JOURNAL OF BACTERIOLOGY, Apr. 1979, p. 60-69
0021-9193/79/04-0060/10$02.00/0

Vol. 138, No. 1

Two-Dimensional Protein Patterns During Growth and Sporulation in *Saccharomyces cerevisiae*

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Received for publication 6 September 1978

Proteins synthesized by *Saccharomyces cerevisiae* in presporulation and sporulation media were compared by using sporulating (**a**/α) and nonsporulating (**a**/**a** and α/α) yeast strains. Total cellular proteins were labeled with [^{35}S]methionine and analyzed by two-dimensional polyacrylamide gel electrophoresis. Autoradiograms and/or fluorograms showed some 700 spots per gel. Nine proteins were synthesized by **a**/α cells which were specific to vegetative, log-phase conditions. During incubation in sporulation medium, sporulating (**a**/α) cells synthesized 11 proteins not present in vegetatively growing cells. These same 11 proteins, however, were synthesized by nonsporulating (**a**/**a** and α/α) cells on sporulation medium as well. Nonsporulating diploids (**a**/**a** and α/α) were also examined with the electron microscope at various times during their incubation in sporulation medium. Certain cellular responses found to be unique to meiotic yeast cells in previous studies were exhibited by the nonsporulating controls. The degree to which all cell types (**a**/α, **a**/**a**, and α/α) were committed to sporulation was also determined by shifting cells from sporulation medium to vegetative medium. Some commitment to the meiotic pathway was observed in both the **a**/α and the **a**/**a**, α/α cells.

In *Saccharomyces cerevisiae*, protein synthesis is essential for sporulation. Inhibition of protein synthesis at any time during sporulation interrupts all measurable physiological events occurring during meiosis and ascospore development with the exception of protein breakdown and RNA synthesis (11). Protein synthesis occurs in both **a**/α and **a**/**a** and α/α cells incubated in sporulation medium, although only the **a**/α cells are actually undergoing sporulation (22). This raises the question as to whether similar proteins are being synthesized by the two types of cells or whether in the **a**/α cells some new, sporulation-specific proteins are produced.

Temperature-sensitive mutants capable of vegetative growth but not sporulation at the nonpermissive temperature suggest the existence of sporulation-specific proteins (3). Despite this expectation, Hopper et al. (9) showed with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis that neither the pattern of proteins synthesized nor their relative intensity was different for heterozygous (**a**/α) and homozygous (**a**/**a**, α/α) mating-type cells. Similar results were found by Friedmann and Egel (5) with the fission yeast *Schizosaccharomyces pombe*.

In this report, we describe the results obtained when proteins from sporulating (**a**/α) and nonsporulating (**a**/**a**, α/α) yeast cells were analyzed with two-dimensional polyacrylamide gel electrophoresis (17, 18). With this technique proteins are separated according to two different parameters, isoelectric point and molecular weight. In addition, we have monitored the cellular responses of **a**/**a** and α/α cells to sporulation medium with an electron microscope. With this method, we can check for the presence of meiotic-specific structures (synaptonemal complexes, meiotic spindle plaques, and prospore walls [15, 16, 27]) which could not be monitored in previous studies by a light microscope (9). The degree to which cells were committed to sporulation was also monitored by returning cells from sporulation medium to vegetative medium (25).

The results indicate that during incubation in sporulation medium, sporulating (**a**/α) and nonsporulating (**a**/**a**, α/α) cells produce at least 11 proteins not present in vegetatively growing cells.

MATERIALS AND METHODS

Yeast strains. Diploid strains AP-1 **a**/α, AP-1 α/α, AP-3 **a**/**a**, and AP-3 α/α were obtained from A. Hopper of the University of Massachusetts. AP-1 **a**/α was obtained by crossing strains A 364A (**a** *ade1 ade2 ura1 his7 lys2 try1 gal1*) and $\alpha_1$131-20 (α *ade2* [noncomplementary with *ade2* from A 364A] *ura3 cyh2 can1 leu1*) (9). The α/α diploid (AP-1 α/α) was derived from AP-1 as described previously (9). Diploid

strains AP-3 **a**/**a** and AP-3 α/α were derived from ultraviolet radiation of the diploid AP-3. AP-3 **a**/α was made by crossing A 364A and $\alpha_3$131-20. $\alpha_3$131-20, like $\alpha_1$131-20, is a haploid derived by sporulation of the diploid 131-20.

Media. Yeast stocks were maintained on yeast extract–peptone–dextrose (YPD) agar (4). The semidefined presporulation medium (SA) has been described previously (4, 25). SA consisted of 0.67% yeast nitrogen base (without amino acids), 0.1% yeast extract, and 1% potassium acetate in 0.05 M phthalate buffer (pH 5.0). The defined presporulation medium (SA-YE) used in these experiments was the same as SA with the exception that no yeast extract was added. To both types of vegetative media 40-μg amounts of adenine and uracil were added per ml of growth medium. Cells were sporulated in a 2% potassium acetate solution (pH 6.0).

Growth and sporulation of yeast. Three- to-five-day-old colonies grown on YPD plates were inoculated in liquid vegetative growth medium at a cell density of 10^5 to 2×10^5 cells per ml. At 30°C, the cell generation times in SA and SA-YE media were 140 and 240 min, respectively. For all experiments, strains AP-1 **a**/α, AP-1 α/α, AP-3 **a**/**a**, and AP-3 α/α were maintained in log phase for 30 h on presporulation medium before transfer to sporulation medium. At a concentration of 10^7 cells per ml, cells were collected from the growth medium by centrifugation, washed with 5 volumes of sporulation medium, and suspended in sporulation medium at a concentration of 10^7 cells per ml.

