Spores from the yeast *Saccharomyces cerevisiae* can germinate and resume their vegetative growth when placed in favorable conditions. Biochemical studies on spore germination have been limited by the difficulty of obtaining a pure population of spores germinating synchronously. Here, we report that spores can be purified and sorted according to their size by centrifugal elutriation and that these spores are able to germinate synchronously. Synchronizing their development has allowed reevaluating certain parameters of germination, and we demonstrate that both transcription and translation are induced very rapidly after germination induction. Spores contain mRNAs that are stable for several months in spores kept at 4 °C; however, they are rapidly degraded when germination induction occurs reevaluating certain parameters of germination. The addition of glucose results in rapid induction of transcription and translation. Spores containing mRNAs that are stable for months when the spores are kept at 4 °C; however, they are rapidly degraded when germination is induced. These mRNAs are capped and polyadenylated, and they co-sediment on sucrose gradients with ribosomes and polysomes and with components of the mRNA degradation machinery. The presence of polysomes in the spores led us to evaluate the activity of the translation apparatus in these cells. We present evidence that there is ongoing transcription and translation in nongerminating yeast spores incubated in water at 30 °C, suggesting that these activities could play a role in spore long term survival.

**Saccharomyces cerevisiae** a/a diploid cells, when deprived of nitrogen in the presence of a nonmetabolizable carbon source, can enter a developmental program known as sporulation (1). Yeast sporulation consists of two overlapping processes, meiosis and spore formation. Meiosis leads to the synthesis of four haploid daughter cells that are the yeast gametes. Meanwhile, these gametes are embedded into a specialized structure, the spore, that can resist a variety of harsh treatments and allow these gametes to survive drought, frost, heat, and chemical exposure (2). When favorable conditions return, the spore germinates and resumes its vegetative growth. This germination program includes the activation of cellular metabolism, extensive morphological changes, and reentry into the cell cycle followed by mating. One haploid cell of the a-mating type will encounter one haploid cell of the a-mating type, and their fusion will lead to the formation of a diploid cell, thus completing the sexual cycle (3). The development of germinating spores has been examined by electron microscopy (4–7), and various physiological parameters have been characterized (8–12). For instance, protein synthesis was reported to start 20 min and RNA transcription 70 min after the addition of glucose (13), therefore suggesting that spores must contain mRNA available for immediate translation. More recently, it has been confirmed that spore germination requires active protein synthesis during the first h and that it depends on the Ras signaling pathway (14). However, biochemical analysis was limited by the poor synchrony of germination of spores obtained by classical methods.

In contrast to spore germination, about which little was known, meiosis had been the subject of numerous studies in yeast, and a large body of information was available, which had been completed by transcriptome analyses (15, 16). Meiosis requires the coordinate expression of a large number of genes that are expressed in a timely fashion (15–17). Regulation of mRNA decay represents a significant part of the post-transcriptional control of gene expression (20). The major mRNA degradation pathway in yeast initiates with 3'-poly(A) tail shortening that is catalyzed by the Ccr4p-Pop2p-Not complex (21, 22). It is followed by the removal of the 5'-cap structure by the Dcp1p decapping enzyme, which exposes the body of the transcript to Xrn1p, a 5'-3'-exonuclease (20, 23). A complex of seven Lsm proteins (including Spb8p/Lsm1p) and Pat1p has been shown to interact with mRNA and to be required for mRNA decapping (20, 23–26).

