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Differential Gene Expression During Aerobic Germination of *Mucor racemosus* Sporangiospores

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Evidence is provided suggesting that several modes of differential gene expression operate concomitantly during the first 60 min of germination of *Mucor racemosus* sporangiospores under air. Protein synthesis was initiated immediately upon exposure of the spores to nutrient medium and accelerated exponentially throughout the period of observation. All translation during the first 30 min of germination occurred using only preformed stable mRNAs as a template. Analysis of the protein products synthesized in vivo was performed by L-[^{35}S]methionine labeling, one- and two-dimensional polyacrylamide gel electrophoresis, and autoradiography. The population of proteins accumulated during spore formation and present at the time of harvest differed significantly from those proteins synthesized during spore germination. Autoradiographs displayed several proteins synthesized during the former but not the latter morphogenetic process. Conversely, other proteins were synthesized during the first 30 min of germination but not during spore formation, even though the mRNA specifying these proteins must have been synthesized and stored in the dormant spore. A post-transcriptional regulatory mechanism that directs selective translation thus appears to exist in the developing spore. In addition, autoradiographs showed that many proteins, although made throughout the intervals examined, displayed significant changes in their relative rates of synthesis. One gene product exemplified a possible case of post-translational modification during the first hour of sporangiospore germination.

Mucor racemosus is a dimorphic zygomycete that has served as a useful system for studying eucaryotic cell differentiation. The conversion of yeasts into hyphae, stimulated by shifting the culture from an anaerobic to an aerobic atmosphere, has been most frequently examined (1, 26). In addition to the two distinct vegetative forms, this organism can produce several types of spores. One kind of spore, called a sporangiospore, is formed asexually in great numbers within sacs (sporangia) at the termini of aerial hyphae when the fungus is grown on a solid medium in air (7). Upon germination, the sporangiospore has the potential to develop into either of the alternative vegetative forms, depending upon the environmental conditions (1, 26). When placed into a nutrient medium under air, the sporangiospore increases several times in diameter via spherical growth, a process dependent upon macromolecular synthesis, before constructing one or more hyphal germ tubes. When placed into a hexose-containing nutrient medium under CO_2 or N_2, for example, the sporangiospore carries out an essentially equivalent amount of spherical growth before initiating the formation of multipolar buds. Several previous studies have dealt with the aerobic germination of *Mucor* sporangiospores (13, 18, 19, 21, 27–29, 33), and this is the system of development we have focused upon in the present work.

One important aspect of spore germination that we have been investigating is the regulation of protein synthesis early in the morphogenetic process. We have previously shown that *M. racemosus* sporangiospores contain a sizable pool of stored mRNA which is translated immediately upon the initiation of germination. Proteins synthesized during the first 30 min of germination are translated only from the stored mRNA (13). In the present study, we compared the proteins synthesized in vivo from the stored mRNA during early germination with the proteins accumulated in vivo during spore formation by means of sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis and two-dimensional isoelectric focusing–SDS-polyacrylamide gel electrophoresis. We provide evidence which suggests that the translation of mRNA in maturing sporangiospores is quite selective. The mRNAs for many proteins were transcribed and stored in a stable form in the dormant spores. Most of these mRNA species were translated in vivo during both spore formation and spore germination. Translation of several populations of stored mRNA, however, took place only after the initiation of germination. We also report the ability of sporangiospores to effect several other qualitative and quantitative changes in gene expression during early germination.

MATERIALS AND METHODS

Organism and spore production. *M. racemosus* (*Mucor lusitanicus*) ATCC 1216B was used in all experiments. The growth medium (YPG) contained 0.3% (wt/vol) yeast extract, 1.0% (wt/vol) peptone, and 2.0% (wt/vol) glucose and was adjusted to pH 4.5 with sulfuric acid. Sporangiospores were produced on YPG solidified with 3.0% (wt/vol) agar and incubated at room temperature (22°C) in air for 7 days.

Germination. Sporangiospores were harvested from 7-day cultures by pouring 10 ml of liquid YPG medium onto the agar plates and gently scraping the mycelial surface with a sterile glass rod. Recovery of the surface liquid resulted in pure spore suspensions containing 5×10^7 to 1×10^8 spores per ml. Purity of spore suspensions and spore concentrations were routinely determined by use of a hemacytometer. Spore suspensions were incubated with shaking (200 rpm) at room temperature (22°C) while water-saturated, sterile air was bubbled through the medium. These conditions resulted in the emergence of hyphal germ tubes from the sporangiospores within 5 to 8 h. Some spore suspensions (2×10^5 spores per ml) were incubated with shaking (200 rpm) at

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room temperature while 100% CO_2 was bubbled through the culture at a flow rate of 0.5 volume of gas per volume of culture fluid per min. All sporangiospores germinated and produced budding yeast cells under these conditions.

Kinetics of protein synthesis. Sporangiospores were harvested into YPG medium containing L-[^{35}S]methionine (final concentration, 4 μCi/ml) and incubated with shaking (200 rpm) at room temperature under air. In some experiments, cycloheximide (200 μg/ml) was included in the growth medium. Samples (100 μl) of culture were withdrawn at 2-min intervals and treated as described by Orlowski and Sypherd (20). Radioactivity residing in the hot trichloroacetic acid-insoluble fraction was quantified with a Beckman LS-200 liquid scintillation spectrometer.