Preparation of labeled samples for gel electrophoresis. Samples for electrophoresis were taken from vegetative medium during log phase and stationary phase and at 2, 4, 6, 8, 12, and 24 h after inoculation in sporulation medium. Cells removed during log and stationary phase were resuspended in 10 ml of fresh presporulation medium containing 100 μCi of [^{35}S]methionine (800 to 1,000 Ci/mmol) (Amersham) for SA or 50 μCi for SA-YE. Samples taken from the sporulation cultures were inoculated in 10 ml of fresh sporulation medium containing 50 μCi of radioactive label. After 1 h of incubation at 30°C, 0.05 mg of nonradioactive methionine per ml of culture medium was added, and the samples were incubated for an additional 10 min. Cells cultured in the presence of [^{35}S]-methionine were washed thoroughly in distilled water and suspended in 500 μl of distilled water. A 25-μl amount of phenylmethylsulfonylfluoride (34 mM stock in absolute ethanol) and 0.3 g of glass beads (0.5-mm diameter) were added to the suspension, and the cells were homogenized for 10 min in a CO_2-cooled Braun homogenizer. Cell lysates were removed from the glass bead mixture, lyophilized, and dissolved in 20 μl of sonic disruption buffer (17). A 1-μl amount of a solution of RNase and DNase (1 mg of each enzyme per ml) was then added to the sample and incubated for 30 min at 0°C. Eighty microliters of lysis buffer (17) was added to the protein solution, blended in a Vortex mixer for 60 s, and then centrifuged in an Eppendorf 3200 centrifuge for 4 min. A 5-μl portion was removed from the supernatant and pipetted into 3 ml of cold 5% trichloroacetic acid, and the acid-insoluble ^{35}S radioactivity was determined in a Nuclear Chicago liquid scintillation counter. Samples containing approximately 10^6 cpm were applied to the first-dimensional gels.

Two-dimensional electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was carried out by a method essentially the same as described by O'Farrell (17) and O'Farrell et al. (18), except that uniform 10% SDS–polyacrylamide gels were used in the second dimension. Electrofocusing was carried out for 7,000 V·h in the case of the isoelectric focusing gels and for 1,600 V·h in the case of the non-equilibrium gradient gels.

Gels were stained with Coomassie brilliant blue as described previously (20) and destained in several changes of 7.5% acetic acid. After destaining, gels were dried immediately and exposed to X-ray film (Ilford Red Seal) or processed for fluorography by the procedure of Bonner and Laskey (1) and then exposed.

Escherichia coli proteins previously identified by two-dimensional gel electrophoresis (19) were used as standard molecular weight markers.

Electron microscopy. Strains of AP-1 **a**/α, AP-3 **a**/**a** and AP-3 α/α were fixed for electron microscopy. Samples of cells were taken during exponential growth and at 2, 4, 6, 8, 12, and 24 h after inoculation into sporulation medium and fixed in 4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.0). After washing, cells were prepared for electron microscopy by the procedure of Zickler and Olson (27) with the exception that Zymolyase 5000 (Kirin Brewery Co., Ltd.) was used to remove cell walls and 0.1 M phosphate buffer (pH 7.0) was used in place of 0.1 M sodium citrate buffer. After dehydration in alcohol, the cells were embedded in Epon. Series of 80 to 120 sections were mounted on Formvar-coated single-holed grids and stained with saturated aqueous uranyl acetate followed by washing and Reynolds lead citrate stain. Electron micrographs were made on 35-mm Kodak fine-grain positive film.

Measurement of commitment to sporulation. Cells were removed after 8 and 13.5 h in sporulation medium, washed with 5 volumes of SA medium, and suspended in SA medium at a concentration of 0.5×10^7 to 1×10^7 cells per ml. Over a 24-h period, 1-ml portions of the culture were removed at hourly intervals and examined under a light microscope for signs of vegetative growth (budding) or commitment to sporulation (percentage of asci). Samples were also removed from the shift-back culture at 0.5-h intervals over the first 4 h and labeled with 1 μCi of [^{35}S] methionine (800 to 1,000 Ci/mmol) per ml of vegetative medium. After 1 h of incubation, the amount of incorporation of radioactive label into protein was determined in a scintillation counter.

RESULTS

Kinetics of ascospore formation. The percentage of cells as asci was determined by light microscopy. The time course of ascus formation observed in two separate experiments for AP-1 **a**/α is shown in Fig. 1. In all experiments conducted with AP-1 **a**/α, the first asci appear 11 h after transfer to sporulation medium, and by 17 h more than 60% of the cells have formed asci. These values are based on the counts of over 200