We present here a method of preparing pure spores that germinate with high synchrony, thus enabling a biochemical analysis of germination. The addition of glucose results in rapid induction of transcription and translation. Spores contain mRNAs that are stable for months when the spores are kept at 4 °C; however, they are rapidly degraded when germination is induced. These mRNAs are capped and polyadenylated in spores, and they co-sediment on sucrose gradients with ribosomes and polysomes. We present evidence that in nongerminating spores there is ongoing transcription and translation whose involvement in spore survival will be discussed.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, Spore Elutriation, and Germination Induction**—The diploid Y55 strain used for this study was provided by J. Haber (Y55-523: *MATa/a ura3*). The two HA-tagged cyclin genes (27) were introduced into the Y55 strain by genetic crossing, followed by five back-
crosses, then a double-tagged strain was constructed to yield strain YBL215 (MATAade2 Δleu2-3 adh1-1 CLN2-2-2A CLB5-2A). Yeast cells were handled essentially as described (28). To induce sporulation, 4 liters of diploid cells were grown at 30 °C in YEPA (YEPA: 1% yeast extract, 2% peptone, 2% potassium acetate) for 72 h. RNA was transferred onto a 0.75-mm thick, 24-cm long 6% SSC. For PAGE, RNA was loaded onto a 0.75-mm thick, 24-cm long 6% agarose slab gel before capillary electrophoresis or by PAGE. For agarose gel electrophoresis, 10 total RNA was separated onto a 1.2% agarose slab gel before capillary electrophoresis and autoradiographed. Separation of the yeast spores according to their size was performed with a Beckman elutriation rotor (JE-5.0), using 0.5% Triton X-100 as a counterbalancing fluid. A0.63 nm ~6,000 cells were loaded into the 30-ml elutration chamber and centrifuged at 3,200 rpm at 4 °C. Once the gradient was established, the flow of the pump was increased progressively, and cellular debris were expelled from the chamber first, as checked by microscopic observation. Then, once all cellular debris had been eliminated, the flow was increased regularly until cells began to exit the chamber. Fractions of 250 ml were collected, and cell size was monitored using a cell analyzer system (CASY-TTC, Scharfe System, Germany). Fractions containing spores (average cell diameter ~3 μm) were pooled and resuspended in 0.5% Triton X-100 at a concentration of A0.63 nm ~10.

To induce germination, spores were equilibrated at 30 °C in YEP minus glucose, at a concentration of A600 nm ~1, and a sample was taken immediately before the addition of glucose (time 0). Then, germination was induced by adding 2% glucose, and samples were taken at time intervals, rapidly cooled down, and washed once in TE before freezing the cell pellets in liquid nitrogen.

For scanning electron microscopy examination, cells were fixed overnight in phosphate buffer containing 3.6% formaldehyde, then washed three times in phosphate buffer, once in 50% ethanol, and once in 100% ethanol before cells underwent critical point drying using CO2. Samples were then covered with carbon and viewed using a Joel GSEM5300P microscope.

RNA Procedures—RNA extraction was performed as described previously (29, 30). RNA samples were separated either by agarose gel electrophoresis or by PAGE. For agarose gel electrophoresis, 10 μg of total RNA was separated onto a 1.2% agarose slab gel before capillary transfer to positively charged nylon (Nyttran plus) performed in 10× SSC. For PAGE, RNA was loaded onto a 0.75-mm thick, 24-cm long gel and electrophoresed for 3 h at 750 V. RNA was transferred onto Nylon membrane (Hybond N+, Amersham Biosciences) by electroblotting in 25 mM phosphate buffer (pH 6.8) overnight at 8 V, in the cold room. Hybridization was subsequently carried out, with or without end labels, with labeled oligonucleotides or with randomly primed double-stranded DNA (Neblot kit, Amersham Biosciences).

Poly(A) tail analysis was performed as described previously (32). For RNase H cleavage experiments, 10 μg of total RNA and 100 ng of mRNA-specific oligonucleotide (OBL137: TTGATCTATCGATTTCAAT) were hybridized in 10 μl of 25 mM Tris-Cl (pH 7.5), 1 mM EDTA, 50 mM NaCl for 10 min at 68 °C. Then, the mixture was slowly cooled down until it reached 30 °C and brought to 20 μl in 20 mM Tris-Cl (pH 7.5), 10 mM MgCl2, 0.5 mM EDTA, 50 mM NaCl, 1 mM dithiothreitol, 30 μg/ml bovine serum albumin, and cleavage was performed with 0.25 unit of RNase H (Invitrogen). The reaction was stopped by adding 300 μl of 10 mM Tris-Cl (pH 7.5), 5 mM EDTA, 0.5% SDS, followed by phenol-chloroform extraction and ethanol precipitation. Immunoprecipitation of capped mRNAs was carried out as described (33), using antibody H20 (34) except that incubation with RNA was performed for 3 h.

Protein Extraction from Yeast Cells, Western Blotting, and Immunoprecipitation—Proteins were prepared and analyzed as described previously (28). For Western blot analysis, primary antibodies were added at the following dilutions: anti-Xrn1p (35), 1/5,000; anti-Pab1p (36), 1/5,000; rabbit polyclonal anti-eIF4E (a gift from J. van den Heuvel), 1/2,500; anti-Que1p (37), 1/1,000, anti-BAH, 1/1,000 (Roche Molecular Biochemicals). Anti-mouse and anti-rabbit antibodies coupled to horseradish peroxidase were obtained from Sigma. Polysomes were prepared as originally described (38), modified according to Ref. 30. Immunoprecipitation was performed using native protein extracts and an anti-Pab1p antibody, as described previously (30).