Free intracellular amino acid pools. Free intracellular amino acids were extracted from cells into 10% (vol/vol) formic acid and quantified with a Beckman model 139C automated amino acid analyzer as previously described (13, 20).

Prelabeling and pulse-labeling of sporangiospore proteins. Proteins were prelabeled during sporangiospore formation at room temperature on YPG agar containing L-[^{35}S]methionine (final concentration, 100 μCi/ml). At 7 days after inoculation, the sporangiospores were harvested directly into aqueous cycloheximide (500 μg/ml). Proteins were pulse-labeled by harvesting spores into YPG medium and exposing them to L-[^{35}S]methionine (final concentration, 160 μCi/ml) for a 15-min interval during germination. Cycloheximide (500 μg/ml) was added to the culture at the end of each pulse. Four successive 15-min pulses during the first 60 min of germination were administered. The radiospecific activity of L-[^{35}S]methionine in the free intracellular methionine pool was measured during each pulse period by a previously described method (13, 20): concentrations of extracted amino acids were quantified with an amino acid analyzer, and radioactivity within the L-methionine pool, which was recovered by paper chromatography, was quantified by scintillation spectroscopy.

Protein assay. Protein was measured by the method of Lowry et al. (16), with crystalline bovine serum albumin used as the standard.

Assay for protein turnover. Protein turnover was assayed by monitoring the release of radioactivity from the hot trichloroacetic acid-insoluble fraction of germinating sporangiospores that had been prelabeled with L-[^{35}S]methionine. The experimental protocol of Orlowski and Sypherd (20, 21) was followed.

Polyacrylamide gel electrophoresis. Proteins were extracted from sporangiospores for two-dimensional isoelectric focusing–SDS-polyacrylamide gel electrophoresis by the modifications of Hiatt et al. (9) applied to the original procedure of O'Farrell (17), except that SDS was excluded from the extraction buffers. Two-dimensional polyacrylamide gel electrophoresis was performed essentially as described by O'Farrell (17). First-dimension isoelectric focusing gels (pH range, 4.5 to 7.1; length, 120 mm; diameter, 3 mm) were subjected to 6,800 V × h. Samples containing 200 μg of protein isotopically labeled with 8×10^4 to 1.6×10^6 cpm of L-[^{35}S]methionine were loaded on the neutral end of the first-dimension isoelectric focusing gels. The resolving portion of the second-dimension SDS-polyacrylamide gel was a linear 8-to-15% polyacrylamide gradient (length, 8 cm; thickness, 1.5 mm). A 4% polyacrylamide stacking gel (length, 5 cm) was poured directly on top of the resolving gel. The gel buffering system and electrode buffers were originally described by Laemmli (12). First-dimension gels were loaded directly on top of the stacking gel and sealed in place with a 1% solution of agarose in SDS sample buffer (see below). The first-dimension gels were electrophoresed in the second dimension without prior equilibration in SDS sample buffer to minimize the loss of proteins during this procedure (17). Total time for electrophoresis in the second dimension was approximately 9 h at a constant current of 20 mA.

Small portions of the protein samples prepared for two-dimensional gel electrophoresis were also processed for SDS-polyacrylamide slab gel electrophoresis. Protein was precipitated by the addition of 10 volumes of 10% (wt/vol) trichloroacetic acid and kept on ice for 30 min. The precipitates were collected by centrifugation and solubilized in 50 μl of SDS sample buffer (25 mM Tris-hydrochloride [pH 7.0], 1.0% [wt/vol] SDS, 2.5% [vol/vol] β-mercaptoethanol, 10% [wt/vol] sucrose) by boiling for 3 min. The resolving and stacking gels for one-dimensional SDS-polyacrylamide slab gels were the same as described for two-dimensional gels, but with added sample wells in the stacking gel. Bromphenol blue tracking dye was added to the samples before they were loaded into the wells. The regimen of electric-field intensity and duration was as described previously (18). Molecular weight standards employed under both conditions of electrophoresis were bovine serum albumin (66,000), ovalbumin (45,000), trypsinogen (24,000), and lysozyme (14,300).

Gels were fixed and stained overnight in a solution of 0.5% (wt/vol) Coomassie brilliant blue R-250 in methanol-water-acetic acid (5:4:1). The gels were destained for 24 h in two to three changes of methanol-water-acetic acid (3:6:1), shaken for an additional 24 h in the above solution plus 3% (vol/vol) glycerol, and dried under vacuum at 65°C onto Whatman 3MM chromatography paper.

Dried polyacrylamide gels containing proteins isotopically labeled with L-[^{35}S]methionine were autoradiographed by exposing them to Kodak XAR-5 X-ray film at −70°C. In the case of two-dimensional gels, exposure was adjusted in such a way that the product of radioactivity and time was equivalent to 10^6 cpm × 150 h (9). In the case of one-dimensional gels, image density as a function of exposure time was determined empirically for each gel and the optimum autoradiograph chosen for analysis and presentation. Autoradiographs were developed in Kodak GBX developer and fixed in Kodak Rapid Fixer.