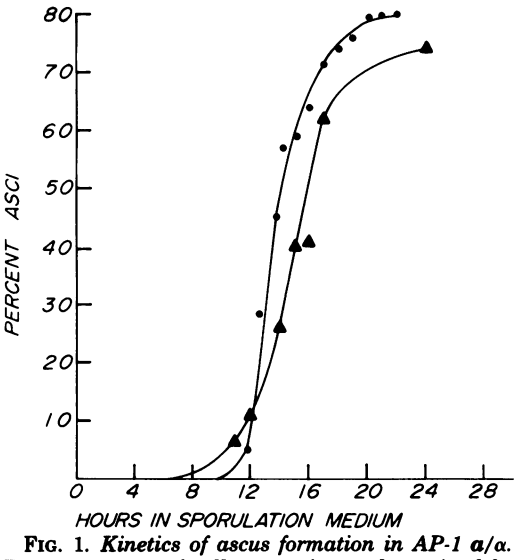

FIG. 1. *Kinetics of ascus formation in AP-1 a/α. The percentage of cells as asci was determined by light microscopy (●, ▲; two separate experiments). Attached and detached buds were not included in the overall cell count.*

cells at each sampling. Cells with a diameter less than 50% of that of a mature separated daughter cell were considered buds and not included in the overall cell count. Previous work by McCusker and Haber (12) showed that when an exponentially growing culture of yeast is exposed to low-pH sporulation medium small buds detach from mother cells. These small daughter cells are viable but do not sporulate and as a consequence tend to lower the estimated percentage of sporulation.

Electron microscopy. The fine structural aspects of "uninuclear" meiosis and ascospore development in **a**/α diploid yeast cells have been described previously (14–16, 21, 27); our observations of AP-1 **a**/α agree with these reports.

The cells of AP-3 **a**/**a** and AP-3 α/α were examined during vegetative log phase and after 2, 4, 6, 8, 12, and 24 h in sporulation medium. Within 2 h on sporulation medium, almost all cells had accumulated as single, unbudded cells. With continued exposure to sporulation medium, the vacuole within the cells became fragmented and diffused, and an increase in numbers of cytoplasmic lipid granules was observed. After cells had been incubated in sporulation medium for 12 h, 8 out of the 11 nuclei examined contained single dense spindle-plaque-bearing microtubules on the nuclear side of the plaque. Microtubules on the cytoplasmic side of the plaque were present in vegetatively growing cells but not obvious in α/α or **a**/**a** cells on sporulation medium. In either of the two strains, at any of the sampling times, there was no evidence of polycomplexes, synaptonemal complexes, or prospore walls.

Commitment to sporulation. After the **a**/α cells had been in sporulation medium for 8 and 13.5 h, 0 and 43% of the cells, respectively, had formed asci. Upon the shift back to vegetative (SA) medium, cells from neither the 8- nor 13.5-h incubation period reverted to normal vegetative growth or continued to sporulate. During this time there was little if any protein synthesis as indicated by the lack of incorporation of radioactive methionine into protein. The **a**/**a** and α/α cells resemble the **a**/α cells in that they too failed to grow and divide or synthesize proteins when returned to vegetative medium.

Two-dimensional electrophoresis. Autoradiograms and/or fluorograms of different exposure times were prepared to minimize autoradiographic spreading and to maximize the resolution of proteins present at low concentrations.

Vegetative medium-specific proteins. Samples were taken during log phase and at stationary phase in SA medium and labeled for 1 h with [^{35}S]methionine as described above. Figure 2 shows the two-dimensional patterns of proteins from labeled vegetative log-phase **a**/α cells. The fluorogram found in Fig. 2A is the result of a combination of isoelectric focusing and SDS electrophoresis (17). By this procedure, proteins with isoelectric points in the range of pH 4 to 7 are distributed across the gel. Figure 2B shows the product of non-equilibrium pH gradient electrophoresis (NEPHGE) (18). This two-dimensional procedure is designed to give high resolution of basic proteins. The black line crossing the gel almost midsection in Fig. 2A correlates with the far left-hand side of the NEPHGE gels shown in this study; the proteins to the right of this line (including those very basic proteins which never enter the gel) are the proteins which appear on the NEPHGE gels. In the NEPHGE gels, resolution of the proteins in the acidic region is poor due to the compression of the pattern; however, these gels resolve approximately 50% more protein spots than those resolved by isoelectric focusing gels. Comparison of the proteins synthesized by cells growing exponentially in SA medium to those synthesized by cells growing in sporulation medium (Fig. 3) reveal that most of the major protein species made vegetatively are also synthesized by sporulating cells. However, nine proteins present in vegetatively growing cells are not synthesized during sporulation (Fig. 2). These proteins, designated vegetative medium-specific proteins, are also synthesized by **a**/**a** and α/α cell types in vegetative (SA) medium (data not shown).