RNA and Protein Labeling—Incorporation of labeled precursors into trichloroacetic acid-precipitable material was used to monitor transcription and translation activities. Cells (2 OD/ml) were labeled with 100 μCi of [35S]methionine for 10 min (germinating spores) or for 0–2 h (resting spores). Total RNA was then extracted, trichloroacetic acid precipitated, and counted. For [35S]methionine incorporation, spores (5 OD/ml) were equilibrated either in YNB or in H2O with or without 100 μg/ml cycloheximide. After 15 min, [35S]methionine was added and incubated for different times as indicated in the figure legends. For incorporation studies, protein extracts were prepared and trichloroacetic acid precipitated. For qualitative analysis, labeled proteins were fractionated onto 12% SDS-PAGE, transferred to nitrocellulose, and autoradiographed.

RESULTS
Preparation of Elutriated Spores—Biochemical analysis of germination has been limited in the past by the difficulty in obtaining large amounts of pure spores germinating synchronously. Indeed, spores prepared using classical methods are contaminated by vegetative cells, and they germinate asynchronously. To overcome these problems, it was necessary to select a yeast strain able to sporulate and germinate efficiently and to design a new method of preparing pure spores able to germinate synchronously. First, we tested several yeast strains for their ability to sporulate and germinate efficiently. For instance, the widely used laboratory strain W303 gave efficient germination but low sporulation rates (~35%). A strain frequently used to study meiosis, SKI, gave a very high sporulation rate (~90%), but the spores thus obtained had a moderate rate and a poor synchrony of germination. We selected for this analysis strain Y55, which combined a high score of sporulation (~75%) with the best rate of germination obtained for the strains that we tested. However, the spores obtained with this strain were still contaminated with vegetative cells, and they germinated asynchronously. Centrifugal elutriation proven to be a powerful tool to separate cells according to their size. For instance, it permits selection of small yeast daughter cells that are able to undergo a full cell cycle synchronously, once returned to the appropriate growth medium (39–41). We reasoned that asynchronous germination of yeast spores also could be because of their size heterogeneity, and we decided to test on this phers the effect of physical separation previously applied to vegetative cells. The Y55 strain was sporulated, and tetrads were enzymatically digested to free the spores from the asc. After several washes and sonication, spores were elutriated, and several fractions were collected and further tested. The major difficulty in handling the spores came from their extreme stickiness, which is likely necessary in their natural environment to attach to their resting and/or feeding substrate. We tested several conditions for elutriation and found that the addition of 0.5% Triton X-100 was sufficient to prevent spore aggregation and clogging of the elutriation apparatus. Once the elutration chamber was filled and the gradient of cells that had formed in the chamber, the flux of the liquid through the chamber was increased progressively to push the smallest spores outside the chamber and to collect them in several fractions. The first fractions, which contained much cellular debris, were tested visually under a microscope and were discarded. Then, spore sorting was monitored by measuring the cell volume using a cell analyzer system (CASY). Before elutriation, the cell suspension was heterogeneous in size (between 2.5- and 7.5-μm diameter) and contained, in addition to the spores, undigested tetrads, diads, vegetative cells, and much cellular debris (Fig. 1A). Pooled elutriated spores had a diameter of ~3 μm (Fig. 1B), and no cellular debris or other cell type could be observed by light microscopy. Spores were also examined by scanning electron microscopy, which confirmed the excellent size homogeneity of the population and the absence of contaminating vegetative cells (Fig. 1C). Starting with A600 nm ~6,000 cells, we routinely obtained A600 nm ~600 eluo...
activity in spores corresponded to 10 min (Fig. 2). Cells were then examined for protein synthesis by measuring incorporation of [3H]uracil for 2 h at 30°C. Incorporation of [3H]uracil reflects transcriptional activity; incorporation of [35S]methionine reflects translational activity. C, proteins synthesized in nongerminating spores were fractionated onto 12% SDS-PAGE, transferred onto membrane, and autoradiographed. VC, vegetative cells incubated for 15 min with or without cycloheximide and then labeled for 5 min with [35S]methionine.