Radioisotopes. $H_2{}^{35}SO_4$ (carrier free; 1,490-Ci/mg atom) and L-[^{35}S]methionine (1,194 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. L-[^{35}S]methionine (approximately 300 Ci/mmol) was also synthesized from $H_2{}^{35}SO_4$ by the procedure of Crawford and Gesteland (4).

Chemicals. Components of the growth medium were obtained from Difco Laboratories, Detroit, Mich. All other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo., or Bio-Rad Laboratories, Richmond, Calif., unless specified otherwise in the text.

RESULTS

Quantitative analysis of protein synthesis during sporangiospore germination. Incorporation of radioactivity into protein commenced immediately after exposure of sporangiospores to nutrient medium containing L-[^{35}S]methionine (data not shown). The kinetics of radioisotope accumulation were exponential for 60 min, being nearly identical to previously observed kinetics of L-[^{14}C]leucine incorporation into a leucine auxotroph of *M. racemosus* (13). Cycloheximide completely inhibited the incorporation of L-[^{35}S]methionine into protein.

TABLE 1. Radiospecific activity of proteins pulse-labeled with L-[^{35}S]methionine during germination of *M. racemosus* sporangiospores[a]

Pulse period (min)	cpm/μg of protein[b]
0–15	340
15–30	747
30–45	875
45–60	1,084

[a] Spores were harvested into YPG medium and incubated with shaking at room temperature under air. L-[^{35}S]methionine was added to the cultures at the appropriate time. Four consecutive 15-min pulse periods were used relative to the initiation of germination by exposure of the spores to YPG. Pulse periods were ended with the addition of cycloheximide. Total cell protein was extracted from the sporangiospores and quantified by the method of Lowry et al. (16). The amount of radioactivity incorporated into hot trichloroacetic acid-insoluble material was measured as described in the text. Radiospecific activities were calculated from the ratios of these two values.

[b] Counts per minute of radioactivity in trichloroacetic acid-precipitable material.

Germinating sporangiospores were pulse-labeled with L-[^{35}S]methionine during each of four separate, consecutive 15-min periods in the first 60 min after the initiation of germination. Determination of the radiospecific activity of protein purified from sporangiospores after each pulse period revealed that the specific rate of protein synthesis increased throughout the first 60 min of germination (Table 1).

Analysis of free amino acid pools during sporangiospore germination. Internal pools of free amino acids within the sporangiospores were comparable to previously reported levels (13, 20, 29) and did not change substantially over the 60-min period of germination (data not shown). The radiospecific activity of L-[^{35}S]methionine in endogenous pools did not fluctuate in an amount or direction that could account for the changes in the rate of isotope incorporation into protein (Table 2). The increase in radiospecific activity of protein must have been due to a genuine increased rate of synthesis rather than an increased availability of label.

Qualitative analysis of protein synthesis during sporangiospore germination. Samples of protein extracted from dormant or germinating sporangiospores were analyzed by SDS-polyacrylamide slab gel electrophoresis (12, 18) and two-dimensional isoelectric focusing–SDS-polyacrylamide gel electrophoresis (9, 17). Isoelectric focusing was performed in a pH gradient of 4.5 to 7.1. Any protein with an isoelectric point outside of this range was therefore not included in the present two-dimensional gel analysis. SDS-polyacrylamide slab gel electrophoresis theoretically separates all proteins strictly on the basis of size. Therefore, changes in any major proteins not displayed on the two-dimensional gels may possibly be seen on the one-dimensional gels.

TABLE 2. Radiospecific activity of L-[^{35}S]methionine in methionine pools of germinating sporangiospores of *M. racemosus*[a]

Time (min)	μmol of methionine per mg (dry wt) (× 10^{-3})	cpm of L-[^{35}S]methionine per mg (dry wt) (× 10^{5})	cpm μmol of methionine (× 10^{7})
15	1.8	1.25	6.9
30	2.5	1.22	4.9
45	2.2	1.17	5.3
60	1.9	0.62	3.3

[a] Sporangiospores were harvested into YPG medium and incubated with shaking at room temperature under air. L-[^{35}S]methionine was addded to separate cultures for four consecutive 15-min pulse periods ending at the indicated times. Amino acid pools were extracted and quantified with an amino acid analyzer. Radioactivity within the methionine pool was assayed by paper chromatography, autoradiography, and scintillation spectroscopy (13, 20).