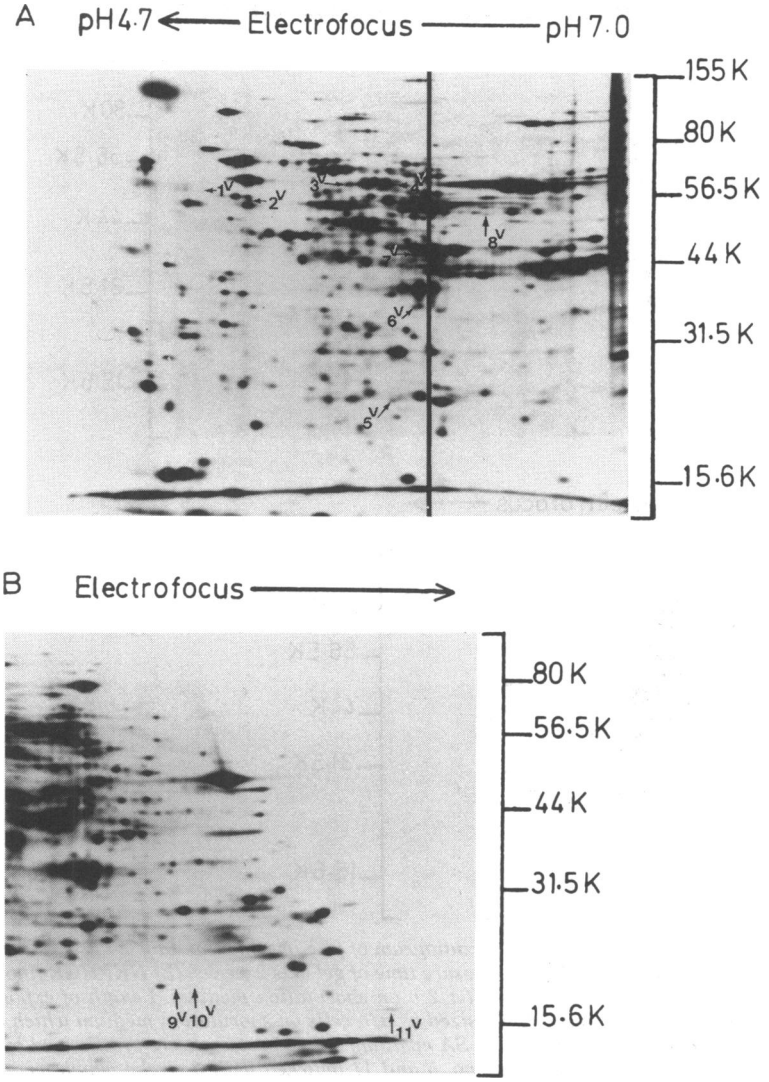

FIG. 2. *(A) Fluorogram of [^{35}S]methionine-labeled proteins synthesized by exponentially growing a/α cells in vegetative growth (SA) medium. The prepared protein sample was subjected to isoelectric focusing in the first dimension at 400 V for 15 h and to SDS electrophoresis in the second dimension at 2.5 W/gel. The second dimension was a uniform 10% SDS–polyacrylamide slab gel. The dried gel was exposed to X-ray film for 5 days. (B) Fluorogram of pulse-labeled proteins of exponentially growing a/α cells in SA medium. The protein sample was prepared for electrophoresis as previously described and subjected to electrofocusing in the first dimension at 400 V for 4 h and to SDS electrophoresis in the second dimension at 2.5 W/gel (NEPHGE electrophoresis). Exposure time of gel was 1 week. Arrows point to the position of protein spots specific to exponentially growing a/α cells in SA medium. Proteins no. 1^v, 2^v, 3^v, 4^v, 7^v, and 8^v in (A) and no. 9^v, 10^v, and 11^v in (B) were also synthesized by a/α cells up to 12 h on sporulation medium; after 24 h on sporulation medium, none of these proteins was present. This considerable time span is partially due to the asynchrony between cells in the sporulating culture. Proteins no. 5 and 6 in (A) were synthesized by a/α cells only under log-phase conditions. E. coli proteins used as molecular weight markers were β subunit of RNA polymerase (molecular weight, 155,000); elongation factor G (molecular weight, 80,000); A protein (molecular weight, 56,500); elongation factor Tu (molecular weight, 44,000); elongation factor Ts (molecular weight, 31,500); ribosomal protein S$_6$ (molecular weight, 15,600) (19).*

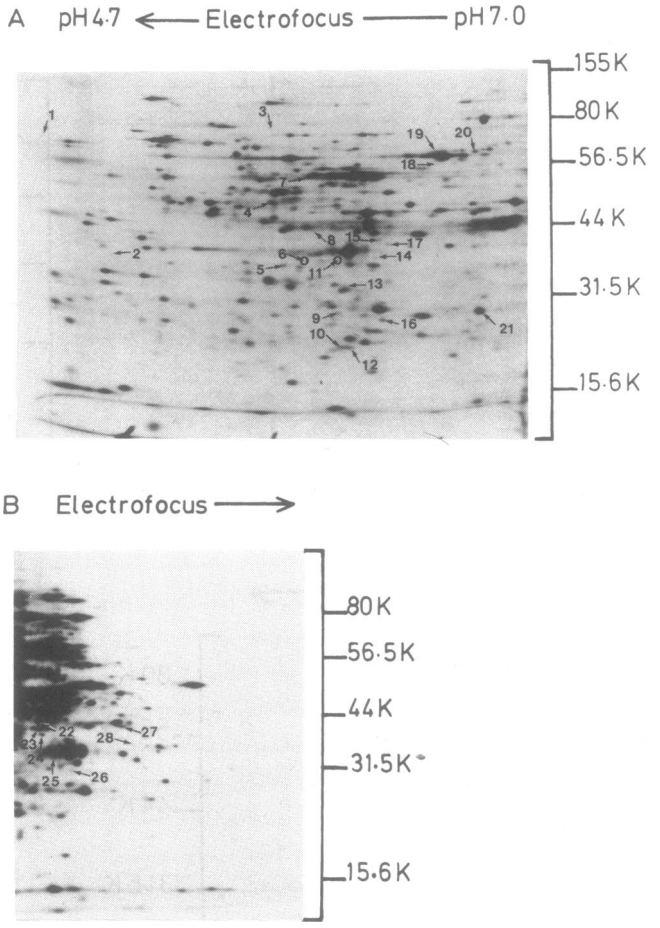

FIG. 3. *(A) Isoelectric focusing-SDS autoradiogram of [^{35}S]methionine-labeled proteins synthesized by a/α cells after 2 h on sporulation medium. Exposure time of gel was 2 weeks. (B) NEPHGE fluorogram of pulse-labeled proteins synthesized by a/α cells after 2 h on sporulation medium. Length of exposure was 5 days. Arrows mark the position of proteins synthesized by a/α cells on sporulation medium which were not present in cells growing exponentially on vegetative SA medium. All 28 proteins were synthesized by the sporulating cells at all sampling times except proteins no. 6 and 11 (marked with a circle) which were not synthesized during the first 2 h in sporulation medium.*