We conclude from these results that yeast spores do possess a basal metabolism when placed in water at 30°C, which corresponds to ~5% of the metabolism of exponentially growing cells.

mRNAs from Spores Cosegregated with Ribosomes, Polysomes, and Components of the Degradation Machinery—To characterize mRNA metabolism in spores further, we first identified mRNAs that were present in spores, and we selected abundant ones to facilitate their analysis. PGK1 mRNA metabolism is well characterized because it has been used in numerous mRNA degradation studies, and it was predicted by a transcriptome analysis to be present in late sporulation (15). Similarly, SPS100 was originally identified by differential screening as a gene induced in late sporulation (43). These two mRNAs turned out to be present in spores, in which they are relatively abundant. Mobilization of mRNA to polysomes reflects their active translational activity (44). Therefore, we analyzed the distribution of PGK1 and SPS100 mRNA after separation of polysomes from free ribosomes on sucrose gradient.

Cellular extracts were fractionated onto linear sucrose gradients, then fractions were tested by Northern blotting to localize the mRNAs and by Western blotting to search for some of the proteins involved in mRNA translation and degradation. Cellular extracts prepared from exponentially growing cells contained large amounts of polysomes, which reflect their high level of translational activity (Fig. 3A). In contrast, cellular extracts from spores contained essentially one type of complex tritiated spores that can still germinate after several months of storage at 4°C in 0.5% Triton X-100.

Nongerminating Spores Exhibit a Significant Level of Metabolic Activity at 30°C—When we initiated this work, it was commonly assumed that spores were resting cells, all of whose metabolic activities were shut off, awaiting a germination signal to resume their vegetative growth (see, e.g., Refs. 10 and 42). We decided to determine carefully whether a basal level of metabolic activity could be detected in elutriated spores prepared from strain Y55. Unexpectedly, when incubated at 30°C in either minimal medium lacking a carbon source or in water, spores were able to incorporate slowly but steadily both uracil and methionine. Incorporation of [3H]uracil for 2 h at 30°C was about half of the level obtained with vegetative cells labeled for 10 min (Fig. 2A and data not shown). Therefore, transcription activity in spores corresponded to ~5% of its level in vegetative cells. Then, we examined protein synthesis by measuring [35S]methionine incorporation rates in spores incubated in water for up to 2 h (Fig. 2B). Incorporation of methionine in spores also corresponded to ~5% of the incorporation in vegetative cells. Similar results were obtained with spores prepared from an SK1 strain. To visualize the synthesized polypeptides, [35S]methionine-labeled proteins were fractionated onto SDS-PAGE, transferred onto membrane, and autoradiographed (Fig. 2C, lanes 1–5). The complex pattern thus obtained revealed that numerous proteins were synthesized in nongerminating spores. Moreover, this pattern is specific for the spore, and it is significantly different from the one obtained with vegetative cells (lane 6). Addition of cycloheximide prevents protein synthesis in vegetative cells (lane 7) but had no effect on spores (data not shown), likely because of the inability of the drug to penetrate the cell wall of nongerminating spores. This last result demonstrates that the translational activity detected in elutriated spores was not caused by contamination by vegetative cells that would have been otherwise inhibited by cycloheximide.

FIG. 1. Spores can be sorted efficiently by centrifugal elutriation. A and B, a diploid Y55 strain was sporulated in liquid medium for 3 days, then spores were freed by digesting the ascus wall with zymolase before being elutriated using a Beckman centrifuge. Samples were taken before and after elutriation and analyzed using a CASY apparatus (respectively, A and B). The cell number is represented as a function of the cell diameter in μm. Likely because of some artifact, the actual peak of elutriated cells (~3 μm) is always preceded by a trace on the record (~2–2.5 μm) which does not correspond to any cells or debris when examined under a microscope. C, scanning electron microscopy picture of elutriated yeast spores.
which likely corresponds to 80 S particles. However, they also contained some larger complexes that are likely polysomes (Fig. 3A). The two mRNAs PGK1 and SPS100 sedimented along with high molecular weight complexes, roughly the size of monosome and small polysome complexes (Fig. 3B). Moreover, Pab1p and eIF4E, the two proteins that bind the poly(A) tails and the cap structure on the mRNA, were also sedimenting with fractions containing the two mRNAs along with monosomes and polysomes, even though a subset of these proteins was also detected in lighter fractions (Fig. 3C). In addition, the exonuclease Xrn1p cosedimented with the two mRNAs and the polysomes. This distribution is very similar to the one observed in exponentially growing vegetative cells, except that the latter possess a much more prominent level of polysomes because of their higher translation rate. In conclusion, in spores mRNAs are engaged on large structures that are likely monosomes and polysomes.

