomes, presumably transcribed from nuclear genes, are essential for development of respiratory activities in the early, pre-emergence phase of germination, and if phase I, chloramphenicol-treated spores are treated with cycloheximide later in the emergence phase (phase II), the aerobic respiratory activity decays fairly rapidly (Fig. 4B), suggesting a requirement for rapid replacement of respiratory components synthesized on cytoplasmic ribosomes. It is possible that the latent respiratory apparatus in the ungerminated spores contains the essential components from the mitochondrial ribosomes and requires only the contribution of the cytoplasmic ribosomes to allow development of aerobic respiration. Cytoplasmic ribosome activity is both necessary and sufficient for this respiration in the first 150 min of germination, while mitochondrial ribosome activity does not become essential (for continued and accelerating respiratory activity) until after 150 min. Experiments are now in progress to test this hypothesis directly.

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