(Note: Faint protein spots on the original autoradiograms are often not visible on the final photograph. To facilitate the detection of those proteins mentioned in the text, especially those proteins too faint to be seen on the print, black arrows have been included in each of the figures to act as position markers.)

Cells in stationary phase in vegetative (SA) medium did not incorporate any radioactivity in protein; therefore, this precluded any comparative analysis.

Sporulation medium-specific proteins. A comparison of the fluorograms of proteins derived from **a**/α, **a**/**a**, and α/α cells growing in SA medium to that from **a**/α cells in sporulation medium revealed 28 proteins which were synthesized exclusively in sporulation medium (Fig. 3). These proteins have been called sporulation medium-specific proteins. However, cells from strains which are genetically incapable of sporulation (**a**/**a**, α/α) synthesized the same 28 proteins when grown in sporulation medium (Fig. 4, 5, and 6), indicating that these proteins may not be sporulation specific or that they may be necessary but not sufficient for sporulation.

Nutritional shift-down-specific proteins. The purpose of the nonsporulating controls in this study was to provide a means of distinguishing proteins specific to sporulation from proteins which were in response to the nutritional shift-down. Sporulating cells (**a**/α) and nonsporulating controls (**a**/**a**, α/α) synthesized 28 proteins

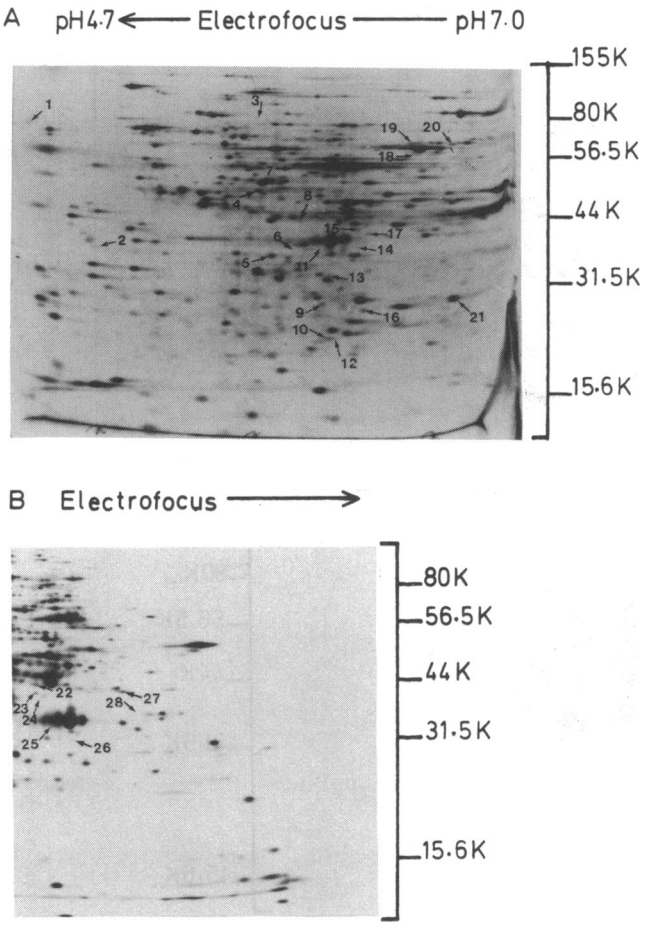

FIG. 4. *(A) Isoelectric focusing–SDS autoradiogram of [^{35}S]methionine-labeled proteins synthesized by AP-1 α/α cells after 2 h on sporulation medium (2-week exposure). (B) NEPHGE autoradiogram of pulse-labeled proteins synthesized by AP-1 α/α cells after 8 h on sporulation medium (2-week exposure). Black arrows mark the positions of the 28 sporulation medium-induced proteins.*

in sporulation medium which were not present in cells grown in enriched presporulation medium (SA). To determine how many of the 28 proteins were indeed nutritional shift-down-specific proteins, **a**/α and α/α cells were grown in an acetate presporulation medium (SA-YE) identical to SA except lacking yeast extract. Cells were grown to log phase and labeled with [^{35}S]methionine as described above. Electrophoresis of the labeled proteins from **a**/α cells (Fig. 7) and α/α cells (data not shown) revealed that 17 out of the 28 sporulation medium-specific proteins were synthesized in the defined presporulation medium and thus presumably were specific for the nutritional shift-down.