Characterization of the Structure of mRNAs Stored in Spores—Spores are able to survive for very long periods of time when kept at 4 °C, and the level of endogenous mRNAs is little affected in spores that are several months old (data not shown). We decided to characterize the structure of mRNAs from spores to determine whether it differs from vegetative cells. The global average size of the poly(A) tails was determined by the RNase A protection assay (32) and found to be similar in spores and in vegetative cells (data not shown). In addition, more than 90% of PGK1 and SPS100 mRNAs were detected in the polyadenylated fraction after oligo(dT) cellulose fractionation (data not shown). Then, we determined specifically the poly(A) tail length of PGK1 mRNA in spores and found that it is comparable with vegetative cells (Fig. 4A). The structure of the mRNA 5'-end from spores was analyzed by the TAP method (45), and the results suggested that mRNA was capped (data not shown). To confirm this observation, we performed immunoprecipitation experiments using an antibody raised against the trim-
ethyl cap structure of small nuclear RNAs which also reacts with the monomethyl cap structure of mRNAs (34). We used the trimethyl capped U3 snoRNA as a positive control for immunoprecipitation, and the uncapped scR1 RNA was used as a negative control for immunoprecipitation (Fig. 4B). From the results of these experiments we concluded that PGK1 and SPS100 mRNAs are mostly capped in spores.

Spore Metabolism Declines Slowly during Incubation at 30°C in Water—Because spores incubated in water were not fed, we expected their metabolic activities to diminish with time. To test this hypothesis, spores were incubated in water at 30°C, and their ability to incorporate [35S]methionine into proteins was tested at different times. Protein synthesis capacity was reduced to ~50% after 24 h and to 25% after 3 days, and some activity could still be detected after 3 weeks of incubation at 30°C (Fig. 5A). Interestingly, spore viability was also reduced after incubation for several weeks at 30°C; however, this diminishing was delayed compared with the decrease in protein synthesis capacity.

Then, we determined whether this reduction in protein synthesis was accompanied by a similar decrease in the mRNA content of spores. Spores were incubated in water at 30°C, and samples were taken at different times as above. Then, RNA was extracted and analyzed by Northern blotting using a probe for PGK1. When spores were incubated at 30°C, PGK1 mRNA was degraded with a half-life of ~10 h, whereas in spores kept at 4°C it was still detected after 6 months of storage (Fig. 5B and data not shown).

Elutriated Spores from Strain Y55 Germinate with High Synchrony—Elutriated Y55 spores were equilibrated in YEP medium at 30°C, then glucose was added, and samples were taken at different times. The size of the spores increased very slowly during the 1st h and then more rapidly, until the first buds appeared after 4 h of germination (Fig. 6A). Western blot analysis revealed that synthesis of the two cyclins Clb5 and Cln2, which are required, respectively, for DNA replication and bud emergence, occurs 240 min after inducing germination (Fig. 6B). Moreover, electron scanning microscopy analysis of hundreds of cells showed that more than 95% of the germinating spores at a given stage have a similar morphology (Fig. 6C).

Taken together, these observations demonstrate that centrifugal elutriation permits selection of spores that are homogeneous in size and germinate with high synchrony until the entry into the S phase.

Glucose Addition Rapidly Boosts Cellular Metabolism—We examined various metabolic activities during synchronous germination of the Y55 spores prepared using the method described above. [3H]Uracil incorporation indicated that RNA synthesis increased within minutes of the addition of glucose until it reached the level of synthesis of vegetative cells after ~3 h of germination (Fig. 7A and data not shown). To confirm
this observation, we examined directly the synthesis of newly formed molecules. Five different selected mRNAs were clearly accumulated at a high level as early as 15 min after the addition of glucose, as demonstrated by Northern blot analysis (Fig. 7B), a result that was in striking contrast to previous studies (13). In addition to this class of early induced genes, several other genes accumulated later during germination, as it is the case for PGK1 (see below) and S phase cyclins (data not shown).