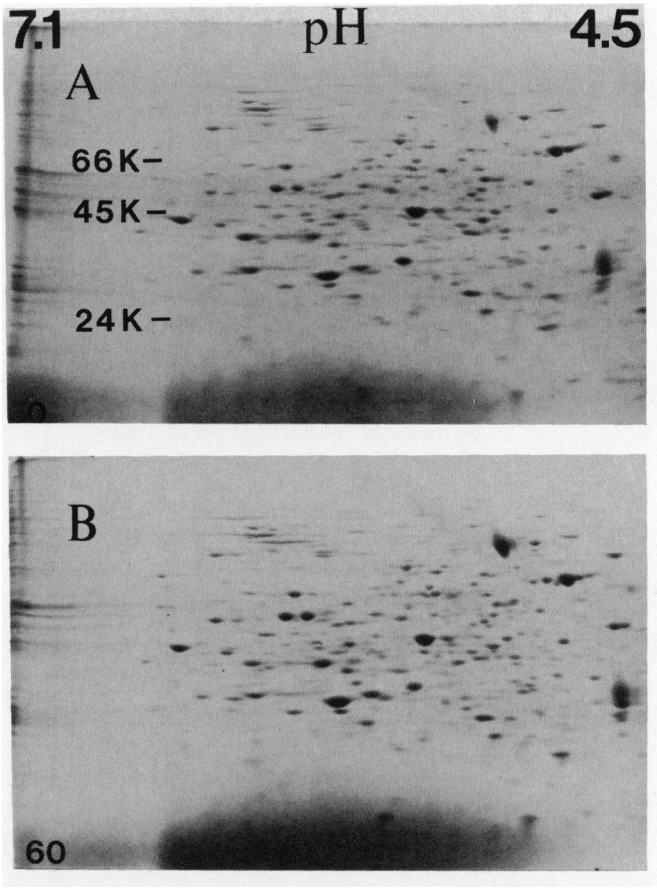

FIG. 1. Coomassie blue- stained, two-dimensional polyacrylamide gels of proteins extracted from germinating sporangiospores of *M. racemosus*. Spores were harvested into sterile water containing cycloheximide or directly into YPG medium and incubated with shaking at room temperature under air. Proteins were extracted and electrophoresed as described in the text. An identical quantity (200 μg) of protein was loaded onto each gel. (A) Proteins extracted from dormant spores; (B) proteins extracted from spores germinated in YPG medium for 60 min. The mobilities of molecular weight standards (bovine serum albumin, 66,000; ovalbumin, 45,000; trypsinogen, 24,000) are indicated at the left of panel A.

Coomassie blue staining of both types of gels indicated only minor changes in the total spectrum of proteins present in sporangiospores during the first 60 min of germination. The total population of proteins extracted from dormant spores (Fig. 1A) and the total population of proteins extracted from spores after 60 min of germination (Fig. 1B) displayed no apparent differences in the constellations of spots visible on two-dimensional gels. The same comparison on an SDS-polyacrylamide one-dimensional slab gel (Fig. 2A, lanes 1 and 5) showed only three bands (arrows) representing an uncertain number of proteins that underwent changes in relative concentrations during the relevant period of development.