DISCUSSION

The purpose of this study was to identify meiotic-specific proteins in *S. cerevisiae* by high resolution two-dimensional polyacrylamide gel electrophoresis (17, 18). That new proteins might be found which are specific for the sporulation process in yeast is suggested by the results of previous investigations. Cytological studies of meiosis and ascospore formation in diploid cells of yeast have identified structures essentially unique to sporulating cells such as meiotic spindle plaques and ascospore walls (16) and synaptonemal complexes (15, 27). These observations lead to the idea that a protein(s) special to meiosis and necessary for the formation of these structures might exist. In addition, certain physiological and biochemical events which are unique to meiosis and ascospore formation and which are dependent on protein synthesis occur during sporulation (11). These sporulation-specific processes include: premeiotic DNA synthesis (9, 24), meiotic recombination (6, 9, 23), gly-

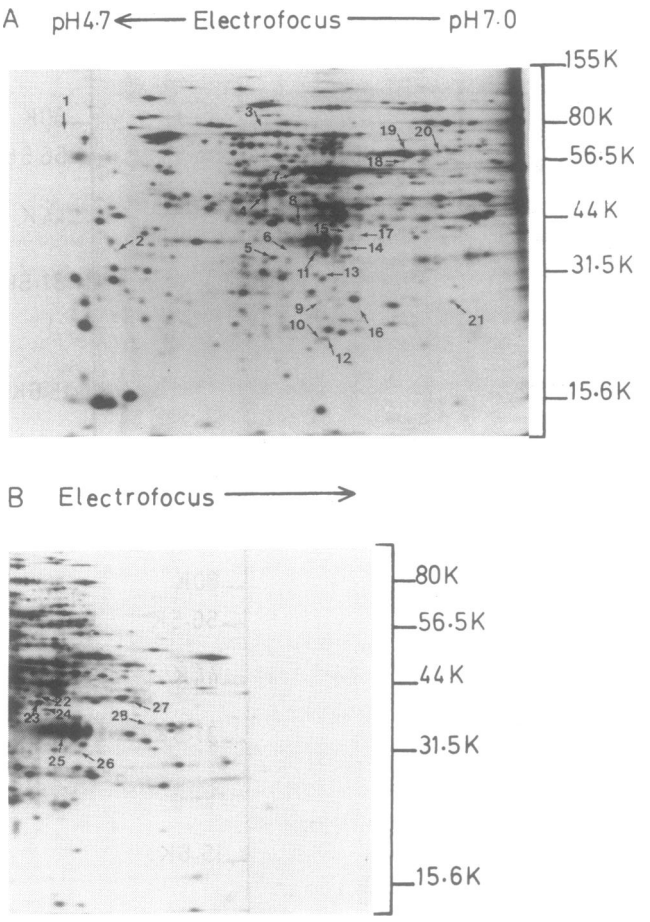

FIG. 5. *(A) Two-dimensional patterns of proteins synthesized by AP-3 a/a. (A) Isoelectric focusing-SDS fluorogram after 12 h on sporulation medium, 3-day exposure; (B) NEPHGE autoradiogram after 2 h on sporulation medium, 2-week exposure. Black arrows serve as position markers to the 28 sporulation medium-induced proteins.*

cogen catabolism (10), and ascus formation (22). The work of Colonna and Magee (2) supports the notion that some of this essential protein synthesis consists of sporulation-specific proteins. From their studies of glycogen catabolism, Colonna and Magee (2) have partially purified a glycogenolytic enzyme with α-1,4-glucosidase activity which appears to be a sporulation-specific gene product.

It is evident from our work that vegetative cells differ from cells incubated in sporulation medium both in the types and the relative amount of proteins being synthesized. Nine proteins were identified which were synthesized in log-phase condition but which were not synthesized by cells in sporulation medium. Conversely, 28 proteins were synthesized by cells in sporulation medium which were absent in cells growing on enriched presporulation medium. Electrophoresis of vegetative log cells grown on a defined presporulation medium revealed that 17 out of these 28 proteins were a generalized response to the nutritional shift-down culture conditions. The remaining 11 proteins (no. 1, 3, 10, 11, 12, 13, 15, 16, 18, and 19 on Fig. 3A; no. 26 on Fig. 3B) were synthesized exclusively in sporulation medium by all cell types, indicating that these proteins may only be a generalized response to nitrogen starvation.

In our experiments radioactive labeling was carried out in fresh sporulation medium adjusted to pH 6.0. Previous work has shown that the pH of the sporulation medium can be adjusted for optimal uptake of radioactive precursors (pH 5.5 to 6.0) with little or no apparent effect on the sporulation process (13). Recently, the tech-