Protein synthesis during germination was monitored by [35S]methionine incorporation (Fig. 7C). Samples were analyzed by SDSPAGE, transferred onto membrane, and autoradiographed (Fig. 7D). As shown on the figure, mRNA translation started within minutes of the addition of glucose to the spores, and the pattern of newly synthesized proteins evolved during germination. Protein synthesis was also followed by immunoprecipitation of newly synthesized proteins that were radiolabeled by incorporation of [35S]methionine. For instance, 35S-radiolabeled Pab1p was immunoprecipitated as early as 20 min after the addition of glucose, although it was not detected when spores were incubated with a nonmetabolizable analog of glucose (2-O-deoxyglucose) (Fig. 7E). Taken together these results demonstrate that protein synthesis was rapidly induced during germination.

mRNAs from Spores Are Rapidly Degraded upon Germination Induction—In nongerminating spores kept at 4°C, PGK1 or SPS100 mRNAs were very stable, whereas incubation in H2O at 30°C led to the decay of PGK1 after a few days (see above and Fig. 5). Data presented above also indicate that mRNA transcription and translation were rapidly boosted during germination. Therefore, we wondered whether mRNA decay was also boosted early after germination induction. We selected PGK1 and SPS100 mRNAs for this analysis for two reasons. First, their abundance in spores facilitates their detection. Second, SPS100 is expressed exclusively during sporulation and not in vegetative cells (43), and PGK1 de novo synthesis starts only after 70 min of germination (see below). Therefore, because neither of these two mRNAs was transcribed during the 1st h of germination, it was possible to analyze their decay solely. scR1, the RNA component of the SRP, is a very abundant and stable cytoplasmic small RNA (46, 47) that is commonly used as an internal standard for normalizing RNA loading. It is noteworthy that scR1, which was known to undergo very little variation in various physiological situations, is also present at a constant level throughout sporulation and spore germination. PGK1 and SPS100 mRNAs were both rapidly degraded upon germination induction. Although PGK1 mRNA has a half-life of about 45 min in vegetative cells (48), it has a half-life of less than 15 min in germinating spores (Fig. 8, —cycloheximide rows). SPS100 mRNA was degraded with similar kinetics. We conclude that spores contain mRNAs that are stable for several months in resting spores stored at 4°C, but whose degradation is induced very rapidly during germination.

To investigate whether this degradation process was comparable with mRNA degradation in vegetative cells, we tested the effect of cycloheximide on the stability of these mRNAs. Cycloheximide is a translation inhibitor that has pleiotropic effects in the cell. It has been reported to stabilize mRNA and to
prevent decapping (49–51). Cycloheximide had no detectable effect on the level of scr1, as is the case in vegetative cells. In contrast, PGK1 and SPS100 mRNAs were highly stabilized by the addition of cycloheximide to the germination medium. For instance, SPS100 half-life was increased from 15 to 180 min (Fig. 8, +cycloheximide rows). This result suggests that the decay pathway for these mRNAs could be similar in germinating spores and in vegetative cells.

**DISCUSSION**

Yeast spore germination provides a good model for studying the mechanisms that govern exit from dormancy and G0 to G1 transition in a simple eukaryotic organism. However, biochemical studies of this cell developmental model have been limited in the past by the difficulty in preparing pure fractions of spores cleared of any contaminating vegetative cells and able to germinate synchronously. Indeed, studying a particular stage of germination implies the ability to isolate components, such as RNA or proteins, which specifically correspond to that stage. Therefore, we first focused our attention on preparing spores devoid of any contaminating vegetative cells and able to germinate synchronously. We demonstrate in this report that centri
gugal elutriation is a powerful tool in achieving this goal. Examination of purified spores by electron scanning microscopy revealed that elutriated spores were homogeneous in size and not contaminated by any detectable vegetative cells. Monitoring cell size increase, budding index, and the accumulation of polysomes and mRNAs, as is the case for vegetative spores, were significantly lengthened. Under these conditions, we detected both RNA transcription and protein synthesis, and spore metabolism was estimated to correspond to ~5% of exponentially growing cells. Because spores incubated in water at 30 °C were not fed, we hypothesized that their metabolic capacity was fading rapidly with time. Indeed, we observed that protein synthesis activity was reduced to less than 25% after 3 days at 30 °C. We concluded from these experiments that, in striking contrast to what was postulated previously and on which we originally based our work, spores are not quiescent cells exhibiting no metabolic activity. Actually, spores do possess a basal level of RNA transcription and translation when placed in water at 30 °C. Previous studies had reported that translation takes place within 20 min of germination, whereas transcription resumes only after 70 min, therefore suggesting that early translation must take place on mRNA already present in spores (13). Thus, we examined the situation with the Y55 strain chosen for this analysis, and with synchronously germinating spores. Results obtained with incorporation of [3H]uracil could be misleading because of the slow turnover of this precursor. However, our preliminary data indicated that transcription was taking off much more rapidly than described previously. Therefore, we examined the appearance of newly formed molecules that were absent or in low amount in spores. Our data clearly show that transcription of several mRNAs takes place very rapidly after germination induction. Similarly, we demonstrate that translation takes place very early during germination, by measuring [35S]methionine incorporation and by immunoprecipitating newly synthesized molecules. In the light of our results, we propose now that these activities exist in spores and are simply highly boosted upon germination induction, thus explaining why they are detected so early in germination.