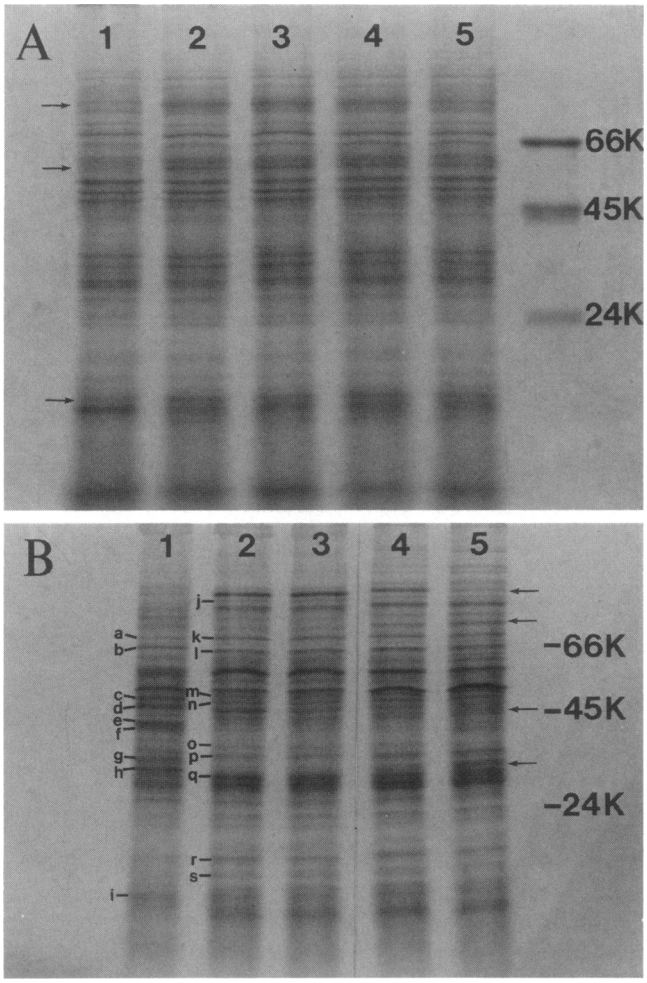

FIG. 2. One-dimensional separation of proteins extracted from dormant and germinating sporangiospores of *M. racemosus*. Proteins of dormant spores were prelabeled by incubation of an aerobic mycelium for 7 days on YPG agar containing L-[^{35}S]methionine. Proteins of germinating spores were pulse-labeled with L-[^{35}S]methionine during four consecutive 15-min pulse periods during the first 60 min of germination. Proteins were extracted from sporangiospores and separated by SDS-polyacrylamide slab gel electrophoresis as described in the text. (A) Total spectrum of proteins made visible by staining with Coomassie blue. Lane 1, Proteins extracted from dormant spores; lanes 2 through 5, proteins extracted after each of the four consecutive pulse periods (0 to 15, 15 to 30, 30 to 45, and 45 to 60 min, respectively). An identical quantity (40 μg) of protein was run in each lane. (B) Autoradiograph of same material as that shown in panel A. An identical amount of radioactivity (35,000 cpm) was run in each lane. Molecular weight standards are the same as those shown in Fig. 1.

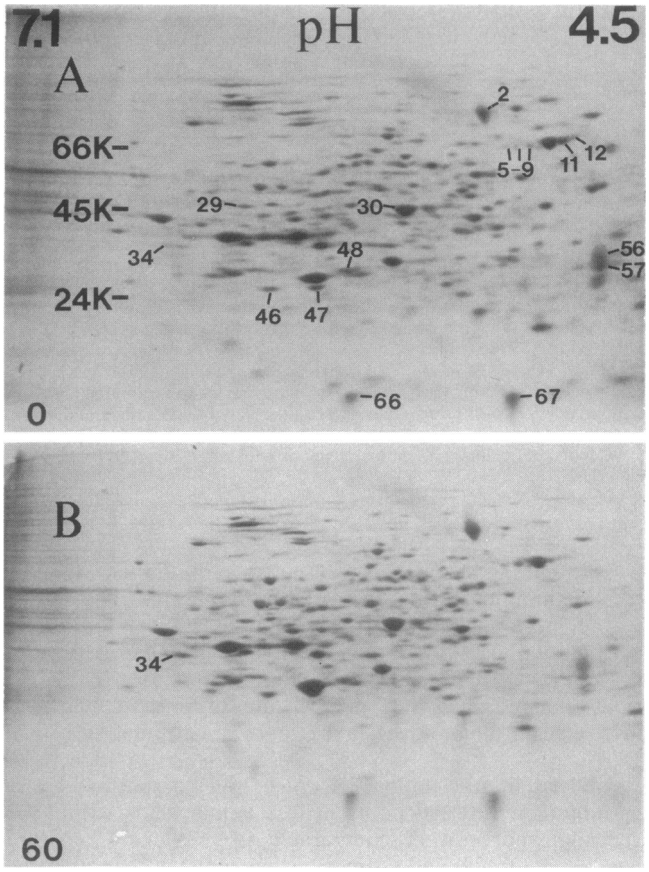

FIG. 3. Two-dimensional separation of prelabeled sporangiospore proteins visualized by autoradiography. Spores prelabeled with L-[^{35}S]methionine were harvested directly into sterile water containing cycloheximide or into YPG medium containing an excess of non-radioactive L-methionine and incubated for 60 min with shaking at room temperature under air. Protein extracts (200 μg) were fractionated by two-dimensional polyacrylamide gel electrophoresis as described in the text. (A) Prelabeled proteins of dormant spores; (B) prelabeled proteins of spores after 60 min of germination in YPG medium. Molecular weight markers are the same as those shown in Fig. 1. Protein 34 may represent an example of post-translational modification of a preexisting polypeptide.

In contrast to the above observation, many changes were noted when the comparison was made between proteins accumulated during formation of the spore and those actively synthesized during the four pulse-labeling periods in the first 60 min of germination. An autoradiograph of an SDS-polyacrylamide slab gel (Fig. 2) showed that prelabeled, dormant spores (lane 1) displayed nine bands of protein (designated a through i) that were present in relatively greater amounts than in the pulse-labeled, germinating spores (lanes 2 through 5). Ten bands of protein synthesized in each of the four pulse periods (j through s) were present in relatively greater amounts than in the dormant spore. Some of these bands appeared to represent newly synthesized proteins (k, l, o, and p), although the resolution here was limiting. In addition, at least four bands implied changes in the relative rates of protein synthesis during the first 60 min of germination (lane 5, arrows).

Two-dimensional isoelectric focusing–SDS-polyacrylamide gel electrophoresis resolved 500 to 600 proteins and showed several striking differences between the population of proteins synthesized during spore formation (Fig. 3A) and those synthesized during the first 60 min of germination (Fig. 4A–D). Several proteins (designated 5-9 and 29), showed increases in relative concentrations in germinating spores, whereas one protein showed a decrease (30). At least eight proteins present in prelabeled, dormant spores were not synthesized during the first 60 min of germination (proteins 2, 11, 12, 48, 56, 57, 66, and 67). Four of these proteins (2, 56, 57, and 67) may be specific to spores since they did not appear in Coomassie blue-stained gels of proteins extracted