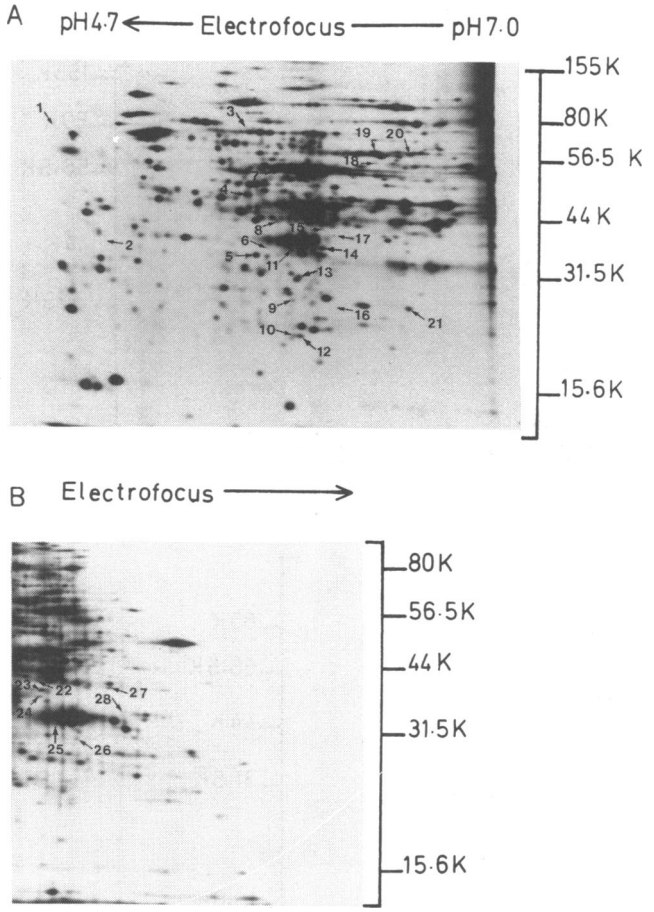

FIG. 6. *(A) AP-3 α/α cells pulse-labeled after 8 h on sporulation medium (isoelectric focusing–SDS fluorogram, 2-day exposure). (B) AP-3 α/α cells after 6 h on sporulation medium (NEPHGE autoradiogram, 2-week exposure). Arrows mark the positions of proteins synthesized exclusively in sporulation medium.*

nique of radioactive labeling with fresh sporulation medium has been criticized by Wejksnora and Haber (26), who showed that sudden change of pH results in abrupt physiological changes within the cell, including an increase in the rates of protein synthesis and RNA processing. And yet it is unlikely that the lack of difference in proteins synthesized in sporulation medium between **a**/α, **a**/**a** and α/α cells is a physiological response to an abrupt shift in pH considering that all 11 sporulation medium-specific proteins (except protein no. 11) appeared within 2 h post-inoculation when the shift in pH from the original inoculum (pH 6.1 to 6.2) (pH profile not shown) to the fresh sporulation labeling medium (pH 6.0) was not abrupt.

The presence of both the **a** and the α alleles of the mating-type gene is essential for meiosis and sporulation to occur (22). Hopper and Hall (8) suggest that the failure to detect proteins specific to sporulating cells is due to the existence of a few "crucial" genes which require both **a** and α for their expression. If so, the sporulation-specific proteins coded by these structural genes may be too few and too low in concentration to be resolved by two-dimensional electrophoresis (10^{-4} to 10^{-5}% of the total protein [17]).

However, the above argument does not rule out the possibility that nitrogen-starved, non-sporulating cells are capable of producing the proteins necessary for sporulation but are blocked from further development because the expression of a few essential gene products is under the control of the **a**/α mating-type locus. In support of this possibility, our studies indicate that **a**/α, **a**/**a**, and α/α diploids enter the meiotic pathway but that **a**/**a** and α/α cells are unable to continue through the pathway. When sporu-

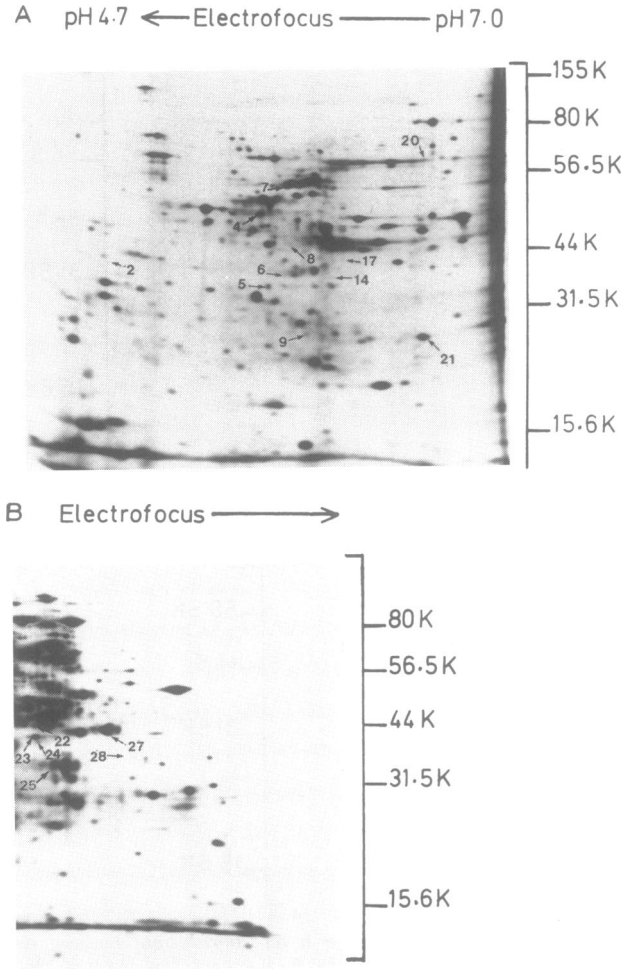

FIG. 7. *Two-dimensional protein patterns of* **a**/α *cells during exponential growth in SA medium containing no yeast extract (SA-YE). (A) Isoelectric focusing–SDS fluorogram, 5-day exposure; (B) NEPHGE fluorogram, 5-day exposure. Seventeen of the 28 sporulation medium-induced proteins synthesized by all cell types (protein position marked by black arrow) were synthesized under these vegetative conditions and therefore were designated nutritional shift-down-specific proteins.*