These results have greatly modified our view on spore germination and have raised several questions. First, how is metabolism down-regulated at the end of the sporulation program, and what are the key controlling elements? Second, how is the transition from a waking state to an active state controlled by the addition of glucose? Third, why do spores maintain a certain level of protein synthesis during their dormancy? It is conceivable that basal level of RNA and protein synthesis is required for spore survival. Our data indicate that there is a significant delay between the decline of protein synthesis and the decrease of spore viability. However, this lag may simply reflect the fact that proteins synthesized in nongerminating spores have long half-lives. In that case, ongoing metabolic activities in spores could play a role in spore survival, a possibility that should be explored in future experiments. Further work will also include a transcriptome analysis of resting and germinating spores, to broaden our view of the different classes of mRNAs that are either degraded or accumulated in various germination conditions. However, transcriptome analysis of sporulation performed with three different yeast strains has yielded significantly different data for each strain, only a fraction of the genes being regulated similarly in the three strains throughout meiosis and sporulation (16). It is likely that a comparison of transcriptome analyses for different yeast strains will be necessary to provide a clear view of the core genes required to achieve this cell developmental program.

**Acknowledgments**—We thank M. Aldea for the strains expressing the HA-tagged cyclins, J. van den Heuvel for the gift of anti-eIF4E, R. Lührmann for anti-cap, and J. Haber and E. Louis for providing several Y55 strains. We thank E. Schub for an introduction to the elutriation method and for numerous fruitful discussions as well as for generously sharing equipment and supplies. We thank R. Parker, C. Bonnerot, and G. Cathala for help and stimulating discussions, and L. Dirick for critically reading the manuscript.
REFERENCES

1. Esposito, R. E., and Klapholz, S. (1981) in The Molecular Biology of the yeast Saccharomyces: Life Cycle and Inheritance (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) Vol. 2, pp. 211–287, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

2. Kupiec, M., Byers, B., Esposito, R. E., and Mitchell, A. P. (1997) in The Molecular and Cellular Biology of the Yeast Saccharomyces: Cell Cycle and Cell Biology (Pringle, J. R., Broach, J. R., and Jones, E. W., eds) Vol. 2, pp. 889–1030, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

3. Herskowitz, I. (1998) Microbiol. Rev. 52, 536–553

4. Rousseau, P., Halvorson, H. O., Bulla, L. A., Jr., and St. Julian, G. (1972) J. Bacteriol. 108, 1232–1238

5. Hashimoto, T., Conti, S. F., and Naylor, H. B. (1958) J. Bacteriol. 76, 406–416

6. Steele, S. D., and Miller, J. J. (1974) Can. J. Microbiol. 20, 929–933

7. Kreg- Van Rij, N. J. (1978) Arch. Microbiol. 117, 73–77

8. Nagashima, T. (1959) Ecolog. Rev. 15, 75–78

9. Rousseau, P., and Halvorson, H. O. (1973) Can. J. Microbiol. 19, 1311–1318

10. Rousseau, P., and Halvorson, H. O. (1973) Can. J. Microbiol. 19, 547–555

11. Rousseau, P., and Halvorson, H. O. (1973) J. Bacteriol. 113, 1289–1295

12. Chio, S. J., Ferro, A. J., and Shapiro, S. K. (1977) J. Bacteriol. 131, 63–68

13. Xu, G., and West, T. P. (1992) Experientia (Basel) 48, 786–788

14. Herman, P. K., and Rine, J. (1997) EMBO J. 16, 6171–6181

15. Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., and Herskowitz, I. (1998) Science 282, 699–705

16. Primig, M., Williams, R. M.,Winzeler, E. A., Tevzadze, G. G., Conway, A. R., Hwang, S. Y., Davis, R. W., and Esposito, R. E. (2000) Nat. Genet. 26, 415–423

17. Baker, B. S., Carpenter, A. T., Esposito, M. S., Esposito, R. E., and Sandler, L. (1976) Annu. Rev. Genet. 10, 53–154