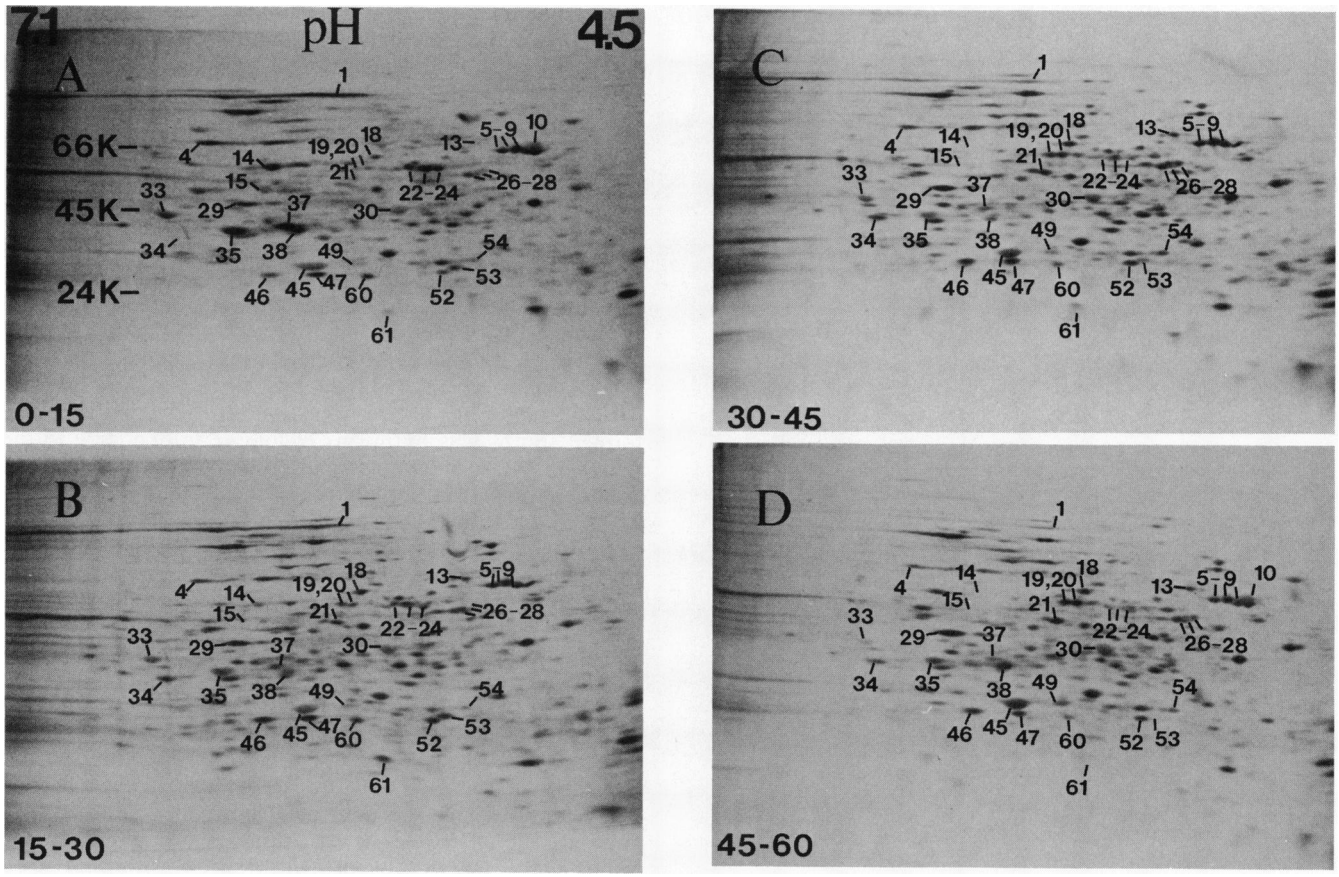

FIG. 4. Two-dimensional separation of proteins pulse-labeled during sporangiospore germination visualized by autoradiography. Proteins of germinating sporangiospores were pulse-labeled with L-[^{35}S]methionine during four consecutive 15-min pulse periods in the first 60 min of germination. Protein samples (200 μg) containing 8.0×10^4 to 1.6×10^6 cpm were subjected to two-dimensional polyacrylamide gel electrophoresis as described in the text. Pulse-labeling was carried out for 0 to 15 min (A), 15 to 30 min (B), 30 to 45 min (C), and 45 to 60 min (D). Molecular weight markers are the same as those shown in Fig. 1.

from either the hyphal or yeast forms of the organism (Fig. 5). Other proteins (4, 13, 60, and 61) were synthesized during the first 30 min of germination or beyond but were absent in the dormant spore. The mRNAs for these proteins were probably synthesized during spore formation but stored without being translated until germination was initiated. The appearance of protein 34 may have resulted from the modification of a preexisting polypeptide molecule (see below). It is interesting to note that protein 60, which was synthesized throughout early germination (Fig. 4A–D), was also found in a Coomassie blue-stained gel of hyphal proteins (Fig. 5A) but was completely absent from dormant spores, suggesting that it may be associated with development of the hyphal morphology. Protein 13 was found in hyphae, yeast cells, and germinating spores, but not in dormant spores.