lating (**a**/α) and nonsporulating (**a**/**a**, α/α) cells are shifted back to presporulation (SA) medium after having been on sporulation medium for 8 and 13.5 h, all cell types are incapable of resuming growth and cell division. We surmise that some commitment to the meiotic pathway has occurred in both the **a**/α and the **a**/**a**, α/α cells. Additional evidence for meiotic development in the nonsporulating controls is provided by our observations made with the electron microscope on the response of these cell types to sporulation medium. In response to the sporulation medium, the vacuole within the **a**/**a** and α/α cells becomes fragmented and diffused, the number of lipid granules is increased, and spindle plaques bearing only nuclear microtubules are observed under an electron microscope. In previous studies, these same cellular responses have been found to be unique to meiotic yeast cells (16, 21). Moreover, we observe that protein synthesis in nonsporulating (**a**/**a**, α/α) cells is greatly decreased during stationary phase in presporulation (SA) medium, but in sporulation medium both **a**/α and **a**/**a**, α/α cells synthesize similar amounts of proteins. This indicates that the **a**/**a** and α/α cells do not act as diploids arrested in stationary phase in sporulation medium—a response expected of yeast cells noninducible for sporulation when placed in a starvation medium (7). Thus, the results from our studies would

imply that the 11 sporulation medium-specific proteins observed in all cell types may indeed be true meiotic-specific proteins.

ACKNOWLEDGMENTS

This work was supported by grants from the National Research Council of Canada (NRC 1901 and NRC A5734) and from the National Cancer Institute of Canada. B.T. was a recipient of an NRC Postgraduate Scholarship.

LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Colonna, W. J., and P. T. Magee.** 1978. Glycogenolytic enzymes in sporulating yeast. J. Bacteriol. **134**:844–853.
3. **Esposito, M. S., and R. E. Esposito.** 1969. The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature-sensitive mutants. Genetics **61**:79–89.
4. **Fast, D.** 1973. Sporulation synchrony of *Saccharomyces cerevisiae* grown in various carbon sources. J. Bacteriol. **116**:925–930.
5. **Friedmann, K. L., and R. Egel.** 1978. Protein patterns during sporulation in fission yeast. Z. Naturforsch. **33**: 84–91.
6. **Friis, J., and H. Roman.** 1968. The effect of mating-type alleles on intragenic recombination in yeast. Genetics **59**:33–36.
7. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle Bacteriol. Rev. **38**:164–198.
8. **Hopper, A. K., and B. D. Hall.** 1975. Mating type and sporulation in yeast. I. Mutations which alter mating-type control over sporulation. Genetics **80**:41–59.
9. **Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall.** 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:619–628.
10. **Kane, S. M., and R. Roth.** 1974. Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118**:8–14.
11. **Magee, P. T., and A. K. Hopper.** 1974. Protein synthesis in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**:952–960.
12. **McCusker, J. H., and J. E. Haber.** 1977. Efficient sporulation of yeast in media buffered near pH 6. J. Bacteriol. **132**:180–185.
13. **Mills, D.** 1972. Effect of pH on adenine and amino acid uptake during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **112**:519–526.
14. **Moens, P. B.** 1971. Fine structure of ascospore development in the yeast *Saccharomyces cerevisiae*. Can. J. Microbiol. **17**:507–510.
15. **Moens, P. B., and E. Rapport.** 1971. Synaptic structures in the nuclei of sporulating yeast, *Saccharomyces cerevisiae* (Hansen). J. Cell. Sci. **9**:665–677.
16. **Moens, P. B., and E. Rapport.** 1971. Spindles, spindle plaques, and meiosis in the yeast *Saccharomyces cerevisiae* (Hansen). J. Cell. Biol. **50**:344–361.
17. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
18. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**:1133–1142.
19. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell **14**:179–190.
20. **Pedersen, S., S. V. Reeh, J. Parker, R. J. Watson, J. D. Friesen, and N. P. Fiil.** 1976. Analysis of the proteins synthesized in ultraviolet light-irradiated *Escherichia coli* following infection with the bacteriophages λdrif^{d}18 and λdfus-s. Mol. Gen. Genet. **144**:339–343.
21. **Rapport, E.** 1971. Some fine structure features of meiosis in the yeast *Saccharomyces cerevisiae*. Can. J. Genet. Cytol. **13**:55–62.
22. **Roman, H., and S. M. Sands.** 1953. Heterogeneity of clones of *Saccharomyces* derived from haploid ascospores. Proc. Natl. Acad. Sci. U.S.A. **39**:171–179.
23. **Roth, R., and S. Fogel.** 1971. A selective system for yeast mutants deficient in meiotic recombination. Mol. Gen. Genet. **112**:295–305.
24. **Roth, R., and K. Lusnak.** 1970. DNA synthesis during yeast sporulation: genetic control of an early developmental event. Science **168**:493–494.
25. **Simchen, G., R. Piñon, and Y. Salts.** 1972. Sporulation in *Saccharomyces cerevisiae*: premeiotic DNA synthesis, readiness and commitment. Exp. Cell Res. **75**:207–218.
26. **Wejksnora, P., and J. E. Haber.** 1976. Influence of pH on the rate of ribosomal ribonucleic acid synthesis during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **127**:128–134.
27. **Zickler, D., and L. W. Olson.** 1975. The synaptonemal complex and the spindle plaque during meiosis in yeast. Chromosoma **50**:1–23.