18. Surdej, P., Riedl, A., and Jacobs-Lorena, M. (1994) Annu. Rev. Genet. 28, 263–292

19. Surdej, P., Riedl, A., and Jacobs-Lorena, M. (1994) Annu. Rev. Genet. 28, 263–292

20. Wilusz, C. J., Wormington, M., and Peltz, S. W. (2001) Nat. Rev. Mol. Cell. Biol. 2, 237–246

21. Tucker, M., Staples, R. R., Valencia-Sanchez, M. A., Muhrad, D., and Parker, R. (2002) EMBO J. 21, 1427–1436

22. Chen, J., Chiang, Y. C., and Denis, C. L. (2002) EMBO J. 21, 1414–1426

23. Tucker, M., and Parker, R. (2000) Annu. Rev. Biochem. 69, 571–595

24. Bouyoret, E., Rigaut, G., Shevchenko, A., Wilm, M., and Seraphin, B. (2000) EMBO J. 19, 1661–1671

25. Boeck, R., Lapeyre, B., Brown, C. E., and Sachs, A. B. (1998) Mol. Cell. Biol. 18, 5062–5072

26. Bonnerot, C., Boeck, R., and Lapeyre, B. (2000) Mol. Cell. Biol. 20, 5939–5946

27. Gallego, C., Gari, E., Colomina, N., Herrero, E., and Aldea, M. (1997) EMBO J. 16, 7196–7206

28. Guthrie, C., and Fink, R. G. (1991) Methods Enzymol. 194

29. Cross, F. R., and Tinkelenberg, A. H. (1991) Cell 65, 875–883

30. Pintard, L., Kressler, D., and Lapeyre, B. (2000) Mol. Cell. Biol. 20, 1370–1381

31. Church, G. M., and Gilbert, W. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1991–1995

32. Sachs, A. B., and Davis, R. W. (1989) Cell 58, 857–867

33. Muhlrad, D., Decker, C. J., and Parker, R. (1994) Genes Dev. 8, 855–866

34. Bochig, P., Reuter, R., Bringmann, P., and Lührmann, R. (1987) Eur. J. Biochem. 168, 461–467

35. Heyer, W. D., Johnson, A. W., Reinhart, U., and Kolodner, R. D. (1995) Mol. Cell. Biol. 15, 2728–2736

36. Adam, S. A., Nakagawa, T., Swanson, M. S., Woodruff, T. K., and Dreyfuss, G. (1986) Mol. Cell. Biol. 6, 2932–2943

37. Tron, T., Yang, M., Dick, P. A., Schmitt, M. E., and Trumpower, B. L. (1995) J. Biol. Chem. 270, 9961–9970

38. Hutchison, H. T., Hartwell, L. H., and McLaughlin, C. S. (1969) J. Bacteriol. 99, 807–814

39. Elliott, S. G., and McLaughlin, C. S. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4384–4388

40. Schwob, E., and Nasmyth, K. (1993) Genes Dev. 7, 1160–1175

41. Johnston, L. H., and Johnson, A. L. (1997) Methods Enzymol. 283, 342–350

42. Harper, J. F., Clancy, M. J., and Magee, P. T. (1980) J. Bacteriol. 143, 958–965

43. Law, D. T., and Segall, J. (1988) Mol. Cell. Biol. 8, 912–922

44. Perry, R. P., and Meyuhas, O. (1990) Enzyme 44, 83–92

45. Couttet, P., Fromont-Racine, M., Steidel, D., Pictet, R., and Grange, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5628–5633

46. Feliz, P., Cesareni, G., and Hughes, J. M. (1989) Mol. Cell. Biol. 9, 3260–3268

47. Bats, R. C., and Walter, P. (1991) Cell 67, 131–144

48. Muhrad, D., Decker, C. J., and Parker, R. (1995) Mol. Cell. Biol. 15, 2145–2156

49. Herrick, T. C., and Jacobs, A. (1990) Mol. Cell. Biol. 10, 2269–2284

50. Beelman, C. A., and Parker, R. (1994) J. Biol. Chem. 269, 9687–9692

51. Hartwell, L. H., Hutchison, H. T., Holland, T. M., and McLaughlin, C. S. (1970) Mol. Gen. Genet. 106, 347–361

52. Dworkin, M. B., Shrutkowski, A., and Dworkin-Rastl, E. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7636–7640

53. Decker, C. J., and Parker, R. (1993) Genes Dev. 7, 1632–1643