Protein 10, which was heavily labeled in the dormant spore, was also synthesized during the first 15 min of germination but disappeared between 15 and 45 min and then reappeared again later. Gene expression was not exclusively an all-or-none phenomenon in the developmental system. Many other proteins were found to have changing relative rates of synthesis during the first 60 min of germination, including proteins 18-21, 26-28, 29, 30, 38, 45, 46, 49, 52, and 54, which displayed increasing rates of synthesis, and proteins 1, 14, 15, 22-24, 33, 35, 37, 53, 60, and 61, which displayed decreasing rates of synthesis.

Assay for protein turnover and post-translational modifications during sporangiospore germination. The apparent changes in protein composition and rates of protein synthesis noted above may result from actual changes in the frequency or rates of translational events, covalent modification of existing proteins, or protein turnover. Dormant sporangiospores prelabeled with L-[^{35}S]methionine were washed free of exogenous isotope and germinated for 60 min in YPG medium containing an excess of non-radioactive L-methionine. Samples were collected every 5 min and assayed for hot trichloroacetic acid-precipitable radioactivity. The incorporated radioactivity did not decrease over the 60-min period, implying that the proteins synthesized during spore formation were not degraded during early germination (data not shown). Protein turnover was thus not likely to be responsible for the observed changes in protein composition or rates of protein synthesis. The experiment described below, in addition to its intended purpose, also supports this notion. An autoradiograph of a two-dimensional gel of prelabeled proteins extracted at the end of the standard 60-min germination period showed no apparent changes from proteins extracted immediately from dormant spores (Fig. 3B). Therefore, post-translational processing did not play a major role in the observed changes in protein composition or rates of protein synthesis. One exception may be protein 34, which appeared after 60 min of germination of prelabeled spores.

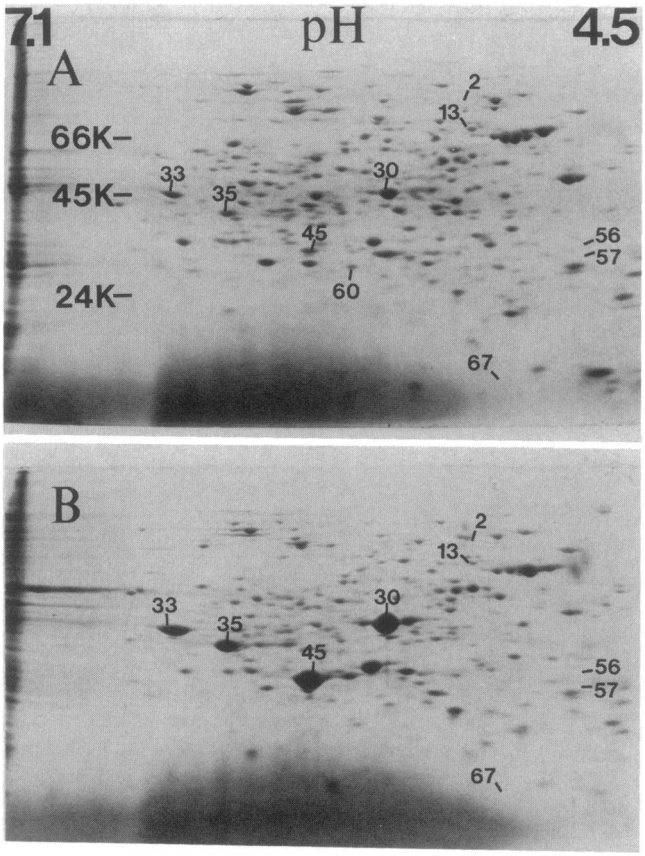

FIG. 5. Two-dimensional separation of *M. racemosus* yeast and hyphal proteins made visible by staining with Coomassie blue. Hyphae were grown from sporangiospores incubated for 16 h with shaking at room temperature under air. Yeasts were grown from sporangiospores incubated for 40 h with shaking at room temperature under 100% CO_2. The inoculum size was 2×10^5 spores per ml in each case. All preparative procedures were as described in the text. Protein samples (200 μg) were subjected to two-dimensional polyacrylamide gel electrophoresis as described in the text. (A) Hyphal proteins; (B) yeast proteins. Molecular weight markers are the same as those shown in Fig. 1.

DISCUSSION

The germination of *M. racemosus* sporangiospores involves the differentiation of a thick-walled, ellipsoid, metabolically quiescent cellular form into a distinctly different morphological form exhibiting very active metabolism and growth. Regardless of whether the spore eventually gives rise to a yeast or hyphal form, one might reasonably predict that the identities, quantities, activities, or distributions of cellular proteins would change as a function of the morphogenetic process. One goal of this investigation was to determine whether any qualitative or quantitative alterations in gene expression evidenced at the level of protein synthesis occur during the early stages of sporangiospore germination under an aerobic atmosphere. A further goal was to determine whether the stable mRNA stored within the dormant sporangiospore (13) plays some special role during early germination, such as specifying the synthesis of a unique population of proteins.

Data presented here and in a previous report (13) show that protein synthesis commenced immediately upon exposure of spores to nutrient medium and that the overall rate of this synthesis increased exponentially throughout the first hour of germination. Since it had been established that de novo synthesis of RNA does not occur for at least the first 20 min of germination and that newly synthesized RNA does not appear in polysomes for at least the first 30 min of germination (13), the stored mRNA must specify all of the protein molecules made during this 30-min interval. To determine whether the observed escalation of synthetic activity simply reflected a nonspecific stimulation of the translational process or reflected preferential synthesis of specific gene products, we analyzed the proteins made and accumulated in vivo during formation of the spore and the proteins actively synthesized in vivo during the first hour of germination by means of radioisotopic labeling, gel electrophoresis, and autoradiography. Since the only way to prelabel uniformly a large population of a synchronously formed spores was by means of long-term exposure to the radioisotope, no inferences can be made as to the relative time of synthesis of specific proteins during this morphogenetic process. Whether a given protein was synthesized early or late during spore formation, however, is not central to the analysis presented here.

To minimize discrepancies and artifacts in the interpretation of polyacrylamide gel electrophoresis, we followed several accepted procedures. Gels to be stained with Coomassie blue for analysis of the total spectrum of proteins present within the cells were loaded with a constant, standard amount of protein so that changes in band or spot intensity reflected real changes in relative protein concentrations. A constant, standard amount of isotopically labeled protein was also loaded on the first dimension of two-dimensional gels later to be analyzed by autoradiography. Since the radiospecific activity of proteins varied with individual samples, the time of X-ray film exposure to the dried gels was adjusted as a linear function of the total radioactivity contained within a given gel to yield the product $10^6 \times 150$ h or its equivalent. In this way, changes in spot intensities reflected real changes in relative rates of protein synthesis. Bands or spots on all gels were identified visually by absolute and relative positions. Bands or spots migrating to the same position on separate gels were assumed to represent the same gene product.

The total spectrum of proteins contained within sporangiospores, as indicated by staining with Coomassie blue, was found to change very little from that preexisting in the dormant spores during the first 60 min of germination. However, the spectrum of proteins synthesized during spore formation vis-à-vis spore germination, as evidenced by the incorporation of L-[^{35}S]methionine, differed significantly. Several proteins synthesized during spore formation were not synthesized at detectable levels during early germination. Some of these proteins were present in dormant spores but not in yeast or hyphal cells. These proteins seem to be specific to spores, although their function is not known. Four proteins were synthesized during the first 30 min of germination but were not synthesized in detectable quantities during spore formation, even though the mRNA for these proteins must have been synthesized and therefore present at this time. Developing sporangiospores thus appear to have the ability not only to store mRNA in a stable form but also to selectively translate mRNAs that are specific for proteins integral to spore maturation and to avoid translating mRNAs that are specific for proteins appropriately expressed during the first 30 min of germination. This represents one example of a post-transcriptional regulatory mechanism. Two proteins newly appearing dur-

ing early germination were identified on Coomassie blue-stained gels of hyphal extracts but not on gels of yeast or dormant spore extracts. These proteins may serve an exclusive role in the correct development of hyphae from spores germinated in air.

In addition to the noted examples of abrupt initiation or cessation of synthesis, the rate of synthesis of many proteins appeared to increase or decrease markedly during the first 60 min of germination. These changes, with one exception, were demonstrated to be due to actual changes in the rates of protein synthesis rather than to protein turnover or post-translational processing. Thus differential gene expression in early germination may be effected by not only qualitative but also quantitative adjustments in protein synthesis.

Although any statements regarding causal relationships between the observed changes and *Mucor* morphogenesis or speculations as to the specific roles of these changes in the morphogenetic process would be premature, we may unequivocally state that differential gene expression, including regulation at the post-transcriptional level, does occur during the initial 60-min period of aerobic *Mucor* sporangiospore germination. Significant follow-ups to the present work will include (i) a characterization of changes accompanying anaerobic germination (leading to budding yeast cells), (ii) an examination of events subsequent to those described here during the actual morphological development of buds or germ tubes, and (iii) a qualitative evaluation of the mRNA content of the spores before and during germination by in vitro protein synthesis.

Stable mRNA populations have been described before in the spores of a few lower eucaryotes, including *Dictyostelium* (3), *Blastocladiella* (14), *Allomyces* (23), *Botryodiplodia* (2, 32), and *Rhizopus* (30) spp. Differential gene expression has been demonstrated during spore germination in some of these systems (5, 6, 24, 25, 31), including evidence for post-transcriptional, translational, and post-translational regulation (6, 8, 10, 11, 15, 22, 24, 25). None of these other organisms, however, possesses the ability to rapidly and directly transform a single cell type into one of two alternative morphologies determined merely by the gaseous environment. *Mucor* sporangiospore germination represents a system in which the implementation of alternative morphogenetic programs can be studied. We have presently shown that the earliest stages of aerobic germination are entirely preprogrammed with stored mRNA. It will prove informative to determine whether the analogous stages of anaerobic germination are also preprogrammed with stored mRNA and, if so, to what extent these two developmental programs share subsets of the total set of stored mRNA.

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LITERATURE CITED

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